ASIATOX
Hangzhou 2021

ASIATOX 2021

The 9th International Congress of ASIATOX
The 8th CST Youth Forum of Science & Technology
第九届亚洲毒理学大会
暨中国毒理学会第八次中青年科技论坛

October 20-23, 2021 Hangzhou · China / Virtual

论文摘要集
Proceedings

CONTENTS

The 9th International Congress of Asian Toxicology (ASIATOX-IX)
8th CST Youth Forum of Science & Technology
第九届亚洲毒理学国际大会暨中国毒理学会
暨第八次中青年科技论坛

Plenary speakers' abstracts

Toxicology in Asia-Past, Present, and Future

Tetsuo Satoh
(ATS, EDJSOT, Emeritus Prof. Chiba University)

Abstract: The Asian Society of Toxicology (ASIATOX), which consists of the eight toxicology member societies of JST, KST, CST, TSTA, TST, TSS, IrSoT, and MySoT, now boasts of more than 3, 000 members from a variety of industries, academia, and regulatory organizations. ASIATOX congresses have been held every three years since its foundation. At the ASIATOX- VIII in Thailand in 2018, the By-Law was amended to space every two years apart. The ASIATOX congresses have been held on the rotation basis among the member societies. In 1995, ASIATOX joined the International Union of Toxicology (IUTOX) as a regional society, and now serves as the scientific voice of toxicology in Asia under the IUTOX umbrella. Since its inauguration, the society has worked diligently to handle matters deemed essential to promoting the vision set fourth by its founders. Future perspectives of ASIATOX include the establishment of education and training programs, and the certification and accreditation of toxicologists. As the leading voice of toxicology in Asia, the society seeks to extend knowledge of toxicological issues to developing nations in Asia based on the following missions and goals：To provide leadership as a worldwide scientific organization that objectively addresses global issues involving the toxicological sciences；To broaden the geographical base of toxicology as a discipline and profession to all countries of the world, and to pursue capacity building in toxicology, particularly in developing countries, while utilizing its global perspective and network to contribute to the enhancement of toxicology education and the career development of young toxicologists.

The progress of clinical trials of COVID-19 vaccine

Feng-cai Zhu
(Jiangsu Provincial center for disease control and prevention)

Abstract: After more than one year of research and development and clinical research on the Cov·id-19, from the earliest vaccine design based on experience, safety and immunogenicity evaluation of the candidate vaccines for emergency use in China and abroad, to scientific interpretation of the effica·cy results of part of the Covid-19; Through a deeper understanding of pathogenic microbial structure, immune response, virus variation and other aspects, in turn, we can see how to accurately design safer and more effective next-generation Covid-19 vaccine. The clinical trial design of the next generation Co·vid-19 vaccine and the new immunization strategy were also introduced.

Data driven candidate-drug safety assessment in large pharma-challenges and opportunities

Lalit Kumar Verma
(Director of Non-Clinical Safety Data Science at Janssen)

Abstract: Bringing transformational, safe therapies to patients takes not only billions of dollars and a decade plus of rigorous, reproducible science but also the cutting-edge scientific innovations involv·ing countless hands & hearts and diversity. It all begins with multi-disciplinary cross-functional research teams diligently carrying out target identification & validation on one hand and therapeutic candidate screening, lead selection & optimization on the other. Through a series of meticulously conducted in-vi·tro assays and in-vivo toxicology & safety studies an NME is born that takes first strides through IND fil·ing and FIH dosing to iteratively develop, advance (through Ph-1/IV clinical trials) and mature into much awaited therapy patients are waiting for. At Janssen R&D, the early pharmaceutical science & methods are producing mind boggling depth & breadth of heterogenous data types and volumes as a digital foot·print of the pharma product draft launch label. This talk will take a deeper dive in highlighting and dis·cussing Janssen applied data science & digital technologies in help solving non-clinical ADME/ T and safety challenges; and turning them into differentiating opportunities hand-in-hand with therapy area leaders, pathologists, toxicologist and in-vivo scientists who answer the questions that matter to de-risk our portfolio. We are on a mission to find better targets and higher quality molecules at economy of scale and accelerated time to NME.

Evolution of food risk assessment: from public policy making to personal healthy choice

Kwon, Hoonjeong
(Department of Food and Nutrition, Seoul National University, 1 Gwanak-ro, Seoul 08826, Korea)

Abstract: Risk Assessment has been a valuable tool for evidence-based policy making for the food-borne hazards. The four steps of risk assessment; hazard identification, hazard characterization, exposure assessment, and risk characterization had been developed and applied for a single chemical, and it is sufficient for the establishment of public policy. In Korea, the consumption of food additives and contamination levels (including heavy metals, POPs, pesticides, mycotoxins, cooking related haz·ards etc.) are continuously monitored every 3~5 years, in order to maintain proper food standards. To·gether with Korean National Health and Nutrition Exanimation Survey (KNHANES) risk assessment da·ta are published every 5 years for the food borne chemicals. The results offer age and gender depen·dent risks, average, median, or high-end risks of individual chemicals. It is certainly valuable asset for a policy maker and contribute to national food safety. These days people focus on personal diet, nutri·tion, and safety. In reality human diet are loaded with chemicals. Announcement like, "Risk of chemical A to average person is negligible", no longer persuade consumers. Since US EPA has published the guideline of the combined risk assessment of the multiple chemical mixture which shares the mode of action, risk assessments of multiple chemicals or multiple routes have been attempted. One of the at·tempts was the combined risk assessments of genotoxic chemicals which are formed during cooking.

Genotoxic carcinogens are measured in total diet study in 4,000 food items and more than 35,000 diet survey data are obtained from KNHANES. Carcinogenic risks are estimated using published oral slope factors or BMDL10 and combined by dishes. Given that the risk is expressed by the dish, the consumer may get insight for the selection. Furthermore, with the development of AI, it may even evolve to personalized dish to incorporate different ingredient. Currently, Korea has public database with 3million dishes with ingredient annotation for machine learning. So, personalized risk assessment is not an unseeable future.

Key words: Food Risk Assessmet

Crresponding author: won, Hoonjeong, E-mail hjkwon@snu.ac.kr

Human Organs-on-a-Chip: a New Trend of Toxicity Testing Alternatives

Zhongze Gu[1,2]

(1. School of Biological Science and Medical Engineering, Southeast University, SiPaiLou #2, Nanjing, Jiangsu 210096, China; 2. Institute of Medical Devices (Suzhou) Southeast University, JinFeng Rd #8, Suzhou, Jiangsu 215163, China)

Abstract: Organs-on-a-chip (OOC) system, or microphysiological system (MPS), is a new type of biomedical research method that aims to recapitulate organ-level tissue structures and functions for drug evaluation and disease modeling. The MPS can be used to simulate the microstructure, microenvironment, and functional features of human organs, and applied in drug screening and clinical diagnosis and treatment. In previous studies, we have developed multiple organ-on-a-chip systems including biomimetic blood vessels, kidney, liver, heart, etc. Our previous work demonstrated that the miniature organs made with advanced microfabrication, 3D printing, microfluidics and tissue engineering techniques could form tissue-specific structures and could maintain some desirable organ functions for drug screening and disease modeling purposes.

In this presentation, we report the development of a two-photon / multi-photon based 3D printing systems for the OOC fabrication and microenvironment formation, and the fabrication of multiple microphysiological systems for disease modeling, and the development of an automated high-content organs-on-a-chip imaging system for automated drug screening together with deep-learning based AI-algorisms for data analysis. The systems that we reported here have been widely applied in drug discovery and toxicity evaluation in collaboration with top-tier pharmaceutical companies in China, and have been used for precision medicine in collaboration with top-tier hospitals. We also report the design and development of a functional Lung-on-a-Chip system for lung bacterial/viral infection, inflammation studies. Lastly, our system and platform have been successfully applied in Covid-19 and other virus infectiousness evaluation, testing of efficacy for drug, neutralizing antibodies (including vaccines from Pfizer, BioNTech, etc.), and other protective measures. In summary, our work demonstrated the usefulness and progressive applications of OOC in multidisciplinary fields in China.

Key words: organs-on-a-chip; tissue engineering; 3D printing; artificial intelligence

Crresponding author: Zhongze Gu, E-mail: gu@seu.edu.cn

Adaptive response and protection against electrophilic stress

Yoshito Kumagai

(Environmental Biology Laboratory, Faculty of Medicine, University of Tsukuba, Japan)

Abstract: We are exposed to numerous xenobiotic electrophiles on a daily basis through living environment, lifestyle, and dietary habits. For example, 1, 4-naphthoquinone is formed by combustion of gasoline and contaminated in PM2.5 and vapor-phase of ambient aerosol. 1,4-Benzoquinone and croton-aldehyde are contained in tobacco smoke. Acrylamide is unexpectedly produced through reaction of sugars with an amino acid such as aspartic acid, during baking food.So, acrylamide is contained in, for example, potato chips. Cadmium is contaminated in rice, and methylmercury is accumulated in fish such as tuna through bio-condensation.(E)-2-Alkenals exist in large quantity in vegetable such as coriander. Since electrophiles have the same chemical property based on electron deficiency, these reactive species covalently modify protein thiols, resulting in formation of protein adducts.

There are a variety of redox signaling pathways consisting of sensor protein with reactive thiols (thiolate ions, S-) and effector molecule such as kinase and transcription factor in cells. Under basal condition, the effector molecule is negatively regulated by sensor proteins. We found that xenobiotic electrophiles activate redox signaling pathways such as PTP1B / EGFR signaling (cell proliferation), Keap1 / Nrf2 pathway (detoxification and excretion of electrophiles), HSP90 / HSF1 signaling (quality control of cellular proteins) and PTEN / Akt signaling (cell growth), through selective modification of sensor proteins at low dose; however, xenobiotic electrophiles cause disruption of the signaling and toxicity through non-selective and extensive modification of cellular proteins at high dose. We also showed that reactive sulfur species (e. g., cysteine persulfide, glutathione (GSH) persulfide, hydrogen sulfide) are able to capture xenobiotic electrophiles, leading to formation of their sulfur adducts, thereby inactivating these electrophiles. Furthermore, it was demonstrated that nuclear factor-erythroid 2-related factor 2 (Nrf2) and cystathionine gamma-lyase (CSE) are involved in decreased risk of electrophilic stress in parallel through GSH and sulfur adduct formation, respectively.

Advanced glycation end products and diabetic retinopathy: The possible molecular mechanisms involved in angiogenesis effects

Meei-Ling Sheu

(Institute of Biomedical Sciences, College of Life Sciences, National Chung Hsing University, Taichung; Department of Medical Research, Taichung Veterans General Hospital, Taichung)

Abstract: Diabetic retinopathy (DR) is a major microvascular complication of diabetes mellitus that can lead to visual impairment and blindness. There is no approved pharmacological treatment for DR; however, laser therapy, steroids and anti-VEGF agents appear to provide some benefit. Nε-(Carboxymethyl)lysine (CML), a majoradvanced glycation end products (AGEs) formed, accumulated under hyperglycemic conditions are thought to play an important role in the pathogenesis of DR. CML can exert their deleterious effects by acting directly to induce vaso-permeability, vascular leakage, inflammation, blood – retinal barrier (BRB) breakdown, capillary degeneration, and neovascularization response of the retinal vasculature. The understanding of the complex cellular and molecular processes involved in BRB leakage has grown rapidly in recent years. However, the exact mechanisms are not yet fully un-

derstood and need further clarification in order to develop new effective drugs for the prevention of reti·nopathy. Appropriate animal models for human conditions like diabetic macular edema are required, these insights have provided tools for rational design of drugs aimed at restoring the BRB as well as for design of effective transport of drugs across the BRB. In serial study, we focus on understanding the biochemical and molecular changes in Endoplasmic reticulum stress(ER stress) and aryl hydrocarbon receptor (AhR), especially early in the diabetic retina may lead to new and effective therapies towards prevention and amelioration of DR, which is important for the millions of individuals who already have or are likely to develop the disease before a cure becomes available.

Key words: Diabetic retinopathy; advanced glycation end products; ER stress; AhR

Crresponding author: Meei-Ling Sheu, E-mail: mlsheu@nchu.edu.tw

Sessions speakers' abstracts

S1 Predictive and Computational Toxicology

Deep Learning on Molecular Structure for Estrogen Receptor Agonism of Chemicals

Wang Liguo, Zhao Lu, Liu Xian, Fu Jianjie, Zhang Aiqian

(State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, P. R. China)

Abstract: Deep learning (DL) offers an unprecedented opportunity to revolutionize the landscape of toxicity prediction based on quantitative structure-activity relationship (QSAR) studies in the big data era. The surprising learning power and flexible structure of deep neural networks allow QSAR models to be provided raw molecular structures, and quantitative descriptors are no longer indispensable. By using data from a set of 18 invitro high-throughput tests for estrogen receptor (ER) activity in ToxCast project, ML methodology study on molecular structure representation has been carried out, aiming to realizing the direct mapping of chemical structure to its ER agonist activity and improve both prediction performance and interpretability of the QSAR model for identification of environmental estrogens. A novel three-dimensional (3D) molecular surface point cloud with electrostatic potential (SepPC) was proposed to describe chemical structures, and a DL architecture SepPCNET was then introduced to di·rectly consume unordered SepPC data of both binary classification and multi-task learning. The ob·tained model recognized the active and inactive chemicals with a total accuracy of 88.3% on the inter·nal test set and 92.5% on the external test set, which outperformed other up-to-date machine learning models. The same network architecture was adopted to successfully establish a multi-task learning model for 18 ER-mediated assays. Additional insights into the toxicity mechanism were gained, which enabled a "black box" model to become more transparent and explainable.

Key words: Deep learning, Toxicity prediction, Structure representation, Model interpretability

Crresponding author: Zhang Aiqian, E-mail: aqzhang@rcees.ac.cn

Development and Improvement of in silico approaches for accelerating regulatory chemical risk assessment

Takashi Yamada
(National Institute of Health Sciences)

Abstract: Risk assessment of a huge number of chemical substances without test data has become a major issue. There is a strong demand for improving the predictive performance and reliability of in silico approaches, expanding the applicability domain, and accelerating the practical use for safety assessment. To meet these regulatory needs, NIHS has conducted developing and improving in silico approaches for major toxicity endpoints for human health effects. Here I show two in silico approaches we work on, one is QSAR for mutagenicity, another is read-across for repeated dose toxicity. NIHS developed a large-scale database for Ames mutagenicity consisting of about 12,000 chemical substances under the Industrial Safety and Health Act in Japan. By sharing this dataset with QSAR vendors all over the world, we conducted international collaborative research for improving the models. Most QSAR tools achieved >50% sensitivity, and accuracy was as high as 80%, almost equivalent to the inter-laboratory reproducibility of Ames tests. In terms of repeated-dose toxicity, NIHS developed case studies of read-across using the category approach, some of which were shared by the OECD IATA Case Studies Project. Through the critical reviews by experts, we figured out several key points for increasing regulatory acceptance, including similarity hypothesis based on possible mechanism, category justification, and endpoint prediction using reliable test data in a transparent and reproducible way. Moreover, we developed a new Adverse Outcome Pathway of histone deacetylase inhibition leading to testicular atrophy via epigenetic changes, which was approved by the OECD in 2021. It is expected to be applicable not only for the prioritization of toxicity testing but also for a mechanism-based integrated approach to toxicity assessment. In recent years, the OECD and other international organizations have been working to develop guidance documents on the use of the in silico methods. The Food Safety Commission of Japan, a risk assessment organization, is developing guidance on the use of QSAR when assessing the mutagenicity of food-related substances. To increase the regulatory acceptance, sufficient reliability needs to be shown. At the same time, efforts should be made to further communicate with stakeholders about the benefits as well as uncertainties and limitations.

Key words: QSAR; Ames mutagenicity; read-across; repeated-dose toxicity

Machine learning as a powerful tool to analyze complicated biological responses

Xiangang Hu
(College of Environmental Science and Engineering, Nankai University, Tianjin China, 300350)

Abstract: The development of machine learning provides solutions for predicting the complicated immune responses and pharmacokinetics of nanoparticles (NPs) *in vivo*, which is critical to the effective design and safe applications of NPs in various fields (e. g., cancer treatment and drug delivery). However, highly heterogeneous data in NP studies remain challenging due to the low interpretability of machine learning. Here, we propose a tree-based random forest feature importance and feature net-

work interaction analysis framework (TBRFA) and accurately predict the pulmonary immune responses and lung burden of NPs, with the correlation coefficient of all training sets > 0.9 and half of the test sets > 0.75. This framework overcomes the feature importance bias brought by small datasets through a multiway importance analysis. TBRFA also builds feature interaction networks that are difficult to iden·tify by machine learning, boosts model interpretability and reveals hidden interactional factors (e.g., var·ious NP properties and exposure conditions). TBRFA provides guidance for the design and application of ideal NPs and discovers the feature interaction networks that contribute to highly complex systems with small-size data, such as human diseases.

Key words: Machine learning; Nanomaterial; AI

Read-across approach for chemical hazard assessment

Wang Ying[a], Kwon Seok[b]

(a. P&G Technology (Beijing) Co., Ltd. No. 35 Yu'an Road, B Zone, Tianzhu Konggang Development Zone, Shunyi District, Beijing, 101312, P.R. China; b. Procter & Gamble (P&G) International Operations, 70 Biopolis Street, Singapore 138547)

Abstract: Structure Activity Relationship (SAR)- based Read-Across is a predictive toxicology ap·proach to establish a safety profile when only limited information is available on a target chemical. When toxicological data are unavailable on a target chemical, but are available on structurally similar chemicals, which are expected to have similar metabolic fate, chem/bio-reactivity and physicochemical properties, those data can be read across to predict the toxicity of the data-deficient chemical. It has been the most widely used animal alternative approach in the past 25 years and is accepted by many regulators globally responsible for chemical registrations including US, Canada, EU, Korea and Japan. Guidance for application of Read-Across has also been published by international authorities, such as OECD and ECHA, as well as literatures1, 2.

The internal P&G integrated framework provides the process for conducting and documenting SAR-based Read-Across assessments, including the considerations of structural similarity, physical- chemical properties, ADME information, reactivity and biology similarity, etc. Firstly, the purpose and data gap(s) of the read across are defined. Then suitable analogues are identified, similarities/suitability of analog(s) are evaluated, toxicity data is collected, and the final read-across hypothesis is verified. Key scientific elements must be considered to complete a read-across assessment. Ultimately the SAR- based read-across should be fully described in the assessment with justification, which provides the sci·entific rationale to support the hypothesis of similarity, explains why the read-across is appropriate and also outlines any areas of uncertainty. The SAR-based read-across assessment is a complex and itera·tive process typically requiring some degree of expert judgement. The weight of evidence (WoE) ap·proach is used to evaluate and integrate multiple information streams to support the read-across. Final·ly, a conclusion is determined for the read-across based on the strength of the WoE, clarity of the justifi·cation, consideration of residual uncertainties, and the original purpose of read-across.

Along with additional trainings and development of new approach methods (NAM), read-across can be ap·plied and accepted more broadly. In this presentation, we will also introduce an interdisciplinary Read-Across work group at Korea Society of Toxicology consisting of safety experts from public and private sectors.

Key words: SAR (structure activity relationship)-based Read Across; Animal Alternative

Crresponding author: Wang Ying, E-mail: wang.yi.13@pg.com; Kwon Seok, E-mail: kwon.s@pg.com

Could superoxide radical be implemented in decontamination processes?

Ruiyang Xiao

(1. Institute of Environmental Engineering, School of Metallurgy and Environment, Central South University; 2. Chinese National Engineering Research Center for Control & Treatment of Heavy Metal Pollution)

Abstract: Contemporary studies emphasize that superoxide radical (O2 • −) exhibits the potential to degrade organic contaminants, but practical application of this radical in engineered waters require an in-depth understanding of its kinetic profiles in a quantitative way. Here, we developed, for the first time, a convenient and reliable approach to generate micromolar level O2 • − in aqueous solution by photolysis of formate and H2O2. The presence of O2 • − was confirmed by comparing the UV spectra under pulse radiolysis and chromogenic reaction. We then constructed an in situ long-path spectroscopy to investigate the kinetics and mechanisms of O2•−-mediated degradation of representative emerging contaminants, including antibiotics and perfluorocarboxylic acids (PFCAs). In addition, we employed the transition state theory to model the reaction rate constants. Both results show that O2•− exhibited low reactivity towards these containments. In addition, the solvation mechanisms for O2•−-mediated degradation of the contaminants were elucidated. The complementary experimental and theoretical approaches provide a mechanistic basis for better understanding aqueous-phase O2 • − chemistry and a holistic evaluation on the application of O2 • − for the degradation of organic contaminants of emerging concern.

Key words: Superoxide radical; In situ long-path spectroscopy; Aqueous solution; Mechanism; Kinetics

S2 Clinical Toxicology

Toxicoepidemiologic studies using poison control center data and other databases

Sahaphume Srisuma

(MD Ramathibodi Poison Center, Mahidol University, Bangkok, Thailand)

Abstract: Poison Control Centers (PCC) give toxicological consultation service to both hospitals and publics. Many toxicological studies report the data from PCC databases including study of natural toxins, medication, drugs of abuse, chemicals, or envenomation. Data from PCC databases can use for both descriptive and analytic studies. Many PCCs can collaborate to do study together. Other databases such as FDA product vigilance data, Health Data System of Department of Health, Forensic databases are also great resource for studies. Social network listening tools are interesting sources for toxicoepidemiologic studies. Using these databases together may give more comprehensive view of the toxicological issues in societies.

pancreatic injury after paraquat ingestion: from a medical center database

Yi Li

(Department of Emergency Medicine, Peking Union Medical College Hospital. Dr. Gao Yanxia, Department of Emergency Medicine, The First Affiliated Hospital of Zhengzhou University)

Abstract: Poisoning diseases rank high in the cause of death. Based on the clinical big data of the First Affiliated Hospital of Zhengzhou University, we established a poisoning disease database. Through analysis, we found that pesticide poisoning accounts for about 40% of poisoning diseases, and paraquat poisoning is the main type of pesticide poisoning (54.6%). Paraquat is of great benefit in agricultural production, but it is highly toxic to humans and animals, with high mortality after exposure and no specific antidote, so it is banned in more and more countries. However, paraquat has an unparalleled advantage in that it causes minimal damage to the environment. If we can fully understand the harm it brings and carry out corresponding research, it can promote the development of agriculture while reducing the hazard to the humans and environment.

The main cause of death induced by paraquat poisoning is multi-organ failure. At present, research on paraquat focuses on its target organ, the lung. Our previous research also proved that paraquat could promote acute lung injury in rats by regulating alveolar macrophage polarization through glycolysis. In the clinical observation study of multiple organ damage caused by paraquat, we found that the incidence of pancreatic injury is second only to gastrointestinal injury, and the initial time of pancreatic injury is earlier than other important organs. We further studied the relationship between elevated pancreatin and the prognosis of paraquat and found that abnormal pancreatic enzymes are useful prognostic markers of death after acute paraquat poisoning. Since then, we have used the paraquat rat model to verify that paraquat can cause pathological damage to the pancreas regardless of whether it is infected through the digestive tract, and the pancreatic damage caused by paraquat intragastric administration is more serious than that caused by intraperitoneal injection. In addition, we also used octreotide, a drug commonly used to treat pancreatitis, to intervene in paraquat poisoned rats, and found that octreotide can antagonize pancreatic injury and alleviate mortality induced by paraquat, which indicates that octreotide has potential clinical value in improving the prognosis of PQ poisoning.

In summary, pancreatic injury plays an important role in paraquat poisoning, and it is worthy of attention in clinical diagnosis and treatment, as well as more in-depth research.

Crresponding author: Yi Li, E-mail: billliyi@126.com

S3 Reproductive and Developmental Toxicology

Assessment of developmental toxicity caused by organophosphate triesters and their metabolites using zebrafish

Kubota Akira[a], Lee Jae Seung[a], Kawai Yusuke K[a], Covaci Adrian[b]

(a. Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine; b. Department of Pharmaceutical Sciences, University of Antwerp)

Abstract: Organophosphate triesters are widely used as additives in plasticizers and flame retar·

dants. Increasing levels of human and environmental exposure to organophosphorus flame retardants (OPFRs) require adequate assessments for their toxicity. Developmental toxicity induced by several OPFRs has already been reported, while studies on developmental toxicity of their metabolites are still limited. The present study was aimed at characterizing the developmental toxicity of OPFR metabolites, in comparison to their parent compounds, in zebrafish embryos (*Danio rerio*) using morphological and molecular assessment. Zebrafish embryos at 72 hours post fertilization (hpf) were exposed to selected OPFRs (TPHP, TDCIPP, TCEP, and EHDPHP) and their metabolites (HO-m / p-TPHP, DPHP, BDCIPP, BCEP, 5-HO-EHDPHP, and EHPHP) for 24 hours, and were used for morphological assessment. RNA-seq was also performed using embryos exposed to EHDPHP and its metabolites to assess molecular mechanisms of developmental toxicity. Significant increases in the percent incidence of pericardial edema and reduction in blood circulation were observed for TPHP, TDCIPP, and some of their metabolites (HO-m-TPHP, HO-p-TPHP, and 5-HO-EHDPHP). None of other OPFR metabolites tested showed any significant circulatory failure even at the highest concentration (30 μM). RNA-seq analysis revealed that genes related to hexose metabolism (*fam3a*, *fbp1b*, *g6pd*, and *gck*) and nutrient level (*gabarapa*, *lepb*, and *ucn3l*) were significantly altered by EHDPHP (3 μM) and 5-HO-EHDPHP (10 μM). On the other hand, EHPHP exhibited significant changes in cellular lipid metabolic process (acot14, lpcat3, ptgis, ptgs2b, and zgc:174917). Glycoproteins such as hexose have been reported to be closely related to repair and necrosis of myocardial cells. Thus, it is indicated that circulatory failure caused by EHDPHP and 5-HO-EHDPHP might be related to altered hexose metabolism but not through lipid metabolism. The present study demonstrated that several OPFR metabolites showed equipotent cardiovascular toxicity as compared to their parent compounds. Furthermore, a hexose metabolism pathway was shown as one of the possible key events for circulatory failure caused by EHDPHP and its metabolite in zebrafish embryos. The current study has established foundation to understand the developmental cardiovascular toxicity induced by OPFRs and their metabolites in zebrafish embryos.

Key words: Organophosphate triesters, circulatory failure, zebrafish embryos

Crresponding author: Kubota Akira, E-mail: akubota@obihiro.ac.jp

Effects of glufosinate-ammonium on male reproductive health: Focus on epigenome and transcriptome in mouse sperm

Ma Xuan, Hu Weiyue, Xia Yankai

(State Key Laboratory of Reproductive Medicine, Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing 211166, China)

Abstract: Glufosinate-ammonium (GLA) is a widely used herbicide with emerging concern over its neural and reproductive toxicity. To uncover potential effects of GLA on male reproductive health in mammals, adult male C57BL/6J mice were administered 0.2 mg/kg·d GLA for 5 weeks, and then copulated with female DBA/ 2 mice. After examination on fertility, testis histology and semen quality in the GLA group, we performed deep sequencing to identify DNA methylation, transcriptionally active (H3K4me3 and H3K27ac) and repressive (H3K27me3 and H3K9me3) histone modifications, and mRNA transcript levels in sperm. Moreover, RNA sequencing was also performed on preimplantation embryos to reveal whether these histone modification alterations would cause any abnormal gene expression after fertilization. We found no significant abnormality either on fertility, testis histology or se·

men quality-related indicators in GLA mice. Next generation sequencing showed alterations of these epigenetic marks and extensive transcription inhibition in sperm. Differential active marks were en·riched at promoters and putative enhancers, while repressive marks were mainly distributed at intergen·ic regions and introns. They were mainly enriched in pathways related to synapse organization. When we zoomed in these regions, increased H3K4me3 overlaps H3K27ac loci at the gene promoter of Phkg2, which was actively expressed in GLA sperm. Additionally, decreased H3K4me3 overlaps H3K27ac at the promoter of Dcn in sperm, which was also down-regulated in GLA preimplantation em·bryos. Subtle differences in genomic imprinting were observed between the two groups. These results suggested that GLA predominantly impaired sperm epigenome and transcriptome in mice, with little ef·fect on fertility, testis histology or semen quality. These alterations in sperm epigenetic marks also dis·rupted normal gene expression after fertilization. Further studies on human sperm using similar strate·gies need to be conducted for a better understanding of the male reproductive toxicity of GLA.

Key words: GLA; DNA methylation; histone modification; male reproduction

Crresponding author: Hu Weiyue, E-mail: weiyuehu@njmu.edu.cn; Xia Yankai, E-mail: yankaix·ia@njmu.edu.cn

Exposure to BPS exerts cytotoxic effect on mouse Leydig cells: involve-ment of mitochondrial dysfunction and defective mitophagy

Zhang Wenjuan, Huang Tao, Sun Zhangbei, Zhang Dalei

(Department of Physiology, School of Basic Medical Sciences, Nanchang University, Nanchang 330006, China)

Abstract: Bisphenol S (BPS), a predominant alternative to bisphenol A, is drawing an increasing attention due to its probable detriment to human health. Epidemiological investigations have shown that exposure to BPS is associated with defective semen parameters. In laboratory animals, it has been linked to testosterone deficiency and male reproductive dysfunction. In this study, TM3 mouse Leydig cells were *in vitro* treated with BPS (100, 200 and 400 μM) for 48 h to examine the cytotoxicity of BPS and to investigate its underlying mechanisms. Our results indicated that exposure to BPS at all concen·trations tested significantly suppressed the viability of TM3 cells in a concentration-dependent manner. Furthermore, BPS challenge triggered oxidative stress manifested by exaggerated generation of reac·tive oxygen species (ROS) andlipid peroxidation product malondialdehyde (MDA) withcompromised ac·tivities of antioxidases superoxide dismutase and catalase. Particularly, BPS incubation evoked an aug·ment of mitochondrial permeability transition pore opening, a dissipation of mitochondrial membrane po·tential and a reduction of ATP production. Targeted metabolomics suggested that exposure to BPS for 48 h resulted in a disorder of energy metabolism and the altered metabolites were mainly involved in the glycolytic pathway. Moreover, BAX expression and caspase-3 activity were markedly elevated and BCL-2 expression were notably inhibited after exposure to intermediate and high doses of BPS. Howev·er,the combined treatment with Mito-TEMPO, a mitochondria-targeted antioxidant, significantly restored the viability, decreased ROS and MDA formation and inhibited apoptosis in BPS-exposed TM3 cells. In addition, BPS-treated TM3 cells exhibited an accumulation of autophagic vacuoles in the cytoplasm, along with up-regulated Beclin1 and P62 expression and increased LC3B-II/LC3B-I ratio. Taken togeth·er, exposure to BPS in vitro elicited cytotoxicity to TM3 Leydig cells by inducing oxidative stress and mi·tochondrial impairment, subsequently resulting in autophagic disturbance and apoptosis. The antioxi·

dant intervention targeting mitochondria can protect against BPS-induced Leydig cell damage through alleviating oxidative stress and apoptosis.

Key words: bisphenol S; oxidative stress; mitochondrial dysfunction; autophagy; apoptosis

Crresponding author: Zhang Dalei, E-mail: zhangdalei@ncu.edu.cn

A Weight of evidence assessment of developmental and reproductive toxicity of diisononyl phthalate under GHS

Zou Hanjun[a]*, Palermo Christine[b], Norman John[b], Green Maia[b]

(a. ExxonMobil (China) Investment Co., Ltd., 1099 Zixing Road, Shanghai, 200241, PRC; b. ExxonMobil Biomedical Sciences, Inc., 1545 US Highway 22 East, Annandale, NJ, 08801, USA)

Abstract: Low Molecular Weight (LMW) phthalates (such as DEHP, BBP and DBP) demonstrate clear toxicity meeting the criteria for GHS classification for reproductive and developmental toxicity. However, evaluations of High Molecular Weight (HMW) phthalate, i.e., DINP, continue to be proposed by different organizations/agencies globally with variable conclusions. The main reasons include discordant views on biological effect versus adversity, misinterpretation of data/data quality, bias on data selection, different rules/framework used for toxicity classification and also a lack of transparency. In this effort a systematic literature review on the reproductive and developmental toxicity data for DINP was carried out in a bid to conclude the classification under globally harmonized classification framework, i.e., UN GHS, following and updating the systematic review conducted by Dekant and Bridges. We screened records from seven bibliographic databases and scored papers using a framework developed by Dekant and Bridges for assessing animal toxicity studies. Papers meeting the criteria (i. e., a minimum score) were used to evaluate DINP against the GHS criteria for the classification of reproductive and developmental toxicity. A weight of evidence analysis indicates DINP did not interfere with sexual function or fertility. Moreover, DINP did not cause effects on fetal development warranting classification, including no effects on male reproductive development (i.e., a lack of association with hypospadias, cryptorchidism, permanent nipple retention or anogenital distance as seen with LWM phthalates) following exposure during the androgen sensitive window of development. Therefore, DINP does not elicit adverse effects warranting classification as a reproductive or developmental toxicant under GHS criteria.

Key word: Phthalate; DINP; GHS Classification; Reproductive and developmental toxicity; Systematic review; Weight of evidence

Crresponding author: Zou Hanjun, E-mail: steven.hj.zou@exxonmobil.com

S5 COVID-19 Vaccines and Therapeutics: from Industry to Regulatory Perspectives

Overview of non-clinical safety assesssment for antiviral drugs development

Hanan Ghantous

(U.S. FDA – Center for Drug Evaluation and Research, USA)

Abstract: Drug Development is an integrated, multidisciplinary process that includes nonclinical

safety assessment, manufacturing, clinical trials, and regulatory submissions. This presentation will provide an introductory overview of drug development and nonclinical safety assessment for drugs and biologics in general and specifically antivirals including COVID-19. Description of some of the challenges that can arise with diseases or conditions with potentially fatal outcomes, and/or for which there are no existing effective treatment options will be discussed. The nonclinical programs for Antivirals like COVID-19 / Ebola indications are designed to be flexible, with the goal of facilitating entry into clinical trials and accelerating the development of promising pharmaceutical candidates while also protecting patients from adverse effects. The FDA lately issued new draft guidance to address challenges related to COVID-19, one to facilitate a sponsor's preparation of, and the FDA review of, a pre-investigational new drug application (pre-IND) meeting request, and another with recommendations to develop drugs with direct antiviral activity, immunomodulatory activity, or other mechanisms of action. Case examples will be discussed.

Non-clinical safety assesssment for preventative vaccines development

Nabil Al-Humadi
(U.S. FDA-Center for Biologics Evaluation and Research, USA)

Abstract: Vaccines are considered to be one of the most cost-effective life-saving interventions in human history. Vaccine development requires pre-clinical toxicology studies, following good laboratory practice (GLP). Vaccines are developed as biological preparations to stimulate the recipient's immune system to recognize targeted aspects of infectious organisms as foreign and generate host mechanisms to control or eliminate them. Toxicology studies are performed to support the establishment of nonclinical safety of vaccines prior to their use in clinical investigations. Careful consideration should be given to the collection of precise information from properly designed toxicology studies. Assessment of safety relies on various endpoints including, but are not limited to: measurement of inflammatory cells at the site of injection, changes in food consumption, body weight changes, body temperature changes, clinical chemistry measurements, and histopathology examination.

Rapid Development of REGEN-COV, an anti-spike antibody cocktail for treatment and prevention of COVID-19

Matt Y. Liu
(Schrodinger Inc., USA)

Abstract: An urgent global quest for effective therapies to prevent and treat COVID-19 disease is still ongoing. Regeneron took parallel efforts using both humanized mice and convalescent patients to generate antibodies against SARS-CoV-2 spike protein. From a large collection of fully-human antibodies, Regeneron selected two potent, neutralizing antibodies targeting distinct noncompeting epitopes on the receptor binding domain (RBD) of the SARS-CoV-2 Spike (S) protein. Regeneron quickly moved the two antibodies cocktail, REGN-COV2 (casirivimab and imdevimab), from preclinical phase to clinical testing and later receiving Emergency Use Authorization (EUA) from the U.S. Food and Drug Administration (FDA) as the first cocktail mAbs for COVID-19. This presentation will cover the area of

the preclinical characterization of the two antibodies, and/or rapid interactions with Agency, and clinical efficacy of REGEN-COV.

S6 Nonclinical Safety Testing Strategy of Novel Therapies

AAV Gene therapy and potential safety considerations: Focus on genome integration

Jing Yuan, PhD
〔 *Drug Safety Research & Development (DSRD), Pfizer Worldwide Research, Development and Medical* 〕

Abstract: Gene therapy is an approach to treating human disorders through the delivery of therapeutic genetic materials to diseased cells. Many different viruses have been explored as viral vectors for gene delivery. Among different viral vectors, adeno-associated virus (AAV) has been the most widely used vector in clinical trials for *in vivo* gene therapy (Lundstrom, 2018). One key reason is its apparent safety profile in humans. AAV is present naturally in human and no clinical diseases have been associated with wildtype AAV infections. Recombinant AAV (rAAV) was developed as a gene delivery vehicle where all AAV protein-coding sequences are replaced with therapeutic gene expression cassettes. rAAV gene therapy is generally considered safe for use in humans. The major adverse events include acute liver toxicity, thrombotic microangiopathy, and possible nervous system toxicity (Colella, Ronzitti et al. 2018). More than two decades of human clinical trial experience and long-term follow-up with AAV has not revealed late toxic effects in humans (Kuzmin, Shutova et al. 2021). However, rAAV is found to integrate at very low frequency into the host cell genome following transduction which caused hepatocellular carcinoma in neonatal mice. The presentation will introduce rAAV gene therapy safety assessment with the focus on evidence of host genome integration in animal models. In addition, clinical experience and regulatory guidance related to AAV genome integration will be discussed.

Safety assessment of CART

Tiomthy Maclachian
Novartis

Abstract: Use of modified T-cells has been an evolving therapy for cancer over the last two decades. An advanced version of this, Chimeric Antigen Receptor-T cells, or CAR-T, has been the basis for five regulatory approvals since 2017. There are clear safety concerns with this modality, categorized as on-target/ on-tumor, on-target/ off-tumor and off-target/ off-tumor. On-target concerns are predominated by cytokine release and neurotoxicity, as well as potential targeting of normal tissue. Off-target concerns have rarely been seen, however are a significant concern to rule out in the preclinical space owing to the potency of the therapeutic. How these safety issues are evaluated non-clinically are in development, focusing on the specificity of the CAR as well as the location of the target. These approaches as well as more recent advances in animal models will be discussed.

S7 ICH M7 Principals and Nitrosamines

ICH M7 Principles and Nitrosamines

Andreas Hartman
(PreClinical Safety Novartis Pharma, Switzerland)

Abstract: The current and evolving knowledge of N-nitrosamine toxicity will briefly be summarized. Since there are important common aspects regarding safety and quality, the inter-relationships between these two disciplines will be highlighted. For ensuring the quality of medicines to patients, current questions on quality and control strategy will be summarized. The presentation will also contain a review of recent scientific meetings between Industry Associations and Regulatory Bodies, specifically, a EMA Safety Working Party meeting with Industry and a meeting organized by PhRMA. Areas of convergence and divergence between Industry and Regulators such as acceptance of bacterial mutagenicity data and requests for *in vivo* follow-up studies will be discussed. The presentation will be concluded by highlighting data and information needed to improve current approaches.

Assessment of nitrosamines in Amesassay

Andreas Czich
(Preclinical Safety Operational Center Sanofi, Germany)

Abstract: The Ames assay is considered as a quick and robust assay for the detection of direct DNA interacting mutagens and thus the detection of potential mutagenic carcinogens. Therefore, many prediction models are built on his data and the assay itself is an integral part of the testing strategies for pharmaceuticals, chemicals, cosmetics, and other classes of compounds. The test design, the strength and the weakness for this assay are described in many publications and in the OECD 471 Guideline.
For pharmaceuticals, the assay plays an important role for the mutagenicity assessment of impurities. Under ICH M7, it is described that impurities are assessed using the bacterial reverse mutation assay in case of a positive in silico prediction followed by expert review. Negative Ames and in silico results allow the impurity to be managed without concern for mutagenic carcinogenicity under ICH Q3. Compounds with positive results are controlled to the Toxicological Threshold of Concern (TTC) or an appropriate acceptable intake (AI) in case of robust carcinogenicity data.

N-Nitrosamines (NAs) have been of recent concern as impurities in pharmaceuticals. This concern is mainly based on a subgroup of Nitrosamines, which are highly potent mutagenic carcinogens in rodent bioassays. Concern has been raised that the Ames test may not be sensitive enough to detect the mutagenic potential of NAs, based on literature data from the 70's and 80's. In this presentation, we will provide results of further investigations about the predictivity of the standard OECD 471 compliant Ames assay.

An analysis to determine the Ames test predictivity for carcinogenic outcomes was conducted with an emphasis on different experimental conditions. In addition, three model NAs (NDMA, NDEA and DIPNA) were tested using different experimental conditions to determine the effect on the Ames test outcome. This analysis found that the assay is sufficiently sensitive when conducted under current OECD 471 guidelines. The choice of solvent, species of metabolic enzyme (i. e., hamster versus rat), and method (plate incubation versus preincubation) made little difference in terms of the sensitivity of

the Ames test for most NAs. However, for short-chain alkyl NAs, preincubation and non-DMSO solvents are recommended as outcome of this analysis. Overall, the data showed, that the Ames Assay performed with Nitrosamines compared to the data from the whole chemical space was equally predictive for mutagenic carcinogenicity.

Derivation of Acceptable Intakes for Nitrosamines: Structural Groups and Compound-Specific Limits

Michelle Kenyon
(Pfizer Global Research and Development, United States)

Abstract: In 2018, a carcinogenic nitrosamine was discovered in some Sartan drug products, leading to a regulatory requirement for pharmaceutical companies to evaluate all active pharmaceutical ingredients (API) and drug products for nitrosamine risk by March 2020. All those "at risk" then require analytical testing to ensure that nitrosamine levels are sufficiently low to minimize excess cancer risk in patients. Due to the large number of complex nitrosamines in Pfizer's portfolio that required assessment in a short period of time, we took a pragmatic approach and classified 66 APIs of concern into 13 structural groups according to the secondary amine substituents and the nitrosamine that might form in the presence of a nitrosating agent. Available carcinogenicity data for the nitrosamines in each structural class were critically reviewed and a conservative acceptable intake (AI) was derived for each structural group.

Although robust carcinogenicity studies are most appropriate for deriving an AI, less robust studies should be considered for understanding relative potency and structure activity relationships. Case studies will be presented to explore situations where a focused read-across on a specific nitrosamine of concern can be valuable and considerations, such as using quantum mechanical data and totality of carcinogenicity data for the appropriate nitrosamine class(es) when deriving the AI.

Considerations for less than lifetime approach

Alejandra Trejo-Martin
(Environmental and Occupational Safety Gilead Sciences, United States)

Abstract: There has been regulatory caution about applying less-than-lifetime (LTL) concepts to cohort of concern (COC) impurities such as N-nitrosamines. The LTL framework described in ICH M7 (R1) guideline is important as many pharmaceutical drugs are not administered for a patient's lifetime and as clinical trials typically involve LTL exposures. This presentation will describe the analysis of N-nitrosodiethylamine empirical data correlating exposure duration (as a percentage of lifespan) and cancer incidence in rodent bioassays indicating that the LTL acceptable intake (AI) as derived using the ICH M7 framework would not exceed a negligible additional risk of cancer over the exposure durations typical of clinical trials and many prescribed medicines.

S8 Alternative Toxicity Testing

Alternative methods for developmental and reproductive toxicity testing regarding ICH S5(R3)

Hajime Kojima

(Japanese Center for the Validation of Alternative Methods, Center for Biological safety and Research, National Institute of Health Sciences, Japan; Hatano Research Institute (HRI), Food and Drug Safety Center (FDSC), Japan)

Abstract: The "Guideline for Reproductive and Developmental Toxicity Studies of Drugs" in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) S5(R3) was revised this year to specify the possibility of using alternative test methods to animal testing (alternatives) for the evaluation of reproductive toxicity in candidate drugs. It is a breakthrough that may reduce either of the two species used in reproductive toxicity studies.

However, it is difficult to say that the contents are understood by everyone, and the alternatives may become a pie in the sky depending on the administrators. They should be used as part of safety pharmacology study by clarifying the mechanism of action. The development and dissemination of the alternatives will be facilitated if it is reasonably accepted that one of the two animal studies may be used as confirmation after the start of clinical studies. On the other hand, there is also a view that wishful thinking is excessive because safety evaluation is the first priority with no alternatives available at present to enter human clinical studies.

In the current situation, Japanese researchers have developed the new approach methods to detect potential hazards to embryo-fetal development for ICH S5 (R3). Our colleagues are on-going development of several type of assays with human induced pluripotent stem cells (iPSC). On the other hand, we are on-going the ring study of the zebrafish embryo assay with academia and the pharmaceutical industries.

Key words: Reproductive and Developmental Toxicity, ICH, Alternative test method

Crresponding author: Hajime Kojima, E-mail: h-kojima@nihs.go.jp

Animal alternatives in neurotoxicological research

Sheikh Raisuddin*, Suhel Parvez

〔*Department of Medical Elementology & Toxicology Jamia Hamdard (Hamdard University), New Delhi 110062, India; * Vice President, Society for Alternatives to Animal Experiments-India (SAAE-I) sraisuddin@jamiahamdard.ac.in*〕

Abstract: In India, in recent years research on alternatives has gained interest with special reference to study of toxic effects of drugs and chemicals and also to understand molecular basis of diseases. With the foundation of Society for Alternatives to Animal Experiments-India (SAAE-I) in 2018 there has been major impetus in this field. SAAE-I has established successful collaboration with leading Asian forums working in this area for quite some time such as Japanese and Korean Societies. There has been exchange of ideas and sharing of experiences at various levels. No doubt, the Mahatma Gandhi – Doerenkamp Centre for Alternatives to Use of Animals in Life Science Education, Bharathidasan University, Tiruchirappalli remains the leading light in alternatives research and education in India. Few

other institutions have emerged as vibrant centres for research in alternatives such as CSIR-Centre for Cellular and Molecular Biology, Hyderabad (zebra fish genomics), CSIR-Central Drug Research Institute, Lucknow (Caenorhabditis elegans in study of neuoronal death), CSIR-Indian Institute of Toxicology Research, Lucknow (Drosophila genotoxicology; *in vitro* toxicology) and Jamia Hamdard, New Delhi (C. elegans and Drosophila in neurobiology of diseases and neurotoxicology). At our Department we have standardized C. elegans and Drosophila to understand the mechanism of neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson disease (PD). None of the existing PD animal models recapitulate all symptoms including those developed in mouse. However, Drosophila melanogaster shows potential of an excellent model to study the same. Drosophila has several powerful attributes for toxicological studies including a high degree of genetic conservation of fundamental signaling and structural protein networks and availability of extensive array of molecular genetics tools for both forward and reverse genetic approaches. We have examined the modulatory effects of nutraceuticals (such as melatonin) in PD and repurposed drugs (such as minocycline and pramiprexole) in AD model of Drosophila. We are also employing Drosophila to assess the neurotoxicological and altered behavioral effects of pesticides (such as paraben). Similarly, we have studied effects of pesticides such as fenpropathrin and paraben in C. elegans and attempted to correlate it with responses of rodent neurobehavioral toxicity biomarkers. With concerted efforts of enthusiastic researchers and collaboration with international groups and other stakeholders working in social sectors it is hoped that research in alternatives will intensify and lead to emergence of much needed accredited centre(s) of research and validation in the field of alternatives in India.

SkinEthic™ HCE Time-to-Toxicity testing method for subcategorization of eye irritation

Yanfeng LIU[1], Nan ZHANG[1], Nathalie ALEPEE[2], Nan LI[1]

(1. L'Oréal Research & Innovation China, Shanghai 201206; 2. L'Oréal Research & Innovation France, Aulnay-Sous-Bois, 93600)

Abstract: Over the past decades, more and more methods were proposed either as a standalone alternative or as part of testing strategy to predict the non-irritation (No category) and serious eye damage (Category 1). However, none of the validated or regulatory accepted single method can predict eye irritation (Category 2).

To address the full range of the eye irritation potential, based on the SkinEthic ™ HCE tissue and eye irritation test method adopted in OECD TG 492, L'Oreal developed the Time-to-Toxicity approach, capable to distinguish NC, Cat. 2 and Cat. 1 chemicals. Briefly, the Time-to-Toxicity of 56 liquids was evaluated by exposing SkinEthic™ HCE tissue to test chemical for different time periods (1). Obtained results demonstrated good predictivity and reproducibility met the OECD required capacity.

The current study, using SkinEthic™ HCE tissue produced in China, evaluated chemicals representing different subcategorizations (NC, Cat. 2, and Cat. 1) with correct predictions and successfully transferred the Time-to-Toxicity approach in China. It provides a solid technical foundation for future extension of application and implementation of alternative testing strategies in China.

Crresponding author: Nan LI, E-mail: nan.li@rd.loreal.com

Novel hepatotoxicity biomarkers of exosomal miRNAs acutely induced by CCl4

Ryuichi Ono
〔 *Division of Cellular and Molecular Toxicology, Center for Biological Safety and Research (CBSR), National Institute of Health Sciences (NIHS), Japan* 〕

Abstract: Recent findings have revealed that exosomes are secreted from cells and circulate in the blood. Exosomes contain mRNAs, microRNAs, and DNAs and have the ability to transfer them from cell to cell. Recently, especially in humans, the diagnostic accuracy of tumor cell type-specific exosomal miRNAs as biomarkers has been found to be more than 90%. In addition, microRNAs contained in exosomes in blood are being identified as specific biomarkers of chemical-induced inflammation and organ damage.

Therefore, microRNAs contained in the exosomes released into the blood from tissues and organs in response to adverse events such as exposure to chemical substances and drugs are expected to be useful as novel biomarkers for toxicity assessment. In this study, C57BL/6J male mice orally dosed with carbon tetrachloride (CCl4) were used as a hepatotoxicity animal model. Here, we report that not only the known hepatotoxicity biomarkers miR-122 and miR-192 but also 42 novel exosomal biomarkers were upregulated in mice dosed with CCl4. Some of these novel biomarkers may be expected to be able to use for better understanding the mechanism of toxicity. These results suggest that our newly developed protocol using as a biomarker would accelerate the rapid evaluation of toxicity caused by chemical substances and/or drugs.

Key words: Exosome; Extracellular vesicle (EV); Hepatotoxicity; miRNA; Biomarker

S9 Chemical Stewardship through Sustainability Lenses

Key considerations on selection of safer chemical alternatives

Eeva Leinala
(Organisation for Economic Cooperation and Development)

Abstract: In 2021 the OECD released Guidance on Key Considerations for the Identification and Selection of Safer Chemical Alternatives (link). The guidance was developed with the aim to advance broader agreement on a general approach and criteria for the selection of safer alternatives, with a focus on chemical substitution. It is intended to advance a consistent understanding of the minimum requirements needed to determine whether a chemical alternative is safer. This presentation will outline the key aspects of the guidance including aspects of the minimum criteria and assessment practices and the self-assessment check-list.

S10 Toxicology & Epigenetics Biomarkers and Cancer

Histone Modification, DNA Methylation, and mRNA Expression Analysis of Murine Liver Repeatedly Exposure to a Chemical

J. Kanno[1,2]*, K. Aisaki[1], R. Ono[1], AND S. Kitajima[1]

(1. Division of cellular & Molecular Toxicology, Center for Biological Safety & Research, National Institute of Health Sciences, Kanagawa, Japan; 2. Environmental Biology Laboratory, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan)

Abstract: The Percellome Project aims at reinforcing and replacing the safety factor in toxicology by comprehensively identifying the transcriptomic networks induced by xenobiotics in murine organs. "Percellome" method was developed (BMC Genomics 7:64, 2006) to generate absolute copy numbers of mRNAs in a "per one cell" basis. Data from the Affymetrix MOE430 2.0 GeneChip are absolutized and visualized in 3-D graphs (time x dose x copy number). Datasets of mouse liver (4 time points x 4 dose levels, triplicate, 48 GeneChip data per chemical/organ) on 160 chemicals are compiled. This database reveals the initial gene network(s) triggered by a chemical.

To analyze the chronic effect of a chemical, a newly designed repeated dose study, insipred by gene knockout mice studies, was developed. To all 48 wild type mice, an equal dose of a chemical is repeatedly exposed for 14 days to create a "chemically-induced transgenic state". Then, on the 15th day, a single dose of a chemical was given and the liver was sampled at 2, 4, 8 and 24 hours thereafter. CCl4, clofibrate, and valproic acid were tested and Percellome mRNA data of the 48 mice, whole genome bisulfite analysis (WGBA) data and ChIP-seq data against H3K4me3, H3K27me3, H3K27Ac, and H3K9me3 for control and 14 day exposed mice were obtained and compared.

Firstly, the effect of repeated dosing on mRNA expression can be interpreted as a combination of two elements, i.e., baseline response (BR: gradual shift of the basal mRNA expression level by repeated dosing) and transient response (TR: alteration of the magnitude and/or pattern of the quick response in 2 to 24 hours). In general, CCl4 data showed that lower BR linked to suppressed TR and vice versa. The links were in some part chemical dependent. Although DNA methylation was not affected by 14-day dosing, ChIP-seq data revealed that BR and TR were, in general, in good correlation with histone modification. Enhanced mRNA response by 14-day repeated dosing was often explained by increases in H3K27Ac and/or H3K4me3 (activation marks), whereas suppressed mRNA response by 14- day repeated dosing was often explained by increases in H3K9me3 and / or H3K27me3 (repression marks). Some examples on the analysis of upstream regulation revealed by the mRNA expression and histone modification will he presented. (Supported by Health and Labor Sciences Research Grant of MHLW, Japan)

Key words: Per cell normalization, gene expression network, toxicity prediction, epigenetics, transcriptomics

Value of twin and family studies in environmental epigenetic research

Shuai Li

(The University of Melbourne)

Abstract: Epigenetic modifications, like DNA methylation, alter gene expression with changing the

underlying DNA sequence and has been proposed to play an important role in linking environmental ex·posures to human health. Population-based environmental epigenetic research involving unrelated indi·viduals are subject to biases of confounding and reverse causation. Twin and family study designs in·volving related individuals could address these issues with their unique value. This presentation will in·troduce the value in investigating the associations between environmental exposures and epigenetic modifications in two aspects: 1) Controlling for confounding; and 2) Making inference for the causation. Relevant study designs and empirical research findings will be introduced.

Epigenotoxicity and cancer and related diseases future perspectives

Mohammad Abdollahi

(Faculty of Pharmacy and the Institute of Pharmaceutical Sciences (TIPS), Tehran University of Medical Sciences, Tehran, Iran)

Abstract: Epigenetic changes are modifications to DNA that regulate whether genes are turned on or off. These modifications are attached to DNA and do not change the sequence of DNA building blocks. DNA methylation, histone changes, and alterations in microRNAs (miRNAs) are among the in·volved mechanisms. Various studies have shown the epigenetic modifications might result in the occur·rence or progression and metastasis of different cancer types. Contrary to the genetic mutations, most changes that occur because of epigenetics can be prevented or are reversible. Therefore, restoring ab·normal epigenetic changes in the neoplastic cells expands treatment for treating or preventing cancer in patients. In this regard, with the development of various drugs aimed at epigenetic regulators, epigen·etic therapy has been used to treat blood malignancies and some solid tumors. Cancer is a multi-stage process that includes: genetic changes in many genes, such as carcinogenic and tumor suppressor genes, repairing damaged DNA, and mechanisms that control cell growth and proliferation. Epigenetic mechanisms regulate gene expression at DNA and chromatin levels.

Epigenetic medicines, despite enjoying numerous advances in this field, still face many challeng·es. These drugs are not selective in inhibiting DNMTs and HDAC isozymes, resulting in side effects, toxicity, and lack of long-term response in the target patient.

This article will discuss the role of epigenetic alterations in the formation and progression of human cancer. This phenomenon has led to the advent of epigenetic drugs or biomarkers in the field of preci·sion medicine. Also, we will describe all the developed transport and release systems of the epigenetic drugs in detail.

Toxicology and Epigenetic Biomarkers for Arsenic-induced Cancer: A Population based study from West Bengal, India

Pritha Bhattacharjee, Ph.D.

(Assistant Professor, Department of Environmental Science, University of Calcutta)

Abstract: Arsenic is a carcinogen and epimutagen. Chronic exposure to arsenic results in cancer·ous outcome. The mechanism of arsenic-induced toxicity and carcinogenicity is very complex. Being a

system toxicant, arsenic affects several systems of a body and causes cancer of lung, liver, urothelial, and other internal organs apart from skin. Arsenic-induced changes in skin lesion like palmar-planter hyperkeratosis, pigmentary changes are the hallmark criteria of identifying arsenicosis across the globe. Studies have been performed in different model system, *in vivo*, *in vitro* and also at population level and identified the mechanism of toxicity. We have critically examined the biological samples (blood, saliva and urine) of population exposed to arsenic for a prolonged period (min of 10 years expo·sure) with a very high exposure (far above the permissible limit of 10ppb) and identified that despite of exposure at a similar extent (through food and drinking water), the phenotypic symptoms as also the metabolism of the studied population greatly vary from each other (skin lesion to cancerous lesion to null). The toxicity of ingested arsenic depends mainly on the metabolic efficiency and the genetic pre·disposition of the population play a major role for such differential susceptibility. It is interesting to note that not necessarily the cancerous outcome are due to permanent changes in DNA sequence. Epigene·tic modifications play a key role in altering the gene expression pattern and such modifications are re·versible in nature. Identifying the epigenetic biomarkers in arsenic toxicity could be further exploited for therapeutic targets. Our team has worked in targeted nuclear as also mitochondrial epigenetics Thus, our study considers a holistic approach to understand the epigenetic interplay in the individuals having prolonged arsenic exposure history.

Crresponding author: Pritha Bhattacharjee, Ph.D., E-mail: 777.pritha@gmail.com

S11 Nanotoxicology

Decision making in nanomaterial induced cell death

Tian Xia

(University of California, Los Angeles, Medicine)

Abstract: Different nanomaterials could induce different types of cell death in the same cell type, including apoptosis or pyroptosis. We screened a library of metal and metal oxide nanoparticles and 2D graphene oxides can found Ag, ZnO, CuO, V_2O_5 could induce apoptosis while GO, fumed silica, and rare earth oxide nanoparticles could induce pyroptosis. A detailed study found SiO_2 and V_2O_5 could both induce caspase 1 and 3 activations, which are responsible for pyroptosis and apoptosis, respec·tively. But how do they decide to go one way but not the other way? We found SiO_2 induced caspase 1 activation could result in pyroptotic cell death faster than caspase 3 induced apoptosis. For V_2O_5, cas·pase 1 activation came after caspase 3 activation, which leads to apoptosis although there is also cas·pase 1 activation and IL-1β production. These data showed that cell death mechanisms by nanomateri·als are dependent upon material properties and the temporal activation of cell death pathways.

Key words: Nanomaterials; cell death; apoptosis; pyroptosis; macrophages

Understanding the aggregation and toxicity of amyloid proteins

Puchun Ke

〔 *Nanomedicine Center at The Great Bay Area National Institute for Nanotechnology Innovation (CanNano) in Guangzhou, China* 〕

Abstract: In this talk, I will present amyloidogenesis inhibition with nanomaterials, a new frontier in

nanomedicine with an integral component in nanotoxicology. After a brief introduction to the key con·cepts in amyloid aggregation and the toxic oligomers,1 I will discuss the cross talk between pathogenic amyloid proteins as well as the cross talk between functional and pathogenic amyloid proteins, 2, 3 to demonstrate the complexity and richness of amyloid protein aggregation and toxicity in a few model systems. Mitigation of amyloidogenesis with tailor-designed nanomaterial-biomolecular complexes for benefiting our strategies against Alzheimer′s disease and type 2 diabetes4,5 will be presented in the end.

The biological effect and safety evaluation method of ingested nanoparticles

Yan Li, Kun Jiang, Hui Cao, Min Yuan, Fei Xu

(School of Medical Instrument and Food Engineering, University of Shanghai for Science and Technology, Shanghai 200093, China)

Abstract: Many recently investigations assessing the toxicological impacts of ingested NPs lack methodological standardization, and results not can be reliably compared between different laborato·ries. In this study, a standardized dietary model based on Chinese Dietary Reference Intakes and Chi·nese Dietary Guidelines from the average for men and women of 18 years or older was developed. Ad·ditionally, an *in vitro* intestinal epithelial model containing intestinal cells, goblet cells and M cells was established. To evaluate the NPs' safety by oral exposure accurately, the impact of food matrix and GIT on physicochemical properties of NPs, the transformations of tristimulus color coordinates, hydro·dynamic size, polydispersity index, agglomeration state, zeta-potential, UV-Vis spectra and solubility of NPs suspensions and their simulated digesta during each step of vitro digestion were measured. The resulting NPs digesta and pristine NPs suspensions at same concentration were then added to the in·testinal epithelial model *in vitro*. The impact of food matrix and GIT on the biological effects NPs was studied by measuring cell viability, cell membrane integrity, inflammatory factor release and cell apoptosis. Overall, this developed standardized dietary model and in vitro intestinal epithelial model could be used to better perform an accurate risk assessment via oral exposure *in vitro* and vivo.

Key words: ingested nanoparticles, safety evaluation method, a standardized dietary model, an *in vitro* intestinal epithelial model

Corresponding author: Yan Li, E-mail: jian_2001@sina.com

Cell-nanoparticle interactions and their implications for nanotoxicity

Angela Ivask

(Institute of Molecular and Cell Biology, Tartu University, Estonia)

Abstract: The potential of nanosize materials to cause cellular and organismal toxicity has been well demonstrated. However, our current understanding of the nanoparticle-cell interactions and how those interactions affect nanoparticle toxicity remains incomplete. We have used a combination of methods that enable us to study the processes taking place in single cells after their exposure to nano·size particles with varying particle size and surface properties. By using those methods we have been

able to quantify the numbers of nanoparticles that have entered the cells or attached onto the cell surfaces, determine the size of those intracellular and cell surface bound fractions along with determination of their biotransformation1,2. Using silver nanoparticles as an example, we demonstrated that cellular uptake of nanoparticles is primarily dependent on their surface properties. Thus, at similar exposure concentrations, the difference between cellular binding and uptake of differently surface functionalized nanosilver differed up to 10-fold. Further, we showed that from the total cell-associated pool of silver nanoparticles, roughly two thirds is bound to the cell surface, and one third is intracellularized. However, the cytotoxic effects are primarily caused by the latter. Using single-particle ICP-MS and synchrotron X-ray absorption spectrometry2 we have been able to demonstrate that both cell surface bound and intracellular silver nanoparticles undergo significant biotransformation. This was evidenced by the changes in particle sizes occurring after cell surface binding or intracellularization as well as by significant changes in chemical speciation of nanoparticles after cell surface association or cellular uptake. Our data demonstrated that biotransformation of silver nanoparticles is dependent on the particle's primary size and surface functionality and, is dominated by sulfidation but also involves formation of other small biomolecule-silver complexes. Evidently, these biotransformation pathways form the basis of (nanoparticulate) silver detoxification pathways in the cells.

S12 Gut microbiota and toxic effects of pollutants

Bidirectional regulation of valproic acid-like compounds on radiation effects of tumor and normal cells and its mechanism

Zhihui Feng

(Department of Occupational and Health and Occupational Medicine, Public health school, Shandong University, Jinan, China)

Abstract: Radiation therapy (RT) is a common clinical treatment for tumors. Due to radiation resistance and RT-induced systemic adverse reactions during the process of tumor treatment, the efficacy of RT treatment is affected. Therefore, finding safe and effective radiosensitizers, which not only can increase the sensitivity of tumor to RT but also effectively avoid RT-induced systemic adverse reactions, would be a major topic in the field of tumor radiotherapy. Both antiepileptic drug valproic acid (VPA) and its derivative 2-hexyl-4-pentynic acid (HPTA), belong to VPA-like compounds and histone deacetylase inhibitors. Through environmental carcinogen-induced a breast cancer animal model and a malignant transformation cell model established by our group, we found that VPA-like compounds had radiosensitization effect on breast tumor tissue and cells. The studies have further revealed that these compounds can promote ubiquitin ligase RFWD3 and inhibit de ubiquitination enzyme UCHL3, which coordinate the regulation of recombinant enzyme Rad51 activity for promoting Rad51 ubiquitination amd reducing the protein level of Rad51 to inhibite homologous recombination mechanism of DNA double strand break damage repair and achieve significant enhancement of radiation antitumor effect. It was also found that VPA-like compounds can reverse tumor immunosuppression and immunoescape for participating in the radiosensitization of tumor cells through activating M1 macrophage-mediated and macrophage-CD8 + T cell-mediated antitumor immune pathways. At the same time, VPA-like compounds can also reduce the radiation toxicity of distal normal tissue cells caused by radiation, inhibit the RFWD3-Rad51 ubiquitination pathway in normal tissue cells to enhance their homologous recombi·

nation ability for improving the radiation protective effect on normal tissue cells. Therefore, VPA-like compounds are effective radiosensitizers as DNA damage repair and immune function regulators, to bi·directionally regulate radiation effects on tumor and normal cells.

Crresponding author: Zhihui Feng, E-mail: fengzhihui@sdu.edu.cn

Exposure to a combination of silica nanoparticlesand low-dose radiation aggravates lung fibrosis in mice via gut microbiota modulation

Jing Xiang[1], Xiaodan Liu[2], Zhao Ju[1], Yao Zhou[1,2], Ruixue Huang[1], Ping-Kun Zhou[2]

(1. Department of Occupational and Environmental Health, Xiangya School of Public Health, Central South University, Changsha, Hunan Province 410078, China; 2. Department of Radiation Biology, Beijing Key Laboratory for Radiobiology, Beijing Institute of Radiation Medicine, AMMS, Beijing 100850, China)

Abstract: Exposure to silica nanoparticles (SNP) causes lung fibrosis and threatens human health. However, it is unknown if low-dose radiation(LDR) exposure exacerbates SNP-induced lung dysfunction. Thus, the aim of our study was to determine the combined effect of SNP and LDR on lung fibrosis, and elucidate the potential mechanisms involved. We used SNP-induced A549 cells and mice models, and detected gut microbiota alteration by 16s rDNA amplicon sequencing. Additionally, lung fi·brosis-related parameters were also detected using hematoxylin and eosin (H&E) staining, immunofluo·rescence (IF) staining, qRT-PCR, and western blot analysis. The histopathological and IF staining as·says illustrated that co-exposure of miceto SNP and LDR had a significant deleterious effect on both lung function and lung fibrosis,in comparison to exposure to SNP or LDR alone. Furthermore, the abun·dance of Bacteroidetes significantly increased while that of Firmicutes significantly decreased following co-exposure to SNP and LDR. Mechanistically, the notch cascade was activated in chronically SNP-ex·posed mice with lung fibrosis and A549 cells. Additionally, the notch pathway-associated proteins showed increased expression levels in the lungs following SNP exposure both in vivo and in vitro. Nota·bly, SNP-induced dysbiosis of the gut microbiota promoted lung epithelial damage by triggering the notch pathway, resulting in SNP-induced lung fibrosis. However, oral administration of probiotics pro·tected the mice from SNP-induced lung injury. Our results strongly indicate that the activation of the gut microbiota-dependent notch pathway in response to co-exposure to SNP and LDR results in lung epi·thelial injury *in vivo*. Probiotics supplementation is a potential way to protect against SNP and LDR-in·duced lung fibrosis.

Crresponding author: Ruixue Huang, E-mail: huangruixue@csu.edu.cn; Ping-Kun Zhou, E-mail: birm4th@163.com

Cancer Risk of Space Radiation Environment and its regulation by lncRNAs

Guangming Zhou, Weiwei Pei, Hailong Pei, Ziyang Guo, Anqing Wu, Huaiyuan Chen, Wentao Hu, Nin·gang Liu, Jing Nie, Bingyan Li

(State Key Laboratory of Radiation Medicine and Protection, Institute of Space Life Sciences, School

of Radiation Medicine and Protection, Collaborative Innovation Center of Radiological Medicine of Jiangsu Higher Education Institutions, Medical College of Soochow University, Suzhou 215123, China)

Abstract: Longitudinal study of astronaut health revealed that space exploration missions even in low orbitposed a risk of carcinogenesis to the astronauts. For long-term manned space exploration,astronauts will confront a much higher accumulated dose and consequently, cancer risk becomes one of the main obstacles. Elucidation of the mechanisms underlying radiation-induced carcinogenesis is essential to the development of effective countermeasures.

Alpha particles are the second most abundant types of space ionizing radiation and contribute much to the effective dose, we irradiated human lung bronchial epithelial BEAS-2B cells to a total dose of 0.2, 0.4, or 0.5 Gy viamultiple exposures with 0.02 Gy alpha particles in every three days. After a subsequent culture of the cells for 150 days, tumor formation ability in NOD/SCID mice was examined. We observed a dose-dependent increase of tumorigenesis as expected. But out of our expectation, low dose rate exposure (0.02 Gy/3 days) didn′ t increase the tumor induction rate but the malignancy of the induced tumors in comparison to the same dose with a relatively higher dose rate exposure (0.02 Gy/min).

We screened the RNA expression profile in human normal cells exposed to different kinds of ionizing radiation and identified a radiation-inducible long non-coding RNA, LNC CRYBG3, and its potential targets. With Pulldown assay, RNA immunoprecipatation, and other biotechniques, we revealed several target proteins of LNC CRYBG3 including G-actin, Bub3, LDHA, and eEF1A1. Low dose irradiation induced LNC CRYBG3 plays an important role in radiation-induced carcinogenesis.

Key words: Space radiation; tumorigenesis; long non-coding RNA; cytoskeleton; Bub3; LDHA

Metabolomicsphenotyping platform-based toxicological study

Fang Zhongze

(School of Public Health, Tianjin Medical University)

Abstract: External exposed xenobiotics can be biotransformed into metabolites by enzymes from gut bacteria and human bodies. Xenobiotics can also disturb the endogenous metabolic network. Metabolomics phenotyping platform-based toxicological study can provide new method to figure out the toxicology/diseases mechanism.

In the first part of this report, the relationship between gut bacteria metabolites (bile acids and trimethylamine (TMA)) and Gastational Diabetes (GDM) was elucidated. Furthermore, the role of circulating lysophosphatidylcholines (LPC) and ceramides in GDM was elucidated, and the interactive effects with bile acids and TMA was established. Furthermore, we explored the influenceof internal exposure ofpolycyclic aromatic hydrocarbons (PAHs) OH-PAHs towards the metabolism of these metabolites. The results showed that OH-PAHs can affect the serum level of bile acids DCA and the ratio of TMAO/TMA.

In the second part, we use animal experiments and metabolic tests to prove the mechanism of metabolic toxicology of cholesterol and protective role of liraglutide. We found Liraglutide treatment increased the biliary concentration of cholesterol, phospholipids and bile acids and thereby decreased the cholesterol saturation index. The expression of Cyp7a1, was significantly increased in liraglutide-treated mice. Mechanistically, liraglutide treatment altered bile acid composition and suppressed FXR activity in the ileum. Genetic ablation or pharmacological inhibition of FXR in the intestine protected

mice against CGD. Intestinal FXR was required for liraglutide-mediated regulation of hepatic expres· sion of Cyp7a1.Our results suggest liraglutide may represent a novel way for treating or preventing cho· lesterol gallstonesin individuals with high risk of CGD.

T01-11-0014

Integrative effects based on behavior, physiology and gene expression of tritiated water on zebrafish

Cui fengmei[a,b], Tu Yu[a,b], Chen na[a,b], Li shengri[a,b], Zhang qixuan[a,b], Zhang yefeng[a,b], Xue huiyuan[a,b], Wan jun[a,b], Sun liang[a,b]

(a. State Key Laboratory of Radiation Medicine and Protection, School of Radiation Medicine and Protection, Soochow University, Suzhou 215123, China; b. Collaborative Innovation Center of Radiation Medicine of Jiangsu Higher Education Institutions, Suzhou 215123, P.R. China)

Abstract: Tritium is a water-soluble hydrogen isotope that releases beta rays during decay. In nature, tritium primarily exists as tritiated water (HTO), and its main source is nuclear power/processing plants. In recent decades, with the development of nuclear power industry, it is necessary to evaluate the impact of tritium on organisms. Zebrafish is an excellent vertebrate biological model and has been applied on several fields such as biomedicine and environmental toxicology assessment. In this study, fertilized zebrafish embryos are treated with different HTO concentrations. After treatment with HTO, the zebrafish embryos developed without evident morphological changes. Nevertheless, the heart rate increased and locomotor activity decreased significantly. In addition, RNA-sequencing shows that HTO can affect gene expressions. The differentially expressed genes are enriched through many physiological processes and intracellular signaling pathways, including cardiac development, nervous system development, cardiovascular system development and the metabolism of xenobiotics by cytochrome P450. Moreover, the concentrations of thyroid hormones in the zebrafish decrease and the expression of thyroid hormone-related genes are disordered after HTO treatment. Our results suggest that exposure to HTO may affect the physiology and behaviors of zebrafish through physiological processes and intracellular signaling pathways and provide a theoretical basis for ecological risk assessment of tritium.

Key words: water contamination, aquatic organisms, gene expression, HTO, locomotor activity level

T01-13-0022

Metabolomics study of plasma and bone marrow cells in G6PD deficient mice with benzene exposure

Wang tong, Wang kun, Sun rongli, Pu Yuepu, Zhang Juan

(Key Laboratory of Environmental Medicine Engineering of Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu, China, 210009)

Abstract: **OBJECTIVE** To investigate the difference of plasma and bone marrow cells metabolites between glucose-6-phosphate dehydrogenase (G6PD) deficient mice and normal mice with benzene exposure. **METHODS**: G6PD deficient mice and normal C3H mice were randomly divided into two

groups included 8 mice in each group. The G6PD deficient and C3H mice were exposed to benzene (0 and 150 mg· kg^{-1} · d^{-1}) by subcutaneous injection for 5 days a week, 4 weeks. The plasma and bone marrow cells were collected on the last day of benzene exposure, and then detected by high-perfor·mance liquid chromatography time-of-flight mass spectrometry. The results were used principal compo·nent analysis (PCA) and orthogonal partial least square analysis (OPLS-DA), combined with the human metabonomics database, Kyoto gene and genome encyclopedia, and metaboanalyst 4.0 to screen, identify and analysis the differential metabolites in plasma and bone marrow cells. **RESULTS** The PCA of metabolites in plasma and bone marrow cells between G6PD deficient and C3H mice showed a sep·aration trend. The results of OPLS-DA showed that the model was not "overfitted", and the current mod·el was reliable. A total of 20 potential differential metabolites were identified in bone marrow cells be·tween G6PD deficient and C3H mice in the control group, and the metabolic pathways included phenyl·alanine, tyrosine and tryptophan biosynthesis pathway, phenylalanine metabolism pathway and purine metabolism pathway. 7 differential metabolites of bone marrow cells and purine metabolism pathway were identified in the exposure group. 17 and 9 metabolites of plasma were separately identified in the control and exposure group. α-linolenic acid metabolism pathway and glycerol phospholipid metabo·lism pathway were identified in the control group after benzene exposure, while a-linolenic acid metabo·lism pathway was found in the exposure group. Cluster analysis showed that plasma and bone marrow cells metabolites between G6PD deficient and C3H mice were significantly different. **CONCLUSION** The deficiency of G6PD can cause the change of plasma and bone marrow cells metabolites with ben·zene exposure, which provides a basis for the study of benzene exposure in G6PD population.

Key words: G6PD; benzene; plasma; bone marrow cells; metabolomics

T01-13-0045

Study on the roles and mechanisms of miR-155-regulated osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) via Smad5 in cadmium-induced osteoporosis

Mo Li-jun[a], Li Dong-li[a], Wang Zhao-jie[a], Wu Lu[b], Wei Qin-zhi[a], Liu Qi-zhan[b], Yang Xing-fen[a,*]

(a. School of Public Health, Southern Medical University, Guangzhou; Guangdong 510515

b. School of Public Health, Nanjing Medical University, Nanjing, Jiangsu 211166)

Abstract: OBJECTIVE To explore the mechanism of miR-155 regulating BMSCs osteogenic differentiation in cadmium-induced osteoporosis, so as to reveal the molecular mechanism of cadmium-induced bone damage, and provide new clues for finding early biomarkers of cadmium-induced health hazards and finding new prevention and treatment measures. **METHODS** First, the BMSCs and hBM·SCs cell lines were treated with different levels of cadmium to observe the osteogenic after different time. Then, intervention experiments were conducted to down-regulate mir-155 with specific inhibitors and a salvage experiment to upregulate mir-155 with specific mimics, ALP staining and alizarin red staining; qRT-PCR was used to detect the expression levels of osteogenic transcription factors Runx2 and Osterix and phenotypic genes ALP, OPN and OCN. On the basis of the above cell (*in vitro*) stud·ies, SD rats observe the changes and regulatory mechanism of non-coding RNA in cadmium-induced osteoporosis,CdCl2 was administrated via drinking water (25,50 ,100 mg·L^{-1}) for 16 weeks, The forma·tion of femur in rats was detected by microCT scanning and immunohistochemical, expression of mir-

155 in serum and femoralwere detected by qRT-PCR. **RESULTS** As determined in the present investi·gation, CdCl2 suppresses the osteogenesis of BMSCs, CdCl2 increased miR-155 levels; Enhancement of miR-155 levels by an miR-155 mimic blocked these CdCl2 - induced changes. Down-regulation of miR-155 blocked the CdCl2-induced increases of miR-155 and the promotion of the osteogenic differ·entiation of BMSCs.*in vitro* experiments showed that cadmium inhibited bone formation in rats.mir-155 was highly expressed in serum and bone tissues of cadmium-exposed rats. **CONCLUSION** In summa·ry, the present findings provide a mechanism by which miR-155 regulates CdCl2 - induced inhibitor of osteogenic differentiation and mir-155 may be an early biomarker of cadmium-induced osteoporosis.

Key words: cadmium; osteoporosis; bone marrow mesenchymal stem cells; microRNA-155

Crresponding Author: Yang Xing-fen, E-mail: xfyang@vip.163.com

T01-14-0030

Behavioral effects of triclocarban at environmental related concentra-tions on the generational toxicity of Caenorhabditis elegans

Dai Cong-hui[1], Lou Han-tian[1], Huang Jie[1], Sun Si-yang[2], Fang Wen-wei[1], Lu Bing-qian[2], Ju Jing-juan[1]

(1. School of Public Health and Management,Wenzhou Medical University, Wenzhou, Zhejiang Province 325035, China; 2. School of Laboratory and medicine and Life science,Wenzhou Medical University, Wenzhou, Zhejiang Province 325035, China)

Abstract: Triclocarban (TCC) is a broad-spectrum and highly active antibacterial agent that is widely used in many personal care products, and has the potential harm to the human body. At pres·ent, most toxicological studies on TCC are based on the concentration range (mostly at mg·L^{-1} level) when producing certain toxicity. However, the concentration of TCC in the environment is mostly in the ng·L^{-1}-g·L^{-1} range, showing low dose and long-term exposure in the environment, which is quite differ·ent from most toxicological studies. In this study, water and sediment samples were collected from sew·age treatment plant and the environmental concentration of TCC was detected by high performance liq·uid chromatography (HPLC). The exposure concentrations were then determined. C. elegans is a free-living nematode, which has the advantages of short experimental period, sensitivity to toxic effects of environmental pollutants, abundant progenies, and relatively conservative genome in mechanism re·search. This study observed the effects of TCC on the parental nematodes and their offspring after dif·ferent exposure patterns. In our study, it was found that parental and offspring′ s exposure of TCC at environmental related concentrations had an interaction effect on the locomotion behavior of offspring. We had studied the motion distance, the length of peristaltic trajectory, the linear distance of motion, the average amplitude and the maximum amplitude, among which the mean amplitude and maximum amplitude decreased the most significantly (P<0.05) compared with the control group. The aim of this study is to establish a rapid evaluation system for generational toxicity of TCC and provide evidences for elucidation of ecological toxicity of TCC.

Corresponding author: Ju Jing-juan, E-mail: jjj0810@wmu.edu.cn

T01-21-0003

The role of Nrf2 on the cell apoptosis in arsenic-induced malignant transformation HaCaT cells

Yang Qian-lei[a], Li Chun-chun[a], Yamanakakenzo[b], An yan[a]

(a.School of Public Health, Medical College of Soochow University, Suzhou, Jiangsu, China
b. Laboratory of Environmental Toxicology and Carcinogenesis, School of Pharmacy,
Nihon University, Chiba, Japan)

Abstract: Arsenic is confirmed human carcinogen and chronic exposure to arsenic can reduce cells apoptosis and cause malignant transformation. However, the exact mechanism remains to be elucidated. According to the malignant transformation model established by our laboratory, HaCaT cells were cultured to 35 generation with 0.0 or 1.0 μmol·L^{-1} $NaAsO_2$. Detect the change in the cellapoptosis rateand apoptosis related proteins in the process, as well as theeffects of Nrf2 on cell apoptosis in the process after transfection Nrf2 siRNA inarsenite-transformed HaCaT cells (T-HaCaT). Compared with passage 0 cells and passage-controlcells (0.0 μmol·L^{-1} $NaAsO_2$), the apoptosis rate, Caspase-3, Cleaved-caspase-3 protein and Cleaved-caspase-3 / Caspase-3showed downward trend. Apoptosis related proteins Cleaved-caspase-8、Caspase-8、Cleaved-caspase-12、Caspase-12protein showed no change in this process. CHOP and Bax protein after infected 21 generationsdecreased. The Bcl-2, Mcl-1 proteins after infected 14 generation increased. The expression of Nrf2 proteins were significantly increasedin T-HaCaT cells. After transfection Nrf2 siRNA to T-HaCaT cells, the cell apoptosis rate was significantly increased. Meanwhile the Cleaved-caspase-3, Caspase-3, CHOP, Bax protein expression were all increased, the Bcl-2, Mcl-1 protein expression were all decreased. $NaAsO_2$ may reduce HaCaT cells apoptosisin the endoplasmic reticulum and mitochondrial apoptosis pathway through Nrf2, leading to malignant transformation.

Key words: $NaAsO_2$; Malignant transformation; Apoptosis; Apoptosis related proteins; Nrf2

T01-26-0041

Transcriptomics-based study of rat silicosis model

Lv JQ, Yu T, Zhang Y, Yang HT, Li YL, Li ZS, Xiao JW*, Li B*

(Toxicology Department, National Institute for Occupational Health and Poison Control,
Chinese Center for Disease Control and Prevention, Beijing 100050, China)

Abstract: Being one of the oldest occupational diseases, silicosis could be dated back to the Hippocratic period, which is a systematic disease induced by inhaling silica particles in workplaces. However, despite its ancient history, patients with silicosis could still not be cured and even the pathogenic mechanism of silicosis has been elusive until now. Therefore, it is necessary and imperative to explore the specific mechanism in the context of state-of-the-arttechnologyof bio-sequencing. In this study, a Wistarrat model for silicosis was established by intratracheal instillation of silica (0, 50, 100 and 200 mg·mL^{-1}, 1 mL); then the lung tissues of rats were isolated, and their RNA were extracted 14 d and 28 d after instillation. The formation of silicotic lesions in rats exposed to silica was determined by Masson staining. RNA· sequencing techniques were used to observe differential expression of mRNAs in lung

tissues of silicotic rats. Prediction of mRNA functions and signaling pathways were conducted using Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.Our research will be conducive toelucidating the specific mechanism of silicosis and determining molecular initiating event (MIE) and adverse outcome pathway (AOP) of silicosis.

Corresponding author: E-mail: xiaojw@niohp.chinacdc.cn; E-mail: libin@niohp.chinacdc.cn

T01-28-0046

Study on differential expression of long non-coding rna in peripheral blood mononuclear cells of cadmium-induced osteoporosis

Li Dong-li[a], Mo Li-jun[a], Wang Zhao-jie[a], Wu Lu[b], Wei Qin-zhi[a], Liu Qi-zhan[b], Yang Xing-fen[a]

(a. Food Safety and Health Research Center, School of PublicHealth, Southern Medical University, Guangzhou 510515, China; b. School of Public Health, Nanjing Medical University, Nanjing 211166, China)

Abstract: OBJECTIVE Long-term environmental cadmium (Cd) exposure can cause osteoporosis and lead to fracture, but the mechanism is not clear. The purpose of this study was to analyze the differential expression of long non-coding RNA (lncRNAs) in peripheral blood mononuclear cells (PBMC) of peoplewith osteoporosis in cadmium-contaminated areas, so as to provide a basis for further exploring the molecular mechanism of cadmium-induced osteoporosis. **METHODS** Epidemiological investigation was carried out in the environmental cadmium pollution area. Spearman correlation analysis and multiple linear regression model were used to analyze the relationship between urinary cadmium level and bone damage index. Select PBMC samples from low cadmium exposure-non-osteoporosis (U-Cd <2 $\mu g \cdot g^{-1}$ Cr.) and high cadmium exposure-osteoporosis (U-Cd ≥20 $\mu g \cdot g^{-1}$ Cr.) for transcriptome sequencing to screen differentially expressed lncRNAs. The differential lncRNAs was verified by real-time fluorescence quantitative PCR between 85 non-osteoporosis and 85 osteoporosis patients. **RESULTS** A total of 438 subjects were enrolled. The average age of the subjects was 60.36 ± 7.89 years old, Urinary Cd concentrations of all studied subjects ranged from 0.18 to 62.27 $\mu g \cdot g^{-1}$ creatinine, with a median of 8.24 $\mu g \cdot g^{-1}$ creatinine. Spearman correlation analysis showed that urinary cadmium was negatively correlated with bone mineral density (BMD) and T value in both males and females. After the confounding factors such as age, BMI and smoking status were adjusted by multiple linear regression, there was a negative correlation between urinary cadmium and T value in both males and females. A total of 250 differentially expressed lncRNAs were screened by transcriptome sequencing, of which 106were up-regulated and 144 were down-regulated. The results of fluorescence quantitative PCR showed that the relative expression of LncRNACTBP1-AS2 in osteoporosis patients was significantly lower than that in non-osteoporosis patients.**CONCLUSION** Long-term environmental cadmium exposure could increase the risk of osteoporosis. In this study, men are more sensitive to cadmium toxicity than that of women. LncRNA CTBP1-AS2 might be closely related to the occurrence of cadmium- induced osteoporosis.

Key words: cadmium; osteoporosis; longnon-codingRNAs; epidemiology

Corresponding author: Yang Xing-fen, E-mail:yangalice79@smu.edu.cn

T01-30-0027

Developmental arsenic exposure induces gut microbiota dysbiosis and plasma metabolites disturbance in offspring mice

Wu Heng-chao, Wu Rui-rui , Chen Xin, Geng Hua-min, Xu Yuan-yuan

(Group of Chronic Disease and Environmental Genomics, School of Public Health, China Medical University, Shenyang 110122, China)

Abstract: Arsenic is a notoriously environmental pollutant. Of note, developmental arsenic exposure has been found to increase risks of various diseases. Gut microbiome is associated with disease development and is readily affected by acute or sub-chronic arsenic exposure. It is still unclear whether developmental arsenic exposure affects the composition of the gut microbiota, and thereby increasing the susceptibility to diseases in later life. The present study established a developmental arsenic exposure model in C57BL / 6 mice to assess the effect of environment-relevant doses (0.5 and 5 ppm) of arsenic on gut microbiota and plasma metabolites. Developmental arsenic exposure altered intestinal morphology and increased gut permeability and inflammation in pups at weaning. 16S rRNA gene sequencing revealed that developmental arsenic exposure significantly perturbed the gut microbiota composition in pups at weaning. The abundance of Muribaculaceae was diminished, the abundance of Akkermansia and Bacteroides were significantly increased at the genus level in pups after developmental arsenic exposure. Moreover, we identified 17 significantly changed plasma metabolites that inhibit genetic pathways associated with glycerophospholipid metabolism and flavonoid biosynthesis in developmental arsenic-exposed pups. Interestingly, the bacterial OTUs belonging to family Muribaculaceae, Akkermansia and Bacteroides showed a strong correlation with plasma metabolites. These findings provide new insights into the mechanisms underlying increased susceptibility to developing diseases due to developmental arsenic exposure.

Key words: Developmental exposure, arsenic, gut microbiota, plasma metabolites

Corresponding author: Xu Yuan-yuan, E-mail: yyxu@cmu.edu.cn

T01-32-0032

Exposure to ambient $PM_{2.5}$ perturbs metabolic profile of maternal serum and placenta in mice

Yanyi Xu[1], Shimin Tao[2], Haidong Kan[1], Weihua Li[2]

〔 *1. Department of Environmental Health, School of Public Health, Fudan University, Shanghai 200032, China; 2. NHC Key Lab of Reproduction Regulation (Shanghai Institute of Planned Parenthood Research), School of Pharmacy, Fudan University, Shanghai 200032, China* 〕

Abstract: Epidemiological and animal studies have shown that maternal fine particulate matter (PM2.5) exposure correlates with low birth weight (LBW) of offspring. However, the underlying biological mechanisms have not been fully investigated. In this study, female C57Bl/6J mice were exposed to concentrated ambient $PM_{2.5}$ (CAP) by inhalation during pre-conception and pregnancy periods, and metabolomics was performed to analyze the metabolic features in maternal serum and placenta. Our results showed that maternal CAP exposure significantly decreased the fetal weight, body length and tail

length at gestation day (GD) 17.5 and birth. Furthermore, PLS-DA analyses demonstrated that CAP exposure significantly altered the metabolic characteristics in both maternal serum and placenta. Metabolic pathway analysis indicated that oxidative stress and inflammation related pathways including vitamin digestion and absorption, arachidonic acid metabolism signaling pathways were disturbed. Meanwhile, sex hormone levels were altered and placental transport capacity of nutrients was impaired by maternal CAP exposure. These results indicate that oxidative stress and inflammation, endocrine disruption and abnormal nutrient transport function of placenta may contribute to $PM_{2.5}$ related LBW in offspring.

Key words: $PM_{2.5}$; low birth weight; metabolomics; oxidative stress; nutrient transportation

Corresponding author: Yanyi Xu, E-mail: yanyi_xu@fudan.edu.cn

T01-34-0007

Inhaled subway $PM_{2.5}$ caused multiple organ injury in mice

Li Qi-dian, Wang Jia-wei, Xue Rou, Shi Jin-gang, He Miao

(Department of Environmental Health, School of Public Health, China Medical University, Key Laboratory of Environmental Health Damage Research and Assessment, Liaoning Province, Shenyang 110122, PR China)

Abstract: Because of the system′s desirable properties, including greater speed and more efficient utilization of urban areas, subways are the most-used general public transport in urban areas worldwide. In this study, we aimed to identify the potential health effects and a toxic mechanism of ambient $PM_{2.5}$ in an underground subway station. 32 BALB/c mice were randomly divided into four groups: ① control group (saline); ② $PM_{2.5}$-low (30 μg/mice); ③ $PM_{2.5}$-mid(100 μg/mice); ④ $PM_{2.5}$-high(300 μg/mice). mice were weekly intratracheal instillation 4 times, and 24 hours after the last exposure, they were killed under anesthesia. $PM_{2.5}$ attenuated lung function in mice. $PM_{2.5}$ induced bronchial epithelial barrier function damage, infiltration of neutrophils and lymphocytes into the airway submucosa, and secretion of mucus in the airway epithelium. $PM_{2.5}$ markedly increased neutrophils and lymphocytes numbers and related cytokines and chemokines in bronchoalveolar lavage fluid (BALF), including interleukin (IL)-12, monocyte chemotactic protein (MCP)-1, tumor necrosis factor (TNF)-α, keratinocyte chemoattractant (KC). $PM_{2.5}$ increased Toll-like receptor (TLR) - 2, TLR 4, myeloid differentiation factor (MyD)-88, and nuclear factor (NF)-κB gene and protein expression in mouse lung tissue. $PM_{2.5}$ increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum of mice. After subway $PM_{2.5}$ treatment, the liver tissue structure was disordered, and with the increase of exposure dose, inflammatory cell including macrophage and neutrophil infiltration increased. $PM_{2.5}$ decreased CD3+cells, CD4+CD8-cells and CD4-CD8+cells in the thymus of mice, but increased CD4+CD8 + cells. Total T lymphocyte CD3 + in mouse spleen decreased in a dose-dependent manner, and CD4+cells in $PM_{2.5}$-high group decreased significantly compared with the control group. Mice were exposed to subway $PM_{2.5}$ through respiration, resulting in inflammatory injury of lung and liver and immunosuppression.

Key words: subway $PM_{2.5}$; inflammatory; multiple organ injury

Corresponding author: He Miao, E-mail: mhe@cmu.edu.cn

T01-38-0010

Tanshinone ⅡA-regulation of ⅠL-6 antagonizes $PM_{2.5}$-induced proliferation of human bronchial epithelial cells via a STAT3/miR-21 reciprocal loop

WenjingXie[a], Min Ling[b], Tian Xiao[b], Zi Fan[c], Dongya Chen[b], Meng Tang[a,*], Qian Bian[b,*]

(a. Key Laboratory of Environmental Medicine Engineering of Ministry of Education, School of Public Health, Southeast University, Nanjing 210009, Jiangsu, PR China; b. Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210009, Jiangsu, PR China; c. School of Public Health, Nanjing Medical University, Nanjing 211166, Jiangsu, China)

Abstract: Particulate matter 2.5 ($PM_{2.5}$), a component of atmospheric particulate matter, leads to changes in gene expression and cellular functions. Epidemiological evidence confirms that $PM_{2.5}$ has a positive correlation with lung injury. However, the molecular mechanisms involved remain poorly understood, and preventive methods are needed. In the present study, with HBE cells in culture, we showed that low concentrations of $PM_{2.5}$ resulted in acceleration of the G1/S transitionand cell proliferation. Consistent with these effects, expression of the pro-inflammatory factor interleukin-6 (IL-6) waselevated in HBE cells exposed to PM2.5. Accordingly, signal transducer and activator of transcription 3 (STAT3) was activated, which down-regulated expression of cyclin D1. In addition, $PM_{2.5}$ exposure led to higher levels of miR-21, and there was a reciprocal loop between miR-21 and STAT3. For HBE cells, tanshinone IIA (Tan IIA) reversed the PM2.5-induced cell cycle alterationand cell proliferation by reducing the IL-6 levels and activating the miR-21 / STAT3 reciprocal loop. These results show that, for HBE cells, tanshinone IIA-regulation of IL-6 antagonizes the $PM_{2.5}$-induced cell cycle alterationand cell proliferation via a STAT3 / miR-21 reciprocal loop, and indicate that it has potential clinical application for $PM_{2.5}$-induced respiratory injuries.

Key words: $PM_{2.5}$; air pollution; proliferation; IL-6; Tanshinone IIA; cell cycle

T01-40-0002

The toxic effects of triclosan on the neurotransmitter systems of Caenorhabditis elegans

Sun Si-yang[a], Wu Jia-hui[b], Huang Jie[b], Chen Qiu-quan[b], Ju Jing-juan[b]

(a. Department of Biotechnology, School of Laboratory medicine and Life Science, Wenzhou Medical University, Wenzhou, Zhejiang Province 325035, China; b. Department of Preventive Medicine, School of Public Health and Management, Wenzhou Medical University, Wenzhou, Zhejiang Province 325035, China)

Abstract: Triclosan is one of the most commonly used antibacterial agents, widely distributed in the environment. It has been reported that triclosan tends to accumulate easily in plants andorgnisms in aquatic environment, which may cause adverse effects on humans through the food chain. At present, the research about the toxicology of triclosan is stillrare, and the exposure concentrations of triclosan used in most studies have far exceeded the environmental levels, which is difficult to truly reflect the

toxic effects of triclosan at environmental concentrations. In this study, Caenorhabditis elegans (C.elegans) was employed to investigate the effect of triclosan at environmental relevant concentrations. C.elegans is a free-living, transparent nematode having several advantages as a model organism owing to its small size (~1 mm), rapid development (3 – 4 days), moderate lifespan (2 – 3 weeks), ease of cultivation and observation, and complete sequencing of its genome. The environmental concentration of triclosan is obtained by collecting wastewater and sediment samples from sewage treatment plantsand measured by using high-efficiency liquid chromatography (HPLC) after solid phase extraction. According to this, the exposure concentration of C. elegans wasdecided, and the toxic effects of triclosan on neurotransmitter systems were observed. After exposing to triclosan for 72 h, the relative fluorescence density of neurons in five neurotransmitter system of C.elegans was observed, and cholinesterol, gamma-amino butyric acid and dopaminergic neurotransmitter systems were affected. The mRNA expression levels of cholinesterol, gamma-amino butyric acid and dopaminergic neurotransmitter system genes were further measured. In Cholinergic system, the expression of all seven genes up-regulated, and the expression of cha-1, unc-17, ace-2, ace-3, ace-4 increased significantly, comparing with the control group ($P<0.01$); All six genes of gamma-amino butyric acid system increased significantly when comparing with the control group ($P<0.01$); in the dopaminergic system, excepting the expression of cat-4 significantly decreased, other genes were significantly increased ($P<0.05$ or $P<0.01$). Therefore, triclosan has a toxic effect on cholinesterol, gamma-amino butyric acid and dopaminergic neurotransmitter systems of C. elegans at environment-related concentrations, including the nervous system and gene expression.

Key words: Triclosan; Caenorhabditis elegans; Environmental relevant concentrations; Neurotransmitter system; Toxicity

T01-42-0029

T-2 toxin induces mitochondrial dysfunction, G1-phase arrest, and autophagy in PC12 cells via the activation of ROS/JNK pathways

Sun Tun, Dai Chong-shan*, Tang Shu-sheng*

(Basic Veterinary Medicine, College of Veterinary Medicine, No.2 Yuanmingyuan West Road, Beijing 100193, P. R. China)

Abstract: T-2 toxin is one of the most toxic mycotoxins produced by Fusarium stains. It is widely detected in food and crops. T-2 toxin exposure can cause potential neurotoxicity in animals; however, the precise molecular mechanism remains poorly understood. In the present study, we investigated the neurotoxicity of T-2 toxin and underlying molecular mechanism using a PC12 neuronal cell model. Cell cycle, cell activity, mitochondrial morphology, and protein expression in JNK/ p38 pathway were measured. Our results showed that T-2 toxin treatment (0-10 ng / mL) significantly decreased cell viability and G1 phase arrest in PC12 cells in a dose-dependent manner. T-2 toxin inhibited mitochondrial biogenesis of PC12 cells by reactive oxygen species(ROS). T-2 toxin treatment also significantly up-regulated the expression of p-JNK and inhibited the expression of cyclin D1, cyclin D3, cyclin B1 and cyclin E1. T-2 toxin treatment also up-regulated the expression of LC3 II protein, and down-regulated the expression of p62 protein. Furthermore, MAPK inhibitors (SP600125 and SB203580) significantly improved T-2 toxin exposure-caused the decrease of cell viability and G1 phase arrest. Furthermore, quercetin, a natural compound could markedly inhibit T-2 toxin-induced cytotoxicity and G1 phase ar·

rest via the inhibition of JNK pathway. Taken together, our results reveal that T-2 toxin-induced cytotoxicity and G1 phase arrest in PC12 cells involve the activation of JNK pathway.

Key words: T-2 toxin, Cell cycleG1, mitochondrial dysfunction, autophagy, JNK pathways

Corresponding author: Dai Chong-shan, E-mail: daichongshan@cau. edu. cn; Tang Shu-sheng, E-mail: tssfj@cau.edu.cn

01-42-0047

The role of glucose and lipid metabolism in cadmium-induced atherosclerosis in ApoE$^{-/-}$ mice

Wan Yu, Song Jia, Huang Hai-bin, Xiong Li-li, Fan Cui-hua, Wei Qin-zhi, Yang Xing-fen
(School of Public Health, Southern Medical University, Guangzhou 510515, China)

Abstract: OBJECTIVE cardiovascular disease (CVD) is a global public health problem. Atherosclerosis is the leading cause of CVD, resulting in high mortality worldwide. Cadmium is proposed to be a risk factor for the development of atherosclerosis, but the underlying mechanism is unknown. Atherosclerosis has been characterized by the progressive accumulation of lipids and inflammatory cells within the intima of large arteries, which is usually accompanied bya series of metabolic disorder like hyperglycemia. Thus, we aimed to investigate the role of dysfunction of glucose and lipid metabolism in cadmium-induced atherosclerosis using apolipoprotein E knockout (ApoE$^{-/-}$) mice. METHOD Sixty ApoE$^{-/-}$ male mice (8-week-old, 25±3 g) were randomized into 4 groups, receiving 0, 50, 100 and 200 mg·L^{-1} of CdCl2through drinking waterfor 160 days, respectively. After treatment, blood samples were collected from the orbit at the 1st and 2nd months and from the inferior vena cava at the 4th month to detect serum triglycerides (TG), total cholesterol (TCHO), low-density lipoprotein (LDL), high-density lipoprotein (HDL), glucose and insulin. At the 4th month, aorta samples were also collected to check the formation of atherosclerotic plaqueby oil red O staining. RESULTS Cadmium-treated mice had higher TG, TCHO and LDL levels at the 4th month than the control (0 mg·L^{-1}). Cadmium exposure decreased the HDL level of ApoE$^{-/-}$ mice at the 2nd and 4th months. Moreover, 200 mg·L^{-1} $CdCl_2$ increased glucose level from the 1st month and all the concentrations of $CdCl_2$ decreased levels of insulin from the 2nd month in ApoE$^{-/-}$ mice. Morphological analysis showed that both 100 and 200 mg·L^{-1} $CdCl_2$ exposure caused aortic atherosclerotic plaque in ApoE$^{-/-}$ mice, but it was more obvious at the latter dose. CONCLUSION Our results suggest that the dysfunction of glucose and lipid metabolism may contribute to cadmium-induced atherosclerosis in ApoE$^{-/-}$ mice.

Key words: cadmium; atherosclerosis; glucose and lipid metabolism

Corresponding author: Yang Xing-fen, E-mail: yangalice79@smu.edu.cn

T01-43-0005

Environmental level arsenic exposure from prenatal and adulthood weakens sperm fertilizing ability of offspring mice

Weixiang Fu[1], Chenkai Cheng[1], Chen Liang[2], Yanjia Tan[1], Ying Xing[1], Xiang Li[1], Jie Yang[1], Xiaohui Tian[1], Yanqin Ma[3], Jundong Wang[1]*, Jianhai Zhang[1]*
(1. Shanxi Key Laboratory of Ecological Animal Science and Environmental Veterinary Medicine,

College of Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi 030801, P.R. China;
2. College of Animal Science, Shanxi Agricultural University, Taigu, Shanxi 030801, P.R. China;
3. College of Life Science, Shanxi Agricultural University, Taigu, Shanxi 030801, P.R. China)

Abstract: **OBJECTIVE** The objective of this study was to investigate the influence of prenatal and early life arsenic exposure close to the environmental level on sperm fertilizing ability of offspring. **METHOD** 168 adult female ICR mice (4-week-old) were randomly divided into four groups. The control group was given distilled water, while the other three groups were treated with 0.02, 0.1 and 0.5 mg·L^{-1} of As_2O_3 via drinking water, respectively. After exposure for 4 weeks, the female mice were randomly bred with the healthy adult male mice (8-week-old) to produce offspring. The offspring male mice were selected and exposed to the same concentrations of arsenic from prenatal period, lactation to adulthood (PND126). *In vitro* fertilization test was used to evaluate the ability of offspring sperm to break down the cumulus cell layer, and the epididymides were processed for RT-PCR, western blotting and immunofluorescence analysis. **RESULTS** Arsenic exposure reduced offspring′s ability of sperm to break down the cumulus cell layer. Compared with the control group, the protein expression levels of PRSS21 and SPAM1 in the epididymis of offspring in all As_2O_3 treatment groups were significantly reduced (P<0.05 and P<0.01), while ACR expression in the epididymis was significantly reduced only exposed to 0.5 mg·L^{-1} As O treatment group (P<0.05). However, the mRNA expression levels of Acr and Spam1 in the testis, as well as Cd9, Cd81, Mfge8 and Hsc70 in the epididymosomes have not been changed in the three As2O3 treatment groups. **CONCLUSIONS** Arsenic exposure close to the environmental level interferes with the sperm fertilizing ability of offspring, and SPAM1, ACR and PRSS21 maybe play key roles during this regulation.

Key words: Arsenic; sperm fertilizing ability; mice; Environmental level

Corresponding author: Zhang Jian-hai, E-mail: jianhaiz@163.com

T01-45-0025

Myclobutanil impairs the locomotor activity in zebrafish larvae through initiation of autophagy and apoptosis

Zhu Jiansheng[a], Liu Chunlan[b], Wang Jingyu[a], Zou Li[a], Xu Qu[a], You Lianghui[a], Ji Chenbo[a], Hong Qin[a], Tong Meiling[a], Chi Xia[a]

(a. Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing 210004, P. R. China; b. Department of Epidemiology, School of Public Health, Nanjing Medical University, 101 Longmian Avenue, Nanjing 211166, P. R. China)

Abstract: Myclobutanil (MYC), a common broad-spectrum triazole fungicide, has the potential effect of neurotoxicity. However, far too little attention was paid to the mechanism of MYC-induced neurotoxicity. In this study, the neurotoxicity of MYC was evaluated in zebrafish and in PC12 cells. Zebrafish embryos were exposed to MYC (0, 0.5 and 1 mg·L^{-1}) from 4 to 96 h post fertilization (hpf) and the morphology and behavior were evaluated. Our results showed that MYC increased the mortality rate and malformation rate of zebrafish. MYC also resulted in neurotoxicity, including abnormal behaviors, disrupted histological structure of brain, decreased level of dopamine and neuronal cell damage in the hb9:egfp transgenic zebrafish model. MYC substantially increased apoptosis via upregulation of genes and proteins related to apoptosis in the hindbrain. Besides, MYC activated autophagy by the enhanced

expressions of LC3- II but the reduction of p62 in both MYC-exposed zebrafish and PC12 cells. Nota·bly, these adverse outcomes were restored by 3-methyladenine (3-MA, an autophagy inhibitor) in ze·brafish and PC12 cells, indicating that MYC might induce neurotoxicity during zebrafish development through initiation of autophagy and apoptosis. Taken together, this study revealed the key role of au·tophagy in MYC-induced neurotoxicity and provided novel insights into strategies to mitigate its toxicity.

Key words: Myclobutanil; Neurotoxicity; Autophagy; Apoptosis; Zebrafish

Corresponding author: Chi Xia, E-mail: chixia2001@njmu.edu.cn

T01-45-0043

An epidemiological study on the association between environmental cadmium exposure and hypertension in middle-aged and elderly non-smokers

Huang Hai-bin, Song Jia, Chen Lin-quan, Wan Yu, Xiong Li-li, Fan Cui-hua, Li Rui-qing, Yang Xing-fen*
(School of Public Health, Southern Medical University, Guangzhou 510515, China)

Abstract: OBJECTIVE Hypertension (HP) is a well-known and preventable risk factor for isch·emic heart disease, stroke and other cardiovascular diseases (CVDs). Cadmium, an independent risk factor for CVDs, is inconsistently associated with HP in the existing studies which cannot exclude the in·fluence of smoking. Therefore, the present study intended to clarify the relationship between urine cad·mium and HP in middle-aged and elderly non-smokers from cadmium polluted andnon-polluted areas. Methods: A total of 734 middle-aged and elderly non-smokers were randomly recruited from cadmium polluted and non-polluted areas (294 in polluted area and 440 in non-polluted area). The main inclusion criteria were (1) aged 40-75 years old,(2) resident years over15 years, and (3) lived on a subsistence diet of rice and vegetables grown in the investigated areas.Occupational exposure was excluded. After obtaining the informed consent of subjects, questionnaire survey (basic information, living behaviors, history of diseases and medication), physical examination (height, weight, blood pressure) and collec·tion of fasting mid-course morning urine samples were carried out. The level of urine cadmium was measured by ICP-MS and corrected by urine creatinine (Cr). Pearson′ s chi-squaredtest, student (inde·pendent) T-test and Mann – Whitney test were used to compare the basic information and urine cadmi·um levels of subjects in cadmium polluted and non-polluted areas. Univariate logistic regression was used to screen the potential influencing factors of HP. After adjustment for age, education level and BMI, multivariate logistic regression was used to analyze the association between urine cadmium and HP. **RESULTS** The overall prevalence of HP was 51.9% in subjects. The prevalence of HPwas signifi·cantly different (P<0.05) between cadmium polluted and non-polluted areas (66.0% vs. 42.5%). The urine cadmium levels of subjects in pollutedand non-polluted areaswere 7.029 (IQR:3.927, 14.941) and 0.121 (IQR: 0.083, 0.185) $\mu g \cdot g^{-1}$ Cr, respectively, with statistical significance (P<0.001). Univariate lo·gistic regression analysis showed that urine cadmium, increased age, low educational level and BMI≥ 24 $kg \cdot m^2$ were risk factors for HP (P<0.001). In the multivariate logistic regression model,urine cadmi·um was positively association with HP after adjustment for age, education level and BMI (OR=1.016, 95% CI: 1.000, 1.032). **CONCLUSION** Cadmium exposure may increase the risk of HP in middle-aged and elderly non-smokers.

Key words: Cadmium; Hypertension; Middle-aged and elderly non-smokers

Corresponding author: Yang Xing-fen, E-mail: yangalice79@smu.edu.cn

T01-51-0020

N21aP as a novel substrate of CYP1A1 induces pulmonary fibrosis by targeting alveolar epithelial typeII cells

Shen jie-miao, Xia rong, Chen chao, Min yue, Wang chao, Wang shou-lin*
(Key lab of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing 211166)

Abstract: Polycyclic aromatic hydrocarbons (PAHs) are the important components of $PM_{2.5}$, leading to COPD, fibrosis and lung cancer, most of which are mediated by cytochrome P450 enzymes (CYP) via metabolic activation. CYP1A1, highly expressed in the lung, is a key metabolic enzyme that activates PAHs to cause respiratory system injury. Compared with other CYP enzyme, CYP1A1 has a higher metabolic efficacy on PAHs, producing the most types and quantities of metabolites. In addition to BaP, many other important toxic PAHs that are metabolized by CYP1A1 are still unknown. Thus, in order to quickly screen out toxic PAHs, we constructed a molecular docking model between PAHs and CYP1A1. Based on the docking model and vitro metabolism, we screened out naphtho[2, 1-a]pyrene (N21aP), a new substrate PAH of CYP1A1. The active metabolites of N21aP metabolized by CYP1A1, including epoxy, diols and epoxy diols compounds, were successfully detected by LC-MS/MS. Moreover, we found that N21aP induced pulmonary fibrosis in WT mice, manifested as collagen deposition and increased hydroxyproline, which could be alleviated by cyp1a1-knockout (KO) mice. Furthermore, given that CYP1A1 has the ability to metabolically activate N21aP, flow cytometry and immunofluorescence were used to determine the expression and localization of CYP1A1, so as to identify critical damaged cells. The experiments showed that CYP1A1 was highly expressed in alveolar type II epithelial cells (AT2). Subsequently, we discovered that N21aP induced pulmonary fibrosis by injuring AT2, exhibiting reduced number, structural destruction and cell senescence. Senescent AT2 cells contributed to the activation of pulmonary fibroblasts via increasing the expression of senescence-associated secretory phenotype (SASP), such as IL-6, IL-1β and CXCL1, which was confirmed by rapamycin, an inhibitor of senescence. Our study first announces N21aP as a new toxic substrate of CYP1A1 to induce pulmonary fibrosis, and provides a new theoretical support for the prevention and source control of PAHs induced pulmonary fibrosis.

Key words: molecular docking model; naphtho[2,1-a]pyrene (N21aP); CYP1A1; alveolar epithelial type II cells (AT2); pulmonary fibrosis; cell senescence

Corresponding author: Wang shou-lin，E-mail: wangshl@njmu.edu.cn

T01-52-0033

Fluvic acid ameliorates cadmium-induced hepatotoxicity through inhibiting oxidative stress and apoptosis in L02 cells

Li Hui, Dai Chong-shan, Tang Shu-sheng
(Department of Basic Veterinary Medicine, College of Veterinary Medicine, China Agricultural University, Beijing 100193)

Abstract: Fulvic acid has several nutraceutical properties, including antioxidant, anti-inflammatory

and detoxification. It also could adsorb and chelate with metal ions. This study aims to use fulvic acid to exert the protective effect on hepatotoxicity against cadmium *in vitro* and illuminate the underlying mechanism. The effect of fulvic was examined by using LDH release assay, fluorescent staining, flow cytometry, spectrophotometer, and western blot. Our results revealed that fulvic acid can significantly decrease cadmium-induced cell death. And we found fulvic acid can decrease the production of ROS and MDA, it also decreased the activities of SOD and CAT. Moreover, fulvic acid treatment alleviated the decrease in mitochondrial membrane potential induced by cadmium. The apoptosis rates also showed a significant decrease after fulvic acid pretreatment. The protein levels of pro-caspase 3 and pro-caspase 9 were up-regulated, and cleaved-PARP-1, cleaved-caspase 3 and Nrf2 were down-regu·lated in the presence of fulvic acid. Taken together, our study demonstrates that fulvic acid ameliorates cadmium-induced hepatotoxicity in L02 cells through inhibiting oxidative stress and apoptosis.

Key words: Fluvic acid; Cadmium; Hepatotoxicity; Oxidative Stress; Apoptosis

Corresponding author: Dai Chong-shan, Email: daichongshan@cau.edu.com; Tang Shu-sheng, E-mail: tssfj@cau.edu.com

T01-52-0040

Multi-omic profiling reveal the mixture toxicity of difenoconazole and tebuconazole in larval and adult zebrafish

Jiang Jin-hua*, Chen Lie-zhong, Wang Lu-yan, Wu Shen-gan, Zhao Xue-ping*

(Institute of Agro-product Safety and Nutrition, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, Zhejiang, China)

Abstract: In current study, an integrated histological, transcriptome, metabonomics and microbiol·ogy was applied to investigate the mixture effects and risk of difenoconazole (DIF) and tebuconazole (TEB) co-exposure on early life stage and adult zebrafish at environmental concentration and aquatic life benchmark. Additive effects have been expected when the substances have similar mode of action. Our results demonstrated that the mixture of DIF and TEB exhibited additive effect on the acute toxicity to embryo-larval and adult zebrafish. The combined toxicity of DIF and TEB on development was less than the additive effect of individual DIF and TEB. It was demonstrated that DIF and TEB co-exposure allayed the inhibition of yolk sac resorption during zebrafish embryo-larvae stage, reduced the toxic effects on liver gonad and intestinal microflora. Transcriptomics and metabolomics showed TEB and DIF mixtures at environmental concentration and aquatic life benchmark showed different response pat·terns in embryo-larval and adult zebrafish. Further integrated analysis showed DIF and TEB exposure had negative effect both on arachidonic acid (AA) metabolism and steroid hormone biosynthesis during early life stage and adult development. DIF, TEB and their mixtures decreased the DEGs and resulted in the metabolites accumulated in larvae zebrafish, while the DIF and TEB mixture at aquatic life bench·mark reduced the levels of accumulated metabolites, and the binary mixture of the low concentration of DIF and TEB had synergistic effect on the accumulation of metabolites involved in AA metabolism and steroid hormone biosynthesis. DIF and TEB at aquatic life benchmark decreased various metabolites levels in the two pathways in adult zebrafish, their mixture up-regulated more DEGs to activate the pathways, futher reduced the intermediates and allayed the toxic effect on liver and gonad. Our results showed the different responses and patterns on transcriptional and metabolic profiles mediated in the

combination effects of DIF and TEB, the environmental concentrations of DIF and TEB mixture had potential risk on metabolism and development during zebrafish early life stage. The results provided a deep mechanistic understanding of the combined effects and mechanism of DIF and TEB mixture on aquatic organisms at environmental concentration and aquatic life benchmarks.

Key words: Difenoconazole; Tebuconazole; Combined effect; Omics; Zebrafish; Early life stages

Corresponding author: Zhao Xue-ping, E-mail: zhaoxueping@tom.com; Jiang Jin-hua, E-mail: jiangjh@zaas.ac.cn

T01-53-0034

Transcriptome and biochemical analyses of rainbow trout (Oncorhynchus mykiss) RTG-2 gonadal cells in response to BDE-47 stress indicates effects on cell proliferation

Li Yuan-yuan[a], Liu Qian[a], Chen Hong-mei[b], Lu Ke-yu[c], Zhou Zhong-yuan

(a. Department of Marine Ecology, College of Marine Life Science, Ocean University of China, Qingdao 266003, China; b. Key Laboratory of Xinjiang Endemic Phytomedicine Resources, Ministry of Education, Pharmacology Department, School of Pharmacy, Shihezi University, Shihezi 832002, China; c. Department of Geography, University College London, London WC1E 6BT, UK)

Abstract: BDE-47 is a biotoxin of PBDEs frequently detected in the environment. In this study, we investigated the effects of BDE-47 on cell viability, morphology, cell cycle and apoptosis. BDE-47 significantly decreased cell viability, and morphological alterations were observed. The significant increase in cells at G1 phase indicated the occurrence of G1 phase cell cycle arrest in RTG-2 cells. An AO / EB staining assay revealed the induction of apoptosis in RTG-2 cells. The results indicated that BDE-47 exposure inhibits cell proliferation. Transcriptome analysis was applied for further evidence. A total of 1300 differentially expressed genes (DEGs) were identified in RTG-2 cells, among which 26 DEGs were associated with the cell cycle and apoptosis. Biochemical analyses also showed the expression of cell cycle- and apoptosis-related proteins and genes. Mapping the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, p53, TNF, MAPK, PI3K-AKT, and ROS-mediated signaling pathways were determined to be the major pathways involved in modulating the cell cycle and apoptosis. Since we demonstrated simultaneous ROS overproduction during BDE-47 exposure in a previous study, we speculated a possible explanation for the observation: BDE-47-induced ROS overproduction was the initiating signal, which activated cell cycle arrest and apoptosis and finally inhibited cell proliferation.

Key words: BDE-47; RTG-2 cells; Transcriptome; Cell cycle arrest; Apoptosis

Corresponding author: Zhou Zhong-yuan, E-mail: 1071253114@qq.com

T01-55-0019

TCDD-induced IL-24 secretion in human chorionic stromal cells inhibits placental trophoblast cell migration and invasion

Liu Ge[a], Jia Jiaoyan[b], Zhong Jianfeng[b], Yang Yongqi[b], Bao Yantao[c], Zhu Qinchang[a,b]

(a. College of Pharmacy, Shenzhen Technology University, Shenzhen 518118, PR China; b. School of Pharmaceutical Sciences, Shenzhen University, Shenzhen 518060, PR China; c. Affiliated Shenzhen Maternity and Child Healthcare Hospital, Southern Medical University, Shenzhen, China)

Abstract: Environmental pollutant dioxins are potentially harmful to pregnant women and can lead to severe adverse outcomes in pregnancy, such as spontaneous abortion. Little is currently known about the toxicological mechanism underlying this severe type of harm from dioxin exposure. Our previ·ous study reported that the IL-24 gene is a dioxin response gene during 2, 3, 7, 8-tetrachlorodibenzo-p- dioxin (TCDD) treatment. Here, we further tested the effect of TCDD on IL-24 expression in human cho·rionic stromal cells. We also investigated the effect of IL-24 on the behaviors of human placental tropho·blast cells and predicted the potential mechanism underlying these behaviors using functional network analysis. We found that TCDD stimulates IL-24 expression in human chorionic stromal cells in an AhR (aromatic hydrocarbon receptor) - dependent manner. We also found that IL-24 inhibits the migration and invasion of human placental trophoblast cells, the possible mechanism of which involves thirteen key proteins and mitochondrial function. These observations provide new insight into the toxicological mechanism whereby dioxin induces adverse pregnancy outcomes in humans.

Key words: TCDD; Abortion; IL-24; Placental trophoblast cells; Mitochondrial

T01-55-0038

Prothioconazole induces cell cycle arrest by up regulation of EIF4EBP1 in extravillous trophoblast cells

Guangzhu Dong[a,b], Rui Zhang[a,b], Qi Hu[a,b], Yufeng Qin[b], Chuncheng Lu[a,b], Yankai Xia[a,b], Xinru Wang[a,b], Guizhen Du[a,b]

(a. State Key Laboratory of Reproductive Medicine, Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing 211166, China; b. Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing 211166, China)

Abstract: Prothioconazole (PTC) is a new broad-spectrum triazole antibacterial agent that is being widely used in agriculture. Whether PTC has adverse effects on embryo implantation and development is yet to be found. Proper trophoblast proliferation and migration is a prerequisite for successful embryo implantation. To elucidate the underlying molecular perturbations, we detect the effect of PTC on extravil·lous trophoblast cells proliferation and migration, and investigate its potential mechanisms. Exposure to different concentrations of PTC (0-500 $\mu mol \cdot L^{-1}$) significantly inhibited the cell viability and migration ability (5 $\mu mol \cdot L^{-1}$ PTC exposure), and also caused the cell cycle arrest at the lowest dose (1 $\mu mol \cdot L^{-1}$ PTC exposure). Through the transcriptome analysis, we observed that PTC exposure disturbed multi·ple pathways including cell cycle and apoptosis pathway, which was consistent with cell phenotype. Mechanistically, we illustrated that eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1, 4E-BP1) was up-regulated after PTC exposure. After knocking down EIF4EBP1, the G1 phase arrest induced by PTC exposure was attenuated, which might be explained by up-regulation of cyclin D1. In summary, our data demonstrated that 4E-BP1 participated in PTC-induced cell cycle arrest in extravillous trophoblast cells by regulating Cyclin D1. These findings shed light on the potential adverse effect of PTC exposure on the embryo implantation.

Key words: Prothioconazole; Extravillous trophoblast cell; Cell cycle; 4E-BP1; Cyclin D1

Corresponding author: Guizhen Du, E-mail: guizhendu@njmu.edu.cn

T01-56-0039

Opposite regulation of JNK and p38 signaling pathways on copper sulfate-induced apoptosis and cell cycle arrest in HepG2 cells

Li Meng, Tang Shu-sheng, Dai Chong-shan

(Department of Basic Veterinary Medicine, College of veterinary medicine, China Agricultural University 100193, P.R. China)

Abstract: Copper is an essential trace element in maintaining cell survival, proliferation, and activities of metabolic enzymes. However, higher dose of copper exposure could induce toxic effects and underlying molecular mechanism is still unclear. This study was aimed to investigate the potential molecular mechanism of copper sulfate-induced liver toxicity *in vitro*. Our results showed that copper-sulfate induced cytotoxicity in dose- and time-dependent manners in HepG2 cells. Copper sulfate exposure significantly induced the production of ROS, followed by inducing cell apoptosis and G2 cell cycle arrest. RNA-seq data showed that copper sulfate-induced cell death involved ER stress, PI3K/AKT, MAPK, and NF-κB pathways. We further detected that copper sulfate increased the expression of p-JNK and p-p38 in time- and dose-dependent manners. Moreover, inhibition of p38 MAPK pathway significantly decreased copper sulfate-induced cell death and G2 cell cycle arrest. On the contrary, inhibition of JNK pathway significantly increased copper sulfate-induced cell death and G2 cell cycle arrest. In conclusions, our results reveal that the activation of MAPK pathway played a critical role in copper sulfate-induced apoptosis and G2 cell cycle arrest. For the first time, our results reveal the opposite regulation of JNK and p38 signaling pathways contributed to copper sulfate-induced apoptosis and cell cycle arrest in HepG2 cells. This study provides an insight on the understanding of copper sulfate-induced liver toxicity.

Key words: MAPK pathway; Apoptosis; Cell cycle arrest; Cytotoxicity; HepG2 cells

Corresponding author: Dai Chong-shan, E-mail: daichongshan@cau.edu.cn; Tang Shu-sheng, E-mail: tssfj@163.com

T01-58-0035

Immunotoxicity Evaluation of Tetrabromodiphenyl Ether (BDE-47) on Mice and RAW264.7 Macrophages

Qian Gao, Tongtan Liu, Shi Dong, Hongmei Chen*

(Key Laboratory of Xinjiang Endemic Phytomedicine Resources, Ministry of Education, Pharmacology Department, School of Pharmacy, Shihezi University, Shihezi 832002, China)

Abstract: As a typical emerging contaminant, Polybrominated diphenyl ethers (PBDEs) exhibit potential negative impacts on human health, especially on immune systems. The research of immunotoxicity and its mechanisms possess great significance in defending against the poison of exogenous pollutants. In this study, the immune toxicity of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47),the most biotoxic PBDEs, was evaluated on mice and murine macrophage RAW264.7 cells. BDE-47 was fed to Mice at concentrations of 0, 0.5, 5, 50 mg·kg^{-1}·day^{-1} for 21 days. The weight, indexes of spleen and thymus of the mice were decreased respectively. Blood routine analysis showed that white blood cells (WBC), lymphocytes (Lym), monocytes (Mon) and granulocytes (GRA) increased in different degrees,

indicating that the immune system of the mice responded to BDE-47 exposure. After BDE-47 exposure, the viability of RAW264.7 cells was decreased in a concentration-dependent manner, accompanied with a reduction of cell phagocytosis. BDE-47 significantly elevated the release levels of nitric oxide (NO), and increased the secretion of inflammatory factors such as TNF-α, IL-6 and IL-1β. However, the secretion of anti-inflammatory factors IL-10 decreased. And furthermore, the results of qPCR showed that BDE-47 inhibited expression of antigen-presenting molecule MHC-II, whereas increased the expression of costimulatory factors CD86, CD80 and CD40. These results indicated that BDE-47 has immunotoxicity effect on RAW264.7 cells. Additionally, Flow cytometry was performed to analyze the expression of M1 and M2 markers on macrophages. The results showed that iNOS in M1-like macrophages was upregulated, while the expression of Arg-1 in M2-like macrophages was downregulated, indicating that the cells had a trend to M1-dominant polarization of macrophages.

In conclusion, in-vivo and in-vitro studies showed that BDE-47 is immunotoxic through stimulating or inhibiting different immune functions of mice and RAW264.7 macrophages. BDE-47 exposure significantly enhanced inflammatory M1 phenotype polarization in RAW264.7 cells.

Key words: 2,2′,4,4′ - tetrabromodiphenyl ether; RAW264.7 cells; immunotoxicity; polarization.

Corresponding author: Hongmei Chen, E-mail: melisasa821@foxmail.com

T01-59-0021

CYP2A5-mediated metabolic activation of 5-hydroxymethylfurfural induces COPD of mice through targeting the alveolar macrophages

Rong Xia, Minghui Ji, Anjie Yuan, Li Wang, Shou-Lin Wang

(Key Lab of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing 211166)

Abstract: Cytochrome CYP450(CYP)- mediated biotransformation is an important way for exogenous pollutants to exert health damage. CYP2A13 is an extrahepatic enzyme mainly expressed in the respiratory system and recognized as the key metabolic enzyme of cigarette smoke, which may play an important role in the respiratory system injury caused by smoking. 5-hydroxymethylfurfural(5-HMF) was identified as a new toxic substrate of CYP2A13/CYP2A5(homologous to human CYP2A13 in mouse) in cigarette smoke. However, the role and mechanism of 5-HMF in cigarette smoke-induced chronic respiratory injuries remains unclear. Our results showed that after long-time aerosolization of 5-HMF, mice developed airflow limitation, enlargement of alveolar spaces accompanied by a large number of inflammatory cell infiltration, presented as emphysema. The number of neutrophils in bronchoalveolar lavage fluid significantly increased and expression levels of inflammatory factor were markedly elevated. The above effects were significantly alleviated after cyp2a5 knockout. These results indicate that 5-HMF can induce the occurrence of chronic obstructive pulmonary disease(COPD), and CYP2A5 plays an important role in the pathogenesis. Based on the above perspective, CYP2A5 was found to be highly expressed in murine alveolar macrophages. Our results also confirmed that CYP2A5 metabolic activation of 5-HMF can cause damage to alveolar macrophages, including decreased proliferation, loss of characteristic molecules, and destruction of cell membrane integrity. The above results suggest that alveolar macrophages could be the target cells of 5-HMF. In addition, through *in vitro* metabolic validation, DFF was found to be a cytotoxic metabolite generated by CYP2A5. Based on proteomics and molecu-

lar modeling docking, we found that 5-HMF and its toxic metabolite DFF could target binding to the cat·alytic domain of CSF1R in alveolar macrophages, causing cell damage, thus initiating COPD, and the binding capacity of DFF was much stronger than that of 5-HMF. The present study is the first to eluci·date that CYP2A5-mediated metabolic activation of 5-hydroxymethylfurfural could induce COPD of mice through targeting the alveolar macrophages. It may play a potential role in cigarette smoke-in·duced COPD.

Key words: 5-hydroxymethylfurfural; Cytochrome P450 2A5; chronic obstructive pulmonary dis·ease; alveolar macrophages; CSF1R

Corresponding author: Shou-Lin Wang, E-mail: wangshl@njmu.edu.cn

T01-60-0037

Proton exposure to bone marrow cell oxidation damage in mice

Yuchen Li[a,b], HonglingZhao[b], Wen Zhang[a,b], YihaoGong[c], Qi Liuc, Qiaojuan Wang[c], Li Sui[c], Hua Guan[b], Pingkun Zhou[b]

(a. Hengyang Medical College, University of South China, Hengyang 421001; b. Beijing Key Laboratory for radiobiology, Institute of Radiation Medicine, Beijing, 100850; c. Radiation Effects Research Laboratory, China Institute of Atomic Energy, Beijing 202413)

Abstract: Recently, mitochondrial ultrastructural changes and damage effects have received more and more attention in the role and mechanism of radiation treatment of tumors.On the one hand, the mi·tochondria of cancer cells themselves have undergone significant changes, and thus play an important role in promoting cancer cell proliferation, tumor radiotherapy and chemotherapy resistance;On the oth·er hand, mitochondrial structure and dysfunction are important factors in cell death and aging.Mitochon·dria will serve as a key target organelle for ionizing radiation outside the nucleus, and it is expected to become a research field of radiation biomedicine that will attract widespread attention in the future. Saenko et al. have found that human leukocytes are irradiated with 12Gy rays, the production of mito·chondrial reactive oxygen species (ROS) and reactive nitrogen is greatly increased. Tohru Yamamori et al. used 10Gy X-rays to irradiate human lung cancer cells and found that mitochondrial ROS produc·tion increased, mitochondrial membrane potential, mitochondrial respiration, and mitochondrial ATP production increased, showing that ionizing radiation can up-regulate the mitochondrial electron trans·fer chain and the number of mitochondria, thereby inducing a large amount of ROS[2]. Based on this phenomenon, we irradiated mice whole body with 90MeV protons to study the production of ROS by bone marrow cells and the protective mechanism of radioprotective drugs (VND3207). The results showed as followed. Proton irradiation had a damaging effect on the bone marrow cells of mice, and the VND3207 before irradiation had a protective effect on the damage caused by the increase of ROS caused by protons. Whether VND3207 can provide protection to the body by reducing radiation-in·duced ROS needs to be further studied. It can provide another option for reducing radiation damage.

Key word: proton irradiation; ROS; Radioprotective drugs

Corresponding author: Hua Guan, E-mail: ghlsh@163. com; Pingkun Zhou, E-mail: zhoupk@nic. bmi.ac.cn

T01-62-0042

Transcriptomics analysis and benchmark concentration estimating to unveil the mode of action of bisphenol A induced damage on human normal prostate epithelial cells at human-relevant levels

Xiaomeng Li, Wei Xiong, Mengmei Ni, Zhirui Yang, Lin Tian, Lishi Zhang, Jinyao Chen
(West China School of Public Health/West China Fourth Hospital and Healthy Food Evaluation Research Center, Sichuan University, Chengdu, China)

Abstract: This study aims to explore the mode of action (MOA) of bisphenol A (BPA) induced damage on human normal prostate epithelial cells based on the strategy of Toxicity Testing in the 21st Century. A physiological based pharmacokinetic model was used to select the administration concentrations according to the BPA concentration in male gonads at human actual exposure level. Human normal prostate epithelial cells RWPE-1 were administrated for 28 days with five BPA groups (1.9×10^{-3}, 1.9×10^{-1}, 1.9×10, 1.9×10^{3} and 1.9×10^{4} nmol·L^{-1}), 0.1% DMSO and 103 nmol·L^{-1} E_2. The possible key signaling pathways that may induce damage on RWPE-1 cells were identified using transcriptome sequencing and analysis, and qPCR test was employed to verify the expressions of mRNA. The protein expressions of possible key signaling pathways and cell cycles were measured using Western blot and flow cytometry methods, respectively. The Bayesian benchmark dose software was used to analyze the concentration-response relationships of key events (KEs). Transcriptomics analysis indicated that the differentially expressed genes were mainly enriched in the endocytosis, cell cycle, cellular senescence, MAPK signaling pathway, TNF signaling pathway, which are possible key signaling pathways. The qPCR results showed that, mRNA expressions of *MAPKAPK2*, *MAP2K3*, *JUN*, *FOS*, *CDKN1A* and *CCND1* increased significantly, and mRNA expressions of *TP53* and *CDC25C* decreased significantly after 28-day BPA exposure. Western blot results showed that the relative protein expressions of phosphorylated MAPKAPK2 and c-fos increased with increasing BPA concentration. In addition, BPA can significantly increase the proportion of G0/G1 and S phase, and reduce the proportion of G2/M phase significantly. All KEs included in concentration-response analysis consisted of relative mRNA expressions of *MAPKAPK2*, *JUN*, *CDKN1A*, *CDC25C* and *CCND1*, and the proportion of G0 / G1 phase, S phase and G2/M phase. Among all the benchmark concentration lower confidence limit (BMCL) values, the G2/M phase ratio yielded the smallest, with the BMCL5 of 110.580 nmol·L^{-1} and $BMCL_{10}$ of 175.862 nM. The BMCL values of mRNA expression of CDKN1A were also relatively small, and the $BMCL_5$ and $BMCL_{10}$ were 142.394 nmol·L^{-1} and 207.648 nmol·L^{-1}, respectively. Our findings suggest that under the present experimental conditions, the MOA of RWPE-1 cell damage caused by the actual human exposure levels of BPA may include: KE1-MAPK pathway activation, KE2-increased expression of CCND1 and CDKN1A, and decreased expression of CDC25C, KE3-cell cycle arrest.

Key words: Transcriptomics; Benchmark concentration; Mode of action; Bisphenol A; Human normal prostate epithelial cells

Corresponding author: Lishi Zhang, E-mail: lishizhang_56@163.com; Jinyao Chen, E-mail: umbrellayy@163.com

T01-66-0006

Exposure to indoor airborne phthalic acid esters is associated with cardiorespiratory responses in healthy young adults: A randomized, crossover trial of air purification

Jiawei Wang[1], Jiazhang Shi[1], Yan Zhao[1], Lijun Xue[1], Guoxing Li[1], Bin Wang[2], Xinbiao Guo[1], Jing Huang[1], Shaowei Wu[3]

(1. Department of Occupational and Environmental Health Sciences, School of Public Health, Peking University, 38 Xueyuan Road, Beijing 100191, China; 2. Institute of Reproductive and Child Health, Peking University/Key Laboratory of Reproductive Health, National Health Commission of the People's Republic of China, 38 Xueyuan Road, Beijing 100191, China; 3. Department of Occupational and Environmental Health, School of Public Health, Xi'an Jiaotong University Health Science Center, 76 Yanta West Road, Xi'an, Shaanxi 710061, China)

Abstract: OBJECTIVE Background: Phthalic acid esters (PAEs) are widely used as plasticizers in industrial process and consumer products. Nowadays, PAEs are ubiquitous in the environment and are reported to be associated with cardiorespiratory diseases. However, studies about the association between indoor airborne PAEs exposure and cardiorespiratory health were limited, and the potential biological mechanism remains under-recognized. **METHODS** A randomized crossover trial was conducted on 57 healthy young adults in Beijing. Repeated health measurements were performed under real and sham indoor air purification with a washout interval of at least 2 weeks. The concentration of indoor air·borne PAEs were determined by gas chromatography-orbit ion trap mass spectrometry. Health indica·tors including blood pressure, lung function, airway inflammation, and circulating biomarkers reflecting blood coagulation and systematic oxidative stress were measured. Linear mixed-effect model was used to examine the between-treatment differences in health indicators, and three models including single-constituent, constituent-PM2.5 joint, and single-constituent residual model were used to estimate the association between indoor airborne PAEs and health indicators. **RESULTS** The indoor airborne PAEs were reduced effectively under real air purification. The total indoor airborne di-2-ethylhexyl phthalate (DEHP), bis(4-Methyl-2-pentyl) phthalate (DMPP), diphenyl phthalate (DPP), and diethyl phthalate (DEP) were identified to be most significantly associated with the increase of blood pressure and air·way inflammation, and decrease of lung function. A doubling increase in DEHP, DMPP, DPP, DEP was associated with the increase of 17.2% (95% CI: 3.9%, 32.2%), 11.7% (95% CI: 3.5%, 20.6%), 7.0% (95% CI: 2.4%, 11.8%), 6.0% (95% CI: 1.8%, 10.4%) in FeNO, respectively, in single-constituent resid·ual model. Significant associations between specific total indoor airborne PAEs and increased levels of health biomarkers including oxidized low-density lipoprotein (ox-LDL), 8-isoprostane (8-isoPGF2α), and soluble P-selectin (sP-selectin) were observed. **CONCLUSION** Indoor airborne PAEs may cause ad·verse cardiorespiratory health effects in young healthy adults, and indoor air purification could amelio·rate the adverse cardiorespiratory effects.

Key words: Phthalic acid esters; Indoor air purification; Oxidative stress; Blood coagulation; Air·way inflammation

Corresponding author: Shaowei Wu, E-mail: shaowei_wu@xjtu.edu.cn

T01-67-0018

Acute ozone exposure induce mitophagy in lung epithelial cells and regulate pyroptosis through the activation of the NLRP3 pathway

Xiaohua Liu, Lei Tian, Jun Yan, Kang Li, Zhuge Xi

(Tianjin Institute of Environmental and Operational Medicine, No.1 Dali Road, Heping District, Tianjin, 300050, China)

Abstract: OBJECTIVE Ozone exposure has gradually become a focus of attention due to its role in multiple lung diseases. However, whether there is a causal relationship between ozone-induced mitochondrial autophagy and cell pyrotosis has not been described in detail. **RESULTS** Male Wistar rats and type I primary alveolar epithelial cells were exposed to different concentrations of ozone. It was found that the levels of oxidative stress markers (GSH-Px, MDA, SOD) in the lung tissues of rats were significantly different in response to different concentrations of ozone, indicating that ozone exposure caused oxidative damage in the lungs. In type I alveolar epithelial primary cells, reactive oxygen species (ROS) content and lactate dehydrogenase (LDH) release increased, while the ATP content and mitochondrial membrane potential significantly decreased. Mitophagy-related markers and PINK1 / Parkin pathway-related proteins were detected, and the mitophagy pathway was activated. The co-localization of LC3, Parkin, and mitochondria in ozone exposure type I alveolar epithelial cells was detected by immunofluorescence. These results concordantly proved that ozone exposure activates the PINK1/Parkin signaling pathway, and triggers mitochondrial autophagy. Oxidative stress and mitophagy were significantly reduced in cells treated with the ROS inhibitor, N-acetylcysteine (NAC). After treating the cells in the 0.5 ppm group with the autophagy inhibitor MDIVI-1, the expression of PINK1, Parkin, and LC3 was significantly decreased; the same was observed for the pyroptosis-related proteins NLRP3, cleaved caspase-1, and N-gasdermin D (N-GSDMD). These results indicate that mitophagy triggered in response to ozone exposure induces NLRP3-mediated cell death. Overexpression and knockdown of NLRP3 confirmed this conclusion. **CONCLUSION** Ozone exposure induces oxidative stress in the lung tissue of rats, which in turn can cause structural and functional damage to mitochondria. ROS induce mitophagy through the activation of the PINK1/Parkin signaling pathway. Subsequently, mitophagy can induce pyroptosis through the activation of NLRP3.

Key words: Ozone; Lung toxicity; Reactive oxygen species; Mitophagy; Pyroptosis

Corresponding author: Xiaohua Liu, E-mail: liuxiaohua1992@sina.com; Zhuge Xi, E-mail: zhugexi2003@sina.com

T01-70-0009

Role of CCL21 regulating Th17 cells passing through blood-cerebrospinal fluid barrier in neuroinflammation of obese mice following lead exposure

Weixuan Wang[a], Shuang Li[b], Shulang Pang[a], Yanshu Zhang[a,b]

(a. School of Public Health, North China University of Science and Technology, Tangshan Hebei 063210,China; b. Laboratory Animal Center,North China University of Science and Technology, Tangshan Hebei 063210, China)

Abstract: Accumulating evidence demonstrates that Th17 cells play critical roles in central nerve system inflammatory diseases, but there is less report regarding the role of Th17 on neuroinflammation of obesity mice following lead exposure. The current study aims to investigate the mechanism of inflammatory injury in obesity mice following lead exposure. Exposure of lead via drinking water ofobesitymicewith high fat diet significantly resulted in theaggravation of neuroinflammation. What's more, we found the ratio of Th17 cells in the brain and IL-17A,IL-22 secreted by Th17 cells also sharply increased. To explain how Th17 cellsenter brain, we measured the chemokines expression by using antibody arrayin Z310, a cell line of choroid plexus epithelium. The results showed thatCCL21 was listed the top change among chemokines. Meanwhile, the protein expression of CCL21 was also higher in hippocampus ofobesitymice and Z310 cells following lead exposure. Then wemade Z310 cellswith CCL21 knocked out, we found chemotaxis index of Th17 cells reduced. Taken together, our finding suggested lead exposure can aggravate the inflammatory reaction in the brain tissue of obesity mice, which mightdue to the regulation of Th17 into brain tissue by CCL21 through blood CSF barrier. Our finding will provide new insight for mechanism of obesity people following lead exposure.

Key words: Lead; Obesity; Neuroinflammation; Th17 cells; Blood-cerebrospinal fluid barrier; Chemokines

Corresponding author: Yanshu Zhang, E-mail: Yanshu_zhang@163.com

T01-72-0023

LncRNA PVT1 regulates hepatocyte necroptosis progression in the inflamed liver of male offspring exposed to paternal nonylphenol through ZBP1

Qiannan Di, Qian Xu

(Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing 210009, China)

Abstract: Nonylphenol (NP) is one of the most worrisome and ubiquitous environmental endocrine disruptors (EDCs), which can be easy to accumulate in organisms, and its biological amplification through the food chain is believed to cause damage to human health. It has been listed as one of the 27 persistent toxic pollutants under priority control by the United Nations Environment Programme (UNEP). Studies have shown that maternal exposure to NP during perinatal and lactation can damage the central nervous system, development, fertility, adipogenesis in offspring. However, there is a lack of research on the intergenerational effects of paternal preconception exposure to NP, although it is increasingly recognized that paternal preconception exposure to EDCs (BPA, DBP, DEHP) affects the health of subsequent offspring. In this study, a rat model of paternal preconception 28-days NP exposure was established. Exposure doses (15, 45 and 135 mg·kg^{-1}·bw/day) were designed to be 1, 3, and 9 times of NP's no observed effect level, representing low, high, and polluted areas population, respectively. According to pathological and biochemical changes, the unclear liver lobular structure, necrotic hepatocytes and inflammatory cell infiltration, the increased level of ALT, AST, TBA, ALP and the decreased level of ALB, the hepatitis in male offspring was determined. The liver samples from male offspring were then subjected to lncRNAsequencing. The results showed that lncRNA Pvt1 expression level in 15 mg· kg^{-1}· bw/ day NP group was not obviously changes, but significant increase in 45 and

135 mg·kg^{-1}·bw/day NP groups. The expression of ZBP1, a protein related to necroptosis, was increased, and the expression of necroptosis downstream proteins RIPK3, p-MLKL were also increased. To verify the mechanism of Pvt1 with hepatitis, primary rat hepatocytes were extracted and identified. Apoptosis results detected by flow cytometry. After determined that Pvt1 is predominantly located in the nucleus, ASO-Pvt1 was chosen for knockdown in the primary hepatocytes of 135 mg·kg^{-1}·bw/day NP group offspring. Compared with NC group, themRNA and protein expression of ZBP1-RIPK3-p-MLKL were significant decreased in ASO-Pvt1 group. Apoptosis assay showed that after ASOtransfection the cell death by necroptosis was inhibited markedly. In conclusion, this study suggested that paternal preconception NP exposure induced hepatitis in male offspring. It was also verified that lncRNA Pvt1 induced necroptosis in hepatocytes through the regulation of ZBP1-RIPK3-p-MLKL pathway. This research was expected to provide a theoretical basis for further assessment of the health risk under NP exposure.

Key words: Nonylphenol ; Paternal exposure ; Pvt1 ; Necroptosis

Corresponding author: Qian Xu, E-mail: qn_di5d@163.com

T01-74-0028

Pubertal exposure to bisphenol-A affects social recognition and arginine vasopressin in the brain of male mice

Jin Shi-zhen, Wang Jin-shan, Fu Wen-shuang, Zheng Jia-qi, Fu Xiao, Xu Gui-ping,
Liang Yu-feng, Xu Xiao-hong
(Chemistry and Life Sciences College, Zhejiang Normal University, Jinhua 321004 P.R. China)

Abstract: Social recognition is an ability of animals to identify and distinguish conspecifics, which is essential for nearly all social species to establish social relationships. Social recognition provides the basis for a variety of social behaviors. Because of modulated by gonadal hormones, it is possible that social cognition is affected by environmental endocrine disruptors (EEDs). Bisphenol-A (BPA), as a plasticizer, not only competes with endogenous estrogens on the receptors, exerting weak estrogenic characteristics, but also changes the synthesis of testosterone (T) and antagonizes androgen receptor (AR)- mediated transcription. In the present study, after being pubertal exposed to bisphenol A (BPA, 0.04, 0.4, and 4 mg·kg^{-1}) for 18 days, adult male mice did not show significant dishabituation to a novel female stimulus in habituation-dishabituation task. The capacity for discriminating the odors between familiar and novel female urine or between male and female urine was suppressed in BPA-exposed male. In addition, BPA (0.4, 4 mg·kg^{-1}) decreased the number of immunoreaction of AVP (AVP-ir) neu·rons in both the bed nucleus of the stria terminalis (BNST) and the medial amygdala (MeA), and BPA (0.04, 0.4, 4 mg·kg^{-1}) reduced the level of V1αR in the lateral septum (LS) of adult male. Further, BPA decreased the levels of testosterone (T) in the brain and androgens receptor (AR) in the LS, the amyg·dala, and BNST, as well the levels of estrogen receptor α and β (ERα/β) in the amygdala and BNST. These results indicate that pubertal exposure to BPA altered the actions of steroid hormones in the brain and thus perturbed the regulation of estrogen and androgen to the AVP system in social circuits and impaired the social cognition ability of male mice. Deficits in social memory and recognition are commonly found in disorders such as autism spectrum disorders, ADHD and learning disabilities. Based on these data, BPA exposure during critical developmental periods may contribute to the risk for social symptoms commonly associated with ADHD and/or autism spectrum disorders.

Key words: bisphenol-A; social recognition; AVP; androgen; estrogen
Corresponding author: Xu Xiao-hong , E-mail: xuxh63@zjnu.cn

T01-75-0008

Prenatal Polycyclic aromatic hydrocarbons exposure and Neurodevelpmental retardation: cohort and mechanism study

Jisheng Nie[1,2], Jinyu Li[1], Lin Cheng[1], Yanning Li[1], Yunjun Deng[1], Zhiwei Yan[1], Lei Duan[1], Qiao Niu[1], Jin Yang[1], Deliang Tang[1,2]

(1. Department of Occupational and Environmental Health, School of Public Health, Shanxi Medical University, Xinjiannan Road 56, Taiyuan 030001, China; 2. Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, 722W. 168th Street, New York, NY 10032, USA)

Abstract: Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous air pollutants generated by combustion of organic material, including fossil fuel. It has been an open question whether prenatal exposure to PAHs significantly increases the risk of offspring Neurodevelpmental retardation.And what is main mechanism in the process. Here, we have examined this hypothesis in a birth cohort in Taiyuan City and a benzo[a]pyrene exposure animal model. We enrolled 400 mother-newborns pairs and collected theirs biological samples after written informed consent. Neonatal behavioral neurological assessment (NBNA) tests were conducted when the infants were three days old. In the follow-up, 221 mother-child pairs with 2-year-old children were included in this study. We used the Gesell Developmental Scales (GDS) for children neurodevelopmental assessments 2 years of age. Multiple linear regressions were used to analyze the associations of maternal urinary PAH metabolites and cord blood BPDE with child neurodevelopmental scores and 5-hmC levels, and restricted cubic spline models were further used to examine the shapes of dose-response relationships. A mediation analysis was also conducted. We observed dose-response associations of maternal urinary 1-hydroxypyrene (1-OH·Pyr) and sum of PAH metabolites (Σ-OHPAHs) with decreased neurodevelopmental scores (NBNA and GDS scores), and the 5-hmC levels of the BDNF and MeCP2 gene promoter (P<0.05). The 1-OHPyr was positively associated with cord blood global DNA 5-hmC levels. The 5-hmC level of BDNF and MeCP2 gene promoter were positively associated with neurodevelopmental scores. Mediation analysis showed the 5-hmC levels of BDNF gene promoter could explained 19.95% and 13.79% of the effect of motor scores change related to prenatal exposure to 1-OHPyr and Σ- OHPAHs (P<0.05), respectively. In animal study, Pregnant rats were exposed to benzo[a]pyrene at three dosages (10 mg· kg^{-1} B[a]P, 20 mg·kg^{-1} B[a]P,40 mg·kg^{-1} B[a]P). Neonatal development was evaluated at ages of 1, 4, 7, 14, 45 and 75 days. Physical development(time of ear stand up, incisor eruption, ear opening and development of fur and body weight), behavior development (Negative geotaxis, Cliff aversion, Forelimb grip, surface righting reflex, olfactory discrimination) and learning and memory in offspring were impaired in a B[a]P dose dependent manner. Further, results shown that 5hmC abundance and the level of Tet in hippocampus isolated from offspring rats were increased after prenatal exposure to B(α)P. As an obvious feature, prenatal B(α)P exposure down-regulated brain-derived neurotrophic factor (BDNF) protein from birth to adulthood. Meanwhile we found that attenuation of BDNF mRNA in PND1-PND7, which was accompanied by increased 5hmC modifications at the gene body of BDNF in PND1 and PND4, but in PND7, 5hmC of BDNF decreased in B(α)P treatment groups. Our results in population cohort and an·

imal model showed that Prenatal PAHs Exposure was the predominant risk factor for offspring neurobehavioral development and elevation of DNA hydroxymethylation and 5hmC modifications at the gene body of BDNF in early development period would be an important molecular mechanism participating in the neurodevelopmental toxicity of PAHs.

Key words: Prenatal Polycyclic aromatic hydrocarbons (PAHs) Exposure; Neurodevelopmental retardation; birth cohort; animal model

Corresponding author: Jisheng Nie, E-mail: niejisheng@sxmu.edu.cn

T01-75-0024

Acrylamide induces apoptosis via ERS and autophagy

Yiqi Wang[a], Ying Liu[a], Xing Zhang[a], Yang Jiao[a], Lian Duan[a], Lingling Dai[b], Hong Yan[a]

(a. Department of Health Toxicology, MOE Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong-Road, Wuhan 430030, PR China 86; b. Experimental Teaching Center of Preventive Medicine School of Public Health, Tongji Medical College, Huazhong University of Science & Technology)

Abstract: Acrylamide (ACR) has obvious neurotoxicity in human by causing apoptosis, endoplasmic reticulum stress, and autophagy, but the relationships among were still unclear. This study aimed to investigate the roles of ERS and autophagy *in vitro*, to further demonstrate the mechanism of ACR neurotoxicity. In present study, different doses of ACR were set, to value ACR toxicity, then the PERK inhibitor and autophagy inhibitor, GSK2606414 and 3-Methyladenine, were used separately to inhibit ERS and autophagy activation under ACR treatment. With the increases of ACR dose, the cell viability and apoptotic rate dose-dependently decreased. After ERS inhibition, the apoptosis and autophagy caused by ACR were alleviated, showing reduced apoptotic rate, and down-regulated levels of Bax, Beclin1, LC3- Ⅱ/LC3- Ⅰ. While the autophagy inhibition deteriorated apoptosis, with up-regulated apoptotic rate, and increased Bax, indicating ACR caused autophagy by activating PERK pathway of ERS, and induced apoptosis finally. This study helps to provide experimental basis for exploring potential molecular targets for the prevention and control of ACR toxicity.

Key words: Acrylamide; Endoplasmic reticulum stress; Apoptosis; Autophagy

Corresponding author: Hong Yan, E-mail: yanhong@mails.tjmu.edu.cn)

T01-75-0026

Polyhexamethylene guanidine aerosol triggers pulmonary fibrosis concomitant with elevated surface tension via inhibiting pulmonary surfactant

Li xin, Zhang jian-zhong, Tang jing-long, Zheng yu-xin

(Department of Environmental and Occupational Health, School of Public Health, Qingdao University, Qingdao 266071)

Abstract: Environmental chemicals inhalation exposure could induce pulmonary fibrosis, which is

characterized by the excessive proliferation of fibroblasts and accumulation of extracellular matrix com·ponents, in which surface tension usually plays vital role. Polyhexamethylene guanidine (PHMG) was first recognized as a potential hazard ingredient in humidifier disinfectants, which caused an outbreak of pulmonary fibrosis in South Korea. Of note, humidifier disinfectant has induced 7336 patients suf·fered from lung disease, including 209 dead patients, reported by the government of South Korea to date (March 2021). Besides, it's particularly worrying that PHMG disinfectant is still widely used (poten·tially used as air disinfectant) in a wealth of countries, including China, even though it has been banned in South Korea. However, the underlying mechanisms involved in PHMG-induced pulmonary fibrosis have not yet been fully elucidated. Therefore, this study mainly focuses on the effect of PHMG on sur·face tension to unveil the influence and involved mechanisms in PHMG-induced pulmonary fibrosis. C57BL / 6J mice were exposed to sub-acute PHMG aerosol for 8 weeks. The results indicated that PHMG aerosol inhalation exposure induced pulmonary fibrosis, increased the respiratory system elas·tic resistance, and reduced respiratory system compliance. Subsequently, constrained drop surfactome·try (CDS) was applied to explore the mechanisms of PHMG-induced surface tension elevated. Results from *in vitro* study further confirmed PHMG elevated surface tension by inhibited pulmonary surfactant. Besides, considering the crucially regulated role of SP-B and SP-C in reducing surface tension, molecu·lar docking was used to further explored structural characteristics and binding sites of PHMG with SP-B and SP-C. Mechanistically, PHMG suppressed SP-B and SP-C by inhibiting protein expression and block their active sites. The present study, for the first time, revealed the molecular mechanism of PHMG-induced pulmonary fibrosis based on pulmonary surfactant inhibition mediated surface tension elevated. And pulmonary surfactant may be a potential target for further intervention to prevent PHMG-induced fibrosis or alleviate the symptom of relevant patients.

Key words: PHMG; pulmonary fibrosis; pulmonary surfactant; surface tension

Corresponding author: Tang jing-long, E-mail: tangjinglong@qdu. edu. cn; Zheng yu-xin, E-mail: yxzheng@qdu.edu.cn

T01-75-0031

Role of exosome-transmitted circRNA circCLIP1 in $PM_{2.5}$-induced asthma

ZHU Huan-huan, TANG Xi-ying, WANG Xi, SUN Guan-ting, WANG Mei-lin,
ZHANG Zheng-dong, CHU Hai-yan
(School of Public Health, Nanjing Medical University, Nanjing 211166, China)

Abstract: OBJECTIVE To reveal the role of exosomal circular RNAs (circRNAs) in $PM_{2.5}$-induced asthma. **METHODS** Exosomal circRNAs regulated by $PM_{2.5}$ was screened by RNA-seq and real-time fl uorescence quantitative polymerase chain reaction (RT-qPCR). Enzyme linked immunosorbent assay (ELISA) and Western blot were used to evaluate the role of exosomal circRNAs in exosomes from PM2.5-treated human bronchial epithelial (HBE) cells ($PM_{2.5}$-Exos) - induced mucus hyper-secretion in HBE cells and contractility of sensitive human bronchial smooth muscle cells (HBSMCs). The loop structure and subcelluar localization of circRNA was determined by Sanger sequencing, fluorescence in situ hybridization (FISH), etc. N6-methyladenosine RNA binding protein immunoprecipitation (MeR·IP) and RT-qPCR were used to explore the effects of PM2.5 treatment and METTL3 on m6A modifica·tion and expression level of circRNA. RNA pulldown, ELISA and Western blot were adopted to screen the RNA-binding protein and appraise its role in $PM_{2.5}$-Exos-induced mucus hyper-secretion in HBE

cells and contractility of sensitive HBSMCs, respectively. **RESULTS** Exosomal circCLIP1 was upregu·lated by $PM_{2.5}$ and promoted mucus secretion in HBE cells and contractility of sensitive HBSMCs, respectively. circCLIP1 was characterized by head-to-tail back splicing, resistant to RNase R and predominantly localized in nuclear. In donor cells (HBE cells), $PM_{2.5}$ upregulated circCLIP1 by METTL3-mediated m6A modification. Knockdown of METTL3 caused a significant decrease of circCLIP1 in cells and exosomes. In recipient cells (HBE cells and sensitive HBSMCs), circCLIP1 bound to SEPT10. Interfering SEPT10 attenuated mucus hyper-secretion in HBE cells and contractility of sensitive HB·SMCs induced by $PM_{2.5}$-Exos. **CONCLUSION** Exosomal circCLIP1 was regulated by METTL3-mediated m^6A modification and promoted PM2.5-induced asthma via targeting SETP10.

Key words: $PM_{2.5}$; Exosomes; METTL3; circCLIP1; Asthma

Corresponding author: CHU Hai-yan, E-mail: chy_grape@njmu.edu.cn

T01-76-0011

Urban fine particulate matter induced Ca^{2+} overload mediated-mitochon-drial bioenergetics inhibition in cardiomyocyte and cardiac hypertrophy in mice heart

Zou Ling-yue[a,b], Li Bin-jing[a,b], Tang Meng[a,b]

(a. Department of Toxicology, School of Public Health, Southeast University, Nanjing 210009, China. bKey Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing 210009, China)

Abstract: The cardiovascular toxicity of urban fine particulate matter ($PM_{2.5}$) has aroused wide·spread concern in recent years. Heart is a tissue rich in mitochondria. Thus knowledge of myocardial mitochondrial dysfunction due to particulate matter exposure is essential for further cardiotoxic effects. However, the involving mechanisms induced by urban fine particles have not been studied well. Here, the mechanism of cardiac hypertrophy through calcium overload and mitochondrial dysfunction induced by urban atmospheric $PM_{2.5}$ was investigated *in vivo* and *in vitro*. In *in vivo* experiments, both male and female BALB / c mice were intratracheally instilled 1.28, 5.5, 11 mg $PM_{2.5}$ / kg bodyweight weekly for 4 weeks and set as low, medium and high dose groups respectively. In the high-dose group, $PM_{2.5}$- caused cardiac edema and rupture, inflammatory infiltration, and cardiac hypertrophy in the left ventri·cle could be observed. Mitochondria were enlarged and ruptured, with abnormal ultrastructural morphol·ogy. *In vitro* experiments on human cardiomyocyte AC16 showed that exposure to $PM_{2.5}$ for 24 h caused hypertrophic single cell surface area, weakened mitochondrial respiratory metabolism capacity, decreased ATP production, and opened mitochondrial permeability transition pore—leading to exces·sive calcium production, also mitochondrial membrane potential decrease, resulting in cells apoptosis. Nevertheless, the administration of calcium chelator ameliorated the mitochondrial damage in the $PM_{2.5}$ treated group. Our *in vivo* and *in vitro* experiments confirmed that calcium overload under $PM_{2.5}$ expo·sure triggered CAMK II activation, leading to mitochondrial respiratory dysfunction and cardiac hyper·trophy.

Key words: Particulate matter; Cardiac hypertrophy; Mitochondria

Corresponding author: Tang Meng, E-mail: tm@seu.edu.cn

T01-82-0048

Cancer Hazard Identification and Exposure Assessment of Perfluorodecanoic Acid

Zhenya Chen

(Proya Cosmetics Co.,Ltd R&D Innovation Center,Proya Cosmetics Co.,Ltd, No.588, Xixi Road, Liuli Street, Xihu District, Hangzhou City, Zhejiang Province 310063)

Abstract: **OBJECTIVE** Perfluorodecanoic acid (PFDA) is a widely used man-made fluorinated organic chemical, which has an extremely long half-life and can bioaccumulate in the environment and human bodies. Numerous studies have identified multiple toxicities of perfluorinated carboxylic acid (PFCAs), however, fewer studies have assessed PFDA. The purpose of the study was to conduct a cancer hazard identification for PFDA and assess the exposure of infants and toddlers to PFDA via breast milk and household dust, respectively. **METHOD** A systematic literature review was performed for cancer hazard identification by searching PubMed, Embase, and Scopus. Search terms from the National Toxicology Program (NTP) were included, and the estimated daily intake dose (EDI) was used for exposure assessment. **RESULTS** Four epidemiological studies, two animal experiments, and the evidence attached to 10 key characteristics of PFDA carcinogenicity were summarized. In terms of exposure assessment, Spain and Belgium showed the highest level of PFDA in breast milk and house·hold dust, respectively. Many factors influenced EDI, including dwelling characteristics, ages and expo·sure routes. **CONCLUSIONS** No information on the potential carcinogenicity of PFDA or environmen·tal risks were found. Further studies are needed to provide sufficient data for hazard identification and the establishment of risk indices.

Key words: Perfluorodecanoic Acid; Hazard Identification; Exposure Assessment; Cancer

Corresponding author: Zhenya Chen, E-mail: chenzhenya@proya.com

T01-84-0012

Effects of triphenyl phosphate at environmentally-related levels on the liver lipid metabolism in mice under different diets

Cui Haiyan, Chang Yeqian, Li Mei

(State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210023)

Abstract: Due to the ubiquitous existence of triphenyl phosphate (TPP) in foodstuffs, human expo·sure to TPP is inevitable, which makes it necessary to assess the human health risk of TPP exposure.1, 2 However, the specific contribution of TPP at environmentally relevant levels to the development of liver lipid metabolism disorders is still unclear, especially in combination with high fructose and fat (HFF) diets. The objectives were to evaluate the effects of TPP exposure at environmentally relevant levels on hepatic lipid metabolism in mice with different diets. Male C57BL/6J mice were chronically ex·posed to an environmentally relevant level (10 $\mu g \cdot kg^{-1}$ body weight per day) of TPP for 12 weeks. Bio·chemical analysis and pathological results showed that TPP exposure induced liver organelle damage and lipid accumulation, and increased glucose and insulin intolerance in mice. Proteomics showed that TPP exposure can affect the peroxisome proliferator-activated receptor (PPAR) signaling pathway by

causing significant expressions of fatty acid binding protein 5 (FABP5), and other PPAR signaling pathway-related proteins. Correspondingly, metabolomics suggested that TPP exposure led to changes in ABC transporters, amino acid metabolism and other differential metabolic pathways. Besides, TPP exposure worsened the abnormal liver damage induced by the HFF diet. Our results showed that TPP exposure could cause liver lipid metabolism disorders by activating PPAR signaling pathway, and liver metabolism disorders also indirectly affect lipid metabolism. These findings provided a reference for future study directions for in-depth exploration of health risk assessment and mechanisms for long-term chronic exposure to TPP.

Key words: Triphenyl phosphate; High fructose and high fat; PPAR; Liver damage

Corresponding author: Li Mei, E-mail: meili@nju.edu.cn

T01-84-0013

Lactobacillus plantarum alleviates lead-induced severer hepatic injury in obese mice, by promoting fecal lead excretion and enhancing antioxidative defense system

Hu Lie-Hai, Zhao Yu, Liu Shan-Ji, You Tao, Zhang Jing-Feng, Xu Heng-Yi
(State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China)

Abstract: Lead (Pb) is a toxic heavy metal which has been intensively studied, but the toxicity in sub-healthy people remains unclear. In order to investigate the potential effects of Pb in obese population, and validate the feasibility of probiotics for the treatment of Pb poisoning and diet-induced obesity, male Kunming mice were fed high-fat/high-sugar (HFHS) diet for 6 weeks, then treated with 1 g/L Pb^{2+} and Lactobacillus plantarum for 2 weeks. After the trail, liver and colon morphology were observed, tissue biochemical indicators and genes expression levels were measured. Here we reported that Pb exposure exacerbated the existing oxidative stress and gut barrier disruption in obese mice, thus caused severer hepatic inflammatory injury compared with normal mice. Present results showed that probiotics treatment significantly reduced Pb accumulation by promoting fecal Pb excretion, and enhanced antioxidant defense system by inhibiting oxidative stress. After Lactobacillus plantarum treatment, obesity-related behaviors were improved, the hepatic injury and gut barrier disruption in mice were alleviated effectively. Our findings indicated that obesity group have lower resistance and face more security risk under Pb pollution, and probiotics supplementation may be a potential strategy for the treatment of both obesity and Pb poisoning.

Key words: Lead exposure; Obesity; Probiotics treatment; Enterohepatic injury; Oxidative stress

Corresponding author: Xu Heng-Yi, E-mail: kidyxu@163.com, E-mail: HengyiXu@ncu.edu.cn

T01-84-0036

Cadmium induces renal inflammation by activating the NLRP3 inflammasome through ROS/MAPK/NF-κB pathway *in vitro* and *in vivo*

Ziyin Li[a], Wei Zhu[b], Guangyu Yang[b], Jia Song[a], Lijun Mo[a], Feifei Xu[a], Zhini He[a,*], Xingfen Yang[a,*]
(a. Food Safety and Health Research Center, Guangdong Provincial Key Laboratory of Tropical

Disease Research, Guangdong-Hongkong-Macao Joint Laboratory for Contaminants Exposure and Health, School of Public Health, Southern Medical University, Guangzhou 510515, P.R. China;
b. Department of Toxicology, Guangzhou Center for Disease Control and Prevention, Guangzhou 510440, Guangdong, P.R. China)

Abstract: Cadmium (Cd) has been reported to induce kidney damage by triggering oxidative stress and inflammation. The NLR family Pyrin Domain Containing 3 (NLRP3) inflammasome has been implicated a role in the pathogenesis of inflammation. However, the connection between Cd and NLRP3 inflammasome in the development of renal inflammation remains unknown. In this study, *in vitro* experiments based on the telomerase-immortalized human renal proximal-tubule epithelial cell line (RPTEC/TERT1) were carried out. Results revealed that CdCl (2-8 μmol·L^{-1}) increased ROS production and activated NLRP3, thereby enhancing secretion of IL-1β and IL-18 (P<0.05). Knock-down of NLRP3 rescued the RPTEC/TERT1 cells from Cd-induced inflammatory damage. Cd activated the MAPK/NF-κB signaling pathway in RPTEC/ TERT1 cells (P<0.05). Additionally, treatment with N-acetylcysteine (NAC) improved inflammation and blocked the upregulation of the MAPK/NF-κB signaling pathway. Pre-treatment with MAPK and NF- κB inhibitors also suppressed NLRP3 inflammasome activation (P< 0.05). Moreover, CdCl2 (25-00 mg· L^{-1}) stimulated the MAPK/ NF- κB signaling pathway, activated the NLRP3 inflammasome, and increased inflammatory response (P<0.05) leading to renal injury in rats. Exposure to cadmium elevated serum levels of NLRP3 and IL-1β in populations (P<0.05). Further analysis found that serum NLRP3 and IL-1β levels were positively correlated with urine cadmium (UCd) and urine N-acetyl-β-D-glucosaminidase (UNAG). Overall, Cd induced renal inflammation through the ROS/ MAPK/ NF- κB signaling pathway by activating the NLRP3 inflammasome. Our research thus provides new insights into the molecular mechanism that NLRP3 contributes to Cd-induced kidney damage.

Key words: Cadmium; Renal; NLRP3 inflammasome; IL-1β; MAPK; NF-κB;

T01-85-0001

Polychlorinated Biphenyl Congener 180 (PCB 180) Regulates Mitotic Clonal Expansion to Enhance Adipogenesis through Modulation of C/EBPβ SUMOylation in 3T3-L1 Cell

Caixia Yu[1,2], Qing Wen[1,2], Qidong Ren[1,2], Yuguo Du[1,2], Xinni Xie[1]
(1. State Key Laboratory of Environmental Chemistry and Eco-Toxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China;
2. College of Chemical Sciences and College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China)

Abstract: PCB 180, a typical non-dioxin-likepolychlorinated biphenyls (NDL-PCBs), isone of the most prevalent PCB-congeners found in human adipose tissue which is believed to be the major depository of PCBs in vivo. Several epidemiological investigations proclaimed the correlation of PCB 180 exposure and the incidence of obesity, however, the role of PCB 180 in obesity development remains poorly understood at the experimental level. This study aims to explore the adipogenic effect and mechanism on PCB 180 treated 3T3-L1 cells in order to get a better understanding in between obesity and PCB 180 exposure. Firstly, we observed significant enhancement on adipogenesis when 3T3-L1 pread·

ipocytes were exposed to PCB 180 in the whole adipogenic differentiation period. Secondly, our study showed that exposure of PCB 180 during the first 2 days was critical tothe adipogenic effect. According to our results from sequential experiments of cell cycle analysis, cell counting, BrdU incorporation, Cyclin D1, Cyclin B1 and P27 proteins quantification, PCB 180 is identified to stimulate progression of mitotic clonal expansion (MCE) which is a required event for early adipogenic differentiation. Molecular mechanistic investigation revealed that PCB 180 promoted accumulation of C/EBPβ, a key regulator to control MCE, specifically at protein level during the early adipogenic stage. Finally, we foundthat PCB 180 could mitigate the degradation of C/EBPβ protein through repressing the SUMOylationand subsequent ubiquitination of C/ EBPβ ascribing to the up-regulation of SUMO-specific protease 2 (SENP2). Collectively, we have disclosed here, for the first time, that PCB 180can facilitate adipogenesis through alleviatingC/EBPβ protein SUMOylation and strengthening MCE progression during the early adipogenic stage. This result provides some novel evidences about obesogenic effect of PCB 180 with respect to its potency to promote adipocyte hyperplasia.

Key words: PCB 180; Obesogen; Mitotic clonal expansion (MCE); SUMOylation; Adipogenesis

Corresponding author: Xinni Xie, E-mail: xnxie@rcees.ac.cn

T01-91-0044

The association between environmental lead exposure and predicted 10-year atherosclerotic cardiovascular disease risk in Chinese middle-aged and older adults

Mo Li-fen, Song Jia, Huang Hai-bin, Wan Yu, Xiong Li-li, Yang Xing-fen

(Food Safety and Health Research Center, School of Public Health, Southern Medical University, 1023-1063 Shatai Nan Road, Guangzhou, Guangdong 510515, China)

Abstract: OBJECTIVE Lead is widely distributed in the environment. Although lead is reported to have cardiovascular toxicity, little is known about its impact on atherosclerotic cardiovascular disease (ASCVD). This study aimed to investigate the relationship between environmental lead exposure and the 10-year ASCVD risk in Chinese middle-aged and older adults. METHODS A total of 838 subjects (301 males, 537 females) aged 35-74 years old were recruited insouthern China, occupational subjects were excluded. Baseline information, history of diseases and medication, living behaviors and data of physical examination were collected by questionnaire and physical examination. Blood lead was detected by ICP-MS. The 10-year ASCVD risk score was calculated through the Chinese-PAR equation model, and classified into low (<5.0%), inter-mediate (5.0-9.9%), and high (≥10%) categories. Generalized linear model (GLM) and logistic regression model were applied to estimateassociations between quartiles (Q) of blood lead and the 10-year ASCVD risk. Subgroup analyses were performed in populations with different characteristics. RESULTS The blood lead of all subjects was 1.92 $\mu g \cdot L^{-1}$ (IQR: 1.16-48.78 ug/L), and predicted 10-year ASCVD risk score was 3.30 % (IQR: 1.70-6.00%). The predicted 10-year ASCVD risk scores increased with the increasing quartiles of blood lead (P<0.001). The higher exposure levels, the more percentage of subjects in the inter-mediate and highrisk of ASCVD. After fully adjusting for age, sex, smoking status, alcohol status, physical activity, hypertension, type 2 diabetes mellitus, gout, dyslipidemia, BMI, heartrate, blood cadmium and carotid plaque, subjects in Q3 of blood lead had an odds ratio (OR) of 2.47 (95% CI: 1.17, 5.18) for grade of 10-year ASCVD risk,compared

with subjects in Q1 of blood lead. Subgroup analyses showed that the OR was higher in females (OR = 4.47, 95% CI=1.26-15.78, P=0.020) and subjects with hypertension (OR=3.25, 95 % CI=1.26-8.37, p= 0.015). **CONCLUSION** Environmental lead exposure is associated with higher predicted 10-year AS·CVD risk in Chinese middle-aged and older adults.

Key words: Environmental lead exposure; 10-year ASCVD risk; Middle-aged and older adults

Corresponding author: Yang Xing-fen, E-mail: yangalice79@smu.edu.cn

T01-91-0048

Discussion on watershed water quality security criteria

Liu Zheng-tao

(Chinese Academy of Environmental Sciences)

Abstract: The scientific implementation of environmental standards is directly related to the high-quality development of national environmental safety strategy and ecological civilization construction, such as the protection of ecological natural resources, the protection of human environmental health and the development of green economic industrial structure etc. The Environmental criteriais the scien·tific basis for formulating environmental standard and determine the scientific property of environmental standard. This paper mainly focuses on the key links of eco-environmental toxicology and fresh water qualitycriteria method, introduces and discusses the technical framework of establishing and verifying environmental safety water quality criteria (WQC) methods in recent years of China. It mainly includes the research of aquatic organismcriteria, the aquatic ecology criteria, the sediment criteria andthe hu·man health water quality criteriaof watershed surface water. It also discusses the ordering of priority control pollutants, the screening of native tested organisms and other technical methods about the WQC. Meanwhile, there are verifiedapplications in some typical watershed such as Liao river, Hai river, Tai Hu and Poyang Hu lakes. Aiming at the implementation of regional water environmental protection objectives, the toxicological safety criteria and risk assessment methods for some typical pollutants which related to water quality criteria as aquatic organisms criteria and human health criteria were dis·cussed, to provideuseful technical support for the deriving and revising of related surface water quality standards.

Key words: Water quality criteria methods; Aquatic organism criteria; Human health criteria; Safety thresholdand risk assessment; Priority control pollutant of native watershed

Corresponding author: Liu Zheng-tao, E-mail: liuzt@craes.org.cn

T01-91-0049

Behavioral effects of triclocarban at environmental related concentrations on the generational toxicity of Caenorhabditis elegans

jie huang, Jiahui Wu, Siyang Sun, Qiuyuan Chen, Jingjuan Ju

(Wenzhou Medical University)

Abstract: Triclocarban (TCC) is a broad-spectrum and highly active antibacterial agent that is

widely used in many personal care products, and has the potential harm to the human body. At present, most toxicological studies on TCC are based on the concentration range (mostly at mg / L level) when producing certain toxicity. However, the concentration of TCC in the environment is mostly in the ng·L^{-1}–g·L^{-1} range, showing low dose and long-term exposure in the environment, which is quite different from most toxicological studies. In this study, water and sediment samples were collected from sewage treatment plant and the environmental concentration of TCC was detected by high performance liquid chromatography (HPLC). The exposure concentrations were then determined. C. elegans is a free-living nematode, which has the advantages of short experimental period, sensitivity to toxic effects of environmental pollutants, abundant progenies, and relatively conservative genome in mechanism research. This study observed the effects of TCC on the parental nematodes and their offspring after different exposure patterns. In our study, it was found that parental and offspring's exposure of TCC at environmental related concentrations had an interaction effect on the locomotion behavior of offspring. We had studied the motion distance, the length of peristaltic trajectory, the linear distance of motion, the average amplitude and the maximum amplitude, among which the mean amplitude and maximum amplitude decreased the most significantly (P<0.05) compared with the control group. The aim of this study is to establish a rapid evaluation system for generational toxicity of TCC and provide evidences for elucidation of ecological toxicity of TCC.

Key words: Triclosan; Caenorhabditis elegans; Environmental relevant concentrations; Neurotransmitter system; T

T01-91-0050

Effects of rats' learning and recognized memory after microwave exposure

Ke Ren, Chuanfu Yao, Liu Sun, You Wu, Yu Liu, Xinping Xu, Binwei Yao,
Hongmei Zhou, Li Zhao,Ruiyun Peng
(Beijing Institute of Radiation Medicine)

Abstract: OBJECTIVE With the rapid development of microwave technology, it was widely applied on communication, navigation, biology, medical and other fields. Microwave not only provided convenience for people's life and work, but also increased the chance of people in the microwave environment. The health hazards have attracted increasing attention. The aim of the research was to investigate the effects of microwave exposure on the spatial memory, working memory and recognition memory function in rats, which could provide a basis for deepening the study of the characteristics and mechanisms of brain damage induced by microwave radiation. **METHODS** Male Wistar rats were exposed to microwaves for 15 min with the average power density of 30 mW/cm^2. At 6 hours to day 4 after exposure, Morris water maze was used to test the learning and spatial memory function of rats. At day 5-10 after exposure, the reversal test of Morris water maze was used to test the spatial working memory of rats. At day 1 after exposure, new object recognition experiment was used to test the rats' recognition and memory ability. **RESULTS** (1) The ability of learning and spatial memory. In the space exploration experiment of Morris water maze, the proportion of the staying time in the quadrant of the original platform was significantly decreased after microwave exposure (P<0.05), and there was no significant difference between two groups of rats in the number of times on the crossing platform area (P>0.05). (2) The ability of spatial working memory. In the positioning and navigation experiment, there was no

significant difference between two groups of rats on the average escape latency (AEL) (P>0.05). The results of Morris water maze reversal showed that in the positioning and navigation reversal experiment, there was no significant difference between two groups of rats on the AEL (P>0.05). In the space exploration reversal experiment, there was no significant difference in times of crossing the platform and the proportion of time on original platform between two groups (P>0.05). (3) The ability of recognized memory. The results of Novel Object Recognition experiment showed that the discrimination index (DI) was significantly decreased at 1 hour after microwave exposure (P<0.05), but there was no significant difference in the discrimination index between two groups of rats at 24 hours after microwave exposure (P>0.05). **CONCLUSION** Microwave exposure with the average power density of 30 mW/ cm^2 could damage the recognition and memory function of rats, but there was no obvious effect on rats' spatial and working memory.

Key words: Microwave radiation; Rat; Spatial memory; Recognition memory

T01-91-0051

Untargeted metabolomics method revealed the mechanism of combined toxicity of hexaconazole and arsenic to mice

Dali Sun[1], Na Yang[1], Qinghai Zhang[1], Zelan Wang[1], Junxiao Pang[2]

(1. Guizhou Medical University; 2. Guiyang University)

Abstract: The high detected ratio increased the co-exited frequency of hexaconazole (Hex) and arsenic (As) in agricultural products. However, the combined toxicity effect and mechanism of action of these two pollutants were still unclear. In this study, an untargeted metabolomics method was used to monitor the changes of endogenous metabolites and metabolism pathways in mice liver. Our study revealed that significant differences in metabolomics profiles were observed after Hex, As, and Hex+ As exposure for 90 d. Hex exposure induced 54 significant differences metabolites 11 pathways altered among them 31% metabolites and 5 pathways were lipid related. For As exposure, 63 metabolites (48% was organic acids and derivatives) and 9 pathways were affected. Hex+ As induced 93 metabolites changed with 34% of lipids and lipid-like molecules and 22% of organic acids and derivatives. Eleven pathways that altered by Hex+As were the combination of Hex and As-altered pathways, indicating that the interaction of Hex and As might be independent action. The data of this study could provide an important insight for understanding the mechanism of combined toxicity for Hex and As and helpful for evaluating their health risk to human.

Key words: Hexaconazole; Arsenic; untargeted metabolomics; Combined toxicity; Mechanism of action

T01-91-0052

Effects of ozone inhalation on the ultrastructures of lungs in mice

Shi Liang[1,2], Ying Xie[1], Yan Sha[1]

(1. 深圳市职业病防治院, Shenzhen Center of Occupational Diseases Control and Treatment

2. Shenzhen Center of Occupational Diseases Control and Treatment)

Abstract: OBJECTIVE To investigate effects of ozone inhalation on the ultrastructure of lungs. **METHODS** ① The ultrasturctures of the lungs and tubes were observed by HE stain and transmission O3 inhalation 2.14 mg·m^3. ② NQO1、Nrf2 and Keap1 expressions in lungs were detected by SP immunohistochemistry. **RESULTS** ① The results of HE staining showed no obvious change in O3 treatment group.② The NQO1 average opical density of O3 treatment group was higher than that of the control (P <0.05), while Nrf2 and Keep1 had no significant difference among these groups (P>0.05). ③ A series of ultrastructural changes were found in O3 treatment group, such as a few bulges in type Ⅰ alveolar cells, the increased evacuation of substance from lamellar bodies in the type Ⅱ pneumocytes, the increased space around the goblet nucleus. **CONCLUSION** Although observation under light microscope did not find that O3 has a significant effect on the structure of mouse lung and bronchus, observation under transmission electron microscope can reveal the changes of ultrastructure.

Key words: Ozone; Mice; Lung; Bronchus; Pathology; Ultrastructure;Electron microscope

T01-91-0053

Bisphenol F induces non-alcoholic fatty liver disease-like changes by affecting mitochondrial morphology and function

Jun Wang, Xuexue Xie, Linwei Zhang

(Department of Toxicology, School of Public Health, Nanjing Medical University, 818 Tianyuan East Road, Nanjing 211166, China)

Abstract: Background Non-alcoholic Fatty Liver Disease (NAFLD), one of the most prevalent chronic liver diseases, is closely associated with the exposure of the endocrine-disrupting chemicals (EDCs). As a potential EDC, bisphenol F (BPF) is increasing replace of bisphenol A (BPA) and causes NAFLD-like changes. Purposes To investigate the effects of mitochondrial dynamics changes in BPF induced NAFLD like changes, and deciphered the mechanism involving BPF induced mitochondrial fission mediated by Drp1. Methods The human normal liver cells L02 were incubated with 10 μmol·L^{-1} BPF for 24 h to establish in vitro exposure model. Meanwhile, the mouse BPF exposure model was established as well. The C57BL / 6J mice were randomly divided into four groups exposed to BPF (0, 50, 200, 500 μg/kg/day), and were fed with BPF for 30 consecutive days. Results BPF treatment elicited a pronounced lipid droplet deposition accompanied by decreased mitochondrial membrane potential, reduced ATP production and increased ROS; Additionally, In terms of cell substructure, BPF treatment obviously increased mitochondrial fragmentation, mitochondrial cristae disappearance and swelling; Mechanistically, BPF exposure dramatically facilitated mitochondrial fission, of note, the key mitochondrial fission protein Drp1 was strikingly upregulated, accompanied by Drp1 activation. In the *in vivo* exposure model, H&E staining and oil red staining results showed that the liver tissue was damaged and the lipid droplets were obviously accumulated, which was characterized as the NAFLD-like changes. The electron microscope results showed that the morphology of mitochondria was obviously damaged. In agreement with the results represented in the in vitro work, the expression of Drp1 is markedly increased and activated after BPF exposure. Conclusions BPF treatment affected the expression and activity of mitochondrial fission protein Drp1, which may promote mitochondrial fission, generate massive immature mitochondria, resulting in ROS accumulation and impair mitochondrial function. These effects, taken together contribute to lipid droplet deposition and finalize NAFLD-like changes.

Key words: Non-alcoholic fatty liver disease; bisphenol F; mitochondrial dynamics

T01-91-0054

Effects of compound microwave exposure on the immune functions of peripheral blood in mice

Chuanfu Yao, Ji Dong, Hui Wang, Haoyu Wang, HongMei Zhou, Ke Ren, Liu Sun,
Yue Yin, Li Zhao, Ruiyun Peng
(Beijing Institute of Radiation Medicine)

Abstract: OBJECTIVE To explore the effects of compound microwave exposure on immune indexes of peripheral blood in mice, the research could provide basis for study on the injury mecha·nism, prevention and treatment targets of immune function. **METHODS** Sixty male C57BL / 6N mice were randomly assigned to sham group, low dose compound group (SAR=3W/Kg) and high dose com·pound group (SAR=15W / Kg). The peripheral blood was collected at 6h and 7d after X-band and S- band microwave compound exposure. The count and proportion of peripheral blood cell were detected by automatic blood cell counter. The ratios of lymphocyte subsets in peripheral blood were measured by flow cytometry. The concentration of IgG, IgM and IgA in serum were detected by ELISA. The con·tents of cytokines including IL-2, IL-4 and TNF - α in serum were measured by multifunctional liquid phase chip analysis platform (Luminex200). **RESULTS** Compared to sham group, the counts of WBC and LYMPH of low and high dose compound groups were increased (P<0.05 or P<0.01) at 6h after exposure. The ratio of $CD19^+$ B cell and $CD3^+$ T cell were increased (P<0.05) of both compound groups, while the ratio of $CD8^+$ T cell and $CD4^+$ T cell were decreased (P<0.01). The concentrations of IgG and IgM in serum of low and high dose group were increased (P<0.05 or P<0.01) compared to sham group, and the concentration of IgA in serum of low dose group was increased (P<0.05) at 6 h after exposure, but was deceased (P<0.01) on 7d after exposure as well as the high dose group. The concentrations of IL-2, IL-4 and TNF-α in serum of low and high dose groups were decreased (P<0.05 or P<0.01) at 6h after exposure compared to sham group, and the concentration of IL-2 of high dose group was decreased (P<0.01) compared to low dose group on 7 d after exposure. **CONCLUSION** Compound microwave exposure could lead to imbalance of immune functions of cellular immunity, and humoral immunity was activated first and then suppressed. The above results showed a positive corre·lation with exposure dosage.

Key words: microwave; compound exposure; mouse; peripheral blood; immune function

T01-91-0055

Curcumin alleviates NG108-15 cell apoptosis triggered by fluorine combined with aluminum

Zhongbi Peng, Chun Xie
(School of Public Health, The Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, Guizhou Medical University, Guiyang 550025, China)

Abstract: To investigate the effects of fluorine combined with aluminum (FA) exposure on the apoptosis of NG108-15 cells and the expression of the PKC-NMDAR signaling pathway, and to analyze whether curcumin has an intervening effect on the aforementioned effects. The NG108-15 cells were

exposed to NaF and AlCl3 at the final concentration of (0, 0), (20, 160), (40, 160), (80, 160) mg· L^{-1}. After treating the cells for 24 h, 16 μmol/L curcumin was used to intervene in each exposure group for 24 h. Cell viability was analyzed using Cell Counting Kit-8; Apoptosis rate was detected by AO/EB fluo·rescent staining; The PKC, NMDAR1, NMDAR2A, and NMDAR2B mRNA expression levels in cells were measured by qRT-PCR; The casepase3, Bcl-2, Bax, PKC, NMDAR1, NMDAR2A, NMDAR2B pro·tein expression levels were evaluated by Western Blot. The results showed that, compared with the control group, the FA groups showed decreased viability of NG108-15 cells (P<0.05). The FA groups showed increased apoptosis rates; the apoptosis rates of the curcumin intervention groups were lower than those in the corresponding FA groups (P<0.05). Compared with the control group, the expression of caspase3 and Bax protein increased, the expression of Bcl-2 protein decreased, and the expression of Bax/Bcl-2 increased. Compared with the corresponding FA group, the expression of Caspase3 pro·tein except the low FA+ curcumin group the other intervention groups all decreased, the expression of Bax protein decreased in the low FA+ curcumin group, the expression of Bcl-2 protein increased, and Bax/Bcl-2 decreased in each intervention group (P<0.05). Compared with the control group, the expres·sions of PKC, NMDAR2B mRNA and NMDAR2A, NMDAR2B protein in the exposure group were all de·creased, and the expressions of NMDAR1, NMDAR2A mRNA and PKC protein were decreased in all the exposed groups except the low FA group (P<0.05); Compared with the corresponding FA group, the expressions of PKC, NMDAR2A, NMDAR2B mRNA and PKC, NMDAR2B protein of each interven·tion group increased, and the expression of NMDAR1, NMDAR2A mRNA and NMDAR2A protein ex·pression of each intervention group increased except the low FA+curcumin group (P<0.05). The results of this study suggested that FA triggered apoptosis of NG108-15 cells, and curcumin may reduce FA-in·duced apoptosis of NG108-15 cells by up-regulating the expression of the PKC-NMDAR pathway.

Key words: Fluorine; Aluminum; Curcumin; PKC; NMDAR; Apoptosis

T01-91-0056

Mitochondrial damage and inflammation activation involved in Perfluorooctane Sulfonate-induced hepatic toxicity in rats

Leilei Tang[1], Xue Han[2], Yaping Deng[1], Jiawen Yu[1], Qiaojuan Shi[2], Guojun Jiang[1]

(1. Affiliated Xiaoshan Hospital, Hangzhou Normal University; 2. Laboratory Animal Center, Hangzhou Medical College)

Abstract: Perfluorooctane sulfonate (PFOS), an environmentally persistent contaminant, have been found to cause hepatic toxicity in animals and humans. However, the underlying mechanism by which it affects the organelle toxicity in liver are not well elucidated yet. In this study, we focused on mi·tochondrial toxicity and inflammation caused by PFOS in rats'liver. Rat was employed to investigate the PFOS-induced hepatic toxicity. Pathological assessment of liver tissues was applied to evaluate PFOS-induced hepatic toxicity. Apoptosis in liver of rats was assessed by TUNEL staining. The struc·ture of mitochondria was assessed by the transmission electron microscope after PFOS treatment. In addition, the oxidative stress was evaluated after PFOS exposure by examinating the activities of NOS, superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX). Finally, whether NLRP3 signaling pathway took part in activating inflammation by PFOS were confirmed by ELISA and western blotting analysis. Briefly, rats were exposed intraperitoneally injected to PFOS (0 mg·kg^{-1}, 1 mg·kg^{-1}, 10 mg·kg^{-1})

every other day for 15 days. PFOS was found to increase liver weight and caused lipid disorder in rats. Meanwhile, PFOS groups exhibited hepatic steatosis and apoptosis in the liver. PFOS destroyed the structure of mitochondria, decreased the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX), and increased the level of Malondialdehyde (MDA) and activity of nitric oxide synthase (NOS). Additionally, PFOS increased expression of NLRP3 and caspase1, thereby increased the expression of pro-inflammatory cytokines IL-1β and TNFα expression in the liver tissues of rats. In conclusion, PFOS caused hepatic steatosis and apoptosis of live tissues, which was correlated to destruction of mitochondrial structure and the activation of inflammation. PFOS destroyed the structure of mitochondria, then activated oxidant stress, resulting in inflammation activation mediated by NLRP3 signal pathway. These results indicated that the abnormal mitochondrial structure and inflammation activation might be the important aspects of hepatotoxicity caused by PFOS, which might provide the experimental basis for further research on PFOS-induced hepatic toxicity.

Key words: Perfluorooctane sulfonate (PFOS); mitochondria; inflammation; oxidation stress; hepatic toxicity

T01-91-0057

Hematopoietic stem cell and immunotoxicity in zebrafish embryos induced by exposure to Metalaxyl-M

Suwen Zeng
(Gannan Normal University)

Abstract: Metalaxyl-M (MM), a protective and therapeutic fungicide, has been shown to be a promising candidate, but its toxicity toward aquatic organisms is unknown. In this study, we evaluated for the first time the immunotoxicity of MM in zebrafish embryos. Phenotypes (heart rate, body length, and yolk area) and the number of neutrophils, macrophages, and T cells in the thymus were analyzed in zebrafi sh embryo after exposure to MM. Our results showed that zebrafish embryos exposed to MM showed a concentration-dependent increase in the yolk area and a significant decrease in the number of neutrophils, macrophages, and thymus T cells. We detected upregulated expression of related immune signaling genes, such as TNF-α, NF- kB, cxcl-c1c, IL-6, MMP-9, and TGF-β. Additionally, we observed a significant decrease in HSCs in zebrafish larvae after exposure to MM. IWR-1 could restore the number of neutrophils and macrophages after exposure to MM. The results indicated that MM exerted developmental toxicity and immunotoxicity to zebrafish embryos, and these phenomena may be caused by MM′ s regulation of WNT signaling pathway.

Key words: Metalaxyl-M; Zebrafish; Immunotoxicity; Wnt signaling pathway;

T01-91-0058

The Role of the Aryl Hydrocarbon Receptor (AhR) on the reproductive injury induced by benzo [k] fluoranthene (BKF)

Wang Dandan[1,2], Wang Furong[1], Zhou Niya[1], Cao Jia[1]
(1. Army Medical University; 2. Ningxia Medical University)

Abstract: OBJECTIVE A large number of studies have confirmed that exposure to polycyclic aro·matic hydrocarbons (PAHs) have harmful effects on male reproductive health inhalation of PAHs in air, especially high molecular weight PAHs (HMW PAHs), may be a potential risk factor for male reproduc·tive health, but the specific mechanism is still unclear. **METHODS** In order to explore the mechanism of HMW PAHs in male reproductive function, we used benzo [k] fluoranthene (BKF) to establish a mouse spermatocyte (GC-2) toxicity model. The dose of BkF was 0, 10, 20, 40 and 80 $\mu mol \cdot L^{-1}$, and the exposure time was 24, 48 and 72 h. ① CCK-8 and EDU were used to detect the effects of BkF on cell proliferation. The production level of intracellular ROS was detected by CM-H2DCFDA fluorescent probe. ② Flow cytometry was used to detect the effect of BkF on cell cycle arrest and apoptosis. ③ The expression of DNA damage markers (γ-H2AX) and the molecular proteins related to the aromatic hydrocarbon receptor pathway (AhR, ARNT, CYP1A1 and Nrf2, etc.) were detected by Western Blot. The expression of genes related to the AhR pathway was further detected by fluorescence quantitative PCR.In addition,the AhR inhibitor CH223191 was used to treat the most toxic group detect the expres·sion of the aryl hydrocarbon receptor (AhR) pathway. **RESULTS** With the increase of BkF exposure dose, the activity of GC-2 cells in the treatment group was significantly lower than that in the control group, the number of cells was significantly reduced after BkF treatment, and the cell morphology was also changed. After BkF treatment, intracellular ROS production was increased ($P<0.05$), suggesting that BkF exposure may lead to oxidative damage of cells. Compared with the control group, the propor·tion of G1 / G0 phase cells increased with the increase of BkF concentration, while the proportion of S phase cells decreased significantly. When the dose of BkF was higher than 40 $\mu mol \cdot L^{-1}$, it was statisti·cally significant ($P<0.05$), suggesting that GC-2 cell division was arrested in the G0/G1 phase. In addi·tion, compared with the control group, the expression of γ-H2AX protein in treatment group was in·creased in a dose-dependent manner ($P<0.05$). This suggests that BkF may induce genetic damage in GC-2 cells. After exposure to BkF, AhR protein accumulated significantly in the nucleus,and the expres·sion of ARNT protein increased significantly in the nucleus, suggesting that BkF could activate AhR and translocation it from cytoplasm to nucleus. After GC-2 was exposed to different concentrations of BkF for 72h, qRT-PCR and Western Blot analysis showed that the mRNA and protein expressions of AhR downstream genes CYP1A1 and Nrf2 were significantly up-regulated ($P<0.05$). After exposure of GC-2 inhibited by CH223191 to BKF, the protein expression of AhR and its downstream genes CYP1A1 and Nrf2 decreased significantly. **CONCLUSION** BkF exposure can cuase DNA oxidative damage and cell cycle arrest by activate the AhR signaling pathway, which in turn induces reprotoxic ef·fects in GC-2 cells.

Key words: Aryl hydrocarbon receptor(AhR); benzo[k]fluoranthene (BkF); spermatogenic cells (GC-2); DNA damage

T01-93-0017

In vitro evaluation of genotoxicity and carcinogenicity potential on $PM_{2.5}$ samples collected from a city in the north of China

Zinan Li[1], Wenjing Zhang[1], Guoyan Zhang[1,2], Peng Zhang[1], Haiming Jin[1], Yanmin Nie[1], Lijuan Qi[1], Nan Zhang[1], Hongju Du[1], Gao Shan[1], Junyu, Ning[1,2]*, Guojun Li[1,2]*

(1. Beijing Center for Disease Prevention and Control, Beijing Research Center for Preventive Medicine, Beijing Key Laboratory of Diagnostic and Traceability Technologies for Food Poisoning,

Beijing 100013; China. 2. School of Public Health, Capital Medical University, Beijing 100069, China)

Abstract: OBJECTIVE To evaluate the he genotoxicity and carcinogenicity potential of $PM_{2.5}$ collected in a northern city of China. **METHODS** $PM_{2.5}$ samples which were collected from a city in north of China were divided into "Heating supply" group (Nov., 2015 to Feb.,2016) and "Post-heating supply" group (Apr., 2016, to Jul., 2016) according to the heat supply period. Ames test (up to 5 mg/dish) and SOS/umu test (up to 500 μg) were used to evaluate their abilities to induce mutation or DNA damage. The oncogenic potential of the two samples was evaluated by malignant transformation assay (up to 21.0 ng / cm^2) based on a human bronchial epithelial cells which is dubbed as BEAS-2B. **RESULTS** Ames test and SOS/umu test showed that two groups of samples possess distinguish characteristics in genotoxicity. Samples in "heat supply" period mainly caused DNA damage, but the mutagenic property was weak; "Post-heat supply" samples showed significant mutagenicity, but were less capable of inducing DNA damage. The results of cell malignant transformation test indicates that long-term exposure (6 months) of both groups of samples could induce malignant transformation on BEAS-2B, which is manifested in increased cell proliferation rate (population doubling time decreased by about one-third), aberrant growth pattern (anchorage-independent), resistance to apoptosis-inducing factors, cell epithelial-mesenchymal transformation (including morphological change, abnormal expression of E-cadherin and other molecular markers). In addition, transcriptome studies have shown that epigenetic changes such as DNA methylation are associated with cell malignant transformation induced by $PM_{2.5}$. **CONCLUSION** $PM_{2.5}$ samples from an urban area in north of China have significant genotoxicity, and carcinogenic potential to a certain degree, but the toxicity characteristics of $PM_{2.5}$ samples between "Heat supply" and "Post-heat supply" period shows great difference.

Key words: Ambient $PM_{2.5}$; Genotoxicity; Carcinogenicity

Corresponding author: Guojun Li, E-mail: ligj@bjcdc.org; Junyu Ning, E-mail: ningjy@gmail.com

T01-93-0018

The therapeutic effect of mesenchymalstem cells on pulmonary fibrosis through BMP-7 signaling in experimental silicosis rats

YanDa Chen[1], Ying Xie[2], Zhijun Chen[2], Jun Luo[2], Xiong Zhang[2], Yan Sha[1],

(1. School of Public Health, Guangdong Medical College, Dongguan 523808, PR China; 2. Shenzhen prevention and treatment center for occupational disease, Shenzhen, Guangdong, China)

Abstract: OBJECTIVE Pulmonary fibrosis induced by silica dust is a chronic irreversible lung disease with no effective treatment at present. Previous studies have shown that mesenchymal stem / stromal cells BMSCs) has positive effect on anti-pulmonary fibrosis caused by silica dust. However, the related mechanism is unclear. In this study, we compared the effects of early intervention and late treatment on different transplantation time. And also explored the possible mechanism in the process. **METHODS** Sprague Dawley (SD) rats were randomly divided into four groups including (1) control group (n=6), (2) silica model group (n=6) which were exposed to silica suspension (1 mL of 50 mg·mL^{-1}), (3) hUC-MSC prevention group (n=6) which received 1 mL hUC-MSC suspension (3×10^6 cells/mL) by tail vein injection on the 1th day after exposure to silica suspension, and (4) hUC-MSC treatment group (n=6) which

received hUC-MSCs from the same cell number by tail vein injection on the 28th day after exposure to silica suspension. First we traced the hUC-MSCs *in vivo* 28 days after transplantation. And compared the dynamic lung fibrosis by computed tomography (CT) on the 60th and75th day after exposure to silica suspension, hematoxylin and eosin (H&E), and Masson's trichrome staining to evaluate the changes in lung tissue. We examined the expression of epithelial-mesenchymal transition (EMT) and BMP-7 pathway-related proteins in lung tissue using immunohistochemistry and western blotting. **RESULTS** Pulmonary fibrosis model was confirmed by H&E and Masson′ s trichrome staining on the 28th day after exposure to silica suspension. The hUC-MSCs were found in the lung of model rat 28days after transplantation. On the 60th and 75th day after exposure, pulmonary CT examination showed a alleviating effect of hUC-MSCs on silica-induced pulmonary fibrosis confirmed by H&E and Masson′ s staining. Treatment of hUC-MSCs reduced the expression α-Smooth actin (α-SMA) after exposure to silica suspension. We also found BMP7 signaling pathway is inhibited in silica-induced pulmonary fibrosis, and transplantation of hUC-MSCs may induce their expression, including BMP7 and Smad5. **CONCLUSIONS** hUC-MSC transplantation inhibits the silica-induced EMT to alleviate pulmonary fibrosis in rats model and the the induced BMP-7 pathway possibly involved in this process.

Key words: Silicosis; human umbilical cord mesenchymal stem cells (hUC-MSC); BMP7

T02-10-0002

Quantitative evaluation of radon and lung cancer association in a cohort study of Chinese Tin miners

Zheng Su[1], Ya-Guang Fan[2], Meng-Na Wei[3], Hui-Jiao Yan[1], Xin-Hua Jia[1], Ru-Fei Duan[1], Fang-Hui Zhao[1], Philip R. Taylor[4], You-Lin Qiao[5]

(1. Department of Epidemiology, National Cancer Center/National Clinical Research Center for Cancer/ Cancer Hospital, Chinese Academy ofMedical Sciences and Peking Union Medical College, Beijing, China; 2. Tianjin Key Laboratory of Lung Cancer Metastasis and Tumor Microenvironment, Tianjin Lung Cancer Institute, Tianjin Medical University General Hospital, Tianjin, China; 3. Breast Tumor Center, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China; 4. Metabolic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD; 5. Center for Global Health, School of Population Medicine and Public Health Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China)

Abstract: OBJECTIVE Background:Globally, residential radon is the second cause after tobacco smoking of lung cancer and most evidences about indoor radon-related lung cancer risks are from the exploration of occupational radon cohorts. Therefore, quantitative evaluation of association between radon, cigarette smoking and lung cancer is of great importance. **METHODS** From 1992 to 1996, 6017 tin miners who had 10 or more years of underground radon exposure were included and exposure information were collected for five consecutive years, and followed up for 28 years. Lung cancer risks were estimated by mathematical formulas modeling both total exposure and intensity. **RESULTS** The cohort experienced 111, 332.63 person-years and 933 lung cancer cases. Excess relative risk increased by 0.96% per cumulative working level month. The risk declined with years since last exposure, attained age, and age at first exposure, while increasing with age at last exposure. There patterns still stable

even adjusting for arsenic exposure or cigarette smoke. In addition, the estimates were increased by a factor of 16.3, 8.1, 4.0 for cumulative exposure, and 6.4, 2.8, 1.7 for concentration with age at first expo·sure (<13, 13-17, 18-24, compared with ≥25); The values decreased to a factor of 2.1, 1.9, 1.5 for total exposure, 2.3, 1.9, 1.3 for intensity with adjustment for tobacco. Finally, our results supported that the most likely model is sub-multiplicative for joint effect between radon, arsenic and smoking on lung can·cer. **CONCLUSION** This study enlighten us that priority measures should be paid to radon prone popu·lation such as childhood, current smoker, and ever smoker and that substantial reductions in the lung cancer burden of population exposed to radon and cigarette could be achieved by reductions in either exposure.

Key words: radon; cigarette smoke; total exposure and intensity; lung cancer; cohort

Corresponding author: Fang-Hui Zhao, E-mail: zhaofangh@cicams.ac.cn; You-Lin Qiao, E-mail: qiaoy@cicams.ac.cn

T02-10-0011

Cooperative coordination-mediated multi-component self-assembly of "All-in-one" nanospike theranostic nano-platform for mri-guided synergistic therapy against breast cancer

Xudong Fan, Xiaojie Chen, Yue Zhang, Ji-Gang Piao, Fanzhu Li

Abstract: Carrier-free multi-component self-assembled nano-systems have attracted widespread attention owing to their easy preparation, high drug-loading efficiency, and excellent therapeutic effica·cy. Herein, MnAs-ICG nanospike is generated by self-assembly of indocyanine green (ICG), manga·nese ions (Mn^{2+}), and arsenate (AsO_4^{3-}) based on electrostatic and coordination interactions, effectively integrating the bimodal imaging ability of magnetic resonance imaging (MRI) and fluorescence (FL) im·aging-guided synergistic therapy of photothermal/ chemo/ chemodynamic therapy within an "all-in-one" theranostic nano-platform. The as-prepared MnAs-ICG nanospike has a uniform size, well-defined nanospike morphology, and impressive loading capacities. The MnAs-ICG nanospike exhibits sensitive responsiveness to the acidic tumor microenvironment with morphological transformation and dimen·sional variability, enabling deep penetration into tumor tissue and on-demand release of functional ther·apeutic components. Meanwhile, glutathione (GSH) overexpressed in tumor sites is used to reduce AsO_4^{3-} to arsenite (AsO_3^{3-}), thereby increasing arsenic toxicity from low to high. In vitro and in vivo re·sults revealthat MnAs-ICG nanospike shows synergistic tumor-killing effect, prolonged blood circulation and increased tumor accumulation compared to their individual components, effectively resulting in syn·ergistic therapy of photothermal/ chemo / chemodynamic therapy with good biocompatibility and excel·lent anti-tumor effect. Taken together, this new strategy may hold great promise for rationally engineer·ing multifunctional theranostic nano-platforms for breast cancer treatment.

Key words: nanospike, tumor microenvironment responsive, synergistic therapy, self-assembly, breast cancer, deep penetration

T02-12-0015

Shikonin synergizes with cisplatin to induce ferroptosis in ovarian carcinomacisplatin-resistancecells

Maowei Ni[1,2,3], Huajun Zhao[1]

〔*1. School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou, China; 2. The Cancer Hospital of theUniversity of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Hangzhou, China; 3. Institute of Basic Medicineand Cancer (IBMC), Chinese Academy of Sciences, Hangzhou, China*〕

Abstract: Cisplatin is the first-line medication for ovarian carcinoma (OC), but it can only extend limited survival because of drug resistance. It isimperative to find a combination strategy to increase cisplatin efficacyincisplatin-resistancecells.Shikonin, a natural naphthoquinone pigment purified from Lithospermum erythrorhizon, exhibits significant cytotoxic activity against multiple cancer cell types. In this study we investigated the combined effect of cisplatin and shikonin in inducing ferroptosis ofcisplatin-resistance cell lines of OC and elucidated the involved molecular mechanisms. Results of CCK-8 and colonyformation showed that shikonin greatly enhanced the anticancer effects of cisplatin against A2780/DDP, SKOV3/DDP and OVCAR4/DDP cell lines in vitro and against A2780/DDP cell xenograft model in Balb/c nude mice. The combination indexmethod confirmed that the combined effect of cisplatin and shikonin was synergistic. Proteomics and bioinformatic analysis revealed that the combination effect associated with ferroptosis. Compared with single drug treatment (cisplatin or shikonin), combination treatment induced significantly exacerbated lipid peroxidation, increased Fe2 + concentration and ferroptosis, which was blocked by ferroptosis inhibitor ferrostatin-1. Furthermore, combination treatment notably activated ferrous ion metabolic pathway refer to TFRC, HOMX1, SLC11A2 and LTF. In conclusion, these results demonstrated that combination treatment prohibited OC cisplatin-resistance cell linesproliferation, and accelerated ferroptosis by activation of ferrous ion metabolic pathway. The findings maybe provide a fresh opinion for cisplatin-resistance medication of OC.

Key words: Shikonin; Cisplatin; Cisplatin-Resistance; Ferroptosis; Ovarian Carcinoma

T02-14-0021

The critical role of polyketide synthase gene on the swainsonine biosynthesis in the fungus Metarhizium anisopliae

Lu Sun[a], Enxia Huang[a], Yu Zhang[a], Ziyu Guo[a], Kexin Wu[a], Yunhao Zhang[a], Chonghui Mo[b], Jinglong Wang[c], Baoyu Zhao[a], Hao Lu[a]*

(a. College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi 712100, China; b. College of Agriculture and Animal Husbandry, Qinghai University, Xining, Qinghai 810016,China; c. Tibet Academy of Agricultural and Animal Husbandry Sciences/State Key Laboratory of Barley and Yak Germplasm Resources and Genetic Improvement, Lhasa, Tibet 850002, China)

Abstract: Swainsonine (SW) is the principal toxic ingredient of locoweeds, and is produced by fungi including Metarhizium anisopliae, Slafractonia leguminicola, and Alternaria oxytropis. While the SW biosynthesis pathway of fungi and the catalytic enzyme genes that regulate synthesis are not cleanly. In this study, we used homologous recombination (HR) to knock out and interfere with the polyketide

synthase gene (pks) of M. anisopliae to determine its effect on the SW biosynthesis pathway. The concentration of SW was measured in the fermentation broth of M. anisopliae at 1 d, 2 d, 3 d, 4 d, 5 d, 6 d or 7 d using LC-MS. The gene for the pks gene was detected by RT-qPCR. Day 5 of M. anisopliae gave the highest content of SW and the highest expression of the pks gene. To determine the role of the pks gene in the SW biosynthesis pathway of M. anisopliae, we used PEG-mediated homologous recombination (HR) to transform a wild-type strain (WT) with a Benomyl (ben)-resistant fragment to knock out the pks gene producing a mutant-type strain (MT) and used PEG-mediated RNAi to transform a wild-type strain (WT) with a Benomyl (ben)-resistant plasmid to interfere with the pks gene. A complemented-type (CT) strain was produced by adding a complementation vector that contains the geneticin (G418) resistance gene as a marker. The content of SW didn't detected in MT strain, and returned to the original level in the CT strain, while the content of SW was significantly decreased in RNAi strain. We suggest that mutation and RNAi in the pks gene affect the cell wall formation of M. anisopliae, while the colony diameters, phenotypes, and growth rates did not change significantly, and no obvious changes in other cellular organelles were noted. These results indicate that the pks gene plays a crucial role in the SW biosynthesis of M. anisopliae, which provides an important theoretical basis for illuminating the SW biosynthesis and solving locoism in livestock.

Key words: M. anisopliae; pks gene; Swainsonine; Gene knockout; RNAi

Corresponding author: Lu H, E-mail: luhao@nwsuaf.edu.cn

T02-16-0007

N6-methyladenosine-mediated downregulation of miR-374c-5p promotes cadmium-induced cell proliferation and metastasis by targeting GRM3 in breast cancer cells

Ping Deng, Yang Yue, Zhengping Yu, Huifeng Pi

〔 *Department of Occupational Health (Key Laboratory of Electromagnetic Radiation Protection, Ministry of Education), Third Military Medical University, Chongqing, China* 〕

Abstract: Cadmium (Cd) is a toxic heavy metal that can facilitate the development and progression of breast cancer (BC). Emerging evidence has indicated that Cd-exposed BC progression is related to the dysregulation of microRNAs (miRNAs). The purpose of our study was to investigate the expression pattern and underlying mechanisms of miR-374c-5p in Cd-mediated BC progression. In this study, T-47D cells were treated with different concentrations of Cd (0.1, 1 and 10 $\mu mol \cdot L^{-1}$) for 72 h. MiR-374c-5p expression was downregulated, and transfection of miR-374c-5p mimics significantly decreased BC cell proliferation, migration and invasion induced by 10 $\mu mol \cdot L^{-1}$ Cd. Importantly, we used the Cytoscape software plugin cytoHubba to analyse the intersected genes between our RNA-Seq results and the mirDIP database, and six hub genes (CNR1, CXCR4, GRM3, RTN1, SLC1A6 and ZEB1) were identified as potential direct targets of miR-374c-5p in our model; however, luciferase reporter assays indicated that miR-374c-5p only repressed GRM3 by directly binding to its 3′-untranslated region (UTR). Of note, we verified that suppression of N6-methyladenosine (m6A) modification led to miR-374c-5p downregulation by decreasing its RNA transcript stability. Together, these findings demonstrated that m6A modification of pri-miRNA-374c blocks miRNA-374c-5p maturation and then activates GRM3 expression, which drives BC cell metastasis after Cd exposure.

Key words: breast cancer; cadmium; miR-374c-5p; m6A; GRM3

Corresponding author: Huifeng Pi, E-mail: pihuifeng2010@163.comn

T02-25-0008

QSAR modeling of rodent carcinogenicity of polycyclic aromatic hydrocarbons: species, sex and interspecies relationship

Feifan Li, Guohui Sun*, Lijiao Zhao, Rugang Zhong, Yongzhen Peng

(Beijing Key Laboratory of Environmental and Viral Oncology, Faculty of Environment and Life, Beijing University of Technology, 100 Pingleyuan, Chaoyang District, Beijing 100124)

Abstract: Polycyclic aromatic hydrocarbons (PAHs) refer to a class of organic compounds with two or more benzene rings. They are a class of harmful substances produced via the incomplete combustion and geochemical process of organic compounds. They are identified as the main organic pollutants affecting human health. However, the existing toxicity test information of PAHs is very limited, especially for carcinogenicity. In order to avoid large-scale animal experiments and reduce the time and capital cost, in silico computational tools, such as quantitative structure-activity relationship (QSAR), are highly praised by various regulatory organizations. In this study, we collected rodent carcinogenicity data (tumorigenic dose, TD50) of PAHs from CPDB (Carcinogenic potency database). Three methods were used to the division of data sets, and we established six sets of carcinogenicities predictive QSAR models (female rat / mouse, male rat / mouse, rat and mouse)using the pTD50(- logTD50) as endpoint based on PaDEL descriptors, Dragon descriptors, and PaDEL descriptors plus Dragon descriptors, respectively. Finally, the best six individual models were evaluated through various internationally recognized validation indicators and OECD principles. Mechanistic interpretation analyzed the detailed association between molecular descriptors in model equations and carcinogenicity. Furthermore, rat-mouse andmouse-rat interspecies quantitative carcinogenicity-carcinogenicity relationship(iQCCR) models were also developed for data gap filling.We also reportedthe predicted carcinogenicity data for true external setsfor each QSAR or interspecies model, the predictive quality of the QSAR and iQCCR modelswere classifiedas "Bad", "Moderate" and "Good" based on thestringent MAE-based criteria. In summary, the proposed models can be used forcarcinogenicity prediction for new or untested or not yet synthesized PAHs fallingwithin the scope of the applicability domain (AD)

Key words: Polycyclic aromatic hydrocarbons; carcinogenicity; quantitative structure-activity relationship; risk assessment

Corresponding author: Guohui Sun, E-mail: sunguohui@bjut.edu.cn

T02-28-0003

Poly(ADP-ribose) glycohydrolase (PARG) silencing protected cells from BaP-induced DNA hypomethylation and regulated the ubiquitination hydrolysis process during BaP carcinogenesis

Haiyan Huang, Yingbin Fu, Wei Gao, Zhuoying Zeng, Wenjuan Dai, Yanxia Den, Jianjun Liu, Xinyun Xu, Desheng Wu, Linqing Yang, Xianru Luo, Jinzhou Zhang, Zhixiong Zhuang

(Key Laboratory of Modern Toxicology of Shenzhen, Shenzhen Center for Disease Control and Prevention, No 8 Longyuan Road, Nanshan District, Shenzhen 518055, P.R. China)

Abstract: Benzo(a)pyrene (BaP) is a well-known genotoxic carcinogens and ubiquitously existing in environment. Many evidences suggest poly(ADP-ribose) glycohydrolase (PARG) inhibitors may sever as

an anti-cancer drug candidate. We previously demonstrated that PARG silencing suppresses BaP induced cell transformation. However, the exact mechanisms involved remain obscure. The present study explored a potential mechanism linking PARG to BaP induced-cell malignant transformation. Here we compared the global DNA methylation between BaP-transformed human bronchial epithelial cells (BTC) and PARG-deficient human bronchial epithelial cells treated with BaP in the same way (B-shPARG). We found significant global DNA hypomethylation in BTC comparison with B-shPARG. To identify charac·terization of differentially methylated genes, DNA methylation co-immunoprecipitation high-throughput sequencing (MeDIP-Seq) analysis was performed in two cell lines (BTC and B-shPARG). Through anal·ysis of the MeDIP-sequence date, we screened the differentially methylated genes, then found a num·ber of genes that involved in the cancer which induced by BaP such as the following: EGFR, EGF, SOS, MAPK, UBA3, WNT2b, WNT5b. These genes were involved in the EGF receptor signaling path·way, MAPK signaling pathway, Wnt signaling pathway and ubiquitin mediated proteolysis. Furthermore, we identified that UBA3, EGFR, WNT5b and WNT2b expression through RT-PCR in vitro, and we found the trend of UBA3, EGFR, WNT5b and WNT2b expression was consistent with the Methylation patterns change. These findings suggest that Wnt signaling pathway and ubiquitin mediated proteolysis are specific factors during BaP carcinogenesis. We further found that the mRNA and protein level of UBA3 wasdecreased in malignant transformation after BaP treatment, whichindicate that the ned·dylation process was inhibited during BaP carcinogenesis. Altogether, the data suggest EGF receptor signaling pathway and Wnt signaling pathway may be activated and promote tumorigenesis in BaP car·cinogenesis. After PARG gene silencing, ADP-ribosylation may regulate the ubiquitination hydrolysis process to inhibit activation of EGF receptor signaling pathway and Wnt signaling pathway, thereby in·hibiting the carcinogenic process induced by BaP.

Key words: poly(ADP-ribose) glycohydrolase; benzo(a)pyrene; DNA methylation;human bronchial epithelial (16HBE) cells; ubiquitination

Corresponding author: Haiyan Huang, E-mail: hhy424@126.com

T02-28-0012

Effects of short-term and long-term exposure of low-dose chrysotile on the malignant phenotype of MeT-5A cells and the carcinogenicity of mesothelioma

Yuan Xiu-yuan, Zhang Fang-fang, Gao Yan-an, Zhu Li-jin

(School of Public Health, Hangzhou Medical College, Xihu District, Hangzhou 310013, China)

Abstract: Chrysotile products are widely used in daily life, and a large amount of inhalable dust can be generated during the production process. At present, there is still controversy in the international community about the safety of chrysotile fibers, and it is not clear whether inhalation of chrysotile dust will cause mesothelioma. In our study, a lower dose (5 $\mu g \cdot cm^2$) of chrysotile was used to explore the toxicity of short-term and long-term exposure to chrysotile asbestos. In this study, three time points of short-term exposure (24 h, 48 h, 72 h) and long-term exposure of 28 w were selected to infect human mesothelial cells MeT-5A to detect the malignant phenotypic changes, including cells Proliferation, mi·gration, invasion, cycle and apoptosis levels, as well as changes in reactive oxygen species (ROS) and mitochondrial membrane potential (MMP), to evaluate the carcinogenicity of chrysotile and its molecu·

lar mechanism in the carcinogenic process of mesothelioma. The results showed that MeT-5A cells showed a certain degree of malignant phenotype after short-term exposure to chrysotile. After 28 weeks of long-term exposure, the cells were anchor-independent manner, and transformed cells (Asb-T MeT-5A) were successfully established. In addition, the CCK-8 experiment was used to detect the cell proliferation ability, and the scratch experiment and Transwell were used to evaluate the cell migra·tion and invasion ability. Flow cytometry is used to detect cell cycle and apoptosis, and flow cytometry is used to detect cell ROS and MMP. The results showed that the migration and invasion capabilities of MeT-5A cells exposed to short-term exposure were significantly enhanced (P<0.05). The number of cells in G1 was significantly lower than that of the control group, but the number of apoptotic cells was significantly higher than that of the control group. Through the transformation of chrysotile, the prolifera·tion, migration and invasion ability of Asb-T MeT-5A cells was significantly enhanced (P<0.01). The results of flow cytometry showed that the number of cells in G1 in the Asb-T MeT-5A group was signifi·cantly lower than that of the control group, and the number of apoptotic cells in the Asb-T MeT-5A group was significantly lower than that of the control group. ROS and MMP level detection results showed that the ROS level of MeT-5A cells exposed to short-term exposure increased, while the ROS of transformed cells Asb-T MeT-5A decreased. The results of the MMP of cells treated at different times were consistent, all showed increasing trend (P<0.05). Chrysotile can induce the malignant trans·formation of MeT-5A cells, enhance the proliferation, migration and invasion ability of MeT-5A cells, and reduce the number of G1 phase and apoptotic cells. Chrysotile asbestos can change the ROS and membrane potential levels of MeT-5A cells.

Key words: Chrysotile; Toxicity; malignant phenotype; ROS; MMP

Corresponding author: Zhu Li-jin, E-mail: lijinzhu@zjams.com.cn

T02-28-0017

Lycorine-induced apoptotic cell death in Raw264.7 cells involves the generation of reactive oxygen, mitochondrial dysfunction, inhibition of IL-1β/IκB-α/NF-κB and Nrf2/HO-1 pathways

Zhang Yuan, Tang Shu-sheng*, Dai Chong-shan*

(Department of Basic Veterinary Medicine, College of veterinary medicine, China Agricultural University 100193, P. R. China)

Abstract: Lycorine, the main phenanthridine Amaryllidaceae alkaloid, exhibits high specificity activ·ities against various cancers both in vivo and in vitro. In the present study, we aimed to investigate the precise molecular mechanisms underlying lycorine-induced apoptotic cell death in Raw264.7 cells. Our results showed that lycorine treatment (0-10 $\mu mol \cdot L^{-1}$) of Raw264.7 cells induced apoptotic cell death and blocked cell proliferation in a dose-dependent manner. Lycorine treatment significantly promoted the production of reactive oxygen species (ROS). Mitochondrial dysfunction was evident by the loss of membrane potential and the increased radio of Bax/Bcl-2, followed to induce the increased expression of cleaved caspase-3. Furthermore, lycorine treatment per se did not change the expression of TNF-α and COX-2 proteins, but significantly decreased the expression of IL-1β, IκB-α, and NF-κB proteins. Meanwhile, Lycorine treatment significantly decreased the expression of Nrf2, and HO-1 protein. In con·clusions, these results indicated that lycorine treatment could induce mitochondrial apoptotic death in

Raw264.7 cells via the inhibition of IL-1β/IκB-α/NF-κB and Nrf2/HO-1 pathways. Our study highlights that lycorine is a promising anti-cancer candidate.

Key words: lycorine; reactive oxygen species(ROS); apoptosis; IL-1β/IκB-α/NF-κB pathway; Nrf2/HO-1 pathway

Corresponding author: Dai Chong-shan, E-mail: daichongshan@cau. edu. cn; Tang Shu-sheng, E-mail: tssfj@cau.edu.com

T02-29-0010

The construction of "arsenic prodrug" loaded multifunctional drugdelivery system and its in situ activation for the synergistictherapy of liver cancer

Zhang Yue, Liu ai-di, Cheng meng-ying, Ji-Gang Piao, Fanzhu Li

Abstract: Primary hepatic carcinoma poses a serious threat to human health with themortality rate ranking the third among all malignant tumors. Traditional Chinesemedicine often treats liver cancer by "fighting poison with poison." Arsenictrioxide (As_2O_3), the active ingredient of traditional Chinese medicine arsenic, is of significant toxicity and has a definite curative effect on liver cancer. However, it can only play a palliative role in clinical application due to itssevere side effect, low targeting efficiency, and limited therapeutic efficacy ofa single drug. On the basis of the relationship among toxicity, potency andvalence of As, the arsenic is designed for "arsenic prodrug" in this project toconstruct the "arsenic prodrug" loaded MnS multifunctional delivery system, innovatively using the H2S generated from MnS multifunctional nano-carrier underthe trigger of the tumor microenvironment as a reductive activator to in situactivate the "arsenic prodrug" into arsenic, which can avoid the toxicity tonormal organs; Simultaneously, MnS multifunctional nano-carrier was activated torelease manganese ions (Mn^{2+}) with chemodynamic therapeutic effect and hydrogensulfide (H_2S) with gas therapeutic effect, respectively, realizing the synergisticeffect of "traditional Chinese medicine, gas therapy, and chemodynamic therapy"with enhanced anti-liver cancer efficacy. This research may provide novel ideas, strategies, and methods for safer and more effective application of toxictraditional Chinese medicine in the synergistic therapy of liver cancer and moderntreatment of traditional Chinese medicine.

Key words: "arsenic prodrug"; multifunctional delivery system; liver cancer; insitu activation; synergistic therapy

T02-32-0013

MMP2-responsive dual-targeting drug delivery system for valence-controlled arsenic trioxide prodrug delivery against hepatic carcinoma

Ke Zhang, ChaoqunLi, XiaoweiXie, Ji-Gang Piao, Fanzhu Li

Abstract: Arsenic trioxide (ATO) is the active ingredient of traditional Chinese medicine, Arsenic, which has shown excellent therapeutic effects on hepatocellular carcinoma. However, due to its poor tumor distribution and high toxicity, the mass adoption of ATO in clinical applications has been severely

impeded. In this study, matrix metalloproteinase 2 (MMP2)-responsive cleaved cell-penetrating peptide (PF) and folate (FA) co-modified liposome coated calcium arsenate nanoparticles (FA / PF-LP-CaAs) were fabricatedin virtue of two considerations: (1) the tumor microenvironment characterized by overexpressed MMP2 in extracellular matrix and folate receptor on the cell membrane can enhance drug accumulation and accelerate endocytosis; (2) leveraging on the different toxicity of arsenic in different valence states, i.e., AsV can be reduced to more toxic AsIII by glutathione in tumor cells. Furthermore, FA/PF-LP-CaAscould be responsively degraded by the mild acidic tumor environment, and was able to escape from lysosomes after endocytosis. More importantly, in light of the invivo biodistribution and pharmacodynamic studies, the vehicle was able to accumulate in the tumor efficiently, and exhibited fabulous anti-tumor efficacy with minimized side effects when compared with single-modified counterparts. Thus, the novel strategy based on the tumor microenvironment proposed in this work can enhance the tumor-targeting efficiency and intratumor toxicity.

Key words: MMP2-responsive; valence-controlled; arsenic trioxide prodrug; dual targeting; hepatocellular carcinoma

T02-37-0009

Role of TP53/mitochondrial pathway in radon induced malignant transformation of BEAS-2B cells and lung injury in mice

Jin Wang, Qian Xu, Jianxiang Li

(School of Public Health, Medical College of Soochow University, China)

Abstract: OBJECTIVE lung cancer is a multifactorial disease, the most critical risk factor is smoking, and residential radon exposure is the second most significant risk factor after smoking. As an essential tumor suppressor, the carcinogenic phenotype of TP53 mutation mainly includes promoting cell proliferation, chemical resistance, apoptosis, migration and invasion. TP53 can regulate many biological processes, including mitochondrial function. Studies have shown that TP53 can interact with members of the BCL2 family and directly participate in the internal apoptosis pathway, thus inducing mitochondrial outer membrane permeability (MOMP). The purpose of this study was to explore the role of the TP53 / mitochondrial pathway in the radon-induced malignant transformation of BEAS-2B cells and lung injury in mice. **METHODS** Our previous study has successfully established the radon-induced malignant transformation cell model by using TP53 wild-type and knockout BEAS-2B cells, and successfully established the lung injury animal model caused by radon inhalation by using BALB/ c mice. For the cell model, we analyzed the malignancy of TP53 knockout and radon exposed cells through soft agar colony formation, EdU, transwellmigration and wound healing assays. Wealso analyzed the mitochondrial activity by analyzing the differentlevels of MOMP related markers inside and outside mitochondria, mitochondrial membrane potential and mitochondrial copy number. For the animal model, we measured the pathological changes, TP53 expression and mitochondrial copy number of lung tissues in radon exposed mice. **RESULTS** Compared with the normal passage BEAS-2B cells, the apoptosis rate of the other three groups was significantly lower, and the cell apoptosis rate of $TP53^{-/-}$+ Rn group was significantly lower than that of Rn and $TP53^{-/-}$ groups. Similarly, the colony formation efficiency, cell proliferation and migration ability of cells in Rn, $TP53^{-/-}$ and $TP53^{-/-}$+ Rn groups were significantly higher than those in the BEAS-2B group, and those malignant related changes in $TP53^{-/-}$+Rn group

were significantly higher than those in Rn and TP53$^{-/-}$ groups. When considering the mitochondrial activity, the results showed that compared with the BEAS-2B group, the mitochondrial membrane po·tential of the other three groups increased significantly, the apoptotic BAX bound to mitochondria de·creased significantly, cytochrome C (Cyt-C) accumulated significantly in the mitochondrial intermem·brane space, and the mitochondrial copy number also increased significantly. Consistent with other changes, the mitochondria-related changes in TP53$^{-/-}$+Rn group changed most significantly. In the *in vivo* model, the results showed that long-term radon exposure could cause adverse pathological changes in lung tissues, decreased TP53 gene expression and increased mitochondrial copy number. **CONCLUSION** TP53 knockout and long-term radon exposure can induce the malignant transformation of BEAS-2B cells, and inhaled radon can cause the adverse pathological changes of lungs in mice; Radon exposure can affect cell mitochondrial activity, and TP53 knockout can significantly enhance the effect induced by radon exposure. The regulatory mechanisms remain to be further studied.

Key words: Radon exposure; Mitochondrion; TP53; Malignant transformation

Corresponding author: Jin Wang, E-mail: Jin.Wang93@outlook.com, E-mail: aljxcr@suda.edu.cn

T02-43-0018

Stk38 regulates the radiation sensitivity of cancer cells by regulating the stability ofcGAS

Lei Gao[1], Xiaoyu Cao[1], Shanshan Gao[2], Hua Guan[2], Pingkun Zhou[2]

(1. The institute of life science, Hebei university, Baoding 071000; 2. Beijjing institute of Radiation Medicine, Beijing 100850)

Abstract: With the development of nuclear technology, its application, detectability and safety have been widely concerned. Ionizing radiation (IR) has been widely used in the clinical treatment of cancer. Cancer cells will be recognized and phagocytosed by autoimmune cells, so immunotherapy has received much attention in the clinical treatment of cancer. Unfortunately, most cancers are insensi·tive to immunotherapy specific drugs PD-1/PD-L1 antibodies, etc. Radiotherapy enhance immunothera·py is usually used in clinical practice. IR stimulates cells to produce double-stranded DNA breaks, which are not repaired in time to prevent cell growth or even death. cGAS, main function is to recognize the DNA of foreign pathogens and detect the stress environment of its own cells, is widely distributed in the cytoplasm and nucleus.Inflammatory response was activated by cGAS/STING pathway or regulate genome replication and homologous recombination repair by independent of STING. Unrepaired DNA generated by ionizing radiation is transmitted to the cytoplasm through micronuclei, which in turn pro·motes the expression of inflammatory factors through the cGAS/ STING pathway. In clinical studies, it has been found that cells can regulate radiation sensitivity by regulating cGAS activity. High-dose irradi·ation (24 Gy) induces more nucleases than multiple low-dose irradiation (8 Gy). Low concentrations of DNA in the cytoplasm inhibit the activity of cGAS, which in turn produces fewer cytokines to activate immune cells. The clinical manifestations are that multiple low-dose irradiation can better achieve the purpose of tumor clearance. Based on this phenomenon, we used mass spectrometry to search the changes of interacting proteins before and after cGAS irradiation to investigate the changes of protein modification before and after cGAS irradiation and the regulatory mechanism of cGAS under emergency conditions. Proteins such as STK38 and PRMT5 were found to be involved in the modification regula·

tion of cGAS. It has been reported that cGAS can be inhibited by PRMT5 methylation from binding to DNA, which in turn regulates the activity of cGAS. STK38 is a serine threonine kinase protein, which has been found to be involved in DNA damage repair and is negatively correlated with the radiosensi·tive type of cells. Whether STK38 can also regulate radiosensitive type through cGAS needs further in·vestigation. It can provide another target for the clinical treatment of cancer.

Key words: Radiotherapy; Immunotherapy; cGAS; STK38

Corresponding author: Hua Guan, E-mail: ghlsh@163. com; Pingkun Zhou, E-mail: zhoupk@nic. bmi.ac.cn

T02-45-0026

Low-dose bisphenol A (BPA) promotes ovarian cancer epithelial-mes-enchymal transition (EMT) progression through CASC15/Smad3 axis

Xiaoyu Yuan, Jing Leng, Hui Lin, Dajing Xia, Yihua Wu

(Department of Toxicology of School of Public Health, and Department of Gynecologic Oncology of Women's Hospital, Zhejiang University School of Medicine)

Abstract: Bisphenol A (BPA) is a typical environmental endocrine disruptor (EDCs) which is widely found in plastic products, and it can interfere with human endocrine regulation function and the develop·ment of the reproductive system, but its correlation with hormone-dependent cancers such as ovarian cancer has remained to be controversial. To understand the possiblemechanisms underlying the effects of BPA, human ovarian cancer cell lines SKOV3 and A2780 were exposed to BPA (100 nmol·L^{-1}), and we foundan upregulated expression of a critical long non coding RNA (lncRNA) CASC15. Previous studies and our recent research indicated that CASC15 has played an important role in epithelial-mes·enchymal transition (EMT) of cancer cells. We furtherly found that 100 nmol·L^{-1} BPA treatment signifi·cantly increased mesenchymal marker N-cadherin expression and decreased epithelial maker ZO-1 expression, which could be partially reversed bythe knockdown of CASC15. In our previous study, Smad3 has been confirmed as a downstream effector of CASC15. Through molecular toxicology exper·iments such as immunoblotting and immunofluorescence, we found that BPA can promote the EMT process of ovarian cancer cells by up-regulating the CASC15/Smad3 axis, while silencing Smad3 also significantly suppressedthe BPA-induced migration, invasion and EMT. In conclusion, environmentally relevant doses of BPA can promote the migration, invasion and EMT of ovarian cancer cells through the CASC15-Smad3 axis. This present study reveals that low-dose BPA promote tumor progression and metastasis, which provides an important evidence for the management and intervention targets of plastic products with potential health risks.

Key words: BPA; Ovarian cancer; Low-dose exposure

Corresponding author: Yihua Wu, E-mail: georgewu@zju. edu. cn; Dajing Xia, E-mail: dxia@zju. edu.cn

T02-46-0024

Exploration on the role of DCdependent T cell activation in radiation-induced lung injury

Li Qian[a,b], Geng Shuang[b], Guo Hao-xin[a,b], Wang Mei-yu[b,c], Wang Zhi-xin[b], Yan Cheng-ming[b], Liu Ben-bo[b], Yang Zhi-hua[b], Wang Yi-long[b], Zhu Mao-xiang[a,b]

(a. Nanhua University, Hengyang 421001, China; b. Beijing Institute of Radiation Medicine, Beijing 100850, China; c. Hebei University, Baoding 071002, China)

Abstract: OBJECTIVE To investigate the role ofT cells inmouse lungat the early stage of gamma- ray chest irradiation and to explore the mechanism of antigen-presenting and T cell activation in radia·tion inducedlung injury. **METHODS** (1)72 C57BL/6 male mice (6-8 weeks, 20±2 g) were randomly di·vided into 6 groups (control groups and irradiation groups,n=12).The irradiation groups received a sin·gle dose of thoracic irradiation (20 Gy) with a 60Co ray source. At 1d, 2dand3dafter irradiation, T cell and theirsubtypes (CD3 +/CD4 +/CD8 +) in bronchialalveolar lavage fluid(BALF) anddraining lymph nodes of micewere detected by flow cytometry. (2) MLE-12 cells irradiated by 6Gy γ - ray were co-cul·tured with DC derived from bone marrow and T cells isolated from spleen. And then antigen presenting of DC cells and T cell activationwere detected by flow cytometry. **RESULTS** (1) Compared with the control group, the proportion of CD3 + T cells in BALF of the irradiation group increased at 1 day; the proportion of CD3+ CD4 + T cell subsets decreased in the irradiation group at 1 and 2 days; the propor·tion of CD3+ CD8+ T cell subsets increased at 1 and 2 days. (2) The proportion of CD3+T cells in the lung draining lymph nodes of the irradiation group was higher than that in the control group at 1-2 days; The proportion of CD3 + CD4 + T cells increased in the irradiation group at 2 and 3 days; but the pro·portion of CD3 + CD8 + T cells decreased at 2 and 3 days. (3) In co-culture experiments,it was found that the number of CD3+T, CD4+T and CD8+T cells in irradiation group(co-cultrue pool including MLE-12 after irradiation, DC and T cells) were significantly lower than that in control group(co-cultrue pool in·cluding MLE-12 without irradiation, DC and T cells) after co-culture for 24 hours. However, Proportion of CD4+T cells in irradiation group were lower than that in control group, and proportion of CD8+T cells in irradiation group were higher than that in control group. Interestinglv, the number and proportion of CD8+ T cells in irradiation group were significantly higher than that in control group after co-culture for 48 hours. Furthermore, The number of DC in irradiation groupwere higher than that in control group af·ter co-culture for 24 and 48 hours. **CONCLUSION** Our study suggest thatT cellsubsets change in dif·ferent way at the early stage after mice receiving thoracic irradiation, and CD8+T cells are activated by pulmonary epithelial cells though DC.

Key words: Ionizing radiation; Dendritic cells; T cell activation; Antigen presentation

T02-52-0022

Brain metastatic breast cancer exosomes based biomimetic brain-targeted "arsenic prodrug" delivery system for anti-glioma treatment with reduced toxicity and enhanced efficacy

Tianxiang Yue, Wenting Gu, Xue Lin, Ji-Gang Piao, Fanzhu Li

Abstract: Glioma is a common primary intracranial malignant tumor. Arsenic trioxide (As_2O_3), the

active ingredient of the traditional Chinese medicine arsenic, has a clear therapeutic effect and mechanism of action against glioma. However, its high toxic side effects, lack of specificity for *in vivo* distribution, and low efficiency of crossing the blood-brain barrier (BBB)have limited its further clinical application. In this study, abiomimetic brain-targeted "arsenic prodrug" delivery system was constructed via self-assembly of arsenate ($AsO4^{3-}$), exosomes, Mn^{2+}, and ICG, efficiently integrating BBB crossing capability of brain metastatic breast cancer cell exosomesand the affinities between the toxicity, efficacy, and valence ofarsenic. The biomimetic delivery system can effectively cross the blood-brain barrier to target gliomas and activate the "arsenic prodrug" at the tumor site in response to the tumor microenvironment, exerting therapeutic effect while avoiding the toxic side effects of arsenic in normal tissues and organs. Meanwhile, in situreleased Mn^{2+} and ICG can synergize with the traditional Chinese medicine arsenic for combined treatment of glioma with traditional Chinese medicine-chemodynamic-photothermal therapy and improve the therapeutic efficacy. This research will provide novel strategies and new methods for targeted therapy of brain diseases.

Key words: brain metastatic breast cancer exosomes; biomimetic brain-targeted delivery system; arsenic prodrug;glioma; reduced toxicity and enhanced efficacy

T02-56-0001

3-Bromopyruvate regulates the status of glycolysis and BCNU sensitivity in human glioma cells and human hepatocellular carcinoma cells

Xiaodong Sun, Guohui Sun*, Lijiao Zhao, Rugang Zhong

(Beijing Key Laboratory of Environmental and Viral Oncology, College of Life Science and Chemistry, Faculty of Environment and Life, Beijing University of Technology, 100 Pingleyuan, Chaoyang District, Beijing 100124)

Abstract: Chloroethylnitrosoureas (CENUs) are bifunctional antitumor alkylating agents, which exert their antitumor activity through inducing the formation of dG-dCinterstrand crosslinks (ICLs) within DNA double strand. However, the complex process of tumor biology enables tumor cells to escape the killing triggered by CENUs, as for instance with the DNA damage repair mediated by O6-methylguanine DNA methyltransferase (MGMT) leading to drug resistance of tumor cells to CENUs. Considering the fact that most tumor cells highly depend on aerobic glycolysis to provide energy for survival even in the presence of oxygen (Warburg effect), inhibition of aerobic glycolysis may be an attractive strategy to overcome the resistance and improve the chemotherapeutic effects of CENUs. Especially, 3-bromopyruvate (3-BrPA) has been emerged as an effective glycolytic inhibitor (energy blocker) in cancer treatment. In this study, we investigated the effects of 3-BrPA on the chemosensitivity of two human glioma cells lines and two human hepatocellular carcinoma cell lines to the cytotoxic effects of l,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and the underlying molecular mechanism. The sensitivity of tumor cells to BCNU was significantly increased by pretreatment with 3-BrPA. Moreover, 3-BrPA decreased the cellular ATP and GSH levels, and extracellular lactate excreted by tumor cells, and the effects were more effective when 3-BrPA was combined with BCNU. Cellular hexokinase-Ⅱ (HK-Ⅱ) activity was also reduced after exposure to the treatment of 3-BrPA plus BCNU. Based on the above results, the effects of 3-BrPA on the formation of dG-dC ICLs induced by BCNU was investigated by HPLC-ESI-MS/MS. The results indicated that BCNU produced higher levels of dG-dC ICLs in tumor cells pretreated with 3-BrPA compared to that without 3-BrPA pretreatment. Notably, in MGMT-deficient cells, the levels of dG-dC ICLs

were significantly higher than MGMT-proficient cells. In general, these findings revealed that 3-BrPA may be considered as a potential clinical chemosensitizer to optimize the therapeutic index of CENUs.

Key words: 3-Bromopyruvate; Chloroethylnitrosoureas; Glycolytic inhibitor; Chemosensitization; dG-dCinterstrand crosslinks

Corresponding author: Guohui Sun, E-mail: sunguohui@bjut.edu.cn

T02-65-0004

Long noncoding RNA lnc-RI regulates DNA damage repair and radiation sensitivity of CRC cells through NHEJ pathway

Ruixue Liu, Qingtong Zhang, Liping Shen, Shuangjing Chen, Junyan He, Dong Wang, Qi Wang, Zhenhua Qi, Meijuan Zhou, Zhidong Wang

(Department of Radiobiology, Beijing Key Laboratory for Radiobiology, Beijing Institute of Radiation Medicine, Beijing, People's Republic of China)

Abstract: A percentage of colorectal cancer (CRC) patients display low sensitivity to radiotherapy, which affects its therapeutic effect. Cancer cells DNA double-strand breaks (DSBs) repair capacity is crucial for radiosensitivity, but the roles of long noncoding RNAs (lncRNAs) in this process are largely uncharacterized. This study aims to explore whether lnc-RI regulates CRC cell growth and radiosensitivity by regulating the nonhomologous end-joining (NHEJ) repair pathway. CRC cells in which lnc-RI has been silenced showed lower cell growth and higher apoptosis rates due to increased DSBs and cell cycle arrest. We found that miR-4727-5p targets both lnc-RI and LIG4 mRNA and inhibit their expression. CRC cells showed increased radiosensitivity when lnc-RI was silenced. These results reveal novel roles for lnc-RI in both DNA damage repair and radiosensitivity regulation in CRC cells. Our study revealed that lnc-RI regulates LIG4 expression through lnc-RI / miR-4727-5p / LIG4 axis and regulates NHEJ repair efficiency to participate in DNA damage repair. The level of lnc-RI was negatively correlated with the radiosensitivity of CRC cells, indicates that lnc-RI may be a potential target for CRC therapy. We also present the first report of the function of miR-4727-5p.

Corresponding author: Zhidong Wang, E-mail: wangzhidong_1977@aliyun.com

T02-71-0014

Preparation and in vitrostudy on reversing tumor drug resistance of mtochondrialtargetedcalcium arsenite/doxorubicin lipid nanoparticles

Zhang Ke[a], Yue Tian-xiang[a], Liu Ai-di[a], Piao Ji-gang[a,b], Li Fan-zhu[a,b]

(a. College of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 310000, China; b.Key Laboratory of Neuropharmacology and Translational Medicine of Zhejiang Province, Hangzhou 310000, China)

Abstract: This study focused on the key role of P-glycoprotein (P-gp) in tumor drug resistance. Taking advantage of ATP-dependant P-gp mediated membrane transport and efflux effect, mitochondrial targeted calcium arsenite/doxorubicin lipid nanoparticles were constructed by hydrothermal synthesis and thin-film dispersion methods. The results showed that the lipid nanoparticles were uniform in size

and well dispersed, with a mean particle size of (261.1±6.5) nm, a zeta potential of (−9.6±1.3) mV, and a DOX loading of 22.6%. The lipid nanoparticles showed pH-dependent in vitro drug release behavior with significantly increased drug accumulation at mitochondria, causing calcium overload, inhibiting P- gp and ATP production and reversing tumor drug resistance. And the simultaneously released arsenic and Doxorubicin (DOX) could synergistically kill the tumor cells. In summary, the lipid nanoparticles pre·pared in this study have uniform particle size, high drug loading, excellent colloidal stability, pH respon·siveness and impressive ability to reverse tumor drug resistance, and have great potential in further clinical applications.

Key words: mitochondrial targeting; doxorubicin; Liposomes; calcium arsenite nanoparticles; re·verse tumor drug resistance

T02-75-0016

Carcinogenicity riskof Tacrolimus in kidney Transplant Recipients: A Systematic Review and Meta-Analysis

Ma Kui-fen, Yao yao, Ye Zi-qi

(Department of Clinical Pharmacy, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China)

Abstract: OBJECTIVE The current anti-rejection program is mainly based on tacrolimus due to the less acute rejection compared with CsA. We aim to determine the carcinogenicity of tacrolimus's optimal regimen for kidney transplant recipients. **METHODS** A systematic literature search of Medline (PubMed and Ovid), EMBASE, CINAHL and the Cochrane Library was conducted from the database created to May 2021. Two hundred and nine studies were included in total, with risk estimates from 11 of these studies able to be pooled for quantitative analysis. Carcinogenicity incidence of tacrolimus was extracted and analyzed in kidney transplant patients according to relevant literatures. **RESULTS** The overall summary estimate showed a significantly increased risk of malignancy in relation to tacrolimus exposure (1.54, 95% CI 1.18-2.01), which was mainly due to comparison of sirolimus (2.58, 95% CI 1.42-4.09). And the skin cancer incidence was consistent with the overall study (2.11 95% CI 1.23- 3.59), owing to an anti-neoplastic action independent of immunosuppression effect. However, there was no different tumor incidence between tacrolimus and cyclosporine A(1.12, 95% CI 0.8-1.56), even in studies with long time follow-up (more than 3 years). **CONCLUSION** Even with greater immunosup·pression, the pooled findings of available evidence did not support the contentionthat treatment with ta·crolimus increasesthe risk of malignancy.

Key words: Tacrolimus; cyclosporin A; sirolimus; malignancy; kidney transplant

T02-76-0023

DNA-PKcs cannot be recruited to DNA damage site in mitosis

Jin Jia[1,2], Hua Guan[1], Chenjun Bai[1], shanshan Gao1, Pingkun Zhou[1]*

(1. Department of Radiation Biology, Beijing Key Laboratory for Radiobiology, Beijing Institute of Radiation Medicine, Beijing 100850, China; 2. School of Medicine, University of South China, Hengyang, China)

Abstract: The DNA-dependent protein kinase (DNA-PK) ,as central to the process of non-homologous end joining (NHEJ) of DNA damage repair, which is atripolymer, including KU70, KU80 and DNA-PKcs. NHEJ can be activated when DNA is damaged byenvironmental hazards insult or endogenous toxic agentssuch as free radicals. Previously researches have suggested the NHEJ starting fromrecognition of the DNA double strand break by the Ku70 / 80 heterodimer, then recruited of the DNA-PKcs and DNA end processing, if required. However, we find an interesting phenomenon by using confocal laser scanning microscopy. We found that ininterphase cellsDNA-PKcs werecolocalized with γH2AX in the nucleus, contrarilyduring mitosis DNA-PKcslocalize at periphery ofmitotic chromosomes, and DNA-PKcs cannot colocalized with γH2AX. Moreover, we use DNA-PKcs p2056 antibody stainingmitosis cell, found the same result. That means DNA-PKcs recruited to DSB in mitosis is supressed. These results imply could exist a different mechanisms to regulate DSB repaire in mitosis, and it will be a potential application to Combination of tumor radiotherapy and chemotherapy.

Key words: NHEJ; DNA repair; DNA damage response; mitosis; KU; DPK-cs

Corresponding author: Pingkun Zhou, E-mail: zhoupk@bmi.ac.cn

T02-84-0025

Title effects of perfluoroalkyl acids exposureon the development of ovarian cancer

Lihuan Zhang, Yuheng Qin, Jiawei Xu, Yihua Wu, Dajing Xia

(Department of Toxicology of School of Public Health, and Department of Gynecologic Oncology of Women's Hospital, Zhejiang University School of Medicine)

Abstract: Perfluoroalkyl acids (PFAAs)is a class of compounds of persistent organic pollutants which have been widely used in aviation science and technology, transportation and electronics industries. Product surface treatment (such as non-stick cookers), convenient food packaging, anti-stick fiber material and fireproof foam contain the abundance of PFAAs. Previous studies have shown that PFAAs could promote the migration and invasion of breast and gynecological tumors. However, theeffects and mechanisms of PFAAs on the development of ovarian cancer have not been clarified. In this present study, we tried to explore the effects of three typical PFAAs—Perfluorooctanoate acid (PFOA), perfluorononanoic acid (PFNA) andperfluorodecanoic acid (PFDA) on the proliferation, invasion and migration of ovarian cancer cells. Here, we found that despite lacking of significant effect on the proliferation, PFOA, PFNA and PFDA can promote the migration, invasion ability and epithelial-mesenchymal transitionof ovarian cancer cells in low concentration. Through molecular toxicology technologies such as western blotting and immunofluorescence assays, we found that PFNA might induce epithelial-mesenchymal transition via causing degradation of SMAD7 in ovarian cancer cells. In addition, PFNA degrades SMAD7 by means of proteasome pathway to increase the phosphorylation and the nuclear localization of SMAD2 / 3so that it can activate TGF - β signaling. In conclusion, our study indicated that PFAAs could promote the progression of ovarian cancer. Furthermore, PFNA might promote EMT of ovarian cancer via TGF-β/SMAD7 pathway.

Key words: PFAAs; Ovarian cancer; Epithelial-mesenchymal transition; TGF-β pathway

Corresponding author: Yihua Wu, E-mail: georgewu@zju. edu. cn; Dajing Xia, E-mail: dxia@zju.edu.cn

T02-86-0005

Based on tumor energy metabolism: lonidamine enhances ACNU sensitivity in human glioma and lung cancer cells

YaxinHuang, Guohui Sun*, Lijiao Zhao, Rugang Zhong

(Beijing Key Laboratory of Environmental and Viral Oncology, College of Life Science and Chemistry, Faculty of Environment and Life, Beijing University of Technology, 100 Pingleyuan, Chaoyang District, Beijing 100124)

Abstract: Chloroethylnitrosoureas (CENUs) are bifunctional antitumor alkylating agents, but their clinical effects are compromised due to the drug resistance of tumors. The development of CENUs resistance in tumor cells involves many factors, the most widely investigated of which is the repair effect of O6-methylguanine-DNA methyltransferase (MGMT) on the DNA guanine O6-damage. In addi·tion to MGMT-mediated DNA repair, there are other internal drug resistance mechanisms in cells, such as glutathione (GSH) mediated drug resistance and ATP-dependent multidrug resistance (MDR). Therefore, our research group tried to find a new chemosensitizer to improve the anti-tumor effect of CENUs. On known chemotherapy drugs when we choice, we found lonidamine (LND) has no common side effects of traditional anticancer drugs, such as bone marrow suppression, alopecia and so on. Sec·ondly, since LND inhibits energy metabolism, induces intracellular acid environment and has multiple cellular targets, so we try to select LND as a sensitizing agent of chemotherapy drug. In this study, we investigated the effects of LND on the chemosensitivity of two human glioma cells lines and one human lung cancer cell line to the cytotoxic effects of nimustine hydrochloride (ACNU) and the underlying mo· lecular mechanisms. The sensitivity of tumor cells to ACNU was significantly increased by a 24 h pre· treatment with LND. LND decreased the cellular ATP, GSH and mitochondrial membrane potential (JC-1) levels, and increased the cellular ROS levels. It was particular to note that LND induced intracellular acidification in tumor cells, which may inactivate MGMT activity. The effects were more effective when LND was combined with ACNU. After LND plus ACNU treatment, the activities of hexokinase II (HK- II) and MGMT enzyme were also decreased. Based on the above results, we concluded that the chemo· sensitivity effect of LND on ACNU is to cut off the energy supply of tumors by interfering with the glycol· ysis and mitochondrial pathway, and reduce MGMT activity by inducing intracellular acidification. Over·all, these findings revealed that LND may be considered as a potential clinical chemosensitizer to opti·mize CENUs therapeutic indicators.

Key words: Lonidamine; ACNU; Tumor energy metabolism; Drug resistance; Combination therapy

Corresponding author: Guohui Sun, E-mail: sunguohui@bjut.edu.cn

T02-94-0019

Prediction of chemical carcinogenicity through toxicogenomics scoring system (TGSS): a novel integrated scoring system

Haohua Lu[1], Dexin Yang[1], Yu Shi[2], Kelie Chen[1], Sisi Huang[1], Dongyu Cui[1], Yuqin Feng[1], Honghe Zhang[3], Jun Yang[4], Xinqiang Zhu[5], Dajing Xia[1], Yihua Wu[1]

(1. Department of Toxicology of School of Public Health, and Department of Gynecologic Oncology of Women's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China; 2. The State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital of

School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China; 3. Department of Pathology, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China; 4. Department of Public Health, Hangzhou Normal University School of Medicine, Hangzhou, Zhejiang, China; Zhejiang Provincial Center for Uterine Cancer Diagnosis and Therapy Research of Women's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China; 5. Central Laboratory of the Fourth Affiliated Hospital, Zhejiang University School of Medicine, Yiwu, Zhejiang, China)

Abstract: OBJECTIVES The study aims to establish a novel scoring system named Toxicoge·nomics Scoring System (TGSS) to predict the potential chemical carcinogenicity using chemical-gene interaction information and gene expression profiles of cancer cases. **METHODS** Using the method of pre-ranked Gene Set Enrichment Analysis (GSEA), the distribution of chemical-regulated gene sets in ranked gene lists generated from differential expression analysis (tumor samples vs. normal samples) were evaluated with normalized enrichment score (NES) and adjusted p-value. Carcinogenicity scores were calculated using outputs of GSEA to establish TGSS. Further, survival analysis based on ssG·SEA score and literature review were conducted to examine the power of TGSS, and a coincidence rate was calculated to quantify the accuracy of prediction. **RESULTS** A number of typical chemicals that met the criteria were examined in the TGSS. Gene ontology enrichment analysis revealed biologi·cal process terms related to response to stimulus and carcinogenesis including DNA damage, reactive oxygen species and endoplasmic reticulum (ER) stress. Carcinogenicity scores were calculated for each chemical-tumor pair. The scores of IARC Group 3 chemicals were significantly lower than those of others (all adjusted $P<0.05$). Otherwise, scores of IARC Group 1 chemicals were significantly higher than those of IARC Group 2A and 2B ($P<0.05$). In validation processes, the assessed carcinogenicity in TGSS seemed to parallel the results of survival analysis as well as literature review (coincidence rate =81.8%). **DISCUSSION** TGSS was successfully established with the chemical-gene relationships obtained from CTD and gene expression profiles obtained from TCGA. This system could play an important role in carcinogenic risk assessment of chemicals, which may greatly contribute to systems toxicology.

Corresponding author: Yihua Wu, E-mail: georgewu@zju. edu. cn; Dajing Xia, E-mail: dxia@zju. edu.cn

T02-97-0020

Aconitine induces cell apoptosis via mitochondriaand death receptor signaling pathways in neuronal cell line HT22

Hui Wang[1], Yanbing Liu[1], Shuhang Zhang[1], Weina Wang[1], Yanli Zhu[1], Bo Li[1], Yanan Tian[2], Baoyu Zhao[1], Hao Lu[1]

(1. College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, 712100, China; 2. Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843, USA)

Abstract: This study sought to investigate the effects of aconitine, a well-known aconitum plant-produced toxin, on growth and apoptosis of hippocampal neuronal cell line (HT22) and to explore the potential mechanisms. HT22 cells were cultured, and cell viability and DA (dopamine) contents was examined in HT22 cells treated with different doses of aconitine. Cell apoptosis was detected by Hoechst 33258 fluorescent staining, Ultrastructural changes were observed by electron microcopy and

Annexin V-FITC / propidium iodide double staining in flow cytometric analysis were performed upon aconitine treatment. Molecular mechanism was analyzed by caspase activity detection and Western blot assay. The results showed that aconitine inhibited HT22 cells growth and increased DA contents in a dose dependent manner. The administration of aconitine in HT22 cells also induced cell apoptosis by upregulating the expression of pro-apoptotic factors Bax, mitochondrial-mediated apoptosis-associated proteins Cyto c, Apaf-1, Caspase9 and death receptor apoptosis-associated proteins Fas, Fas-L, Fadd, Caspase8, Caspase3 and by decreasing the anti-apoptotic Bcl-2 and adaptor protein Bid expression. Collectively, results suggest that aconitine induce apoptosis through mitochondrial-mediated and death receptor signaling pathways in HT22 cells.

Key words: aconitine; cell apoptosis; apoptotic pathway; HT22 cells; aconitum

Corresponding author: Hao Lu, E-mail: luhao@nwsuaf.edu.cn

T02-97-0027

A novel antitumor peptide inhibits proliferation and migration and promotes apoptosis in glioma cells by regulating the MKK6/p38 signaling pathway

Feng Zhi

(Changzhou First People's Hospital)

Abstract: Protein-or peptide-based therapeutics have emerged as an innovative strategy for the treatment of cancer. Our previous research demonstrated that tripartite motif 9 short isoform (TRIM9s) is a tumour suppressor in glioma. In this report, we investigated whether a new peptide derived from TRIM9s, named T9sP, inhibits glioma progression and determined the possible molecular mechanism. METHODS The CCK-8 proliferation assay was performed in LN229 and U251 glioma cells. The scratch-wound assay was used to determine migration of the cells. Apoptosis was assessed by flow cytom· etry using Annexin V-FITC / PI double staining method. The relative protein expression levels were detected by immunoblot analysis. RESULTS The cell penetrating peptide TAT was fused with T9sP to form TAT-T9sP. TAT-T9sP efficiently penetrated through the cell membrane of both LN229 and U251 cells. TAT-T9sP inhibited proliferation and migration and promoted apoptosis of glioma cells. TAT-T9sP activated p38 signaling by upregulating MKK6, and a p38 inhibitor, SB203580, reversed the inhibitory effects of TAT-T9sP on glioma cells. CONCLUSIONS These results indicated the potential of TAT-T9sP for development into a new anti-glioma medicine.

Key words: T9sP; glioma; p38; MKK6

T02-97-0028

IMP3, a potential biomarker for predicting prognosis in various cancers

Dexi Zhou, Jiajie Luan

(Yijishan Hospital of Wannan Medical College)

Abstract: IMP3, an insulin-like growth factor 2 mRNA-binding protein, is correlated with prognosis and metastasis. This meta-analysis investigated the association between IMP3 expression and clinical

outcomes. The primary endpoints were overall survival (OS), lymph node metastasis (LNM) or distant metastasis (DM). The literature collection was conducted by searching electronic databases PubMed, Cochrane Library, OVID, Web of Science, and China National Knowledge Infrastructure (CNKI) (up to May 9, 2017). Strength of association between IMP3 and cancer prognosis was assessed by computing the hazard ratios (HR) with its corresponding 95% confidence interval (CI). According to the inclusion and exclusion criteria, a total of 4477 patients from 23 studies were included in this meta-analysis. In our study, the types of tumors are included as follows: digestive system neoplasms, urinary system tumors, melanoma, cervical squamous cell carcinoma, neuroblastoma, oral squamous cell carcinoma, ovarian cancer, lung adenocarcinoma, hepatocellular carcinoma, and mucoepidermoid carcinoma. The result showed that over expression of IMP3 could predict poor OS in cancer patients, with pooled HR of 0.74, 95% (CI=0.47-1.01, P<0.0001 random-effects model). Likewise, we also found that high IMP3 expression led to lymph node metastasis (OR=2.78, 95% CI:1.53–5.07, P<0.001 random-effects model) and distant metastasis (OR=5.50, 95% CI: 3.24-9.32, P<0.001 fixed-effects model). In conclusion, the present meta-analysis demonstrated that IMP3 might be served as a novel biomarker for predicting prognosis in various cancers.

Key words: IMP3, Prognosis; Lymph node metastasis; Distant metastasis; Meta-analysis

T02-97-0029

Probiotics may be associated with reduced incidence of anxiety in cancer patients: a retrospective cohort study

Ziqi Ye, Yanfang Zhang, Mengfei Du, Shaojia Lu, Qingwei Zhao, Si Yang

(The First Affiliated Hospital, Zhejiang University School of Medicine)

Abstract: OBJECTIVE Studies have shown a correlation between gut microbiota and the development of anxiety and depression. However, there is still a lack of relevant studies in cancer patients. The main objective of this trial was to analyze the relationship between probiotics and the incidence of mental disorders (anxiety and depression) in cancer patients. **METHODS** A total of 190 cancer patients (taking probiotics, as cohort A) and 82 cancer patients (not taking probiotics, as cohort B) with complete medical records at the First Affiliated Hospital, Zhejiang University School of Medicine in May 2020 were enrolled. We followed up these two cohorts of patients by phone in June 2021. Due to reasons, 108 patients in cohort A dropped out, while the number of patients who dropped out in cohort B was 29. Ultimately, the number of patients enrolled in cohort A and B were 82 and 53, respectively. The 17-item Hamilton Depression Scale (HAMD-17) questionnaire was used to measure the depression levels of the patients, and we also used Hamilton Anxiety Scale (HAMA) questionnaire to assess the patients' anxiety levels. **RESULTS** Demographic and clinical characteristics of all cancer patients enrolled in cohort A and B were similar. We performed segmental statistical analysis for patients with HAMA scores <14 (no anxiety) and HAMA scores ≧ 14 (anxiety). Interestingly, the proportion of patients with HAMA scores ≧14 was significantly lower in cohort A compared to cohort B (6.1% and 18.9% in cohort A and B, respectively, P=0.021). However, we performed segmental statistical analysis for patients with HAMD-17 scores ≤7 (no depression) and HAMD-17 scores >7 (depression). The results demonstrated that there was no statistical difference in the proportion of patients with HAMD-17 scores >7 between cohort A and B (30.5% and 26.4% in cohort A and B, respectively, P=0.610). **CONCLUSIONS** Our results suggest that probiotics play a beneficial role in the incidence of anxiety in cancer patients.

However, whether probiotics affect anxiety incidence in cancer patients by improving gut microbiota remains to be studied. Moreover, high-quality RCTs are also needed to further confirm the conclusions of this study.

T02-97-0030

Proteomic analysis of exosomes associated with radiation injury in A549 lung adenocarcinoma cells

Ke Wen[1], Ping-Kun Zhou[1], Chenjun Bai[1], Ping Xu[2]

(1. Department of Radiation Biology, Beijing Key Laboratory for Radiobiology, Beijing Institute of Radiation Medicine, AMMS, Beijing 100850, PR China; 2. State Key Laboratory of Proteomics, National Center for Protein Sciences (Beijing), Beijing Institute of Lifeomics, Beijing 102206, China)

METHOD: (1) Exosomes were extracted from the supernatant of irradiated and non-irradiation A549 lung adenocarcinoma cells by super-centrifugation with gradient velocity. (2) The diameter and concentration of exosome particles were analyzed by nanoparticle tracking analysis, the morphology of exosomes were observed by transmission electron microscopy, and the marker proteins of exosome were identified by western blot analysis. (3) The changes of exosome proteins related to irradiation were analyzed by LC-MS and then analysis of quantitation and function was also performed. (4) The data were bioinformatically analyzed. **RESULTS** (1) The exosomes derived from irradiated A549 cells were determined and confirmed by particle size analysis and concentration measurement, morphological observation under transmission electron microscope and identification of exosome marker protein. (2) TMT (tandem mass tags) quantitative analysis showed that 2257 peptides related to 687 proteins were identified, of which 662 proteins could be quantified. Cluster analysis and Cluster trend analysis were performed on the 662 quantitative proteins, and the results showed that there were 6 major clusters, among which the trend of Custer 5 category was consistent with the expected. We took the protein of Cluster 5 as the differentially expressed proteins associated with irradiation for subsequent functional analysis. (3)The identified proteins were compared in the Human Exosome Database, and 604 of the 662 exosome proteins identified were previously identified. In addition, 58 new exosome proteins were identified. 84 proteins were identified in the Top100 exosomal protein database. (4) The cluster trend analysis of exosomal differentially expressed proteins indicated that they were mainly enriched in biological pathways such as cell migration, extracellular matrix and cell adhesion, and was enriched in Phagosome and ECM-receptor interaction KEGG pathway. **CONCLUSION** The composition of exosome proteins secreted by irradiated lung cancer cell A549 was significantly changed, among which 662 exosome proteins were quantified. Cluster analysis and functional analysis revealed that these pro·teins were involved in a variety of cell biological processes.

Key words: ionizing radiation; exosomes; exosomal proteins; proteomics

T02-97-0031

Cooperative coordination-mediated multi-component self-assembly of "All-in-one" nanospike theranostic nano-platform for mri-guided synergistic therapy against breast cancer

Xudong Fan, Xiaojie Chen, Yue Zhang, Jigang Piao, Fanzhu Li
(Zhejiang Chinese Medical University)

Abstract: Carrier-free multi-component self-assembled nano-systems have attracted widespread attention owing to their easy preparation, high drug-loading efficiency, and excellent therapeutic efficacy. Herein, MnAs-ICG nanospike is generated by self-assembly of indocyanine green (ICG), manganese ions (Mn^{2+}), and arsenate ($AsO4^{3-}$) based on electrostatic and coordination interactions, effectively integrating the bimodal imaging ability of magnetic resonance imaging (MRI) and fluorescence (FL) imaging-guided synergistic therapy of photothermal/ chemo/ chemodynamic therapy within an "all-in-one" theranostic nano-platform. The as-prepared MnAs-ICG nanospike has a uniform size, well-defined nanospike morphology, and impressive loading capacities. The MnAs-ICG nanospike exhibits sensitive responsiveness to the acidic tumor microenvironment with morphological transformation and dimensional variability, enabling deep penetration into tumor tissue and on-demand release of functional therapeutic components. Meanwhile, glutathione (GSH) overexpressed in tumor sites is used to reduce $AsO4^{3-}$ to arsenite ($AsO3^{3-}$), thereby increasing arsenic toxicity from low to high. *In vitro* and *in vivo* results reveal that MnAs-ICG nanospike shows synergistic tumor-killing effect, prolonged blood circulation and increased tumor accumulation compared to their individual components, effectively resulting in synergistic therapy of photothermal/ chemo/ chemodynamic therapy with good biocompatibility and excellent anti-tumor effect. Taken together, this new strategy may hold great promise for rationally engineering multifunctional theranostic nano-platforms for breast cancer treatment.

Key words: nanospike, tumor microenvironment responsive, synergistic therapy, self-assembly, breast cancer

T02-97-0032

MMP2-responsive dual-targeting drug delivery system for valence-controlled arsenic trioxide prodrug delivery against hepatic carcinoma

ke zhang, Chaoqun Li, Jigang Piao, Fanzhu Li
(Zhejiang Chinese Medical University)

Abstract: Arsenic trioxide (ATO) is the active ingredient of traditional Chinese medicine, Arsenic, which has shown excellent therapeutic effects on hepatocellular carcinoma. However, due to its poor tumor distribution and high toxicity, the mass adoption of ATO in clinical applications has been severely impeded. In this study, matrix metalloproteinase 2 (MMP2)-responsive cleaved cell-penetrating peptide (PF) and folate (FA) co-modified liposome coated calcium arsenate nanoparticles (FA / PF-LP-CaAs) were fabricated in virtue of two considerations: (1) the tumor microenvironment characterized by overexpressed MMP2 in extracellular matrix and folate receptor on the cell membrane can enhance drug accumulation and accelerate endocytosis; (2) leveraging on the different toxicity of arsenic in different

valence states, i.e., AsV can be reduced to more toxic AsIII by glutathione in tumor cells. Furthermore, FA/PF-LP-CaAs could be responsively degraded by the mild acidic tumor environment, and was able to escape from lysosomes after endocytosis. More importantly, in light of the in vivo biodistribution and pharmacodynamic studies, the vehicle was able to accumulate in the tumor efficiently, and exhibited fabulous anti-tumor efficacy with minimized side effects when compared with single-modified counterparts. Thus, the novel strategy based on the tumor microenvironment proposed in this work can enhance the tumor-targeting efficiency and intratumor toxicity.

T02-97-0033

The epigenetic regulator BRD4 is involved in cadmium-induced proliferation, migration and invasion of lung cancer cells

Zhonggui Gong[1,2,3], Wenxuan Dong[1,2,3], Kanglei Zhang[1,2,3], Wenjing Liu[1,2,3], Hui Zou[1,2,3], Zongping Liu[1,2,3]

(1. College of Veterinary Medicine, Yangzhou University; 2. Joint International Research Laboratory of Agriculture and Agri-Product Safety of the Ministry of Education of China; 3. Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses)

Abstract: Cadmium (Cd) has been described as a potential human carcinogen, while increasing studies demonstrate that chronic Cd exposure promoted the occurrence and development of lung cancer. Bromodomain-containing 4 (BRD4) is an important epigenetic regulator involved in the pathological processes of many cancers, but its regulatory roles in Cd-triggered lung cancer remain to be clarified. Here, we found that treatment with low-level Cd (2 μmol·L^{-1}, 24 hours) induced the proliferation, migration and invasion of human non-small cell lung cancer cells (A549 cells). Our data indicated that Cd exposure obviously increase the expression level of proto-oncogene protein MYC in A549 cells. Meanwhile, Cd increased the expression level of BRD4 and the acetylation level of H3K27ac, by which enhanced the recruitment of BRD4 to MYC gene promoter regions to mediate the transcription level of MYC. Next, the inhibition of BRD4 by JQ1 (BRD4 inhibitor, 0.5 μmol·L^{-1}, 24 hours) and BRD4 small interfering RNA (siBRD4) were significantly abrogated the recruitment of BRD4, thus decreased the expression level of MYC and inhibited the proliferation, migration and invasion of Cd-exposed A549 cells. In summary, our study suggests that BRD4 is involved in Cd-induced carcinogenic effect by increasing itself binds to acetylated H3K27 and mediating the over expression of MYC in lung cancer cells, therefore, BRD4 could be a potential therapeutic target for Cd-promoted occurrence and development of lung cancer cells.

Key words: cadmium; lung cancer; BRD4; MYC; JQ1

T02-97-0034

Brain metastatic breast cancer exosomes based biomimetic brain-targeted "arsenic prodrug" delivery system for anti-glioma treatment with reduced toxicity and enhanced efficacy

Yue TianXian, Gu WenTing, Lin Xue, Piao Ji-Gang, Li FanZhu

(Zhejiang Chinese Medical University)

Abstract: Glioma is a common primary intracranial malignant tumor. Arsenic trioxide (As2O3), the active ingredient of the traditional Chinese medicine arsenic, has a clear therapeutic effect and mechanism of action against glioma. However, its high toxic side effects, lack of specificity for in vivo distribution, and low efficiency of crossing the blood-brain barrier (BBB) have limited its further clinical application. In this study, a biomimetic brain-targeted "arsenic prodrug" delivery system was constructed via self-assembly of arsenate ($AsO4^{3-}$), exosomes, Mn^{2+}, and ICG, efficiently integrating BBB crossing capability of brain metastatic breast cancer cell exosomes and the affinities between the toxicity, efficacy, and valence of arsenic. The biomimetic delivery system can effectively cross the blood-brain barrier to target gliomas and activate the "arsenic prodrug" at the tumor site in response to the tumor microenvironment, exerting therapeutic effect while avoiding the toxic side effects of arsenic in normal tissues and organs. Meanwhile, in situ released Mn^{2+} and ICG can synergize with the traditional Chinese medicine arsenic for combined treatment of glioma with traditional Chinese medicine-chemodynamic-photothermal therapy and improve the therapeutic efficacy. This research will provide novel strategies and new methods for targeted therapy of brain diseases.

Key words: brain metastatic breast cancer exosomes; biomimetic brain-targeted delivery system; arsenic prodrug

T02-97-0035

EChLESs, an effective fraction isolated from Eupatorium chinense L. , inhibites liver cancer cells by NCOA4-dependent Ferroptoisis

Jing tao Yuan, Zhi Hui Zhu, Bo Yang, Hua Jun Zhao
(Zhejiang Chinese Medical University)

Abstract: Ferroptoisis is a new form of programmed cell death with characteristics like iron-dependent lipid peroxidation, which also occurs in cancer cells including hepatocelluar. It is a potential therapeutic target which has attracted more and more attention. In this study, we proved for the first time that the effective part isolated from Eupatorium chinense L. increased intracellular reactive oxygen species, lipid peroxidation and iron ion level, decreased glutathione level, and caused ferroptoisis of liver cancer cells. Afterwards, the results showed that the EChLESs up regulated NCOA4 aggrevated ferroptoisis in liver cancer cells. In general, our study demonstrated that EChLESs regulated ferritinophagy dependent ferroptoisis and a series of cell damage inhibited the viability of liver cancer cells, and we also found that it had a good synergistic effect with sorafenib, which is hopefully served as an adjuvant drug for liver cancer treanment.

Key words: ferroptoisis; autophagy; NCOA4: ferritinophagy

T02-97-0036

Eupaformosanin induces ferroptosis through ubiquitination of mutant p53 in triple negative breastcancer

Wei Yingying, Yang Bo, Zhao Huajun
(Zhejiang Chinese Medical University)

Abstract: Ferroptosis is a novel form of cell death due to missed control of iron-dependent lipid peroxidation, which is a potential strategy for the survival of triple·negative breast cancer (TNBC). The mutant p53 plays a vital role in the control of cell survival and division under various stresses, including ferroptosis. Here, we showed the eupaformosanin (Eup), a natural compound isolated from Eupatorium cannabinum Linn., exerted its anticancer activity via inducing cell death in TNBC cells. Afterwards, ferroptosis-induced cell death was demonstrated in p53 mutant TNBC cells MDA-MB-231 and MDA-MB-468, accompanied by lipid ROS accumulation, GSH depletion and intracellular iron increase. These effects were blocked by ferroptosis inhibitors Fer-1 and DFO, which indicates that ferroptosis facilitate Eup - induced cell death. Meanwhile, Eup regulated mutant p53 ubiquitination by directly binding to mutant p53. Moreover, mutant p53 signaling participates in Eup-ferroptosis which was rescued when mutant p53 was silent in TNBC cells. Taken together, the data show that the natural compound Eup is a potential therapeutic agent for TNBC by inducing ferroptosis through ubiquitination of mutant p53.

T02-97-0037

The VEGFR2/mTOR/S6K1 signal pathway in the angiogenesis promotion induced by roxarsone

Meng Zhang, Xin Chen, Yumei Zhang

(Yangzhou University)

Abstract: Roxarsone (Rox), an organic arsenic compound, is still widely used as a feed additive in some developing countries. It is reported that Roxarsone has a low absorption rate and mostly excreted with feces, which could poses a risk to human health through environmental and animal food routes. It is demonstrated that Roxarsone have a tumor-promoting and proangiogenic effects. We report that the mechanism of VEGFR2 / mTOR / S6K1 in roxarsone promoting vascular endothelial cell, Matrigel plug and the mouse B16 cell transplantation tumor. ECs were exposed to 0.1-10.0 $\mu mol \cdot L^{-1}$ roxarsone or with 1.0 $\mu mol \cdot L^{-1}$ roxarsone plus Rapamycin or SU5416 to examine their role in cell activity, proliferation, migration, tube formation and the related protein expression. The ECs treatment with Rox or interfereed shRNA targeting Vegfr2 were injected into the mouse subcutaneously to form a rubber plug to examine the role of roxarsone in angiogenesis *in vivo*. The mouse were gavaged with roxarsone or injected intraperitoneally SU5416 after B16 tumor cells injected into the axillary to explore the role of roxarsone in tumor growth. Rox significantly increase the activity, proliferation, migration and tube formation of rat vascular endothelial cells. In addition, 1.0 $\mu mol \cdot L^{-1}$ Rox up-regulate the protein expression of mTOR, p-mTOR, S6K1, p-S6K1 and VEGFR2 and significantly increase the expression of mTOR and S6K1 mRNA. Rapamycin and SU5416 was found significantly combat the effects of 1.0 $\mu mol \cdot L^{-1}$ roxarsone on cell growth. The weight, volume and CD31 expression of B16-F10 xenografts and matrigel plug is up-regulated by 25 mg · kg^{-1} roxarsone. Furthermore, the protein and mRNA levels of mTOR, S6K1 and its phosphorylated proteins in tumor tissue were significantly increased in roxarsone treatment group. SU5416 and shRNA significantly reduced the promoting effect of Rox on tumor and Matrigel plug. In summary, our study indicate that VEGFR2/mTOR/S6K1 signaling plays a regulatory role in roxarsone promoting angiogenesis and enhancing the tumor growth.

Key words: Roxarsone; Angiogenesis; Endothelial cells; Tumor; Matrigel

T03-13-0002

Obesity-induced male mouse reproductive toxicity and mitochondrial autophagy participation

Zhao Jian[a], Zhai Ling-ling[b], Xu Gao-yang[a]

(a. Department of Pharmacology, Shenyang Pharmaceutical University, No. 103 Wenhua Road, Shenhe District, Shenyang,Liaoning 110016, China; b. Department of Maternal and Child Health, School of Public Health, China Medical University, Shenyang, Liaoning 110001, China)

Abstract: OBJECTIVE The purpose of this experiment is to study the effects of obesity on the reproductive system of male mice and the role of autophagy in obesity on the offspring embryos. **METHODS** 48 male C57BL/6J mice aged 4-5 weeks were randomly divided into two groups, 12 in the normal diet group and 36 in the high-fat diet group. After 19 weeks of feeding, the 36 mice in the high-fat diet group gained the first 1 / 3 (DIO mice) and the last 1 / 3 mice (DIO-R mice) were used.The mice of the common feed group was defined as the Control group. A total of 36 male rats in the Control group, DIO group and DIO-R group were paired with 36 female mice of the same strain fed with 8-week-old common feed in a 1: 1 pair and cage mating for 2 weeks, and the vaginal plug or vaginal smear was checked every morning. After mating, all mice were calculated. **RESULTS** At weeks 21, the body weight of the DIO group was significantly higher than that of the control group. The results of organ co·efficient measurement showed that compared with the control group, the relative testis weight of the mice in the DIO group were significantly reduced, and the relative weight of the testicular fat and the rel·ative retroperitoneal fat were significantly increased. The results of sperm quality measurement showed that compared with the control group, the sperm motility of the DIO group was significantly reduced.The reproductive capacity and fetal development of female mice showed that there were no significant dif·ferences in the conception rate, live birth rate, stillbirth rate, absorbed fetal rate, and average corpus lu·teum number of litters among the groups. The results of ELISA showed the levels of testosterone and luteinizing hormone were significantly reduced. Western blot results showed that compared with the control group, the expression levels of LC3 - Ⅱ/LC3-Ⅰ, Beclin 1, Atg 5 protein in the testis tissue of the DIO group were significantly increased, and the expression level of P62 protein was significantly re·duced. Western blot results showed that compared with the control group, the expression level of Pink 1 protein in the testis tissues of the DIO group and DIO-R group decreased significantly, and the ex·pression level of Parkin protein showed a downward trend. **CONCLUSIONS** The fertility rate of male obese mice did not change significantly.Obesity can cause reproductive system damage in male mice. The damage includes changes in hormone levels in male mice, pathological changes in testicular tis·sue and changes in autophagy level. The mechanism of obesity-induced reproductive system damage in male mice may be related to mitochondrial autophagy in testis tissue.

Key words: Obesity; male reproductive; autophagy

Corresponding author: Jian Zhao, E-mail: zll0625@sohu.com

T03-16-0008

Flaxseed powder attenuates non-alcoholic steatohepatitis via modula-tion of gut microbiota and bile acid metabolismthrough gut-liver axis

Chao Yang, Min Wan, Dengfeng Xu, Ligang Yang, Guiju Sun

Abstract: NASH is gradually becoming one of the most common and health-endangering diseases, so it is very important to prevent the occurrence of NASH and prevent NAFL from further developing into NASH.We fed mice the high-fat diet (HFD, 60% fat) for 14 weeks to induce simple hepatic steato·sis, and then fed different doses of flaxseed power 〔low (10%), middle (20%) and high (30%)〕 to the mice for 28 weeks. After the animal experiment, we analyzed fecal BA profiles of the HFD mice, flax·seed-fed (FLA-fed) mice and control mice with normal diet (10% fat) using a targeted metabolomics ap·proach, andwe analyzed the gut microbiota at the same time. We also investigated the mechanistic role of BAs in NASH, and identified whetherthe altered BAs strongly bounding to colonic FXR or TGR5. In present study, we found that 28-week FLA-fed treatment notably alleviated NASH development in NAFL-model mice fed with HFD, the beneficial effects may be attributed to the regulation and improve·ment of gut flora and microbiota related BAs, which then activated intestinal FXR-FGF15 and TGR5-NF - κB pathway. Our data indicates that FLA-fed might be a promising functional food for preventing NASH through regulating microbiomes and BAs.

T03-25-0010

Portable food freshness prediction platform based on colorimetric barcode combinatorics and deep convolutional neural networks

Lingling Guo[a], Ting Wang[b], Zhonghua Wu[c], Jianwu Wang[b], Ming Wang[b], Zequn Cui[b], Shaobo Ji[b], Jianfei Cai[c], Chuanlai Xu[b], Xiaodong Chen[b]

(a. State Key Lab of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, People's Republic of China; b. Innovative Center for Flexible Devices (iFLEX), Max Planck-NTU Joint Lab for Artificial Senses, School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798 Singapore; c. School of Computer Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798 Singapore)

Abstract: Artificial scent screening systems (known as electronic noses, E-nose) have been researched extensively. A portable, automatic, and accurate, real-time E-nose requires both robust cross-reactive sensing and fingerprint pattern recognition. Few E-noses have been commercialized because they suffer from either sensing or pattern recognition issues. Here, we combined cross-reactive colorimetric barcode combinatorics and deep convolutional neural networks (DCNN) to form a meat freshness monitoring system that concurrently provides scent fingerprint and fingerprint recognition. The barcodes-compris·ing of 20 different types of porous nanocomposites of chitosan, dye and cellulose acetate-form scent fingerprints that are identifiable by DCNN. A fully supervised DCNN trained using 3475 labeled barcode images predicted meat freshness with an overall accuracy of 98.5%. Incorporating DCNN into a smart·phone application formed a simple platform for rapid barcode scanning and food freshness identifica·tion in real time. Our system is fast, accurate and non-destructive, enabling consumers and all stake·

holders in the food supply chain to monitor food freshness.

Key words: food freshness, colorimetric barcode combinatorics, deep convolutional neural networks

Corresponding author: Chuanlai Xu, E-mail: xcl@jiangnan.edu.cn; Xiaodong Chen, E-mail: chenxd@ntu.edu.sg; Jianfei Cai, E-mail: Jianfei.Cai@monash.edu

T03-33-0007

Validation, optimization, andapplication of the zebrafish developmental toxicity assay for pharmaceuticals

Zhang Yong

(Hunter Biotechnology, Inc.)

Abstract: The zebrafish as an alternative animal model for developmental toxicity testing has been extensively investigated, but its assay protocol was not harmonized yet. This study has validated and optimized the zebrafish developmental toxicity assay previously reported by multiple inter-laboratory studies in the United States and Europe. In this study, using this classical protocol, of 31 ICH-positive compounds, 23 compounds (74.2%) were teratogenic in zebrafish, five had false-negative results, and three were neither teratogenic nor non-teratogenic according to the protocol standard; of 14 ICH-negative compounds, 12 compounds (85.7%) were non-teratogenic in zebrafish and two had false-positive results. After we added an additional TI value in the zebrafish treated with testing compounds at 2 dpf along with the original 5 dpf, proposed a new category as the uncategorized compounds for those TI values smaller than the cutoff both at 2 dpf and 5 dpf but inducing toxic phenotypes, refined the testing concentration ranges, and optimized the TI cut-off value from 10 to 3 for compounds with refined testing concentrations, this optimized zebrafish developmental assay reached 90.3% sensitivity (28/31 positive compounds were teratogenic in zebrafish) and 88.9% (40/45) overall predictability. Our results from this study strongly support the use of zebrafish as an alternative in vivo method for screening and assessing the teratogenicity of candidate drugs for regulatory acceptance.

Key words: Zebrafish; Developmental toxicity; Teratogenicity; Malformation

Corresponding author: Zhang Yong, E-mail: zy@zhunter.com

T03-39-0006

Puerarin alleviates cadmium-induced mitochondrial loss by inhibiting PINK1-Parkin and Nix-mediated mitophagy in rat cortical neurons

Wen Shuang-quan[a,b,c], Yuan Yan[a,b,c], Liu Zong-ping[a,b,c]

(a. College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, Jiangsu, China; b. Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou 225009, Jiangsu, China; c. Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China, Yangzhou University, Yangzhou 225009, China)

Abstract: Cadmium (Cd) has well-known central nervous system toxicity, and mitochondria are direct targets of Cd-induced neuronal toxicity. However, how Cd induces mitochondrial loss in terms of its neurotoxic effects remains unknown. Puerarin, an isoflavone extracted from kudzu root, can cross

the blood – brain barrier and exert protective effects in nervous system disease. The purpose of the study was to determine the mechanism of Cd-induced mitochondrial loss and the protective role of puerarin in rat cortical neurons. The results indicated that Cd induced mitochondrial loss by activating mitophagy mediated by the PTEN-induced putative kinase protein 1 (PINK1)-E3 ubiquitin ligase (Parkin) and Nip3-like protein X (Nix) pathways in rat cortical neurons. Puerarin improved the Cd-induced decrease in mitochondrial membrane potential (MMP) *in vitro*, and blocked PINK1 – Parkin and Nix-mediated mitophagy, inhibiting Cd-induced mitochondrial loss in rat cortical neurons *in vitro* and *in vivo*. In summary, our data clearly indicated that puerarin protects rat cortical neurons against Cd-induced neurotoxicity by ameliorating mitochondrial damage, inhibiting mitophagy-mediated mitochondrial loss. Puerarin appears to have great potential as a neuroprotective agent.

Key words: puerarin; cadmium; mitochondrial loss; mitophagy; neuron

Corresponding author: Yuan Yan, E-mail: yuanyan@yzu.edu.cn; Liu Zong-ping, E-mail: liuzongping@yzu.edu.cn

T03-41-0001

Arachidonic acid facilitateM2 macrophage polarization through PPARG inhibition

Xu Miao[a], Wang Xiao hong[b], Zhang Li-shi[a]*, Yang Hui[b]*, Jia Xu-dong[b]*

(a. Department of Nutrition, food safety and toxicology, West China School of Public Health, Sichuan University, No.16, section 3, Renmin South Road, Chengdu, Sichuan 610041; b. NHC Key Laboratory of Food Safety Risk Assessment, China National Centerfor Food Safety Risk Assessment, No.7 PanjiayuanNanli, Beijing 100021)

Abstract: Macrophages polarization was a context-dependent process which was precisely controlled by sometranscription factors include PPARγ. The phenotype shift between classically activated macrophages (M1) and alternatively activated macrophages (M2) always accompanied by metabolism reprogramming. Although, the relationship between PPARγand lipid metabolism has been widely accepted, how their interactions contribute to macrophage polarization has not been clarified clearly. We examined the major differences in lipid profiles between M1 and M2. Joint pathway analysis of transcriptomics and lipid metabolomics revealed that arachidonic acid metabolism was the most different lipid metabolism pathway between M1 and M2 macrophages. Arachidonic acid and its important metabolites (lipoxins, prostaglandins) were higher in M2 macrophages. We next investigated macrophage polarization in THP-1 derived macrophages. Interestingly, PPARγactivated by its agonist Rosiglitazone significantly inhibitedM2 polarization figured by CD209 expression, and PPARγinhibited by its specific inhibitor T0070907 promoted M2 polarization. Extra addition of arachidonic acid also promoted M2 polarization, which was similar with T0070907 but opposed to Rosiglitazone. Further, Rosiglitazone pre-incubation blunted arachidonic acid mediated M2 polarization, while T0070907pre-incubation enhanced its effect dramatically. In addition, pretreatment withPD146176 or Indomethacin, important enzyme inhibitors of arachidonic acid metabolism, synergistically increased CD209 along with arachidonic acid, suggesting that elevation of arachidonic acid facilitated M2 polarization. In this study, we elucidated that arachidonic acid interacted with PPARγand accelerated macrophage polarized to M2 through PPARγinhibition. To our knowledge, this is the first time to reveal a role of PPARγin inhibiting M2 macrophage polarization and a role of arachidonic acid in facilitating M2 macrophage polarization through

PPAR γ inhibition, indicating potentials of dietary lipids on immune suppression.

Key words: PPARG; arachidonic acid; metabolism; macrophages polarization

Corresponding author: Yang Hui, E-mail: yanghui@cfsa.net.cn

T03-46-0005

Combination prevents acute liver injury of Houttuynia cordata polysaccharide and Lactobacillus plantarum by regulating gut microbiota in mice

Xu Xiao-wei, Wang Meng-qi, Liu Shan-ji, Zhao Yu, Xu Heng-yi

(State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China)

Abstract: Polysaccharides and probiotics can play an excellent role in the treatment of liver dis· ease throughregulating the gut microbiota. Recently, the combined therapeutic effect of probiotics and polysaccharides has attracted the attention of researchers. Here, *Houttuynia cordata* polysaccharide (HCP) from *Houttuynia cordata* hay was used in combination with *Lactobacillus plantarum* was used to prevent acute liver injury (ALI) induced by CCl4 in miceto explore the effect on the regulation of gut microbi·ota. The results showed that HCP combined with *L. plantarum* significantly alleviated oxidative stress injury and inflammation injury in the liver of mice with beneficial changes in gut flora composition. Corre·lation analysis showed that the expression levels of *Nrf2* and TLR4 / NF - κB were correlated with the abundance of beneficial and pro-inflammatory bacteria in the gut. HCP combined with L. plantarum might mediate the gut-liver axis to regulate the gut microbiota, activate the antioxidant pathway and inhibit the inflammatory response, thereby alleviating CCl_4-induced ALI. This study provided a new perspec· tive for polysaccharides combined with probiotics to treat liver disease by regulating gut flora.

Key words: *Houttuynia cordata* polysaccharide; Lactobacillus plantarum; Gut microbiota; Oxida· tive stress; Inflammation

Corresponding author: Xu Heng-yi, E-mail: kidyxu@163.com, E-mail: HengyiXu@ncu.edu.cn

T03-52-0012

Gold nanoparticle-based immunosensor assay for the rapid detection of spinosad and spinetoram in rice, tea, and onion samples

Lei Xian-lu[a,b], Xu Xin-xin[a,b], Liu Li-qiang[a,b], Kuang Hua[a,b], Xu Li-guang[a,b], Xu Chuan-lai[a,b]

(a. State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, People′ s Republic of China; b. International Joint Research Laboratory for Biointerface and Biodetection, and School of Food Science and Technology, Jiangnan University, Wuxi, People′ s Republic of China)

Abstract: Spinosad (SPI) and spinetoram (Et-SPI) are currently one of the most popular new insecticides because of their high efficiency and low toxicity. However, excessive residues in food still pose a potential risk to public health. Therefore, it is necessary to strengthen residue monitoring of the drugs based on a simple and repaid method. In this study, a highly sensitive mAb (6G9) against SPI and Et-SPI was prepared using the hapten SPI-HS and used to develop a colloidal gold nanoparticle-

based immunochromatographic strip for the detection of SPI and Et-SPI in samples. The quantitative range of the developed strip for SPI and Et-SPI were 7.41–1494 ng·g^{-1} and 16.31–4344 ng·g^{-1} in rice, 35.12–703.6 ng·g^{-1} and 86.43–1754 ng/g in tea, and 10.27–343.4 ng·g^{-1} and 22.73–937.2 ng·g^{-1} in onions, respectively. In addition, recovery rates ranged from 85.7% to 112.7% with a coefficient of variation <9.5%. Therefore, our developed method was sensitive and valid as a quantitative tool for the rapid screening of SPI and Et-SPI in foods.

Key words: antibody; immunochromatographic; strip; spinetoram; spinosad

Corresponding author: Kuang Hua, E-mail: kuangh@jiangnan. edu. cn; Xu Chuan-lai, E-mail: xcl@jiangnan.edu.cn

T03-67-0009

Gender differences in metabolism and blood coagulation among rats after a fungicide sodium dehydroacetate exposure

Xin Chen, Cunkai Wang, Meng Zhang, Binlin Chen, Zhiqiang Zhou, Yumei Zhang

(College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu, China)

Abstract: Sodium dehydroacetate (Na-DHA), a fungicide used in food, feed, and medicine, has been found to cause coagulation aberration accompanied by the inhibition of vitamin K epoxide reductase (VKOR) in the liver in rats. VKOR complex 1 (VKORC1) and VKORC1 like-1 (VKORC1L1) are two homologous VKOR proteins. Little information is available on the effect of Na-DHA on VKORC1L1 in the liver or VKORC1/VKORC1L1 in extrahepatic tissue and gender differences in Na-DHA metabolism. Prothrombin time (PT) and activated partial thromboplastin time (APTT) of the animals were determined after the administration of 200 mg · kg^{-1} Na-DHA by gavage. Na-DHA concentrations in blood and tissues were determined using high-performance liquid chromatography, and VKORC1 / VKORC1L1 expression was evaluated by RT-PCR and western blot. Furthermore, cytochrome P450 (CYP) activity was investigated using the cocktail probe method. Significant inhibition of VKORC1 or VKORC1L1 in tissues, as well as prolonged PT and APTT, were observed. Serum or tissue Na-DHA concentrations were significantly higher in females than in males; specifically, the concentrations were 2- to 3-fold higher in tissues and 1.1- to 1.4-fold higher in serum than the corresponding concentration in males. The pharmacokinetic parameters (HL, Cmax, AUC0~24 h, and MRT0~24 h) of Na-DHA in female rats were significantly higher than those in male rats. Na-DHA exhibited an inductive effect on CYP1A2, 2D1/2, and 3A1/2 activities by changing the main pharmacokinetic parameters of four probe drugs in male rats. However, no significant change in CYP2E1 activity was found. There were gender differences in the metabolism and coagulation in rats exposed to Na-DHA. The lower metabolism and higher blood Na-DHA concentration in females may be the reasons for higher coagulation sensitivity in female rats.

Key words: Sodium dehydroacetate; Gender difference; CYP; Metabolism; VKOR; Coagulation

Corresponding author: Yumei Zhang, E-mail: ymzhnet@sina.com

T03-78-0003

Lanthanum induced neurotoxicity on C. elegans

Gao-chao Han, Hai-ming Jing, Wen-jing Zhang, Nan Zhang, Zi-nan Li, Guo-yan Zhang, Shan Gao, Jun-yu Ning, Guo-jun Li

(Beijing Center for Disease Prevention and Control/Beijing Center of Preventive Medicine Research, Beijing Key Laboratory of Diagnostic and Traceability Technologies for Food Poisoning, Beijing 100013)

Abstract: As rare earth elements (REEs) are widely used in industry, agriculture, biomedical, the number of studies focusing on their biological properties is increasing. However, environmental and health risks of REEs are still poorly understood. While some studies have attempted to evaluate the toxicity of REEs towards animals, the in vivo toxic effects on the nervous system and the molecular mechanisms are unclear. In this study, neurotoxic effects and the underlying mechanisms of lanthanum(III) nitrate hexahydrate ($La(NO_3)_3 \cdot 6H_2O$) were evaluated using L1 and L4 stages Caenorhabditis elegans (C. elegans) as the assay system. Median lethal concentrations (48 h) of $La(NO_3)_3 \cdot 6H_2O$ were 93.163 and 648.0 mg · L^{-1} for L1 and L4 stages nematodes, respectively. Our results showed that $La(NO_3)_3 \cdot 6H_2O$ induced growth inhibition and behavioral defects, including alterations to body length, body width, body bending, head thrashing and pharyngeal pumping on C. elegans at L1 and L4 stages, while the former nematodes are more sensitive to the toxicity of lanthanum than the latter nematodes. Using transgenic nematodes, we found $La(NO_3)_3 \cdot 6H_2O$ resulted in loss of dendrite and soma of neurons in L1 and L4 stages C. elegans; as well as α-synuclein aggregation in L1 stage C. elegans. It indicates that La can lead to damage of dopaminergic and GABAergic neurons. Further investigations suggested that $La(NO_3)_3 \cdot 6H_2O$ exposure inhibited or activated the transporters and receptors of glutamate, serotonin and dopamine in C. elegans at the genetic level within 48 h. Moreover, excessive reactive oxygen species (ROS) generation was observed in the La-treated L4 stage C. elegans. Our data suggest that exposure to lanthanum caused neurotoxicity of inducing behavioral deficits and neural damage. These findings provide useful information for understanding neurotoxicity mechanism and health risk of REE materials.

Key words: Caenorhabditis elegans; lanthanum; neurotoxicity; real-time PCR

Corresponding author: Guo-jun Li, E-mail address: ligj@bjcdc.org

T03-92-0013

Bioaccessibility and bioavailability adjusted dietary exposure of cadmium for local residents from a high-level environmental cadmium region

Xu Fei-fei[a], Li Yue-qi[b], Lai Yue-fei[a], Lin Jun[a], Chi Hui-qin[a], Wang Yan[b], Li Zi-yin[a], Wu Wei-liang[a], Yang Xing-fen[a]

(a. Food Safety and Health Research Center, Guangdong Provincial Key Laboratory of Tropical Disease Research, Guangdong-Hongkong-Macao Joint Laboratory for Contaminants Exposure and Health, School of Public Health, Southern Medical University, Guangzhou 510515, P. R. China; b. Department of Preventive Medicine, Faculty of Medical Science, Jinan University, Guangzhou 510632, P. R. China)

Abstract: The critical health risks caused by cadmium (Cd) dietary exposure are commonly assessed employing the detected concentrations of Cd in foods. Differently, the bioaccessibility and bioavail·ability of Cd in major local foodstuffs were introduced in this study for dietary exposure of local resi·dents from a high-level environmental Cd region. The results showed that a part of Cd released into diges·tive juice after in vitro digestion with the bioaccessibility of 20%~63% for rice and 3%~32% for leafy vegetables, and the released portion was also partially absorbed by CaCO-2 cell with the bioavailability of 2%~21% for rice and 0.2%~13% for leafy vegetables. The findings obtained from the toxicokinetic model showed that the predicted urinary Cd values from estimated daily intake (EDI) of Cd, which taken the bioaccessibility and bioavailability into account, were closer to the actual measured values, and the EDIs were considerably lower than the acceptable daily intake. It suggested that dietary Cd exposure adjusted with its bioaccessibility and bioavailability should be more precise. The key issue should be concerned in our study appeals that a potential health risk could not be neglected for those people with a high consumption of rice from high-level zone.

Key words: Gastrointestinal simulated model; Bioaccessibility; Bioavailability; Local food; Urinary cadmium

Corresponding author: Yang Xing-fen, E-mail: yangalice79@smu.edu.cn

T03-96-0011

Ultrasensitive detection of seventeen chemicals simultaneously using paper-based sensors

Zhongxing Wang, Chuanlai Xu*

(International Joint Research Laboratory for Biointerface and Biodetection, andSchool of Food Science and Technology, Jiangnan University, Wuxi, People's Republic of China)

Abstract: Today, many steroid hormones and nonsteroid hormones are widely used in animal feed. The advantages of the hormones for accelerating growth, increasing yield, and preventing or treating disease, however, are accompanied by an increasing risk from veterinary drug and animal feed residues. Hormones assimilated into the human body from the food chain can produce a range of health problems such as developmental disorders and birth defects. Moreover, most of them are poten·tial human carcinogens. A previous study has reported that multiple hormones represent various ef·fects on many cancers. Therefore, to eliminate the illicit use of hormones and resist the diseases caused by hormones, simultaneous detection of these multiple hormones is necessary. In this study, we developed an ultrasensitive gold nanoparticle-based multicomponent lateral-flow strip assay for the simultaneous detection of seventeen hormone drugs from three classes: nandrolone (NR) and itsana·logues, dexamethasone (DEX) and its analogues, and hexestrol (HES) and its analogues. The antibod·ies and coating antigens were selected for the assay and the multi-immunochromatographic strip (multi-ICS) exhibited a very low limit of detection (LOD). Quantitative results were obtained using a strip scan reader, giving LOD values of 0.06-4.85 ng mL1 for NR and its analogues, 0.005-2.85 ng mL1 for DEX and its analogues, and 0.03-2.14 ng mL1 for HES and its analogues. The recovery rates from milk samples were 85.8-93.3% for NR, 78.8-87.5% for DEX, and 89.4-99.4% for HES. This multi-ICS assay therefore provides a useful tool for on-site detection and rapid initial screening of hormone drugs in biological samples.

Key words: Immunochromatographic strip; Simultaneous detection; hormone; milk

T03-96-0014

Residue levels and profiles of PBDEs, PCBs and OCPs in sediments and fish tissues of river nile at greater cairo, egypt

Ahmed ElKady[1], Nguyen Minh Tue[2], Tatsuya Kunisue[2]
(1. National Research Centre, Food Toxicology & Contaminants, Egypt; 2. Ehime University, Center for Marine Environmental Studies, Japan)

Abstract: Research on Brominated Flame Retardants (BFRs) is a new subject in Egypt, where there is very limited information about it. So, residue profiles of polybrominated diphenyl ethers (PB·DEs), as well as PCBs and OCPs, were studied in 20 sediments and 12 tilapia fish to assess and eluci·date their spatial distribution in the aquatic environment of River Nile at Greater Cairo. GC/MS (Agilent 5975C) and MS/MS (Agilent 7000) have been used to determine PBDEs/PCBs and OCPs, respective·ly. For PBDEs, the concentrations of Σ 44 PBDEs in sediments ranged from 150 to 17, 000 pg/ g dw. BDE-209 was detected in all sediments followed by BDEs-206, 207, and 208 with similar detection fre·quencies (79%). The vast majority of PBDEs was BDE-209, which constituted 65 to 100% of total PB·DEs in all sediments. For tilapia fish, the concentrations in the muscle ranged from 12 to 200 pg/g ww. BDE-47 was detected in all the tissues and constituted 65 to 100% of ΣPBDEs. The detection frequen·cies of BDEs-100, - 154, and - 28 were 75, 67, 58%, respectively. The concentrations of Σ 62 PCBs ranged from 56 to 400,000 pg/g dw and from 150 to 1,200 pg/g ww for sediment and fish samples, re·spectively. Relatively high concentrations of ΣDDTs and ΣHCHs were found in sediment, particularly at Al-Rahawy drain station, which has uncontrolled inputs of untreated domestic, agricultural and indus·trial wastes. DDT levels were between 13 and 130,000 pg/g dw in sediments and between 220 and 3, 100 pg/g ww in fish. Ratios of DDT to its metabolites suggest that the source of DDT is from past usage of technical DDT in the regions surrounding the investigated area. HCHs were detected in almost all sediment and fish samples with concentrations ranged from 306 to 3,185 pg/g dw and from 14 to 92 pg/ g ww, respectively. The effect range low (ERL) for ΣDDTs (1.58 ng/g dw) in three out of twenty sedi·ments exceeded sediment quality guidelines. Sediment in front of Al-Rahawy drain exceeded the proba·ble effect level (PEL) for ΣPCBs, DDE, and DDD isomers 2, 4′ and 4, 4′ by 1.44, 17, and 1.26 times. The levels of ΣDDTs and ΣPCBs in all fish samples were much lower than tolerance levels set by US EPA.

Key words: PBDEs, PCBs, OCPs, sediment, fish, River Nile

T03-96-0015

Safety evaluation and anti-inflammatory effect of Lactobacillus paracasei PS23

Tai-Ying Chen[1,2], Shu-Mei Chang[1], Jaw-Jou Kang[1], Po-Lin Liao[1]
(1. National Yang Ming Chiao Tung University; 2. Taiwan University)

Abstract: Probiotics are extensively available to consumers; However, the use of probiotics may not always be safe and there are few reports on their side effects, including those of Lactobacillus. Lac·tobacillus paracasei strain PS23TM isolated from spontaneously fermented mustard greens in Taiwan has recently been reported to exhibit probiotic properties. In this study, we aimed to assess the safety

of strain PS23TM for use in humans via examining genotoxic and oral toxic effects using *in vitro* and *in vivo* testing. Five strains of Salmonella typhimurium were evaluated by the Ames test; no signs of increased reverse mutation were observed following exposure to PS23TM. Additional testing of Chinese hamster ovary cells exposed to PS23TM revealed that the frequency of chromosomal aberra·tions *in vitro* had not increased. PS23TM treatment also did not affect the proportion of immature to total erythrocytes or the number of micronuclei in the immature erythrocytes of ICR mice. Moreover, follow·ing a 28-day study involving repeated oral dose toxicity tests (4000, 400, and 40 mg·kg^{-1} body weight) utilizing an ICR mouse model, no observable adverse level was found at any of the doses. PS23TM was sensitive to antibiotics; however, genes related to the production of biogenic amines were absent. While further research is required, these toxicological assessments suggest that PS23TM could be safe for human consumption.

Key words: 28-day repeated oral dose toxicity; genotoxicity test; Lactobacillus paracasei PS23TM; NOAEL

T03-96-0016

Bacteriostasis test and bioactive components of secondary metabolites were analysis from Monascus ruber

Qi Chen, Lijun Ge, Xiwen Jia, Yue Wang, Shuyuan Liu, Jun Wan

(Zhejiang Chinese Medical University)

Abstract: OBJECTIVE To study the bioactive components and functions of secondary metabo·lites of Monascus ruber Mr-1. **METHODS** The content of secondary metabolites were determined by HPLC. The active components were separated and purified by silica gel column and Sephadex LH-20. The structures of the compounds were analyzed by NMR and HR-ESI-MS. and the antioxidant, antibac·terial activities were determined *in vitro*. **RESULT** There were 16 kinds of amino acids and γ-aminobu·tyric acid, ergosterol and monacolin K were isolated. Four new active compounds were identified as three flavonoids, Luteolin (1), Hesperetin (2), Glycoitin (3), and one terpenoid, Ursolic acid (4). Com·pounds 1, 2 and 4 were isolated from Monascus for the first time. Compound 1 had strong scavenging ability to ABTS+ , DPPH and OH－, with IC50 of 13.36, 8.74 and 32.75 μg·mL^{-1}, respectively; Com·pound 4 showed moderate antibacterial activity against Staphylococcus aureus and Listeria monocyto·genes, and the diameter of inhibition zone was 13.4 and 11.9 mm, respectively. **CONCLUSION** The metabolites of Monascus ruber Mr-1 has the potential to be developed into functional food materials.

Key words: Monascus ruber Mr-1; Antibacterial; Antioxidant

T03-96-0017

An immunochromatographic sensor for ultrasensitive and direct detection of histamine in fish

Zeng Lu

(Jiangnan University)

Abstract: To ensure food quality and prevent histamine (HA) toxicity, a rapid and direct method of detecting HA is required. In this work, we prepared a monoclonal antibody (mAb) against HA using a

hapten produced by the introduction of a phenyl-containing linker. The novel mAb exhibited high sensitivity against HA as determined by ELISA, with a half-maximal inhibitory concentration of 21.51 ng/mL. A gold nanoparticle-based immunosensor was fabricated for rapid detection of HA in fish samples. After optimizing the immunosensor, a visual limit of detection (LOD) and a calculated LOD were 0.25 mg / kg and 10.48 μg/kg for HA, respectively. Recovery rates from the spiked fish samples ranged from 87.33% to 104.67% with the coefficient of variation below 10.82%. Concurrently, the whole process in testing real sample was completed within 15 min, and all results were well confirmed and comparable by liquid chromatography-mass spectrometry and the commercial test strip. These data revealed that the proposed immunosensor could be used as a monitoring tool for the rapid and direct detection of HA in fish samples.

Key words: Histamine; monoclonal antibody; gold nanoparticle-based immunochromatographic assay

T03-96-0018

An ultrasensitive colloidal gold immunosensor to simultaneously detect 12 beta (2)-adrenergic agonists

Xu Xiao-xin

(Jiangnan University)

Abstract: In this study, we first prepared a selective monoclonal antibody against 12 beta (2) -adrenergic agonists (Salbutamol, Clenbuterol, Brombuterol, Clenpenterol, Mabuterol, Carbuterol, Cimbuterol, Mapenterol, Pirbuterol, Terbutaline, Cimaterol, and Clenproperol). Then three haptens were designed and derived, among which, haptenS3 used the amino group of the salbutamol analog to derive a carboxyl group containing a spacer, which is unique to this study. The half-maximal inhibitory concentration (IC50) values were 0.35 ng / mL (Salbutamol), 0.42 ng / mL (Clenbuterol), 0.78 ng / mL (Brombuterol), 0.88 ng/mL (Clenpenterol), 1.34 ng/mL (Mabuterol), 1.38 ng/mL (Carbuterol), 1.71 ng/mL (Cimbuterol), 2.24 ng/mL (Mapenterol), 2.25 ng/mL (Pirbuterol), 2.27 ng/mL (Terbutaline), 3.49 ng/mL (Cimaterol), and 4.89 ng / mL (Clenproperol). We further developed a monoclonal antibody-based colloidal gold immunochromatographic test strip to detect 12 beta (2) - adrenergic agonists in swine urine and lamb samples. The immunochromatographic method developed in this study is a suitable tool for the on-site rapid detection and screening of beta (2)- adrenergic agonists in swine urine and lamb samples.

Key words: Beta (2)-adrenergic agonists; Colony selective monoclonal antibody; Cross reaction; Collo

T03-96-0019

Comparison of the functional effect of VVC protein synthesized by prokaryotic expression system under different purification strategies

Liu Jian-fei[a,b], Zhan Chen[a], Qin Ke-wei[a,b], Wu Cheng-lin[a,b], Zhang Chang-jian[a,b], Zhou Li-jun[a,b]*

[a. Central Laboratory, b. College of Otolaryngology Head and Neck Surgery, the Sixth Medical Centre, Chinese PLA (People's Liberation Army) General Hospital, Beijing, China]

Abstract: Background Vibrio vulnificus is a species of pathogenic bacterium that causes human foodborne and wound inflammation, leading to rapid and severe progress. As one of the important toxicity factors, VVC dominates the infection progress. To better understanding its mechanism, mass-produced VVC protein with stable biological activity is the basic and critical part. **OBJECTIVE** To screen a stable preparation method that is satisfied with the mass requirement and high biological activity of VVC protein. Methods Targeted gene sequence was amplificated. A prokaryotic expression method was performed. Several renaturation methods including dialysis and dilution refolding were used. A hemolytic activity assay was used to verify the biological activity. Cell viability assay was used to detect the in vitro cytotoxicity. Survival analysis was performed, and hematoxylin-eosin staining assays on the infected mice′ s skin tissue were used to evaluate the *in vivo* virulence. **RESULTS** Ni-chelating affinity chromatography with SDS-PAGE analysis showed a clear VVC expression with high purification efficiency. VVC protein using dialysis renaturation showed no or little hemolytic activity; VVC protein obtained and performed dilution refolding renaturation was proved to have an evident hemolytic activity. and the activity showed a close positive correlation with time and concentration effects. Comparing to VVC protein renaturing with dilution refolding method, VVC with dialysis renaturation showed little cytotoxicity ability to GES-1 cell regardless of the salinity buffer concentration. Regarding VVC protein renaturing with dilution refolding method, cell viability results showed VVC showed broad-spectrum cytotoxicity to different cell types with obvious preference. Comparing the virulence in vivo between VVC protein renaturing with dialysis renaturation and dilution refolding method, VVC protein renaturing with dilution refolding method showed much stronger virulence to mice, causing coarse disorderly, diarrhea and mental fatigue, and HE staining results confirmed distinct inflammatory reaction, which was not showed under the interference of VVC protein prepared with dialysis renaturation. **CONCLUSION** Via comparative study, our research established a stable and efficient prokaryotic expression, purification, and renaturation method for VVC protein quantity production. This study revealed the importance of biological activity after fusion protein was expressed, which laid the material foundation for the further study of the VVC mechanism.

Key words: Vibrio vulnificus; VVC; biological activity; inflammatory

Corresponding author: Zhou Li-jun, E-mail: hzzhoulj@126.com

T04-16-0002

Correlation analysis between virulence genes distribution and phylo-groups of *Escherichia coli* in dairy breedings

TANG Min-jia, ZHANG Xue-jing, HE Zhuo-lin, PU Wan-xia*

(Key Laboratory of New Animal Drug Project, Gansu Province/Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture and Rural Affairs/ Lanzhou Institute of Husbandry and Pharmaceutical Sciences of Chinese Academy of Agriculture Sciences, Lanzhou 730050, China)

Abstract: To provide a reference for the research and prevention of Escherichia coli (E. coli), the phylogenetic grouping and virulence genes of E. coli which isolated from two large-scale dairy farms in Gansu Province were identified, and the correlation between phylo-groups and virulence genes of E. coli was analyzed. The phylogenetic grouping and virulence genes detecting of the E. coli strains were carried out by PCR. 281 E. coli strains were assigned to eight phylo-groups, and the phylo-groups B1,

A, E, F, D, C, B2 and clades I or II were 51.96 %, 28.47 %, 11.39 %, 2.85 %, 1.78 %, 1.07 %, 0.71 % and 0.36 %, respectively. The detection rates of *ompA*, *ibeB*, *ompT*, *iroN*, *traT*, *iucD*, *fyuA* and *irp2* were 100%, 98.58%, 76.16%, 71.53%, 68.33%, 64.06%, 30.96% and 30.25%, respectively. There were significant differences in the distribution of some virulence genes in different years and dairy breedings. The strains carrying all identified virulence genes were mainly distributed in B1 and B2 phylo-groups and fecal-soil sourced samples. The distribution of traT in 2017 was significantly lower than that in 2018 and 2019. The distribution of *traT* and *iroN* in fecal-soil sourced isolates was higher than that of sewage sourced isolates. Phylo-group A was negatively correlated with *ompT* and *traT*, whereas phylo-group B1 was positively correlated with *ompT*, *irp2*, *fyuA*, and *iucD*. Phylo-group E had a negative correla·tion with *irp2*, *fyuA*, and *iucD*, and a positive correlation with the *iroN*. Phylo-group B1 was more preva·lent than other phylo-groups. There were significant differences in the distribution of some virulence genes in different years and breedings. The distribution of some virulence genes was associated with phylo-groups.

Key words: *Escherichia coli*; dairy breedings; virulence genes; phylogenetic grouping

Corresponding author: PU Wan-xia, E-mail: puwanxia@caas.cn

T04-33-0003

Subinhibitory concentration of colistin promote the conjugative transfer rate of *mcr-1* and bla_{NDM-1} positive plasmid

Xiao Xia, Zeng Fu-xing, Wang Zhi-qiang*

(College of Veterinary Medicine, Yangzhou University, Yangzhou, China)

Abstract: Antibiotic resistance (AMR) has been a growing global threat to public health security. Horizontal gene transfer (HGT) plays a major role in spearing of antibiotic resistance genes (ARGs) in the environment. To investigate the potential role of colistin in facilitating the dissemination of ARGs through plasmid conjugation, an *in vitro* mating model was established. *Escherichia coli* (*E. coli*) DH5α carrying plasmid RP4-7 was the donor strain, and *E. coli* J53 was the recipient strain, respectively. Mechanisms of the HGT promoted by colistin were unveiled by detecting oxidative stress, cell mem·brane permeability, and the expression level of the corresponding genes. Results: Exposure to Sub-MIC concentrations of colistin (1/8MIC) significantly stimulated the conjugation transfer rate of plasmid with an increasing of conjugation frequency of 38 times compared with the blank control. Meanwhile, when exposure to 1/8MIC colistin, the conjugation frequency of wide-type IncI2 plasmid bearing impor·tant resistance gene *mcr-1* and *IncX3* plasmid bearing bla_{NDM-5} was also considerably increased. Through scanning electron microscopy, the cell membrane was shrunken after colistin exposure. The cell membrane permeability was increased through PI and NPN fluorescent probe. The expression lev·el of the outer membrane protein (*ompF* and *ompC*) were increased. The expression of the global regu·latory genes (*korA*, *korB*, *trbA*) were inhibited when exposure at 1/8 MIC colistin. And the expression of mating pair formation gene (*trbBp*) was promote. Colistin can promote plasmid transfer rate at sub-in·hibitory concentrations. The possible machanisms are that the morphology of the cell membrane was changed after the colistin exposure; the permeability of the bacterial cell membrane was increased; the mating pairing machine was producted.

Key words: Colistin; Plasmid; conjugation; Horizontal gene transfer

Corresponding author: Wang Zhi-qiang, E-mail: zqwang@yzu.edu.cn

T04-62-0004

Effects of florfenicol combined with copper on biofilm formation and nitrogen regulatory gene expression in rhizobia

Mei Wang, Tong Zhou, Yi Liang, Fulin Li, Yongxue Sun*

(a. The Guangdong Provincial Key Laboratory of Veterinary Pharmaceutics Development and Safety Evaluation, South China Agricultural University, Guangzhou, China; b. National Laboratory of Safety Evaluation (Environmental Assessment) of Veterinary Drugs, South China Agricultural University, Guangzhou, China; c. National Risk Assessment Laboratory for Antimicrobial Resistance of Animal Original Bacteria, South China Agricultural University, 483 Wushan Road, Guangzhou 510642, China)

Abstract: Nitrogen fixation mediated by nitrogen-fixing bacteria is the main nitrogen input pathway in soil ecosystem. Antibiotics and heavy metal residues may affect nitrogen-fixing bacteria. In this study, the rhizobia were isolated from the rhizosphere and identified as Rhizobium pusense strain RpEC2071 by morphological observation, carbon source utilization, 16S rDNA sequencing and whole genome sequencing. Florfenicol and copper were selected respectively to evaluate their effects on rhizobia. Blank group (0 $\mu g \cdot mL^{-1}$), florfenicol group (40 $\mu g \cdot mL^{-1}$), copper group (200 $\mu g \cdot mL^{-1}$) and florfenicol mixed with copper sulfate group (40 $\mu g \cdot mL^{-1}$+200 $\mu g \cdot mL^{-1}$) were set respectively. After treatment, samples were collected at 120 h, 132 h, 144 h, 156 h, 168 h to determine the contents of extracellular polysaccharide and biofilm, and the mRNA expression levels of nitrogenase structure gene (*nifH*), nitrogen metabolism regulation genes (*ntrY*, *ntrX*, *glnK*, *nnrR*) and biofilm-related genes (*flaF*, *fliL*, *flhA*, *fliQ*) in each group. The results showed that florfenicol and copper alone promoted the formation of biofilms while mixed treatment inhibited that. The change of extracellular polysaccharide content was consistent with biofilm formation ability. Correlation analysis showed that biofilm-related genes (*flaF*, *fliL*, *flhA*, *fliQ*) were significantly positively correlated with nitrogen metabolism regulation gene (*ntrX*) (r=0.691-0.775, $P<0.01$). Copper treatment significantly increased the expression of *nifH*, while florfenicol treatment promoted the expression of *fliQ*. In addition, mixed treatment had significant antagonistic effect on the expression of *fliQ* gene ($P<0.05$), but there was a significant synergistic effect on the expression of nnrR gene ($P<0.05$).

Key words: Rhizobia; Florfenicol; Copper sulfate; Soil

Corresponding author: Yongxue Sun, E-mail: sunyx@scau.edu.cn

T04-83-0006

Characteristics of polycyclic aromatic hydrocarbon pollution in atmospheric $PM_{2.5}$ and health risk assessment in Shenyang, 2016–2018

Liu Huo[a], Zhao Chen-kai[a], Wang Jun-long[b], Sun Li[b], Cui Zhong-ming[b], He Miao[a]

(a. School of Public Health, China Medical University, Key Laboratory of Environmental Health Damage Research and Evaluation in Liaoning Province, Shenyang 110122, China; b. Liaoning Center for Disease Control and Prevention, Shenyang 110050, China)

Abstract: OBJECTIVE To investigate the pollution characteristics of polycyclic aromatic hydrocarbons (PAHs) in atmospheric $PM_{2.5}$ in Shenyang and their health risks to the population. **METHODS** Atmo·

spheric $PM_{2.5}$ in Heping and Shenhe districts of Shenyang City from January 2016 to December 2018was collected, and the concentrations of 16 PAHs in $PM_{2.5}$ were detected by inductively coupled plasma mass spectrometry to analyze their concentration change patterns, identify their main sources. The health risk of a population is assessed based on four indicators: toxic equivalents, mutagenic equivalent, average daily dose, excess risk of carcinogenic diseases in the population, and loss of life expectancy. **RESULTS** From 2016 to 2018, the annual average mass concentrations of $PM_{2.5}$ in Hep·ing District were 71.22 μg·m^3, 63.19 μg·m^3, 46.37 μg·m^3; the annual average mass concentrations of 16 PAHs were 45.86 ng · m^3, 52.37 ng · m^3, 45.57ng / m3; the annual average mass concentrations of PM in Shenhe District were 79.55 μg·m^3, 73.59 μg·m^3, 51.43 μg·m^3; the annual average mass con·centrations of 16 PAHs were 89.09 ng·m^3, 89.11 ng·m^3, 58.72 ng·m^3. The concentration of 16 PAHs and atmospheric $PM_{2.5}$ concentrations varied consistently as winter>spring>autumn>summer. The mean values of toxic equivalent quantity in Heping District were 8.11 ng·m^3, 7.31 ng·m^3, 6.03 ng/m3, and the mean values of mutagenic equivalent quantity were 7.5 ng· m^3, 7.14 ng· m^3, 4.83 ng· m^3; the mean values of toxic equivalent quantity in Shenhe District were 9.17 ng·m^3, 11.72 ng·m^3, 7.81 ng/m3, and the mean values of mutagenic equivalent quantity were 8.19 ng·m^3,11.49 ng·m^3,6.51ng/ m3.The excess lifetime carcinogenic risk of PAHs for adults and children between 1.45×10^{-6} to 4.04×10^{-6}, which is within acceptable levels. **CONCLUSION** The concentration of PAHs pollution in atmospheric $PM_{2.5}$ in Shenyang from 2016-2018 has seasonal variability and is most severe in winter, which does not cause obvious carcinogenic risk to the population.

Key words: $PM_{2.5}$; polycyclic aromatic hydrocarbons; health risk assessment

Corresponding author: He Miao, E-mail: meh@cmu.edu.cn

T04-97-0001

Use of GreenScreen Ⓡ assessment to Screen out safer UV absorbers

Chen Shui-juan, Jiang Ping, Wang Shuang-jie, Wu Qi-rou

(REACH24H Consulting Group China)

Abstract: GreenScreenⓇ for Safer Chemicals (GreenScreen) is a publicly available and transpar·ent chemical hazard assessment methodology developed by Clean Production Action for safer chemi·cals, to help move our society quickly and effectively toward the use of greener and safer chemicals. Based on the GreenScreen method, we selected 68 organic UV absorbers commonly used in the inter·national market, compared and analyzed their hazard. Generally, 18% of the total 68 chemical UV ab·sorbers are classified as "Chemical of High Concern", and they are already on the lists of substances of high concern in many countries and should be avoided using. Another 35% of the substances analy·sis results are "possibly chemical substances of high concern", which have appeared on the non-au·thoritative high concern list, but the hazard risk is uncertain, and a full GreenScreen assessment is re·quired to confirm its risk. The overall safety of other substances cannot be confirmed due to a lack of necessary data or systematic hazard analysis. We divided these 68 UV absorbers into five categories, benzophenone, phenyl salicylates, benzotriazole, triazine, and other compounds, and found that 61% of the substances classified as "Chemical of High Concern" and "Possible High Concern Chemical Sub·stances" is benzophenone and phenyl salicylates. When analyzing the hazard results of these two types of UV absorbers, it is showing out that the high hazards mainly lie in their environmental fates and dose-related acute-local human health hazard. Importantly, two UV absorbers are mutagenic,

which need to be strictly restricted for use. By applying the GreenScreen method, we quickly screened out UV absorbers that may have hazards and need to be substituted by the market. Manufacturers can perform GreenScreen assessment on the candidate chemicals before, then screen out greener and safer UV absorbers with a GreenScreen® certification to comply with the development trend of safety, sustainability, and environmentally friendly, and increase their competitiveness.

Key words: UV absorber; GreenScreen assessment; hazard assessment; sustainable development

Corresponding author: Chen Shuijuan, E-mail: chenshuijuan@reach24h.com

T04-98-0005

Physiologically based pharmacokinetic (PBPK) modeling in supporting cumulative risk assessment

Zhang Tao, Wang Qi

(Department of Toxicology, School of Public Health, Peking University, Beijing 100191, China)

Abstract: Cumulative risk assessment (CRA) has been developed to assess the risks from combined exposures to chemicals and other stressors in humans. A prerequisite before embarking on a cumulative risk assessment is determining the external or internal exposure levels of multiple chemicals. Physiologically based pharmacokinetic (PBPK) modeling is a mechanism-based approach to simulate and predict the kinetic behaviors of chemicals and/or their metabolites. With the greater power of extrapolations across dose, species and exposure regimen, PBPK modeling has been proposed and developed by EPA, EFSA, OECD, ATSDR and WHO/IPCS frameworks. PBPK reverse dosimetry could convert human biomonitoring concentrations of chemicals in plasma, urine or other target tissues to external exposure levels, or convert *in vitro* toxicity testing's bioactive concentrations to in vivo points of departure (PODs), supporting the derivations of hazard indexes (HIs) or Margins of exposure (MOEs). Combining PBPK modeling with probabilistic approaches like Monte Carlo (MC) analysis provides population distributions of internal doses or external exposures, supporting population-scale risk assessment of chemicals. In recent years, there has been a surge in developing and applying PBPK models for special populations including pregnant women, children, the elderly, and patients with renal impairment, liver cirrhosis or other diseases. These models provide accurate predictions for chemical pharmacokinetics and hold exciting promise for cumulative risk assessment in a well-defined population. A challenge for estimating cumulative risks is to evaluate the hypothesis of dose additivity among chemicals. Therefore, it is necessary to conduct a quantitative cumulative risk assessment for describing the interactions in the pharmacokinetics (PK) and/or pharmacodynamics (PD) of chemical mixtures. PBPK models have been widely used to simulate the binary interactions induced by hepatic enzyme inhibition or induction, which is the most common single mechanism of interaction investigated. Furthermore, several studies have indicated the ability of PBPK models in predictions of interactions from binary to more complex chemical mixtures, facilitating the development of interaction-based risk assessment for chemical mixtures. Future efforts will be focused on integrating PBPK models with exposure models and dose-response models to better describe the transport and transformation processes of multiple chemi·cals, and support the experimental designs and evaluations conducted in cumulative risk assessment.

Key words: physiologically based pharmacokinetic model; cumulative risk assessment; mixtures

Corresponding author: Wang Qi, E-mail: wangqi@bjmu.edu.cn

T04-98-0007

Health risk assessment of paraquat contaminated in Spirogyra spp. (tao) in Chiang Mai, Thailand.

Preechaya Tajai, Assawin Daducale, Sarunya Chuanphongpanich
(Payap University)

Abstract: Spirogyra spp. (tao) are freshwater green algae which are consumed as an uncooked food especially in the north of Thailand. Aquatic plants especially algae are easily contaminated by pes·ticide residues from soil and water. Paraquat (1,1′-dimethyl, 4,4′-bipyridinium dichloride; PQ), a widely used herbicide among Thai farmers, is toxic, mutagenic, and carcinogenic to mammals through inges·tion, inhalation and skin contact. This study aims to determine the concentrations of PQ contaminated in Spirogyra spp. and risk assessment on the consumption of Spirogyra spp. in Chiang Mai province. The levels of PQ were analyzed by using a simple, sensitive, and reliable method which was high per·formance liquid chromatography (HPLC). Seven samples were collected from the different districts. The levels of PQ contamination were 5.07 ± 0.00 to 12.69 ± 0.10 mg· kg^{-1}. The results showed that the concentrations of PQ in samples were lower than the standard level of the European Commission Reg·ulation. Risk assessment of PQ found that the hazard quotient was in the range of 0.18–0.46. It indicat·ed that the consumption of Spirogyra spp. was expected to have no adverse health effects. However, the consumption of Spirogyra spp. should be a concern in terms of chronic exposure to toxic herbicide contaminated in the environment.

Key words: Spirogyra spp. (tao); Paraquat; Risk assessment; High performance liquid chromatog·raphy

T04-98-0008

Comprehensive chemical characterization of the aerosol generated by a heated tobacco product

Mark Bentley, Daniel Arndt, Arno Knorr, Gerhard Lang, Pavel Pospisil, Serge Maeder
(Philip Morris International)

Abstract: It has been demonstrated that, in principle, heated tobacco product (HTP) aerosols con·tain significantly lower levels of harmful and potentially harmful constituents (HPHC) than cigarette smoke. Upon applying targeted analysis for the most extensive list of known HPHCs issued by the US FDA (containing more than 93 chemicals) to the aerosol of the Tobacco Heating System (THS), an HTP developed by Philip Morris Products S.A., the average reduction in HPHC levels was found to be greater than 90% relative to the smoke from a 3R4F reference cigarette, on a per-stick basis. However, the formation of other toxicologically relevant compounds specific to HTPs could not be excluded by these targeted analyses. Therefore, advanced untargeted screening methods were applied to compre·hensively characterize the composition of THS aerosol and to identify compounds that may be present in THS aerosol in higher concentrations than in 3R4F smoke. Upon applying a reporting threshold (100 ng/item) which optimized the proportion of substances that could be identified by using reasonable ef·forts, untargeted screening demonstrated that THS aerosol was significantly less complex than 3R4F

smoke, with a ca. 10-fold reduction in the total number of constituents present (532 versus ca. 4800) and no compounds unique to THS aerosol being observed at concentrations of 100 ng/item or higher. Untargeted differential screening, which did not involve a reporting threshold, revealed that 85 compounds (total number across all 3 heated tobacco variants tested) were more abundant in THS aerosol than in 3R4F smoke; of these, 9 were unique to THS aerosol. The observed differences between THS aerosol and 3R4F smoke are likely attributable to differences in tobacco blend and the presence of flavors (3R4F is an unflavored reference cigarette). The FDA's overall assessment regarding the yield of HPHCs analyzed by targeted analysis and the chemicals identified by untargeted differential screening that were not in the FDA's established list of HPHCs concluded that "the yields of potential carcinogens, respiratory toxicants, and reproductive/developmental toxicants were considerably lower in Heat-Stick aerosols compared with combusted cigarette smoke"1.

It has been demonstrated that, in principle, heated tobacco product (HTP) aerosols contain significantly lower levels of harmful and potentially harmful constituents (HPHC) than cigarette smoke. Upon applying targeted analysis for the most extensive list of known HPHCs issued by the US FDA (containing more than 93 chemicals) to the aerosol of the Tobacco Heating System (THS), an HTP developed by Philip Morris Products S. A., the average reduction in HPHC levels was found to be greater than 90% relative to the smoke from a 3R4F reference cigarette, on a per-stick basis. However, the formation of other toxicologically relevant compounds specific to HTPs could not be excluded by these targeted analyses. Therefore, advanced untargeted screening methods were applied to comprehensively characterize the composition of THS aerosol and to identify compounds that may be present in THS aerosol in higher concentrations than in 3R4F smoke. Upon applying a reporting threshold (100 ng / item) which optimized the proportion of substances that could be identified by using reasonable efforts, untargeted screening demonstrated that THS aerosol was significantly less complex than 3R4F smoke, with a ca. 10-fold reduction in the total number of constituents present (532 versus ca. 4800) and no compounds unique to THS aerosol being observed at concentrations of 100 ng/item or higher. Untargeted differential screening, which did not involve a reporting threshold, revealed that 85 compounds (total number across all 3 heated tobacco variants tested) were more abundant in THS aerosol than in 3R4F smoke; of these, 9 were unique to THS aerosol. The observed differences between THS aerosol and 3R4F smoke are likely attributable to differences in tobacco blend and the presence of flavors (3R4F is an unflavored reference cigarette). The FDA's overall assessment regarding the yield of HPHCs analyzed by targeted analysis and the chemicals identified by untargeted differential screening that were not in the FDA's established list of HPHCs concluded that "the yields of potential carcinogens, respiratory toxicants, and reproductive/developmental toxicants were considerably lower in Heat-Stick aerosols compared with combusted cigarette smoke"1.

Key words: Heated Tobacco Product; HTP; Tobacco Heating System; THS; HPHC

T04-98-0009

Incidence of venous thromboembolism and hemorrhage in Chinese patients after pulmonary lobectomy: mechanical prophylaxis or mechanical prophylaxis combined with pharmacological prophylaxis, a randomized controlled trial

Yun Hong

(First Affiliated Hospital of Zhejiang University School of Medicine)

Abstract: OBJECTIVE Venous thromboembolism (VTE) and postoperative bleeding are important complications of lung resectionsurgery. We investigated the preventive effect of mechanical prophylaxis versus pharmacological prophylaxis after lobectomy, and evaluated the effect of both on the incidence of hemorrhagic events. **METHODS** A prospective study of 424 lobectomies with moderate to high risk of VTE (Caprini risk score <5) in a single center was performed from April 2020 to March 2021. Patients were randomly allocated to mechanical prophylaxis or to the low-molecular-weight heparin (LMWH) - combination-prophylaxis. The incidenceof postoperative thrombotic and bleeding events and relevant factors of the two groups were analyzed. **RESULTS** A total of 410 participants, with 202 and 208 in the mechanical prophylaxis and LMWH-combination-prophylaxisgroups respectively, were selected for analysis. Both groups had similar baseline and clinical characteristics. There were no cases of VTE or major bleeding during the study, but the incidence rate of minor bleeding in theLMWH-combination-prophylaxis group was significantly higher than mechanical prophylaxis group (odds ratio 0.035, 95% confidence interval0.011-0.113). **CONCLUSIONS** A case-by-case risk assessmentof VTE and hemorrhage remains necessary to determine the most appropriatemethod of thrombosisprophylaxis for patients undergoing pulmonary surgery. Mechanical prophylaxis may be preferable for lung cancer patients with moderate to high risk of VTE (Caprini risk score <5) undergoing lobectomy.

Key words: Bleeding events; prophylaxis; postoperative; venous thromboembolism (VTE)

T04-98-0010

Interpretation for Technical Guidance for Cosmetics Safety Assessment

Xinrong Pei

(National Institutes for Food and Drug Control)

Abstract: Cosmetics safety assessment refers to the use of existing scientific data to scientifically evaluate the known or potential adverse effects on human health in cosmetics, which can effectively reflect the potential risks of cosmetics. It is an important technical means of cosmetic safety assessment. The Regulations on the Supervision and Administration of Cosmetics (here in after referred to as the Regulations) issued in June 2020 clearly stipulates that before the registration and filing of new cosmetic ingredients and cosmetic products, the registration applicants and the filing applicants shall conduct safety assessment by themselves or entrust a professional organization. In order to implement the new provisions and requirements for cosmetics safety assessment proposed in the Regulations, National Medical Products Administration has issued the Technical Guidance for Cosmetics Safety Assessment (2021 Edition) (here in after referred to as the Guidance), which systematically stipulates the principles and procedures of cosmetics safety assessment. The Guidance is used to standardize and guide cosmetics safety assessment.

The formulation of the guidelines followed the principles of scientificity and feasibility, and combined with the current situation of the cosmetics industry, introduced advanced safety assessment technologies such as Threshold of Toxicological Concern and Grouping/Read Across, which provided assessment tools and ways for cosmetics enterprises to face the lack of safety data of some ingredients and risk substance in safety assessment; At the same time, sort out the historical use information of ingredients of registered products, expand the types of safety assessment evidence, and apply it to the simplified safety assessment report.

The main body of the guidelines consists of ten parts, which mainly defines the principles and personnel of safety assessment, and specifies the assessment procedures, safety assessment requirements for ingredients and products, contents of assessment reports, simplified safety assessment report requirements, etc. The appendix includes the contents of safety assessment report for the ingredients and cosmetic product, and examples of the full version and simplified version of the product safety assessment report.

The guidelines are technical documents to guide cosmetic manufacturers to scientifically carry out cosmetic safety assessment. Cosmetics registration applicants and filing applicants shall carry out safety assessment on new cosmetic ingredients and products in accordance with the guidelines, and be responsible for the authenticity and accuracy of the safety assessment materials submitted.

Key words: Cosmetics Safety Assessment, Technical Guidance

Corresponding author: Xinrong Pei, E-mail: rongpx@163.com

T04-98-0011

Industrial View: Principles and Application of Comprehensive Safety/Risk Assessment and Relevant Key Points

Tingting Zhu[1]*

(1 P&G Technology (Beijing) Co., Ltd. No. 35 Yu'an Road, B Zone, Tianzhu Konggang Development Zone, Shunyi District, Beijing, 101312, P.R. China)

Abstract: Comprehensive safety/risk assessment is a systematic way to identify and analyze all available safety information to achieve scientific and consistent decisions. The modern safety assessment approach, also called as "4-step method" including hazard identification, dose-response relationship, exposure assessment and risk characterization, has been developed for over 60 years globally and applied to establish regulations to protect public health in multiple areas such as food, cosmetic, drinking water, medical device, chemical and etc. The results of risk assessment are eventually to guide risk management and then communicate towards the public. Decades of accumulation have enriched toxicological data of chemicals and the improvement of safety assessment methodologies, which have transformed the work of safety assessors (toxicologists) from data producers to data analysts, from conducting required toxicological tests to analyzing available toxicological data to enable assessment. In addition, under 3R principle (Reduction, Replacement and Refinement) and Animal-ban regulation in certain regions, many animal alternative methods and NGRA (Next-Generation Risk Assessment) have become more interest in the work of International Cooperation of Cosmetics Regulation (ICCR). In China, the risk assessment concept and framework have been developed rapidly and incorporated in regulations of food, chemicals and in particular cosmetics. Recently, the advance of cosmetic regulations and policies significantly promote the establishment of safety assessment system and development of safety assessors. Regulatory acceptance and supervision transformation both empower the whole cosmetic industry to learn the principles and methodologies of safety assessment and develop toxicological expertise and hands-on practice to ensure product safety. Stricter requirements are needed for safety assessors on their technical capability, data reservation and familiarity to the products and manufacture for future. The main principles of safety assessment such as Weight of Evidence (WoE), data reliability, exposure assessment, expert judgement and some typical misunderstandings about risk assessment were clarified with examples. The relevant hot topics like

animal test ban, data interpretation of animal alternatives, predictive methods/modeling and filling in data gaps as well as the key aspects of safety assessment to speed up its development are discussed.

Key words: safety assessment, risk assessment, risk management, cosmetics, Weight of Evidence (WoE), animal alternatives

Corresponding author: Tingting Zhu, E-mail: zhu.tt.1@pg.com

T04-98-0012

Overview of control strategies for genotoxic impurities in pharmaceutical chemicals

Hua Chen

(National Institutes for Food and Drug Control)

Abstract:

I Overview of control strategies for genotoxic impurities in pharmaceutical chemicals

Genotoxic impurities (GTIs) refer to the impurities that can cause genotoxicity, including mutagenic impurities and other types of non-mutagenic impurities, also known as genotoxic impurities. It mainly comes from the production process of Active Pharmaceutical Integrant (API) or preparation, including starting raw material reactants, catalysts, reagents, solvents, intermediates, by-products, degradation products, etc. The relevant guidelines issued by the regulatory authorities of various countries mainly focus on the genotoxic impurities related to the mutagenic mechanism. This kind of impurities is different from the general impurities in drugs and has significant safety risks. These impurities can induce DNA mutation on very small levels. Therefore, it is necessary to establish stricter limits than the general impurities to ensure the safety and effectiveness of drug quality. ICH M7 guidelines for genotoxic impurities provides a feasible framework for the identification, classification, identification and control of genotoxic impurities. The 2020 edition of Chinese Pharmacopoeia officially also updated the homologous guideline for the control of genotoxic impurities (general chapter 9306).

II Establishment of analytical method for genotoxic impurities

Compared with general impurities, genotoxic impurities can cause damage to genetic materials in human body at minor concentration. Therefore, the analysis and detection of GTIs is a challenging for researchers. At present, the limit of genotoxic impurities in drugs is usually controlled at a very low level, which requires high sensitivity and selectivity of analytical instruments and methods. In order to meet these challenges, it is necessary to explore various analytical strategies, select reasonable sample pre-treatment methods and analytical instruments, and develop and optimize analytical methods according to the characteristics of different genotoxic impurities, so as to accurately measure and control the level of GTIs in drugs.

HPLC-MS and GC-MS are universal analytical methods with a wide detection range. Because they have higher sensitivity and specificity than other detectors, they are more often used for trace detection of various genotoxic impurities than other analytical methods. The analytical methodology verification of genotoxic impurities should follow the guidelines for analytical method verification (general chapter 9101 in Chinese Pharmacopoeia).

The Institute for chemical products of NIFDC has carried out research on detection methods for some types of genotoxic impurities, including detection methods for NDMA related drugs, sulfonates and diazo (azide) compounds.

III Data integration of genotoxic impurities

The detection of genotoxic impurities involves different fields, including toxicological data retrieval, QSAR software evaluation, in vivo and in vitro mutagenicity experiments, limit evaluation of genotoxic impurities, detection of micro (trace) chemicals, etc., and these works are scattered in different drug fields at present. The existing data integration from identification, retrieval, evaluation to analysis will be very conducive to the relevant workers of drug R&D, production, quality control, evaluation and supervision departments to improve their work efficiency and further ensure the drug safety of the people.

T04-98-0013

Risk assessment of trace chemicals in consumer products

Haixia Sui *

(Department of risk assessment, China National Center for Food Safety Risk Assessment.

No 37, Guangqu Road, Chaoyang District, Beijing, China,100022)

Abstract: With the development of modern technology and analytical tools, more and more substances can be detected in consumer products. Trace chemicals are referred to the substances with very low level which could be sourced from impurities in raw materials, processing aids, chemical reaction derivatives within products, package under normal storage conditions and etc. Taking food as an example, the trace chemicals could include naturally present impurities in raw materials, residuals left over from the manufacturing process (e. g. solvents and catalysts), degradations by products and transformations of active substances in pesticides, unintentionally added substances from food contact materials. Exposure-based risk assessment is the well-known approach to evaluate safety of products and widely used to establish regulations to protect public health globally. Following the same principle and practice of active or main components of products, the exposure-based risk assessment is applicable and must be done for safety evaluation of trace chemicals. For those trace chemicals with safety benchmark such as health-based guidance values (HBGV) and well-established “acceptable safety dose”, if the total exposure is below the benchmark, then the risk is acceptable. For trace chemicals whose toxicity has no threshold, then margin of exposure approach can be used to characterize the risk. For those applicable to threshold of toxicological concern (TTC) approach, TTC decision tree method can be used to screen the risk. On the other hand, the risk assessment results could determine the limits of trace chemicals especially impurities to help controlling the quality and specification of their raw materials.

In summary, the presence of trace chemicals in consumer products is inevitable but can be controlled from thorough understanding of raw materials, production process as well as other potential source of trace chemicals. Its potential risk to humans needs to be decided by exposure-based risk assessment approach rather than the hazard itself.

Keywords: Exposure-based risk assessment, trace chemicals, consumer products

References

[1] Principles and methods for the risk assessment of chemicals in food (EHC 240, 2009).

T05-24-0005

Euphobiasteroid disrupts the cell junction and suppresses energy metabolism of Caco-2cells

Zhu An[1,2], Gao Yadong[1], Zhao Jingwei[1], Wang Qi[1]*

(1. Department of Toxicology, School of Public Health, Peking University; Beijing 100191, China

2. Key Laboratory of Ministry of Education for Gastrointestinal Cancer; School of Basic Medical Sciences, Fujian Medical University, Fuzhou 350108, China)

Abstract: OBJECTIVE *Euphorbia semen* is the seed of Euphorbia lathyris Linnaeus, exerts the effect of diurese, but also has strong stimulating effect on the intestinal tract, thus causes abdominal pain and diarrhea. Euphobiasteroid (EFL1) is its main toxic component, but its toxic effect and mechanism are still unclear. This study intends to use human colon adenocarcinoma cells (Caco-2) to reveal the intestinal toxicity and molecular mechanism caused by EFL1 from the perspective of intestinal permeability and energy metabolism. **METHODS** MTT was used to detect the survival rate of Caco-2 cells treated with 0-200 $\mu mol \cdot L^{-1}$ EFL1 for 72 h. Transwell was used to construct the monolayer model of intestinal cells to detect the changes in the transmembrane resistance after EFL1 treatment. ELISA method was used to detect the content of TGF-β1 in cell culture supernatant. The intracellular ATP content and ATPase activity was determined by biochemical kits. Molecular docking was performed to predict whether EFL1 can bind to intercellular junction proteins. Cell immunofluorescence and western blotting were used to observe the expression levels of tight junction proteins and skeleton proteins. Western blotting detected the protein expression levels of ion and water channels, as well as AMPK energy metabolism pathways. **RESULTS** After 72 hours of EFL1 treatment in Caco-2 cells, the cell survival of the 200 $\mu mol \cdot L^{-1}$ group decreased to 77.9% of the control. Compared with the initial TEER of the control group at 0 h, the 50, 100, and 200 $\mu mol \cdot L^{-1}$ EFL1 groups decreased to 92.6%, 73.4%, and 66.7%, respectively. The content of TGF-β1 increased from 0.34 ng/ml in the control group to 0.40, 0.41, and 0.44 ng/ml in the 50, 100, and 200 $\mu mol \cdot L^{-1}$ groups. The intracellular ATP content decreased from 2590 $\mu M/gprot$ in the control to 1453 $\mu mol \cdot L^{-1}/gprot$ in the 200 $\mu mol \cdot L^{-1}$ group. The activities of Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase decreased significantly. EFL1 directly bind to Occludin, Claudin-4, ZO- 1, JAM-1, and E-cadherin proteins, and the total score of docking were higher than 5. After EFL1 treatment, the tight junctions of Occludin, Claudin-1, ZO-1 and the skeleton protein F-actin have weaker fluorescence and blurred boundaries, and the expression of JAM protein was down-regulated. The protein expressions of Na^+ channel NHE1, Cl^- channel CFTR, water channel AQP1, AQP2, AQP4 and AQP8 were all down-regulated. The phosphorylation level or total expressions of AMPK, SIRT1, PGC-1 and NRF2 in energy metabolism pathways were down-regulated. **CONCLUSION** After EFL1 treated Caco-2 cells for 72 hours, the tight junctions between cells were injured, the cytoskeleton was abnormal, so the toxic components could pass through the intercellular space. The mechanism involved decreased expression levels of ion and water channels, and decreased intestinal water reabsorption. The intracellular ATP content decreased, as well as the energy supplied by the sodium pump and calcium pump, and the energy metabolism pathway AMPK/ PGC-1 was inhibited, which exacerbated the state of intracellular energy deficiency.

Key words: Euphobiasteroid; Intestinal toxicity; Permeability; Energy metabolism

Corresponding author: Zhu An, E-mail: zhuan@fjmu. edu. cn; Wang Qi, E-mail: wangqi@bjmu. edu.cn

T05-31-0007

Elucidate the potential mechanism of Eucommiae Cortex against osteoporosis by network pharmacology

Liu Yun[a,b], Tan Jian-bin[a], Xie Cheng-liang[c], Yang Xing-fen[d], Huang Wei-ling[a], Lu Zhi[e], Lin Hong[a], Luo Man-si[a], Jiang Ying[a], Wang Hong-xia[a], Wang Ke-xin[a], Zhao Min[a]

(a. Center for Disease Control and Prevention of Guangdong Province, Guangzhou 511430, China; b. Guangdong Provincial Institute of Biological Products and Materia Medica, Guangzhou 510440, China; c. School of Pharmaceutical Science (Shenzhen), Sun Yat-Sen University, Guangzhou 518107, China; d. School of Public Health, Southern Medical University, Guangzhou 510515, China; e. Infinitus (China) Company Ltd., Guangzhou 510610, China)

Abstract: OBJECTIVE Osteoporosis is a systemic skeletal disease that leads to high risk of bone fractures. Eucommiae Cortex (EC) has been used as multiple curative effect traditional Chinese medi·cine (TCM) in China. However, the mechanism of EC as an anti-osteoporotic herb remains unknown. **METHODS** Network pharmacology approach was applied to explore the potential action mechanisms of EC in osteoporosis treatment. Cytotoxicity assay and osteogenesis assay were adopted as the ex·periment approach. Active compounds of EC and their potential osteoporosis related targets were re·trieved from databases of TCMSP, TCMID, BATMAN TCM, Pubchem, SwissTargetADME, SwissTar·get Prediction, DisGeNET, GeneCards and OMIM. A protein-protein interaction network was built to an·alyze the target interactions. The DAVID database was used to carry out GO enrichment analysis and KEGG pathway analysis. **RESULTS** A total of 19 active compounds and 128 anti-osteoporosis targets of EC were selected for analysis. The KEGG pathway enrichment analysis showed that EC prevents osteoporosis through the Estrogen signaling pathway, Thyroid hormone signaling pathway and VEGF signaling pathway. Moreover, the genes Akt1, MAPK3, EGFR, CASP3, SRC, MAPK1, STAT3, PTGS2, ESR1, CXCL8 and MMP9 were considered as the key targets regulated by EC. The GO enrichment analysis results indicated that the anti-osteoporosis targets of EC mainly in the response to phosphati· dylinositol-mediated signaling and steroid binding. **CONCLUSION** Osteogenesis effect of EC was demonstrated by in vitro experiments and the molecular mechanism was investigated by network phar·macology. Results showed that Estrogen signaling pathway, Thyroid hormone signaling pathway and VEGF signaling pathway were the vital pathway of EC against osteoporosis.

Key words: osteoporosis; network pharmacology; traditional Chinese medicine; Eucommiae Cor·tex; osteogenesis

Corresponding author: Zhao Min, E-mail: 1181796446@qq.com; Xie Chengliang, E-mail: xiechl·iang@mail.sysu.edu.cn

T05-38-0006

Study on the mechanism of heart pharmacological safety of the main components of Yunnan Baiyao

Cheng dongrong, Chen yuan

(Department of Biology, Collegeof Forestryand Biotechnology, Zhejiang A&F University, 666 Wusu Street, Lin'an District, Hangzhou City, Zhejiang Province, China, 311300)

Abstract: Yunnan Baiyao is a well-known Chinese patent medicine. It has a wide range of phar·macological effects.Among them, *Aconitum kusnezoffii* Reichb. is a common Chinese herbal medicine with high medicinal value and strong toxicity. Studies have shown that one of the key ion targets for arrhythmia induced by aconitine, the main active ingredient in *Aconitum kusnezoffii* Reichb., may be the potassium channel of hERG. However, the study on the cardiotoxicity of water extract of Aconitum kusnezoffii Reichb. is not deep enough. The blocking effect of water extract of *Aconitum kusnezoffii* Reichb. on potassium channel current of hERG was studied in this experiment. Panax notoginseng in Yunnan Baiyao is also a common Chinese herbal medicine. Studies have shown that *panax notogin- sengsaponins*, the main active ingredient of *panax notoginseng*, have extensive and obvious antagonistic effects on ion channels and arrhythmia. On this basis, the effects of water extract of *panax notoginseng* and several main components of *panax notoginseng* saponins on potassium channel of hERG were studied, and the cardiac safety of panax notoginseng was explored. The structure of hERG channel is special, and it is often regarded as an important target for drug cardiotoxicity. In this paper, we studied the effects of the main components of Yunnan Baiyao, namely the water extract of Radix Aconiti, the water extract of panax notoginseng and the total saponins of panax notoginseng, on the potassium channel of hERG in the heart, and discussed the cardiac pharmacological safety mechanism of Yunnan Baiyao.

In this paper, the whole cell patch clamp technique was used to study the effects of water extract of *Aconitum kusnezoffii* Reichb, water extract of *panax notoginseng* and several main components of total saponins of *panax notoginseng* on potassium channel of hERG. Through experiments, the follow·ing conclusions are drawn: 1. Aqueous extract of A*conitum kusnezoffii* Reichb inhibited potassium channel of hERG in a concentration-dependent manner, IC50=23.42 mg·mL^{-1}. 2. The water extract of *panax notoginseng* could enhance the potassium channel of hERG at 0.1 mg · mL^{-1}. The potassium channel of hERG was not stable at 0.3 mg/mL, but inhibited at higher concentration. 3. 10 μmol·L^{-1} gin·senoside Rb1 significantly enhanced potassium channel of hERG, and 10 μmol·L^{-1} ginsenoside Rd sig·nificantly enhanced potassium channel of hERG, while 10 μmol·L^{-1} ginsenoside Rg1 significantly inhibited potassium channel current of hERG, while 10 μmol·L^{-1} notoginsenoside R1 had no significant effect on potassium channel of hERG.

Key words: *Aconitum kusnezoffii* Reichb.; *Panax notoginseng* ; hERG potassium channel; whole cell patch clamp technique

T05-42-0002

Antioxidant activity and the potential mechanism of the fruit from Ailanthus altissima Swingle

Ya-nan Mo[a], Feng Cheng[a,c], Zhen Yang[a], Xiao-fei Shang[a], Jian-ping Liang[a], Ruo-feng Shang[a], Bao-cheng Hao[a], Xue-hong Wang[a], Hong-juan Zhang[a], Ahmidin Wali[c], Chun-fang Lu[c], Yu Liu[a,b]

(a. Key Laboratory of New Animal Drug Project, Gansu Province; Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture and Rural Affairs; Lanzhou Institute of Husbandry and Pharmaceutical Sciences of Chinese Academy of Agriculture Sciences, Lanzhou 730050, P.R. China; b: College of Veterinary Medicine, Gansu Agricultural University, Lanzhou 730070, P. R. China; c: Key Laboratory of Plant Resources and Chemistry in Arid Regions, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi 830011, P.R. China)

Abstract: The fruits of Ailanthus altissima Swingle (AS) possess a variety of pharmacological activities. Its antioxidant activity and the potential mode of action have not yet been investigated. Antioxidant assay *in vitro* proved AS possessed fairly strong antioxidant capacity. Meanwhile, AS increased the activities of SOD, CAT, GSH-Px and decreased the MDA level compared with the H2O2 group, suggesting it relieved oxidative stress of RAW264.7 cells significantly. Network pharmacology analysis screened the core targets of AS like threonine kinase1 (AKT1), mitogen-activated protein kinase 1 (MAPK1), sirtuin-1(SIRT1), mechanistic target of rapamycin kinase (MTOR) and the key pathways involved PI3K-AKT and FoxO signaling pathway. Besides, qRT-PCR revealed AS preconditioning significantly upregulated the expression level of AKT1, SIRT1, MAPK1, MTOR in model cells, and the effect was related to the regulation of FoxO and PI3K/ AKT signaling pathway. In summary, AS revealed significant antioxidant activity and its potential mechanism was regulating FoxO and PI3K/ AKT signaling pathway.

Key words: Ailanthus altissima Swingle; antioxidant activity; RAW264.7 cell; network pharmacology

Corresponding author: Yu Liu, E-mail: liuyu8108@163.com

T05-89-0004

Inhibition mechanism of YC-004 from magnolia officinalis on hERG potassium channel

Lanying Pan, Jiaxin Yang, Wei Zhao, Jintao Wang, Huan Liu, Xiaofeng Wei, Yuan Chen

(Chinese Herb Medicine Division, Zhejiang Agriculture and Forestry University, Zhejiang Provincial Key Laboratory of Resources Protection and Innovation of Traditional Chinese Medicine, 88 North Circle Road, Lin'an 311300, P. R., China)

Abstract: HERG (human *Ether- à- go-go* Related Gene, Kv11.1) channel mediates rapid delayed rectifier potassium current, which is crucial during the repolarization phase of the cardiac action potential. Any obstruction of hERG channel may prolong K^+ repolarization process and the QT interval, resulting in various heart diseases. As a traditional Chinese medicine, *Magnolia officinalis* has a wide range of applications, however, the toxicity mechanism of it remains unclear. To address this, whole-cell patch clamp technique was applied on hERG channels to explore the effect of YC-004, an active ingredient in *M. officinalis*, by using different pulse programs. Moreover, binding selectivity studies based on molecular docking were explored. The results showed that YC-004 had a concentration-dependent inhibitory effect on hERG channels with IC_{50} reaching 8.50 $\mu mol \cdot L^{-1}$. Molecular docking results showed that the inhibition was due to the hydrogen bondings or other interactions between YC-004 and V625 on the pore helix, and also was due to the hydrogen of YC-004 at S621, M645 sites on the S6 helix, which can prevent passing of K^+ through the selective filter and thus reduce hERG current. On the other hand, steady-state activation curve of hERG channels significantly shifted to left under the exposure of YC-004, which may be related to the hydrogen bond formed between YC-004 and W412. Thus the force might disrupt the D411-S4 interactions and destabilize the closed states leaving hERG channels being able to activate more rapidly. In addition, YC-004 could accelerate the rate of inactivation and reduce inactivation via interaction with S631, P632, S641, F617 and other residues.

In summary, the inhibition of YC-004 on hERG channels may be related to the binding mechanism of the central cavity, or of the trans-membrane helixes which could influence the activation and inactivation rate of hERG channels, thus leading to the cardiotoxicity of YC-004. Further research on the mechanism will be carried out, which would benefit to the development and utilization of YC-004. Further, establishing a complete accurate evaluation of *M. officinalis* is necessary since we speculate that other ingredients in it may reduce the cardiotoxicity of YC-004.

Key words: YC-004; hERG potassium channel; whole-cell patch clamp; molecular docking; cardiotoxicity evaluation

Corresponding author: Yuan Chen, E-mail: ychen@zafu.edu.cn

T05-94-0001

Autophagy promotes GSDME-mediated pyroptosis via apoptosis pathway in aristolochic acid-induced acute kidney injury model

Wang Li-meng[a], Cao Hui-xia[a], Chen Song[b], Shao Feng-min[a]

(a. Henan Provincial Key Laboratory of Kidney Disease and Immunology, Henan Provincial People's Hospital, Zhengzhou, Henan 450003, China; b. Translation Research Institute, Henan Provincial

People's Hospital, Zhengzhou, Henan 450003, China)

Abstract: Aristolochic acids (AAs) are representative nephrotoxins in Chinese traditional medicines, which are widely used in Asia. Meanwhile, AAs are environmental pollutants to underground water, soil, wheat and maize plants in Balkan regions. AAs induces acute kidney injury (AKI), of which the essential pathological basis are the death of proximal tubular epithelial cells (PTEC) and inflammation. Pyroptosis, one inflammatory programmed cell death, which plays an essential role in connecting cell death and inflammation, probably provides a novel target for AKI. But to date, the role of pyroptosis in AKI and related mechanism are not investigated. We established the AKI mouse model by injecting 5 mg AAI / kg b. w. /day intraperitoneally for 5 days. The H&E staining showed significant cell death of PTEC. The western blot and immunofluorescence results indicated that Gasdermin E (GSDME)-mediated pyroptosis was involved in the cell death. The CCK-8 and LDH release results indicated AA induced acute cell death in HK-2 cell line. The western blot and immunofluorescence results showed that AA induced dose-dependent and time-dependent elevation of pyroptosis under the exposure to 0-50 μg/mL AAI within 48 h. Knocking down of GSDME by siRNA transfection obviously attenuated AA-induced cell damage and cytokines' expression. Mechanism research showed that intrinsic and extrinsic apoptosis pathways were involved in the cleavage of GSDME. Inhibiting the activities of cleaved-caspase 8, cleaved-caspase 9, or cleaved-caspase 3 significantly protected the cells from GSDME-mediated pyroptosis. Meanwhile, knocking down autophagy-related genes significantly inhibited apoptosis, and blocked GSDME-mediated pyroptosis. Results indicated that autophagy promotes GSDME-mediated pyroptosis via apoptosis pathway in aristolochic acid-induced AKI model.

Key words: Aristolochic acid; Pyroptosis; GSDME; autophagy

Corresponding author: E-mail: fengminshao@126.com

T05-98-0003

Modulating the gut microbiomeusing *Lactobacillus plantarum* in symbiotic combinationwith catechins alleviate CCl_4-induced liver injury

Wang Meng-qi, Xu Xiao-wei, Zhao Yu, Liu Shan-ji, Wen Si-yue, Xu Heng-yi*

(State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China)

Abstract: This study was aimed to investigate the synergy effect and explore the mechanism of *Lactobacillus plantarum* and catechins (EGCG) from preventing liver injury induced by carbon tetrachloride (CCl4). There are five groups—control group, model group, probiotic treatment group (108 CFU / mL resuspended in 200 μL PBS), EGCG group (EGCG 7 g· mL^{-1}dissolved in 200 μL PBS) and P+ E group (Lactobacillus plantarum resuspended in 7 g/mL EGCG). Mice in control group and other groups were intraperitoneally injected with 3 mL·kg^{-1} BW olive oil or CCl_4/olive oil (20% v/v) after the 21th day intragastric administration 2 h. By activating the oxidative stress signal pathway of *Nrf2 / Keap1* and down-regulating the inflammatory signal pathway of *TLR4/ NF-κB*, *Lactobacillus plantarum* and catechins alleviated liver damage caused by CCl_4. The intestinal microbiota analysisresultsrepresented that the synergy of Lactobacillus plantarum and catechins increased the abundance of beneficial bacteriaand decreased the abundance of harmful bacteria. In addition, the intestinal microflora was well correlatedwith inflammationin our study. Mainly, *Alistipes*, *Prevotellaceae UCG-001*, *Rikenellaceae RC9* gut

group showed significant positive correlation with pro-inflammatory cytokines, while *uncultured bacterium f Desulfovibrionaceae* showed negative correlation. The study suggested that theprophylaxis function ofprobioticsand catechins to reduce liver damage was demonstrated through the gut microbiome. It is of great significance to study the synbiotic role of probiotics and natural plant ingredients in human health care by vigorously exploring the regulatory effect on gut microbiome.

Key words: Probiotics; Catechins; Gut microbiota; Liver damage

Corresponding author: Xu Heng-yi, E-mail:kidyxu@163.com, HengyiXu@ncu.edu.cn

T05-98-0008

A preliminary study of emodin-induced phototoxicity

Pei-Ching Cheng, Jia-Wei Shen, Chiao-Xin Chang, Hsiu-Mei Chiang
(China Medical University)

Abstract: Phototoxicity is defined as a toxic reaction to a substance caused after exposed to light, and it usually produces obvious reactions at low doses. Phototoxicity is a non-immune light-induced reaction, which means it can be induced immediately after a single exposure, without an induction period. Although phototoxicity easily cause severe skin symptoms, it can also be used in photodynamic therapy to treat skin diseases such as vitiligo and psoriasis.

Emodin is a natural anthraquinone derivative that exists in Rheum rhabarbarum, Polygonum multiflorum,Aloe veraand other commonly used Chinese herbal medicines, which are readily available to the public. According to the literature, emodin is not photostable and it is speculated to exhibit phototoxicity. The results shown that emodin under 50 $\mu mol \cdot L^{-1}$ is not toxic to HaCaT cells. However, emodin exhibit phototoxicity at 250 $nmol \cdot L^{-1}$ in HaCaT cells after UVA exposure. In addition, emodin significantly increase the intracellular oxidative stress after UVA irradiation, resulting in a decrease survival rate of HaCaT cells. Moreover, the PIF value is 107.99, inferring emodin has the potential of phototoxicity.

In conclusion, we proved emodin is phototoxicity in HaCaT cells, but the mechanism of phototoxicity induced by emodin need to be explored further.

Key words: Phototoxicity; Emodin; oxidative stress; UVA irradiation

T05-98-0009

Network pharmacology-based analysis of the material basis and mechanism of danhong injection against ischemic brain damage

Yue Wang, Lijun Ge, Shuyuan Liu, Xiwen Jia, Qi Chen, Huilong Liu
(Zhejiang Chinese Medical University)

Abstract: OBJECTIVE To explore the active compounds and the potential mechanism of Danhong injection(DHI)for the treatment of ischemic brain damage (IBD) by the network pharmacology. **METHODS** Traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) and Bioinformatics Analysis Tool for Molecular mechanism of Traditional Chinese Medicine (BATMAN-TCM) and related literature were retrieved for the action targets of Chemical components from DHI. TCMSP, Batman-TCM, ETCM, STITCH and Swiss Target Prediction were used to query all the

collected active ingredients one by one, mining and sorting out the Target information corresponding to each ingredient. And disease targets were screened by Gene Card and UniProtKB database. Protein-Protein Interaction (PPI) network was established by String Version 10.5 database. Gene ontology(GO) functional enrichment analysis and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis were carried out with the help of Meta-platform, to study the mechanism of DHI against ischemic brain damage. Cytoscape 3.6.0 software was used for visualized analysis of drugs-active ingredient- disease targets and active ingredient- disease targets-signaling pathway. **RESULT** Total 70 active ingredients of DHI and 49 therapeutic targets. The main active components of Danhong injection to protect cerebral ischemia include Tanshinone IIA, Quercetin, Kaempferol, beta-sitosterol, Hydroxysafflor yellow A, HYSA, etc. The core therapeutic targets include ALB, PTGS2, AKT1, IL6, NOS3 and mainly involving Neuroactive ligand-receptor interaction pathway, PI3K-Akt signaling pathway and TNF signaling pathway, etc. Involved in biological process (BP) including regulation of body fluid levels and adenylate cyclase-modulating G protein-coupled receptor signaling pathway and others. **CONCLUSION** Tanshinone II A, Quercetin, Kaempferol, β - Sitosterol and Hydroxysafflor A in DHI may play a role on anti-cerebral ischemic injury by acting on PTGS2, PTGS1, AKT1, NOS3 and other targets.

Key words: Danhong injection; Ischemic brain damage; Network pharmacology; Protein-protein interaction; Signal

T05-98-0010

Response surface methodology to optimize the ultrasonic-assisted extraction ofartemisinin

Shuyuan Liu, Lijun Ge, Jun Wan, Xiwen Jia, Yue Wang, QI Chen

(Zhejiang Chinese Medical University)

Abstract: OBJECTIVE To obtain the optimal technology for ultrasonic extraction of Artemisinin. **METHODS** Using response surface methodology to optimize the extraction process of Artemisinin. Based on the single-factor experiment, the ultrasonic time, ultrasonic power, and material-to-liquid ratio were selected for a three-factor three-level experimental design, and the Artemisinin extraction rate was used as the response value for response surface analysis. **RESULT** The best extraction process of Artemisinin was ultrasound time 42 min, ultrasound power 82 W, material-to-liquid ratio 4.2 g/50mL, and the extraction rate under these conditions was 0.688%. The predicted value was in line with the verification test result. **CONCLUSION** Based on the response surface model analysis, the method of optimizing the extraction process of Artemisinin is valid and doable.

Key words: RSM; Ultrasonic method; Extraction; Artemisinin

T05-98-0011

Euphobiasteroid disrupts the cell junction and suppresses energy metabolism of Caco-2 cells

An Zhu[1,2], Yadong Gao[1], Jingwei Zhao[1], Qi Wang[1]

(1. Department of Toxicology, School of Public Health, Peking University, Beijing 100191, China;

2. Key Laboratory of Ministry of Education for Gastrointestinal Cancer, School of Basic

Medical Sciences, Fujian Medical University, Fuzhou 350108, China)

Abstract: OBJECTIVE Euphorbia semen is the seed of Euphorbia lathyris Linnaeus, exerts the effect of diurese, but also has strong stimulating effect on the intestinal tract, thus causes abdominal pain and diarrhea. Euphobiasteroid (EFL1) is its main toxic component, but its toxic effect and mecha·nism are still unclear. This study intends to use human colon adenocarcinoma cells (Caco-2) to reveal the intestinal toxicity and molecular mechanism caused by EFL1 from the perspective of intestinal permea·bility and energy metabolism. **METHODS** MTT was used to detect the survival rate of Caco-2 cells treated with 0-200 $\mu mol \cdot L^{-1}$ EFL1 for 72 h. Transwell was used to construct the monolayer model of intes·tinal cells to detect the changes in the transmembrane resistance after EFL1 treatment. ELISA method was used to detect the content of TGF-β1 in cell culture supernatant. The intracellular ATP content and ATPase activity was determined by biochemical kits. Molecular docking was performed to predict whether EFL1 can bind to intercellular junction proteins. Cell immunofluorescence and western blotting were used to observe the expression levels of tight junction proteins and skeleton proteins. Western blotting detected the protein expression levels of ion and water channels, as well as AMPK energy metabo·lism pathways. **RESULTS** After 72 hours of EFL1 treatment in Caco-2 cells, the cell survival of the 200 $\mu mol \cdot L^{-1}$ group decreased to 77.9% of the control. Compared with the initial TEER of the control group at 0 h, the 50, 100, and 200 $\mu mol \cdot L^{-1}$ EFL1 groups decreased to 92.6%, 73.4%, and 66.7%, respective·ly. The content of TGF-β1 increased from 0.34 ng/ml in the control group to 0.40, 0.41, and 0.44 ng/ml in the 50, 100, and 200 $\mu mol \cdot L^{-1}$ groups. The intracellular ATP content decreased from 2590 $\mu mol \cdot L^{-1}$/gprot in the control to 1453 $\mu mol \cdot L^{-1}$/gprot in the 200 $\mu mol \cdot L^{-1}$ group. The activities of Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase decreased significantly. EFL1 directly bind to Occludin, Claudin-4, ZO-1, JAM-1, and E-cadherin proteins, and the total score of docking were higher than 5. After EFL1 treatment, the tight junctions of Occludin, Claudin-1, ZO-1 and the skeleton protein F-actin have weaker fluorescence and blurred boundaries, and the expression of JAM protein was down-regulated. The protein expres·sions of Na^{+} channel NHE1, Cl^{-} channel CFTR, water channel AQP1, AQP2, AQP4 and AQP8 were all down-regulated. The phosphorylation level or total expressions of AMPK, SIRT1, PGC-1 and NRF2 in energy metabolism pathways were down-regulated. **CONCLUSION** After EFL1 treated Caco-2 cells for 72 hours, the tight junctions between cells were injured, the cytoskeleton was abnormal, so the toxic components could pass through the intercellular space. The mechanism involved decreased expres·sion levels of ion and water channels, and decreased intestinal water reabsorption. The intracellular ATP content decreased, as well as the energy supplied by the sodium pump and calcium pump, and the energy metabolism pathway AMPK/PGC-1 was inhibited, which exacerbated the state of intracellu·lar energy deficiency.

Key words: Euphobiasteroid; Intestinal toxicity; Permeability; Energy metabolism

T05-98-0011

Endoplasmic reticulum stress and autophagy contributed to cantharidin-induced nephrotoxicity in HK-2 cells using untargeted metabolomics

Jianyong Zhang, Tianmu He, Xiaofei Li

(School of Pharmacy, Zunyi Medical University, Zunyi 563000, China)

Abstract: Cantharidin (CTD) is the major bioactive compound in Mylabris and has been shown to

exhibit antitumor activity. However, its clinical application is relatively limited due to its potential toxic effects, especially nephrotoxicity. In this study, a UHPLC-QE/ MS based metabolomics approach and molecular experiments was used to investigate the mechanism of CTD-induced nephrotoxicity in HK-2 cells. Sustained exposure of CTD was shown to cause cell viability loss and LDH activity. A total of 76 potential biomarkers and 28 disturbed metabolic pathways were identi fi ed in HK-2 cells exposed to CTD using UHPLC-QE / MS technology. Apoptosis with time-dose dependence was observed using Hoechst 33342 and flow cytometry. Nuclear translocation and fluorescence distribution of ATF4, CHOP, and LC3 was observed in HK-2 cells. Level of endoplasmic reticulum stress(ERS) gene PERK, eIF2α, ATF4 and CHOP mRNA were increased in HK-2 cells exposed to CTD. And ERS, autophagy and apoptotic protein expression levels of GRP78, ATF4, CHOP, LC3, Beclin-1, Atg 3, Atg 7, Caspase 3 and Bax/Bcl-2 ratio were increased in HK-2 cells exposed to CTD. These observations indicate that ERS, autophagy and apoptosis signaling were activated in CTD-induced nephrotoxicity. These may represent potential diagnostic markers and therapeutic targets, and may also lead to a strategy for reducing CTD-induced toxicity in the clinic.

Key words: Cantharidin; nephrotoxicity; endoplasmic reticulum stress; autophagy; metabolomics

Corresponding author: Jianyong Zhang, E-mail: zhangjianyong2006@126.com

T06-29-0001

Identification of potential crucial genes and key functions in type 2 diabetic hearts by bioinformatic analysis

Xin Huang[a,b]*, Kai-jie Zhang[c,d], Jun-jie Jiang[c,d], Shou-yin Jiang[e], Jia-bin Lin[f], Yi-jia Lou[c]*

(a. Cardiovascular Key Laboratory of Zhejiang Province, The 2nd Affiliated Hospital, School of Medi- cine, Zhejiang University, Hangzhou 310009, China; b. Biotherapy Research Center, The 2nd Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, China; c. Institute of Pharmacolo- gy and Toxicology, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, Chi- na; d. Chu Kochen Honors College, Zhejiang University, Hangzhou 310058, China; e. Department of Emergency Medicine, The 2nd Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, China; f. Clinical Research Center, The 2nd Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, China)

Abstract: Type 2 diabetes (T2D) patients with SARS-CoV-2 infection rapidly emerge an acute car· diovascular syndrome. It is urgent to illuminate the underlying mechanism associated with acute cardi· ac injury in T2D hearts. In the current study, we employed identification of the potential pathogenic and prognostic differentially expressed genes (DEGs) in T2D hearts through bioinformatic analysis of public datasets based on researches from our group and others. Cardiac RNA-sequencing data from *db / db* T2D or BKS mice were updated to Gene Expression Omnibus (GEO) (GSE161931). Together with public datasets from GEO for autopsy heart specimens with COVID-19 (5/6 have T2D, GSE150316), or dead healthy persons (free from any major disease, GSE133054) were used for the bioinformatic analysis. DEGs and overlapping homologous DEGs were identified using R package edge R. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes analyses were conducted for event enrichment through R package cluster profile. The protein-protein interaction (PPI) network of the DEGs was estab·lished and visualized by Cytoscape. In total, 542 up-regulated and 485 down-regulated DEGs in mice,

and 811 up-regulated and 1399 down-regulated DEGs in human were identified, respectively. There were 74 overlapping homologous DEGs across both species. Mitochondria inner membrane in human module and serine-type endopeptidase activity in mouse module were the major enriched functions for overlapping homologous DEGs. The PPI network was constructed with 30 interactions in overlapping homologous DEGs module. ACTN4, MYH9 and CAPNS1 appear as the most potential pathogenic targets, which may contribute to the major compensatory role in T2D hearts. Further validations of these novel potential events in adverse prognostics should be carried out in db/db mice.

Key words: type 2 diabetes; acute cardiac injury; COVID-19; differentially expressed genes; bioinformatics; calpain small subunit 1; self-protective role

T07-24-0008

90-day subchronic toxicological study of *Methylococcus Capsulatus* protei

Gao Yan-jun, Nie Ya, Wang Zhen, Wang Xiao-bo, Bu Shi-jin*

(Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Veterinary Medicine College, Yangzhou University, Yangzhou Jiangsu China, 225009)

Abstract: The experimental studied the 90-day subchronic oral toxicity of *methylococcus capsulatus* protein. A total of 80 Wistar rats were included and randomly divided into 4 groups (10 males and 10 females per group) andcorresponding to three treatment groups at levels of 50 000 mg/ kg, 25000 mg/kg and 10 000 mg/kg in the diet and one negative control group, administering continuously for 90 days. All rats were monitored for clinical changes and mortality on a daily basis. Body weight and food intake were assessed on a weekly basis. At the middle and end of the study, 10 rats (half male and half female) were selected from each group for autopsy after collecting blood samples which used for hematological and blood biochemical examination. Meanwhile complete gross necropsy was conducted. The heart, liver, kidneys, spleen, stomach, lung and testes or ovary were collected and weighed. Organ-to-body weight ratios (relative organ weight) were determined. In addition, major organ samples from control and high dose animals were fixed and subjected to histopathological evaluation. The results showed that subchronic study of *methylococcus capsulatus* protein administration was not associated with any changes in rat food intake, weight, hematological parameters, organ weight, or organ histology. Although there were statistical differences ($P<0.05$) in the individual parameters relative to negative control animals, the parameter levels were within the normal range reported in literature, and no biological significance changes were found. No pathological changes related to the test substances were observed in all organs. The average daily intake of male rats in low, medium and high dose groups was 582, 1601 and 3281 mg/kg, respectively. The average daily intake of female rats in low-dose, medium- dose and high-dose groups was 588, 1561 and 3138 mg/kg, respectively. The no-observed-adverse effect level of *methylococcus capsulatus* protein is greater than 3100 mg/kg.

Key words: Subchronic toxicological study; *methyllococcus capsulatum* protein

Corresponding author: Bu Shi-jin, E-mail: sjbo@yzu.edu.cn

T07-37-0001

Rac1-independent methuosis mediates maduramicin-induced cardiotoxicity

Zheng Yu-ling, Ji Chun-lei, Wang Jun-qi, Ji Hui, Guo Dwei, Jiang Shan-xiang, Gao Xiu-ge
(College of Animal Medicine, Nanjing Agricultural University, Nanjing, Jiangsu 210095)

Abstract: Maduramicin often causes severe cardiotoxicity in target and nontarget animals. Apoptotic and non-apoptotic cell death mediate the cardiotoxicity induced by maduramicin. The effect and mechanism of non-apoptotic cell death are largely unknown. To understand the role of non-apoptotic cell death in maduramicin-induced cardiotoxicity, the rat cardiomyocytes (H9c2) were used as an *in vitro* model in present study. We found maduramicin induced a large number of cytoplasm vacuoles in H9c2 cells, which were time-dependent and concentration-dependent. The vacuoles were phase-lucentcoated with monolayer membrane. In a certain time range, the vacuolation of H9c2 cells induced by maduramicin is reversible. Organelle staining showed that the cytoplasmic vacuoles induced by maduramicin did not originate from the swelling of mitochondria, endoplasmic reticulum and Golgi apparatus, but there was partial co-localization between vacuoles and lysosomes. The dextran uptake assay indicated that the cytoplasmic vacuoles mainlygenerated from macropinocytosis. However, there was no obvious co-localizationbetweenmaduramicin-induced cytoplasm vacuoles and early endosome marker protein EEA1, late endosome marker protein Rab7, lysosome marker protein LAMP1 as well as autophagy marker protein LC3B. H-Ras and Rac1 were significantly upregulated ($P<0.05$ or $P<0.01$) after maduramicin treatment for 24 h-72 h, along with the significant increased expression of activated Rac1. After silencing Rac1 gene, the death rate of H9c2 cells was significant reduced, but the number of cytoplasm vacuolesdid not decrease. These findings demonstrate a non-apoptotic cell death type methuosis mediates mduramicin-induced severe cardiotoxicity in Rac1-independent way.

Key words: Nonapoptotic cell death; Maduramicin; Methuosis; Cardiotoxicity

Corresponding author: Gao Xiu-ge, E-mail: vetgao@njau.edu.cn

T07-42-0006

Study on the safety of Diclofenac Injection for Target Cows

Li Shi-hong, Qin Zhe, KongXiao-jun, Li Jian-yong*
(Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS/Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture/Key Laboratory of New Animal Drug Project, Gansu Province, Lanzhou 730050, China)

Abstract: To evaluate the safety of Diclofenac Injection for target animal cows,24 healthy lactating cows were randomly divided into 4 groups, including the control group (without any treatment), the commended dosage (2.2 mg·kg^{-1}·bw) group, three times of recommend dosage (6.6 mg·kg^{-1}·bw) group and five times of recommend dosage (11.0 mg·kg^{-1}·bw) group. Intramuscularinjection were performed in the neck once a day for 3 days. During the experiment, the health condition,blood routine and serum biochemical indexes of cows were observed and measured. The results showed that no obvious adverse reactions were found in clinical observation during the whole experiment by using Diclofenac Injection at the recommended dose (2.2 mg·kg^{-1}·bw) to 5 times the recommended dose (11.0 mg·kg^{-1}·bw), and

there was no significant difference in 18 indexes in blood routine examination compared with the control group. Serum biochemical tests showed there were significant differences in total protein, globulin, aspartate aminotransferase,creatine kinase and phosphorus after 3 days of administration compared with the control group, and all indexes returned to normal after 7 days of administration. The results show that Diclofenac Injection is safe to be used fortarget cows.

Key words: Diclofenac Injection; Safety; Target Cow

Corresponding author: Li Shihong, E-mail: lzlishihong@163. com; Li Jian-yong, E-mail: lijy1971@163.com

T07-42-0007

Effect of sodium dehydroacetate on cytochrome P450 of rats

Wang Cun-kai, Xiao Jin-zha, Xiao Yi-rong, Zhang Yuan, Zhang Yu-mei

(Department of veterinary pharmacology and toxicology, College of Veterinary Medicine, Yangzhou University, Yangzhou Jiangsu 225009, China)

Abstract: OBJECTIVE Sodium Dehydroacetate (Na-DHA) with CAS number 4418-26-2 appeared broad-spectrum antibacterial and antifungal effect. It is widely used in food or feed, personal care products industries used as a preservative or fungicide. It is still involved in some pharmaceutical drugs such as oral liquid and soft medicinal extract. There is more potential chance of Na-DHA to coexist with other drugs. No information about the interaction of it and other drug up to now. The study was to determine whether Na-DHA influences the cytochrome P450 (CYP450) system involved in drug metabolism or drug-drug interaction. **METHODS** Rats were randomly divided into two groups (Na-DHA treated and control). They were administered 200 $mg \cdot kg^{-1}$ or physiological saline for consecutive five days. On day 6^{th}, rats were administrated cocktail drugs as probe substrates of the four CYP or liver samples were collected for determination of CYP450 content. The four-drug cocktail was composed of phenacetin (PHE), omeprazole (OME), chlorzoxazone (CHL) and dapsone (DAP) (all administered concomitantly) to phenotype for rat CYP1A2, -2D1/2, -2E1 and -3A1/2, respectively. The expression of CYP isoforms were detected by RT-PCR and ELISA methods. **RESULTS** Na-DHA displayed significantly decreased the metabolism of PHE, OME and DAP in female than in male rats and no clear change in metabolism of CHL, indicating Na-DHA remarkably inhibited the activity of CYP1A2, - 2D1 / 2 and - 3A1 / 2 of rats in female and no significantly effect on activity of CYP2E1. The level of CYP2D1/2 and CYP3A1 mRNA in male treated by Na-DHA were both significantly higher than that in male of control, or than that in female in Na-DHA group ($P<0.05$) in RT-PCR analysis. There were no significant effect of Na-DHA on CYP450 enzyme contents of rats by ELISA analysis. **CONCLUSION** Na-DHA was found significantly inhibited the activity of CYP1A2, - 2D1 / 2 and - 3A1 / 2 of rats in female than in male, may induce the expression of CYP2D1/2 and CYP3A1 mRNA in male.

Key words: Sodium Dehydroacetate; Cytochrome P450; Cocktail probes; Rats

Corresponding author: Zhang Yu-mei, Email: zym@yzu.edu.cn

T07-43-0004

Recent progress on the recognition and evaluation of cardiotoxicity in early drug discovery

Chuan Yu

(Yangtze River Pharmaceutical Group Guangzhou Hairui Pharmaceutical Co., Ltd)

Abstract: Cardiotoxicity is usually life-threatening and it is one of the most common reason that lead to the discontinuation of preclinical drug development and the withdrawals of marketed drug. Although the current application of cardiac ion channels and action potential detection, and electrocar·diogram (ECG) telemetry technology has greatly improved the accuracy of drug induced arrhythmia,the detection method and technology for drug induced other cardiotoxicities such as myocardial contraction dysfunction, myocardial ischemia, myocardial injury and heart failure are still insufficient. To decrease the cost of failure and to allow more safe drug candidates having the opportunity to enter the clinical trials and eventually benefit the patients, increasing the accuracy, specificity and sensitivity of cardiotox·icity prediction and evaluation and more integrative human-relevant platforms in early drug discovery are still in urgent need. This article reviews the recent progress and application on cardiotoxicity recog·nition and assessment such as in silico prediction, in vitro assays using human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) and adult human primary cardiomyocytes (CMs), ex vivo assays using full heart slices, and in vivo assays during preclinical study of drug candidates, in hoping to provide a clear understanding of cardiotoxicity, and comprehensive, systematic methods and strategies to predict cardiotoxicity.

Key words: Cardiotoxicity; CiPA; in silicon prediction; hERG; hiPSC-CMs

T07-47-0011

The rat subchronic inhalation toxicity evaluation of xylitol

Yushan Tian, Hongjuan Wang, Huan Chen, Yaning Fu, Shulei Han,Tong Liu, Hongwei Hou, Qingyuan Hu

(China National Tobacco Quality Supervision and Test Center, Zhengzhou, PR China)

Abstract: Xylitol, a pentahydroxy sugar alcohol, has reported to decrease gingival inflammation and nasopharyngeal pneumonia. It can inhibit different types of pathogenic bacteria, reduce the adhe·sion of oral streptococcus bacteria to teeth, prevent and treat dental caries. Moreover, it also showed to decrease the microbial load through anti inflammation, which indicates that xylitol may have potential application in respiratory diseases. Although some studies have reported the inhalation toxicity of xyli·tol, however, the longest period tested was only for 14 days. The inhalation toxicity of xylitol is insuffi·cient. Therefore, our study aims to explore the potential subchronic inhalation toxicity of xylitol accord·ing to the OECD TG 413. In our study, rats were randomly divided into the control group and different dosage groups (0.8 g·m^3, 2 g·m^3, 5 g·m^3), and exposed for 6 hours/day, 5 days/week for 13 weeks. At the end of the exposure or recovery period, clinical signs, mortality, body weight, food consumption, hematology, blood biochemistry, gross pathology, organ weight, and histopathology were examined. During exposure period, rats of treatment groups exhibited no mortality or remarkable clinical signs for in-life observations. Compared with the control group, rats in the exposure groups exhibited no signifi·

cant changes in body weight, organ mass, food uptake and respiratory physiology. After the exposure, several indicators of hematology and blood biochemistry showed significant difference, while during recovery period, these indicators were restored, suggesting that xylitol exerted no observable toxic effect on blood. For BALF analysis, except for LDH (0.8 g·m^3), which is not dose dependent or adverse effect. Infiltration of inflammatory cells were observed in different groups. The pathological changes were relieved after recovery for 28 days and in the high group, the inflammatory infiltration was still exerted. Therefore, it is concluded that the no observable adverse effect level (NOAEL) was 2 g·m^3 in rat exposed to xylitol for subchronic inhalation.

Key words: Xylitol; Aerosol inhalation; Subchronic toxicity; NOAEC

Corresponding author: Yushan Tian, E-mail: yushantian@126.com

T07-52-0002

Application of optimized inhalation exposure technology in safety evaluation of Indacaterol bromide inhalation powder spray mixed powder

Zi Fan[a], Bo Zhou[b], Yujia Liu[c], Wu Sun[b], Yuntao Fang[b], Hongguo Lu[d], Dongya Chen[e], Kuikui Lu[e], Xinyue Wu[e], Tian Xiao[e], Wenjing Xie[f], Qian Bian[a,e]

(a. The Key Laboratory of Modern Toxicology, Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing 211166, China; b. Jiangsu Center for Safety Evaluation of Drugs, Jiangsu Province Institute of Materia Medica, School of Pharmaceutical Sciences, Nanjing Tech University, Nanjing 211800, China; c. Medical School, Nanjing University, Nanjing 210093, China; d. Respirent Pharmaceuticals Co.,Ltd, No.5, Yuntu Road, Beibei District, Chongqing 400714, China; e. Institute of Toxicology and Risk Assessment, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210009, China; f. Key Laboratory of Environmental Medicine Engineering of Ministry of Education, School of Public Health, Southeast University, Nanjing 210009, China)

Abstract: Inhalation is a promising and challenging method in pharmaceutical and biological science research. Indacaterol glycopyrronium bromide inhalation powder (IM/GP mixed powder) is composed of two bronchodilators to treat chronic obstructive pulmonary disease (COPD). Besides, establishing stable conditions is critical in dynamic inhalation administration. The aim of this study is to build suitable inhalation conditions and then evaluate the pulmonary safety of this drug in Sprague-Dawley (SD) rats. In the research, through the coordination of the atomization flow, air pump flow, and scraper speed to realize the aerosols were stabilized at 200±20% mg/m3 and then rats were nose-only administered with the IM/GP mixed powder (Test), Ultibro (Positive Control) and Lactose-magnesium stearate mixed powder (Vehicle Control) at 2.6 mg/kg/day for 14 days. After exposure, hematology, inflammatory cytokines in rats Bronchoalveolar lavage fluid (BALF) and serum, histopathological examination were performed. Results showed that the stability of powder aerosols can realize under the atomization generation flow rate: 10 L/min, sampling flow rate: 2 L/min, system pumping capacity: 10 L/min and powder scraper speed: 8-10 L/min and there were no significant adverse effects on body weight, clinic signs, hematology and pathology in rats. Overall, the results suggested that the IM/GP mixed powder inhalation at the dose of 2.6 mg/kg/d can be reached when the aerosol concentration within the range of 200±20% mg/m^3, and there were no pulmonary toxicity effects in rats.

Key words: Inhalation exposure; Toxicity; Pulmonary delivery; Aerosol; Safety

Corresponding author: Qian Bian, E-mail: tox@jscdc.cn

T07-60-0010

Protective effect and mechanisms of diazoxide on pancreatic β cell in diabetes mellitus

Abstract: **OBJECTIVE** Type 2 diabetes mellitus is a common metabolic disease characterized by hyperglycemia, with high morbidity and mortality worldwide. Chronic hyperglycemia in diabetes can lead to dysfunction and failure of various organs, among these, diabetic nephropathy is one of the most severe complications of diabetes. To our knowledge, during insulin secretion, a window current is formed when the voltage-gated calcium channels in the β cells of the pancreas are stimulated. Window current of ion channels is the current that cannot be closed, and islet β cells in the state of window current for a long time will cause the apoptosis of islet β cells. Here, we aim to explore the potential protective role of diazoxide, a stimulator of ATP-sensitive potassium (KATP) channel,in diabetes mellitus and diabetic nephropathy. **METHODS** Streptozotocin (STZ) - induced diabetic rats were established, then the indexes such as triglyceride, cholesterol, body weight, and pathological results of the pancreas were detected. Besides, microalbuminuria (mALB), serum creatinine (SCr), urine creatinine (UCr), urea nitrogen (BUN), β2-microglobulin (β2-MG) and retinol-binding protein 4 (RBP4) were checked to analyze the effect of diazoxide in diabetic nephropathy, the pathological changes of rat kidney were analyzed by renal tissue section. Then, a series of experiments were designed based on the assumption that window current is a potential mechanism causing diabetes. Toluene sulfonylurea, an antagonist of KATP, was selected to make the window current model. Low, medium and high doses of nifedipine effectively inhibiting calcium ion channels and thus inhibiting window currents were conducted respectively to evaluate the effect of window current, which can be regulated by diazoxide in diabetes. **RESULTS** diazoxide could effectively control the increase of body weight, triglyceride, cholesterol and glycosylated hemoglobin in rats. Immunohistochemical (IHC) results of pancreatic tissue showed that the percentage of β cells was 50.41 ± 16.07% in the diazoxide group, significantly higher than the control group (7.55 ± 2.78%) ($P<0.05$). There were significant differences in mALB, SCr, UCr, BUN and RBP4 between diazoxide and the control group (all $P<0.05$). The time course of mALB abnormality in the diazoxide group was occurred 3 months later than that in the negative control group ($P<0.05$). Moreover, the diazoxide evaluation experiments of window current indicated that a 9-month window current was not enough to cause diabetes injuries in rats, although the stimulation effect could cause slight damage to the pancreatic β cells. **CONCLUSION** This study demonstrated that diazoxide can effectively control body weight, reduce blood lipids, and prevent pancreatic β cell apoptosis, thus playing a protective role on pancreatic β cells. In addition, diazoxide also significantly delayed the progression of diabetic nephropathy.

Key words: diazoxide; pancreatic β cell; diabetes mellitus; diabetic nephropathy; window current

T07-61-0005

Role of mitochondrial Cx43 nitrosylation in GSNO-induced *in vitro* differentiation of mouse ES cells into cardiomyocytes

Qi Jia-yu, Chen Li-ting, Shao Ying, Zhu Dan-yan

(Institute of Pharmacology and Toxicology, College of Pharmaceutical Sciences, Zhejiang University, Zhejiang Hangzhou, 310058)

Abstract: The model of embryonic stem (ES) cell differentiated into cardiomyocytes is being con·

sidered as an alternative system to study the highly simulated embryonic myocardial development *in vitro*. Mitochondrial-dependent energy pathway, as is known to all, plays a dominant role in initiating the differentiation of ES cells towards cardiomyocytes. Mitochondrial Cx43 (mtCx43) is closely related to mitochondrial function and energy metabolism. S-nitrosoglutathione (GSNO), considered vital to S-nitrosylation of proteins[4], has been recognized to be of fundamental importance to the regulation of various biological processes, including transcription, cell growth, differentiation and apoptosis. In our previous studies, we found that S-nitrosylation of 104 protein molecules were promoted by GSNO during the differentiation of ES cells into cardiomyocytes. However, whether mtCx43 is involved in GSNO-induced myocardial differentiation of ES cells via S-nitrosylation has not been fully elucidated. In this study, we showed that GSNO significantly up-regulated mitochondrial transmembrane potential, ATP production, mtCx43 hemichannel permeability, ROS concentration and respiratory chain complex Ⅰ activity in embryoid bodies (EBs). Additionally, it was found that S-nitrosylation of mtCx43 was increased after the adoption of GSNO. Furthermore, overexpression of mtCx43 contributed to the promotion of GSNO on mitochondrial maturation and ES cells differentiation into cardiomyocytes, while overexpression of mtCx43C271A did not. Pretreatment of Gap19, mtCx43 hemichannel-specific blocker, followed by GSNO, revealed a remarkable decrease in expression of Mfn2, inhibiting the mitochondrial maturation and its functions. Meanwhile, the pro-myocardial differentiation effect of mtCx43 S-nitrosylation was also antagonized by Gap19, as is seen from the reduced beating rate of EBs, the decreased expression of α-actinin and the lessened formation of sarcomere structures. The above results validated the significance of opening mtCx43 hemichannels on GSNO-induced mtCx43 S-nitrosylation-mediated mitochondria regulation and cardiomyogenesis from ES cells. In conclusion, GSNO-activated S-nitrosylation of mtCx43 could regulate the mitochondria function in EBs by boosting the opening of mtCx43 hemichannels, thus participating in the targeted differentiation of mouse ES cells into cardiomyocytes.

Key words: S-nitrosoglutathione; embryonic stem cells; cardiomyocytes; S-nitrosylation; mitochondrial connxin 43

Corresponding author: Zhu Danyan, E-mail: zdyzxb@zju.edu.cn

T07-61-0009

Vancomycin-induced cytotoxicity involves the generation of reactive oxygen, mitochondrial apoptotic and JNK pathways in HEK293 cells

Yue Liu, Shusheng Tang, Chongshan Dai

(Department of Basic Veterinary Medicine, College of veterinary medicine, China Agricultural University 100193, P. R. China)

Abstract: Vancomycin is a glycopeptide antibiotic, which is often used as the last defense against pan drug-resistant Gram-positive bacteria. Nephrotoxicity is one of vancomycin-induced adverse effects in patients, however, the precise molecular mechanism is unclear. This study aims to investi·gate the molecular mechanism using a HEK293 cell line. Our results showed that vancomycin treat·ment significantly decreased the cell viability of HEK293 cells in time-dependent and dose-dependent manner. Meanwhile, vancomycin treatment significantly induced the production of ROS, decreased the mitochondrial membrane potential, upregulated the level of anti-apoptotic Bax protein expression and downregulated the level of pro-apoptotic Bcl-2 protein expression. Vancomycin treatment also significantly upregulated the levels of cleaved-caspase 3 and cleaved-PARP-1 protein expressions, finally

lead to apoptotic cell death in HEK293 cells. Furthermore, vancomycin treatment significantly increased the expression of p-JNK protein in a dose-dependent manner, and inhibition of JNK expression signifi·cantly decreased the expression of HO-1, the production of ROS, and reduce vancomycin treatment - induced cell death. In conclusion, our study shows that vancomycin induced-cytotoxicity in HEK293 cells involved the production of ROS, activation of mitochondrial apoptotic pathway and JNK pathway. Our study highlights the understanding the molecular mechanism of vancomycin-induced nephrotoxicity and provides information for nephron-protection agents.

Key words: Vancomycin; Mitochondrial apoptotic pathway; JNK pathway; Nephrotoxicity

Corresponding author: Chongshan Dai, E-mail: daichongshan@cau. edu. cn; Shusheng Tang, tssfj@163.com

T07-63-0003

Target animal safety evaluation of a novelformation of moxidectin for sheep

Xiangchun Ruan[1,2], Chengbo Yu[1], Jidong Hu[1], Youwei Wang[1], Xiaoling Deng[1], Ying Sun[1], Meiling Tan[1], Qing Wei[3]

(1. Laboratory of Veterinary Pharmacology and Toxicology, College of Animal Science and Technology, Anhui Agricultural University, Hefei, Anhui Province, 230036, China; 2. Anhui Province Key Laboratory of Veterinary Pathobiology and Disease Control, Hefei Anhui Province 230036, China; 3. College of Eco-Environmental engineering, Qinghai University, Xining, Qinghai Province 810016, China)

Abstract: Moxidectin is useful for controllingparasitic diseases in humans and animals.The safety profile of moxidectin in-situ hydrogel, a novel formationfor the treatment and prevention of internal and external parasites, was evaluated inHu sheep. In the safety studies, Hu sheep were dosed with multi·ples of the exposure dose (1X, 3X and 5X) one times at one-day intervals. The sheep were treated with a continuous subcutaneous administration of moxidectin gel for 3 days.Theblood samples(antico·agulantandnon-anticoagulant)and urine sampleswere collected in the morning at day 0, 1, 3 and 10 of each group. Meanwhile, the Weight, body temperature, respiration and pulse rate of sheep were mea·sured. The sheep selected randomly from control (4), 1Xgroup (4), 3X group (6) and 5X group (10) wereslaughtered. Physical examinations and clinical pathology analyses were performed throughout the studies, followedby necropsy and detailed histopathological evaluation in studies. The results showed that the moxidectin gel at 5 times the clinically recommended dose did not showany adverse effects on the tested sheep. No significant pathological changes were observed, either. In conclusion, moxidectin in-situ hydrogel wasdemonstrated safe in Hu sheep following repeated injection administra·tions and very highinjection doses of moxidectin were well tolerated.

Key words: Moxidectin; Safety evaluation; in-situ hydrogel; Novel formation; Hu sheep

Corresponding author: Qing Wei, E-mail: xwq3519@sina.com

T07-63-0012

Recent progress on the recognition and evaluation of cardiotoxicity in early drug discovery

Chuan Yu

(Yangtze River Pharmaceutical Group Guangzhou Hairui Pharmaceutical Co.,Ltd)

Abstract: Cardiotoxicity is usually life-threatening and it is one of the most common reason that lead to the discontinuation of preclinical drug development and the withdrawals of marketed drug. Although the current application of cardiac ion channels and action potential detection, and electrocardiogram(ECG)telemetry technology has greatly improved the accuracy of drug induced arrhythmia, the detection method and technology for drug induced other cardiotoxicities such as myocardial contraction dysfunction, myocardial ischemia, myocardial injury and heart failure are still insufficient. To decrease the cost of failure and to allow more safe drug candidates having the opportunity to enter the clinical trials and eventually benefit the patients, increasing the accuracy, specificity and sensitivity of cardiotoxicity prediction and evaluation and more integrative human-relevant platforms in early drug discovery are still in urgent need. This article reviews the recent progress and application on cardiotoxicity recognition and assessment such as in silico prediction, in vitro assays using human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) and adult human primary cardiomyocytes (CMs), ex vivo assays using full heart slices, and in vivo assays during preclinical study of drug candidates, in hoping to provide a clear understanding of cardiotoxicity, and comprehensive, systematic methods and strategies to predict cardiotoxicity in human.

Key words: Cardiotoxicity; CiPA; in silicon prediction; hERG; hiPSC-CMs

T07-63-0013

Safety evaluation of ponazuril suspension in target animal suckling piglets

Wang Zhen[1], XU Qinse[2], ZHANG Wei[2], GAO Yanjun[1], NIE Ya[1], HUO Haoyuan[1], XIAO Wenhua[2], PU Shijin[1]

(1. Yangzhou University; 2. Jiangsu Agri-animal Husbandry Vocational College)

Abstract: OBJECTIVE To study the safety of ponazuril suspension on target piglets, and to provide basis for clinical safe drug use. **METHODS** 32 sucking piglets aged 3-5 day-old were divided into 4 groups with 8 piglets (half male and half female) in this experiment. Swine safety studies were performed with multiple dose levels of the drug given. The dose levels of 5% ponazuril suspension included 0 (for the control group), 1× (15 mg·kg^{-1} for test group Ⅰ), 3× (45 mg·kg^{-1} for test group Ⅱ), and 5× times (75 mg·kg^{-1} for test group Ⅲ). The tested drug was orally administered 3 consecutive times with each interval of 3 days. The control group was given 5×, the maximum recommended dose of normal saline. The experiment lasted for 17 days; during which periodic weighting, clinical observation, and physical examinations were performed; hematology, blood and serum chemistries, as well as gross autopsy and histopathological examination were carried out in predetermined periods of time. **RESULTS** The results showed that ,until the end of the trial, there had been no death of the piglets, all of which were clinically normal. No pathologic lesions were found at autopsy for all the test animals.

The histopathological examinations in the 5 × recommended dose group and the control group did not identify any abnormal tissue lesions. **CONCLUSION** The oral administration of 5% ponazuril suspen·sion was of greater safety for piglets according to the recommended dosing schedule.

Key words: ponazuril suspension; target animal safety; suckling piglets

T07-63-0014

Mapping used in cardiac safety evaluation

Gongxin Wang[1], Guoliang Hao[1,2]

(1. Henan SCOPE Electrophysiology Research Institute Co., LTD; 2.Department of Physiology, Anatomy and Genetics, University of Oxford, UK, OX1 3PT)

Abstract: During new drug development, timely and effective preclinical cardiac safety evaluation is an important guarantee for the advance of drug candidates. The existing pharmacologic evaluation of cardiac safety is mainly based on the framework guideline S7A/B document formulated by the Interna·tional Coordination Committee for Registration technology of Medicinal Products for Human Use (ICH). However, with the wide application of the guideline document, a series of problems have been exposed in this evaluation method. Currently in the critical stage of S7A/B revision, the application of Multi-Elec·trodes Mapping technology can make up for many key issues in cardiac safety evaluation and provide guarantee for drug candidates to move forward.

Previously, preclinical cardiac safety evaluation of drugs was mainly achieved by patch clamp and electrocardiogram (ECG) recording. Patch clamp technology is the "gold standard" for studying ion channel signals, and plays an important role in cell and ion channel level research. HERG channel detection is recorded in whole-cell level, whereas new studies suggest that heterogeneity of action potential duration or repolarization dispersion is an important basis for arrhythmias. QT recorded by body surface ECG is less sensitive to repolarization heterogeneity. Cardiac mapping could play an irre·placeable role in the detection of cardiac heterogeneity. Mapping technology can play an important role in the simultaneous recording of multi-point electrical signals, the study of electrical conduction charac·teristics and action potential duration heterogeneity in cell to cell and whole myocardial tissue, which makes up for the insufficiency of patch clamp and ECG technology in tissue level. The combined appli·cation of patch clamp, ECG and mapping technology can make the cardiac safety evaluation of drugs more thorough and comprehensive.

Mapping can record multi-point electrical signals synchronously on the whole heart or part of the heart tissue, and monitor action potential activity frequency, pacemaker position, conduction direction, conduction velocity, conduction dispersion, repolarization dispersion, QT interval dispersion, etc. Map·ping technology can not only be used in anti-arrhythmic drug screening, arrhythmia mechanism research, cardiac electrophysiological research, etc., but also in cardiac safety evaluation of drugs.

Key words: Mapping; cardiac safety evaluation; drug development

T08-76-0002

Prediction of sting potential of cosmetic materials based on *in vitro* TRPV1 channel activation

Yin Qinfei[b], Zhou Lihong[a], Guo Yiguang[b], Cui wenguang[a], Chen Tian[a]

(a. Shanghai Municipal Center for Disease Control and Prevention, Shanghai 200336;

b. Research&Development Center, Shanghai Jahwa United Co., Ltd., Shanghai 200336)

Abstract: To investigate the potential sting risk evoked by various alkanolamides used as cosmet·ic ingredients, human, rabbit, and rat TRPV1-stable transfected SHSY5Y cell lines were constructed successfully. The EC50 values of dihydrocapsaicin in human, rabbit and rat TRPV1 overexpression cell lines were 1.35 nM, 354.7 nM and 0.42 nM, respectively. The EC50 values of cocamide MEA were 34.37 μM, 2251 μM and 28.35 μM in aforementioned cell lines, respectively. Similarly, cocamide MIPA, lauramide MEA and cocamide DEA activated two or three of human, rabbit and rat TRPV1 receptors. Nevertheless, palmitamide MEA and lactamide MEA could not activate any of the three TRPV1 recep·tors. It was very interesting that alkanolamides able to activate TRPV1 receptors did not induce vascu·lar reaction in choriollantoic membrane assay at the same dose, which is often used to evaluate eye irri·tation potential. It can be concluded that the sensitivities of mammalian TRPV1 receptors are different and it seems that human and rat TRPV1 receptors are more sensitive, what is more, some alkanol·amides are proved to be of agonist activity for TRPV1 receptors, and should be paid more attention when used in cosmetic and personal care products for babies and people with sensitive skin.

Key words: Cosmetic; Sting; TRPV1 receptor; Alkanolamides

Corresponding author: Chen Tian, E-mail: chentian@scdc.sh.cn

T08-82-0001

Apply Threshold of Toxicological Concern (TTC) Approach as a Tool to Help Chemical Analysis and Safety Assessment of Baby Diapers

Tingting Zhu[1], Kara Woeller[2], Joan Abbinantenissen[2], Susan Felter[2],

Vincent Sica[2], Carrie Spitzmueller[2], Kady Krivos[2]

(1. P&G, Beijing, China; 2. P&G, Cincinnati, OH, United States)

Abstract: The Threshold of Toxicological Concern (TTC) has been developed to assess low level exposures to compounds with limited toxicology data. In safety assessment, if human exposure to a substance is below the TTC value, the likelihood of adverse effects is considered to be very low. The TTC approach has been well-established and accepted by regulatory bodies in different areas and achieved breakthrough in China recently. In particularly, genotoxic TTC was included in 9306 Control Guideline of Genotoxic Impurities of Chinese Pharmacopeia 2020. In Apr of 2021, TTC approach was officially accepted as a method in Technical Guidelines for Cosmetic Safety Assessment. TTC provides a pragmatic way to assure safety while minimizing the time and efforts spent on assessing low level ex·posures without generating additional toxicity data which reduces unnecessary animal tests. On the oth·er hand, TTC can also be used as a threshold tool to define analytical detection limits for constituents, extractables and contaminants. Recently, the use of TTC has broadened to include the establishment

of lower limits of quantitation sufficient to detect constituents of botanicals or complex mixtures. US FDA included TTC in the ISO 10993 standards for biocompatibility for medical device evaluation, where TTC was used as an exposure threshold of extractables and/or leachables for some biological endpoints. Based on TTC, analytical detection limit is established to identify constituents that would exceed the TTC exposure limit and require further evaluation. For baby diaper products, analytical chemistry is leveraged to enable targeted analysis of low-level constituents that could transfer from the diaper to the skin under physiologically relevant in-use wear conditions in order to refine human exposure and to augment supplier disclosure information to enable a comprehensive safety assessment. TTC was leveraged to develop an analytical threshold of extractables of raw materials and rewet constituents of finished diapers for chemical characterization. The peaks above TTC-based Limit of Detection (LOD) were quantified and identified and more appropriately assessed within physiological relevant consumer exposure. This work demonstrates a case study on the use of TTC for this threshold application approach.

Key words: TTC; threshold of toxicological concern, baby diapers; chemical analysis, detection limit

Corresponding author: Tingting Zhu, E-mail: zhu.tt.1@pg.com

T10-38-0002

Exposure to cadmium induces neuroinflammation and impairs ciliogenesis in hESC-derived 3D cerebral organoids

Yan Huang[a], Qian Bu[a,b]

(a. West China School of Public Health and West China Fourth Hospital, SichuanUniversity, Chengdu 610041, China; b. National Chengdu Center for Safety Evaluation of Drugs, West China Hospital, Sichuan University, Chengdu 610041, China)

Abstract: Cadmium (Cd) is an environmental heavy metal toxicant with central nervous system toxicity and has a greater negative impact on fetal neurodevelopment. However, the causative mechanisms for the neurodevelopmental toxicity of Cd have remained unclear. The human cerebral organoids can better mimic the three-dimensional structure of the early fetal nerve tissue, which can be used to study the developmental neurotoxicity under the condition of maternal exposure to Cd. Our study identified that Cd exposure specifically induced apoptosis in neurons and inhibited the proliferation of neural progenitor cells, but neural differentiation was not significantly affected in cerebral organoids. Cd exposure also elicited overexpression of GFAP, a marker of astrocytes and resulted in IL-6 release. This study revealed that mineral absorption was significantly disturbed with metallothioneins expression up-regulation. Moreover, we found Cd exposure inhibited cilium-related gene ex- pression and reduced ciliary length with increasing dose. In conclusion, our study has shown that Cd exposure regulated neural cell proliferation and death, induced neuroinflammation, enhanced metal ion absorption, and impaired ciliogenesis, which hinder the normal development of the fetal brain.

Key words: Cadmium; Cerebral organoid; Mineral absorption; GFAP; Cilium

Corresponding author: Qian Bu, E-mail: buqian7978@scu.edu.cn

T10-48-0001

Nanomaterials-induced cardiotoxicity and emerging toxicity assessment techniques

Cheng Yanping[a], Chen Zaozao[b], Yang Sheng[a], Liu Tong[a], Yin Lihong[a], Pu Yuepu[a], Liang Geyu[a]*

(a. Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu, China P.R. 210009; b. State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing, Jiangsu, China P.R. 210096)

Abstract: The extensive production and use of nanomaterials have resulted in the continuous release of nano-sized particles into the environment, and the health risks caused by exposure to these nanomaterials in the occupational population and the general population cannot be ignored. Studies have found that particle exposure is closely related to cardiovascular disease. In addition, there have been many reports that nanomaterials can enter the heart tissue, accumulate and then cause damage. Therefore, in the present article, literature related to nanomaterials-induced cardiotoxicity in recent years was collected from the PubMed database, and then organized and summarized to form a review. This article mainly discusses heart damage caused by nanomaterials from the following three aspects: Firstly, we summarize the research progress on the cardiotoxicity of nanomaterials widely present in the environment, mainly involving nano-TiO2, nano-SiO2, nano-iron oxide, nano-silver, carbon nanotubes, etc. Secondly, we discuss in depth the possible underlying mechanism of the damage to the heart caused by nanoparticles. And oxidative stress damage, mitochondrial damage, inflammation and apoptosis have been found to be key factors. Finally, we summarize the current research models used to evaluate the cardiotoxicity of nanomaterials, and highlight reliable emerging technologies and in vitro models that have been used for toxicity evaluation of environmental pollutants in recent years, with special attention to the huge application prospects of organoids and organ-on-chips.

Key words: Cardiotoxicity; Environmental toxicology; Nanomaterials; Mechanism; Organ-on-chips

Corresponding author: Geyu Liang, E-mail: 2250152552@qq.com

T10-54-0004

Development of tissue-engineered blood vesselsand heart-on-a-chip for disease modeling and toxicological studies

Zaozao Chen[1,2], Zhongze Gu[1,2]

(1. School of Biomedical Science and Engineering, Southeast University, Nanjing, Jiangsu 210096, China; 2. Institut of Medical Devices (Suzhou) Southeast University, JinFeng Rd #8, Suzhou, Jiangsu 215163, China)

Abstract: Advances in vascular tissue engineering offer an alternative to the inaccessibility of human blood vessels. Human cell-based tissue-engineered blood vessels (TEBV) have been demonstrated as predictive *in vitro* models to facilitate drug toxicity/efficacy testing and disease modeling. Recent studies demonstrated the feasibility of studying drug responses in those TEBVs. However, another important feature of blood vessel function – leukocyte-endothelium interactions – has not been carefully characterized in these researches. As a new research area, there are very limited studies have

tackled the leukocytes interaction with TEBV. Most of existing works were using so called "end-stage" observations. The most desired information-the dynamics of leukocyte adhesion and trans-endothelial migration into the TEBV-has yet to be acquired.

In this work, we developed a perfusion and imaging chamber and deep penetrating two-photon la·ser microscopy to perform a dynamic observation of leukocyte and TEBV interaction. With this setup, we successfully captured the leukocyte behavior inside of TEBV under flow conditions and observed leukocyte-TEBV endothelium interactions such as leukocyte adhesion, migration, and trans-endothelial migration. We further investigated drug-induced alternation in leukocyte behavior change. Anti-inflam·matory drugs attenuated this endothelium activation in TEBV and significant reduced monocyte adhe·sion and transmigration. We further developed branched TEBVs (brTEBV) for atherosclerosis modeling and monitored the endothelial cell activation and ox-LDL accumulation in bifurcation area in brTEBV. Lastly, we generated Heart-on-a-Chip with iPSC derived cardio-myocyte organoids and tested drug effectiveness and toxicity. All together, we developed multiple technologies and microphysiological sys·temsin this research. This work is the first demonstration of real-time imaging of immune cellsinterac·tion with TEBV, itpaves the road for studying the mechanisms of immune cell-related vascular and heart diseases, and provides multiple organs-on-a-chip system for drug-screenings and toxicological studies.

Key words: Organs-on-a-Chip; Tissue Engineered Blood Vessel; Heart-on-a-Chip; Drug Screening

Corresponding author: Zaozao Chen, E-mail: 101012282@seu.edu.cn

T10-96-0003

The joint effect of polystyrene microplastics and bisphenol A on the liver organoids: implication of hepatotoxicity and lipotoxicity

Wei Cheng[a], Yue Zhou[a], Yan Li[a], Hengyi Yu[a], Yichun Xie[a], Hui Wang[a,b,c], Yan Feng[a], Yan Wang[a,d]

(a. School of Public Health, Shanghai Jiao Tong University School of Medicine, China; b. State Key Laboratory of Oncogenes and Related Genes, Shanghai Jiao Tong University School of Medicine, China; c. Center for Single-Cell Omics,Shanghai Jiao Tong University School of Medicine, China; d. The Ninth People's Hospital of Shanghai Jiao Tong University School of Medicine, China)

Abstract: Microplastic particles (MP) has been detected in the environment widespread. Inevitably, human beings are exposed to MP via multiple routines, together with various kinds of environmental pollutants being absorbed surround the surface of MP. Many studies suggest the liver is a potential tar·get organ, but currently less is known regarding the MP on human liver. In this study, we used a novel in vitro 3D model, the liver organoids (LOs) generated from human pluripotent stem cells, as an alterna·tive model to the human liver, to explore the adverse biological effect of 1μm polystyrene-MP (PS-MP) and its joint toxic effect with bisphenol A (BPA).

When the LOs were exposed to 0.25, 2.5 and 25 $\mu g \cdot mL^{-1}$ PS-MP (the lowest one was relevant to the environmental concentrations). The potential mechanisms of PS-MP induced hepatotoxicity and lipotoxicity, in aspects of cytotoxicity, expressionof key molecular markers, ATP production, alteration in lipid metabolism, ROS generation, oxidative stress and inflammation response, were determined. Spe·cifically, it has been firstly observed that PS-MP could elevate the hepatic HNF4A and CYP2E1.

Further, when non-cytotoxic BPA (8 $ng \cdot mL^{-1}$) were exposed combined with 0.25 $\mu g \cdot mL^{-1}$ PS-MP, significant higher levels of lipid accumulation, IL-6 secretion, aspartate amino transferase (AST)

release and malondialdehyde (MDA) could be observed in PS-MP + BPA group than those of PS-MP group and BPA group individually, while no significant change in cytotoxicity was found.Based on these findings, the potential adverse outcome pathways (AOPs) relevant to PS-MP were proposed. Further studies are anticipated to validate the hepatotoxic molecular mechanism of PS-MP and PS-MP+ BPA, to investigate their joint toxic effect for human beings.

Key words: Microplastics; Bisphenol A; Human embryonic stem cells; Hepatotoxicity; Joint toxic effect

Corresponding author: Yan Wang, E-mail: wangyan@shsmu.edu.cn

T10-96-0004

Construction of a high fidelity epidermis-on-a-chip for *in vitro* irritation evaluation

Jing Zhang

(Institute of Medical Devices (Suzhou), Southeast University Suzhou 215010 PR China)

Abstract: 3D skin equivalents have been increasingly used in pharmaceutical and cosmetic industries as in vitro models in safety evaluation or scientific research. We grew and differentiated human keratinocytes within a specially designed microfluidic chip to construct an epidermis-on-a-chip system. After 14-day′s culture at the air-liquid interface (ALI), the constructed epidermis-on-a-chip demonstrated histological features similar to those observed in normal human epidermis: a proliferating basal layer and differentiating spinous, granular and cornified layers, especially the transepithelial electrical resistance value reached 3 kΩ·cm^2 and prevented more than 99% of cascade blue-607 Da permeation owing to the enhanced barrier function. Additionally, the epidermis-on-a-chip can distinct all the 10 known toxins and non-toxins (according to OECD 439) in irritation measurement by MTT assay. This high fidelity epidermis-on-a-chip could be a potential alternative to animal and be applied in preclinical studies for toxicological evaluation.

Corresponding author: Jing Zhang, E-mail: zhangjing@i-bmd.org

T11-10-0010

Advances of reproductive toxicity of main nanomaterials to *Caenorhabditis elegans* and influencing factors

Yongshuai Yao, Meng Tang

(Key Laboratory of Environmental Medicine and Engineering, Ministry of Education; School of Public Health, Southeast University, Nanjing, Jiangsu 210009, China)

Abstract: In recent decades, nanotechnology has developed rapidly; however, the safety evaluation of nanomaterials has lagged behind the development of the application. The nematode *Caenorhabditis elegans* (*C. elegans*) provides nanotoxicology an efficient alternative method and has been widely used to study the toxicity of nanomaterials from the whole animal level to the single-cell level, especially in the reproductive toxicity. In this review, we have discussed the reproductive toxicity of common nanomaterials to *C. elegans*, including metal-based nanomaterial (e.g., silver nanoparticles (NPs), gold NPs, zinc oxide NPs, copper oxide NPs), carbon-based nanomaterial (e.g., graphene oxide, multi-walled carbon

nanotubes, fullerene nanoparticles), polymeric NPs, silica NPs, quantum dots. We focused on by what mechanism do these nanomaterials affect *C. elegans* reproduction. The above provide insights into the toxicity of existing nanomaterials to the human reproductive system. In addition, for future optimization of nanomaterials, we also summarized how the physicochemical properties (e.g., size, charge, surface modification, shape) of nanomaterials influence their reproductive toxicity. Overall, using *C. elegans* as a platform to develop rapid detection techniques and prediction methods for nanomaterial toxicity is expected to reduce the gap between biosafety evaluation of nanomaterials and their application.

Key words: *C. elegans*; Nanomaterial; Reproductive toxicity; Influence factors; Signaling Pathways; Multi-generation

Corresponding author: Meng Tang, E-mail: tm@seu.edu.cn

T11-10-0014

Cadmium telluride quantum dots induced redox imbalance and ferroptosis of macrophages

Liu Na, Wei Ting-ting, Zou Ling-yue, Bai Chang-cun, Huang Xiao-quan, Hu Yuan-yuan, Wang Zhi-hui, Li Bin-jing, Yao Yong-shuai, Wang Xiao-li, Niu Yi-ru, Qiao Dong, Li Cong-cong, Tang Meng

(Key Laboratory of Environmental Medicine & Engineering, Ministry of Education; School of Public Health, Southeast University, Nanjing 210009, P.R. China)

Abstract: Cadmium telluride quantum dots (CdTe QDs) caused inflammatory reactions in organisms, and the intermediate mechanism is worthy of in-depth exploration. When RAW264.7 macrophages were exposed to 1 μM CdTe QDs for 24 h, the content of reactive oxygen and nitric oxide increased, and CdTe QDs disturbed glutathione (GSH) homeostasis and changed the activity of antioxidant enzymes. After treatment, the macrophages undergo lipid peroxidation with an increase of malondialdehyde and lipid peroxidation, and cytosolic Fe2 + increased. Additionally, changes in proteins that regulated GSH homeostasis and lipid metabolism were confirmed. CdTe QDs reduced the levels of SLC7A11 and GPX4 but increased the level of NRF2. Similarly, CdTe QDs reduced the antagonistic SCD level, increased their expression of ACSL4, NOX4, and COX2. Then, the decrease in the cell viability of macrophages inhibited by CdTe QDs can be rescued by the specific and selective inhibitor of ferroptosis Ferrostatin-1 and Liproxstain-1 HCl, and iron chelate Deferoxamine mesylate, so 1 $\mu mol \cdot L^{-1}$ CdTe QDs educed ferroptosis of macrophages. These findings provided useful insight into understanding and reducing the toxicity of CdTe QDs from control ferroptosis, and shared valuable information for the safe use of QDs.

Key words: CdTe QDs; Nanotoxicology; Macrophages; Ferroptosis; Oxidative stress

Corresponding author: Tang Meng, E-mail: tm@seu.edu.cn

T11-14-0013

Silver nanoparticles induced ferroptosis of HT22 cells and its mechanism

Shuyan Niu, Xiaoru Chang, Mengting Shang, Wenli Zhang, Yuying Xue*

(Key Laboratory of Environmental Medicine and Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing 210009, China)

Abstract: Silver nanoparticles (AgNPs) have excellent physicochemical properties and are widely used in various antibacterial products. There are many opportunities for human body exposure. Ferroptosis is a newly discovered, iron-mediated cell death that is involved in the development and progression of some neurological diseases. The purpose of this study is to explore ferroptosis induced by AgNPs on mouse hippocampal neuron cell line (HT22) and its mechanism. Cell viability was determined by CCK-8 analysis. Total GSH was measured by a commercial GSH kit. MDA level was measured by a commercial MDA kit. The protein expression levels related ferroptosis was measured by Western blotting assay. The results showed that (1) AgNPs-induced cell death involved ferroptosis. Ferroptosis inhibitors desferrioxamine (DFO) and ferrostatin-1 (Fer-1) could significantly inhibit the cytotoxicity caused by AgNPs; (2) AgNPs could cause iron homeostasis disorder in HT22 cells by affecting the expression levels of ferritin heteropolymers ferritin light chain (FTL), ferritin heavy chain (FTH) and iron importers transferrin receptor protein 1 (TFRC); (3) AgNPs-triggered ferroptosis was related to the increase of lipid peroxide MDA, the depletion of reduced glutathione (GSH), the down-regulation of glutathione peroxidase 4 (GPx4) and the up-regulation of the system Xc-transporter subunit cystine/glutamate transporter (SLC7A11). Our data provided a new mechanism basis for ferroptosis as a new cell death phenotype induced by AgNPs. The deeper mechanism and its relationship with the neurotoxicity of AgNPs need to be further studied.

Key words: Silver Nanoparticles; Ferroptosis; Lipid Peroxidation

Corresponding author: Yuying Xue, E-mail: yyxue@seu.edu.cn

T11-14-0023

Comparison of plasma borne bioactivity increase lung cancer risk in Chronic carbon black VS. diesel exhaust particles exposure

Jianzhong Zhang, Xin Li, Jinglong Tang, Yuxin Zheng
(School of Public Health, Qingdao University, Qingdao 266071, China)

Abstract: The health effect of occupational nanoparticles exposure has attracted great attention, especially in the respiratory and circulation system. The most available mechanism information about nanoparticle toxicity is from *in vivo* or *in vitro* studies. However, few studies concerned the nanoparticle's real-ambient exposure causing systemic change and further affecting the target organ. Here, we used an ex vivo biosensor assay to investigate the transcriptome change of primary bronchial epithelial cells after treatment with the plasma from workers with long-term occupational nanoparticles exposure history. Characteristics of the systemic environment after exposure to two kinds of nanoparticles and their effects on cellular function were assessed in this study. Based on biosensor assay and transcriptome sequencing, we found the effect of systemic environment on cells after carbon black exposure was an inflammatory response, which mainly activates cell cycle-related pathways. However, the activated pathway in the diesel exhaust particle group was mainly related to autoimmunity. After exposure to both nanoparticles, the systemic environment could activate cancer-related pathways like epithelial-mesenchymal transition, hypoxia, TNF-α signaling via NF-κb. Furthermore, the prognosis models based on hallmarks of nanoparticles exposure were constructed by COX regression to reveal the rela·

tionship between nanoparticles exposure and lung cancer. The hub genes in the carbon black group (CDC20 and PLK1) or diesel exhaust particle group (FOS and JUN) and their correlation with the sys·temic environment were uncovered by constructing the protein-protein interaction network and calculat·ing the Spearman correlation coefficient. Inflammatory cytokines, particularly CRP, influenced the expression of CDC20 and PLK1, whereas FOS and JUN were affected by polycyclic aromatic hydrocar·bon metabolites. Our results demonstrate that the systemic environment after exposure to carbon black and diesel exhaust nanoparticles will have different effects on cell function in dissimilar ways. After exposure to both nanoparticles, the systemic environment could activate cancer-related pathways, increasing the risk and affecting the prognostic of lung cancer. These attempts might provide a further understanding of the indirect effect of occupational inhaled nanoparticles exposure on pulmonary.

Key words: Nanotoxicology; Carbon Black; Diesel exhaust particles; Systemic environment; Bio·sensor

Corresponding author: Jinglong Tang, E-mail: tangjinglong@qdu. edu. cn; Yuxin Zheng, E-mail: yxzheng@qdu.edu.cn

T11-16-0001

Intercellular transfer of mitochondria via tunnelling nanotubes protects against cobalt nanoparticle-induced neurotoxicity and mitochondrial damage

Fuli Zheng[1,2,3], Zhousong Luo[2,3,4], Xinpei Lin2[,3], Wei Wang[2,3], Michael Aschner[5], Wenya Shao[1,2,3], Guangxia Yu[1,2,3], Zhenkun Guo[2,3], Siying Wu[2,3,6], Huangyuan Li[1,2,3]

(1. Department of Preventive Medicine, School of Public Health, Fujian Medical University, Fuzhou 350122, China; 2. Fujian Provincial Key Laboratory of Environmental Factors and Cancer, School of Public Health, Fujian Medical University, Fuzhou 350122, China; 3. The Key Laboratory of Environ- ment and Health, School of Public Health, Fujian Medical University, Fuzhou 350122, China; 4. Fujian Key Laboratory of Molecular Neurology, Fujian Medical University, Fuzhou 350005, China; 5. Depart- ment of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461, USA; 6. De- partment of Epidemiology and Health Statistics, School of Public Health, Fujian Medical University, Fu- zhou 350122, China)

Abstract: The broad applications of cobalt nanoparticles (CoNPs) have raised increasing concern regarding their potential toxicity. However, the underlining mechanism / s of their potential toxicity has yet to be characterized. Here, we first evaluated neurotoxicity and mitochondrial toxicity of CoNPs with cell viability kit-8 and lactate dehydrogenase assays. CoNPs induced oxidative stress, as shown by the generation of reactive oxygen species (ROS) with the involvement of hypoxia induced factor 1 alpha. Moreover, CoNPs led to mitochondrial ROS generation as determined by MitoSOX probe. Interestingly, exogenous mitochondria and tunneling nanotubes (TNTs) were observed between neurons and astro·cytes upon CoNPs exposure by scanning and transmission electron microscopy, suggesting intercellu·lar mitochondrial transfer, a newly discovered crosstalk phenomenon between cells. We further con·firmed by confocal microscopy that TNTs were utilized for this transfer. Moreover, we illustrated that the inhibition of TNTs intensified apoptosis triggered by CoNPs. Our study demonstrates, for the first time, that the inhibition of intercellular mitochondrial transfer via TNTs aggravates CoNPs-induced cellular and mitochondrial toxicity in neuronal cells, implying a novel intercellular protection mechanism.

Key words: Cobalt nanoparticles (CoNPs); Mitochondrial transfer; Tunneling nanotubes (TNTs); Neurotoxicity; Astrocytes

Corresponding author: Huangyuan Li, E-mail: lhy@fjmu.edu.cn; Siying Wu, E-mail: sywu@fjmu.edu.cn

T11-16-0018

Silver nanoparticles induced neurotoxicity viaimbalance of mitochondrialdynamics

Xiaoru Chang, Jiangyan Li, Mengting Shang, ShuyanNiu, Wenli Zhang, Zuoyi Sun, Yunjing Li, Meng Tang, Yuying Xue

(Key Laboratory of Environmental Medicine and Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing 210009, China)

Abstract: Silver nanoparticles (AgNPs) have been reported to accumulated in the central nervous system (CNS) and induce neurotoxicity. While previous studies have reported toxicity in neuronal cell lines after AgNPs exposure, mechanistic research is largely unknown in biological safety and neurotoxicity, especially in the hippocampus. This study aimed to investigate mechanism of hippocampal neuronal apoptosis and role of reactive oxygen species (ROS) and Ca^{2+} levels in mitochondrial dynamics. As a result, apoptosis was observed in hippocampal neuronal HT22 cell line and hippocampus of mice intravenously injected with AgNPs. Additionally, mitochondria could be severely disturbed by AgNPs in morphology and function, characterized by mitochondrial fission and disappearance of cristae, ROS overproduction and significant reduction in mitochondrial ATP and mitochondrial membrane potential (MMP). Mechanistically, this effect is mediated by phosphorylation and translocation to mitochondria of dynamin-related protein 1 (Drp1) at a serine 616 (S616) by Ca^{2+}/calmodulin-dependent kinase II (CaMKII), which partlydependent on the ROS-mediated Ca^{2+} overload. Fortunately, additional antioxidants and calcium inhibitors pretreatment could mitigate apoptosis and cytotoxicity through improvement of mitochondrial function. This is based on the evidence that N-acetyl-L-cysteine (NAC) and BAPTA-AMinhibited the Ca^{2+} overload and activation of CaMKII while attenuating Drp1 phosphorylation. In conclusion, our data basically revealed that AgNPs induced apoptosis by promoting excessive Drp1 phosphorylation and mitochondrial fission. ROS-mediated intercellular Ca^{2+} level was the key effector mediating AgNPs-induced Drp1 phosphorylation and mitochondrial dysfunction. Prevention of oxidative stress and Ca^{2+} overload by antioxidants and calcium inhibitors might confer protective effects on neurotoxicity of AgNPs.

Key words: Silver nanoparticles; Mitochondria; Drp1; Oxidative stress; Ca^{2+}

Corresponding author: Yuying Xue, E-mail: yyxue@seu.edu.cn

T11-16-0022

ZnO Nanoparticles induced KK1 cell apoptosis via oxidative stress-mediated ERS pathway

Luo Feng-xian, Feng Xiao-yan, Zhao Yu, Liu Shan-ji, Xu Heng-yi *

(State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China)

Abstract: ZnO Nanoparticles (ZnO NPs) is a widely used nanoscale material, which could have adverse effects on the human reproductive system. However, the mechanism thatZnO NPs has an effect on female germ cellsis unclear. Therefore, this study aims to explore the effect and mechanism of ZnO NPs on KK1 cells(ovarian granulosa cells). The results showed that ZnO NPs decreased the cell viability in a time dependent manner. In addition, the exposure of ZnO NPs could induce endoplasmic reticulum damage andapoptosis of KK1 cells. Furthermore, ZnONPs increased the content of malondialdehydeand decreased the activity ofsuperoxide dismutase. Additionally, ZnO NPs activated the endoplasmic reticulum stress (ERS)signaling (i.e., p-PERK, BIP, p-eIF2α, CHOP). These effects were reversed by eIF2α siRNA and sal (eIF2α inhibitor). These results indicated that ZnO NPs inducedKK1 cells apoptosis viaoxidative stress-mediated ERS signaling pathway.

Key words: ZnO Nanoparticles; KK1 cells; apoptosis; oxidative stress; endoplasmic reticulum stress

Corresponding author: Xu Heng-yi, E-mail: kidyxu@163.com, E-mail: HengyiXu@ncu.edu.cn

T11-16-0025

The triterpenoid CDDO-imidazolide protects against zinc oxide nanoparticle-induced acute lung inflammation in mice

Tingyue Guo[a], Xin Fang[a], Yiting Liu[a], Yihui Ruan[a], Yu Hu[a], Xuening Wang[a], Yuxin Hu[b], Gang Wang[b], Yuanyuan Xu[a,c]

(a. Group of Chronic Disease and Environmental Genomics, School of Public Health, China Medical University, Shenyang 110122, China; b. Experimental Teaching Center, School of Public Health, China Medical University, Shenyang 110122, China; c. The Key Laboratory of Liaoning Province on Toxic and Biological Effects of Arsenic)

Abstract: Zinc oxide nanoparticles (ZnONPs) are widely used worldwide. Human inhalation exposure to ZnONPs induces acute lung inflammation (ALI), however, the preventive and therapeutic target for pulmonary toxicity of ZnONPs is limited. CDDO-imidazolide (CDDO-Im) is a synthetic triterpenoids, which againsts oxidative stress and inflammation through increasing the transcriptional activity of NRF2. To explore whether CDDO-Im confers protective effects on ZnONPs-induced ALI. Mice were exposed to 20 μg ZnONPs on day (0) by intratracheal instillation (IT), administrated with CDDO-Im (2 mg·kg^{-1}) on day (-1 and 1) intraperitoneally and sacrificed on day 3. CDDO-Im significantly decreased the number of macrophages and concentrations of total protein in bronchoalveolar lavage fluid, inhibited expression of lung proinflammatory cytokines including Il-6 and Il-8 in ZnONPs+CDDO-Im group compared with ZnONPs group. Lung pathology showed ameliorated infiltration of inflammatory cells and interstitial thickening, and reduced number of F4 / 80 - and Ly6g-positive cells after CDDO-Im intervention. Treatment with CDDO-Im upregulated protein levels of NRF2 and its downstream genes (GCLC, GCLM and NQO1). Collectively, these results suggest that CDDO-Im acts against pulmonary toxicity caused by nanoparticles, and it may serve as a potential pharmacologic strategy to prevent and treat nanoparticle-induced ALI.

Key words: ZnONPs; ALI; NRF2; CDDO-Im; lung

Corresponding author: Yuanyuan Xu, E-mail: yyxu@cmu.edu.cn, E-mail: laurel1214@hotmail.com

T11-20-0006

In vitro toxicity of VO_2 nanoparticles on human liver HepG2 cells

Li, Jia-Bei[a], Xi Wen-Song[a], Tan Shi-Ying[a], Liu Yuan-Yuan[a], Wu Hao[a], Liu Yuanfang[a,b], Cao Aoneng[a], Wang Haifang[a]

(a. Institute of Nanochemistry and Nanobiology, Shanghai University, Shanghai 200444, China; b. Beijing National Laboratory for Molecular Sciences, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China)

Abstract: Vanadium dioxide (VO_2) nanomaterial displays a fully reversible phase transition accompanied with distinct changes in electrical and optical properties, and thus are used to develop smart devices, including optical/ electrical switching devices, batteries and supercapacitors, and intelligent windows for buildings and vehicles for energy saving. Therefore, VO_2 nanomaterials may appear in many aspects of our lives, and enter the environment via various routes, during production, use and disposal. Primary studies indicate that VO_2 nanoparticles display toxicities to animals and cells. Liver is the primary accumulation organ of nanoparticles after entering the body via various routes. However, the knowledge of the effects of VO_2 nanoparticles on liver is almost blank at present. Herein, we comprehensively evaluated the cytotoxicity, genotoxicity and metabolism disorder induced by a commercial VO_2 nanoparticle, S-VO_2, in a human liver cell line HepG2. We found that S-VO_2 was cytotoxic and genotoxic, and it also interfered the glucose and lipid metabolism. Both of S-VO_2 particle and its dissolved species produced ROS, induced mitochondrial dysfunction, decreased ATP content, and set off DNA damage, consequently arrested the cell cycle in G2/M phase and inhibited the proliferation of HepG2 cells, thus leading to proliferation inhibition. Meanwhile, S-VO_2 enhanced glucose uptake of HepG2 cells through oxidative stress, and blocked lipophagy process, which led to lipid accumulation. Our findings provide insights in liver toxicity of VO_2-based nanomaterials, benefiting their safety assessment and safely practical applications.

Key words: VO_2; nanoparticle; HepG2 cell; nanotoxicology; cytotoxicity; genotoxicity; metabolism

Corresponding author: Cao Aoneng, E-mail: ancao@shu.edu.cn, Wang, Haifang, E-mail:hwang@shu.edu.cn

T11-29-0021

Plasma-borne bioactivity increases lung cancer risk in chronic carbon black nanoparticles exposure cohort: Implications from an *ex vivo* biosensor assay

Jinglong Tang, Yuxin Zheng

(Department of OccupationalandEnvironmentalHealth, School of Public Health, Qingdao University, Qingdao, China, 266071)

Abstract: Carbon black (CB, Chemical Abstracts Service Registry No.1333-86-4) is a nano-scale fine black powder or a pellet appearance with an increase in popularity as a virtually pure elemental carbon (EC) applied into research, commercial, and manufactured products which developed over a century that are composed under controlled conditions by incomplete combustion or thermal decomposition of gaseous or liquid hydrocarbons. In February 2006, an IARC Monographs Working Group reevaluat·

ed the carcinogenic hazards to humans of carbon black. Due to the epidemiological studies among workers in carbon black production and in the rubber industry provided inadequate evidence of carcinogenicity, the Working Group ranked carbon black as possibly carcinogenic to humans, Group 2B.

Herein, we and collaborators developed a novel ex vivo biosensor assay to evaluate the potential lung risk from inhaled carbon black nanoparticles. The primary HBEpiCs were obtained from the bronchi of normal people with a bronchial brush. Modified ex vivo biosensor assays were carried out using HBEpiCs between 4 and 6 generations. The HBEpiCs were first starved with plasma-free medium for 4 hours and then cultured in BEBM, containing 10% plasma of the subjects for 24 hours. The control and non-control cells were treated with random and blind methods according to a certain proportion.Based on transcriptome sequencing, we found the effect of systemic environment on cells after carbon black exposure was an inflammatory response, which mainly activates cell cycle-related pathways. In contrast, the diesel exhaust particle (Group 1 carcinogenic) exposure activated pathway was mainly related to autoimmunity and oxidative stress. Our results demonstrate that the systemic environment after exposure to carbon black and diesel exhaust nanoparticles will have different effects on cell function in dissimilar ways. After exposure to both nanoparticles, the systemic environment (plasma-borne bioactivity) could activate cancer-related pathways, increasing the risk and affecting the prognostic of lung cancer. These attempts might provide a further understanding of the carcinogenicity effect of occupational inhaled nanoparticles exposure on pulmonary.

Key words: Carbon black nanoparticles; carcinogenic; *Ex vivo* biosensor; Lung cancer

Corresponding author: Jinglong Tang, E-mail: tangjinglong@qdu.edu.cn

T11-35-0004

RNA m6A epitranscriptome reveals the toxicological mechanisms of metal oxide nanoparticles

RuanFengkai, Changqian Liu, Yi Wang, Xisen Cao, Zhen Tang,Jiaying Xu, Jiahua Gao, Jie Zeng, Hanying Yin, Naying Zheng, Chunyan Yang, ZhenghongZuo, Chengyong He*

(State Key Laboratory of Cellular Stress Biology, School of Life Sciences, Shenzhen Research Institute of Xiamen University, Xiamen University, Xiamen, Fujian 361005, China)

Abstract: Metal and metal oxide nanoparticles (nMOx) have broad applications; however, the increased exposure is associated with higher health risk.RNA N6-methyladenosine (m6A) modification regulates cell stress response and homeostasis. It is unclear whether nMOxcauses alterations in m6A epitranscriptomic, and the underlying mechanisms are also unknown.

In this study, we find that nMOx ($nTiO_2$, nAg, nZnO, nFe_2O_3, and nCuO) exposure significantly alter the expression of m6A methyltransferases or demethylases. We further focused on the question of how $nTiO_2$ upregulated the global m6A level. First, we found that $nTiO_2$ mainly increased the global m6A level and the expression of METTL3, but not other methylases or demethylases. In fact, nTiO2 increased METTL3 protein levels by promoting METTL3 stability but not the mRNA level of METTL3. However, a recent study showed that activated ERK signalling enhances the stability of METTL3 in both stem cell differentiation and carcinogenesis. In our study, similarly, $nTiO_2$ activated ERK signalling in both lung cells and tissues. Furthermore, inhibition of ERK1 / 2 restored METTL3 protein levels in lung cells exposed to $nTiO_2$. Numerous studies have shown that ERK1/2 can be activated by ROS. Many nMOx, including $nTiO_2$, have been reported to produce ROS generally by their electronic structures and surface

defects. In this study, $nTiO_2$ exposure also increased the ROS level, and its scavenger impaired ERK1/ 2 activation and METTL3 upregulation following TGF - β signalling activation and the inflammatory re·sponse in lung cells. These studies suggest that $nTiO_2$ triggers the inflammatory response in the lung through the ERK/ METTL3 / m6A pathway, which is further validated in a mouse model. In conclusion, our study demonstrates thatm6A epitranscriptomicsis a novel parameter for nanosafety evaluation and that m6A is also a potential intervention target to alleviate the adverse effects of nanoparticles, both of which have far-reaching implication for protecting human health and improving sustainability of nano·technology.

Key words: RNA m6A; Epitranscriptomics; Nanomaterials; Nanosafety; METTL3

Corresponding author: Chengyong He, E-mail: hecy@xmu.edu.cn

T11-38-0029

Study on the toxicity of organically modified montmorillonite in mice

Jingyi Liu[a], Shizhao Wu[a], Jing Gao[a,b], Guohua Li[a,b]

(a. School of Chemical Engineering, Zhejiang University of Technology, Hangzhou 310032, P.R.

b. State Key Breeding Base of Green Chemistry Synthesis Technology, Hangzhou 310032, P.R.)

Abstract: Montmorillonite (Mnt) and its organically modified nanomaterials are widely used in biological toxicology research, and have been attracted widespread attention from the scholars all over the world for the reason that Mnt prominent with adsorption and gelation properties, and it has the advantages of protecting the digestive tract and adsorbing various viruses. Herein, a nano-Mnt was exfoliated by lithium chloride and then modified by hexadecylamine (OMnt). A nano-Mnt labeled with sulfo-cyanine5 nhs ester (Cy5) fluorescence was fabricated by stirring a certain proportion of Mnt sus·pension and Cy5 at 20°C in the dark for 12 h, and then freeze-drying it at -50°C for 48 h to obtain a Cy5 fluorescent labeled nano-Mnt (Mnt-Cy5) and gavage it to mice at different time intervals of 0.5 h, 1 h, 2 h, 4 h, 12 h, 24 h and 48 h, then take a in vivo fluorescence imaging test on mice to observe the distri·bution of Mnt-Cy5 in the organs of mice over time and whether OMnt could poison mice. The chemical composition and phase composition were characterized by FTIR and the distribution of Mnt-Cy5 in the organs of mice was characterized by in vivo fluorescence imaging system. The results show that the peaks near the 3635 cm^{-1} ~3430 cm^{-1} and 1665 cm^{-1} ~1560 cm^{-1} in the FTIR spectra can be assigned to the stretching vibration of the N-H and C=O bond, which indicates that the composite nanomaterials constituted of Mnt and Cy5. Furthermore, test results of in vivo fluorescence imaging show that Mnt-Cy5 mainly distributed in the stomach of mice at 1 h; then one part of it stays in the stomach of mice, and the other part is transferred to the liver and digestive tract at 4 h; while after 12 h, there is still a small part of it in the stomach, a small part in the liver and digestive tract, and the others are excreted from the body; and a small part of it stays in the digestive tract of the mice, and the rest has been excreted from the body during 24 to 48 h. So that the erimental results prove that OMnt is not toxic in mice.

Key words: organically modified montmorillonite; toxicology; *in vivo* fluorescence imaging

T11-54-0012

Mechanisms of regulating autophagy by different nanoparticles

Qiao Dong, Tang Meng*

(Key Laboratory of Environmental Medicine Engineering of Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu, 210009)

Abstract: Autophagy serves as a protective mechanism that can be activated by externalfactors such as physical, chemical, and biological. It maintains intracellular homeostasis by degrading its own damaged organelles and removing aggregates of defective proteins through lysosomes. An increasing number of studies have demonstrated that different types of nanoparticles are capable of modulating the autophagy process in cells, which indicates the potential of nanoparticles in the development of therapeutics for autophagy-related diseases. Nanoparticles can both provoke autophagy and block the autophagy process, resulting in different biological outcomes, depending on their composition, dose of action, and cellular model. In this review, we discuss the effects and biological consequences of various nanoparticles on autophagy. Besides, the mechanisms regulating autophagyare highlighted, including organelle damages, oxidative stress, autophagy-inducing factors, and signaling pathways. It provides a theoretical basis for the development of therapeutic tools for autophagy-related diseases.

Key words: Autophagy; Nanoparticles; Oxidative stress; Lysosomal damage; Endoplasmic reticu·lum stress.

Corresponding author: Tang Meng, E-mail: tm@seu.edu.cn

T11-60-0003

Carbon Nanotubes promote the development of intestinal organoids through regulating extracellular matrix viscoelasticity and intracellular energy metabolism

Bao Lin[1,2], Cui Xuejing[1,3], Chen Chunying[1,3]

(1. CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety & CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology of China, Chinese Academy of Sciences (CAS), Beijing 100190, China; 2. University of Chinese Academy of Sciences, Beijing 100049, China; 3. The GBA National Institute for Nanotechnology Innovation, Guangdong 510700, China)

Abstract: The biological effect of engineered carbon nanotubes (CNTs) as beneficial biomaterials on the intestine, especially on their development, remains unclear. Here we investigated the profitable effect of CNTs with different graphene layer and surface modification using the intestinal organoids 3D model after exposure for different time period (0-5 days) and demonstrated that CNTs (50 $\mu g \cdot mL^{-1}$) pro·moted the development of intestinal organoids. The mechanisms involve the modulation of extracellular matrix (ECM) viscoelasticity and intracellular energy metabolism. In particular, CNTs reduced the hard·ness of extracellular matrix through decreasing the elasticity and increasing the viscosity as a result of elevated metalloproteinase and binding to protein scaffold, which activated the mechanically mem·brane sensors of cells, Piezo, and downstream P-p38-yes-associated protein (YAP) pathway. More·

over, CNTs altered the metabolic profile of intestinal organoids and induced increased mitochondria activity, respiration, and nutrient absorption. These mechanisms cooperated with each other to promote the proliferation and differentiation of intestinal organoids. In addition, the promoted effect of CNTs is highly dependent on the number of graphene layer, manifested as multi-walled CNTs>single-walled CNTs. Our findings highlight a new research paradigm of nano-intestine interaction and imply the potential of CNTs as biomaterials for intestine-associated tissue engineering.

Key words: Carbon nanotubes; Intestinal organoids; Development; Extracellular matrix viscoelasticity; Intracellular energy metabolism

Corresponding author: Chen Chunying, E-mail: chenchy@nanoctr.cn; cuixj@nanoctr.cn

T11-61-0011

Research progress of nanomaterials regulating cell pyroptosis

Li Congcong, Tang Meng

(Key Laboratory of Environmental Medicine Engineering of Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu, 210009)

Abstract: Pyroptosis is a newly discovered method of programmed cell death that relies on inflammatory caspase, which is mainly through the shear activation of gasdermin family members and causes cell membrane perforation and cell content release. In addition to the rupture of the plasma membrane, the typical characteristics of pyrolysis are accompanied by the release of pro-inflammatory factors and cell contents, including IL-1β, IL-18. Caspase-1 mediates the pyrolysis in the classical pathway, while the pyrolysis caused by the direct recognition of lipopolysaccharide (LPS) by caspase-4, 5, and 11 is defined as a non-classical pathway. Numerous human diseases are closely related to pyrexia, especially inflammatory diseases, immune diseases, and malignant tumors. In this review, we discussed the latest research findings of cell pyrolysis caused by nanomaterials, as well as the various possible effects of nanomaterials on pyrolysis-mediated diseases and cell pyrolysis. Despitepyroptosis is usually harmful to normal organs and tissues, it has been proven to be of great significance for the treatment of malignant diseases such as cancer by specifically activating the site of action of programmed cell death.Nanomaterials can regulate the occurrence and development of cell pyroptosis. They can effectively eliminate malignant cells through the controllable induction of cell pyroptosis, providing new strategies for the treatment of many diseases. Although the mechanism of pyroptosis has been fully revealed, the specific process of pyroptosis caused by nanomaterials in changing the diseases remains to be clarified, which may provide new directions for the targeted treatment of cancer and various diseases.

Key words: Nanomaterials; Pyroptosis; Caspase; Gasdermin; Cancer

Corresponding author: Tang Meng, E-mail: tm@seu.edu.cn

T11-75-0016

Application of combined omics in nano-neurotoxicity studies

Wang Zhi-hui, Tang Meng*

(Key Laboratory of Environmental Medicine and Engineering, Ministry of Education; School of Public Health, Southeast University, Nanjing, People's Republic of China)

Abstract: Nanotechnology has a wide range of applications and is closely related to our lives. Quantum dots (qds) can be extensively used in biomedicine because of their unique photoelectric properties, which may bring technological innovation to neuroscience research such as time-lapse imaging, tracking bioactive molecules, and multicolor immunofluorescence assay. However, studies have shown that nanomaterials can penetrate through the blood-brain barrier to reach the brain or migrate along the olfactory nerves, and then induce cytotoxicity which can be explored in depth by means of omics. The omics analyses concentrate on the molecular profiles of biological systems to evaluate nanotoxicity. Moreover, instead of focusing on a single compound, analyses of omicscan provide detailed profiles of multiple biological systems. Whereasthe understanding of biological issues in a single omics study is not comprehensive. Combined omics also allows for the evaluation of various changes in the toxicity of compounds through in-depth analyses. This review emphasizes theanalysis of different omics and their combination as a new method to evaluate the neurotoxicity of nanomaterials. We found that the combination of omics includes various types that aim to detect and identify various molecules at different levels, study their functions and the interactions with one another, which is an essential method to discover the pathways and the network to build biological modules.

Key words: Nanomaterials; Quantum dots; Omics; Nano-neurotoxicity

Corresponding author: Meng Tang, E-mail: tm@seu.edu.cn

T11-76-0026

Defect-rich adhesive molybdenum disulfde/rGO VerticalHeterostructures with enhanced nanozyme activity forsmart bacterial killing application

Fen e Gao, Jing Liu, Chunying Chen

(National center for Nanoscience and Technology, No.11.Beiyitiao Alley, Zhongguancun, Haidian District, Beijing 100190, China)

Abstract: Nanomaterials with intrinsic enzyme-like activities, namely "nanozymes, " areshowing increasing potential as a new type of broad-spectrum antibiotics. However, theirfeasibility is still far from satisfactory, due to their low catalytic activity, poor bacterial capturing capacity, and complicated material design. Herein, afacile synthesis of a defect-rich adhesive molybdenum disulfde (MoS_2)/rGO vertical heterostructure (VHS) through a one-step microwave-assisted hydrothermalmethod is reported. This simple, convenient but effective method for rapidmaterial synthesis enables extremely uniform and well-dispersed MoS_2/rGOVHS with abundant S and Mo vacancies and rough surface, for a performanceapproaching the requirements of practical application. It is demonstrated experimentally and theoretically that the as-prepared MoS_2/rGO VHS possesses defectand irradiation dual-enhanced triple enzyme-like activities (oxidase, peroxidase, and catalase) for promoting free-radical generation, owing to much more activeedge sites exposure. Meanwhile, the VHS-achieved rough surface exhibits excellent capacity for bacterial capture, with elevated reactive oxygen species (ROS) destruction through local topological interactions. As a result, optimized efcacyagainst drug-resistant Gram-negative and Gram-positive bacteria can be exploredby such defect-rich adhesive nanozymes, demonstrating a simple but powerfulway to engineered nanozymes for alternative antibiotics.

Key word: bacterial capture; defect-rich materials; microwave-assisted synthesis; MoS_2/rGO vertical heterostructures; nanozyme antibacterial therapies

T11-77-0005

Cytotoxicity induced by co-exposure of ZnO and TiO_2 nanoparticles

Liang Yan, SimaitiAili, Xu Mingxuan, LvShenchong, Cai Xiaobo, Yu Peilin*
(Department of Toxicology, Zhejiang University School of Public Health, Hangzhou, 310000)

Abstract: ZnO and TiO_2 nanoparticles (NPs) are the main components of sunscreens, and are often used in different brands of sunscreen products with different proportions. However, the secure formula and the toxic effects of co-exposureof NPs have not been reported explicitly. In this study, we employed immortalized humankeratinocytes HaCaT cells on behalf of epidermis to investigate the cytotoxicityinduced by ZnO and TiO_2 NPs, and to analyzethe concrete mechanism oftheir co-exposureeffects. The results showed that: (1) TiO_2 NPs had no significant cytotoxicity toHaCaT cellseven the concentration reached up to 300 $\mu g \cdot mL^{-1}$; (2) ZnO NPs significantly inhibited cell proliferation in a dose-dependent manner starting at concentration of 20 $\mu g \cdot mL^{-1}$; (3) The cell viability decreased by ZnO NPs wasrescued withco-exposure of TiO_2 NPs. Further mechanism research by flame atomic absorption spectrum (AAS) and flow cytometric analysis revealed that TiO_2 NPs restricted the cellular uptakeof ZnO NPs by competing for the caveolin-dependent pathway, thus reducing the ZnO NPs released Zn^{2+} which is mainly responsible for the cytotoxicity. By combining fluid dynamic analysis and solubility tests, it is further showed that TiO_2 NPs increased the aggregations of ZnO NPs, which also reduced the releaseof Zn^{2+}. Taken together, the currentresearch contributes new insights into understandingthe co-exposure effects of NPsand providesvaluable reference to secure formula of NPs in sunscreens.

Key words: ZnO NPs, TiO NPs; co-exposure, Zn^{2+} concentration

Corresponding author: Yu Peilin, E-mail:yupeilin@zju.edu.cn

T11-79-0009

Biological implications of macrophages in nanoparticles exposure

Niu YiRu, Tang Meng
(Key Laboratory of Environmental Medicine and Engineering of Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu 210009, China)

Abstract: In recent years, the wide application of nanoparticles (NPs) has caused inevitable human exposure and environmental pollution, and its safety has attracted more and more attention. Since the cells most directly exposed to NPs invaded by multiple approaches in the body are macrophages, it is necessary to understand the interaction between NPs and macrophages to clarify the *in vivo* toxicity and mechanism of NPs. A considerable number of studies have reported the toxic effects of NPs on macrophages *in vitro*. However, there is a lack of relevant *in vivo* research and integration of *in vitro* research results. This paper reviews the cytotoxicity and interaction of macrophages after exposure to nanoparticles, especially QDs, and focuses on the cell changes during the interaction, such as internalization of cells, the production of ROS and cytokines, DNA damage and cell death. The internalization of NPs by macrophages is a crucial step for NPs to induce macrophage toxicity, the production of ROS and cytokines in macrophages are early events of NPs-induced oxidative and inflammatory damage, while DNA damage and cell death of macrophages are the irreversible cell changes induced by NPs. These discussions aims to improve people′s current understanding of the biological signifi·

cance of macrophages in nano-exposure, and to provide new ideas for reducing the toxic and side effects of nano-exposure.

Key words: Nanoparticles; QDs; Macrophages; Cytotoxicity; interaction; internalization

Corresponding author: Tang Meng, E-mail: tm@seu.edu.cn

T11-81-0024

Chronic toxic effects of quantum dots cadmium sulfide CdS on the Scenedesmus obliquus

Chang Wenrui, Geng Qihan, Yang Sai, Shu Xinyue, Xia qingyi, Guo Simiao, Yue Jianing, Xiong Li*

(Hubei Key Laboratory of Genetic Regulation and Integrative Biology, School of Life Science, Central China Normal University, Wuhan, Hubei 430079, China)

Abstract: Quantum dots are widely used in nanomedicine, biosensors, and deep tissue imaging due to their unique optical properties, which has led to an increasing interest in the study of the toxic effects of quantum dots, but less research has been proposed on their chronic toxic effects. In this paper, the chronic (42d) toxicity effects of CdS quantum dots on Scenedesmus obliquus were evaluated comprehensively in terms of growth inhibition and cell morphology, photosynthesis and cell damage. The results showed that the specific growth rate of algal cells gradually decreased with increasing treatment concentrations, the cell volume increased, the color became lighter, the cell membrane was irreversibly damaged, and the cell metabolism level decreased, accompanied by high reactive oxygen levels, but the corresponding indexes of cells in the low concentration treatment group could return to normal levels after a period of time. Quantum dot exposure at low concentrations (0.01 mg·L^{-1}) had a certain degree of low concentration stimulation effect on the synthesis of photosynthetic pigments in algal cells, while other concentration treatment groups showed concentration-dose effects. Toxic inhibition response to quantum dots is earlier for light-response-related genes (psaB and psbE) than for dark-response-related gene (rbcL), and phytochrome synthase genes (PCS) showed an inhibitory effect. Some of the phenomena identified in the study provide some scientific basis for a comprehensive and objective evaluation of the toxicological effects of nanoparticles.

Key words: Scenedesmus obliquus; chronic toxic effects; quantum dots

Corresponding author: Xiong Li, E-mail: xionglily@mail.ccnu.edu.cn

T11-83-0015

Nanoplastics induces ferroptosis in human lung epithelial cells through iron overload and redox imbalance

Yang Sheng[a], Cheng Yan-ping[a], Chen Zao-zao[b], Liu Tong[a], Yin Lihong[a], PuYue-pu[a], Liang Ge-yu[a]

(a. Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu,China P.R. 210009; b. State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing, Jiangsu, China P.R. 210096)

Abstract: Nanoplastics, including polystyrene nanoplastics (PS-NPs), are widely existed in the

atmosphere, which can be directly and continuously inhaled into the human body, posing a serious threat to the respiratory system. Therefore, it is urgent to understand the potential mechanism of PS-NPs induced lung cell death. In this research, we used two types of human lung epithelial cells BEAS-2B and HPAEpiC to investigate the association between ferroptosis and PS-NPs. We found PS- NPs could significantly reduce cell viability in a dose-dependent manner. In addition, we evaluated intra·cellular iron content, ROS release and lipid peroxidation, as well as biomarkers of ferroptosis were detected, respectively. As a result, PS-NPs treatment could increase cellular iron content and ROS pro·duction, damage to lipids, decrease GSH-Px and NADPH. Moreover, significantly changed expression of ferroptosis-related genesfurther hinted that irondysfunction and redox imbalance are major inducers of ferroptosis by microarray data, qRT-PCR and Western blot analysis. Importantly, index monitored above can be partially rescued by lipid peroxidation inhibitor ferrostatin-1 and iron chelator deferox·amine mesylate, which mediated anti-ferroptosis activity mainly depends on the restoration of antioxi·dant activity and iron metabolism. In conclusion, the present study basically show that PS-NPs enhances ferroptosis sensitivity with increased ferroptosis in lung cells, in which iron overload, lipid peroxidation and redox imbalance act pivotal roles.

Key words: Nanoplastics; ROS; iron overload; lipid peroxidation; ferroptosis

Corresponding author: Geyu Liang, E-mail: lianggeyu@163.com

T11-87-0017

Study on the mechanism of mitochondrial oxidative damage and dysfunction of nerve cells by Silica nanoparticles

Ning Xiao-fan, Ma Kai, Li Xin-yue, Hao Hui-fang, Hou Shan-shan, Zhang Xia-yu, Wu Hao, Li Chunrui
(Department of health laboratory, School of public health, Jilin University, Changchun 130021)

Abstract: OBJECTIVE Silica nanoparticles (SiNPS) have been widely used in industry, electron·ics and pharmaceutical industries. It is widely used in medicine, tumor treatment and diagnosis, and other biomedical and biotechnology fields. The opportunities for people to the exposure to SiNPS through iatrogenic, occupational and environmental exposureare gradually increasing. The damage and biological effects of SiNPS on the nervous system have attracted widespread attention in the field of toxicology. Central nerve cells are rich in mitochondria. The maintenance of membrane potential of nerve cells, the synthesis and operation of neurotransmitters, and the transmission of nerve impulses, etc., require energy and information support, which will all be provided by mitochondria. When cells are exposed to foreign compounds, the balance between oxidation and anti-oxidation in the cells is broken, and the function of mitochondria changes accordingly. However, the specific mechanism of SiNPS damage to nerve cell mitochondria is still unclear. In this study, SH-SY5Y cells were used as the target cells to investigate the effect and mechanism of SiNPSon the oxidative damage and dysfunc·tion of nerve cell mitochondria. **METHODS** The MTT method was used to detect the influence of SiNPS on the survival rate of SH-SY5Y cells; the biochemical method was used to detect the SOD and GSH-Px activities in the cells and mitochondria; the intracellular ROS content was observed by fluores·cence microscope; the flow cytometry was used to detect apoptosis and mitochondria changes of mem·brane potential; Western blot method was used to detect the expression of Mn-SOD, VDAC1, COXI, UCP2, PRDX3 relatedto mitochondrial damage induced by SiNPS in cells and mitochondria. **RESULTS** Different doses of SiNPS exposed to cells for 24 hours can reduce cell survival; after the cellswere

treated with SiNPS for 3 hours, the mitochondrial membrane potential decreased and reactive oxygen species increased; intracellular SOD activity decreased, mitochondrial Mn-SOD activity increased, intracellular and mitochondrial GSH-Px activity increased; SOD protein expression levels in cells decreased, VDAC1, COXI, UCP2, PRDX3 related protein expression levels increased, Mn-SOD, VDAC1, COXI, UCP2, PRDX3 related protein expression levels in mitochondria increased with the increase of SiNPS concentration. **CONCLUSIONS** SiNPS has a toxic effect on SH-SY5Y cells, and leads to oxidative damage to cells, as well as oxidative damage to cell mitochondria and changes in the expression of mitochondrial function-related proteins, which may eventually induce SH-SY5Y nerve cell apoptosis.

Key words: Silica nanoparticles; SH-SY5Ycells; Mitochondria; Oxidative damage; Apoptosis

Corresponding author: Jin Ming-hua, E-mail: jinmh@jlu.edu.cn

T11-90-0027

Understanding the Aggregation and Toxicity of Amyloid Proteins

Pu Chun Ke[1,2], Aleksandr Kakinen[2], Yunxiang Sun[3], Nicholas Andrikopolous[4], Feng Ding[5]

(1. The GBA National Institute for Nanotechnology Innovation, Guangzhou, China; 2. Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Australia; 3. Ningbo University, China; 4. Monash Institute of Pharmaceutical Sciences, Monash University, Australia; 5. Clemson University, USA)

Abstract: In this talk, the amyloid aggregation of proteins associated with the pathologies of Alzheimer′ s disease, Parkinson′ s disease and type 2 diabetes will be discussed. Specifically, the structural and toxicity profiles of the heterogeneous oligomers, the cross seeding of human islet amyloid polypeptide by its primary and secondary amyloidogenic fragments, the pathogenesis of amyloid beta elevated by bacterial protein FapC, and the mitigation of a range of amyloidosis by inorganic and polymeric nanomaterials as well as bio-nanocomposites will be presented. This talk intends to highlight a complex nano-bio interface in nanomedicine, where nanotoxicology plays an indispensable role against dementia and metabolic diseases.

T11-94-0007

Cadmium telluride quantum dots triggered mTORC1-dependent TFEB activation and promoted M1 polarization in THP-1 derived macrophages

Wei Tingting, Zou Lingyue, Liu Na, Bai Changcun, Huang Xiaoquan, Yao Yongshuai, Wang Zhihui, Li Binjing, Tang Meng *

(Laboratory of Environmental Medicine Engineering of Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu 210009, China)

Abstract: Cadmium telluride quantum dots (CdTe-QDs) have been increasingly applied in biomedical fields for their excellent performance. Monocytes/ macrophages play critical roles in the innate immune system. The risk assessment of QDs to the immune system attracts increased attention. Here, we found CdTe-QDs impeded monocyte-macrophage differentiation as evidenced by reduced expres·

sion of macrophage surface marker CD11b. Moreover, the CdTe-QDs treatment promoted the M1 polarization in differentiated THP-1 (dTHP-1) via augmenting transcription factor EB (TFEB) nucleus translocation. Meanwhile, TFEB nucleus translocation promoted the autophagy-lysosomal pathway in macrophages, enhancing autophagy and lysosomal function. The mTOR signaling pathway regulated the activation of TFEB but was independent of both the ERK1 / 2 and AMPK pathways. Our results pinpointed TFEB as a potential target for developing future practical CdTe-QDs based immunotoxicity assessment in biomedical applications.

Key words: Transcription factor EB (TFEB); Autophagy-lysosome pathway (ALP); Macrophage polarization; Immunotoxicity

Corresponding author: Tang Meng, E-mail: tm@seu.edu.cn

T11-95-0002

Amino-functionalized graphene quantum dots trigger ferroptosis via Hippo/YAP signaling pathway in the hippocampus of mice and cultured microglia

Wu Tian-shu, Wang Xin-yu, Chen Min, Cheng Jin, Zhang Xiao-meng, Kong Lu, Tang Meng
(Key Laboratory of Environmental Medicine and Engineering, Ministry of Education; School of Public Health, Southeast University, Nanjing 210009, P.R. China)

Abstract: In recent years, amino-functionalized graphene quantum dots (A-GQDs), as a class of novel luminescent nanomaterials, have been attracting extensive interest in a widespread of fields for their extraordinary optical properties, but the biosafety assessment of A-GQDs in the central nervous system (CNS) is limited which would restrict their applicability in the field of neuroscience. In this study, we found that the intranasal administration of A-GQDs enhanced the number of neuronal cell death and triggered ferroptotic activities, including increases in ferrous iron levels, lipid peroxidative product MDA contents and $NADP^+$ coenzyme, and obviously inflammatory responses in the hippocampus of mice, which all could be alleviated by a specific ferroptosis inhibitor ferrostain-1 (Fer-1). In *in vitro* experiments, A-GQDs treatment induced ferroptotic alternations of ferrous iron overload, excessive ROS generation, GSH depletion and lipid peroxidation in microglia, which were efficiently reversed by pre-treatments of Fer-1 and an iron chelator deferoxamine mesylate (DFOM). According to the microarray data, the Hippo/YAP signaling pathway might participate in mediating the A-GQDs-caused ferroptosis in microglia. Firstly, both decreased phosphorylated YAP expression and nuclear translocation of YAP were observed in the hippocampus of mice and microglia treated with A-GQDs. When YAP expres·sions were knocked down by lentivirus transfection, the ferroptotic activates elicited by A-GQDs in the hippocampus of mice and microglia were obviously attenuated. Meanwhile, these ferroptotic alterna·tions became more noticeable in YAP-overexpressed microglia treated with A-GQDs, but they could be reversed by Fer-1 and DFOM. Owning to the lentivirus transfection techniques again, we further found that two YAP target genes *tfrc* encoding transferrin receptor protein (TFRC) and aloxe3 encoding Ara·chidonate lipoxygenase 3 (ALOXE3) were involved in the regulation of A-GQDs triggering ferroptosis in microglia. The former one was activated to enhance the content of intracellular ferrous iron, while the later one was up-regulated to produce excessive oxylipin. In conclusion, A-GQDs triggered ferroptosis in hippocampus of mice and microglia via promoting the nuclear translocation of YAP to regulate the transcriptional outputs. This study not only highlights the importance of neurotoxicological study on

QDs containing low toxic component like A-GQDs, but also provides a new idea for the mechanism investigation of novel cell death induced by A-GQDs in the treatment of brain tumors.

Key words: neuroinflammation; iron overload; lipid peroxidation; TFRC; ALOXE3

Corresponding author: Wu Tianshu, E-mail: ninatswu@126.com, E-mail: ninatswu@seu.edu.cn

T11-97-0008

Research Progress on toxic effects of different kinds of quantum dots on lung cells

Wang, Xiaoli, Tang Meng

(Key Laboratory of Environmental Medicine Engineering of Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu 210009, China)

Abstract: Quantum dots (QDs) refer to water-soluble nanoparticles with a size of 1-10nm, which are generally composed of II -VI and III-V elements. Due to the nano-size and surface functionaliza·tion of QDs, it can be dispersed in the air in the form of suspended liquid or powder. Therefore, inhala·tion has become an important exposure way, including occupational and life environment. This paper mainly reviews pulmonary cytotoxicity of 4 types of QDs, including Cd-containing QDs, grapheme QDs, black phosphorus QDs and indium phosphide QDs. And the mechanism of pulmonary cytotoxicity will be summarized, such as oxidative stress, endoplasmic reticulum stress, inflammation, etc. Besides, we will discuss how to reduce pulmonary cytotoxicity from components, surface groups and sizes of QDs.

Key words: Quantum dots; Lung; Toxic effects; Mechanism; Influencing factors

Corresponding author: Tang Meng, E-mail: tm@seu.edu.cn

T11-97-0019

AgNPs impairs autophagic flux and contributes to M1 macrophage polarization in BV2 cells

Mengting Shang, Xiaoru Chang, Shuyan Niu, Jiangyan Li, Wenli Zhang, Tianshu Wu, Ting Zhang, Meng Tang, Yuying Xue

(Key Laboratory of Environmental Medicine and Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing 210009, China)

Abstract: As the release of silver nanoparticles (AgNPs) in the environment continues to increase, great concerns have been raised about their potential toxicity to humans. It is urgent to assess the pos·sible toxicity of AgNPs to the immune cells of the central nervous system due to the continuous accu·mulation of AgNPs in the brain. This study aimed to evaluate the neurotoxicity of AgNPs and the regula·tory mechanism of autophagy in AgNPs-induced inflammation by using mouse microglia BV2 cell lines. AgNPs decreased the microglia cell activity in a concentration and time-dependent manner. The expo·sure of BV2 cells to AgNPs at a noncytotoxic level of 5 $\mu g \cdot mL^{-1}$ resulted in increase of pro-inflammatory cytokines and decrease of mRNA expression of anti-inflammatory cytokines. AgNPs exposure increased M1 markers of iNOS expression and decreased the expression of M2 markers of CD206 in a time-dependent manner. Meanwhile, the expression of inflammatory proteins IL-1β and NF-κB increased

significantly. Additionally, AgNPs induced an increase in autophagosome and upregulation of LC3 Ⅱ, Beclin1, and p62 expression levels. Pretreatment by an autophagy inhibitor, 3-Methyladenine, caused more AgNPs-treated microglia to polarized into proinflammatory phenotypes. Inhibition of autophagy also increased the expression of inflammation-associated mRNA and proteins in BV2 cells. These results indicated that AgNPs could induce pro-inflammatory phenotypic polarization of microglia and the autophagy could play a key regulatory role in the proinflammatory phenotypic polarization of microglia induced by AgNPs.

Key words: AgNPs; Microglia; Autophagy; Polarization; Inflammation

Corresponding author: Yuying Xue, E-mail: yyxue@seu.edu.cn

T11-97-0020

Review of intestinal exposure and toxicity of nanomaterials

Xiaoquan Huang, Meng Tang

(Key Laboratory of Environmental Medicine Engineering of Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu 210009, China)

Abstract: Nanomaterials are widely used in various fields, such as food processing, daily necessities and medicine, which may contact with the gastrointestinal tract through multiple exposure routes. The intestine is an important digestion-absorption and barrier organ. However, for a long time, studies on the toxicity of nanomaterials have mostly focused on organs such as liver, kidney and brain. The intestinal safety evaluation of nanomaterials is rare. Recently, there is evidence *in vivo* that nanoparticles entering into the intestine will be distributed in different sub-structures, leading to structural and functional damages, in which the gut microbiota and its metabolites play important roles. In addition, due to the complex physiological environment in the gastrointestinal tract, nanoparticle will undergo complex biotransformation. Therefore, in this review, we make results of mammalian experiments in vivo as evidence to discuss the damages and their possible mechanisms of nanoparticles to intestinal structure and function. In addition, the exposure routes, biodistribution and biotransformation of nanoparticles in the intestine are also considered. We hope this review will arouse people′s attention to intestinal safety of nanomaterials and guide the research of intestinal nanotoxicology *in vivo*.

Key words: Nanomaterials; Nanoparticles; Gut microbiota; Gut barrier

Corresponding author: Meng Tang, E-mail: tm@seu.edu.cn

T11-97-0030

Silver nanoparticles induced neurotoxicity via imbalance of mitochondrial dynamics

Xiaoru Chang, Jiangyan Li, Mengting Shang, Shuyan Niu, Wenli Zhang, Zuoyi Sun, Yunjing Li, Meng Tang, Yuying Xue

(Key Laboratory of Environmental Medicine and Engineering, Ministry of Education, School of Public Health, Southeast University)

Abstract: Silver nanoparticles (AgNPs) have been reported to accumulated in the central nervous

system (CNS) and induce neurotoxicity. While previous studies have reported toxicity in neuronal cell lines after AgNPs exposure, mechanistic research is largely unknown in biological safety and neurotoxicity, especially in the hippocampus. This study aimed to investigate mechanism of hippocampal neuronal apoptosis and role of reactive oxygen species (ROS) and Ca^{2+} levels in mitochondrial dynamics. As a result, apoptosis was observed in hippocampal neuronal HT22 cell line and hippocampus of mice intravenously injected with AgNPs. Additionally, mitochondria could be severely disturbed by AgNPs in morphology and function, characterized by mitochondrial fission and disappearance of cristae, ROS overproduction and significant reduction in mitochondrial ATP and mitochondrial membrane potential (MMP). Mechanistically, this effect is mediated by phosphorylation and translocation to mitochondria of dynamin-related protein 1 (Drp1) at a serine 616 (S616) by Ca^{2+}/calmodulin-dependent kinase II (CaMKII), which partly dependent on the ROS-mediated Ca^{2+} overload. Fortunately, additional antioxidants and calcium inhibitors pretreatment could mitigate apoptosis and cytotoxicity through improvement of mitochondrial function. This is based on the evidence that N-acetyl-L-cysteine (NAC) and BAPTA-AM inhibited the Ca2+ overload and activation of CaMKII while attenuating Drp1 phosphorylation. In conclusion, our data basically revealed that AgNPs induced apoptosis by promoting excessive Drp1 phosphorylation and mitochondrial fission. ROS-mediated intercellular Ca^{2+} level was the key effector mediating AgNPs-induced Drp1 phosphorylation and mitochondrial dysfunction. Prevention of oxidative stress and Ca^{2+} overload by antioxidants and calcium inhibitors might confer protective effects on neurotoxicity of AgNPs.

Key words: Silver nanoparticles; Mitochondria; Drp1; Oxidative stress; Ca^{2+}

T11-97-0031

AgNPs Impairs autophagic flux and contributes to M1 macrophage polarization in BV2 cells

Mengting Shang, Xiaoru Chang, Shuyan Niu, Jiangyan Li, Wenli Zhang, Tianshu Wu, Ting Zhang,Meng Tang, Yuying Xue

(Southeast University)

Abstract: As the release of silver nanoparticles (AgNPs) in the environment continues to increase, great concerns have been raised about their potential toxicity to humans. It is urgent to assess the possible toxicity of AgNPs to the immune cells of the central nervous system due to the continuous accumulation of AgNPs in the brain. This study aimed to evaluate the neurotoxicity of AgNPs and the regulatory mechanism of autophagy in AgNPs-induced inflammation by using mouse microglia BV2 cell lines. AgNPs decreased the microglia cell activity in a concentration and time-dependent manner. The exposure of BV2 cells to AgNPs at a noncytotoxic level of 5 $\mu g \cdot mL^{-1}$ resulted in increase of pro-inflammatory cytokines and decrease of mRNA expression of anti-inflammatory cytokines. AgNPs exposure increased M1 markers of iNOS expression and decreased the expression of M2 markers of CD206 in a time-dependent manner. Meanwhile, the expression of inflammatory proteins IL-1β and NF - κB increased significantly. Additionally, AgNPs induced an increase in autophagosome and upregulation of LC3II, Beclin1, and p62 expression levels. Pretreatment by an autophagy inhibitor, 3-Methyladenine, caused more AgNPs-treated microglia to polarized into proinflammatory phenotypes. Inhibition of autophagy also increased the expression of inflammation-associated mRNA and proteins in BV2 cells. These results indicated that AgNPs could induce pro-inflammatory phenotypic polarization of microglia

and the autophagy could play a key regulatory role in the proinflammatory phenotypic polarization of microg·lia induced by AgNPs.

Key words: AgNPs; Microglia; Autophagy; Polarization; Inflammation

T11-97-0032

Skin damage induced by zinc oxide nanoparticles combined with UVB is mediated by activating cell pyroptosis via the NLRP3 inflammasome-authophagy-exosomal pathway

Ying-Jan Wang, Yu-Ying Chen
(National Cheng Kung University, College of Medicine, Tainan)

Abstract: Zinc oxide nanoparticles (ZnONPs) are widely used nanomaterial in personal cosmetics, such as skin creams and sunscreens, due to their whitening properties and strong UV light absorption. However, the safety issues and the hazards of ZnONPs, which can be taken up by the skin and cause skin toxicity, are still unclear. This study aims to determine the skin toxicity and the potential mecha·nisms of UVB and ZnONPs exposure. The co-exposure of UVB and ZnONPs elicit NLRP3 inflamma·some activation and pyroptosis in keratinocytes. Furthermore, exposure to both UVB and ZnONPs also disrupts cellular autophagy, which increases cell exosome release. *In vivo* UVB and ZnONPs exposure triggers skin toxicity, as indicated by increased histological injury, skin thickness and transepidermal water loss (TEWL). Notably, the NLRP3 inflammasome-mediated pyroptosis are also activated during exposure. Topical application of pterostilbene (PT) attenuates NLRP3 inflammasome activation and py·roptosis by decreasing ROS generation and mitochondrial ROS (mtROS) levels. In addition to its anti·oxidant effect, PT also reversed autophagy abnormalities by restoring normal autophagic flux and de·creasing NLRP3 inflammasome-loaded exosome release. In conclusion, our findings provide new in·sight into the interplay of inflammasomes, pyroptosis, autophagy dysfunction and exosomes in skin tox·icity and show that PT alleviates skin inflammation by regulating the inflammasome-autophagy-exosom·al pathway.

Key words: zinc oxide nanoparticles; NLRP3 inflammasomes; pyroptosis; autophagy; exosomes

T11-97-0033

Biotransformation modulates the penetration of metallic nanomaterials across the blood brain barrier

Zhiling Guo, Iseult Lynch
(University of Birmingham)

Abstract: Although brain is protected by a tight physiological guardian named blood brain barrier (BBB), deposition of engineered nanomaterials (ENMs) in brain1and consequent neurotoxicity has been reported2, 3. To date, it is still unclear whether and how ENMs enter the brain by crossing the BBB. Understanding the potential of ENMs to cross the BBB as a function of their physicochemical properties and subsequent behavior, fate, and adverse effect beyond that point is vital for evaluating

the neurological effects arising from their unintentional entry into the brain, which is yet to be fully explored. This is not only due to the complex nature of the brain but also the existing analytical limitations for characterization and quantification of NMs in the complex brain environment. Herein, we conducted an interdisciplinary study by using a novel analytical workflow and in vitro BBB model, as a complex biological barrier, to determine and quantify the biotransformation of metallic NMs as a function of their physicochemical properties and correlate the influence of the biotransformation to the BBB-penetration ability and transport pathways. We found metallic ENMs transform in the BBB as affected by their shape, size and intrinsic solubility, which in turn modulates their transport form, efficiency and pathways through the BBB, and consequently their neurotoxicity. Very little was transcytosed to the basolateral (brain) side of the BBB model, with significant amounts being recycled back to the apical (bloodstream) side and limited retention in the BBB cells. Paracellular transport was only observed at the higher concentration tested and was associated with membrane damage and NM dissolution. The generated data about biotransformation modulated uptake and transport of NMs through BBB open a new horizon for medical application of NMs, e. g. targetable drug delivery systems for brain diseases and also for biological fate assessment of NMs in brain to support their risk assessment.

Key words: nanomaterials; blood brain barrier; neurotoxicity; single particle ICP-MS; synchrotron

T11-97-0034

Subchronic toxicity evaluation of food related titanium dioxide nanoparticles orally administered to rats

Hong Lin[1,2], Jianbin Tan[2], Jing Wang[2], Mansi Luo[2], Yun Liu[2], Weiling Huang[2], Hongxia Wang[2], Ying Jiang[2], Kexin Wang[2], Min Zhao[2], Ciyong Lu[1]

(1. Sun Yat-sen University; 2. Guangdong Provincial Center for Disease Control and Prevention)

Abstract: OBJECTIVE Titanium dioxide nanoparticles (TiO2 NPs) have been widely used in food industry, and existed in food grade TiO2, which was used for whitening and brightening foods, especially candies, chewing gums. A wider population of consumers risk long term exposure to TiO2 NPs via oral consumption of food. However, the researches about safety assessment of food related TiO2 NPs have been limited. METHODS We conducted a vivo study to investigate the potential effect of oral expo·sure to TiO2 NPs (anatase, 40 nm), which was selected based on the minimum particle size and com·mon crystal structure of TiO2 NPs additive in food. Eighty Sprague-Dawley rats were randomly divided into 4 groups (10 animals/sex/group), and were treated with TiO2 NPs suspended in ultrapure water by gavage at doses of 0, 10, 100, 1000 mg · kg^{-1} BW daily for 90 days. In addition, two satellite groups including control and maximum dose group (8 animals / sex / group) were orally administered to TiO2 NPs for 45 days, and allowed to recover for 28 days following 90-day exposure, respectively. During the experiment, the animals were observed for general clinical signs, ophthalmological examinations, body weight, food consumption. After termination of the repeated-dose 45-day, 90-day, and recovery studies, various experimental endpoints were examined such as hematology indexes, clinical biochem·istry parameters, urinalysis, organ weights, gross and microscopical pathology, endocrine changes, dis·tribution of Ti contents. RESULTS No systemic toxicological effects were related with the TiO2 NPs after 45-day and 90-day exposure in rats. For endocrine function, there were no marked toxicities in terms of endocrine related organ weights, histopathology, lipid and protein metabolism controlled by thyroid hormone, and serum hormone among rats after subchronic exposure. In the distribution analysis,

no obvious differences of Ti contents were detected in brain, liver, spleen, kidney, mesenteric lymph nodes, ovary/testis and urine of control and high-dose males/females after 90-day exposure. **CONCLUSION** The no-observed-adverse-effect level (NOAEL) of anatase TiO2 NPs (40 nm) was 1000 mg/kg/ day, the highest administered dose, in male or female SD rat.

Key words: titanium dioxide nanoparticles; oral exposure; subchronic toxicity; NOAEL; safety assessment

T11-97-0035

Subchronic Co-exposure of Titanium Dioxide Nanoparticles and Cadmium alleviated Cadmium-Induced Nephrotoxicity in Rats

Mansi Luo[1,2], Jianbin Tan[2], Jing Wang[2], Hong Lin[2], Yun Liu[2], Weiling Huang[2], Ying Jiang[2], Hongxia Wang[2], Kexin Wang[2], Min Zhao[2], Ying Lin[1]

(1. School of Biology and Biological Engineering, South China University of Technology; 2. Institute of Toxicology, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou)

Abstract: Researches have found that cadmium (Cd) and titanium dioxide nanoparticles (nTiO2) have interaction in vitro and in aquatic organisms. Cadmium has been a contaminant in food for a long time, and the safety of E171 (Titanium Dioxide) as a food colourant has been on the spotlight recently due to its nanoparticles component. In these cases, we investigated the oral combined toxicity of cadmium chloride (CdCl2) and nTiO2 (40 nm, anatase) in rats for 90 days. Forty female Sprague-Dawley (SD) rats (5-week-old) were equally divided into 4 groups: control group (Control), CdCl2 exposure group (Cd), nTiO2 exposure group (nTiO2) and combined exposure group (Cd+nTiO2). CdCl2 was administered in drinking water at 50 mg Cd/L, and nTiO2, dispersing in ultrapure water by ultrasonic, was given at 100 mg/ kg BW per day by gavage. In our study, there was no significant difference in body weight and serum biochemistry between any dosed group and control group. Nevertheless, in monthly urine routine test and urine biochemistry, biomarkers of renal injury elevated in both Cd group and Cd+ nTiO2 group, but compared to Cd group, the increase of Cd+ nTiO2 group was slighter. According to factorial analysis, co-exposure of Cd and nTiO2 had significant antagonistic effect on urea and creatinine after 1-month exposure. The pathological results verified that acute tubular injury and inflammatory infiltration was observed in Cd group, meanwhile the renal tubular lesion was milder in Cd + nTiO2 group with only moderate epithelial edema. We had measured the Cd concentration in 24h feces, and found an increasing trend of Cd excretion in Cd + nTiO2 group. In conclusion, after 90-day exposure, nTiO2 did not exert adverse effects on rats, while Cd caused significant nephrotoxicity to rats, whereas the nTiO2 ameliorated Cd-caused renal damage. Therefore, nTiO2 (40 nm, anatase, 100 mg/ kg BW) has antagonistic effect on the nephrotoxicity induced by CdCl2 (50 mg Cd/L) in female SD rats after 90- day oral exposure.

Key words: Co-exposure; Titanium dioxide nanoparticles; Cadmium; Nephrotoxicity; Antagonistic effect

T11-97-0036

The apoptosis induced by CdTe quantum dots through the mitochondrial pathway in dorsal root ganglion cell line ND7/23

Changcun Bai, Tingting Wei, Lingyue Zou, Na Liu, Xiaoquan Huang, Meng Tang
(Southeast University)

Abstract: Recently, the use of CdTe quantum dots in the field of biomedicine, such as biological imaging, biosensors, cell markers, and drug carriers, is increasing due to their special physical and chemical properties. However, their biosafety assessment lags far behind their rapid application. In this study, we observed that CdTe quantum dots with certain exposed doses and time decreased the cell viability while increased the apoptosis rates in ND7/23 cells. In general, CdTe quantum dots exposure could promote the accumulation of ROS in cells, decrease the mitochondrial membrane potential, which led to subcellular organelle damages such as mitochondrial swelling and pyknosis of the nucleus. The results suggested that CdTe quantum dots exposure increased the expression levels of three mitochondrial pathway markers, e. g. caspase-3, cytochrome c and Bax while decreased Bcl-2 protein expression, followed by cytochrome c falling out of the inner membrane of mitochondrial and releasing into the cytoplasm. The application of caspase-3 protein inhibitor Ac-DEVD-CHO could decrease apoptosis rates in ND7 / 23 cells. The results, taken together, demonstrated that CdTe quantum dots could induce apoptosis of ND7 / 23 cells through the mitochondrial pathway. Our findings provide a novel insight for researchers to explore the peripheral neurotoxicity mechanisms of CdTe quantum dots.

Key words: Neurotoxicity; Quantum dots; ND7/23 cells; Cell apoptosis; Reactive oxygen species

T11-97-0037

Toxicity mechanism of nanomaterials: focus on endoplasmic reticulum stress

Binjing Li
(Southeast University)

Abstract: The wide application of nanomaterials not only brings convenience to people's life, but also makes people worry about its safety. Therefore, exploring the toxicity mechanism of nanoparticles has always been a key research content of nanotoxicology. At present, generally recognized toxic mechanisms include oxidative stress, inflammatory response, autophagy, DNA damage and so on. In recent years, endoplasmic reticulum stress (ERS) is regarded as another toxic mechanism of nanomaterials by more and more people. Endoplasmic reticulum (ER) is an important place for the formation of protein and lipid and for the storage of Ca^{2+}. It participates in a variety of physiological processes in organisms. When the internal environment changes, it may cause ERS and trigger a series of biological reactions. Many studies believe that ERS is related to the occurrence and development of nervous system diseases, cardiovascular diseases and kidney diseases, so it is necessary to understand ERS deeply. This paper reviews the mechanism of ERS in the toxic effect of nanomaterials, briefly introduces the process of ERS and its related unfolded protein response, summarizes the factors affecting the ability of nanoparticles to induce ERS, and expounds the changes of ER morphology after exposure to nanoparticles. Finally, the specific role and molecular mechanism of ERS under the action of different nanoparticles were deeply analyzed, including the relationship between ERS and inflammation, oxida·

tive stress, lipid metabolism and apoptosis. It provides a reference for a better understanding of the tox·ic mechanism of nanoparticles, and also provides some new ideas for the safer application of nanoparti·cles and the treatment of diseases.

Key words: nanoparticles; endoplasmic reticulum stress; unfolded protein response; inflammation; apoptosis

T11-97-0038

Review of intestinal exposure and toxicity of nanomaterials

Xiaoquan Huang
(Southeast University)

Abstract: Nanomaterials are widely used in various fields, such as food processing, daily necessi·ties and medicine, which may contact with the gastrointestinal tract through multiple exposure routes. The intestine is an important digestion-absorption and barrier organ. However, for a long time, studies on the toxicity of nanomaterials have mostly focused on organs such as liver, kidney and brain. The in·testinal safety evaluation of nanomaterials is rare. Recently, there is evidence *in vivo* that nanoparticles entering into the intestine will be distributed in different sub-structures, leading to structural and func·tional damages, in which the gut microbiota and its metabolites play important roles. In addition, due to the complex physiological environment in the gastrointestinal tract, nanoparticle will undergo complex biotransformation. Therefore, in this review, we make results of mammalian experiments *in vivo* as evi·dence to discuss the damages and their possible mechanisms of nanoparticles to intestinal structure and function. In addition, the exposure routes, biodistribution and biotransformation of nanoparticles in the intestine are also considered. We hope this review will arouse people′ s attention to intestinal safety of nanomaterials and guide the research of intestinal nanotoxicology *in vivo*.

Key words: Nanomaterials; Nanoparticles; Gut microbiota; Gut barrier

T11-97-0039

Understanding the aggregation and toxicity of amyloid proteins

Pu Chun Ke1,2, Aleksandr Kakinen2, Yunxiang Sun3, Nicholas Andrikopoulos4, Feng Ding5
(1. The GBA National Institute for Nanotechnology Innovation; 2. Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Australia; 3. Ningbo University; 4. Monash Institute of Pharmaceutical Sciences, Monash University; 5. Clemson University, USA)

Abstract: In this talk, the amyloid aggregation of proteins associated with the pathologies of Al·zheimer′ s disease, Parkinsons′ disease and type 2 diabetes will be discussed. Specifically, the struc·tural and toxicity profiles of the heterogeneous oligomers, the cross seeding of human islet amyloid polypeptide by its primary and secondary amyloidogenic fragments, the pathogenesis of amyloid beta elevated by bacterial protein FapC, and the mitigation of a range of amyloidosis by inorganic and poly·meric nanomaterials as well as bio-nanocomposites will be presented. This talk intends to highlight a complex nano-bio interface in nanomedicine, where nanotoxicology plays an indispensable role against

dementia and metabolic diseases.

Key words: amyloid aggregation; the oligomer; cross seeding; toxicity; inhibition

T11-97-0040

Integrative effects based on behavior, physiology and gene expression of tritiated water on zebrafish

Shengri Li[a,b], Yefeng Zhang[a,c], Huiyuan Xue[a,b], Qixuan Zhang[a,b], Na Chen[a,b], Jun Wan[a,b], Liang Sun[a,b], Qiu Chen[a,b], Ying Zong[a], Fenghui Zhuang[a], Pengcheng Gu[a], Anqi Zhang[a], Fengmei Cui[a,b]*, Yu Tu[a,b]

(a. State Key Laboratory of Radiation Medicine and Protection, School of Radiation Medicine and Protection, Soochow University, Suzhou 215123, China; b. Collaborative Innovation Center of Radiation Medicine of Jiangsu Higher Education Institutions, Suzhou 215123, P.R. China; c. School of Public Health, Soochow University, Suzhou 215123, China)

Abstract: Tritium is a water-soluble hydrogen isotope that releases beta rays during decay. In nature, tritium primarily exists as tritiated water(HTO), and its main source is nuclear power / processing plants. In recent decades, with the development of nuclear power industry, it is necessary to evaluate the impact of tritium on organisms. Zebrafish is an excellent vertebrate biological model and has been applied on several fields such as biomedicine and environmental toxicology assessment. In this study, fertilized zebrafish embryos are treated with different HTO concentrations. After treatment withHTO, the zebrafish embryos developed without evident morphological changes. Nevertheless, the heart rate increased andlocomotor activity decreased significantly. In addition,RNA-sequencing shows that HTOcan affect geneexpressions. The differentially expressed genes are enriched through many physiological processes and intracellular signaling pathways, including cardiac development, nervous system development, cardiovascular system development and the metabolism of xenobiotics by cytochrome P450.

Moreover, the concentrations of thyroid hormones in the zebrafish decrease and the expression of thyroid hormone-related genes aredisorderedafter HTO treatment.Our results suggest that exposure to HTO may affect the physiology and behaviors of zebrafish through physiological processes and intracellular signaling pathways and provide a theoretical basis for ecological risk assessment of tritium.

Key words: nuclear waste, water contamination, aquatic organisms, gene expression, HTO,locomotor activity level

Corresponding author: Shengri Li, E-mail: cuifengmei@suda. edu. cn; Yu Tu, E-mail: tuyu@suda. edu.cn

T11-97-0041

Effects of Micro-and nano-particles of foodborne titanium dioxide on gut microbiota-host Co-Metabolites in juvenile mice

Jun Yan, Lei Tian, Kang Li, Xiaohua Liu*, Zhuge Xi *

(Tianjin Institute of Environmental & Operational Medicine, China. No. 1, Dali Road, Heping District, Tianjin 300050, China)

Abstract: The wide use of TiO_2 particles in food and the high exposure risk to children have

prompted research into the health risks of TiO_2. We used targeted metabolomics to explore the potential mechanism of intestinal toxicity of foodborne TiO_2 micro -/nanoparticles after oral exposure for 28 days in juvenile mice. A total of 121 gut microbiota-host co-metabolites were measured. The results showed that the gut microbiota-host co-metabolites changed significantly. Compared with the control group, 24 different metabolites were screened out in the micro-TiO_2 groups. Among these, there were 20 different metabolites in the micro-TiO 10 mg·kg^{-1} bw group and 10 different metabolites in the micro-TiO 40 mg·kg^{-1} bw dose group. Six metabolites—homocysteine, L-glutamic acid, myristic acid, palmitic acid, phenylacetic acid, and pyroglutamic acid—were shared by the two groups with different doses of micro-TiO_2, and may be potential biomarkers of exposure to micro-TiO_2. Compared with the control group, nine metabolites were screened out in the nano-TiO_2 group. Among these, there were four different metabolites in the nano-TiO 10 mg·kg^{-1} bw group and seven different metabolites in the nano-TiO 40 mg·kg^{-1} bw group. Pyroglutamic acid and 3-hydroxyphenylacetic acid were differential metabolites shared by the two doses of nano-TiO_2. Pyroglutamic acid, L-glutamic acid, phenylacetic acid, 3-hydroxyphenylacetic acid, and homocysteine were common differential metabolites among the groups exposed to the two kinds of TiO_2 (micron and nanoscale). Differential metabolic pathway analysis showed that Glutathione metabolism and propionic acid metabolism were the different metabolic pathways shared by the groups exposed to the two types of TiO_2 particles. In addition, micro-TiO_2 significantly affected the biosynthesis of unsaturated fatty acids, and nano-TiO_2 significantly affected the metabolic pathways of D-glutamine and D-glutamate metabolism. Changes in these metabolites and pathways may be an important metabolic mechanism for the toxic effects of TiO_2 micro-/nanoparticles.

Key words: TiO_2 micro-/nanoparticles; gut microbiota-host co-metabolites; metabolic mechanism

Corresponding author: Xiaohua Liu, E-mail: liuxiaohua1992@sina.com; Zhuge Xi, E-mail: zhugexi2003@sina.com

T12-12-0001

Chemometric QSAR modeling of acute oral toxicity of polycyclic aromatic hydrocarbons (PAHs) to Rat Using Simple 2D descriptors and interspecies toxicity modeling with mouse

Guohui Sun*, Yifan Zhang, Luyu Pei, Yuqing Lou, Yao Mu, Lijiao Zhao, Rugang Zhong

(Beijing Key Laboratory of Environmental and Viral Oncology, College of Life Science and Chemistry, Faculty of Environment and Life, Beijing University of Technology, Beijing 100124, P. R. China)

Abstract: The information of the acute oral toxicity for most PAHs in mammals are lacking due to limited experimental resources, leading to a need to develop reliable in silico methods to evaluate the toxicity endpoint. In this study, we developed the quantitative structure-activity relationship (QSAR) models by genetic algorithm (GA) and multiple linear regression (MLR) for the rat acute oral toxicity (LD50) of PAHs following the strict validation principles of QSAR modeling recommended by OECD. The best QSAR model comprised eight simple 2D descriptors with definite physicochemical meaning, which showed that maximum atom-type electrotopological state, van der Waals surface area, mean atomic van der Waals volume, and total number of bonds are main influencing factors for the toxicity endpoint. A true external set (554 compounds) without rat acute oral toxicity values, and 22 limit test compounds, were firstly predicted along with reliability assessment. We also compared our proposed

model with the OPERA predictions and recently published literature to prove the prediction reliability. Furthermore, the interspecies toxicity (iST) models for PAHs between rat and mouse were also estab·lished, validated and employed for filling data gap. Overall, our developed models should be applicable to new or untested or not yet synthesized PAHs falling within the AD of the models for rapid acute oral toxicity prediction, thus being important for environmental or personal exposure risk assessment under regulatory frameworks.

Key words: PAHs; Acute oral toxicity; QSAR; Interspecies toxicity model; Toxicity prediction

Corresponding author: Guohui Sun, E-mail: sunguohui@bjut.edu.cn

T12-28-0004

Read-across approach for chemical hazard assessment

Wang Ying[a], Kwon, Seok[b]

(a. P&G Technology (Beijing) Co., Ltd. No. 35 Yu'an Road, B Zone, Tianzhu Konggang Development Zone, Shunyi District, Beijing, 101312, P.R. China; b. Procter & Gamble (P&G) International Operations, 70 Biopolis Street, Singapore 138547)

Abstract: Structure Activity Relationship (SAR) - based Read-Across is a predictive toxicology approach to establish a safety profile when only limited information is available on a target chemical. When toxicological data are unavailable on a target chemical, but are available on structurally similar chemicals, which are expected to have similar metabolic fate, chem/bio-reactivity and physicochemical properties, those data can be read across to predict the toxicity of the data-deficient chemical. It has been the most widely used animal alternative approach in the past 25 years and is accepted by many regulators globally responsible for chemical registrations including US, Canada, EU, Korea and Japan. Guidance for application of Read-Across has also been published by international authorities, such as OECD and ECHA, as well as literatures.

The internal P&G integrated framework provides the process for conducting and documenting SAR-based Read-Across assessments, including the considerations of structural similarity, physical- chemical properties, ADME information, reactivity and biology similarity, etc. Firstly, the purpose and data gap(s) of the read across are defined. Then suitable analogues are identified, similarities/suitability of analog(s) are evaluated, toxicity data is collected, and the final read-across hypothesis is verified. Key scientific elements must be considered to complete a read-across assessment. Ultimately the SAR- based read-across should be fully described in the assessment with justification, which provides the sci·entific rationale to support the hypothesis of similarity, explains why the read-across is appropriate and also outlines any areas of uncertainty. The SAR-based read-across assessment is a complex and itera·tive process typically requiring some degree of expert judgement. The weight of evidence (WoE) ap·proach is used to evaluate and integrate multiple information streams to support the read-across. Final·ly, a conclusion is determined for the read-across based on the strength of the WoE, clarity of the justifi·cation, consideration of residual uncertainties, and the original purpose of read-across.

Along with additional trainings and development of new approach methods (NAM), read-across can be applied and accepted more broadly. In this presentation, we will also introduce an interdisciplin·ary Read-Across work group at Korea Society of Toxicology consisting of safety experts from public and private sectors.

Key words: SAR (structure activity relationship)-based Read Across, Animal Alternative

Corresponding author: Wang Ying, E-mail: wang.yi.13@pg.com, Kwon, Seok, E-mail: kwon.s@pg.com

T12-60-0003

Mutagenic risk prediction of PAH mixtures originated from metabolic activation characteristics by CYP450 conformations and QSAR approach

Chao Chen, Jiemiao Shen, Rong Xia, Yue Min, Di Zhang, Shou-Lin Wang*

(Key Lab of Modern Toxicology of Ministry of Education, Center for Global Health, School of Public Health, Nanjing Medical University, 101 Longmian Avenue, Nanjing 211166, PR China)

Abstract: PAHs and their derivatives are the main sources of mutagenicity in airborne particular matter and cause serious public health and environmental problems. Toxicity mechanism research and risk assessment of PAHs are challenged by the mixed nature and deficiency of toxicity data of most PAHs and their derivatives. Cytochrome P450 enzymes (CYPs) play important roles in PAH-induced carcinogenicity via metabolic activation. P450 protein conformations of either different enzymes or different stages in metabolic cycle determine the metabolic fate of binding PAHs. In this study, typical CYP protein conformations were simulated and combined with QSAR descriptor analysis for metabolic activation characteristics identification of PAHs. Specific Binding stage and Compound I stage conformations of CYP1A1 were simulated by QM / MM calculation. With these conformation models, key structural properties that affect metabolic processes of PAHs by CYP1A1 were identified using quantitative structure-activity relationship (QSAR) strategy. Several properties including van der Waals interaction, binding distance, binding affinities and HOMO energy showed significant influence on the metabolic processes. Further analysis of CYP1B1 and 2A13 compound I conformations showed that a common optimum binding distance for PAHs clearance may exist among different CYPs. By quantifying the behaviors of PAHs interacting with CYP1A1, 1B1, 2C19 compound I conformations, which derived from BaP epoxide generation process, mutagenicity values of PAHs and derivatives were predicted with a metabolic conformation fitness (MCF) approach. Then, the prediction model for the mutagenicity risk of PAH and derivative mixtures was established based on the relative potential factor (RPF) approach and mutagenicity prediction, which was then successfully validated by the mutagenesis of PAH and derivative mixture mimic samples of PM2.5 collected in eastern China. The models derived from this study provide useful tolls for screening, classifying and predicting PAHs for their metabolism-related toxicities and risk assessment of their complex mixtures in the environment.

Key words: Computational toxicology; Polycyclic aromatic hydrocarbons; Cytochrome P450; Mutagenicity; Risk assessment

Corresponding author: Chao Chen, E-mail: chenchao1990@njmu. edu. cn, Shou-Lin Wang, E-mail: wangshl@njmu.edu.cn

T12-65-0006

Development of PBPK model for two active compounds in Spatholobi Caulis in rats and humans

Liu Xiao-yan, Zhang Tao, Wang Qi

(Department of Toxicology, School of Public Health, Peking University, Beijing 100191, China)

Abstract: 3′-methoxydaidzein and 8-O-methylretusin are two phytochemicals from Spatholobi

Caulis (SPC) with anti-inflammatory activities. Here, the objective of our study is to develop physiological-based pharmacokinetic (PBPK) models in rats for these two active compounds in SPC with the assistance of ADMET Predictor and GastroPlus software. To predict the pharmacokinetic properties after orally administrated different doses of SPC to rats, then the PBPK models were extrapolated to humans to predict the kinetic characteristics in different species.

Based on the physicochemical and pharmacokinetic parameters obtained from the ADMET Predictor and our previous studies, PBPK models of 3′-methoxydaidzein and 8-O-methylretusin in SPC in rats were constructed and optimized, respectively. The model accuracy was assessed by fold error (FE =10 log (predicted/observed). When FE<2, the model is considered to be accurate). As shown in Figure 1 (A1 and B1), the predicted and observed concentration-time curves fit well when rats were orally administered 3′-methoxydaidzein and 8-O-methylretusin in SPC with doses at 0.062 mg·kg^{-1} and 0.11 mg·kg^{-1}, respectively. The FE of C_{max} (μg·mL^{-1}), T_{max} (h) and AUC0→12 (μg·h·mL^{-1}) for 3′-methoxydaidzein were 1.00, 1.04 and 1.11, and for 8-O-methylretusin were 1.06, 1.12 and 1.02, respectively, indicating the accurate and reliable models that can be used for future prediction. Moreover, the concentration-time curves of 3'-methoxydaidzein and 8-O-methylretusin in rats with different doses were simulated by the well-constructed PBPK models, and the curves and pharmacokinetic parameters of these two compounds in humans were also extrapolated by using these models.

Key words: PBPK model, 3′-methoxydaidzein, 8-O-methylretusin, pharmacokinetics

Corresponding author: Wang Qi, E-mail: wangqi@bjmu.edu.cn

T12-77-0002

Target analyses and preclinical validation of repositioned antiviral drugs based on host-directed antiviral strategy

Xie Dafei[a], He Song[b], Han Lu[c], Tao Huan[b], Wu Lianlian[d], Zhou Pingkun[a], Bai Hui[b,e], Bo Xiaochen[b]

(a. Department of Radiation Biology, Beijing Institute of Radiation Medicine, Beijing, China, 100850; b. Department of Biotechnology, Beijing Institute of Radiation Medicine, Beijing, China, 100850; c. Beijing Institute of Pharmacology and Toxicology, Beijing, China, 100850; d. Academy of Medical Engineering and Translational Medicine, Tianjin University, Tianjin, China, 300072; e. BioMap (Beijing) Intelligence Technology Limited, Beijing, China, 100005)

Abstract: Inhibiting host-cell protein functions using established drug compounds with excellent safety profiles produces promising antiviral effects with greater flexibility for candidate targets, decreased incidence of resistant variants and favorable balance of costs and risks. Recent genomic methods generated a large number of robust host dependency factors, providing numerous candidates for discovery of novel drug targets and antivirals. However, there is a lack of a global view of the landscape of different viruses and their potential host factors targeted by known drugs. Development of repositioned antivirals is currently limited to discrete study of single virus. Here, we integrated comprehensive published data among viruses, host factors and known drugs and presented a large-scale drug-virus network (DVN) to predict novel antiviral therapeutics on the basis of matching host factors for viral infections and drug targets. We grouped viral infections from a new perspective based on virus targeted host proteins (VTHPs) according to their enriched signaling pathways. Elucidating superiority of non-essential membrane and hub proteins as potential targets of repositioned drugs, we proposed 543 druggable candidate VTHPs. We then predicted new antiviral indications and therapeutic potentials for 703

FDA-approved small-molecule drugs, exploring their potential as broad-spectrum antivirals. The *in vitro* and in vivo tests confirmed the repositioned indications for bosutinib, maraviroc and dextromethorphan against herpes simplex virus-1 (HSV-1), hepatitis B virus (HBV) and influenza A virus (IAV), respectively for their potency in non-toxic concentrations. In addition, efficacy of their combinations with antivirals already in clinical use have been investigated and confirmed. Dextromethorphan in low doses turned out to be superior to high doses in both single and combined treatments. This study offers a systematic and efficient bioinformatics way to discover novel host targets with higher potential and new antiviral indications for established drugs, which may facilitate their translation into clinical therapies for combating viral infections.

Key words: Virus-host interactome; network analysis; drug target; drug repositioning; HBV

Corresponding author: Zhou Pingkun, E-mail: zhoupk@bmi.ac.cn; Bai Hui, E-mail: huibai13@hotmail.com; Bo Xiaochen, E-mail:boxc@bmi.ac.cn

T12-85-0007

Ensemble machine learning approach to predict antibiotic resistance genes with low sequence conservation

Kunkai Su[1], Xin Huang[2], Danhua Zhu[1], Lanjuan Li[1], Yonghong Xiao[1]

(1. State Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University; 2. Biotherapy Research Center, The Second Affiliated Hospital, College of Medicine, Zhejiang University)

Abstract: Antimicrobial resistance (AMR) has been observed since the first antibiotics were discovered, yet antimicrobial abuse and misuse has resulted in increasing levels of clinical resistance. Current online databases of AMR genes mainly rely on manual bio-curation of fast accumulated literatures. Algorithm employing sequence alignment is constrained by the lack of solid standard of cut-off to define AMR. PSI-blast was employed retrieve potential AMR genes, especially those showing low similarity to the experimental validated AMR genes. Based on our thorough examination of feature engineering, algorithm choosing and ensemble evaluation, and meanwhile to keep the model concise and time-saving, a final prediction model employing composition of k-spaced amino acid pairs (CKSAAP) and Random Forrest (RF) was screened out. The AUROC were >0.999 and 0.983 for 5-fold validation and independent test, respectively. All the reviewed protein sequences of bacteria from Unirpot were evaluated by the model, and allocated into multidrug resistance and other nine major concerned AMR categories. Conclusions: PARD (PSSM-based Antimicrobial Resistance Database) was established to keep track of the curated and retrieved AMR genes and provide an online machine learning approach.

Key words: Antibiotic resistance; machine learning; support vector machine; random forest; ensemble learning

Corresponding author: Kunkai Su, E-mail: ksu@zju.edu.cn

T12-98-0005

Machine learning as a powerful tool to analyze complicated biological responses

Xiangang Hu

(College of Environmental Science and Engineering, Nankai University, Tianjin China, 300350)

Abstract: The development of machine learning provides solutions for predicting the complicated immune responses and pharmacokinetics of nanoparticles (NPs) in vivo, which is critical to the effective design and safe applications of NPs in various fields (e. g., cancer treatment and drug delivery). However, highly heterogeneous data in NP studies remain challenging due to the low interpretability of machine learning. Here, we propose a tree-based random forest feature importance and feature network interaction analysis framework (TBRFA) and accurately predict the pulmonary immune responses and lung burden of NPs, with the correlation coefficient of all training sets >0.9 and half of the test sets >0.75. This framework overcomes the feature importance bias brought by small datasets through a multiway importance analysis. TBRFA also builds feature interaction networks that are difficult to identify by machine learning, boosts model interpretability and reveals hidden interactional factors (e.g., various NP properties and exposure conditions). TBRFA provides guidance for the design and application of ideal NPs and discovers the feature interaction networks that contribute to highly complex systems with small-size data, such as human diseases.

Key words: Machine learning; Nanomaterial; AI

T12-98-0008

Correlation between local discontinuity of network-like structure-activity landscape and prediction errors of QSAR models

Wang Zhongyu, Chen Jingwen

(Key Laboratory of Industrial Ecology and Environmental Engineering (Ministry of Education), Dalian Key Laboratory on Chemicals Risk Control and Pollution Prevention Technology, School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024)

Abstract: QSAR models have been widely employd as an inexpensive and pragmatic instrument to fill data gaps in safety assessment of chemicals. For a defined toxicity endpoint, performance of the QSAR models based on carefully curated data sets could still be disappointing, which were characterized by coined indices such as modelability and rivality index. The indices are closely related to "activity cliffs" in structure-activity landscapes (SALs). The SAL could be depicted as a network-like similarity graph (NSG), where nodes and edges represent chemicals and pairwise similarity between the chemicals, respectively. From the NSG, local discontinuity (LD) could be calculated for each nodes, reflecting the "ruggedness" of the local SAL. This study took previously established QSAR models on human PPARγ activity as examples, showed that LD significantly correlated with the cross-validation prediction errors of the QSAR models. Furthermore, several modified LD indices were invented to explain both magnitude and direction (sign) of the prediction errors. These indices enhanced understanding of the holistic performance and per-chemical prediction reliability of QSAR models.

Key words: QSAR; modelability; network; structure activity landscapes

Corresponding author: Chen Jingwen, E-mail: jwchen@dlut.edu.cn

T12-98-0009

Development and Improvement of in silico approaches for accelerating regulatory chemical risk assessment

Takashi Yamada

(National Institute of Health Sciences)

Abstract: Risk assessment of a huge number of chemical substances without test data has become a major issue. There is a strong demand for improving the predictive performance and reliability of in silico approaches, expanding the applicability domain, and accelerating the practical use for safety assessment. To meet these regulatory needs, NIHS has conducted developing and improving in silico approaches for major toxicity endpoints for human health effects. Here I show two in silico approaches we work on, one is QSAR for mutagenicity, another is read-across for repeated dose toxicity. NIHS developed a large-scale database for Ames mutagenicity consisting of about 12,000 chemical substances under the Industrial Safety and Health Act in Japan. By sharing this dataset with QSAR vendors all over the world, we conducted international collaborative research for improving the models. Most QSAR tools achieved >50% sensitivity, and accuracy was as high as 80%, almost equivalent to the inter-laboratory reproducibility of Ames tests. In terms of repeated-dose toxicity, NIHS developed case studies of read-across using the category approach, some of which were shared by the OECD IATA Case Studies Project. Through the critical reviews by experts, we figured out several key points for increasing regulatory acceptance, including similarity hypothesis based on possible mechanism, category justification, and endpoint prediction using reliable test data in a transparent and reproducible way. Moreover, we developed a new Adverse Outcome Pathway of histone deacetylase inhibition leading to testicular atrophy via epigenetic changes, which was approved by the OECD in 2021. It is expected to be applicable not only for the prioritization of toxicity testing but also for a mechanism-based integrated approach to toxicity assessment. In recent years, the OECD and other international organizations have been working to develop guidance documents on the use of the in silico methods. The Food Safety Commission of Japan, a risk assessment organization, is developing guidance on the use of QSAR when assessing the mutagenicity of food-related substances. To increase the regulatory acceptance, sufficient reliability needs to be shown. At the same time, efforts should be made to further communicate with stakeholders about the benefits as well as uncertainties and limitations.

Key words: QSAR; Ames mutagenicity; read-across; repeated-dose toxicity

T12-98-0010

Could superoxide radical be implemented in decontamination processes?

Ruiyang Xiao

(1.Institute of Environmental Engineering, School of Metallurgy and Environment, Central South University, Changsha 410083, China; 2. Chinese National Engineering Research Center for Control & Treatment of Heavy Metal Pollution, Changsha 410083, China)

Abstract: Contemporary studies emphasize that superoxide radical ($O_2^{\cdot-}$) exhibits the potential to degrade organic contaminants, but practical application of this radical in engineered waters require an in-depth understanding of its kinetic profiles in a quantitative way. Here, we developed, for the first

time, a convenient and reliable approach to generate micromolar level $O_2^{\cdot-}$ in aqueous solution by photolysis of formate and H_2O_2. The presence of $O_2^{\cdot-}$ was confirmed by comparing the UV spectra under pulse radiolysis and chromogenic reaction. We then constructed an in situ long-path spectroscopy to investigate the kinetics and mechanisms of $O_2^{\cdot-}$-mediated degradation of representative emerging contaminants, including antibiotics and perfluorocarboxylic acids (PFCAs). In addition, we employed the transition state theory to model the reaction rate constants. Both results show that $O_2^{\cdot-}$ exhibited low reactivity towards these containments. In addition, the solvation mechanisms for $O_2^{\cdot-}$-mediated degradation of the contaminants were elucidated. The complementary experimental and theoretical approaches provide a mechanistic basis for better understanding aqueous-phase $O_2^{\cdot-}$ chemistry and a holistic evaluation on the application of $O_2^{\cdot-}$ for the degradation of organic contaminants of emerging concern.

Key words: Superoxide radical; In situ long-path spectroscopy; Aqueous solution; Mechanism; Kinetics.

T12-98-0011

Deep learning on molecular structure for estrogen receptor agonism of chemicals

Wang Liguo, Zhao Lu, Liu Xian, Fu Jianjie, Zhang Aiqian*

(State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, P. R. China)

Abstract: Deep learning (DL) offers an unprecedented opportunity to revolutionize the landscape of toxicity prediction based on quantitative structure-activity relationship (QSAR) studies in the big data era. The surprising learning power and flexible structure of deep neural networks allow QSAR models to be provided raw molecular structures, and quantitative descriptors are no longer indispensable. By using data from a set of 18 *in vitro* high-throughput tests for estrogen receptor (ER) activity in ToxCast project, ML methodology study on molecular structure representation has been carried out, aiming to realizing the direct mapping of chemical structure to its ER agonist activity and improve both prediction performance and interpretability of the QSAR model for identification of environmental estrogens. A novel three-dimensional (3D) molecular surface point cloud with electrostatic potential (SepPC) was proposed to describe chemical structures, and a DL architecture SepPCNET was then introduced to directly consume unordered SepPC data of both binary classification and multi-task learning. The obtained model recognized the active and inactive chemicals with a total accuracy of 88.3% on the internal test set and 92.5% on the external test set, which outperformed other up-to-date machine learning models. The same network architecture was adopted to successfully establish a multi-task learning model for 18 ER-mediated assays. Additional insights into the toxicity mechanism were gained, which enabled a "black box" model to become more transparent and explainable.

Key words: Deep learning; Toxicity prediction; Structure representation; Model interpretability

Corresponding author: Zhang Aiqian, E-mail: aqzhang@rcees.ac.cn

T12-98-0012

Machine learning models for predicting lung inflammation induced by metal oxide nanoparticles

Li Xue-hua[1], Huang Yang[1], Li Rui-bin[2], Chen Jing-wen[1]

(1. Key Laboratory of Industrial Ecology and Environmental Engineering, School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China; 2. State Key Laboratory of Radiation Medicine and Protection, Collaborative Innovation Center of Radiological Medicine of Jiangsu Higher Education Institutions, School for Radiological and Interdisciplinary Sciences (RAD-X), Soochow University, Suzhou 215123, China)

Abstract: Risk assessment of emerging substances such as engineered nanomaterials (ENMs) is essential to protect human health and the environment. Non-testing approaches in hazard assessment is necessary, considering cost and time efficiency to assess potential risks. Lung inflammation caused by inhaled nanoparticles is an endpoint of high concern. Development of in silico models can fill gaps in lung toxicity data, avoid testing of each new nanoparticle formulation from scratch and facilitate safe nanotechnology. However, no previous attempt has been made to predict the inflammatory potential of nanoparticles. Thus, machine learning models were built to relate metal oxide nanoparticle (MeONPs)' physicochemical properties to their inflammation in an alveolar-macrophage-like cell (THP-1), which is the main route to remove inhaled insoluble fine particulates. A comprehensive dataset of 30 MeONPs was built to screen a pro-inflammatory cytokine (IL-1β) release in THP- 1 cells. The in vitro toxicity data were validated in mouse lungs by oropharyngeal instillation of six representative MeONPs. Inflammatory potential was correctly predicted with predictive accuracy (ACC) exceeding 90% and further validated experimentally with ACC reaching 86%. DFT computations further revealed the underlying mechanisms: MeONPs with lower metal electronegativity and positive ζ-potential were more likely to cause lysosomal damage and substantial inflammation. The models can help to address the ethical, economic, and time constraints of traditional toxicology while also advance mechanistic understanding.

Key words: engineered nanomaterials; computational toxicology; machine learning; health risk assessment

Corresponding author: Li Xue-hua, E-mail: lixuehua@dlut.edu.cn

T13-13-0010

The epigenetic alteration and correlation analysis of premature senescence cell induced by oxidative stress

Fan Wu[a, c], Susu Yu[a, c], Xinyue Peng[a], Jianji Gao[a], Caiyun Lai[a], Yan Wang[a], Yueqi Li[a], Gaoqiang Zhang[a], Bo Zhang[b], Wenjuan Zhang[a]

(a. Department of Public Health and Preventive Medicine, School of Medicine, Jinan University, Guangzhou, Guangdong 510632, P.R. China; b. School of Public Health, Southern Medical University, Guangzhou, Guangdong 510515, P. R. China; c. These authors contributed equally to this article)

Abstract: Oxidative stress brings devastating effects to organism, including senescence and related diseases, mainly due to the excess reactive oxygen species (ROS). The epigenetic alterations of ox·

idative stress-induced premature senescence possibly providing insights into target treatment in medi·cal. Using the human embryonic lung fibroblasts as the cell senescence model, which were divided into three groups: the young group (22PDL) as the control; the premature senescence initiation (PSi) group, which were exposed to 400 μmol·L^{-1} H_2O_2 for 4 days, 2 hours each time; and the premature aging per·sistence (PSp) group, which were cultured complete medium for 7 days after periodically H_2O_2 expo·sure. It was showed that exogenous oxidative stimulation resulted in the rapid accumulation of mito·chondrial ROS (mtROS) in PSi cells, while the compensatory reduction in PSp group promoted the oc·currence of premature senescence. In premature senescence cells (PSi and PSp), the mRNA expres·sion of *SOD2* decreased but the protein expression increased, however the expressions of *SOD1*, *SOD3* and *CAT* had no significant alteration. Furthermore, both PSi and PSp cells had the lower lever of the mtDNA methylation than the young cells, besides the PSp cells were under a lower m6A methyla·tion. In addition, there were negative correlations between the methylation levels and mtROS. Overall, the oxidative stress damaged cells by ascending SOD2 protein expression and reducing methylations of mtDNA as well as m6A RNA modification, eventually leaded cellular premature senescence. These epigenetic alterations could be able to provide the potential treatment target for the intervention of se·nescence and its related diseases.

Key words: Oxidative stress; Premature senescence; Mitochondrion; m6A; Epigenetics;

Corresponding author: Bo Zhan, E-mail: zhangbo2018@smu. edu. cn; Wenjuan Zhang, E-mail: zwj2080@126.com

T13-15-0018

The function and mechanism of protein crotonylation modifications in Radiation DNA damage repair

Yang Han[a], Gang Li[b], Hejiang Guo[a], Hua Guan[a], Chenjun Bai[a], shanshan Gao[a], Pingkun Zhou[a]

(a. Department of Radiation Biology, Beijing Key Laboratory for Radiobiology, Beijing Institute of Radiation Medicine, Beijing 100850, China; b. Institute for Environmental Medicine and Radiation Hygiene, School of Public Health, University of South China, Hengyang, Hunan 421001)

Abstract: OBJECTIVE Protein post-translational modification plays vital roles in DNA damage response (DDR) signal transduction, DNA damage repair and other aspects. Lysine crotonylation(Kcr) modifica·tion is a newly post-translational modification that is initially identified by the laboratory of Professor Yingming Zhao from the University of Chicago, is mainly mediated by acetylases CBP, PCAF, P300, etc., and participates in regulating reproduction, DNA damage repair and other life activities. In previ·ous reports, CBP, PCAF, P300, etc. regulate DNA damage response through acetylation of their down·stream substrates, but the function and regulation of Kcr activity mediated by them in DDR is still un·clear. METHODS 1. Mass spectrometry modified omics to screen the changes of protein Kcr in MCF7 cells before and after radiation; 2. Bioinformatics excavated modified omics data to select the most valuable DNA damage repair protein; 3. Use Western Blot and Co-IP (Co-Immunoprecipitation) for veri·fication; finally, its function is verified by methods such as site-directed mutagenesis.Results: 1. Kcr par·ticipates in the NHEJ repair pathway; 2. MRE11 can undergo Kcr modification, and the Kcr level of MRE11 was reduced after irradiation; 3. The crotonylation modification site of MRE11 is identified by modification omics data; 4. the Kcr modification site of MRE11 has been clarified by the Western Blot and Co-IP. Conclusion: MRE11 can bemodified by Kcr, and the modification level of MRE11 was re·

duced after irradiation. MRE11 is an important protein in DNA damage repair. Therefore, Kcr modification may play an important role in DNA damage repair.

Key words: lysine crotonylation modification; MRE11; NHEJ; DNA repair; DNA damage response

Corresponding author: Pingkun Zhoua, E-mail: zhoupk@bmi.ac.cn

T13-22-0020

Inhibiting TRPV1-mediated autophagy attenuates nitrogen mustard-induced dermal toxicity

Mingliang Chen[1,2,3], Xunhu Dong[1,3], Haoyue Deng[4], Feng Ye[1,3], Yuanpeng Zhao[1,3], Jin Cheng[1,3], Guorong Dan[1,3], Jiqing Zhao[1,3], Yan Sai[1,3], Xiuwu Bian[2], Zhongmin Zou[1,3]

〔*1. Department of Chemical Defense Medicine, School of Military Preventive Medicine, Third Military Medical University (Army Medical University), 30 Gaotanyan Street, ShapingbaDistrict, Chongqing 400038, China; 2. Institute of Pathology and Southwest Cancer Centre, Southwest Hospital, Third Mili- tary Medical University (Army Medical University),Chongqing 400038, China; 3. Institute of Toxicology, School of Military Preventive Medicine, Third Military Medical University (Army Medical University), 30 Gaotanyan Street, Shapingba District, Chongqing 400038, China; 4. State Key Laboratory of Trauma, Burns and Combined Injury, Second Department of Research Institute of Surgery, DapingHospital, Third Military Medical University (Army Medical University), Chongqing 400042, China*〕

Abstract: Nitrogen mustard (NM) causes severe vesicating skin injury, which is lack of effective and targeted therapies. The major limitation is that the specific mechanism of NM-induced skin injury has not been well understood. Recently, autophagy has been found to play important roles in physical or chemical exposure-caused cutaneous injuries. However, whether autophagy contributes to NM-induced dermal toxicity is unclear. Herein, we initially confirmed that NM dose-dependently caused cell death and induced autophagy in keratinocytes. Suppression of autophagy by 3-methyladenine, chloroquine and bafilomycin A1 or ATG5 siRNA attenuated NM-caused keratinocyte cell death. Furthermore, NM increased transient receptor potential vanilloid 1 (TRPV1) expression, intracellular Ca^{2+} content, and the activity of Ca^{2+}/Calmodulin-Dependent Kinase β (CaMKKβ), AMP-activated protein kinase (AMPK), ULK1 (unc-51-like kinase 1) and mammalian target of rapamycin (mTOR). NM-induced autophagy in keratinocytes was abolished in the presence of inhibitors of TRPV1 (capsazepine), CaMKKβ (STO-609), AMPK (compound C) or ULK1 (SBI-0206965) as well as TRPV1, CaMKKβ, and AMPK siRNA transfection. Additionally, mTOR inhibitor (rapamycin) had no significant effect on NM-stimulated autophagy and cell death of keratinocytes. Finally, the results of *in vivo* study in NM-treated skin tissues were consistent with the findings of *in vitro* study. In conclusion, NM caused dermal toxicity by overactivating autophagy partially through the activation of TRPV1-Ca^{2+}- CaMKKβ - AMPK-ULK1 signaling pathway. These suggest that blocking TRPV1-dependent autophagy could be a potential treatment strategy for NM-caused cutaneous injury.

Key words: Nitrogen mustard; autophagy; dermal toxicity; TRPV1; keratinocytes

T13-24-0004

Circadian clock proteinsregulate ovarian reproductive dysfunction under altitude environmental conditions

Mengnan Ding, Xin Huang, Chen Xing, Kun Liu, Lun Song

(Department of Neuroimmunology, Beijing Institute of Basic Medical Sciences, 27 Taiping Road, Beijing 100850, P. R. China)

Abstract: According to the results of epidemiological research, the reproductive function of women working at the high altitude is significantly disturbed, mainly manifested as menstrual cycle disorder, abnormal hormone fluctuation, etc. Data from the animal experiments further confirmed that female mice exposed to the high altitude environmental stressors, such as cold and hypoxia, showed great changes in ovarian function. However, the reason leading to female reproductive dysfunction under altitude conditions have not been fully elucidated. Due to the high incidence of sleep disorders at high altitude, we therefore propose that circadian clock proteins might be involved in regulating female reproductive dysfunction under high altitude conditions. Here we found that acute exposure of mice to either cold (4°C, ZT3-ZT7, 3 days) or hypoxia (simulated 6000 meters, ZT0, 2 days) condition resulted in prolonged estrous cycle and follicular atresia, along with reduction of luteinizing hormone receptor (LHCGR) expression in the ovary and granulosa cells under hypoxia exposure and increase of STAR-dependent progesterone (PG) synthesis in the female mice under cold exposure. We further demonstrated that circadian clock proteins, CLOCK and BMAL1 functioned as the transcriptional activator for LHCGR; while E4BP4 was acted as the transcriptional suppressor for LHCGR. Hypoxia exposure inhibited CLOCK and BMAL1 expression, while enhanced E4BP4 expression, and therefore coordinately mediated down-reg·ulation of LHCGR expression in the ovary. Furthermore, we also observed the decreased expression of ovulation-related gene, epiregulin (EREG), in the granulosa cells followed by LHCGR reduction after hypoxia exposure, which resulted in impaired ovulation and estrous cycle disorder in the female mice. Thus we have demonstrated circadian clock proteins-dependent female reproductive dysfunction induced by hypoxia exposure. At low temperature, the expression level of the gap junction protein, connexin-43 (CX43), were significantly up-regulated in the ovary and the granulosa cells. Knockdown CX43 expression significantly inhibited the induction of STAR in the granulosa cells, accompanying with the suppression of progesterone synthesis in response to cold exposure. We further revealed that circadian protein E4BP4 was up-regulated under cold exposure, which promoted the activation of ER stress sensor protein, PERK, and its downstream target, NRF2, and then increased the transcription of CX43. These data indicated that cold exposure disturbed the expression of the circadian clock gene E4BP4, which lead to PERK/NRF2/CX43-dependent abnormal progesterone secretionin the granulosa cells and thereforeimpair follicular development. Taken these data together, we have elucidated circadian clock proteins controlled female reproductive dysfunction at high altitude environmental conditions.

Key words: altitude; hypoxia; cold; circadian clocks; female reproduction

Corresponding author: Lun Song, E-mail: lunsong0752@163.com

T13-24-0009

Study on the mechanism of high expression of HIF-1α alleviating benzene-induced hematopoietic toxicity and immunosuppression

Huang Jiawei, Pu Yunqiu, Xu Kai, Sun Rongli, Zhang Juan, Pu Yuepu*

(Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing 210009)

Abstract: Benzene is a recognized hematopoietic toxic substance, and long-term benzene exposure may lead to bone marrow hematopoietic suppression. Under the hypoxic environment of bone marrow, the degradation of the alpha subunit of hypoxia-inducible factor HIF-1 is inhibited, which can regulate the transcription of many genes in the nucleus. This study aimed to explore the regulatory effects of HIF-1α on the hematopoietic and immune system in the early stage of benzene exposure, in order to provide a new theoretical basis for the prevention and treatment of benzene toxicity. C57BL/6 mouse model with high HIF-1α expression was constructed by Tet-on system. The HIF-1α high expression and wild-type C57BL/ 6 mice were exposed to 150 mg/kg benzene respectively by subcutaneous injection, and the exposure period was set to 1, 3, 7, 10, 14 and 28 days. After the exposure period, the mice were sacrificed, and organs or tissues were collected for analysis and evaluation. Pancytopenia occurred in the wild-type mice on the 10th day, and there was stable hematopoietic toxicity on the 14th and 28th days. During the 28-day exposure period, the level of HIF-1α gradually decreased, and the levels of its target genes EPO and VEGF also reduced. At the same time, the spleen and thymus coefficient were decreased. The results of ELISA and RT-qPCR showed that the levels of IFN-γ, IL-2, and IL-17 decreased, while the levels of IL-10 increased. Compared with wild-type mice exposed to benzene for the same exposure time, HIF-1α mice had higher levels of white blood cells, red blood cells, platelets, and hemoglobin. HE staining showed that the bone marrow, spleen and thymus of HIF-1α mice were better than wild-type mice exposed to benzene during the same period. On the other hand, the levels of HIF-1α, EPO and VEGF were also higher than those of wild-type mice, and the degree of immunosuppression was weakened. In conclusion, benzene may damage the hematopoietic microenvironment and affect the differentiation of T helper cell populations by depleting the level of HIF-1α in the bone marrow of mice, thereby causing hematopoietic toxicity and immunosuppression. The high expression of HIF - 1α could alleviate the hematopoietic toxicity and immunosuppression of benzene, which may be a new strategy for the prevention and treatment of benzene toxicity.

Key words: benzene, HIF-1α, hematopoietic toxicity, immunosuppression

Corresponding author: Pu Yuepu, E-mail: yppu@seu.edu.cn

T13-29-0019

Targeting SIRT3 by Vitamin D3 ameliorates nitrogen mustard-induced cutaneous inflammation via inactivating the NLRP3 inflammasome

Xunhu Dong[1,2], Ying He[4], Feng Ye[1,2], Yuanpeng Zhao[1,2], Jin Cheng[1,2], Jingsong Xiao[1,2], Wenpei Yu[1,2], Jiqing Zhao[1,2], Yan Sai[1,2], Guorong Dan[1,2], Mingliang Chen[1,2,3], Zhongmin Zou[1,2]

(1. Department of Chemical Defense Medicine, School of Military Preventive Medicine, Third Military Medical University (Army Medical University), Chongqing, China; 2. Institute of Toxicology, School of

Military Preventive Medicine, Third Military Medical University (Army Medical University), Chongqing, China; 3. Institute of Pathology and Southwest Cancer Centre, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China; 4. Department of Ultrasound, Xinqiao Hospital, Third Military Medical University (Army Medical University), Chongqing, China)

Abstract: Nitrogen mustard (NM) causes severe skin injury with an obvious inflammatory response, which is lack of effective and targeted therapies. Vitamin D3 (VD3) has excellent anti-inflammatory properties and is considered as a potential candidate for the treatment of NM-induced dermal toxicity; however, the underlying mechanisms are currently unclear. In the present study, we observed that NM induced inflammation in HaCaT cells and skins of C57BL/6J mice. NM promoted NLRP3 expression and caspase-1 activity *in vitro* and *in vivo*. Notably, treatment with a caspase-1 inhibitor (YVAD), NLRP3 inhibitor (MCC950) and NLRP3 or caspase-1 siRNA attenuated NM-induced NLRP3 inflammasome activation, with subsequent suppression of inflammation in keratinocytes. Meanwhile, NM increased mitochondrial reactive oxygen species (mtROS), decreased manganese superoxide dismutase 2 (SOD2) and sirtuin 3 (SIRT3) activities. Mito-TEMPO (a mtROS scavenger) ameliorated NM-caused NLRP3 inflammasome activation in keratinocytes. Notably, VD3 improved SIRT3 and SOD2 activities, decreased mtROS contents, inactivated the NLRP3 inflammasome and attenuated cutaneous inflammation induced by NM *in vitro* and *in vivo*. The beneficial activity of VD3 against NM-triggered cutaneous inflammation was enhanced by Mito-TEMPO, YVAD, MCC950, caspase-1 and NLRP3 siRNAs and abolished in the presence of 3 -(1H-1, 2, 3-triazol-4-yl) pyridine (a selective SIRT3 inhibitor) and SIRT3 siRNA, indicating that the effects of VD3 on NM-caused cutaneous inflammation were dependent on SIRT3, mtROS, NLRP3 and caspase-1. In conclusion, VD3 ameliorated NM-induced cutaneous inflammation by inactivating the NLRP3 inflammasome, which was partially mediated through the SIRT3-SOD2-mtROS signaling pathway.

Key words: Vitamin D3; Nitrogen mustard; SIRT3; NLRP3 inflammasome; Cutaneous inflammation

T13-32-0017

Pyrroloquinoline quinone ameliorates diabetic cardiomyopathy by inhibiting the pyroptosis signaling pathway in AC16 cells

Xue-feng Qu, Yin Wang, Bing-zhong Zhai

(Institute of Food Science and Engineering, Hangzhou Medical College, Tianmushan Road 182th, Hangzhou, 310013)

Abstract: Diabetic cardiomyopathy (DCM), a common complication of diabetes mellitus and is characterized by myocardial hypertrophy and myocardial fibrosis. Pyrroloquinoline quinone (PQQ), a natural nutrient, exerts strong protection against various myocardial diseases. Pyroptosis, a type of inflammation-related programmed cell death and is vital to the development of DCM. However, the protective effects of PQQ against DCM and the associated mechanisms are not clear. This study aimed to investigate whether PQQ protected against DCM and to determine the underlying molecular mechanism. Diabetes was induced in mice by intraperitoneal injection of streptozotocin, after which the mice were administered PQQ orally (10, 20, or 40 mg · kg^{-1} body weight / day) for 12 weeks. AC16 human myocardial cells were divided into the following groups and treated accordingly: control (5.5 mmol · L^{-1} glucose), high glucose (35 mmol · L^{-1} glucose), and HG + PQQ groups (1 and 10 nmol · L^{-1}

PQQ). Cells were treated for 24 h. PQQ reduced myocardial hypertrophy and the area of myocardial fibrosis, which was accompanied by an increase in antioxidant function and a decrease in inflammatory cytokine levels. Moreover, myocardial hypertrophy - (ANP and BNP), myocardial fibrosis - (collagen Ⅰ and TGF - β1) and pyroptosis-related protein levels decreased in the PQQ treatment groups. Furthermore, PQQ abolished mitochondrial dysfunction and the activation of NF - κB/ IκB, and decreased NLRP3 inflammation-mediated pyroptosis in AC16 cells under high-glucose conditions. PQQ improved DCM in diabetic mice by inhibiting NF - κB/ NLRP3 inflammasome-mediated cell pyroptosis. Long-term dietary supplementation with PQQ may be greatly beneficial for the treatment of DCM.

Key words: diabetic cardiomyopathies; inflammation; NF-kappa B; pyroptosis; Pyrroloquinoline quinone

Corresponding author: Bing-zhong Zhai, E-mail: zhbingzhong@163.com

T13-33-0013

Maltol aluminum induced hippocampal neuron damage and reduced learning and memory function in rats

HuanLi[1,2], Jing Zhang[1], Chunzhi Zhang[1], Qiao Niu[2], Li Lin[1]

(1. Department of Occupational Health, School of Public Health, Jining Medical University, China;

2. Department of Occupational Health, School of Public Health, Shanxi Medical University, China)

Abstract: Background: Aluminum, the most abundant metal element in nature, has a clear neurotoxic effect, the main mechanism is inducing neuronal apoptosis and synaptic plasticity decline. **METHODS** New object recognition experiment was performed to observe the changes of learning and memory function in rats exposed to maltol aluminum intraperitoneally. Golgi staining was used to stain rat brain tissue to observe the morphological and structural changes of neurons. The internal exposure dose (including plasma and brain) of rats was determined by ICP-MS. Electron microscopy (SEM) was used to observe the damage of synaptic structure and mitochondria in rats exposed to aluminum. **RESULTS** Preference index results showed that compared with the control group, all aluminum exposure groups had a certain decreasing trend, and the difference was statistically significant (all $P<0.05$). The results showed that the preference index decreased with the increase of aluminum exposure dose, indicating that maltol aluminum exposure reduced the time for rats to explore new objects and weakened their preference for exploring new objects. Discrimination index results showed that compared with the control group, all maltol aluminum exposure groups had a certain reduction, the difference was statistically significant (all $P<0.05$), indicating that with the increase of aluminum exposure dose, the discrimination index has a downward trend, resulting in a weakened ability of rats to distinguish new objects.These data suggestthat maltol aluminum exposure could reduce learning and memory function in rats.The axons of neurons in the hippocampal CA1 region of rats exposed to maltol aluminum showed bead-like changes. By observing groups exposed to maltol aluminum at different concentrations and under high-power microscope, it was found that with the increase of exposure dose, the occurrence of bead-like changes in neurons increased, indicating that aluminum may cause axon damage of neurons in the hippocampal CA1 region. And the number of dendritic spines decreased significantly ($P<0.05$). The plasma and brain aluminum contents of rats were significantly increased by ICP-MS ($P<0.05$). With the increase of the dose, the synaptic structure of neurons in the hippocampal CA1 region was damaged, the presynaptic membrane vesicles were reduced, the postsynaptic dense became thinner, and the synaptic func-

tion might be affected accordingly. Therefore, subchronic aluminum exposure may damage the mitochondrial structure of neurons in the hippocampal CA1 region of rats, and synaptic function may also be affected. **Conclusion** Maltol aluminum can decrease the learning and memory function and damage the neuron structure in rats exposed to subchronic maltol aluminum.

T13-38-0011

The role and molecular mechanism of m6A modification in pulmonary fibrosis development induced by atmospheric particulate matter

Ding Ji[a], Jie Ning[a], Rong Zhang[a,b]

(a. Department of Toxicology, School of Public Health, Hebei Medical University, Shijiazhuang 050017, PR China; b. Hebei Key Laboratory of Environment and Human Health, Shijiazhuang 050017, PR China)

Abstract: It has been reported that particulate matter with an aerodynamic diameter of <2.5 μm ($PM_{2.5}$) could induce epithelial – mesenchymal transition (EMT)- and extracellular matrix (ECM)- related pulmonary fibrosis (PF). The transcription factor Nrf2 alleviated $PM_{2.5}$-induced PF by antagonizing oxidative stress. The N6-methyladenosine (m6A) modifications play a significant role in the stress response. However, the effect of m6A modification on the mechanisms of Nrf2-mediated defense against PM2.5-induced PF remain unknown. Here, we investigated the role and the underlying molecular mechanisms of m 6 A methylation of Nrf2 mRNA in $PM_{2.5}$-induced PF. Male C57BL/6 mice were exposed to filtered air (FA), unfiltered air (UA) and concentrated air (CA)for 16 weeks. 16HBE cells were treated with 0, 50, or 100 μg·mL^{-1} $PM_{2.5}$ for 24 h. Our data showed that chronic $PM_{2.5}$ exposure could induce fibrosis in lung and increase Nrf2 signals. In Nrf2 deficient cells, α-SMA expression was significantly upregulated whereas E-cadherin decreased compared with WT cells after $PM_{2.5}$ treatment which implied the aggravated fibrosis. m6A methyltransferase METTL3 was upregulated after $PM_{2.5}$ treatment. m 6 A-methylated RNA immunoprecipitation (MeRIP) and qRT-PCR results showed that METTL3 improved the m 6 A modification of Nrf2 mRNA in $PM_{2.5}$-exposed 16HBE cells. MeRIP-Seq and single-base T3 ligase-based PCR results showed that the m6A-modified sites of Nrf2 mRNA were 1317, 1376, and 935 in lung of mice after $PM_{2.5}$ exposure. RIP results suggested that the m6A binding proteins YTHDF1 / IGF2BP1 promoted Nrf2 translation by binding to Nrf2 mRNA m 6 A residues. Our results revealed the mechanism by which m6A regulated the activities of the Nrf2-mediated signaling pathway against $PM_{2.5}$-induced PF.

Key words: $PM_{2.5}$; m6A; METTL3; Nrf2; pulmonary fibrosis

Corresponding author: Rong Zhang, E-mail: rongzhang@hebmu.edu.cn

T13-46-0006

The role and underlying mechanisms of NOX2 in environmental toxins-induced dopaminergic neurodegeneration

Liyan Hou, Dongdong Zhang, Zhengzheng Ruan, Ruixue Huang, Qingshan Wang*

(School of Public Health, National-Local Joint Engineering Research Center for Drug-Research and

Development (R & D) of Neurodegenerative Diseases, Dalian Medical University)

Abstract: Gradual and irreversible loss of dopamine neurons in the substantia nigra is one of the pathological hallmarks of Parkinson′ s disease (PD), the most common neurodegenerative movement disorder. However, the underlying mechanisms of the progressive nature of neurodegeneration in PD remain unclear. Epidemiological studies revealed that chronic exposure to environmental toxins, such as infectious agents, pesticides, heavy metals and solvents, has been implicated to be associated with increased risk of developing PD. We recently found that mice intoxicated with endotoxin LPS, pesticides rotenone and paraquat or 2, 5-hexanedione (HD), the toxic metabolite of organic solvent n-hexane, displayed progressive dopaminergic neurodegeneration in the substantia nigra. Interestingly, time course studies revealed that the activation of microglia preceded neurodegeneration in environmental toxins-intoxicated mice. Moreover, depletion of microglia markedly reduced environmental toxins-induced dopaminergic neurotoxicity, indicating a critical role of microglial activation in progressive dopaminergic neurodegeneration. Furthermore, NADPH oxidase 2 (NOX2) was identified as a common target to be critical for environmental toxins-induced microglial activation. Environmental toxins activated microglial NOX2 by inducing membrane translocation of NOX2 cytosolic subunit, p47phox. Src and Erk were subsequently verified as the potential signaling to mediate environmental toxins-induced NOX2 activation. Finally, genetic or pharmacological inactivation of NOX2 mitigated environmental toxins-elicited microglial activation and progressive dopaminergic neurodegeneration. Altogether, our findings revealed a key role of NOX2-mediated microglial activation in the progressive dopaminergic neurodegeneration in PD, providing novel mechanisms and potential therapeutic target to combat this neurodegenerative disorder.

Key words: Environmental toxins; Microglia; NOX2; Parkinson′ s disease

Corresponding author: Qingshan Wang, E-mail: wangq4@126.com

T13-47-0001

Novel insights into the role of mitochondria dynamics and redox balance in regulating macrophagesinflammationresponse

Yu Wei-hua, Zi Long, Li Wen-li, Hai Chun-xu

(Department of Toxicology, Fourth Military Medical University, Xi'an 710032, China)

Abstract: Macrophages are monocyte-derived innate immune cells that participate in regulating inflammation response and various pathologies. Macrophages are usually divided into two categories, pro-inflammatory (M1) and anti-inflammatory (M2) macrophages. However, the mechanisminvolvedremainsobscure. Here, we aim to investigate the role ofmitochondrial dynamics and redox balance in regulating macrophage polarization. In LPS-treated macrophages, mitochondriashow increased mass, shortenlength and looser cristae, which indicated enhanced mitochondrial fission. We prove that mitochondriain M1 macrophagesshifttheir function from ATP synthesis to ROS generation, and ROSmodulatesNF κ B-dependent pro-inflammatoryresponse. Dynamin-related protein 1 (Drp1) is a key GTPase that plays critical role in regulating mitochondrial fission. Our data showthat Drp1-dependent mitochondrial fission promotesmacrophages pro-inflammatory differentiation. Knockdown or inhibition of Drp1 alleviates LPS-induced macrophages pro-inflammatory differentiationand mice sepsis. As a key member of the STAT family, signal transducers and activators of transcription 2 (Stat2)is indispensable for IFN-

mediated anti-viral and anti-tumor. We prove that Stat2 enhances LPS-induced mitochondrial fission through regulating Drp1 S616 phosphorylation. Knockdownof Stat2 bluntstheincrease of pro-inflamma·tory cytokines in LPS-stimulated macrophages. Therefore, these results suggested that Stat2-Drp1 me·diated mitochondrial fission modulates the initiation of macrophage pro-inflammatory response. More· over, weobserve an obvious increase of mitochondrial fusion and decrease of ROS generation in IL4- activated M2 macrophages. Enhance mitochondrial fusionalso promotes the expression of anti-inflam· mation cytokines, including Arg1, Fizz1 andIL-10. It has been reported that ROS dose influences the inflammatory course, and ROS reduction promotes macrophages anti-inflammatory response. Hence, weprove that mitochondrial fusion boosts anti-inflammatory response through inhibiting mtROS genera· tion. In conclusion, the crosstalk of mitochondrial dynamicsand redox signaling may control macro· phages polarization. Increased mitochondrial fission and ROS generation facilitates M1 macrophages pro-inflammatory response, while enhanced mitochondrial fusion and decreased ROS promotes M2 macrophages anti-inflammatory response.

Corresponding author: Yu Wei-hua, E-mail: yuweihua1@fmmu.edu.cn

T13-48-0007

Repressionof miR-486-3p by ionizing radiation promotes the formation of radiation-induced pulmonary fibrosis

Xinxin Liang[1], Ziyan Yan[2], YuhaoLiu[2], PingWang[2], Chenjun Bai[2], Pingkun Zhou[2], Yongqing Gu[1,2]

(1. Hengyang Medical School,University of South China, Hengyang 421001;

2. Beijing Institute of Radiation Medicine, Beijing 100850)

Abstract: Inthe course of radiotherapy,thelungasanorganwith a greater impact on chest radiothera·py is extremely sensitive to radiation, and its self-healing ability is relatively poor, so the incidence of Radiation induced pulmonary fibrosis (RIPF) is very high during the treatment of chest tumors, which greatly reducesthesurvivalrateofpatients. RIPF is usually alate-stage complication of lung injury. Inthe process ofRIPF,some scholars proposed that lung tissue injury was accompanied by changes in the mi· croenvironment of lung epithelial cells, leading to the transformation of epithelial cells into fibroblasts and myofibroblasts in the form of mesenchymal cells. This change process of epithelial cells is called epithelia mesenchymal transition(EMT).

MicroRNA(miRNA)referstonon-coding small molecule RNA with a length of 19 to 25nt, which can participate in a variety ofbiological processes such as cellproliferation,differentiation,apoptosisandsoon. In the condition of ionizing radiation, the mRNAs of many differentially expressed miRNAs are shown to participate in the signaling pathway of the occurrence and development of EMT, which indicates that the change of miRNAmay be related to the promotion or inhibition of EMT, thus influencing the forma· tion or inhibition ofRIPF. There have been many reports that ionizing radiation cancause changes in miRNA contentin the body, but there are few studies on the pathways of action, and the specific mecha· nisms have not been clarified.

Inordertorevealthe mechanism of miR-486-3pcontent change after ionizing radiation induction and theaction pathway of triggering EMT, we first irradiatedA549 cells by 6Gy of 60Co γ ray, cultured and extracted miRNA, and verified its expression with real-time RT PCR. The results showed that the ex· pression of Mir-486-3p decreased significantly after the induction of ionizing radiation. Next, we trans·

fected miR-486-3p inhibitors into A549 cells. After 48 hours, the EMT-related protein was verified by western blot, and the results showed that miR-486-3p can promote EMT.Our results suggest that radiation may inhibit the expression of miR-486-3p, promoting the EMT process and causing radioactive pulmonary fibrosis. We look forward to using this way to create more ways to prevent and treat radioactive pulmonary fibrosis.

Key words: Radiation-induced pulmonary fibrosis; epithelia mesenchymal transition; miRNA; miR-486-3p

Corresponding author: Yongqing Gu, E-mail: yqgu96@163.com; Pingkun Zhou, E-mail: zhoupk@nic.bmi.ac.cn

T13-48-0015

Mechanism of Ku80 crotonylation on DNA damagerepair after irradiation

Gang LI[a,b], Yang HAN[b], He-jiang GUO[b], Chen-jun BAI[b], Hua GUAN[b], Shan-shan GAO[b], Ping-kun ZHOU[a,b]

(a. School of Public Health,University of South China, No. 28, Changsheng West Road, Zhengxiang District, Hengyang City, Hunan Province 421200; b. Beijing Key Laboratory for Radiobiology, Institute of Radiation Medicine, 27 Taiping Road, Haidian District, Beijing 100850)

Abstract: OBJECTIVE Protein post-translational modification play a key role in DNA repair. Ku80, an important DNA repair protein, has been identified to participate in the regulation of DNA damage repair through diverse post-translational modifications, such as ubiquitination, SUMOylation and phosphorylation. Lysine Crotonylation (Kcr), since its discovery, has been studied on its histone and non-histone, chromosome spatial structure, physiological functions. Studies found Kcr plays a fundamental role in DNA damage repair through interaction with acetyltransferases CBP/p300. However, the molecular mechanisms underlying function and regulation of Ku80 Kcr are still elusive. **METHODS** Changes of Kcr in MCH-7 before/after radiation were screening by Mass spectrometry. Bioinformatics and data mining were used to find the most promising protein on DNA damage repair. Western Blot and Co-IP were used to verify Kcr, and site-directed mutational was for its function. **RESULTS** Kcr was involved in NHEJ repair pathway. Ku80 was post-translationally modified byKcr, and Kcr was downregutated by irradiation. Mass spectrometry showed there were two Kcr-sites on Ku80. Kcr was significantly downregulated upon mutation of these two sites. The interaction between Ku80, Ku70 and DNA-PKcs was increased after radiation and point mutation. **CONCLUSION** Kcrcould occur on Ku80, and irradiation and point mutation negatively regulated Kcr of Ku80. Ku80 is one of the most critical functional proteins, so we speculate that Kcr of Ku80 plays a role on DNA damage repair.

Key words: Kcr; Ku80; NHEJ; DNA damage repair; DNA damage response

Corresponding author: Ping-kun ZHOU, E-mail: zhoupk@bmi.ac.cn

T13-50-0002

Prenatal chronic stress impairs the learning and memory ability via inhibition of the NO/cGMP/PKG pathway in the hippocampus of offspring

Youjuan Fu[a,b], Hongya Liu[a,b], Ling He[c], Shuqin Ma[c], Xiaohui Chen[a,b], Kai Wang[a,b],

Feng Zhao[a,b], Faqiu Qi[a,b], Suzhen Guan[a,b,d]*, Zhihong Liu[a,b]*

(a. School of Public Health and Management, Ningxia Medical University, No. 1160, Shengli Street, Xingqing District, Yinchuan, Ningxia; b. Key Laboratory of Environmental Factors and Chronic Disease Control, No. 1160, Shengli Street, Xingqing District, Yinchuan, Ningxia; c. Obstetrics and gynecology center, General hospital of Ningxia Medical University, No.804, Shengli Street, Xingqing District, Yinch- uan, Ningxia; d. Ningxia Key Laboratory of Cerebrocranial Disease, Incubation Base of National Key Laboratory, Ningxia Medical University, No.1160, Shengli Street, Xingqing District, Yinchuan, Ningxia)

Abstract: Numerous clinical and animal studies have found that chronic antenatal stress can lead to pathological changes the hippocampal development from embryos to fetuses, but the mechanisms are not well understood. In this study, gestating rats were subjected to chronic unpredictable mild stress (CUMS), the learning and memory capacity, behavioural performance and protein changes in the hippocampus of the offspring were measured. It was found that chronic prenatal stress led to post· natal growth retardation and impaired spatial learning and memory ability in the offspring. In addition, 26 significantly differentially expressed proteins (DEPs) were found between the two groups using an isoquantitative tag-based relative and absolute quantification (iTRAQ) proteomics analysis. Further analyses of these DEPs showed that involved in different molecular functions and in several biological processes, such as biological regulation and metabolic processes. Among these, the KEGG pathway enrichment showed that learning and memory impairment was mainly associated with the cyclic guano· sine monophosphate protein kinase G (cGMP-PKG) pathway. At the same time, the NO, nNOS and cGMP level were significantly decreased, and the expression of PKG protein was also dropped, and chronic stress during pregnancy causes various degrees of damage to neurons, niche, mitochondria and synaptic structures in the hippocampus of the offspring. All of these results suggest that pregnant rats exposed to chronic psychological stress may impair spatial learning and memory in their offspring, and the effects of this impairment were mediated through inhibition of the NO / cGMP / PKG signaling pathway.

Ke ywords: chronic stress; iTRAQ; learning and memory; offspring; NO/cGMP/PKG pathway

Corresponding author: Suzhen Guan, E-mail: guansz_nx2017@sina. com; Zhihong Liu, E-mail: lzh_2580@sina.com

T13-59-0021

The role of mitochondrial DNA-cGAS /AIM2 signaling pathway in hematopoieticinjuryinduced by ionizing radiation

Wen Zhang[a,b], Yuchen Li[a,b], Hua Guan[b], Pingkun Zhou[b]

(a. Hengyang Medical College, University of South China, Hengyang, 421001; b. Beijing Institute of Radiation Medicine, Beijing, 100850)

Abstract: OBJECTIVE To study the activation of the signaling pathway induced by the recogni· tion of cytoplasmic DNA receptor cGAS/AIM2 by different doses of ^{60}Co γ -ray irradiation.And toexplore the biological effect of activating cGAS/AIM2 signaling pathway on hematopoietic injury and analyze its mechanism. **METHODS** FDC-P1 cell wasexposed to 2Gy^{60}Co γ - ray, and the expression of cGAS / AIM2 signaling pathway related proteins induced by mitochondrial DNA (mtDNA) was detected by Western blotting 12 h later. FDC-P1 cell was irradiated with 2Gy^{60}Co γ-rayand apoptosis was detected

by flow cytometry 48 h after irradiation. Mice were exposed to ^{60}Co γ- ray, and the expression level of mtDNA in the cytoplasm of mouse bone marrow cells was detected by real-time quantitative PCR 6 h after exposure. Mice were exposed to 6Gy^{60}Co γ-ray,The apoptosis of bone marrow cells in mice was detected by flow cytometry 48h after exposure. **RESULTS** Compared with the control group (0Gy), the expression of mtDNA in cytoplasm of irradiated mice (6Gy^{60}Co γ - ray) was increased. Compared with the irradiation group (6Gy^{60}Co γ-ray), the expression of mtDNA in cytoplasm of the treated group (DIDS combined with 6Gy^{60}Co γ-ray) was decreased. Irradiation group (6Gy^{60}Co γ-ray) promoted the apoptosis of bone marrow in mice, and the apoptosis rate was significantly higherthan that in the control group (0Gy). Compared with irradiation group, the apoptosis rate of treated group (DIDS combined with 6Gy^{60}Co γ-ray) decreased significantly.Irradiation group (2Gy^{60}Co γ-ray) promoted apoptosis, and the apoptosis rate was significantly increased compared with the control group (0Gy). Compared with the irradiation group (2Gy^{60}Co γ - ray), the apoptosis rate of treated group (DIDS combined with 2Gy^{60}Co γ-ray)was reduced. Compared with the control group (0Gy), phosphorylation levels of downstream proteins TBK1 and IRF3 in cGAS signaling pathway increased after 6Gy ^{60}Co γ-ray irradiation of FDC-P1. The phosphorylation levels of TBK1 and IRF3 decreased when DIDS was administered before irradiation compared with the irradiation group. Compared with the control group, the expression of downstream protein C-GSDMD and mature IL-1β in AIM2 signaling pathway was increased when FDC-P1 was irradiated with 6Gy ^{60}Co γ- ray. The expression of C-GSDMD and mature IL-1β was decreased in the treated group (DIDS combined with 6Gy^{60}Co γ-ray) compared with the irradiation group (6Gy^{60}Co γ-ray). **CONCLUSION** ^{60}Co γ - rayirradiation can promote the release of mtDNA into the cytoplasm and be recognized by cGAS/AIM2, and induce inflammatory reaction and cell pyrodeath. Thus it is involved in regulating hematopoietic injury, but the related mechanism remains to be further explored.

Key words: mitochondrialDNA; ^{60}Co γ-ray; CyclicGMP-AMP synthase (cGAS); AIM2; Hematopoietic injury

Corresponding author: Pingkun Zhou, E-mail: 2542170542@qq.com

T13-67-0005

Study on the molecular mechanism of Manganese-induced S-nitrosylation of autophagy regulatory proteins disturbing autophagic activation

Zhuo Ma, Can Wang, Chang Liu, Dong-Ying Yan, Xuan Tan, Kuan Liu, Meng-Jiao Jing, Yu Deng, Wei Liu, Bin Xu

(Department of Environmental Health, School of Public Health, China Medical University, No.77 Puhe Road, Shenyang North New Area, Shenyang, Liaoning Province 110122, People's Republic of China)

Abstract: OBJECTIVE Exposure to excess levels of manganese (Mn) may lead to nitrosative stress and neurotoxic effects on the central nervous system (CNS). The dysfunction of autophagy correlates with Mn-induced nitrosative stress; however, the exact mechanism of Mn-mediated autophagy dysfunction is still unclear. Three S-nitrosylated target proteins, namely, JNK, Bcl-2, and IKKβ, were classified as the pivotal signaling pathway mediators that could play a role in the regulation of autophagy. **METHODS** To reveal whether these three proteins were involved in Mn-mediated autophagy dysregulation, we studied the effects of Mn on C57BL/6J mice and human neuroblastoma cells. Then we used 1400 W, an iNOS-specific inhibitor, to neutralize the nitrosative stress induced by Mn. **RESULTS** Exposing the mice or cells, to 300 μmol·kg^{-1} or 200 μmol·L^{-1} Mn, inhibited the degradation system of

the autophagy-lysosome pathway (P<0.01). Additionally, in Mn-treated mice or cells, S-nitrosylated JNK, Bcl-2, and IKKβ increased while the level of their phosphorylation reduced (P<0.01). The interac·tion of Beclin1 and Bcl-2 significantly increased in response to 200 μmol·L^{-1} Mn, whereas the decrease in phosphorylation of AMPK activated the mTOR pathway (P<0.01). Our results also show that 20 μmol·L^{-1} 1400 W reduced the S-nitrosylated JNK, Bcl-2, and IKKβ and relieved their downstream sig·naling molecular functions (P< 0.01). Moreover, pretreatment with 20 μmol·L^{-1} 1400 W alleviated Mn-in·duced autophagic dysregulation and nerve cell injury (P<0.01). **CONCLUSION** The outcomes of this study demonstrate that Mn could induce nitrosative stress through activating iNOS and subsequently S-nitrosylated JNK, Bcl-2, and IKKβ. S-nitrosylated Bcl-2 increased the affinity of Bcl-2 / Beclin1 complex due to the lack of phospho-Bcl-2, which led to autophagy dysregulation. Moreover, S-nitrosylated Ikkβ reduced the phosphorylation of AMPK, which affected the regulation of autophagy through the mTOR signaling pathway.

Key words: Manganese; Nitrosative stress; Neurotoxicity; Autophagy dysregulation

Corresponding author: Bin Xu, E-mail: bxu10@cmu.edu.cn

T13-76-0016

Effect of ^{60}Coγ rays on cGAS-STING signaling pathway and its mechanism

Xiao-yu Cao[a], Shan-shan Gao[b], Ping-Kun Zhou[b]

(a. College of Life Sciences, Hebei University, Baoding,China 071000; b. Department of Radiation Toxicology and Oncology, Beijing Key Laboratory for Radiobiology, Beijing Institute of Radiation Medicine, Beijing, China 100850)

Abstract: OBJECTIVE In recentyears, the importanceof cGAS-STING signaling pathway has been discovered and studied by more and more people. The main mechanism of this pathway is that cyclic GMP-AMP synthase (cGAS) in cytoplasm can recognize the double-stranded DNA of foreign vi·ruses, bacteria and other organisms so as to activate cGAS-STING downstream signaling pathway to play immune function. This research experiment attempts to combine the immune function of irradiation and cGAS-STING signaling pathway,so as to find a new wayto kill tumor cells.Methods:1.In this study, Hela and A549 tumor cells commonly used in the laboratory were selected as the main experimental objects, and ^{60}Coγ rays were used for ionizing radiation. The changes of cGAS protein level and mRNA level of tumor cells at 1 h, 6 h and 12 h after irradiation were studied. 2. Hela and A549 cancer cells were administered with cycloheximide (CHX) (100 μg·ml^{-1}) before irradiation to study the half-life of cGAS protein and the effect of irradiation on the half-life of cGAS protein in tumor cells; 3. The ubiqui·tination of cGAS and the effect of irradiation on ubiquitination were verified by immunoprecipitation; 4. Proteins interacting with cGAS were screened by mass spectrometry; 5. The interaction between cGAS and protein screened by mass spectrometry was verified by immunoprecipitation. **RESULTS** 1.The lev·els of cGAS protein in Hela and A549 tumor cells at 1 h, 6 h and 12 h after irradiation were significantly higher compared with those in non-irradiation group. 2. The mRNA levels of cGAS in Hela and A549 tu·mor cells at 1 h, 6 h and 12 h after irradiation showed no significant changes compared with the non-ir·radiation group; 3. The half-life of cGAS protein could be prolonged by irradiation. 4. The cGAS protein can undergo significant ubiquitination modification and irradiation can reduce the ubiquitination level of cGAS; 5. Mass spectrometry showed that UBC might be the interaction protein of cGAS. 6. The interac·

tion between UBC and cGAS was confirmed by immunoprecipitation assay. **CONCLUSION** Irradiation can improve the stability of cGAS protein and inhibit the ubiquitination modification level of cGAS. How to screen the protein UBC that interacts with cGAS and can regulate ubiquitinationneeds to be further studied.

Key words: cGAS; irradiation; ubiquitination; UBC

T13-77-0012

Defects of DNA-PKcs promotes radiation-induced lung fibrosis

Ziyan Yan[1], Xinxin Liang[2], YuhaoLiu[1], PingWang[1], Xingkun Ao[2], Chenjun Bai[2], Pingkun Zhou[1], Yongqing Gu[1,2]

(1. Beijing Institute of Radiation Medicine, Beijing 100850; 2. Hengyang Medical School, University of South China, Hengyang 421001)

Abstract: OBJECTIVE The study found that DNA damage was closely related to the process of tissue fibrosis, but its specific molecular mechanism is not clear. To investigate the role and molecular mechanism of DNA-PKcs in radiation-induced epithelial-mesenchymal transition (EMT) and radiation - induced lung injury (RIPF). **METHODS** Human type II alveolar epithelial cells A549 were infected with lentivirus to obtain DNA-PKcs stable knock-down cell line. Western blot was used to detect the expression of epithelial marker protein E-cadherin, interstitial marker Vimentin, transcription factor twist1 and snail after 6 Gy ^{60}Co γ-ray irradiation. The interaction between DNA-PKcs and twist was detected by co-immunoprecipitation. The co-localization of DNA-PKcs and twist was detected by immunofluorescence. The half-life of twist in DNA-PKcs knockdown cells was detected by cycloheximide. The ubiquitination level of twist in DNA-PKcs knockdown cells was detected by ubiquitination assay. *In vivo*, the DNA-PKcs gene knockout mice were constructed by CRISPR/cas9 and the RIPF model was constructed by 20 Gy ^{60}Co γ - ray irradiation. The pathological changes of lung structure in mice were observed by HE staining and Masson staining. The expression of twist in knockout mice was detected by immunohistochemistry. **RESULTS** 1. Knockdown of DNA-PKcs promoted the expression of E-cadherin and inhibited the expression of Vimentin in A549 cells after radiation. 2. Knockdown of DNA-PKcs promoted twist expression in irradiated A549 cells without affecting snail expression. 3. DNA-PKcs co-localized with twist and could interact with each other. 4. Knockdown of DNA-PKcs inhibits twist degradation and prolongs its half-life. 5. Knockdown of DNA-PKcs inhibits the ubiquitination degradation of twist. 6. High twist expression in lung tissue of DNA-PKcs knockout mice. 7. Knockdown of DNA-PKcs promotes the damage of lung tissue structure caused by irradiation and accelerates the deposition of collagen. **CONCLUSION** DNA-PKcs deficiency inhibits normal ubiquitination and accumulation of twist, which promotes EMT of alveolar epithelial cells and aggravates RIPF in mice.

Key words: ionizing radiation; pulmonary fibrosis; EMT; DNA-PKcs; Twist

Corresponding author: Yongqing Gu, E-mail: yqgu96@163.com; Pingkun Zhou, E-mail: zhoupk@nic.bmi.ac.cn

T13-86-0003

Relationship between Sema 3A or 4A and arsenic induced cardiotoxicity in mice

Yang Yuan*, Song Shuang

(School of Public Health, Guizhou Medical University, Guiyang 550025, Guizhou, China)

Abstract: OBJECTIVE To explore the association of Semaphorin 3A/4A (Sema 3A/4A) with arsenic-induced cardiotoxicity in mice. **METHODS** Healthy male C57BL/6 mice were used as experimental subjects, which were divided as 6 groups, that was control group, sodium arsenite (SA) exposure group (10.0 mg·kg^{-1}.bw), Sema 3A antibody treatment group (80 μg·kg^{-1}), Sema 4A antibody treatment group (80 μg·kg^{-1}), Sema 3A antibody intervention+ SA exposure group, Sema 4A antibody intervention+ SA exposure group, respectively. After 7 days (once a day) of mice experiments, hematoxylin-eosin (HE) was used to evaluate the characteristics of cardiac pathological damage; Enzyme-linked immunosorbent assay (ELISA) or Western blotting (WB) was used to measure the levels of cardiac troponin I (cTnI), creatine kinase (CK) in serum, Sema 3A/4A, IL-1β, IL-6, p-AKT2, and NF-κB p65 in heart tissues. **RESULTS** Compared with control group, SA exposure group showed pathological myocardial damage and the elevated levels of serum cTnI, CK, while the SA induced cardiotoxicity were antagonized significantly by the intervention with Sema 4A antibody (P<0.05). ELISA or WB experiments showed the antagonistic effects of Sema 4A antibody down-regulated the levels of the Sema 4A, IL-1β or IL-6, and up-regulated the level of Sema 3A compared with that of SA exposure group (P<0.05). And, the intervention with Sema 4A antibody down-regulated the expressions of Sema 4A, p-AKT2, NF-κB p65, and up-regulated Sema 3A in heart tissues compared with that of SA exposure group (P<0.05). **CONCLUSION** The intervention with Sema 4A antibody alleviates arsenic induced cardiotoxicity in mice, which may be associated with the inhibition of AKT2 or NF-κB inflammatory signaling pathway mediated by Sema 4A or Sema 3A.

Key words: Arsenic; Cardiotoxicity; Semaphorin 3A; Semaphorin 4A; Inflammation

Corresponding author: Yang Yuan, E-mail: yang1977yuan@sohu.com

T13-96-0014

p21 governs the protective effect of berberine on colistin-induced nephrotoxicity through modulating AMPK/Akt/Nrf2/HO-1 axis

Dai Chongshan, Tang Shusheng, Shen Jianzhong

(College of Veterinary Medicine, China Agricultural University, Beijing 100193, P. R. China)

Abstract: Nephrotoxicity is the major adverse effect patients experience during colistin therapy. The development of effective nephroprotective agents that can be co-administered during polymyxin therapy remains a priority area in antimicrobial chemotherapy. In this study, we performed a high-throughput screening via a small molecule compound library and the results showed that berberine exhibited the most obviously protective effect on colistin-induced cytotoxicity in HEK293T cells. In a mouse model, our results showed that berberine supplementation at 100 mg·kg^{-1} significantly improved colistin treatment-induced oxidative stress, mitochondrial dysfunction and renal pathology damage. In HEK293T cells, our results showed thar berberine treatment significantly inhibited colistin-in·

duced production of mitochondrial ROS, loss of mitochondrial membrane potential, and apoptotic cell death. Drug structure-activity network interaction analysis showed that cyclin-dependent protein serine / threonine kinase regulator activity pathway is ranked in the first and its targets contain p21, CCND1 and HO-1 proteins. Then, we found that berberine treatment significantly upregulated the expression of p21, HO-1, Nrf2, phosphorylation (p)-AMPK (Thr172), and p-Akt (Ser473) proteins, and downregulated the expression of CCND1 protein in a dose-dependent manner. Compared to colistin alone treatment, berberine co-treatment significantly increased the expression of p21, p-AMPK (Thr172), p-Akt (Ser473), Nrf2 and HO-1 proteins, but did not rescue the decrease of CCND1 protein caused by colistin treatment. Gene knockout of p21 by CRISPR partly improved colistin-induced cell death, but completely abolished the regulated effect of berberine on the expression of p- AMPK (Thr172), p-Akt (Ser473), and Nrf2 proteins. Pharmacology inhibitions of Akt, Nrf2, and HO-1 protein expression significantly promot·ed colistin-induced cytotoxicity in HEK293T cells. In conclusions, our study for the fist time reveals that p21 plays a critical role in the protective effect of berberine on colistin-induced nephrotoxicity, which may involve the modulation of AMPK/Akt/Nrf2/HO-1 axis. Our study highlights that oral berberine could potentially ameliorate nephrotoxicity in patients undergoing polymyxin therapy.

Key words: Colistin; AMPK/Akt/Nrf2/HO-1 axis; Nephrotoxicity; Berberine

Corresponding author: Dai Chongshan, E-mail: daichongshan@cau.edu.cn; Shen Jianzhong, E-mail: sjz@cau.edu.cn

T13-96-0022

Therapeutic approaches target on unfolded protein response in kidney disease and fibrosis

Chih-Kang Chiang, Jia-Huang Chen

(Graduate Institute of Toxicology, Taiwan University College of Medicine)

Abstract: Pathological conditions of cells usually disturb its protein folding capacity and lead to the accumulation of misfolded proteins in the endoplasmic reticulum (ER). This accumulation will lead to so-called "ER stress". Increasing evidence indicated that ER stress is a considerable trigger factor of many types of kidney diseases. The unfolded protein responses (UPRs), a set of molecular signaling activated under ER stress, is thought to participate in the progression of chronic kidney disease and fi·brosis. And the idea that target UPRs for disease cure had been well discussed in the past decade. In this review, we summarize the existing literature regarding studies on the relationship between the UPRs, systemic fibrosis, and renal diseases. We also address the potential therapeutic possibilities of renal diseases based on modulating UPRs and/or ER protein homeostasis. Finally, we list some of the current UPR modulators and their beneficial effects.

Key words: unfolded protein responses; endoplasmic reticulum stress; fibrosis; kidney

T13-96-0023

Advanced glycation end products activated endothelial-to-mesenchymal transition in pancreatic islet endothelial cells and triggered islet fibrosis in diabetic mice

Shing-Hwa Liu[1], Pei-Shan Tsai[1], Chen-Yuan Chiu[4], Meei-Ling Sheu[2], Ching-Yao Yang[1], Kuo-Cheng Lan[3]
(1. Taiwan University; 2. National Chung Hsing University; 3. Tri-Service General Hospital; 4.Center for Drug Evaluation)

Abstract: Advanced glycation end products (AGEs) are associated with the pathogenesis of diabetic vascular complications. Induction of the endothelial-to-mesenchymal transition (EndMT) is associated with the pathogenesis of fibrotic diseases. The roles of AGEs in islet EndMT induction and diabetes-related islet microvasculopathy and fibrosis remain unclear. This study investigated the pathological roles of AGEs in islet EndMT induction and fibrosis in vitro and in vivo. Non-cytotoxic concentrations of AGEs upregulated the protein expression of fibronectin, vimentin, and α-smooth muscle actin (α-SMA) (mesenchymal/myofibroblast markers) and downregulated the protein expression of vascular endothelial (VE)-cadherin and cluster of differentiation (CD) 31 (endothelial cell markers) in cultured mouse pancreatic islet endothelial cells, which was prevented by the AGE cross-link breaker alagebrium chloride. In streptozotocin-induced diabetic mice, the average islet area and islet immunoreactivities for insulin and CD31 were decreased and the islet immunoreactivities for AGEs and α-SMA and fibrosis were increased, which were prevented by the AGE inhibitor aminoguanidine. Immunofluorescence double staining showed that α-SMA-positive staining co-localized with CD31-positive staining in the diabetic islets, which was effectively prevented by aminoguanidine. These results demonstrate that AGEs can induce EndMT in islet endothelial cells and islet fibrosis in diabetic mice, suggesting that AGE-induced EndMT may contribute to islet fibrosis in diabetes.

Key words: advanced glycation end products; diabetes; islet fibrosis; endothelial-to-mesenchymal transition

T13-96-0024

Modulation of diabetic retinopathy by the aryl hydrocarbon receptor

Meei-Ling Sheu
(1.Institute of Biomedical Sciences, College of Life Sciences, National Chung Hsing University, Taichung; 2. Department of Medical Research, Taichung Veterans General Hospital, Taichung)

Abstract: Rationale Diabetic retinopathy (DR) is a pathophysiologically vasculopathic process, which the exact mechanisms remain obscure and effective therapeutic strategies are still limited. **OBJECTIVE** Aryl hydrocarbon receptor (AhR) is an important regulator of xenobiotic metabolism and environmental sensor. This study investigated the role of AhR in the development of diabetic retinopathy and elucidated the molecular mechanism of its downregulation. **METHODS** and **RESULTS**: Retinal expression of AhR was determined in human donor and mouse eyes by immunofluorescence. AhR activity was decreased in diabetes. AhR knockout (AhRKO) mice were used to induce diabetes with streptozotocin, high-fat diet, or genetic double knockout with diabetes spontaneous mutation ($Lepr^{db}$) (DKO; $AhR^{-/-} \times Lepr^{db/db}$) for investigating structural, functional, and metabolic abnormalities in vascular and epithelial retina. Compared to diabetic control mice, the alterations in retinal vasculature from diabetic AhRKO mice were aggravated, which amplified hallmark feature of DR, including vasopermeability, vascular leakage, inflammation, blood-retinal barrier breakdown, capillary degeneration, and neovascularization. Structural molecular docking simulation was used to survey the pharmacological AhR agonists targeting on phosphorylated AhR (Tyr245). AhR agonists effectively inhibited inflammasome formation

and promoted AhR activity in primary human retinal microvascular endothelial cells and pigment epithe·lial cells. Diabetic mice downregulated AhR activity and protein expression, resulting in a decrease in DNA promoter binding site of pigment epithelium-derived factor (PEDF) by gene regulation in transcrip·tional cascade, which could be reversed by these AhR agonists. **CONCLUSIONS** Strategies targeted the protective AhR/PEDF axis possess potentially maintain retinal vascular homeostasis, providing op·portunities to retard the development of DR during progression to diabetes.

Key words: diabetic retinopathy; aryl hydrocarbon receptor; pigment epithelium-derived factor (PEDF)

T13-96-0025

Aggravation of pulmonary fibrosis after knocking down the Aryl hydro-carbon receptor in the Insulin-like growth factor 1 receptor pathway

Sheng-Mao Wu[1], Meei-Ling Sheu[1,2]

(1. National Chung Hsing University; 2. Taichung Veterans General Hospital)

Abstract: OBJECTIVE Idiopathic pulmonary fibrosis (IPF) is a devastating disease with multiple contributing factors. For the development of airway inflammation, insulin-like growth factor 1 receptor (IGF1R), with a reciprocal function to Aryl hydrocarbon receptor (AhR), is well known to be involved. However, the exact relationship between IGF1R and AhR in lung fibrogenesis is unclear. Here, we aimed to investigate the cascade pathway between the role of IGF1R and AhR in idiopathic lung fibro·sis. **METHODS** AhR and IGF1R expressions were determined in the lungs of IPF patients and rodent fibrosis model. Pulmonary fibrosis was evaluated in bleomycin (BLM)- induced lung injury in wild type and AhR knockout (AhR-/-) mice. We also examined the effect of IGF1R inhibition and AhR activation in vitro on TGF - β1 induced Epithelial-mesenchymal transition (EMT) in Beas2B cells and in vivo on BLM-exposed mice. **RESULTS** Increased IGF1R levels and diminished AhR expression was found in the lung tissues of IPF patients and BLM-induced mice. AhR knockout aggravated lung fibrosis, while IGF1R inhibitor and AhR agonist significantly attenuated such effect, and inhibited TGF - β1 induced EMT in Beas2B cells. Specifically, TGF-β1 and BLM markedly suppressed AhR expression through the activation of the endoplasmic reticulum (ER) stress, and consequently IGF1R activation. Furthermore, the activation of TGF - β1 signal pathway was reversed by IGF1R inhibitor and specific knockdown of IGF1R. **CONCLUSION:** AhR and IGF1R play opposite roles in the development of IPF via the TGF-β/Smad/STAT signaling cascade. AhR/IGF1R axis is a potential target for treating lung injury and fibrosis.

Key words: IGF1R; AhR; bleomycin; pulmonary fibrosis; ER stress; epithelial-mesenchymal transition

T13-96-0026

The role of autophagy regulated-NLRP3 inflammasome inactivation in the therapy of alcoholic liver disease

YU HSUAN LEE[1,2], Yu-Hsuan Huang[2], Hui Wen Chiu[3]

(1. Department of Cosmeceutics, China Medical University, Taichung; 2. Department of Food Safety/ Hygiene & Risk Management, College of Medicine, National Cheng Kung University, Tainan; 3. Graduate Institute of Clinical Medicine, College of Medicine,

Taipei Medical University, Taipei)

Abstract: Alcoholic liver disease (ALD) is one of the major causes of liver disease that results in significant morbidity and mortality in the world. According to the Lancet journal, alcohol is associated with 2.8 million deaths each year worldwide. Despite the high mortality of alcoholic hepatitis (AHS), there are still limited medical therapies available. Recent studies have shown that resveratrol (Res) could ameliorate alcoholic liver injury. Nevertheless, resveratrol has poor bioavailability, which hinders its immense potential. Addressing the issue, we attempt to used resveratrol-loaded nanoparticles (nanoRes) for improving the bioavailability and further investigate whether alcohol activates NLRP3 in·flammasome by down-regulating autophagy in hepatocytes. *In vivo* study, C57BL / 6 male mice were treated with ethanol liquid diet alone or combined with Res or nanoRes. The liver tissues and serum were collected for toxicity analysis. *In vitro* study, normal mouse hepatocytes (AML-12 cells) were used to mimic liver organ exposure. *In vivo* study, ethanol significantly increased the relative liver weights over the 10 weeks' treatment. In addition, the mice treated with ethanol liquid diet also significantly ele·vated serum AST, ALT and TCHO levels and hepatic TG contents. The results of immunohistochemis·try staining also showed that the protein expressions of NLRP3, and TGF-β were increased in the etha·nol liquid diet group. The administration of nanoRes effectively improved the lipid accumulation, inflam·mation, and fibrosis caused by ethanol in both *in vivo* and *in vitro* results. Taken together, our findings demonstrated that ethanol would regulate autophagy-NLRP3 inflammasome pathway to cause lipid ac·cumulation and inflammation. The new mechanisms may be a new target to treat ALD. Otherwise, the treatment of resveratrol-loaded nanoparticles also could rescue those ethanol-induced damages by in·hibiting the NLRP3 inflammasome pathway. In the future, resveratrol-loaded nanoparticles could be a potential drug to treat ALD.

Key words: Alcoholic liver disease (ALD); NLRP3 inflammasome; autophagy; resveratrol-loaded nanoparticles

T14-22-0009

Polystyrene microplastics induced male reproductive toxicity via initiation of reticulum stress signaling pathway

Wen Siyue, Tang Yizhou, Zhao Yu, Liu Shanji, You Tao, Xu Hengyi*

(State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China)

Abstract: The ubiquitous microplastics (MPs) have aroused great attention on human health, but relatively scarce are reproductive toxicity combined with underlying mechanism of MPS. In present study, we intended to investigate the adverse effects of 10 μm polystyrene microplastics (PS-MPs) on male reproductive system and focused on the endoplasmic reticulum stress (ERS) for illustration of mo·lecular mechanism. The results showed that the number and viability of sperm significantly decreased and the malformation rate was increased after PS-MPs ingestion. For evaluation of testicular damage, H&E staining displayed the vacuolization, atrophy and even shedding of germ cells in seminiferous tu·bule. Consistently, the testosterone in serum was detected to obviously decreased after PS-MPs treat·ment. Moreover, the molecular analysis indicated that PS-MPs up-regulated the trait genes for ERS (BIP, IRE1α, XBP1s, JNK, CHOP) and downstream apoptotic regulator genes (Caspase12, 9, 3) in tes·

tis. Nevertheless, the down regulation of StAR, the mediator of testosterone biosynthesis, was also observed in study. With the subsequent supplementation of ERS inhibitor salubrinal (sal), the MPs-induced semen and testicular damage were alleviated, and testosterone secretion was also improved to almost normal level. Taken above together, our findings suggested that PS-MPs could generate reproductive toxicity possibly via the activation of ERS and apoptosis signaling pathway.

Key words: Microplastics; Male reproductive toxicity; Endoplasmic reticulum stress

Corresponding author: Xu Hengyi, E-mail: kidyxu@163.com, E-mail: HengyiXu@ncu.edu.cn

T14-43-0005

Arsenic exposure diminishes ovarian follicular reserve and induces abnormal steroidogenesis by DNA methylation: conclusion from epidemiological investigation to laboratory studies

Chen yiqin, Wu siyi, Liu weili, Wang wenxiang

(Fujian Province Key Laboratory of Environment and Health, School of Public Health, Fujian Medical University, Fuzhou, Fujian, China, 350108)

Abstract: Arsenic contamination is a worldwide public health problem. The effect of arsenic on male reproduction has been extensively studied. However, less data was available on the biotoxicity of arsenic to female reproduction. In this study, a human-cell-animal translational strategy was applied to explore the effect of arsenic exposure on ovarian steroidogenesis and its potential mechanism. We conducted a 1:1 propensity score matching case-control study, involving 127 DOR cases and 127 healthy controls. The ovarian follicular fluid levels of 21 metal elements including As were measured. The results showed that there were significant differences in follicular fluid metal profiles between DOR patients and normal women, and arsenic played an important role in DOR progression. In the primary ovarian granulosa cell culture model, we found that the E2 level was significant decreased after arsenic exposure, which was dependent on the alteration of key enzymes in steroidogenesis. In addition, we also established the model of sodium arsenite exposure through water in rats from weaning to sexual maturity. We evaluated ovarian development by monitoring vaginal opening time, determining estrus cycles, observing ovarian pathology, and calculating follicular proportion. RT-PCR, western blot, and BSP were used to investigate the effect of arsenic exposure on ovarian steroidogenesis and its possible mechanism. The results indicated that SF-1 was an important target of steroidogenesis disorder induced by arsenic exposure. Arsenic significantly increased the DNA methylation level in the promoter region of SF-1 to reduce its mRNA expression. However, SF-1 may be regulated by a post-transcriptional regulation mechanism to significantly increase its protein expression, thus promoting the secretion of E2 in the ovary, and leading to premature depletion of ovarian follicles.

Key words: Arsenic; Diminished ovarian reserve; Ovary; Follicular fluid; Steroidogenesis

T14-45-0011

Early-life exposure to lead changes single-cell transcriptome profiles and compromises cardiac development

Liu Qian, Gu Aihua*

(Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing, 211166)

Abstract: Lead (Pd) is ubiquitous in the environment because of widespread human use. People with prenatal exposure to lead may be at risk for heart disease, but the underlying mechanisms remain largely unknown. Here we surveyed the single-cell transcriptomic atlas of the embryonic heart and revealed the cellular key events caused by early-life lead-exposure during heart development. The reduced cardiomyocytes proportion and increased epicardial cells proportion were found in Pb group compared with the control group. Histologically, early-life lead exposure predisposes offspring mice to cardiomyocytes incomplete differentiation by sarcomere dysplasia and mitochondrial degeneration, endothelial cells dysfunction and myocardial fibrosis. Notably, the early life pathological changes have a long-term health hazard to the heart of adult offspring, rendering them more susceptible to cardiac stress induced by Angiotensin II infusion, even lead exposure has stopped at the 4th week postnatally. Mechanistically, Pb ion may directly inhibit metabolic enzymes so as to disrupt cellular energetics and compromise cell-type specific functions, especially for cardiomyocytes and endothelial cells. The influenced cardiac subsets proportion and function are used as early key events for abnormal heart development. Collectively, the present study first demonstrates that early-life low dose lead exposure compromises cardiac development in embryonic stage and exacerbates second hit-induced cardiac pathological responses in adulthood. These results suggest that effective prevention of lead exposure in early life is critical for amelioration of not only congenital heart defects but also susceptibility to cardiovascular diseases in adults.

Key words: Lead (Pd), Early-life exposure, Cardiac development, Sarcomere, Mitochondria, Single-cell RNA sequencing (scRNA-Seq)

Corresponding author: Liu Qian, E-mail: qianliu@njmu.edu.cn, Gu Aihua, E-mail: aihuagu@njmu.edu.cn

T14-46-0001

Cholesterol metabolism homeostasis programming mechanism and potential early warning marker of dexamethasone inducedhippocampal synaptic injury in offspring

Shiyun Dai, Tingting Wang, Hui Wang, Dan Xu*

(Department of Pharmacology, School of Basic Medical Sciences, Wuhan University, Wuhan 430071, China; Hubei Provincial Key Laboratory of Developmentally Originated Disease, Wuhan 430071, China)

Abstract: The common biological basis of cognitive and affective disorders is synaptic transmission and its plasticity, which is mainly related to central cholesterol metabolism homeostasis. Based on previous studies in our laboratory, prenatal dexamethasone exposure (PDE) can cause cognitive and

emotional deficits and synaptic impairment in male offspring. This study aims to explore the common programming mechanism and potential early warning markers of hippocampal synaptic injury in PDE offspring from the perspective of cholesterol metabolism homeostasis. Wistar pregnant rats were given subcutaneous injection of normal saline or dexamethasone (0.2 mg·kg^{-1}·d) at 9-20 days of gestation. The hippocampi and the primary astrocytes of the hippocampi of 20 days of gestation (GD20), 6 weeks after birth (PW6) and PW12 were obtained for morphological and related indexes detection. The primary hippocampal astrocytes of male Wistar fetal mice were treated with different concentrations of dexamethasone at the cellular level to clarify the molecular mechanism. The total cholesterol in GD20, PW6 and PW12 hippocampus of male PDE offspring rats was increased, the synaptic vesicles were decreased, and the lipid raft of neurons was damaged. By extracting GD20, PW6 and PW12 hippocampal primary astrocytes, we observed that intracellular total cholesterol content increased significantly while extracellular total cholesterol content decreased, and the expression and acetylation level of cholesterol transporter ABCG1 in astrocytes continued to decrease from intrauterine to postnatal in PDE group. In combination with bioinformatics analysis and experimental results, we found that the elevated expression of miR450a-3p in the hippocampus persisted from intrauterine to postnatal and was closely related to the disturbance of cholesterol metabolism in the hippocampus. After treatment with different concentrations of dexamethasone in the hippocampal primary astrocytes of Wistar male fetal mice, intracellular cholesterol levels were increased and ABCG1 expression and acetylation levels were decreased. Treatment with GR antagonist RU486 and miR450a-3P inhibitor reversed the reduction of ABCG1 expression and acetylation induced by dexamethasone. These results suggest that miR-450a-3p may be an early warning target for fetal-derived cognitive and emotional disorders, as PDE activates hippocampal GR in male fetal mice, and decreases the cholesterol transport from hippocampal astrocytes to neurons mediated by the cascade effect of miR450a-3p / ABCG1, thereby causing synaptic damage.

Key words: Dexamethasone; Hippocampus; Cholesterol metabolism; Cynapse

T14-46-0007

A metabolomic study on male reproductive toxicity induced by molybdenum exposure and its mechanism

Guan Yu-sheng, Zhou Jing-jing, Huang Rui, DuanJia-wei, Xu Yun-tian, Tang Miao-miao, Chen Min-jian

(State Key Laboratory of Reproductive Medicine, Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing 211166, China)

Abstract: Heavy metal exposure has become a widespread concern. Increasing evidence shows that the exposure to some heavy metals can induce reproductive toxicity. Our previous study used high-throughput elementomics technology found that molybdenum showed high exposure among many heavy metals and showed the most significant potential male reproductive toxicity. As an essential trace element, the safe dose range of molybdenum exposure is worth exploring. Due to its extensive use and serious pollution, its human exposure burden is gradually increasing, and its health hazards are worthy of attention. In this study, male C57 mice were exposed to sodium molybdate at doses of 0.001, 10 and 100 mg/ kg/ day to establish a male youth (8 weeks old) and late reproductive age (56 weeks old) mouse model. The effects of molybdenum exposure on male reproductive toxicity were studied by histopathological evaluation and computer-aided semen parameter analysis (CASA); the ex·

posure levels of molybdenum in urine and testis were determined by inductively coupled plasma mass spectrometry (ICP-MS); metabolomic analysis covering hypothalamus / serum / testis was used to ex· plore the mechanism of male reproductive toxicity induced by molybdenum exposure. The results showed that molybdenum induced male reproductive toxicity in a dose-dependent manner, including testicular tissue damage, decreased number of round spermatids, decreased sperm number and sperm motility. The sperm deformity rate was increased in 8-week-old mice, and the number of Sertoli cells were decreased in 56-week-old mice. The exposure dose-dependently increased the content of molybdenum in urine and testis. Metabolomic analysis showed that the main metabolic pathways in· volved in male reproductive toxicity induced by molybdenum exposure included glutathione metabolic pathway, β-Alanine metabolism pathway, pantothenic acid and CoA synthesis pathway, riboflavin me· tabolism pathway and steroid hormone synthesis pathway. 8-week-old mice showed oxidative stress - related metabolic disorder and essential (conditionally) amino acid metabolic disorder in hypothalamus/ pituitary/testis axis; 56-week-old mice showed oxidative stress-related metabolic disorder and essential amino acid metabolic disorder in testis, as well as hormone disorder in hypothalamus and blood. Exces· sive exposure to molybdenum could cross the blood testis barrier, and had a specific testicular toxicity, which significantly affected spermatogenesis. Its effects generally were consistent among mouse models at different ages, but there were also some differences. Toxic mechanism of molybdenum was related to the oxidative stress in hypothalamus / pituitary / gonad axis and the disturbance of estrogen synthesis. This study provides a new scientific understanding of the health hazards and the metabolic mechanism of the exposure to molybdenum, which is a potential male reproductive toxicant.

Key words: Molybdenum; Metabolomics; Spermatogenesis; Male reproductive toxicity; Metabolic mechanism

Corresponding author: Chen Min-jian, E-mail: minjianchen@njmu.edu.cn

T14-51-0003

Flurochloridone induces apoptosis in Sertoli cells mediated by ER stress and p-eIF2α/ATF4/CHOP signaling pathway

Fen Zhang, Zhijing Ni, Shuqi Zhao, Yanna Wang, Xiuli Chang, Zhijun Zhou

(Department of Occupational Health and Toxicology, School of Public Health, Fudan University, Shanghai 200032, China)

Abstract: To understand mechanism of Flurochloridone (FLC) - induced damage in testis, adult C57BL/6 mice were exposed to FLC by intragastric perfusion at gradient dosesof 0 (0.5% sodium car· boxymethyl cellulose), 3, 15, 75, 375 mg·kg^{-1} for 28 days respectively. Histological analysis, the ultra· structure of endoplasmic reticulum (ER), apoptosis, and proteins related with ER stress, unfolded pro· tein response (UPR) pathway and apoptosis in testis were evaluated by hematoxylin-eosin staining, electron microscope observation, TUNEL staining and Western Blot. To verify these finding, mouse Sertoli cell line TM4 cells were treated with FLC (0, 40, 80,160 μmol·L^{-1}) for 6, 12, 24 h and similar indi· cators were observed. Furthermore, the inhibitor of eIF2α phosphorylation, ISRIB, was used to explore the role of UPR pathway in FLC-induced apoptosis in TM4 cells. *In vivo*, FLC caused Sertoli cells vacu· olation in testis, and inducedER dilatation and apoptosis of Sertoli cells. The proteins levels of ER stress, UPR and apoptosis were up-regulated in dose-dependent way. In vitro, FLC induced TM4 cells· apoptosis by triggering ER stress and up-regulating the proteins levels of phosphorylated-eIF2α, ATF4,

CHOP. The activation of p-eIF2α/ATF4/CHOP signaling pathway and apoptosis induced by FLC could be efficiently reversed by pre-treatedwithISRIB. While the FLC-induced cytotoxicity and cell viability were improved. The present study provided the evidence that FLC induced apoptosis in TM4 Sertoli cells and this process may involve ER stress and p-eIF2α/ATF4/CHOP signaling pathway.

Key words: Flurochloridone; testicular toxicity; TM4 Sertoli cells; endoplasmic reticulum stress; unfolded protein response; apoptosis

Corresponding author: Zhijun Zhou, E-mail: zjzhou@fudan.edu.cn

T14-55-0004

Semen quality and sperm DNA methylation in relation to long-term exposure to six air pollutants in fertile men-A cohort study

Yuting Cheng[a, b], Qiuqin Tang[c], Yiwen Lu[a, b], Peihao Wu[a, b], Yijie Zhou[a, b], Mei Li[a, b], Minjian Chen[a, b], Chuncheng Lu[a, b], Xinru Wang[a, b], Yankai Xia[a, b], Wei Wu[a, b]*

(a. State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing, 211166; b. Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing, 211166; c. Department of Obstetrics, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital 210004)

Abstract: Few studies have examined the association between long-term exposure to six air pollutants and semen quality. And potential mechanisms haven't been confirmed. We aimed to investigate the association between long-term exposure to air pollutants and semen quality and to explore the effect of sperm DNA methylation in such association. We enrolled 1554 fertile men from 2014-2016 to evaluate 14 parameters of their semen quality. Exposure window was defined as one-year before semen sampling. Multivariable linear regression and WQS regression model were used to investigate the association between six air pollutants and semen quality. Semen samples were randomly selected from 200 participants to detect the levels of genomic 5mC and 5-hmC in sperm by ELISA kits. PM_{10}, $PM_{2.5}$, SO_2 and NO_2 were in negative associations with sperm total motility (PM_{10}: β =- 2.67, P=0.009; $PM_{2.5}$:β=-2.86, P=0.004; SO_2:β=-2.32, P=0.011; NO_2: β=-2.21, P=0.012). WQS regression results also showed that co-exposure of six air pollutants caused sperm total motility to decrease (β =- 1.64, P=0.003). Wherein, PM10 accounted for the proportion of 43.4%. The level of 5-hmC was associated with exposure to PM10 (β=0.002, P<0.001). Long-term exposure to air pollution may reduce semen quality in fertile men by affecting DNA methylation levels in sperm.

Key words: air pollution; co-exposure; PM10; semen quality; sperm motility; 5mC; 5-hmC

Corresponding author: Wei Wu, E-mail: wwu@njmu.edu.cn, E-mail: t19871004@sina.com

T14-60-0013

Effects of cadmium exposure during pregnancy on estrogen and progesterone synthesis in offspring's ovarian granulosa cells and its maternal intergenerational effects

Li Jing-wen, Li Qing-yu, Liu Zhang-pin, Lv Ya-ke, Zhang Wen-chang
(Department of Preventive Medicine, School of Public Health, Fujian Medical University, Xueyuan Road No.1, Minhou County, Fuzhou, China, 350108)

Abstract: Cadmium(Cd) has been shown to interfere with the synthesis of hormones (such as progesterone and testosterone),and hormonal changes induced by Cd may have long-term effects.In this study, pregnant SD rats were orally dosed with Cd (0, 0.5, 2.0, and 8.0 mg/kg/day) from gestation day 1 until birth.F1 and F2 female rats were mated with untreated males to produce F2 and F3 generations. After prenatal Cd exposure, hormone secretion examination showed a significant decrease in serum progesterone (Pg) and estradiol (E2) in the F2 female rats, while the expression level of Pg and E2 were increased in the F3 female rats. In addition, we found that in those estrogen and progesterone synthesis-related genes, the mRNA expression levels of steroidogenic acute regulatory protein (StAR) and cytochrome P450 side chain cleavage enzyme (CYP11A1) were increased in F2 and F3 generations; the expression levels of cytochrome P450 aromatase (CYP19A1) were decreased in the medium dose group of F2 generation female rats, while were increased in F3 generation female rats. Moreover, the related protein detection results showed that the expression levels of StAR in the F2 and F3 generation 8.0 mg / kg dose groups was decreased; the expression levels of cytochrome P450 hydroxylase (CYP17A1) and CYP19A1 increased in both generations;the expression level of CYP11A1 was decreased in F2 generation, F3 generation 0.5 mg/kg and 2.0 mg/kg dose groups. Overall, this study suggest that the effect of cadmium exposure during pregnancy on serum estrogen and progesterone in the offspring has a trans-generational effect, and their changes are inconsistent between generations. Besides, the transcription and protein levels of genes related to estrogen and progesterone synthesis are also different. The specific mechanism of transgenerational genetic need to be explored further in future research.

Key words: Cadmium; Maternally Inherited; Trans-Represented; Epigenetic

T14-71-0006

The critical role of CYP3A65 in BDE47 induced developmental malformation of zebrafish

Chao Wang, Yinyin Liang, Jiansheng Zhu, Lu Yang, Yuhuan Hu, Shou-lin Wang*
(State Key Lab of Reproductive Medicine, Institute of Toxicology, School of Public Health, Nanjing Medical University, Nanjing 211166)

Abstract: Among PBDE congeners, 2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE47) is the predominant congener found in the environment and in particular in human blood, placenta, urine and breast milk. Our previous studies reported that CYP3A-mediated metabolic activation of BDE47 plays an important role in reproductive and developmental toxicity. Zebrafish CYP3A65 is regarded as a homolog to human CYP3A4. The present study intends to investigate the critical role of CYP3A65 in BDE47-in·

duced zebrafish developmental malformation. We generated a CYP3A65 knockout zebrafish model using TALEN technology. Toxicological assessments of BDE47 exposure on zebrafish were evaluated. (i) CYP3A65 was mainly expressed in the liver and intestinal tissues from 72hpf and reached the peak at 144hpf. Induction of CYP3A65 by environmentally relevant doses of BDE47 had been shown in developing zebrafish. (ii) BDE47 could be metabolized by CYP3A65 to produce more toxic OH- and MeO-metabolites, which could aggravate zebrafish developmental deformities, such as spinal dorsal curvature, eye dark adaptation injury and behavioral changes. BDE47-induced deformities had trans-generational effects in the offspring (F1). CYP3A65 knockout significantly reversed the developmental deformities of zebrafish. The structure of BDE47 and its metabolites was similar to that of thyroxine. 3-MeO-BDE47, a main metabolite of BDE47 by CYP3A65, reduced the level of thyroid hormone by up-regulating the expression of dio3a and dio3b, resulting in developmental toxicity. (iii) Transcriptome analysis revealed that CYP3A65 was also an important endogenous metabolic enzyme besides exogenous metabolism, involving the retinoic acid homeostasis. Up-expression of CYP3A65 induced by BDE47 could accelerate the clearance of retinoic acid and reduce the expression of rhodopsin in zebrafish, resulting in the impairment of dark adaptation. Zebrafish visual injury could be restored with CYP3A65 KO, thyroxine (T3) and retinoic acid (RA) intervention. For the first time, this study confirmed that BDE47 could be metabolized by CYP3A65 in zebrafish. BDE47 treatment induced spinal dorsal curvature, abnormal dark adaptation and behavioral changes. The role that the CYP3A65 plays was therefore potentially twofold. One cause was exogenous metabolic pathway. BDE47 was metabolized by CYP3A65 into more toxic active metabolites OH-BDE47 and MeO-BDE47. Another cause was the endogenous metabolic pathway. Over-expression of CYP3A65 accelerated the retinoic acid clearance rate and lead to the impairment of dark adaptation. These results will provide an important theoretical basis for the systematic evaluation of the reproductive and developmental effects of brominated flame retardants in the environment.

Key words: BDE47; CYP3A65; zebrafish; metabolic activation; developmental toxicity

Corresponding author: Shou-lin Wang, E-mail: wangshl@njmu.edu.cn

T14-71-0010

The effects of bisphenol AP exposure during pregnancy on behavior of offspring

Zhang Mei-jia[a], Wu Xiao-rong[a], Li Shiqi[a], Qin Yufeng[a,b]

(a. Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing 211166, China; b. Department of Microbes and Infection, School of Public Health, Nanjing Medical University, Nanjing 211166, China)

Abstract: OBJECTIVE Bisphenol compounds are widely used and have adverse effects on human health. Bisphenol AP(BPAP), as substitutes for bisphenols, is synthetic compound detected increasingly frequently in plastics and resins. BPAP is endocrine disruptors with unclear effects on development and social behavior. METHODS To investigate the effects of maternal exposure to BPAP during pregnancy on development and social behavior of offspring mice, C57BL/6 pregnant mice were given BPAP at 0.4 mg/kg/d in drinking water containing. RESULTS We didn′t find significant differences in general birth outcomes in control and BPAP group, including sex ration, littler numbers and birth weight between control and BPAP group. However, maternal BPAP exposure altered the social behav·

ior in offspring. Open Field Test showed that compared with the control group, maternal BPAP exposure significantly decreased the movement distance and speed of in female offspring. Light-Dark-Box Test showed that the time of staying in the bright box and the distance of movement of the offspring mice were significantly increased in maternal BPAP exposure group. Social Interaction Test suggested the contact time was significantly changed by maternal BPAP exposure. Rotarod Test showed that mice in BPAP group dropped earlier than those in the control group. **CONCLUSION** Our study indicated that maternal exposure to BPAP resulted in impaired social interaction, motor coordination, and learning ability in offspring mice. The underline mechanisms required further studies.

Key words: Bisphenol AP; Maternal Exposure; Behavioral experiment

Corresponding author: Qin Yufeng, E-mail: qiny@njmu.edu.cn

T14-74-0008

Effects of glufosinate-ammonium on male reproductive health: Focus on epigenome and transcriptome in mouse sperm

Ma Xuan[a], Hu Weiyue[a], Xia Yankai[a]

(State Key Laboratory of Reproductive Medicine, Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing 211166, China)

Abstract: Glufosinate-ammonium (GLA) is a widely used herbicide with emerging concern over its neural and reproductive toxicity. To uncover potential effects of GLA on male reproductive health in mammals, adult male C57BL/6J mice were administered 0.2 mg·kg^{-1}·d GLA for 5 weeks, and then copulated with female DBA/ 2 mice. After examination on fertility, testis histology and semen quality in the GLA group, we performed deep sequencing to identify DNA methylation, transcriptionally active (H3K4me3 and H3K27ac) and repressive (H3K27me3 and H3K9me3) histone modifications, and mRNA transcript levels in sperm. Moreover, RNA sequencing was also performed on preimplantation embryos to reveal whether these histone modification alterations would cause any abnormal gene expression after fertilization. We found no significant abnormality either on fertility, testis histology or semen quality-related indicators in GLA mice. Next generation sequencing showed alterations of these epigenetic marks and extensive transcription inhibition in sperm. Differential active marks were enriched at promoters and putative enhancers, while repressive marks were mainly distributed at intergenic regions and introns. They were mainly enriched in pathways related to synapse organization. When we zoomed in these regions, increased H3K4me3 overlaps H3K27ac loci at the gene promoter of Phkg2, which was actively expressed in GLA sperm. Additionally, decreased H3K4me3 overlaps H3K27ac at the promoter of Dcn in sperm, which was also down-regulated in GLA preimplantation em·bryos. Subtle differences in genomic imprinting were observed between the two groups. These results suggested that GLA predominantly impaired sperm epigenome and transcriptome in mice, with little ef·fect on fertility, testis histology or semen quality. These alterations in sperm epigenetic marks also dis·rupted normal gene expression after fertilization. Further studies on human sperm using similar strate·gies need to be conducted for a better understanding of the male reproductive toxicity of GLA.

Key words: GLA; DNA methylation; histone modification; male reproduction

Corresponding author: Hu Weiyue, E-mail: weiyuehu@njmu.edu.cn; Xia Yankai, E-mail: yankaix·ia@njmu.edu.cn

T14-90-0014

Exposure to BPS exerts cytotoxic effect on mouse Leydig cells: involvement of mitochondrial dysfunction and defective mitophagy

Zhang Wen-juan, Huang Tao, Sun Zhang-bei, Zhang Da-lei

(Department of Physiology, School of Basic Medical Sciences, Nanchang University, Nanchang 330006, China)

Abstract: Bisphenol S (BPS), a predominant alternative to bisphenol A, is drawing an increasing attention due to its probable detriment to human health. Epidemiological investigations have shown that exposure to BPS is associated with defective semen parameters. In laboratory animals, it has been linked to testosterone deficiency and male reproductive dysfunction. In this study, TM3 mouse Leydig cells were *in vitro* treated with BPS (100, 200 and 400 μmol·L^{-1}) for 48 h to examine the cytotoxicity of BPS and to investigate its underlying mechanisms. Our results indicated that exposure to BPS at all concentrations tested significantly suppressed the viability of TM3 cells in a concentration-dependent manner. Furthermore, BPS challenge triggered oxidative stress manifested by exaggerated generation of reactive oxygen species (ROS) and lipid peroxidation product malondialdehyde (MDA) with compromised activities of antioxidases superoxide dismutase and catalase. Particularly, BPS incubation evoked an augment of mitochondrial permeability transition pore opening, a dissipation of mitochondrial membrane potential and a reduction of ATP production. Targeted metabolomics suggested that exposure to BPS for 48 h resulted in a disorder of energy metabolism and the altered metabolites were mainly involved in the glycolytic pathway. Moreover, BAX expression and caspase-3 activity were markedly elevated and BCL-2 expression were notably inhibited after exposure to intermediate and high doses of BPS. However, the combined treatment with Mito-TEMPO, a mitochondria-targeted antioxidant, significantly restored the viability, decreased ROS and MDA formation and inhibited apoptosis in BPS-exposed TM3 cells. In addition, BPS-treated TM3 cells exhibited an accumulation of autophagic vacuoles in the cytoplasm, along with up-regulated Beclin1 and P62 expression and increased LC3B - Ⅱ/LC3B - Ⅰ ratio. Taken together, exposure to BPS *in vitro* elicited cytotoxicity to TM3 Leydig cells by inducing oxidative stress and mitochondrial impairment, subsequently resulting in autophagic disturbance and apoptosis. The antioxidant intervention targeting mitochondria can protect against BPS-induced Leydig cell damage through alleviating oxidative stress and apoptosis.

Key words: bisphenol S; oxidative stress; mitochondrial dysfunction; autophagy; apoptosis

T14-91-0002

Title:a weight of evidence assessment of developmental and reproductive toxicity of diisononyl phthalate under GHS

Zou Hanjun[a]*, Palermo Christine[b], Norman John[b], Green Maia[b]

(a. ExxonMobil (China) Investment Co., Ltd., 1099 Zixing Road, Shanghai 200241, PRC; b. ExxonMobil Biomedical Sciences, Inc., 1545 US Highway 22 East, Annandale, NJ, 08801, USA)

Abstract: Low Molecular Weight (LMW) phthalates (such as DEHP, BBP and DBP) demonstrate clear toxicity meeting the criteria for GHS classification for reproductive and developmental toxicity. However, evaluations of High Molecular Weight (HMW) phthalate, i.e., DINP, continue to be proposed

by different organizations/agencies globally with variable conclusions. The main reasons include discor·dant views on biological effect versus adversity, misinterpretation of data / data quality, bias on data selection, different rules / framework used for toxicity classification and also a lack of transparency. In this effort a systematic literature review on the reproductive and developmental toxicity data for DINP was carried out in a bid to conclude the classification under globally harmonized classification frame·work, i. e., UN GHS, following and updating the systematic review conducted by Dekant and Bridges. We screened records from seven bibliographic databases and scored papers using a framework devel·oped by Dekant and Bridges for assessing animal toxicity studies. Papers meeting the criteria (i. e., a minimum score) were used to evaluate DINP against the GHS criteria for the classification of reproduc·tive and developmental toxicity. A weight of evidence analysis indicates DINP did not interfere with sex·ual function or fertility. Moreover, DINP did not cause effects on fetal development warranting classifica·tion, including no effects on male reproductive development (i.e., a lack of association with hypospadi·as, cryptorchidism, permanent nipple retention or anogenital distance as seen with LWM phthalates) fol·lowing exposure during the androgen sensitive window of development. Therefore, DINP does not elicit adverse effects warranting classification as a reproductive or developmental toxicant under GHS criteria.

Key word: Phthalate; DINP; GHS Classification; Reproductive and developmental toxicity; System·atic review; Weight of evidence

Corresponding author: Zou Hanjun, E-mail: steven.hj.zou@exxonmobil.com

T14-91-0012

Blood trace elements associated with miscarriage in early pregnancy

Yingying Lu[a], Ting Chen[b, c, d], Qing Xu[a, c, d]

(a. Department of Obstetrics and Gynecology, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing, China, 210004; b. Department of Science and Technology, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing, China, 210004; c. Key Laboratory of Modern Toxicology of Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing, China, 211166; d. State Key Laboratory of Reproductive Medicine, School of Public Health, Nanjing Medical University, Nanjing, China, 211166)

Abstract: An increasing number of epidemiologic studies have estimated associations between trace elements and miscarriage. However, these studies are limited in scope and have produced incon·sistent results. This cross-sectional study collected whole blood samples from 195 women with 92 pa·tients with miscarriage and 103 controls before 12 gestational weeks, and measured concentrations of 20 trace elements using inductively coupled plasma mass spectrometry (ICP-MS). The limits of detec·tion (LOD) for the trace elements ranged from 0.0036 (Re) to 77.5747 (Zn) $\mu g \cdot L^{-1}$. For some trace ele·ments, >90% of the subjects had blood levels that were higher than LOD, and these trace elements were further analyzed. We found several trace elements had significantly statistical association with miscarriage. Conclusions: several trace elements had significantly statistical association with miscar·riage during early pregnancy.

Key words: Miscarriage; trace elements; inductively coupled plasma mass spectrometry (ICP-MS)

Corresponding author: Qing Xu, E-mail: xq079054@163.com

T14-91-0015

Bisphenol A induced spermatogenic cell damage by regulating Chchd10 mediated mitochondrial dynamics in mice

Xiao Jiang[a], Li Yin[a,b], Fei Han[a], Xi Ling[a], Yang-xi Zhou[a], Jia Cao[a], Jin-yi Liu[a]

(a. Institute of Toxicology, College of Preventive Medicine, Army Medical University, Chongqing, China; b. College of Pharmacy and Bioengineering, Chongqing University of Technology, Chongqing, China)

Abstract: Mitochondrial fusion and fission (mitochondrial dynamics) are homeostatic processes that safeguard normal cellular function and seems to be critical for spermatogenesis. So far, the effects and mechanisms of Bisphenol A (BPA) on mitochondrial dynamics in spermatogenic cells are less-well understood. Here, we utilized BPA exposure in vivo and in vitro model to assess the effects of BPA on spermatogenic cell and explored the molecular mechanism involved in. The results showed that the sperm motility decreased and the head abnormality of sperm increased significantly compared to the control after BPA (30 mg/kg/d) exposure for 35 days. Meanwhile, some pathological changes including the vacuoles, exfoliated as well as abnormal mitochondria were found in seminiferous tubules or germ cells in mice. In addition, we found that the number of long tubular mitochondria decreased significantly in BPA treated GC-2 cells. Affymetrix GeneChip results showed that multiple mitochondrial related genes were differentially expressed after BPA treatment, and the expression of Chchd10, a mitochondrial inner membrane structural protein was significantly up-regulated after BPA treatment, which was verified both *in vivo* and *in vitro* model. Furthermore, interfering with the expression of Chchd10 can improve the fragmented mitochondria caused by BPA induced mitochondrial fusion and fission imbalance in GC2 cells. Together, these results suggested that mitochondria are the important target organelles in BPA induced spermatogenic cell damage and Chchd10 mediated mitochondrial fission/fusion imbalance in BPA induced spermatogenic cell damage in mice.

Key words: Bisphenol A; Chchd10; Mitochondrial dynamics

Corresponding author: Jin-yi Liu, E-mail: jinyiliutmmu@163.com

T14-91-0016

Lifelong exposure to low-dose pyrethroid insecticide cypermethrin impairs ovarian development and function in female mice

Xiaochen Ma, Jing Liu

(MOE Key Laboratory of Environmental Remediation and Ecosystem Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou, China, 310058)

Abstract: Our recent epidemiological study of Chinese women reported that pyrethroid insecticide exposure was positively associated with the risk of primary ovarian insufficiency (POI), which is a major cause of female infertility due to loss of ovarian follicles before age 40 [1]. In this study, we utilized cypermethrin (CP) as a representative pyrethroid insecticide to identify how lifelong and low-dose exposure to pyrethroid affects the ovarian functions and the underlying mechanisms.

Female mice were exposed to CP at doses of human dietary intake of 6.7 μg/kg/day, an acceptable daily intake (ADI) of 20 μg/kg/day, or the chronic reference dose (RfD) of 60 μg/kg/day, starting

from gestational day 0.5 until 44 weeks old (W) which phase correlates to humans about 40 years old. Female mice exposed to CP produced less pups, showing reduced fertility. Developmental exposure to CP at doses of ADI and RfD reduced the size of the primordial follicle pool on postnatal day (PND) 5. When females were continuously exposed to CP until 44W, the number of all types of follicles significantly decreased. Chronic exposure of female mice to CP at doses of ADI and RfD caused significantly lower estrogen and higher follicle-stimulating hormone (FSH). We identified BMF, a pro-apoptotic protein, was significantly up-regulated in CP-exposed neonatal ovaries based on transcriptome data on PND5. We further found that p27, a factor inhibits the progression of the cell cycle, was highly expressed in the low-dose-exposed ovaries in 44W mice. The remarkable reduction in cell proliferation and an increase in apoptosis were observed in ovarian follicles in PND5 and 44W mice. Two key genes that catalyze estrogen synthesis, CYP19A1 and HSD17B1 were significantly decreased in developing follicles and corpus luteum by CP at dose of RfD. These data suggest that lifelong exposure of female mice to CP at safe human dose could induce the expression of pro-apoptotic protein and cell cycle inhibitor that promote apoptosis in ovarian cells to lead to loss of primordial follicles, and dysregulate ovarian genes of key enzymes involved in steroidogenesis to result in the insufficient estradiol and increased gonadotropin levels, which in turn lead to reduced fecundity and show POI phenotype.

Key words: pyrethroids; primary ovarian insufficiency; fertility; ovarian follicles

T15-17-0003

In vitro and *in vivo* evaluation of montmorillonite for paraquat poisoning

Guo Xiang, Guo Wei, Li Tian-di, Liu Fen, Zhou Jin-peng, Guo Mei-qiong

(Shen Zhen Prevention and Treatment Center for Occupational Disease, Shen Zhen 518020)

Abstract: Background Evaluation of montmorillonite for paraquat poisoning by *in vitro* and *in vivo* test. METHODS *In vitro* test were evaluated by a batch test, taking the paraquat concentration, adsorbents, reaction environment and time as indices, the absorption rate was screened by orthogonal design. *In vivo* test was executed with rabbits. Group 1：4 rabbits dosed with montmorillonite. Group 2：8 rabbits dosed with 200 mg·kg^{-1} paraquat. Group 3：6 rabbits dosed with 200 mg·kg^{-1} paraquat then gavage with montmorillonite 5 min later. Group 4：6 rabbits dosed with 200 mg · kg^{-1} paraquat then gavage with montmorillonite 30 min later. Blood paraquat concentration, serum cytokines, blood gas analysis and histopathology of lung were implemented. RESULTS *In vitro* test found that all the four factors influence the absorption rate of paraquat (P<0.05). *In vitro* test found that oral montmorillonite could change toxicokinetics parameters of paraquat (P<0.05); decrease raised serum TGF - β1 and HMGB1 (P<0.05) and alleviate the histopathology damage of lung. CONCLUSIONS Montmorillonite might exert its protective effects on paraquat induced damage.

Key words: paraquat; poisoning; montmorillonite; toxicokinetics; histopathology; in vivo; in vitro; rabbit

Corresponding author: Li Tian-di, E-mail: flight027@126.com

T15-34-0004

SIK2 Functions in radiation-induced alveolar epithelial-mesenchymaltransition

Ping Wang[1], Yuhao Liu[1], Xingkun Ao[2], Ziyan Yan[1], Xinxin Liang[2], Shanshan Gao[1], Pingkun Zhou[1], Yongqing Gu[1,2]

(1. Beijing Institute of Radiation Medicine, Beijing 100852; 2. Hengyang Medical School, University of South China, Hengyang 421001)

Abstract: Radiation induced lung injury is one of the most important and serious complications in patients with chest tumor after radiotherapy. It is mainly divided into early acute radiation pneumonitis and late radiation-induced-pulmonary-fibrosis (RIPF). It is characterized by excessive proliferation of fi·broblasts and excessive deposition of collagen and extracellular matrix (ECM). In recent years, relevant studies have shown that ionizing radiation can induce epithelial-mesenchymal transition (EMT), which is one of the main sources of fibroblasts.

Salt induced kinase 2 (SIK2) is an ionizing radiation-induced protein found in our laboratory. It has serine/ threonine (Ser/ the) kinase activity and can mediate PI3K/ Akt signaling pathway. In our current research, we showed that ionizing radiation can increase the expression of SIK2inalveolarepithelial cells. And overexpression of SIK2 significantly increased the expression of EMT related marker pro·teins such as N-cadherin and vimentin, meanwhile knockdown of SIK2 by siRNA could significantly reverse radiation-induced EMT and decrease the expression of mesenchymal markers. Finally, the increase of SIK2 expression induced by radiation is significantly related to the aging of type II alveolar epithelial cells and the activation of fibroblasts, which aggravates the occurrence and development of radiation-induced lung injury.

Key words: ionizing radiation; SIK2; EMT; pulmonary fibrosis

Corresponding author: Yongqing Gu, E-mail: yqgu96@163.com

T15-38-0001

Meta-analysis for the anesthesia effects of pentobarbital sodium and chloral hydrate on rats

Hu Xiongfei, Yang Xiuhong, Yuan Shu

(Hunan Prevention and Treatment Institute of Occupational Diseases, Changsha, 410007)

Abstract: OBJECTIVE To evaluate the anesthesia effects of pentobarbital sodium and chloral hy·drate on rats through Meta-analysis. **METHODS** Randomized controlled trail about the theme had been searched in several databases including Wanfang, Weipu, CNKI, Cochrance library, Pubmed, et al. The studies that met inclusion criteria were analyzed by using RevMan 5.3. Result 9 studies were chosen. **RESULTS** Meta-analysis were as follows: The induction time of chloral hydrate on rats was less than that of pentobarbital sodium ($P<0.01$). The mortality rate of chloral hydrate on rats was lower than that of pentobarbital sodium ($P<0.05$). There was no significant difference in duration time on rats between the two groups. **CONCLUSION** The anesthesia effects of chloral hydrate is better than that of pentobarbital sodium. Because of the heterogeneity of most indicators and inadequate number of rats in many studies, more larger sample size and high-quality studies are needed to evaluate the anesthe·

sia effects of the two substances.

Key words: pentobarbital sodium; chloral hydrate; rat; anesthesia

T15-69-0002

Atypical antipsychotics alters spliceosome signaling in mouse heart: implications from proteomic and transcriptomicanalyses

Jing Wang, Xiaoqing Li, Xinyi Lin, Zheng Liu, Xinru Tang, Liliang Li
(Department of Forensic Medicine, School of Basic Medical Sciences, Fudan University, Shanghai 200032, China)

Abstract: Atypical antipsychotics (AAs) are first-line drugs that are prescribed for mental disorders in clinic. Severe cardiotoxicity has been widely reported and thus limits their clinical application. This study aimed to identify the common mechanism underlying AAs-induced cardiotoxicity using dual-omics analyses. Balb / C mice were intraperitoneally injected with two representative AAs, olanzapine (2.5 $mg \cdot kg^{-1}$) and clozapine (25 $mg \cdot kg^{-1}$), at clinically comparable doses for 0, 7, 14 and 21 days. Our results showed that both AAs induced cardiomyocyte degeneration, inflammation infiltration, and cardiac fibrosis, all of which worsened with time. Proteomic analysis showed that 22 differentially expressed (DE) proteins overlapped in olanzapine and clozapine-treated hearts. These proteins were significantly enriched in muscle contraction, amino acid metabolism and spliceosomal assembly by GO term analysis and spliceosome signaling was among the top enriched pathways by KEGG analysis. Among the 22 DE proteins, three spliceosome signal proteins were validated in a dynamic detection, and their expres·sion significantly correlated with the extent of AAs-induced cardiac fibrosis. Following the spliceosome signaling dysregulation, RNA sequencing revealed that alternative splicing events in the mouse heart were markedly enhanced by AAs treatments, and the production of vast transcript variants resulted in dysregulation of multiple pathways that are critical for cardiomyocytes adaptation and cardiac remodeling. The use of pladienolide B, a specific inhibitor of spliceosome signaling, successfully corrected AAs - induced alternative splicing, and significantly attenuated the secretion of pro-inflammatory factors and cell deaths induced by AAs exposure. Our study concluded that the spliceosome signaling was a com·mon pathway driving AAs cardiotoxicity in mice.

Key words: Antipsychotics; Spliceosome; Cardiotoxicity; Proteome; Transcriptome

T15-82-0005

Safety evaluation of chloroquine phosphate in type 2 diabetic rats

Xian Jing, Yuling Zheng, Junqi Wang, Shiheng Hu, Hui Ji, Dawei Guo,
Shanxiang Jiang, Xiuge Gao
(College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, Jiangsu)

Abstract: The coronavirus disease 2019 (COVID-19) patients complicated with underlying diseases are at increased risk of death. Using the officially recommended anti-COVID-19 drug chloroquine phos·phate (CQ) for treatment of severe patients with type 2 diabetes (T2D) may induce potential hazards. We aimed to understand the safety of CQ on type 2 diabetes in this study by administrating the recom·

mended dosage (63 mg · kg^{-1} twice daily for 7 days) and high dosage (126 mg · kg^{-1} twice daily for 7days) of CQ in T2D rats. We found that CQ increased the mortality of diabetic rats at day 5. The mor·tality of T2D rats in the recommended dosage and higher dosage group were 40% and 80%, respec·tively, compared to the control group. Hematotoxicity was observed only in high dosage group other than the normal dosage group. The hepatic enzymes of CQ-treated T2D rats were significant higher than T2D rats and healthy rats. CQ changed the electrocardiogram and prolonged QTc interval of T2D rats. Leukocyte in urine of T2D rats were confirmed by CQ treatment for 7 days. Ocular toxicity was ob·served in two CQ dosage groups at day 5. Histopathology analysis showed severe damage of heart, liv·er, kidney, jejunum, spleen and retina of T2D rats after CQ administration for 7 days. However, CQ treatment significant decreased blood glucose level of T2D rats and inhibited the production of several inflammatory cytokines, including IL-1β and IL-6. In conclusion, based on preclinical safety evaluation of CQ in T2D rats, the dosage and regimen must be reconsidered in treating COVID-19 patients due to its safety concerns, especially using in patients combined with diabetics.

Key words: Chloroquine phosphate; Type 2 diabetes; Safety evaluation; Toxicology

Corresponding author: Xiuge Gao, E-mail: vetgao@njau.edu.cn

T15-82-0006

Atypical antipsychotics induce cardiotoxicity through dysregulating spliceosome signaling in mouse heart: implications from proteomic and transcriptomic analyses

Liliang Li, Jing Wang, Xiaoqing Li, Xinyi Lin, Zheng Liu, Xinru Tang
(Fudan University)

Abstract: Atypical antipsychotics (AAs) are first-line drugs that are prescribed for mental disorders in clinic. Severe cardiotoxicity has been widely reported and thus limits their clinical application. This study aimed to identify the common mechanism underlying AAs-induced cardiotoxicity using dual-omics analyses. Balb / C mice were intraperitoneally injected with two representative AAs, olanzapine (2.5 mg·kg^{-1}) and clozapine (25 mg·kg^{-1}), at clinically comparable doses for 0, 7, 14 and 21 days. Our results showed that both AAs induced cardiomyocyte degeneration, inflammation infiltration, and cardi·ac fibrosis, all of which worsened with time. Proteomic analysis showed that 22 differentially expressed (DE) proteins overlapped in olanzapine and clozapine-treated hearts. These proteins were significantly enriched in muscle contraction, amino acid metabolism and spliceosomal assembly by GO term analy·sis and spliceosome signaling was among the top enriched pathways by KEGG analysis. Among the 22 DE proteins, three spliceosome signal proteins were validated in a dynamic detection, and their ex·pression significantly correlated with the extent of AAs-induced cardiac fibrosis. Following the spliceo·some signaling dysregulation, RNA sequencing revealed that alternative splicing events in the mouse heart were markedly enhanced by AAs treatments, and the production of vast transcript variants result·ed in dysregulation of multiple pathways that are critical for cardiomyocytes adaptation and cardiac re·modeling. The use of pladienolide B, a specific inhibitor of spliceosome signaling, successfully correct·ed AAs-induced alternative splicing, and significantly attenuated the secretion of pro-inflammatory fac·tors and cell deaths induced by AAs exposure. Our study concluded that the spliceosome signaling was a common pathway driving AAs cardiotoxicity in mice.

Key words: Antipsychotics; Spliceosome; Cardiotoxicity; Proteome; Transcriptome

T15-82-0007

Higher preoperative total protein is associated with post-transplant diabetes mellitus in Chinese Han kidney transplant recipients treated with tacrolimus-based immunosuppression

Yanfang Zhang

(The First Affiliated Hospital, Zhejiang University School of Medicine)

Abstract: OBJECTIVE Post-transplant diabetes mellitus (PTDM) is one of common comorbidities in kidney transplant recipients (KTRs). Because of the high incidence of PTDM in Chinese KTRs, more studies need to be conducted to explore new risk factors. The aim of our study is to explore the incidence and new predictors for PTDM. **METHODS** A total of 227 KTRs without previously diagnosed diabetes were included. They all utilized a triple-immunotherapy scheme including tacrolimus, mycophenolate and glucocorticoid after kidney transplantation (KT). Their clinical and anthropometric data together with tacrolimus concentrations within 3 months were collected. Student′ s t test or Mann – Whitney U test was used for continuous variables to compare the differences between PTDM patients and non-PTDM, and chi-square test for categorical variables. Then, univariate and multivariate logistic regression models were used to identify significant predictive variables associated with PTDM. **RESULTS** Overall, 37 (16.3%) KTRs developed PTDM within 3 months after KT. Patients with PTDM had older age (P< 0.0001), a higher proportion of deceased donors (P=0.013), higher levels of monocyte ratio (P=0.048), hemoglobin (P=0.030), total protein (P=0.002), alkaline phosphatase (P=0.033) and glutamyl transpep· tidase (P=0.025) than non-PTDM. Multivariate logistic regression analysis showed that preoperative total protein was an independent risk factor for the development of PTDM, together with age. **CONCLU- SIONS** Preoperative total protein and age were independent predictors for PTDM in Chinese Han KTRs.

T15-82-0008

Ppp1r3b as a master regulator of Tacrolimus caused new onset diabetes mellitus by inducing hepatic insulin resistance

Lu Li

(The First Affiliated Hospital, Zhejiang University School of Medicine)

Abstract: New onset diabetes mellitus (NODM) is one of the most common complications after transplantation. As one of the most commonly used immunosuppressants, tacrolimus is a high-risk factor of NODM after transplantation. Previous studies suggested that pancreatic β cell dysfunction was the main cause of tacrolimus induced NODM. Insulin resistance also take a role in tacrolimus induced hyperglycemia. In the present study, balb/c mice were treated with 2.0mg/kg/d tacrolimus for 12 weeks. As a result, we found that the tacrolimus-treated mice had higher blood glucose levels and larger areas under the curve after glucose tolerance test. Moreover, the increased the cholesterol level and triglyceride level. Tacrolimus caused higher HOMA-IR index, which indicated the presence of hepatic insulin resistance. We then analysis the gene expression profiles of tacrolimus-treated mice livers using RNA-sequencing. We identified 506 differentially expressed genes (DEGs), including 230 up-regulated DEGs and 276 down-regulated DEGs. KEGG functional pathway annotation showed that the DEGs mainly en·

riched in insulin resistance, TNF signaling pathway, IL-17 signaling pathway, retinol metabolism and so on. Hepatic insulin resistance is an important mechanism in the development of diabetes. In order to further explore the mechanism of tacrolimus induced NODM, we analyzed the insulin resistance path·way related DEGs. Mlxip, G6pc, Insr, Rps6ka3, Gsk3b, Pik3cb, Trib3, Socs3, and Ppp1r3b were DEGs enriched in insulin resistance pathway. Among these DEGs, Mlxip was the DEG displayed highest fold decrease while Ppp1r3b was the DEG displayed highest fold increase. We then next checked the pro·tein expression of Mlxip and Ppp1r3b in tacrolimus-treated mice liver. We demonstrated that the protein expression of Ppp1r3b was significant increased in tacrolimus group while the expression of Mlxip was not changed. Ppp1r3b involved in regulating glycogen synthesis in liver, therefore might be a key regu·lator in tacrolimus-induced NODM.

Key words: Tacrolimus; diabetes; hepatic insulin resistance; PPP1r3b

T15-82-0009

Mechanism of analyzing the acute liver toxicity of tripterygium glycosides by the proteomic-molecular pathway

GAO Xiao-xin, LI Xuemei, ZHU Chun-yue, Zhang Qian-qian, WANG Jia-shuai,
Cao Chun-ran, HU Yuchi, WANG Shu
(Beijing Institute for Drug Control, NMPA Key Laboratory for Safety Research and Evaluation of Innovative Drug,Beijing Key Laboratory of Analysis and Evaluation on Chinese Medicine, Beijing 102206, China)

Abstract: OBJECTIVE To investigate the mechanism and potential targets of tripterygium glyco·sides acute hepatotoxicity by proteomic and molecular pathway analysis. METHODS 70 mice were randomly divided into 7 groups, including blank control group and 6 dose groups (11800 mg·kg^{-1}, 1332 mg·kg^{-1}, 986 mg·kg^{-1}, 730 mg·kg^{-1}, 540 mg·kg^{-1}, 400 mg·kg^{-1}), with 10 mice in each group and half male and half female. LD50 values were calculated. Livers of three dying animals were collected for proteomic molecular pathway analysis, and livers of the remaining living and dead animals were collect·ed for pathological examination. RESULTS A single dose of tripterygium glycosides was administered to mice. On the day of administration, some mice showed drowsiness and inactivity, but did not die. On the first day after administration, the mice in the administration group showed weight loss, hair erection and lethargy, and some animals died in the administration group. The above reactions had a dose-re·sponse relationship. Toxicity lasted for several days, especially the longest duration of hair erection, and death lasted up to the fourth day. The calculated LD50 value was 830.794 mg·kg^{-1}. In the dead an·imals, liver cords were disarranged, hepatic sinuses were narrowed, liver cells were necrotic and vacuo·lar degeneration, accompanied by bleeding and inflammatory cell infiltration. Proteomics showed 112 differentially expressed proteins, including 45 up-regulated proteins and 67 down-regulated proteins. Bi·ological enrichment results include the metabolic process of cell lipids, lipids, sterols, cholesterol, ste·roids, ethanol, secondary alcohol and synthesis of sterols, secondary alcohol, cholesterol, etc. The ex·pression of related differential proteins was down-regulated in Scarb1, Npc1l1 and Scd1, and up-regu·lated in Hmgcs1, Acss2, Mvd, Pmvk, Ttr, Thrsp, Idi1, Aacs, Acly, Sc5d and Acaca. The enrichment re·sults of cell components included extracellular space, region, foreign body, organelles, vesicles and in·tracellular membrane, cytoplasm, surface vesicles and blood particles, etc. There were significantly

more differentially expressed proteins in extracellular space and region than intracellular. The expres·sions of relevant differential proteins H2-Q10 and Serpina3k were up-regulated, while the expressions of Serpina3n, Serpina3i and Serpina3g were down-regulated. The enrichment results of molecular func·tion include oxidoreductase, aromatase, steroid hydroxylase, arachidonic acid monooxyase, paired community, monooxidase activity, reduced molecular oxygen, arachidonic acid, iron channel, heme, tet·rapyrrole binding, etc. The expressions of relevant differential proteins were up-regulated by Cyp2a4, Cyp2a5, Cyp2c70, Sc5d and Tf, and down-regulated by Cyp2c37, Cyp2c44, Cyp3a11, Scd1, Cyp2b10, Cyp3a25 and Hrg. KEGG pathway enrichment results include linoleic acid, retinol (also known as vita·min A), arachidonic acid metabolism, terpenoid skeleton biosynthesis, etc. This is related to the abnor·mal expression of tripterygium glycosides protein and related cholesterol and lipid anabolism. **CON-CLUSION** Acute toxicity test in mice confirms that tripterygium glycosides is moderately toxic and has liver injury. Large doses of tripterygium glycosides can induce changes in protein expression profile, which involves biological process, cell composition, KEGG pathway and molecular function, and is relat·ed to multiple metabolic pathways such as cholesterol and lipids. Active components in mitochondria and endoplasmic reticulum are active. The differentially expressed proteins Npc1l1, Hmgcs1 and Cyp2a5 have potential research value in acute hepatotoxicity.

Key words: Hepatotoxicity; Acute Hepatotoxicity; Tripterygium Glycosides; Proteomics

T16-62-0001

APreliminary study on the mechanism of subacute toxicity of Aconitum kusnezoffii Reichbbased on intestinal metagenomicsequence

Wurihan, Li-li, Bilige, Meng Xiang-hua, Bolor, Han Xiao-jing, Caolumengerile, Wurihan, Bai Mei-rong

(Key Laboratory of Mongolian Medicine Research and Development Engineering Ministry of Education, Tongliao 028000, China)

Abstract: OBJECTIVE Preliminary study on the subacute toxicity and mechanis of Aconitum kus·nezoffii Reichb. **METHODS** Use Aconitum kusnezoffii Reichbas as an example drug and SDrats as the research objects, except the normal group, the corresponding drug solution was intragastrically admin·istered for 28 days, and observing the effects of each group on heart, liver and kidney function and on pathomorphology. After the last administration, the fecal samples were collected and the fecal DNA was extracted for macrogenomic sequencing of intestinal flora. **RESULTS** Compared with the normal group, on the 14th day after poisoning, serum ALT, AST, CK, CKMB, ALP and UREA increased in CG group (P<0.05), CK and CKMB increased in CZ and CD groups (P<0.05 respectively). On the 21th day, AST and Urea increased in CG group (P<0.05), CKMB decreased (P<0.05), ALT and ALP increased in CZ group (P<0.05); On the 28th day, ALT increased in CG group (P<0.05), CK and CKMB decreased significantly (P<0.05). Histopathological observation showed that Aconitum kusnezoffii Reichb caused hepatocyte edema in different degrees, myocardial fibers arranged disorderly loosely and part of cell edema; edema of renal tubular epithelial cells. The results of flora abundance analysis and difference analysis showed that, at different levels, Aconitum kusnezoffii Reichb makes the relative abundance of flora composition change to a certain extent, especially at the lower level.There were significant differ·ences between the groups, and 34 different microflora were found in CG group. Correlation analysis showed that different bacteria were involved in the regulation of serum ALT, AST, UREA, CK, CKMB and ALP factors. KEGG enrichment analysis showed that CG group was mainly involved in flagellar as·

sembly, bacterial chemotaxis, taurine and hypotaurine metabolism and so on. **CONCLUSION** Aconi·tum kusnezoffii Reichb had certain subacute toxicity. It is preliminarily suggested that Aconitum kusne·zoffii Reichb can causeClostridium perfringens, Desulfovibriodesulfuricans, Desulfovibrio fairfieldensis and Streptococcus suiswere significant enriched andinduce cardiotoxicity, hepatotoxicity and renal tox·icity. The mechanism is related to flagella assembly and bacterial chemotaxis pathway.

Key words: Aconitum kusnezoffii Reichb; Subacutetoxicity; heart; liver; kidney

T16-97-0002

Lactobacillus plantarum alleviated lead-induced severer colonic injury in HFD mice, by repairing gut barrier and microbial homeostasis

Xu Heng-Yi *, Hu Lie-Hai, Zhao Yu, Liu Shan-Ji, You Tao, Zhang Jing-Feng

(State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China)

Abstract: Existing studies on lead (Pb) toxicity are mostly based on healthy population, in order to investigate the toxicity of Pb in the population on high fat diet (HFD), male C57BL/6 mice were fed HFD and 1 g/L Pb^{2+}-containing water for 8 weeks. Results showed that Pb exposure caused severer gut bar·rier injury in HFD mice. In particular, the colon length was significantly decreased, the level of serum li·popolysaccharide was increased, the expression of tight junction related genes was downregulated. The alteration of gut microbiota composition can be observed by 16S rDNA sequencing analysis. Dur·ing the experiment, fecal microbiota transplantation (FMT)was performed in antibiotics treatment mice. The recipient mice showed the similar damage as above, which indicated that gut microbiota played an important role in Pb-induced colon injury in HFD mice. Furthermore, the gut barrier and microbial ho·meostasis was repaired after ***Lactobacillus plantarum*** treatment, which indicated that probiotic can ef·fectively alleviate Pb-induced colonic injury in HFD mice. Our research highlighted the importance of evaluating the effects of heavy metals in sub-healthy population, and showed the possibility of using probiotics to combat heavy metal toxicity.

Key words: Lead exposure; High fat diet; Colonic injury; Gut microbiota; Fecal microbiota trans·plantation; Probiotics treatment

Corresponding author: Xu Heng-Yi, E-mail: kidyxu@163.com, E-mail: HengyiXu@ncu.edu.cn

T16-97-0003

Alterations to the immunity and gut microbiome of mice following oral exposure to 2,6-dichloro-1,4-benzoquinone, an emerging water disinfection byproducts

Jinhua Li

(School of Public Health, Jilin University)

Abstract: 2, 6-dichloro-1, 4-benzoquinone (2, 6-DCBQ), as an emerging water disinfection byprod·ucts (DBPs), has posed potential risks via the digestion system. However, little is known about the tox·

icity of 2, 6-DCBQ on the gut microbiome, which plays a critical role on human health. This study has comprehensively investigated the impact of 2,6-DCBQ on the intestinal microbiome and immunity after the mice orally exposure to 2,6-DCBQ for 28 days. Our results indicated that DCBQ exposure has perturbed the balance between T helper (Th) 1 mediated pro-inflammatory response and Th2 mediated anti-inflammatory response in mice, especially inducing the activation of immune system toward a Th2 response. DCBQ group has induced gut microbiota dysbiosis, and at phylum level, Proteobacteria was relatively less abundant compared with that in the control group. In addition, there was a significant difference in the composition of gut microbiota between DCBQ-treated group and control group, where DCBQ treatment group showed an overgrowth of Prevotellaceae, Alloprevotella, Actinomycetales, and Gemella, and decrease of Escherichia_Shigella, Enterobacteriales, and Enterobacteriaceae. In particular, the altered gut microbiota showed strong correlations with the altered immunological variables after DCBQ exposure. This study provides evidence that 2,6-DCBQ can impact the immune system and gut microbiome, highlighting the adverse effects and potential mechanisms of 2, 6-DCBQ, and providing new insight into the potential relationship between exposure to DBPs and cancer risks.

Key words: disinfection byproducts; 2,6-dichloro-1,4-benzoquinone; gut microbiota; anti-inflammatory response

T17-19-0001

Downregulation of m^{6}A Reader YTHDC2 promotes the proliferation and migration of cigarette smoke-induced malignant lung cells via CYLD/NF-κB

Jin Wang, Lirong Tan, Beibei Jia, Jianxiang Li

(School of Public Health, Medicine College, Soochow University)

Abstract: Lung cancer is one of the most common types of carcinoma worldwide. Cigarette smoking is considered the leading cause of lung cancer. Aberrant expression of several YT521-B homology (YTH) family proteins has been reported to be closely associated with multiple cancer types. The present study aims to evaluate the function and regulatory mechanisms of the YTH domain containing 2 (YTHDC2), a binding-protein of RNA m^{6}A methylation, through *in vitro*, *in vivo* and bioinformatics analyses, especially the effect of smoking. The results revealed that YTHDC2 was reduced in lung cancer and cigarette smoke-exposed cells. Notably, bioinformatics and tissue arrays analysis demonstrated that decreased YTHDC2 was highly associated with smoking history, pathological stage, invasion depth, lymph node metastasis and poor outcomes. The *in vitro* studies revealed that YTHDC2 overexpression inhibited the proliferation and migration of lung cancer cells. YTHDC2 overexpression markedly attenuated the growth of tumors in nude mice compared with the findings in the blank group. Reduced YTHDC2 expression is associated with gene copy loss in DNA. Furthermore, YTHDC2 decreased expression was modulated by copy number deletion in lung cancer. The xenograft animal model validated the tumor-suppressor effect of YTHDC2 on lung cancer cell tumorigenesis *in vivo*. Importantly, the cylindromatosis (CYLD)/NF - κB pathways were confirmed as the downstream signaling of YTHDC2, and this axis was mediated by m^{6}A modification. The present results indicated that smoking-related downregulation of YTHDC2 was associated with enhanced proliferation and migration in lung cancer cells, and appeared to be regulated by DNA copy number variation. Importantly, YTHDC2 func·

tions as a tumor suppressor through the CYLD/ NF - κB signaling pathway, which is mediated by m6A modification.

Key words: Cigarette Smoke; lung cancer; YTHDC2; m^{6}A RNA methylation; CYLD/NF-κB

Corresponding author: Jianxiang Li, E-mail: aljxcr@suda.edu.cn

T17-41-0004

SUMOylation of Smad2 regulates the TGF-β-mediated endothelial mesenchymal transition

Qi Su, Xinyi Yang, Congcong Du, Xu Chen, Wenbin Lu, Qiqi Wang, Hong Yuan, Zhenzhen Zhang, Hongmei Wu, Yitao Qi

(College of life sciences, Shaanxi Normal University, Xi'an, Shaanxi, 710119)

Abstract: Endothelial-mesenchymal transition (EndoMT) is a complex biological process in which endothelial cells are transformed into mesenchymal cells, and excessive EndoMT causes a variety of pathological processes. Here, we show that SUMOylation of Smad2 regulates TGF-β-mediated EndoMT. It was identified that Smad2 is predominantly modified by SUMO1 on two major SUMOylation sites K156 and K383, and PIAS2α is the major E3 ligase for its SUMOylation, whereas SENP1 mediates deSUMOylation of Smad2. In addition, SUMOylation significantly enhances the transcriptional activity of Smad2. SUMOylation also increases the phosphorylation of Smad2 and the formation of Smad2-Smad4 complexes, ultimately promotes the nuclear translocation of Smad2. Finally, the SUMOylation of Smad2 significantly promotes the TGF- β - mediated EndoMT process in endothelial cells. We found that the SUMOylation of Smad2 plays critical roles in TGF - β mediated EndoMT process, providing a new theoretical basis for the occurrence and potential drug targets for EndoMT-related clinical diseases.

Key words: Endothelial mesenchymal transition; Smad2; TGF-β; SUMOylation

T17-69-0002

The expression and function of N6-methyladenosine (m^{6}A) methyltransferase METTL3 in 1-methyl-3-nitroso-1-nitroguanidine (MNNG)-induced malignant transformation in GES-1

Tong Liu, Sheng Yang, Yi-ling Ge, Lu-yan Feng, Si-yi Xu, Yan-ping Cheng, Yi-yi Reng, Li-hong Yin, Yue-pu Pu, Ge-yu Liang

(Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University)

Abstract: **OBJECTIVE** Accumulating evidence indicated that dysregulated m^{6}A methylation was closely associated with the onset and development of cancers. However, the m6A core regulator' s role in gastric tumorigenesis induced by environmental carcinogens remains largely unknown. In this study, we investigated m6A methyltransferase METTL3 expression level in human gastric epithelial cells (GES-1) after the long-term low dose exposure N-methyl-N-nitro-N-nitrosoguanidine (MNNG). We also explored the biological function of METTL3 on MNNG-induced malignant transformed cells. **METHODS**

The soft agar colony formation assay, Western blotting, and wound healing assay were used to evaluate the malignant characteristics of MNNG-induced transformed GES-1 cells (MC cell). The EpiQuikTMm6A RNA kit was used to measure the m6A content of total RNA in MC cells. We construct plasmid and lentivirus stably transfected into MC cells to knock down the expression of METTL3. Quantitative real-time PCR and Western blotting were performed to detect the transfected efficiency. The effect of knockdown METTL3 on proliferation, migration, and invasion in MC cells was analyzed using Cell Counting Kit-8, wound healing assay, and the TransWell assay, respectively. Western Blot analysis was used to detect the effect of knocking down METTL3 on the expression level of key epithelial-mesenchymal transition (EMT) proteins. **RESULTS** We found a progressive time-dependent increase in METTL3 expression and m^6A content of total RNA in MC cells following MNNG exposure. Knockdown of METTL3 significantly affected the proliferation, migration, and invasion of MC-40 (40 generation of MC cell) cells. In addition, down-regulated of METTL3 also inhibited the colony formation assay rate and EMT protein expression level in MC-40 cells. **CONCLUSION** Dysregulated METTL3 was involved in the gastric tumorigenesis induced with MNNG.

Key words: m6A; METTL3; GES-1; MNNG; EMT

Corresponding author: Ge-yu Liang, E-mail: lianggeyu@163.com

T17-87-0003

MicroRNA-145-5p suppresses the progression of breast cancer via inhibiting SENP2-ERK2signaling

Xu Chen, Yuanyuan Qin, Hong Yuan, Qiqi Wang, Congcong Du, Zhenzhen Zhang, Wenbin Lu, Yitao Qia, Hongmei Wu

(College of life science, Shaanxi Normal University, Shaanxi Xi'an,710119, China)

Abstract: OBJECTIVE Breast cancer remains a public-health issue on a global scale and a serious threat to human health due to the highly metastatic which result in remarkable short survival and poor prognosis. Therefore, the search for new therapeutic targets has always been the hot spot and leading-edged researchin breast cancer treatment. SENP2 is a SUMO-specific protease that is involved in the maturation of SUMO proteins and maintained the homeostasis of substrate proteins SUMOylation status. SENP2 has been demonstrated to regulate tumor progression in a variety of cancers, but its role in breast cancer has been rarely reported yet. In this report we aim to explore the molecular mechanism of SENP2 regulation role in breast cancer procession, hoping to provide new horizon for cancer treatment. **METHODS** and **RESULTS** We explored the public bioinformatic data combined withfurtherassay indicated that SENP2 is essential and highly expressed in breast cancer. The molecular and biochemistry assay showed that SENP2 acted as acarcinogenic factorpromoting the proliferation, migration and invasion of breast cancer.Further exploration identified thatERK2 activity was upregulated by SENP2 in breast cancer. Then we proved that ERK2 is SUMOylated by SUMO2 at K99 and K272 in vivo and vitro and is deSUMOylated by SENP2. Interestingly, further experimental evidences determined that ERK2 SUMOylation inhibited the proliferation and migration of breast cancer cells, indicating that SENP2-ERK2 signaling is essential for breast cancer procession. Furthermore, microRNA-145-5p is lowly expressed in breast cancer, it bound to 3'-untranslated region of SENP2 mRNA to regulate the SENP2 expression. MicroRNA-145-5p was predicted and confirmed as a direct upstream regu·

lator of SENP2 in breast cancer cellsthrough inhibition of SENP2-ERK2 axis. **CONCLUSION** In conclu·sion, we explored the molecular mechanism of SENP2 affecting the growth and metastasis of breast cancer, and further confirmed its upstream microRNA-145-5p and downstream ERK2. We identified the oncogenic role of SENP2 in breast cancer and identified microRNA-145-5p as upstream regulator of SENP2.Moreover, we further confirmed the SUMOylation of ERK2 and its critical roles in promoting the growth and metastasis of breast cancer. In conclusion, the study showed the regulatory role of microR·NA-145-5p / SENP2 / ERK2 axis in breast cancer, providing a new horizon for the further treatment of breast cancer.

Key words: Breast cancer; SENP2; ERK2; microRNA-145-5p

Corresponding author: Hongmei Wu, E-mail: Hq8479@snnu.edu.cn

T17-87-0005

Epigenetic effects of trichloroethylene and its metabolite trichloracetic acid on the hepatic L-02 cells

Xinyue Peng[1], Susu Yu[1], Hui Lin[2], Fan Wu[1], Jiani Yang[1], Cheng Zhou[1], Luyun Zhang[1], Jianping Yang[3],Wenjuan Zhang[1]

(1. Jinnan University; 2. Guangdong Provincial People's Hospital; 3. Shenzhen Boruikang Tech. Co., Ltd)

Abstract: Trichloroethylene (TCE) is an important volatile organic solvent. Trichloroacetic acid (TCA) is one of the main metabolites of TCE in the human body and it is a common non-volatile by- product of chlorination disinfection for drinking water, which cause a series of toxic damage to health. Conventional genetic mechanisms cannot fully explain their toxicity and carcinogenicity, indicative of the possible involvement of epigenetic mechanisms. Our study was intended to investigate the epigene·tic toxicity and underlying the mechanisms of TCE and TCA. Data showed that TCE (0.3 mmol·L^{-1}) and TCA (0.1 mmol·L^{-1}, 0.3 mmol·L^{-1} and 0.9 mmol·L^{-1}) had inhibitory effects on hepatic L-02 cells growth with no significant changes in morphology. Subacute exposure to TCE and TCA induced the genomic DNA hypomethylation and histone hyperacetylation. The levels of DNMTs and HDACs were abnormal in L-02 cells when treated with TCA. DNA hypomethylation in the promoter regions of tumor-related genes including c-Myc, c-Jun and IGF-II, further promoted their protein levels in a time-dependent man·ner. TCE and TCA exposure could increase the level of H-Ras protein, with no significant DNA methyla·tion in the promoter region. The change of the level of N-ras and C-fos genes had no significance in the TCA-induced cells, but increased in the TCE-induced cells, and DNA hypomethylation was involved in the promoter regions of these two genes. These results revealed that there might be some relation·ships among DNA hypomethylation, histone hyperacetylation and protein expression in tumor-related gene after TCE and TCA exposure under specific epigenetic microenvironment, serving as early bio·markers for TCE and TCA-associated diseases.

Key words: Trichloroethylene; Trichloracetic acid; Epigenetics; Tumor-related genes

T17-87-0006

Epigenetic mechanism of cell migration and invasion promoted by exposure to endosulfan in human prostate cancer cells

Dan Xu*, Yue Wang, Yanyuan Lu, Yubing Guo, Yeqing Sun
(Institute of Environmental Systems Biology, Environment Science and Engineering College, Dalian Maritime University, Linghai Road 1, Dalian 116026, China)

Abstract: Endosulfan is a persistent organic pollutant that bioaccumulates in human body through the food chain and thus represents a potential risk to public health. Despite epidemiological studies, the molecular mechanisms underlying the carcinogenic effects of endosulfan in the prostate remain poorly understood. In this study, we investigated the effects of endosulfan on epithelial-mesenchymal transi·tion (EMT) in human prostate cancer PC3 and DU145 cells. Endosulfan induced alterations of EMT bio·markers and affected Notch and TGF - β signaling pathway. Protein-tyrosine Phosphatase 4A3 (PTP4A3) was upregulated by endosulfan. Specific inhibitor of PTP4A3 reversed the changes of EMT biomarkers, the increased expression levels of p-Smad2 / Smad2, but did not affect Notch signalling pathway when exposure to endosulfan. Endosulfan promoted cell migration and invasion, which were rescued by PTP4A3 inhibitor as well as inhibitors for Notch and TGF-β signaling pathway (LY2109761 and DAPT), respectively. PTP4A3 overexpression promoted cell migration and invasion. PTP4A3 is a direct target gene of miR-137-3p, which was identified by bioinformatics analysis and luciferase report·er assay. Furthermore, pull-down and RNA immunoprecipitation assays demonstrated that KCNQ1OT1 acts as a miRNA sponge of miR-137-3p to mediate PTP4A3 on cell migration and invasion. KC·NQ1OT1 knockdown also alleviated cell migration and invasion, which were inhibited by anti-miR-137- 3p. In addition, KCNQ1OT1 knockdown or miR-137-3p overexpression abolished the effects of endosul·fan on EMT biomarkers, TGF-β signaling pathway, cell migration and invasion. These findings suggest that endosulfan triggers EMT to promote cell migration and invasion through KCNQ1OT1/miR-137-3p/ PTP4A3 axis-mediated TGF-β signaling pathway in prostate cancer cells.

Key words: endosulfan; prostate cancer; epithelial-mesenchymal transition; PTP4A3; KCNQ1OT1

Corresponding author: Dan Xu, E-mail: jotan1995@163.com

T17-87-0007

Analysis of correlation between endosulfan and human cancers and key oncogenes promoting cancer cell migration

Yue Wang, Yanyuan Lu, Yubing Guo, Yeqing Sun, Dan Xu*
(Institute of Environmental Systems Biology, Environment Science and Engineering College, Dalian Maritime University, Linghai Road 1, Dalian 116026, China)

Abstract: Endosulfan is a persistent organic pollutant that bioaccumulates in human body through the food chain and thus represents a potential risk to public health. Despite several pesticides were as·sociation with human cancers, the relationship between endosulfan and human cancers remain poorly understood. In this study, we reveal the correlation between exposure to endosulfan as typical environ·ment pollutants and carcinogenesis and study the key oncogenes promoting cancer cell migration. Based on the comparison and analysis of previous gene microarray data and existing research data,we used NextBio software to predict that endosulfan exposure was related to human breast cancer, pros·tate cancer, leukemia and uterine cancer. Endosulfan increased the expression of PTP4A3 oncogene and promoted cell migration, which were rescued by PTP4A3 Inhibitor. Furthermore, the PTP4A3

mRNA expression was upregulated by dechlorane plus and downregulated by perfluorooctane sulpho·nate in DU145 cells. These findings indicated that PTP4A3 might act as the key oncogene and partici·pate in cancer metastasis promoted by endosulfan.

Key words: endosulfan; prostate cancer; breast cancer; leukemia; terine cancer; PTP4A3

Corresponding author: Dan Xu, E-mail: jotan1995@163.com

T18-90-0001

Nonclinical evaluation studies of gene therapy products (Regulatory Declaration and R&D Cases)

Quanjun Wang

(Institute of Toxicology and Drug Research, National Beijing Center for Drug Safety Evaluationand Research, Beijing, China 100850)

Abstract: The idea of altering genes to treat or even cure a disease was out of the question in the past. However, the current concept "gene is drug" has also become a reality, and combined with other high-end technical methods, jointly remodels the approach of drug treatment and disease cure.

Gene and cell therapy (GCT) refers a medical field which focuses on the introduction of exogenous normal genes into target cells to correct or compensate diseases caused by gene defects and abnor·malities for the purpose of treatment. GCT is called advanced therapy medicinal products (ATMP) by the European Medicines Agency (EMA), which refers to gene therapy, somatic cell therapy, tissue-engi·neered product, and combined ATMP for the treatment, prevention, and / or diagnosis of diseases for which there is no effective therapy. GCT is called cell and gene therapy product (CGTP) in the U. S., and the FDA's CGTP product classification is quite different from the EMA ′ s classification. The U. S. FDA manages GCTs as Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) as per the Public Health Service Act, which are regulated by the Office of Tissues and Advanced Thera·pies (OTAT) under the Center for Biologics Evaluation and Research (CBER), and prophylactic GCT products are reviewed by the Office of Vaccines Research and Review (OVRR) under the CBER. In Ja·pan, the name of GCT is "再生医療等製品" (regenerative medical products). In China, the management of GCT is more scientific and reasonable. On February 26, 2019, the National Health Commission draft·ed the Regulations for the Administration of Clinical Application of New Biomedical Technologies. After implementation, some cell therapy technologies and gene therapy technologies (where the final prod·uct is neither a drug nor a medical device as oriented per the results) will be used by biomedical tech·nologies for clinical research and translational application, and subjected to hierarchical management. For such products, the national drug regulatory system adopts the new drug review and management mode designed for class 1 new biological products.

Compared with traditional micromolecular chemical drugs and macromolecular biotechnological drugs, the particularity, advancement and research and development difficulty of GCT are mainly re·flected in the unprecedented treatment mode, the target of action directly aiming at the root cause, the unmet treatment needs, as well as the unique ecosystem and the emerging research and development paradigms.

Risk is a combination consideration of the likelihood of a harm occurrence and the severity of that harm. In general, the risk of a GCT product comes from the following aspects, concretely, defects in the scientific understanding of the product, quality control of the manufacturing process, and the inherent

characteristics of the product including factors such as amplification of normal physiological functions. Based on the existing risks of GCT products, it is necessary to carry out non-clinical evaluation studies, determine pre-clinical evaluation strategies and objectives and establish biological rationality (proof of concept), determine the biologically active dose, provide the basis for the selection of initial dose, dose escalation regimen and dosing regimen for clinical trials, provide support for the feasibility and safety of proposed clinical route of administration, provide the basis for the eligibility criteria of clinical trial popu·lation, identify the possible risks in clinical trials, provide reference for monitoring of clinically relevant risks, and provide safety information that cannot be provided or cannot be fully evaluated due to ethical and safety issues in clinical trials.

For the safety of GCT products, it is required to mainly take into account vector/virus types, biological distribution of vectors / viruses in non-target tissues, virus replication degree and retention duration in the non-target tissues, adverse immune reactions of vectors and exogenous genes/RNAs, potential in·sertional mutation and carcinogenicity of vectors, adverse cell proliferation/differentiation and migration of genetically modified cells and the impact on the microenvironment of non-target tissues, and risks re·lated to specific gene editing techniques.

For non-clinical safety evaluation trial of GCT products, primary factors to be taken into consider·ation include proposed clinical trial (indications, administration route, population, dosing regimen), pub·lished (known) non-clinical/ clinical information of like products, non-clinical/ clinical information of pro·posed drug-carrying system/stent materials, available pharmacological or proof-of-concept data of GCT product or its like products, biological reactivity of selected animal species to GCT product, possible mechanism of action, pharmaceutical attributes (source, type, process, non-cellular components) of GCT product as well as pathophysiological profile of disease/injury animal models (if used); secondary factors to be taken into consideration include number of animals (sufficient for evaluation, random allo·cation), appropriate control group (sham operation arm, vehicle control, stent control, etc.), dose which covers (includes) the proposed dose range of the clinical trial, dosing regimen which should simulate the clinical dosing regimen as much as possible, and multiple necropsies at different time points recom·mended to maximize toxicity exposure; in addition to routine endpoints, study endpoints also include humoral/cellular immune response, distribution of GCT products, neurobehavioral testing, heart assess·ment (MRI, ultrasound, imaging), ectopic growth (hyperplasia, tumors), and interaction with host tissues (immunohistochemistry, recognized markers). At present, FDA-approved drug products granted IND in·clude EDIT-101, a drug for the treatment of inherited retinal degenerative disease LCA, and LUXTUR·NA, an adeno-associated virus vector-based gene therapy indicated for the treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy.

T19-11-0008

Maduramicin induces chicken hepatotoxicity via apoptosis and nonapoptotic cell death *in vitro* and *in vivo*

Junqi Wang, Xiuge Gao, Yuling Zheng, Xinhao Song, Shanxiang Jiang

(College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, Jiangsu)

Abstract: Drug-induced liver injury is a major cause of hepatic dysfunction in veterinary clinical practice. Maduramicin, an anticoccidial drug, frequently induces liver damage of broilers due to over·dose. However, the underlying mechanism of maduramicin of maduramicin-caused liver damage in

chicken remains unclear. Hence, in this study, primary chicken hepatocytes and broilers were used as *in vitro* and *in vivo* model to uncover the hepatotoxicity and its mechanism of maduramicin. Chicken primary hepatocytes were exposed to a series of concentrations of maduramicin (0.1-1 $\mu g \cdot mL^{-1}$) for 24 h to 72 h, and 21 day-old broiler chickens were orally administrated with different doses of maduramicin (5-15 $mg \cdot kg^{-1}$) for 21 days, followed by cell activity assay, microscopy, flow cytometry, RT-qPCR, immunofluorescence, hepatic enzymes test and histopathological examination. We found that chicken primary hepatocytes treated by maduramicin (0.1-1 $\mu g \cdot mL^{-1}$) for 24 h to 72 h showed severe cell damage and significant decrease in cell viability ($P<0.01$) in a concentration and time dependent manner, cells gradually shrunk and detached from the substrate of dish. Maduramicin exposure increased apoptosis rate significantly ($P<0.01$), compared with the control group, which were demonstrated by hoechst33342 and DAPI staining. Furthermore, the apoptotic genes caspase-3/8/9 were upregulated significantly ($P<0.01$) after maduramicin exposure. Histopathological examination and transmission electron microscopy observation exhibited maduramicin induced obvious liver damage, including the scattered liver cords, necrotic and inflammatory cell infiltration, the swollen mitochondria, the disorganized mitochondrial ridges, as well as cytoplasmic vacuolization. Taken together, apoptosis mediates maduramicin-induced hepatotoxicity of chicken *in vitro* and *in vivo*. The manipulation of caspases will provide effective way to attenuate maduramicin-triggered chicken hepatotoxicity.

Key words: Maduramicin; Hepatotoxicity; Chicken; Apoptosis.

Corresponding author: Shanxiang Jiang, E-mail: nauvy@sina.com

T19-19-0006

Construction of the lung-on-a-chip and its toxicological application

Sheng Yang[1], Zaozao Chen[2], Yanping Cheng[1], Geyu Liang[1]*

(1. Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu, China P.R. 210009; 2. State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing, Jiangsu, China P.R. 210096)

Abstract: OBJECTIVE The aim of the study is to build the lung-on-a-chip, a micro physiological system that can reflect the physiological function of human lung, as a reliable, effective toxicological evaluationmethod. METHODS The chip body is comprised of four layers and a porous membrane layer. The four layers are label as upper channel layer, upper cell culture unit layer, bottom cell culture unit layer and bottom channel layer. Human umbilical vein endothelial (HUVEC) cells and human lung epithelial cells (HPAEpiC) are respectively seeded on the two sides of the membrane to reproducethe alveolar capillary barrier. Hydrogen peroxide (H_2O_2) ornanoplastic particles (NPs) were applied to the upper channel to simulate lung exposure. We then detected tight junction protein expression, transepithelial electrical resistance (TEER) value, cytotoxicity, reactive oxygen species production and inflammatory response to evaluate alveolar capillary barrier injury and potential pulmonary dysfunction. RESULTS The lung-on-a-chip can be stably cultured for at least 10 days. The complete structure of alveolar capil·lary barrier was reconstructed and confirmed by confocal imaging of HPAEpiC and HUVEC with tight junction protein. In addition, the complete function of alveolar capillary barrier was confirmed by living and dead cell staining and TEER values. After exposure to H_2O_2 or NPs for 24 hours, the dead cells in the lung-on-a-chip increased significantly, and the TEER values decreased in a dose-dependent man·

ner. The fluorescence image also showed that the integrity of alveolar capillary barrier was destroyed. The levels of total reactive oxygen species, superoxide anion, IL-6 andTNF-α increased with the increase of exposure concentration, indicating that the damage of alveolar capillary barrier may be caused by redox imbalance. **CONCLUSION** Lung-on-a-chip can simulate the physiological activity and structural characteristics of human lung, and reflect the lung function *in vitro*. Moreover, it can visualize and quantitatively analyze a variety of biological processes of exogenous chemicals affecting lung organs, which provides a good *in vitro* platform and a strong technical support for toxicological evaluation.

Key words: lung-on-a-chip; alveolar capillary barrier; toxicology application

Corresponding author: Geyu Liang, E-mail:lianggeyu@163.com

T19-19-0013

Retrospective analysis of recombinant human interferon-α1b in treatment of respiratory syncytial virus in children

Jia-Wen Yu, Guo-Jun Jiang

(Department of Pharmacy, Zhejiang Xiaoshan Hospital, Hangzhou 311202, Zhejiang, China)

Abstract: OBJECTIVE To observe the clinical efficacy of recombinant human interferon-α1b(IFN-α1b) atomization in treatment of respiratory syncytial virus in children. **METHODS** In retrospective study, 55 children with bronchopneumonia caused by respiratory syncytial virus from January 2019 to January 2020 were selected and divided into two groups: 26 children in the group of conventional treatment (control group) and 29 children in the group of IFN - α1b combined with conventional treatment (IFN group). The clinical efficacy was observed and compared of two groups before the treatment and after the treatment for 5-10 days. The drug adverse reactions were recorded. **RESULTS** The total effective rate of the IFN group significantly higher than that of the control group (P<0.05). A total of 55 children were included in the retrospective study, which were including 29 in the IFN group and 26 in the control group. There was no significant difference of the onset time of main symptoms and signs, including body temperature, tachypnea and pulmonary rales recovered between the IFN group and the control group. In the hospitalization time, the onset time of cough recovered in the IFN group was significantly shorter than that in the control group (P<0.05). In both groups , there were no drug adverse reactions were found during the treatment. **CONCLUSION** Recombinant human interferon-α1b is effective in treating respiratory syncytial virus in children, which can significantly shorten the relieving time of cough. The treatment of nebulizing inhaled is safe and reliable.

Key words: Respiratory syncytial virus infection; children; recombinant human interferon-α1b

T19-29-0016

Effect and mechanism of DNA-PKcs on regulating cGAS activity in DNA radiation damage

Shuting Lai[a], Shanshan Gao[b], Pingkun Zhou[a,b]

(a. Institute for Environmental Medicine and Radiation Hygiene, School of Public Health, University of South China, Hengyang, Hunan 421001; b. Department of Radiation Biology, Beijing Key Laboratory for Radiobiology, Beijing Institute of Radiation Medicine, Beijing 100850)

Abstract: OBJECTIVE Radiotherapy is an important method in cancer treatment. Recently, it has been found that cyclic guanylate-adenylate synthase (cGAS) plays an important role in the innate immune response induced by DNA radiation damage. cGAS is a DNA sensor, genomic DNA damage can lead to cGAS activation. The activated cGAS further activates the downstream cGAMP-STING pathway, promotes the production of inflammatory cytokines such as type I interferon, and mediates anti-tumor immunity, aging and inflammatory responses. DNA-PK has been reported to target the cGAS-STING signaling pathway, phosphorylate cGAS and inhibit its enzyme activity, thereby inhibiting antiviral innate immunity, while its role and mechanism in regulating cGAS activity after DNA radiation damage remain to be studied. **METHODS** (1) Co-immunoprecipitation to verify the interaction between DNA-PKcs and cGAS; (2) Immunofluorescence was used to verify the cell co-localization of DNA-PKcs and cGAS; (3) A549 cells were irradiated with γ-rays produced by cobalt-60 source radiation, and ELI·SA kit was used to detect the expression of cGAS-induced inflammatory factor IL-6 after DNA radiation damage; (4) Co-immunoprecipitation to detect the phosphorylation of cGAS by DNA-PKcs after irradiation; (5) The expression changes of related inflammatory factors induced by DNA radiation damage were detected after transfection of cGAS phosphorylation site mutants in cGAS knockout cell lines. **RESULTS** (1) Co-immunoprecipitation confirmed that DNA-PKcs interacted with cGAS, and its interaction was weakened after irradiation; (2) DNA-PKcs interacted with cGAS and co-localized in the nucleus; (3) Compared with the control group, the secreted IL-6 was significantly increased in both cell lines with knockdown of DNA-PKcs and cells treated with DNA-PKcs inhibitor after irradiation, while this effect was reversed in cell lines with DNA-PKcs and cGAS double knockdown. **CONCLUSION** After DNA radiation injury, DNA-PKcs targets cGAS signaling pathway and inhibits cGAS-mediated innate immune response, which further deepens the understanding of the activation mechanism and signaling pathway of cGAS and provides a new possible target and idea for the application of cGAS in tumor radiotherapy.

Key words: DNA radiation damage; DNA-PKcs; cGAS; innate immune response; radiotherapy

T19-31-0004

Discussion on the investigation procedure of OOS

Wen Peng

〔 *Safety Evaluation Center of Shenyang SYRICI Testing Co.,Ltd; National Research Center for New Drug Safety Evaluation(Shenyang); No.600 Shenliao Road,Tiexi District, Shenyang, Liaoning Province China (Post code:110141)* 〕

Abstract: OOS investigation can confirm whether the results are valid or not, and can also guide the discovery of defects in the test process, rectification and taking corresponding preventive measures. The procedure is as follows: Miscalculation investigation: Recalculate the calculation to determine if it was a miscalculation. Investigation Sample: Check the integrity and handling of the original sample, while investigating the sampling process (including sampling methods, sampling tools, etc.) to determine whether the original sample is representative. Analysis process investigation: confirm that test process and method are correct; Verify that the instruments used have been calibrated and operated correctly; Verify that reagents, solvents and standards are used correctly; Make sure there is no visible contamination, etc. Retesting: The same samples originally used should be retested and the process of handling the samples should be repeated, but should be performed by another experienced tester. Resampling: When the results of the first retest show significant differences from the original test, a

repeat sample may be required in this particular case. Calculate new data: the test results of each sam·ple should be evaluated separately and not averaged during reinspection. If the results obtained from multiple measurements are both compliant and non-compliant, then even if the mean is compliant, the abnormal results should be investigated to clarify the cause. If the test process meets the relevant re·quirements after investigation, the preliminary test results will be directly issued; If abnormal data is found to be due to laboratory causes, it should be corrected and retested, and corrective and preven·tive actions should be developed and regularly followed up.

Key words: OOS, procedure

Corresponding author: Wen Peng, E-mail:wenpeng@sinochem.com

T19-32-0001

Association of polymorphisms of telomere maintenance genewithtelomere length in coke oven worker

Shuai Cheng

(Department of Occupational health and occupational diseases, College of Public Health, Zhengzhou University, Zhengzhou, China)

Abstract: OBJECTIVE Polycyclic aromatic hydrocarbons, the main component of coke oven emissions, have high genotoxic. This study aimed to investigatethe association between coke oven emissions exposure and telomere length, and explore whether polymorphisms in telomere mainte·nance gene can modify these associations in coke oven workers. METHODS We measured leukocyte telomere length by real-time polymerase chain reaction (RT-PCR) in blood DNA from 544 coke oven workers and 238 office workers. Polymerase chain reaction and restriction fragment length polymor·phism (PCR-RFLP) were used to detect POT1rs1034794, POT1 rs10250202, TERF1 rs3863242, and TERT rs2736098. The covariance was used to analyze the effects of genetic polymorphisms or environ·ment factors on telomere length and the differences in telomere length between genotypes at each lo·cus. The gene-environment interactions were analyzed with generalized linear model method. RE- SULTS The peripheral blood relative telomere length inexposure group were significantly lower than that of the control group (P<0.001). The relativetelomere lengths with GG genotypes in TERT rs2736098 were longercompared to AG genotype in the control group (P=0.032). The interaction be·tween the AG+AAgenotypes in TERT rs2736098 and coke oven exposure had significant effect on telo·merelength (P<0.05). CONCLUSIONS The effect of telomere length shorting is associated with inter· actions betweenTERT rs2736098 polymorphism and coke oven emissions exposure.

Key words: Coke oven emissions; Telomere length; Polymorphisms; TERT

Corresponding author: Shuai Cheng, E-mail: 254184086@qq.com

T19-34-0014

Methuosis: an excessive cytoplasm vacuolization related cell death mediatesmaduramicin-induced cardiotoxicity

Gao Xiu-ge, Ji Chun-lei, Zheng Yu-ling, Guo Da-wei, Peng Lin, Ji Hui, Jiang Shan-xiang

(College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, Jiangsu)

Abstract: Maduramicin frequently triggers severe cardiotoxicity in domestic animals as well as human accidentally. Apoptotic and non-apoptotic cell death contribute to the serious myocardial damage in clinic. The mechanisms underlying maduramicin-induced non-apoptotic cell death remain largely unknown. In present study, we aimed to elucidate the morphological characteristics and regulatory mechanisms of cytoplasmic vacuolization related cell death induced by maduramicin. H9c2 cells and primary chicken myocardial cells, as well as broiler chickens and rats were used as experimental models. We found that maduramicintriggered numerous phase-lucent vacuoles, which occupied large area of cytoplasm in primary and passage myocardial cells. The cytoplasmic vacuoles induced by maduramicin were generated in a concentration and time dependent manner, along with increased cell death rate. Furthermore, maduramicin-induced cytoplasmic vacuoles are generated from micropinocytosis, rather than the swelling of mitochondria, lysosome, endoplasmic reticulum and Golgi apparatus, demonstrat·ing by the internalization of Dextran-AF 488 into myocardial cells. Intriguingly, the vacuoles acquired some characteristics of late endosomes and lysosomes rather than early endosomes and autophago·somes. Vacuolar H^+-ATPase inhibitor bafilomycin A1 efficiently prevented the generation of cytoplasmic vacuoles and decreased the cardiotoxicityinduced by maduramicin. Mechanism studying indicated that maduramicin activated H-Ras-Rac1 signaling pathway. However, the pharmacological inhibition and siRNA knockdown of Rac1 rescued maduramicin-induced cytotoxicity of H9c2 cells but did not alleviate cytoplasmic vacuolization. Moreover, maduramicin-induced methuosis was confirmed in rats and chick·ens with extensive vacuolar degenerationof myocardium. Proteomic analysis further indicated differen·tially expressed proteinswere significant enriched in SNARE interactions in vesicular transport, ECM-re·ceptor interaction, PPAR signaling pathway, cell adhesion, regulation of actin cytoskeleton and Jak-STAT signaling pathway. In summary, methuosis, a novel non-apoptotic cell death, mediatesmadurami·cin-induced cardiotoxicity in noncanonical programmed pathways.

Key words: Methuosis; Nonapoptosis; Cardiotoxicity; Cytoplasmic vacuolization; Cell death

Corresponding author: Jiang Shan-xiang, E-mail: nauvy@sina.com

T19-41-0017

Development of a lateral flow immunoassay strip for rapid detection of SARS-CoV-2 antibodies after vaccination

Ye Liya

(International Joint Research Laboratory for Biointerface and Biodetection, and School of Food Science and Technology, Jiangnan University, Wuxi 214122, China)

Abstract: Vaccination interventions is considered an important preventive measure to block the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and protect the organ·ism from pathogen infection effectively. However, a quick and accurate technique to evaluate the im·mune efficacy of the SARS-CoV-2 inactivated vaccine remains scarce. In this paper, an full antibodies detection colloidal gold immunochromatography assay kit was optimized and developed, which can as·sess the efficacy of the inactivated SARS-CoV-2 vaccine. We collected sera samples (70 cases) from vaccinees who received their two doses of the COVID-19 vaccine. The results showed that the propor·tion of antibody was high after the second shots immunization. The strip detected neutralizing antibody with a detection limit of of 8 $ng \cdot mL^{-1}$. This strip provides an effective method in preliminary evaluation of the antibody effect of vaccine recipients, and provide a reference index for the potential clinical appli·

cation value of the vaccine.

Key words: SARS-CoV-2 inactivated vaccine; full antibodies; colloidal gold immunochromatography

Corresponding author: Ye Liya, E-mail: yeliiyaaa@163.com

T19-58-0009

Preliminary study on the mechanism of reducing hepatotoxicity of Terminalia chebula to Toosendan fruit

Wurihan[1], Bilige[1], Li-li[1], Meng Xiang-hua[2], Bolor[2], Han Xiao-jing[2], Caolumengerile[3], Wurihan[3], Bai Mei-rong[3]

(1, 2, 3 Key Laboratory of Mongolian Medicine Research and Development Engineering Ministry of Education, Tongliao 028000, China)

Abstract: OBJECTIVE Preliminary study on the mechanism of terminalia chebula palliating hepa·totoxicity induced by toosendan fruit. METHODS Use the compound Sanzitang containing the medici·nal flavor of Terminalia chebula and toosendan fruit, and the drug pairs of Terminalia chebula, toosend·an fruit, gardenia,toosendan fruit plus terminalia chebula , toosendan fruit plus gardenia,terminalia che·bula plus gardenia as an example drug, rats as the research objects, except for the normal group, each administration group was given corresponding solution continuous administration for 21 days, observe the effect of each administration group on liver function and pathomorphology, and metabolic profile of the serum samples of rats were detected by the method of serum metabolomics. Indicative biomarkers were screened in each group. RESULTS Compared with the normal group, the content of ALT and AST could be remarkably improved by fuetus toosendan (P<0.05), and serum ALT、AST content in oth·er groups was not increased. HE staining showed that there was no abnormal change in the liver cells of Sanzitang and terminalia chebula group,except for the small amount of hepatocyte swelling in fruc·tus toosendan group and the cytoplasm of hepatocytes in fructus toosendan plus terminalia chebula group was loose and light stained. The results of metabolomics analysis shows that the LPC, pyrrole-2-carboxylic acid and L-valine, which were associated with hepatotoxicity, were significantly increased in serum samples of toosendan group. But the contents of the differential metabolites in terminalia chebula, fructus toosendan plus terminalia chebula and Sanzitang were lower than those in fructus toosendan group, and were obviously reversed to normal level. CONCLUSION Terminalia chebulia can reduce the liver toxicity of fructus toosendan. The detoxification mechanism of terminalia chebulia may be relat·ed to the decrease of serum LPC18:2. Terminalia chebula has the compatibility and attenuating effect.

Key words: Terminalia chebula; toosendan fruit; hepatotoxicity; attenuated role

T19-64-0007

Investigation of the uptake and transport of aspirin eugenol ester in Caco-2 cells model

Qi Tao†, Zhen-Dong Zhang†, Zhe Qin, Xi-Wang Liu, Shi-Hong Li, Li-Xia Bai, Ya-Jun Yang, Jian-Yong Li

(Key Lab of New Animal Drug Project of Gansu Province, Key Lab of Veterinary Pharmaceutical Development of Ministry of Agriculture and Rural Affairs, Lanzhou Institute of Husbandry

and Pharmaceutical Sciences of CAAS, Lanzhou 730050, China)

Abstract: Aspirin eugenol ester (AEE) is a new medicinal compound synthesized by esterification of aspirin with eugenol using the prodrug principle. AEE has anti-inflammatory, antipyretic, analgesic, anti-cardiovascular diseases, and anti-oxidative stress pharmacological activity. However, its oral bioavailability is poor, and its intestinal absorption and transport characteristics are still unknown. The purpose of this study is to investigate the uptake and transport mechanism of AEE in human intestinal Caco-2 cells. AEE was proved to be non-toxic to Caco-2 cells when its concentration is less than 256 $\mu mol \cdot L^{-1}$. Because higher concentrations of such AEE metabolite as salicylic acid were detected in the supernatant of the cell lysate and cell culture fluid while AEE could be not detected, the change in the content of salicylic acid was used to indicate the change in the content of AEE. The result showed the uptake and transport of AEE were related to time, concentration, and temperature. In the uptake experiment, the uptake of SA reached the maximum at 30 min when Caco-2 cells were incubated with 64$\mu mol \cdot L^{-1}$ AEE from 0 to 120 min. Low temperature can significantly reduce the uptake of SA in Caco-2 cells. In the transport experiment, the concentration of AEE in the Caco-2 cell monolayer was constant and the transport volume increases with time within 120 minutes. The results showed that the transepithelial transport of salicylic acid from the AP-BL and BL-AP sides was time-dependent. Interestingly, the transport amount of salicylic acid in Caco-2 cells increases with the increase in concentration, but transmembrane was no certain correlation between transport rate and concentration. The apparent permeability coefficient (Papp) of low concentration was higher than that of high concentration Papp, which may be the saturation phenomenon of high concentration. The apparent permeability coefficient (Papp) of aspirin eugenol ester (64 $\mu mol \cdot L^{-1}$) from AP to BL was determined to be 0.034 ($\pm$0.012)$\times 10^{-6}$ cm/s, which was considered to have poor permeability and absorptive *in vivo* rate. Efflux ratio (ER)<1, indicating that their intestines transport mechanism was passive transport. In addition, the temperature had a significant effect on the transport of aspirin eugenol ester. In summary, the results indicated that the intestinal absorption of AEE through the Caco-2 cell monolayer involves passive transport. This experiment illustrates the absorption and transport properties of AEE. All these results may help to explore the mechanism of chemically synthesized drugs *in vitro* absorption and transport.

Key words: Aspirin eugenol ester; Metabolite; Caco-2 cells; Uptake; Transport

Corresponding author: Ya-Jun Yang, E-mail: yangyue10224@163.com; and Jian-Yong Li, E-mail: lijy1971@163.com

T19-64-0015

Establishment of an *in vitro* method of rabbit embryo toxicity with toxicokinetics study

Jun Guo[2,3], Qiuyang Zhu[1,2,3], Yuling Jia[2,3], Xiang Meng[2,3], Liming Chong[2,3], Li Xu[2,3], Li Zhou[2,3], Zuyue Sun[2,3]

(1. School of Pharmacy, Fudan University, Shanghai, China; 2. National Health Commission (NHC) Key Laboratory of Reproduction Regulation, Shanghai Institute for Biomedical and Pharmaceutical Technologies, Shanghai, China; 3. Reproductive and Developmental Research Institute, Fudan University, Shanghai, China)

Abstract: This research introduces a novel method, rabbit whole embryo culture (WEC) combined

with toxicokinetics (TK) study, for embryonic toxicity testing. Rodent WEC has been extensively used for in vitro screening of developmental toxicity. To improve the reliability of *in vitro* data, it is important to consider TK and species specificity. To test the utility and effectiveness of this method, we investigated the toxic effect of thalidomide on rabbit embryos and its behavior in test systems both *in vitro* and *in vivo* under the same experimental condition. The data showed that thalidomide induced embryo malformations such as embryonic brain hypoplasia, short limb buds, and declined embryonic growth both *in vitro* and *in vivo*. The toxic effect increased with the increasing exposure of the embryo to thalidomide. In addition, we observed similar toxic effects and exposure-effect relationships both in vivo and in vitro. Therefore, we preliminarily conclude that this new method can effectively predict and explain the developmental toxicity of thalidomide. Furthermore, we investigated the behavior of test compounds in the WEC system for the first time, and this method is expected to be an important technique for *in vitro* developmental toxicity study after extensive verification.

Key words: animal testing alternatives; developmental toxicity; rabbit whole embryo culture; thalidomide; toxicokinetics

Corresponding author: Zuyue Sun, E-mail: sunzy64@163.com

T19-65-0012

The toxic effect of pulse Pb exposureon the microstructureand bacterial composition ofskin in adult Pelophylax nigromaculatus

Liu Yang, Zhao Qiang, Cao Xiao-hong, Xu xiang, Wan Yu-yue, Duan-Ren-yan, Huang Min-yi*

(College of Agriculture and Biotechnology, Hunan University of Humanities, Science and Technology, Loudi 417000, Hunan, China)

Abstract: Lead (Pb) is a toxic heavy metal often present in the environment as a pulse in water. Traditional toxicity tests are usually carried out under conditions of continuous concentration, without considering the impact of pulse exposure on aquatic organisms. This study aimed to evaluate the effects of short-term continuous and pulse Pb exposures on the skin bacteria and histomorphological structure of Pelophylax nigromaculatus. Results showed that Pb exposure changed the numbers and diameter of skin glands and affected the thickness of the skin epidermis and dermis. Compared to the control (CON) and Pb continuous exposure group (CEPb), the Pb pulse exposure group (PEPb) showed the smallest size of granular glands. Pb exposure significantly changed the composition and diversity of skin bacteria. Compared to the CON and CEPb groups, the PEPb group showed a significant increase in the abundance of harmful bacteria (e. g. Bacteroidetes and Chryseobacterium) and a decrease in the abundance of beneficial bacteria (e.g. Pseudomonas). PICRUSt software showed that there were differences in the metabolic pathway of skin bacteria among the three groups (CON, CEPb and PEPb). Overall, this study indicates that Pb pulse exposure can aggravate the toxicity of Pb for frog skin, providing a new framework for simulating short-term heavy metal exposure in the context of frog health.

Key words: Environmental pollutants, Pulse exposure, 16S rRNA, High-throughput sequencing, Skin bacteria

Corresponding author: Huang Min-yi, E-mail: huang.m.y@163.com

T19-68-0011

TNKS1BP1 Play Roles in Radiation-induced Epithelial Mesenchymal Transition in A549 cancer cells

Xingkun Ao[1], Ping Wang[1], Yuhao Liu[1], Xinxin Liang[2], Ziyan Yan[1], Shanshan Gao[1], Pingkun Zhou[1], Yongqing Gu[1,2]

(1. Hengyang Medical School,University of South China, Hengyang 421002; 2. Beijing Institute of Radiation Medicine, Beijing 100850)

Abstract: Radiotherapy is an important method for the treatment of many kinds of malignant tu·mors. Its goal is to completely eliminate the tumor or to reduce the tumor load while preserving normal tissue. Most patients with chest tumors will receive radiotherapy for cure, local tumor control or symp·tom relief. Lung is one of the most easily damaged organs in chest radiotherapy, and lung is one of the most easily damaged organs in chest radiotherapy. Chest radiotherapy is easy to cause radiation-in·duced lung injury, which becomes the main factor limiting the chest radiation dose of tumor patients, and is one of the inevitable complications of chest radiotherapy. RILI significantly affects the treatment of patients with lung cancer, breast cancer, lymphoma or bone marrow transplantation, and reduces tu·mor control probability, which may lead to dyspnea, pulmonary fibrosis and affect the quality of life of patients. TAB182 is a terminal anchored polymerase 1 (tankyrase1) binding protein. It is reported that TAB182 is located in nucleus and cytoplasm. In cytoplasm, it is a new protein found in screening tan·kyrase1 binding protein. Its molecular weight is 182kD. It can be modified by adenosine diphosphate ri·bosylation (PAR) of tankyrase1 *in vitro*. In our previous study, we found that radiation is closely related to TAB182. The results of previous studies are as follows: (1) radiation increases tab182. (2) Local irra·diation of C57/B16 mice and tab182 knockout mice with 60Co radiation source showed that the degree of lung injury in TAB182 knockout mice was lower than that in C57/B16 mice. (3) In A549 cells, knock·down of TAB182 by siRNA significantly attenuated radiation-induced epithelial mesenchymal transition. In addition, we found that TAB182 is closely related to cell aging.

Key words: radiation-induced lung injury; epithelia mesenchymal transition; TAB182

Corresponding author: Yongqing Gu, E-mail: yqgu96@163.com

T19-69-0020

Establishment of LC-MS/MS method for detection of diflubenzuron in swine plasma

Yu-Zhen Xu, Zhe Qin, Xi-Wang Liu, Shi-Hong Li, Li-Xia Bai, Ya-Jun Yang*, Jian-Yong Li*

(Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Lanzhou 730050, China)

Abstract: Diflubenzuron (DFB) is an insect growth regulator that inhibits the synthesis of insect chi·tin synthase, preventing insect larvae from forming new epidermis during molting, which also results in stunted insect body development, deformity, and mortality, impacting an entire generation of insects. Domestically, the commonly used anti-fly and mosquito drugs in farms mainly include cyromazine, or·ganophosphorus and pyrethroid drugs, as well as traditional Chinese medicine preparations such as *Boluosan*. DFB has been approved as an oral anti-fly maggot medicine for swine, cattle, sheep and oth·

er animals in United States, Germany and Brazil. To characterize the absorption, distribution and excre·tion of DFB after administration to swine, an LC-MS/MS method was developed to determine DFB con·centration in swine plasma. 500 μL plasma was spiked with 10 μL DFB-$^{13}C_6$ internal standard working solution and mixed with a vortexer. 2 mL methanol was added into plasma, followed by vortex and cen·trifuge. The supernatant was dried. The residue was completely dissolved with 500 μL methanol-water (1+1), filtered and tested. The chromatographic conditions included with Agilent Zorbax Eclipse plus C_{18} column (3.0×50 mm, 1.8 μm); mobile phase was 5 mmol·L^{-1} ammonium formate solution as the aque·ous phase (containing 0.1% formic acid), methanol as the organic phase, gradient elution, with flow rate of 0.4 mL min^{-1}. The injection volume was 10 μL, temperature of column was 35°C. Mass spectrom·eter with an electrospray ionization source interface operated in the positive ion of MRM mode was used for LC-MS/ MS analysis. The product ions were 311.10→158.10*, 141.10 and 317.10→158.10*, 141.10 for DFB and internal standard, respectively. The LOD and LOQ for DFB using this method was 0.5 ng · mL^{-1} and the 1 ng · mL^{-1}, respectively. The method linearity range was 1-1000 ng · mL^{-1}, R^2= 0.9959; the matrix effect was 97.57%~113.88%. The method' s accuracy was between 85% and 120%, the intra-assay coefficient of variation was 3.83% to 9.26%, and the inter-assay coefficient of variation was 1.95% to 10.07%. Good stability was observed in all tests. The above results met the Guidelines' requirements for the Verification of Quantitative Analysis Methods for Biological Samples in Appendix 9012 of the Chinese Pharmacopoeia and create a foundation for the pharmacokinetic study of difluben·zuron premix in swine.

Key words: Diflubenzuron; Swine; Plasma; LC-MS/MS; PK

Corresponding author: Ya-Jun Yang, E-mail: yangyue10224@163.com; Jian-Yong Li, E-mail: li·jy1971@163.com

T19-70-0005

Hierarchically structuredmicrocapsules for oral delivery of emodin and tanshinone IIAto treat renal fibrosis

Jiang Sun, Zhishi Xu, Yu Hou, Wenjie Yao, Xudong Fan, Hangsheng Zheng, Jigang Piao, Fanzhu Li,Yinghui Wei

(School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 311402, China)

Abstract: Renal fibrosis is the expected outcome of many chronic kidney diseases, and effective treatment is needed. Emodin (EMO) and tanshinone IIA (Tan IIA), active ingredients in traditional Chi·nese herbs, have been effective in treating renal fibrosis. However, their application is greatly limited by inferior oral absorption, unexpected drug-drug interactions, and influence on the pharmacokinetic pro·file of each other. For this,a new co-delivery approach based on a nano-in-micro system was designed by embedding Tan IIA-loaded nanoparticles (Tan IIA-NPs) in EMO-containing microcapsules to treat re·nal fibrosis. Microcapsules were prepared using the sharp flow technique, showing uniform spherical morphology high encapsulation efficiency and drug loading. Furthermore, the encapsulated Tan IIA-NPs inside the microcapsules exhibited superior cellular internalization and transmembrane transport due to the modification of cell-penetrating peptides and polyethylene glycol, further promoting the oral absorption of Tan IIA. Additionally, this nano-in-micro system achieved an evident sequential drug re·lease. The oral bioavailability of EMO and Tan IIAwas significantly improved when loaded in the hierar·

chically structured microcapsules, contributing to superior therapeutic outcomes in unilateral ureteral obstruction rats. Therefore, the nano-in-micro carrier designed in this study could be an efficient strate·gy for oral delivery of combined therapies to treat renal fibrosis effectively.

Key words: hierarchical structure; nanoparticle-in-microparticle system; sequential release; oral delivery; renal fibrosis;

T19-80-0003

The neurotoxicity and underlying mechanisms of antimony

Yu Sha-li, Li Zhi-jie, Wang Xiao-ke, Zheng Yu-dan, Zhao Xin-yuan*

(Depeartment of Occupational Medicine and Environmental Toxicolgy, School of Public Health, Nantong University, Seyuan Road, Nantong 226019)

Abstract: Antimony (Sb), a naturally occurring metal present in air and drinking water, has been found in the human brain, and there is evidence of its toxic effects on neurobehavioral perturbations, suggesting that Sb is a potential nerve poison. We provide the first study on the molecular mechanism underlying Sb-associated neurotoxicity. Mice exposed to antimony potassium tartrate hydrate showed significantly increased neuronal apoptosis. *In vitro*, Sb triggered apoptosis in PC12 cells in a dose-de·pendent manner. Mechanically, Sb triggered autophagy as indicated by increased expression of micro·tubule-associated protein 1 light chain 3-II (LC3 - II) and accumulation of green fluorescent protein-tagged LC3 dots. Moreover, Sb enhanced autophagic flux and sequestosome 1 (p62) degradation. Subsequent analyses showed that Sb treatment decreased phosphorylation of protein kinase B (Akt) as well as the mammalian target of rapamycin (mTOR), while an Akt activator protected PC12 cells from autophagy. Moreover, the antioxidant N-acetylcysteine attenuated Sb-induced Akt / mTOR inhibi·tion and decreased autophagy and apoptosis, with autophagy inhibition also playing a cytoprotective role. *In vivo*, mice treated with Sb showed higher expression of LC3- II and p62 in the brain, consistent with the *in vitro* results. In summary, Sb induced autophagic cell death through reactive oxygen species- mediated inhibition of the Akt/ mTOR pathway. Under Sb treatment, β- catenin was dramatically down·regulated *in vivo* and *in vitro*. Moreover, overexpression of β-catenin effectively attenuated Sb-induced survivin gene expression suppression and subsequent apoptosis in PC12 cells. In addition, Sb stimualt·ed glycogen synthase kinase-3β (GSK-3β) activation, shown as decreased phosphorylation levels at Ser 9 both in PC12 cells and mice brain. Paramacological inhibition of GSK-3β using lithium chloride (LiCl) significantly rescued β- catenin expression. For upstream pathway analysis, we found Sb treat·ment decreased Akt phosphorylation, and Akt activator protected PC12 cells from GSK-3β activation and subsequent β- catenin suppression. Therefore, Sb-decreased Akt phosphorylation induced neuro·nal apoptosis by both autophagy activation and β-catenin downregulation. Besides of neuoronal apopto·sis, Sb also triggered astrocyte proliferation as well as increase of inducible nitric oxide synthase (iN·OS) and glial fibrillary acidic protein (GFAP) expression, two critical protein marks of reactive astroglio·sis, indicating that Sb induced astrocyte activation. Sb exposure upregulated inflammatory factor ex·pression. Moreover, Sb induced nuclear Factor kappa B (NF-κB) signalling activation. NF-κB inhibition effectively attenuated Sb-induced astrocyte activation. Finally, Sb also phosphorylated TGF-β-activated kinase 1 (TAK1), while its inhibition alleviated Sb-induced astrocyte activation and NF-κB activation.

Key words: antimony, neurotoxicity, neuronal apoptosis, astrocyte activation

Corresponding author: Zhao Xinyuan, E-mail: zhaoxinyuan@ntu.edu.cn

T19-81-0002

Compositional analysis of preservative systems in cosmetics intended for infants and sensitive population

Chang Huailong[a], Chen Tian[b]

(a. R&D Center, Shanghai Jahwa United Co., Ltd., 527 Baoding Rd, Shanghai 200082, China; b. Division of Public Health Service and Safety Assessment, Shanghai Municipal Center for Disease Control and Prevention, 1380 West Zhongshan Rd, Shanghai 200336, China)

Abstract: The increasing safety concern over preservatives in cosmetic products has led the industry to search for safer substitutions, especially for products intended for infants and sensitive population. Our study focused on investigating the preservative systems, emphasized on multifunctional ingredients with antimicrobial activities, in cosmetics intended for infants and sensitive population. Product labels of 29 infant products and 13 soothing products were reviewed for traditional preservatives and ingredients with antimicrobial properties, of which the actual contents were measured by chromatographic methodologies. Lastly, selected systems were evaluated for preservative efficacy via the preservative challenge test. Traditional preservatives including phenoxyethanol (PE), sodium benzoate (SB), sorbic acid and potassium sorbate (SA/PS), benzyl alcohol (BeOH), and methylparaben (MP); and ingredients with antimicrobial activities including glycols (from propylene (PG) to hexylene glycol (HG) plus caprylyl (CG) and decylene glycol (DG)), p-anisic acid (*p*-AA), aminomethyl propanol (AMP), and ethylhexylglycerin (EHG) were identified from across the product labels. PE and CG were the most abundantly employed traditional preservative and glycol, respectively. The contents for PE, SB, SA/PS, BeOH, MP, p-AA, HG, CG, DG, AMP and EHG in infant products were 0.01-0.99%, 0.08-0.59%, 0.06-0.13%, 0.33-0.65%, 0.01-0.25%, 0.08-0.16%, 0.19-1.23%, 0.03-0.56%, 0.30% (*n*=1), 0.35% (*n*=1), and 0.03-0.39%, respectively. In soothing products, the contents of PE, SA/PS, MP, p-AA, PG, butylene glycol (BG), pentylene glycol (PeG), HG, CG, and EHG ranged between 0.19-0.29%, 0.09% (*n*=1), 0.2- 0.29%, 0.12% (*n*=1), 1.11-3.23%, 0.22-4.82%, 0.48-2.21%, 0.25% (*n*=1), 0.19-0.52%, and 0.22-0.51%, respectively. Moreover, the preservative systems consisted of PeG (<LOQ) and HG (1.23%); BS (0.08%) and PS (0.08%); PE(0.51%), CG(0.11%), and EHG (0.05%); and PeG (1.34%), CG (0.28%), and PE (0.19%) were found to fail the challenge test. In conclusion, our data reveals the trend in selection of preservative strategies for cosmetic products intended for infants and sensitive population.

Key words: Cosmetics; Infant skin; Sensitive skin; Preservatives; Antimicrobial activities

Corresponding author: Chen Tian, E-mail: chentian@sh.cdc.cn

T19-83-0018

Low dose radiaiton generates RET/PTC in human thyroid cells after *in Vitro* exposure to γRadiation

Yuhao Liu[1], Ping Wang[1], Xingkun Ao[2], Ziyan Yan[1], Xinxin Liang[2], Dafei Xie[1], Hua Guan[1], Yongqing Gu[1,2]*, Pingkun Zhou[1]*

(1. Beijing Institute of Radiation Medicine, Beijing 100850; 2. Hengyang Medical School, University ofSouth China, Hengyang 421001)

Abstract: The biological effects of ionizing radiation on DNA can be divided into two types, direct

and indirect effects. DNA double-strand breaks (DSB) is a severe result of ionizing radiation. When DSB occur, in general the cell will have two types of repairment, error-free homologous recombination repair (HR) pathway and the error-prone nonhomologous end-join repair (NHEJ). And to ensure that the cells can survive after the DSB, most fixes will choose the consequences-free NHEJ approach over HR, and this is one of the reasons why chromosomal rearrangements occur. Thyroid cancer(TC), which develops from malignant carcinoma of the follicular epithelium of the thyroid gland. The inci·dence of thyroid cancer is increasing worldwide. The majority of pathological types of TC are mostly papillary carcinomas. Coincidentally, after the atomic bombings in Japan and catastrophe occurs in Chernobyl, there was a dramatic increase in the incidence of thyroid cancer among survivors, and the vast majority of pathological types were papillary thyroid cancer (PTC) with the proto-oncogene RET re·arrangement on chromosome 10. Rearrangement of the RET proto-oncogene is seen as the initiating factor for thyroid carcinogenesis. The RET proto-oncogene is located on the long arm of chromosome 10 (10q11.2). The protein RET encodes is a cellular tyrosine kinase transmem-brane receptor. Activa· tion of RET stimulates multiple downstream pathways that promote cell growth, proliferation, survival and differentiation. According to the literature, PTC associated with ionizing radiation is mostly RET / PTC1 and RET/PTC3. But it is difficult to be exposed to such high doses of ionizing radiation in our daily life as after the atomic bomb explosion, while low-dose of ionizing radiation are more common instead. We used a cell line N-thy-ori-3-1 (human normal thyroid follicular epithelial cells), and a γ radiation source60Co to simulate a low-dose ionizing radiation environment where cells were irradiated with a dose of 0.1 Gy. After 14 days, we detected the occurrence of RET/ PTC1 rearrangements in 72 inde·pendent samples by nested PCR. Although the probability of rearrangement is relatively low, this confirms that rearrangement occurs in the presence of low dose of ionizing radiation. We will investigate in more depth the mechanisms of how chromosomal rearrangements occur due to low dose of ionizing radiation.

Key words: low-dose ionizing radiation; γ Radiation; PTC; RET proto-oncogene; RET / PTC rear·rangement

Corresponding author: Yongqing Gu, E-mail: yqgu96@163.com; Pingkun Zhou, E-mail: zhoupk@nic.bmi.ac.cn

T19-98-0019

Detection and analysis of safety signals of ribavirin based on FDA adverse event database for COVID-19 therapy

Yang Hongyu[a,b], Hong Lina[a,c], Li Ying[a], Hu Xi[a,b], Zhang Xingguo[a,b], Hong Dongsheng[a,b]*, Zhao Qingwei[a,b]*

(a. Department of Clinical Pharmacy, the First Affiliated Hospital, Zhejiang University School of Medi- cine, Hangzhou 310003; b. Zhejiang Provincial Key Laboratory for Drug Evaluation and Clinical Research, Hangzhou 310003; c. College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058)

Abstract: OBJECTIVE To analyze and evaluate the safety signals of ribavirin in the patients with novel coronavirus pneumonia(COVID-19), and provide a valuable reference for its rational use in antivi·ral therapy. **METHODS** The reporting odds ratio (ROR) method and Bayesian confidence propagation neural network (BCPNN) were applied to analyze safety signals of ribavirin from US Food and Drug Ad·ministration (FDA) Adverse Event Reporting System (FAERS) database ranging from 2004 to 2020. All

ADEs data were encoded with the preferred terms (PT) of MedDRA. Time-scanning diagram is conducted to clarify the warning signals on new unexpected adverse drug reaction (ADR). Meanwhile, ADEs distribution in SARS and MERS therapy were systematically assessed. **RESULTS** Among 91638 reports of ADEs in the analysis, 76167 were reported with ribavirin as suspected drug. The reported occurrence of ADEs in male patients (39726, 52.16%) was more than that in females (30090, 39.51%), and the proportion of serious adverse events was higher (25730, 33.78%). 20 safety signals of drug adverse events were found in 8 systems, among which 12 high-risk signals were not listed in the drug label of ribavirin, such as anorectal discomfort, renal impairment, dehydration, pancytopenia , thrombocytopenia, syncope, loss of consciousness, ascites and hepatic cirrhosis, of which ascites, cirrhosis, syncope and renal injury were highly correlated with high-risk signals. Furthermore, the distribution and frequency of ADEs were similar between SARS and MERS. **CONCLUSION** ADEs signal analysis of ribavirin based on FAERS leads to a comprehensive understanding of its safety characteristics and will be helpful for clinical decision-making in COVID-19 treatment.

Key words: Ribavirin; COVID-19; Safety signals detection; Data mining; FDA Adverse Events Reporting System; Adverse drug events

Corresponding author: Qing-Wei ZHAO, E-mail: qwzhao@zju.edu.cn and Dong-Sheng HONG; E-mail: hdswell@zju.edu.cn

T19-98-0021

Resveratrol alleviates methylmercury-induced cognitive deficits via miR-9-targeted regulation of FOXP2 in rats

Caiyun Deng, Li Zhang, Fang Chen, Aihua Zhang, Wenjuan Wang*

(The Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, School of Public Health, Guizhou Medical University, Guiyang 550025, Guizhou, PR China)

Abstract: During the fetal development stage, the Central Nervous System (CNS) is particularly sensitive to methylmercury (MeHg). However, the mechanism underlying the antagonistic effect of resveratrol (Res) on MeHg toxicity is still not fully understood. In this study, female rat models with MeHg and Res co-exposure were developed. Thirty-six Wistar rats (male: female=1∶2) were randomly divided into control group (NaCl: 4 mg/kg/d), MeHg-treated group (MeHg: 0.6 mg/kg/d; 1.2 mg/kg/d; 2.4 mg/kg/d) d); antagonist group (MeHg: 1.2 mg/Kg/d+ Res: 20 mg/kg/d). Res group: 20mg/kg/d. Morris water maze was used to observe the learning memory ability of the offspring rats, HE staining was used to observe the alteration of hippocampal tissue structure, proteomics techniques were used to screen the differential expression profiles of hippocampal proteome, transmission electron microscopy was used to observe the alteration of hippocampal synaptic structure, Golgi staining was used to observe the alteration of dendritic spine density in CA1 region of hippocampus, RT-qPCR was used to detect the relative expression of miR-9, Western blot was used to detect the expression level of related proteins.Compared with the control group, the incubation period of localization voyage and the time of stage penetration were significantly increased while the number of stage penetration was significantly reduced in the MeHg-treated group ($P<0.05$). The proteomic results showed that a total of 74 different proteins, 42 up-regulated and 32 down-regulated, were identified in the 2.4 mg/kg/d dose group compared to the control group. We further observed the antagonistic effect of Res and results showed that the thickness of

the post-synaptic and length of active zone were reduced in the 2.4 mg/kg/d dose group, while the resveratrol antagonist group significantly alleviated (P<0.05). The dendritic spine density in the MeHg-treated group was significantly reduced, and Res antagonist alleviated the decreased of dendritic spine density in the hippocampus of methylmercury-induced rats. Furthermore, the results confirmed that the expressions of miR-9, FOXP2, GRIN2B, NMDAR1, NMDAR2A, NMDAR2B, SYP and PSD-95 were lower in hippocampal tissues in the MeHg-treated group than in the control group (P<0.05), while Res effectively antagonized the toxic effects of MeHg (P<0.05). In conclusion, Res provides a potential intervention strategy for MeHg toxicity, miR-9 and its target-regulated FOXP2 are the key molecules for Res to exert antagonistic effects.

Key words: Methylmercury; Resveratrol; Hippocampus; Synaptic Plasticity

Corresponding author: Wenjuan Wang, E-mail: reality0337@126.com

T19-98-0022

Arsenic-induced lung inflammation and fibrosis in a rat model: Contribution of the HMGB1/RAGE, PI3K/AKT, and TGF-β1/SMAD pathways

Fanyan Zheng, Wenjuan Wang, Aihua Zhang*

(The Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, School of Public Health, Guizhou Medical University, Guiyang 550025, Guizhou, PR China)

Abstract: It is well know that chronic arseinc exposure continues to be a global public health concern and that the human body causes toxic reactions through ingestion of contaminated water and food. At present, an increasing number of studies have shown that arsenic exposure increases the risk of lung cancer as well as a variety of non-malignant respiratory diseases, including bronchitis and tracheobronchitis. HMGB1 is a ubiquitous non-histone DNA binding protein in the nucleus,which is widely expressed in a variety of tissues and cells and is involved in the pathological processes of many lung diseases through binding to the corresponding receptors and activating the downstream signaling pathways. However, the exact role of HMGB1/RAGE in arsenic-induced lung injury remains unknown. The aim of this study was to investigate whether HMGB1/RAGE and its activated downstream pathways are involved in the process of arsenic exposure-induced lung injury in rats. In this study, we performedan animal model of oral exposure to arsenic through gave Wistar rats 2.5, 5 and 10 mg·kg^{-1} $NaAsO_2$. After the exposure, the bronchoalveolar lavage fluid and lung tissue were collected for analysis.The number of inflammatory cells, protein content and enzyme activity in BALF were analyzed by Wright-Giemsa staining and kit detection. Histopathological and ultrastructural changes were observed by H&E staining,masson stainingand electron microscope. The expression of related proteins in lung tissue was detected by immunofluorescence and Westernblotting. The results showed that capillary permeability (LDH, TP, ACP, and AKP) was increased in the arsenic exposure groups, resulting in cell damage; this was accompanied by acute inflammation marked by significant neutrophil infiltration. Meanwhile, obvious histopathological damage, including thickening of the lung epithelium, increased infiltration of inflammatory cells, rupture of the alveolar wall, swelling of the mitochondria, and chromatin agglutination was observed by H&E staining and transmission electron microscopy. Furthermore, the results confirmed that the expressions of HMGB1 and RAGE in lung tissue were enhanced, and protein expression of PI3K, p-AKT, IL-1β, IL-18, and MMP-9 was increased in lung homogenates from the arsenic-exposed groups compared to the control group. Finally, Masson′s staining results revealed arsenic-in·

duced fibrosis and collagen deposition. Moreover, a significant increase in key fibrosis factors, includ·ing TGF- β1, p-SMAD2, p-SMAD3, and SMAD4 was observed in the lung homogenates in arsenic-ex·posed groups. In conclusion, the current study demonstrates that sub-chronic arsenic exposure triggers the inflammatory response and collagen fiber deposition in rat lung tissue, and then promote lung inju·ry. The potential mechanism may be closely related to activation of the pro-inflammatory-related HMGB1/RAGE pathway and initiation of the PI3K/AKT and TGF-β1/SMAD pathways.

Key words: Arsenic; lung; inflammation; fibrosis; HMGB1

Corresponding author: Aihua Zhang, E-mail: 97349238@qq.com, E-mail: aihuagzykd@163.com

T19-98-0023

Quality assurance affairs of traditional Chinese medicine injection in non-clinical and clinical research

Mo Yang, Xiaodong Zhang, Tianyu Hu, Jiedan Wang, Yaxian Wang
〔*Medicilon Preclinical Research (Shanghai) LLC*〕

Abstract: The first Chinese medicine injection has been found more than 60 years. Chinese medi·cine injection has shown outstanding curative effect in the first aid of viral infectious diseases, myocardi·al infraction and apoplexia. Some are irreplaceable, some are used in the treatment of acute critical dis·eases. These drugs are developed according to ancient prescriptions, and have good clinical effects for several decades. But there are also adverse reactions in clinical phase, the main reasons are the differ·ent quality level of drugs, the abuse of drugs in clinical. Therefore, safety evaluation plays an important role in the research and development and application of traditional Chinese medicine injection. This pa·per discusses the matters needing attention from the non-clinical and clinical aspects. In non-clinical studies, active and passive allergic reactions need to be studied. In these studies, we need to pay at·tention to the following points: first, for different batches of studies, batch information should be clearly defined in the protocol. At the same time, check the instructions and evaluate whether the solvent is used properly. Secondly, in the animal experiment stage, select appropriate enough species and num·ber of animals for allergic evaluation. Due to the particularity of traditional Chinese medicine, it may be necessary to increase the evaluation index of allergic reaction in the detection, so as to lay a founda·tion for the establishment of animal model. Third, in the allergic evaluation, summarize the symptoms that may appear in animals, and formulate a table to record. In the inspection, focus on the time when symptoms appear and the accuracy of symptom description. Fourth, check the guidance and principles and put forward opinions and requirements on the adequacy of inspection of test indicators. Fifthly, whether or not to add adjuvant has a certain influence on the diameter of blue spot, and the addition of adjuvant should be adjusted appropriately. In clinical research stage, Chinese medicine injections should not only improve the proportion of measurable components and their quality control, but also do a good job in basic research. Special research on pyrogen, allergens and their anaphylactoid reaction components, and quality control of allergic reaction, pyrogen reaction and anaphylactoid reaction are the key points of improving the quality of Chinese medicine injection. At present, the detection methods of injection allergy and pyrogen are backward, and the detection of anaphylactoid reaction is not enough. Therefore, it is necessary to research new high sensitive detection methods, such as bioinfor·matics, network pharmacology, system biology, pharmacokinetics research and other new technologies and new methods comprehensive research, in the quality assurance work, comprehensively improving

the inspection of safety detection methods and standards is fundamental.

Key words: Quality Assurance; Traditional Chinese Medicine Injection Non-clinical; Clinical

T19-98-0024

Preliminary analysis of corona virus disease 2019 treatment drugs

Jin Lu

(湖北省鄂州市中心医院)

Abstract: With the outbreak of Corona Virus Disease 2019, the epidemic spread from Wuhan to the whole country and spread abroad. there are no effective antivirus medicines for SARS-CoV-2 treatment , A small number of drugs with clinical effect are recommended for selection in the new scheme of COVID-19 diagnosis and treatment that Issued by National Health and Health Commission of the people's Republic of China. Now, the recommended drugs are analyzed and Introduced the difficulties of current drug treatment and the research progress in Anti SARS-CoV-2 drugs is summarized. In view of the novel coronavirus's attack on human body, it can cause multiple organ damage, aiming at COVID-19 there is no specific medicine at present, Therefore, in the treatment, it is necessary to treat empirically with antiviral therapy, at the same time, relieve cough and phlegm, moisten lung and liver, nourish heart and kidney, etc. Reduce the damage of multi organ function, avoid critical situation, reduce mortality, isolate potential infectious sources and reduce the number of patients which is perhaps the most effective preventive and treatment method at present.

Key words: SARS-CoV-2,COVID-19; Drug research; Curative effect

T19-98-0025

Compositional analysis of preservative systems in children and soothing cosmetic products

Huailong CHANG[1], Tian Chen[2]

(1. Shanghai Jahwa United Co., Ltd.; 2. Shanghai Municipal Center for Disease Control and Prevention)

Abstract: The toxicological concern in the traditional preservatives presented in cosmetic products has led the industry to search for safer substitution of preservatives, especially for products intended for children and consumers with sensitive skin. The current study focused on investigating the compositions of preservatives and their preservation efficacy in different cosmetic products intended for children and sensitive skin, aiming to provide insights for optimizing preservative systems in future formulation design. A total of 42 cosmetic products were purchased from the market, including 29 children products and 13 soothing products. After excluding data below the limit of quantification, the contents for phenoxyethanol, sodium benzoate, sorbic acid, benzyl alcohol, and methylparaben in children products were 0.01-0.99%, 0.08-0.59%, 0.06-0.13%, 0.33-0.65%, and 0.01-0.25% respectively. The content of p-anisic acid in children products ranged from 0.08% to 0.16%. The contents for polyols were 0.19-1.23% for 1,2-hexanediol, 0.03-0.56% for caprylyl glycol, 0.35% for aminomethyl propanol, 0.03-0.39% for ethylhexylglycerin, and 0.30% for decylene glycol respectively. In soothing products, the contents of phenoxyethanol, sorbic acid, and methylparaben ranged between 0.19-0.29%, 0.09%, and 0.2-0.29%

respectively. p-Anisic acid was labelled and detected in only one product with a content of 0.12%. In the case of polyols, the content of propylene glycol, butylene glycol, pentylene glycol, 1, 2-hexanediol, caprylyl glycol, and ethylhexylglycerin was 1.11-3.23%, 0.22-4.82%, 0.48-2.21%, 0.25%, 0.19-0.52%, and 0.22-0.51% respectively. Moreover, the preservative systems consisted of pentylene glycol (< LOQ) and 1,2-hexanediol (1.23%); sodium benzoate (0.08%) and potassium sorbate (0.08%); phenoxyethanol (0.51%), caprylyl glycol (0.11%), and ethylhexylglycerin (0.05%); pentylene glycol (1.34%), caprylyl glycol (0.28%), and phenoxyethanol (0.19%) were found to fail the challenge test. Our data reveals the trend in selection of preservative strategy for cosmetic products intended for children and sensitive skin.

Key words: Cosmetics; Children skin; Sensitive skin; Preservatives; Polyols

T19-98-0026

Correlation of vibration-induced HAVD Vascular endothelial damage and vascular biomarkers

Xiu Wen Hu, Zi yu Chen, Nuo yan Wei, Jiajie Li, Yuan Wei, Hong yu Yang, Qing song Chen, Yun Xia
(Guangdong Pharmaceutical University)

Abstract: Introduction Occupational hand-arm vibration disease (HAVD) refers to a disease with the main clinical manifestations of hand peripheral circulation, arm nerve dysfunction and bone, joint and muscle injury caused by workers engaged in hand-transmitted vibration for a long time. The typical manifestation is the vibrating white finger (VWF).Studies at home and abroad have shown that the occurrence of HAVD is closely related to the changes of vascular regulators. **OBJECTIVE** This study aim to explore the correlation between LTB4, 5-HT, CGRP, IL-1β, MLC2 and VEGF six vascular regulators and HAVD. **METHODS** A judge sampling method was used to select 60 workers with VWF as the HAVD group, 60 workers who exposed to hand-arm vibration without VWF as the vibration exposure group,60 workers without hand-arm vibration exposure as the control group. A one-way chi-square test was used to analyze the basic characteristics of the research object and the symptoms caused by WVF; Enzyme-linked immunosorbent assay (ELISA) was used to detect the expression levels of 6 vasodulators in plasma.Logistic regression analysis was used to screen HAVD's associated indicators, to construct a new multivariate model indicator $\hat{Y}$, and to judge HAVD indicators through (ROC) curve screening. **RESULTS** The expression levels of LTB4, 5-HT, IL-1β, CGRP, and MLC2 in plasma were different among the three groups, and the levels from high to low were HAVD group>vibration exposure group>control group (P<0.01). There was no significant difference in plasma VEGF levels among the three groups (P>0.05). The logistic regression analysis results showed that after adjusting confounding factors such as age, length of service, smoking, alcohol drinking and hand symptoms, the higher the level of LTB4, 5-HT, IL-1β and the adverse effects on life, the higher risk of HAVD (P<0.05) and the lower MLC2 plasma level, the higher risk of HAVD (P<0.05). The area under ROC curve (AZ) of HAVD was determined by the above effective indicators, and $\hat{Y}$ was constructed to determine the AZ value of HAVD. The AZ values from high to low was $\hat{Y}$(0.980) >IL-1β(0.907) >LTB4(0.876) >5-HT (0.858) >adverse effects on life (0.717)>MLC2 (0.178) (P<0.001). **CONCLUSION** The levels of LTB4, 5-HT, IL-1β and MLC2 in plasma of HAVD patients were correlated with HAVD. The constructed $\hat{Y}$ was the best biomarker for HAVD screening. This study suggests that the above vasodulators may play a role in the pathogenesis of VWF, which is an indicator of the impact of vibration exposure on health, providing a

basis for the early prevention and treatment of HAVD.

Key words: Hand-arm vibration disease; Leukotriene B4; Serotonin; ROC curv

T19-98-0027

Construction and preliminary study of carboxymethyl panax notoginseng polysaccharide-chitosan nanoparticles loaded with Salvianolic acid B/Doxorubicin

ZHANG Yue-lin
(Zhejiang Chinese Medical University)

Abstract: the combination of drugs and excipients as a carrier for drug delivery is an emerging direction of nano-preparation research in recent years. There is also the concept of "medicine and supplement in one" in traditional Chinese medicine preparations, but there are few related basic researches. Panax notoginseng polysaccharide is a natural polymer compound with anti-oxidation, anti-tumor and other pharmacological activities. Therefore, a new type of nano-medicine carrier was prepared by combining panax notoginseng polysaccharide after carboxylation with chitosan in this project. The combination of chemical drugs and natural products can reduce toxic and side effects while ensuring the therapeutic effect, and successfully prepared polyelectrolyte complex nanoparticles loaded with chemotherapeutic drug doxorubicin[1] and salvianolic acid B[2], an active ingredient of traditional Chinese medicine with myocardial protective effect. The optimization of drug loading process, characterization of physical and chemical properties, preliminary evaluation of biological safety and investigation of drug release behavior under different pH conditions were carried out. It enriches the concept of "medicine and supplement in one" and provides support for related research on the combination of chemical drugs (doxorubicin) and natural products (salvianolic acid B). In this study, the concept of medicine and supplement in one was applied to the construction of new polyelectrolyte complex preparations. Panax notoginseng polysaccharide is a natural polymer compound with anti-oxidation, anti-tumor[3] and other pharmacological activities. It is an ideal substance for "medicine and supplement in one". Panax notoginseng polysaccharide is carboxymethylated as a polyanion and can form a polyelectrolyte complex carrier through electrostatic interaction with polycations, such as chitosan. And carried charged drugs (salvianolic acid B and doxorubicin) through electrostatic interaction. In this way, the concept of "medicine and supplement in one" that uses carboxymethyl panax notoginseng polysaccharide as medicine and excipients to construct a polyelectrolyte complex carrier and enhance the activity.

Key words: Toxicology; Polyelectrolyte complex nanoparticles; Salvianolic acid B; Doxorubicin; Carboxymethyl pa

T19-98-0028

Effects of different frequency and time of vibration on the activity of vascular endothelial cells and vasoactive substances

Xiu wen Hu, Zi yu Chen, Nuo yan Wei, Jia jie Li, Yuan Wei, Hong yu Yang, Qing song Chen, Yun Xia
(Guangdong Pharmaceutical University)

Abstract: Introduction Occupational hand-arm vibration disease (HAVD) refers to a disease with the main clinical manifestations of hand peripheral circulation, arm nerve dysfunction and bone, joint and muscle injury caused by workers engaged in hand-transmitted vibration for a long time. The disturbance of vasoactive substances secretion in vascular endothelial cells caused by vibration may be an important mechanism of vascular endothelial damage caused by vibration. The purpose of this study was to investigate the effects of different frequency and time of vibration exposure on HUVEC activity and vasoactive substances *in vitro* environment. **OBJECTIVE** To explore the effect of different frequency and time of vibration on the activity of HUVEC cells and vasoactive substances. Different frequencies (10Hz, 20Hz, 40Hz, 70Hz, 100Hz, 125Hz, 200Hz, 500Hz) and different time (1 h, 1.5 h, 2 h, 2.5 h, 3 h) were applied to HUVEC *in vitro* by cell vibrator, and they were divided into vibration group and control group. Cell viability was detected by CCK8 assay. Enzyme-linked immunosorbent assay (ELISA) was used to detect the expression levels of myosin light chain 2 (MLC2), endothelin-1 (ET-1) and prostaglandin (PCL2) in cells and medium. **RESULTS** Within a time gradient of 1-2 h, with the extension of the action time, 10Hz, 20Hz, 40Hz, 70Hz, 100Hz, 125Hz, 200Hz and 500Hz vibration exposure caused a gradual increase in cell viability. In the time gradient of 2.5-3 h, with the extension of the exposure time, 20Hz, 125Hz, 200Hz vibration caused a gradual decrease in cell viability. Compared with the control group, the expression level of ET-1 in both cells and medium was increased after the cells were treated with 10Hz, 20Hz and 125Hz vibration for 2.5 h. The expression level of PCL2 was decreased.Vibration at 10Hz and 125Hz can reduced the expression level of MCL2 in cells without affecting the level in the medium,A 20Hz vibration can caused a reduction in the MCL2 medium without affecting the level in cells. When the vibration exposure time was 1h, the expression level of ET-1 in cells and medium was increased by 10Hz and 20Hz vibration compared with the control group. Compared with the control group, the expression level of PCL2 in the 10Hz vibration group was decreased. Compared with the control group, the expression level of PCL2 in the vibration-induced group with frequency of 10Hz and 20Hz was decreased, and the above differences were statistically significant ($P<0.05$). **CONCLUSION** Vibration of particular frequency could change the activity of human umbilical vein endothelial cells and affect the expression level of vasoactive substances. MLC2, ET-1 and PCL2 may be involved in the vibration-induced injury of vascular endothelial cells.

Key words: Vibration; HUVEC; ET-1

T19-98-0029

The critical role of polyketide synthase gene on the swainsonine biosynthesis in the fungus Metarhizium anisopliae

Lu Sun[a], Enxia Huang[a], Yu Zhang[a], Ziyu Guo[a], Kexin Wu[a], Yunhao Zhang[a], Chonghui Mo[b], Jinglong Wang[c], Baoyu Zhao[a], Hao Lu[a]*

(a. College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi 712100, China; b. College of Agriculture and Animal Husbandry, Qinghai University, Xining, Qinghai 810016, China; c. Tibet Academy of Agricultural and Animal Husbandry Sciences/State Key Laboratory of Barley and Yak Germplasm Resources and Genetic Improvement, Lhasa, Tibet 850002, China)

Abstract: Swainsonine (SW) is the principal toxic ingredient of locoweeds, and is produced by fungi including Metarhizium anisopliae, Slafractonia leguminicola, and Alternaria oxytropis. While the SW biosynthesis pathway of fungi and the catalytic enzyme genes that regulate synthesis are not cleanly.

In this study, we used homologous recombination (HR) to knock out and interfere with the polyketide synthase gene (pks) of M. anisopliae to determine its effect on the SW biosynthesis pathway. The concentration of SW was measured in the fermentation broth of M. anisopliae at 1 d, 2 d, 3 d, 4 d, 5 d, 6 d or 7 d using LC-MS. The gene for the pks gene was detected by RT-qPCR. Day 5 of M. anisopliae gave the highest content of SW and the highest expression of the pks gene. To determine the role of the pks gene in the SW biosynthesis pathway of M. anisopliae, we used PEG-mediated homologous recombination (HR) to transform a wild-type strain (WT) with a Benomyl (ben)-resistant fragment to knock out the pks gene producing a mutant-type strain (MT) and used PEG-mediated RNAi to transform a wild-type strain (WT) with a Benomyl (ben)-resistant plasmid to interfere with the pks gene. A complemented-type (CT) strain was produced by adding a complementation vector that contains the geneticin (G418) resistance gene as a marker. The content of SW didn′ t detected in MT strain, and returned to the original level in the CT strain, while the content of SW was significantly decreased in RNAi strain. We suggest that mutation and RNAi in the pks gene affect the cell wall formation of M. anisopliae, while the colony diameters, phenotypes, and growth rates did not change significantly, and no obvious changes in other cellular organelles were noted. These results indicate that the pks gene plays a crucial role in the SW biosynthesis of M. anisopliae, which provides an important theoretical basis for illuminating the SW biosynthesis and solving locoism in livestock.

Key words: M. anisopliae; pks gene; Swainsonine; Gene knockout; RNAi

Corresponding author: Hao Lu, E-mail: luhao@nwsuaf.edu.cn

T19-98-0030

Aconitine induces cell apoptosis via mitochondriaand death receptor signaling pathways in neuronal cell line HT22

Hui Wang[1], Yanbing Liu[1], Shuhang Zhang[1], Weina Wang[1], Yanli Zhu[1], Bo Li[1],
Yanan Tian[2], Baoyu Zhao[1], Hao Lu[1]
(1. College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi 712100, China; 2. Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843, USA)

Abstract: This study sought to investigate the effects of aconitine, a well-known aconitum plant-produced toxin, on growth and apoptosis of hippocampal neuronal cell line (HT22) and to explore the potential mechanisms. HT22 cells were cultured, and cell viability and DA(dopamine) contents was examined in HT22 cells treated with different doses of aconitine. Cell apoptosis was detected by Hoechst 33258 fluorescent staining, Ultrastructural changes were observed by electron microcopy and Annexin V-FITC / propidium iodide double staining in flow cytometric analysis were performed upon aconitine treatment. Molecular mechanism was analyzed by caspase activity detection and Western blot assay. The results showed that aconitine inhibited HT22 cells growth and increased DA contents in a dose dependent manner. The administration of aconitine in HT22 cells also induced cell apoptosis by upregulating the expression of pro-apoptotic factors Bax, mitochondrial-mediated apoptosis-associated proteins Cyto c, Apaf-1, Caspase9 and death receptor apoptosis-associated proteins Fas, Fas-L, Fadd, Caspase8, Caspase3 and by decreasing the anti-apoptotic Bcl-2 and adaptor protein Bid expression. Collectively, results suggest that aconitine induce apoptosis through mitochondrial-mediated and death receptor signaling pathways in HT22 cells.

Key words: aconitine; cell apoptosis; apoptotic pathway; HT22 cells; aconitum

Corresponding author: Hao Lu, E-mail: luhao@nwsuaf.edu.cn

T19-98-0031

SUMOylation of Smad2 regulates the TGF-β-mediated endothelial mesenchymal transition

Qi Su, Xinyi Yang, Congcong Du, Xu Chen, Wenbin Lu, Qiqi Wang, Hong Yuan, Zhenzhen Zhang, Hongmei Wu, Yitao Qi

(College of life sciences, Shaanxi Normal University, Xi'an, Shaanxi 710119)

Abstract: Endothelial-mesenchymal transition (EndoMT) is a complex biological process in which endothelial cells are transformed into mesenchymal cells, and excessive EndoMT causes a variety of pathological processes. Here, we show that SUMOylation of Smad2 regulates TGF-β-mediated EndoMT. It was identified that Smad2 is predominantly modified by SUMO1 on two major SUMOylation sites K156 and K383, and PIAS2α is the major E3 ligase for its SUMOylation, whereas SENP1 mediates deSUMOylation of Smad2. In addition, SUMOylation significantly enhances the transcriptional activity of Smad2. SUMOylation also increases the phosphorylation of Smad2 and the formation of Smad2-Smad4 complexes, ultimately promotes the nuclear translocation of Smad2. Finally, the SUMOylation of Smad2 significantly promotes the TGF-β-mediated EndoMT process in endothelial cells. We found that the SUMOylation of Smad2 plays critical roles in TGF-β mediated EndoMT process, providing a new theoretical basis for the occurrence and potential drug targets for EndoMT-related clinical diseases.

Key words: Endothelial mesenchymal transition; Smad2; TGF-β; SUMOylation

T19-98-0032

Study on the safety of diclofenac injection for target cows

Li Shi-hong, Qin Zhe, Kong Xiao-jun, Li Jian-yong*

(Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS / Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture/Key Laboratory of New Animal Drug Project, Gansu Province, Lanzhou 730050, China)

Abstract: To evaluate the safety of Diclofenac Injection for target animal cows, 24 healthy lactating cows were randomly divided into 4 groups, including the control group (without any treatment),the commended dosage (2.2 mg/kg-bw) group, three times of recommend dosage (6.6 mg/kg-bw) group and five times of recommend dosage (11.0 mg/kg-bw) group. Intramuscularinjection were performed in the neck once a day for 3 days. During the experiment, the health condition, blood routine and serum biochemical indexes of cows were observed and measured. The results showed that no obvious adverse reactions were found in clinical observation during the whole experiment by using Diclofenac Injection at the recommended dose (2.2 mg/kg-bw) to 5 times the recommended dose (11.0 mg/kg-bw), and there was no significant difference in 18 indexes in blood routine examination compared with the control group. Serum biochemical tests showed there were significant differences in total protein,globu-

lin, aspartate aminotransferase, creatine kinase and phosphorus after 3 days of administration compared with the control group, and all indexes returned to normal after 7 days of administration. The results show that Diclofenac Injection is safe to be used fortarget cows.

Key words: Diclofenac Injection; Safety; Target Cow

Corresponding author: Li Jian-yong, E-mail: lijy1971@163.com; Li Shi-hong, E-mail: lzlishihong@163.com

T19-98-0033

Orally administrated halofuginone-loaded TPGS polymeric micelles against triple negative breast cancer: enhanced absorption and efficacy accompanied by reduced toxicity and metastasis

Runan Zuo[a], Yan Zhang[a], Xiaorong Chen[a], Shiheng Hu[a], Xinhao Song[a], Xiuge Gao[a], Jiahao Gong[a], Hui Ji[a], Fengzhu Yang[a], Kun Fang[b], Junren Zhang[a], Shanxiang Jiang[a],*, Dawei Guo[a],*

(a. Engineering Center of Innovative Veterinary Drugs, Center for Veterinary Drug Research and Evaluation, MOE Joint International Research Laboratory of Animal Health and Food Safety, College of Veterinary Medicine, Nanjing Agricultural University, 1 Weigang, Nanjing 210095, PR China; b. The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, 17 Lujianglu, Hefei 230001, PR China)

Abstract: Background Halofuginone hydrobromide (HF) - TPGS polymeric micelles (HTPMs) were successfully fabricated using thin-film hydration technique. Our previous studies have demonstrated HTPMs exert the excellent anticancer effect against triple-negative breast cancer (TNBC) cells and subcutaneous xenografts via intravenous injection. In the present study, we further explored the treatment potential of HTPMs orally administered alone or in combination with surgical therapy toward TNBC in subcutaneous or orthotopic mouse models. METHODS Herein, the stability and in vitro release behaviors of HTPMs were firstly evaluated in the simulated gastrointestinal (GI) fluids. Caco-2 cell monolayers were then applied to investigate the absorption and transport patterns of HF with/ without encapsulation of TPGS-polymeric micelles. Subsequently, the therapeutic effect of orally administrated HTPMs was checked against subcutaneous xenografts of TNBC in nude mice. Ultimately, HTPMs orally administrated combined with surgical therapy were utilized to treat orthotopic TNBC of nude mice. RESULTS Our data confirmed HTPMs exhibited the good stability and sustained release in the simulated GI fluids. HF was authenticated to be a substrate of efflux transporter P-glycoprotein (P-gp), and its permeability across Caco-2 cell monolayers was markedly enhanced via heightening intracellular absorption and inhibiting P-gp efflux of HF due to encapsulation of TPGS-polymer micelles. HTPMs showed stronger tumor-suppressing effects against subcutaneous xenografts of MDAMB-231 cells compared to HF when orally administrated. Moreover, peroral HTPMs integrated with partial surgical excision could synergistically retard the growth of orthotopic TNBC in comparison to HTPMs or surgical therapy alone. Fundamentally, HTPMs orally administrated at the therapeutic dose didn't cause locally and systemically pathological injury of experimental mice while HF would lead to the weight loss and jejunal bleeding. CONCLUSIONS Taken together, HTPMs could be applied as a potential anticancer agent toward TNBC by oral administration.

Key words: Triple-negative breast cancer; Halofuginone hydrobromide; TPGS polymeric micelles;

Subcutaneous xenografts; Orthotopic xenografts; Oral administration

Corresponding author: Dawei Guo, E-mail: gdawei0123@njau. edu. cn; Shanxiang Jiang, E-mail: nauvy@sina.com are the

T19-98-0034

Aristolochic Acid A-induced hepatotoxicity in Tianfu broilers relates to oxidative stress-mediated apoptosis and mitochondrial damage

Dan Xu[1], Gang Shu[2], Chonglin Ran[1], Xi Peng[3], Huaqiao Tang[2], Wei Zhang[2], Haohuan Li[2], Funeng Xu[2], Juchun Lin[2], Hualin Fu[2], Xiaoling Zhao[1]

(1. Farm Animal Genetic Resources Exploration and Innovation Key Laboratory of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan, China; 2. Department of Basic Veterinary Medicine, Sichuan Agricultural University, Chengdu, Sichuan, China; 3. Sichuan Industrial Institute of Antibiotics, School of pharmacy, Chengdu University, No. 2025,Chengluo Road,Chengdu 610106, China)

Abstract: OBJECTIVE Aristolochic acid (AA), one of the components of some traditional Chinese medicines, is commonly used in domestic poultry industry due to its various therapeutic effects. However, over-exposure to AA has been statistically associated with hepatotoxicity, but the underlying mechanism still remains unknown. Furthermore, AA-Iwas the main toxic component of AA. In the current study, a subchronic toxicity test was conducted to evaluate the hepatotoxicity of AA-I on Tianfu broilers and provide medication reference to clinic in poultry industry. **METHODS** In the current study, a subchronic toxicity test based on our previous acute toxicity testand the value of LD50 obtained was conducted to evaluate the hepatotoxicity of AA-I on Tianfu broilers.A total of 40, 1-day-old male Tianfu broilers were randomly divided into four groups. Broilers in the control group (CG), low-dose group (LAG), middle-dose group (MAG) and high-dose group (HAG) were injected intraperitoneally with normal saline, AA-I solution (1/100 LD50),AA-I solution (1/50 LD50) and AA-I solution (1/10 LD50) every 24 h, respectively. After acclimation for day one (d 0, birth), the experiment was commenced from d 1 to 28. Moreover, the methods of biochemical analysis, histopathology and ultrastructure observation, Flow Cytometry, and QRT-PCR were used to evaluated the molecular mechanism. **RESULTS** According to the results, AA-I induces hepatotoxicity in Tianfu broilers reflected by the histological abnormalities and function damage, as elevated liver relative weight, increased levels of serum glutamic oxalacetic transaminase (GOT) and glutamic-pyruvic Transaminase (GPT) were observed. Additionally, AA-I induced histopathological changes in a dose-dependent manner, which were characterized as hepatocyte degeneration and necrosis. Both fatty degeneration and hydropic degeneration were observed in hepatocytes, showing as red granular substances, irregular or circular vacuoles in the cytoplasm. Single necrotic hepatocytes were occasionally seen, with pyknosis, karyorrhexis, and karyolysis. For the molecular mechanism, AA-I elevated Reactive Oxygen Species (ROS) production and break the balance of oxidation and antioxidant systemto arouse oxidative stress, meanwhile, the marked downregulation of Nrf2-HO-1 / NQO1 pathway also provides evidence for that AA-I caused liver lesion by inducing oxidative stress. Further, theoxidative stress may leadto excessive apoptosis which was featured by higher mitochondrial depolarization and upregulated levels of Bax, whereas down-regulated Bcl-2 expression. Also, the ultrastructural lesionsrecorded in this work also verified such damage mechanism. After exposure to AA-I, local glycogen aggregation and numerous low electron density lumpiness with fuzzy internal structure could be easily found in the liver cytoplasm, containing some suspected glycogen and

membrane components. The mitochondria outer membrane expanded, thereby leading to local vacan·cy. Meanwhile, a large number of secondary lysosomes with uneven electron density were observed. Locally, some double-layer membrane circular cavity which contained striated structural materials pre·sented in the liver cytoplasm.Altogether, abovementioned results indicated that the mechanism underly·ing AA-I induced hepatotoxicity was relative to oxidative stress-mediated apoptosis and mitochondrial damage. CONCLUSION To conclude, the current work demonstrated that AA-I induces hepatotoxicity in Tianfu broilers reflected by the histological abnormalities and function damage, thus triggering oxida·tive stress-mediated apoptosis and mitochondrial damage. This study has enriched information about the clinical dosage of Chinese medicines containing AA-I in broilers.

Corresponding author: Xiaoling Zhao, E-mail: zhaoxiaoling@sicau.edu.cn

T19-98-0035

90-day subchronic toxicological study of *methylococcus capsulatus* protein

Gao Yan-jun, Nie Ya, Wang Zhen, Wang Xiao-bo, Bu Shi-jin*

(Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Veterinary Medicine College, Yangzhou University, Yangzhou Jiangsu China, 225009)

Abstract: The experimental studied the 90-day subchronic oral toxicity ofmethylococcus capsula·tus protein.A total of 80 Wistar rats were included and randomly divided into 4 groups(10 males and 10 females per group)and corresponding to three treatment groups at levels of 5000 mg·kg^{-1}, 2500 mg· kg^{-1}and 1000 mg · kg^{-1}in the dietand one negative control group, administering continuously for 90 days. All rats were monitored for clinical changes and mortality on a daily basis. Body weight and food intake were assessed on a weekly basis. At the middle and end of the study, 10 rats (half male and half female) were selected from each group for autopsy after collecting blood samples which used for hema·tologicaland blood biochemical examination. Meanwhilecomplete gross necropsy was conducted. The haert, liver, kidneys, spleen, stomach, lungand testes or ovary were collected and weighed. Organ-to-body weight ratios (relative organ weight) were determined. In addition, majororgan samples from con·trol and high dose animals were fixed and subjected to histopathological evaluation. The results showed that subchronic study of *methylococcus capsulatus* protein administration was not associated with any changes in rat food intake, weight, hematological parameters, organ weight, or organ histolo·gy. Although there were statistical differences (P<0.05) in the individual parametersrelative to negative control animals, the parameter levels were within the normal range reported in literature, and no biologi·cal significance changes were found.No pathological changes related to the test substances were ob·served in all organs. Based on these results, the no-observed-adverse effect level of *methylococcus capsulatus* protein is greater than 5000 mg·kg^{-1}.

Key words: Subchronic toxicological study; *methyllococcus capsulatum* protein

Corresponding author: Bu Shi-jin, E-mail:sjbo@yzu.edu.cn

T19-98-0036

The study on the toxicity of 8:2 FTOH and its mechanism

Chen Min, Chen Zhen, Chen Dongmei

(National Reference Laboratory of Veterinary Drug Residues (HZAU) and MAO Key Laboratory for Detection of Veterinary Drug Residues, Wuhan, Hubei 430070, China)

Abstract: As a widely used surfactant, fluorotelomer alcohols (FTOHs), especially 8:2 FTOH, are the most widely used and researched. It is reported to difficultly degrade in the environment and has high bioaccumulation, which has aroused people′s attention to its environmental and health-related risks. Considering its numerous biological toxicity mechanism has not yet been elucidated. In this study, we researched the oral acute toxicity and oral subacute toxicity of 8:2 FTOH to ICR mice and Wi·star rats, respectively. And the effects of 8:2 FTOH exposure on rat liver metabolism were comprehen·sively evaluated through metabolomics. Our results showed that the oral and sexual toxicity of 8: 2 FTOH to ICR mice was low and obvious gender differences, whose LD_{50} was 9270 $mg \cdot kg^{-1}$ and 4780 $mg \cdot kg^{-1}$, respectively. During the experiment, the body weight of female rat with high-dose decreased significantly or extremely significantly during the administration period, and there were no adverse signs and clinical reactions in the other dose groups. The blood test results illustrated that low, medium and high doses of 8: 2 FTOH caused a significant decrease in red blood cell (RBC) and platelet count (PLT) indicators in male and female rats. And the blood biochemical indicators and organ pathological analysis indicated that exposure to 8: 2 FTOH leaded multiple organ damage, including liver, kidney, heart, spleen, uterine ovarian and testicular. The liver may be the main toxicity target tissue of 8: 2 FTOH. Meanwhile, the serum indexes of liver, kidney and spleen injury TB, ALB, ALT, AST, UREA, RBC and PLT all showed significant or extremely significant changes. Non-targeted metabolomics and lipid metabolomics further revealed significant regulatory abnormalities in liver metabolic pathways, in·cluding glutathione, glyceride, arachidonic acid, linoleic acid, glycerophospholipid, fatty acid elongation and fatty acid degradation. These indicated that the multi-organ toxicity induced by 8: 2 FTOH may be caused by the disorder of lipid metabolism caused by oxidative stress. Simultaneously, it may reduce the antioxidant capacity of liver through the disorder of glutathione metabolism, and further induce oxi·dative stress in the body. Therefore, multiple organ damage in the body may be the result of oxidative stress and decreased antioxidant capacity.

Key words: 8:2 FTOH; rat; acute and subacute toxicity; metabolomics; toxicity mechanism

T19-98-0037

Past and future of genomics in the evaluation of combined toxicity

ZhaoJun-jie[1], Cheng Lin-li[1,2,3], Tang Shu-sheng[1,2,3], Chen Yan-an[1]

(1. Veterinary Medicine College of China Agricultural University, Beijing 100093; 2. National Veterinary Drug Residue Reference Laboratory, Beijing 100093; 3. Beijing Key Laboratory of Animal Source Food Safety Testing Technology, Beijing 100093)

Abstract: Investigators and regulatory agencies are aware that new food safety and environmen·tal risk assessments should take into account the impact of co-exposure to chemicals. Joint toxicity as·sessment has also been greatly developed as an essential method for evaluating the nature as well as

risk of chemical interactions. Correspondingly, the traditional toxicological evaluation methods have been unable to meet the current research requirements. With the rapid development of molecular biology, computer science, mathematics and bioinformatics, various genomics technologies have emerged. Based on genomics technologies to assess the combined toxic effects of chemicals and explore the relevant mechanisms has become a current hotspot. Compared with pesticides and heavy metal ions, the study of combined toxicity of antibacterial drugs will certainly attract more extensive attention in the future, and it is also worth looking forward to. At the same time, this field is bound to get out of the quagmire of studying binary mixtures, and realize the transition from effects description to mechanistic research.

Key words: Toxicology; Genomics; Combined toxicity; Risk assessment

Corresponding author: Cheng Lin-li, E-mail: chenglinli@cau.edu.cn

T19-98-0038

Evaluation of 30-day oral toxicity of amoxicillin baicalein intramammary suspension in rats

Chen Ting-ting, Tang Shu-sheng

(Department of Basic Veterinary Medicine, College of veterinary medicine, China Agricultural University, 100193, P. R. China)

Abstract: Amoxicillin Baicalein Intramammary Suspension was a self-developed preparation for the treatment of clinical dairy cow mastitis. To evaluate the 30-Day oral toxicity of the suspension, 80 SD rats were divided into four groups: high-dose group (5000 mg/kg·b.w.), medium-dose group (2000 mg/kg·b.w.), low-dose group (200 mg/kg·b.w.) and the control group. Rats in all treatment groups were exposed to the suspension orally for 30 consecutive days. The results indicated that there was no significant difference in appetite, weight, hematological index, blood biochemical index and pathological index between the treatment rats exposed to 200, 2,000, and 5,000 mg·kg^{-1} suspension and the control group. Amoxicillin Baicalein Intramammary Suspension did not exhibit subchronic toxicity in the range of 200-5,000 mg·kg^{-1} in the rats and the maximum no-observed effect level (NOEL) was 50,000 mg· kg^{-1}, which preliminarily provided guidance for safe use of the suspension in the treatment of mastitis.

Key words: amoxicillin; baicalein; mastitis; oral toxicity; NOEL

Corresponding author: Tang Shu-sheng, E-mail: tssfj@cau.edu.com

T19-98-0039

Lycorine-induced apoptotic cell death in Raw264.7 cells involves the generation of reactive oxygen, mitochondrial dysfunction, inhibition of IL-1β/IκB-α/NF-κB and Nrf2/HO-1 pathways

Zhang Yuan, Tang Shu-sheng, Dai Chong-shan

(Department of Basic Veterinary Medicine, College of veterinary medicine, China Agricultural University 100193, P. R. China)

Abstract: Lycorine, the main phenanthridine Amaryllidaceae alkaloid, exhibits high specificity activ·

ities against various cancers both in vivo and in vitro. In the present study, we aimed to investigate the precise molecular mechanisms underlying lycorine-induced apoptotic cell death in Raw264.7 cells. Our results showed that lycorine treatment (0-10 $\mu mol \cdot L^{-1}$) of Raw264.7 cells induced apoptotic cell death and blocked cell proliferation in a dose-dependent manner. Lycorine treatment significantly promoted the production of reactive oxygen species (ROS). Mitochondrial dysfunction was evident by the loss of membrane potential and the increased radio of Bax/Bcl-2, followed to induce the increased expression of cleaved caspase-3. Furthermore, lycorine treatment per se did not change the expression of TNF-α and COX-2 proteins, but significantly decreased the expression of IL-1β, IκB-α, and NF-κB proteins. Meanwhile, Lycorine treatment significantly decreased the expression of Nrf2, and HO-1 protein. In conclusions, these results indicated that lycorine treatment could induce mitochondrial apoptotic death in Raw264.7 cells via the inhibition of IL-1β/IκB-α/NF-κB and Nrf2/HO-1 pathways. Our study highlights that lycorine is a promising anti-cancer candidate.

Key words: lycorine; reactive oxygen species(ROS); apoptosis; IL-1β/IκB-α/NF-κB pathway; Nrf2/HO-1 pathway

Corresponding author: Dai Chong-shan, E-mail: daichongshan@cau.edu.cn; Tang Shu-sheng, E-mail: tssfj@cau.edu.com

T19-98-0040

Determination of piperazine residues in chicken muscle and pig tissues

XiaXiao, Fu-Xin Zeng, Dong-Qing Zhou, Zhi-Qiang Wang
(College of Veterinary Medicine, Yangzhou University)

Abstract: Piperazine has been approved as an anthelmintic for use in most livestock, but its residues in animal-derived foods pose a potential risk to human health. Therefore, a method for the quantitative analysis of piperazine residues in animal products must be established. This study reports a method combining precolumn derivatization with high-performance liquid chromatography for piperazine determination in chicken muscle and pig tissues (muscle, liver, and kidney). Piperazine was extracted with acetonitrile and perchloric acid, and purified using a mixed-mode cation exchange (MCX) cartridge. Piperazine was then derivatized with dansyl chloride (DNS-Cl), separated on a C18 column using acetonitrile and water (78:22, v/v) as the mobile phase, and detected at a wavelength of 278 nm using a UV detector. The limits of detection (LOD) and quantification (LOQ) were 0.03 and 0.05 mg/kg in different tissues, respectively. The recoveries, and intra-day and inter-day coefficient variations, for all tissues tested in this study were >76.33%, <6.98%, and <7.81%, respectively. The selectivity and sensitivity of this method make it efficient for the detection of piperazine residues in chicken muscle and pig tissues.

Key words: Piperazine; Residue; Chicken; Pig; HPLC

T19-98-0041

Subinhibitory concentration of colistin promote the conjugative transfer rate of *mcr-1* and bla_{NDM-1} positive plasmid

Xiao Xia, Zeng Fu-xing, Wang Zhi-qiang*

(College of Veterinary Medicine, Yangzhou University, Yangzhou, China)

Abstract: Antibiotic resistance(AMR) has been a growing global threat to public health security. Horizontal gene transfer (HGT) plays a major role in spearing of antibiotic resistance genes (ARGs) in the environment. To investigate the potential role of colistin in facilitating the dissemination of ARGs through plasmid conjugation, an *in vitro* mating model was established. *Escherichia coli* (*E. coli*) DH5α carrying plasmid RP4-7 was the donor strain, and E. coli J53 was the recipient strain, respectively. Mechanisms of the HGT promoted by colistin were unveiled by detecting oxidative stress, cell membrane permeability, and the expression level of the corresponding genes. **RESULTS** Exposure to Sub-MIC concentrations of colistin (1/8MIC) significantly stimulated the conjugation transfer rate of plasmid with an increasing of conjugation frequency of 38 times compared with the blank control. Meanwhile, when exposure to 1/8MIC colistin, the conjugation frequency of wide-type IncI2 plasmid bearing important resistance gene *mcr-1* and IncX3 plasmid bearing bla_{NDM-5} was also considerably increased. Through scanning electron microscopy, the cell membrane was shrunken after colistin exposure. The cell membrane permeability was increased through PI and NPN fluorescent probe. The expression level of the outer membrane protein (*ompF* and *omp*C) were increased. The expression of the global regulatory genes (*korA*, *korB*, *trbA*) were inhibited when exposure at 1/8 MIC colistin. And the expression of mating pair formation gene (*trbBp*) was promote. Colistin can promote plasmid transfer rate at sub-inhibitory concentrations. The possible machanisms are that the morphology of the cell membrane was changed after the colistin exposure; the permeability of the bacterial cell membrane was increased; the mating pairing machine was producted.

Key words: Colistin; Plasmid; conjugation; Horizontal gene transfer

Corresponding author: Wang Zhi-qiang, E-mail: zqwang@yzu.edu.cn

T19-98-0042

Safety evaluation of chloroquine phosphate in type 2 diabetic rats

Xian Jing, Yuling Zheng, Junqi Wang, Shiheng Hu, Hui Ji, Dawei Guo,
Shanxiang Jiang, Xiuge Gao
(College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, Jiangsu)

Abstract: The coronavirus disease 2019 (COVID-19) patients complicated with underlying diseases are at increased risk of death. Using the officially recommended anti-COVID-19 drug chloroquine phosphate (CQ) for treatment of severe patients with type 2 diabetes (T2D) may induce potential hazards. We aimed to understand the safety of CQ on type 2 diabetes in this study by administrating the recommended dosage (63 $mg \cdot kg^{-1}$ twice daily for 7 days) and high dosage (126 $mg \cdot kg^{-1}$ twice daily for 7days) of CQ in T2D rats. We found that CQ increased the mortality of diabetic rats at day 5. The mortality of T2D rats in the recommended dosage and higher dosage group were 40% and 80%, respectively, compared to the control group. Hematotoxicity was observed only in high dosage group other than the normal dosage group. The hepatic enzymes of CQ-treated T2D rats were significant higher than T2D rats and healthy rats. CQ changed the electrocardiogram and prolonged QTc interval of T2D rats. Leukocyte in urine of T2D rats were confirmed by CQ treatment for 7 days. Ocular toxicity was observed in two CQ dosage groups at day 5. Histopathology analysis showed severe damage of heart, liver, kidney, jejunum, spleen and retina of T2D rats after CQ administration for 7 days. However, CQ

treatment significant decreased blood glucose level of T2D rats and inhibited the production of several inflammatory cytokines, including IL-1β and IL-6. In conclusion, based on preclinical safety evaluation of CQ in T2D rats, the dosage and regimen must be reconsidered in treating COVID-19 patients due to its safety concerns, especially using in patients combined with diabetics.

Key words: Chloroquine phosphate; Type 2 diabetes; Safety evaluation; Toxicology

Corresponding author: Xiuge Gao, E-mail: vetgao@njau.edu.cn

T19-98-0043

Vancomycin-induced cytotoxicity involves the generation of reactive oxygen, mitochondrial apoptotic and JNK pathways in HEK293 cells

Yue Liu, Shusheng Tang, Chongshan Dai

(Department of Basic Veterinary Medicine, College of veterinary medicine, China Agricultural University, 100193, P. R. China)

Abstract: Vancomycin is a glycopeptide antibiotic, which is often used as the last defense against pan drug-resistant Gram-positive bacteria. Nephrotoxicity is one of vancomycin-induced adverse effects in patients, however, the precise molecular mechanism is unclear. This study aims to investigate the molecular mechanism using a HEK293 cell line. Our results showed that vancomycin treatment significantly decreased the cell viability of HEK293 cells in time-dependent and dose-dependent manner. Meanwhile, vancomycin treatment significantly induced the production of ROS, decreased the mitochondrial membrane potential, upregulated the level of anti-apoptotic Bax protein expression and downregulated the level of pro-apoptotic Bcl-2 protein expression. Vancomycin treatment also significantly upregulated the levels of cleaved-caspase 3 and cleaved-PARP-1 protein expressions, finally lead to apoptotic cell death in HEK293 cells. Furthermore, vancomycin treatment significantly increased the expression of p-JNK protein in a dose-dependent manner, and inhibition of JNK expression significantly decreased the expression of HO-1, the production of ROS, and reduce vancomycin treatment-induced cell death. In conclusion, our study shows that vancomycin induced-cytotoxicity in HEK293 cells involved the production of ROS, activation of mitochondrial apoptotic pathway and JNK pathway. Our study highlights the understanding the molecular mechanism of vancomycin-induced nephrotoxicity and provides information for nephron-protection agents.

Key words: Vancomycin; Mitochondrial apoptotic pathway; JNK pathway; Nephrotoxicity

Corresponding author: Chongshan Dai, E-mail: daichongshan@cau.edu.cn; Shusheng Tang, E-mail: tssfj@163.com

T19-98-0044

Baicalin Alleviates *Mycoplasma gallisepticum*-induced Oxidative stress and Inflammation via modulating NLRP3 Inflammasome-Autophagy pathway

Muhammad Ishfaq[a, b], Jichang Li[b], Yurong Guan[a], Zhihua Hu[a]

(a. Computer Pharmacology, College of Computer Science, Huanggang Normal University, Huanggang

438000, China; b. Heilongjiang Key Laboratory for Animal Disease Control and Pharmaceutical Development. Faculty of Basic Veterinary Science, College of Veterinary Medicine, Northeast Agricultural University, Harbin 150000, China)

Abstract: Baicalin is a well-known flavonoid compound, possess therapeutic potential against inflammatory diseases. Previous studies reported that *Mycoplasma gallisepticum* (MG) induced inflammatory response and immune dysregulation inside the host body. However, the underlying molecular mechanisms of baicalin against MG-infected chicken-like macrophages (HD11 cells) are still illusive. The present study investigated the preventive effects of baicalin on MG-infected HD11 cells with the aim to identify molecular targets that might be helpful for the prevention of MG infection. The effect on MG-induced oxidative stress and inflammation-related signaling pathways including TLR-2-NF-κB, NLRP3 inflammasome and autophagy pathway were evaluated that might contribute to the therapeutic effects of baicalin. Oxidant status and total reactive oxygen species (ROS) were detected by ELISA assays and flow cytometry respectively. Mitochondrial membrane potential (ΔΨM) was evaluated by immunofluorescence microscopy. Transmission electron microscopy was used for ultrastructural analysis. The hallmarks of inflammation and autophagy were determined by western blotting. Oxidative stress and reactive oxygen species (ROS) were significantly enhanced in the MG-infected HD11 cells. MG infection caused disruption in the mitochondrial membrane potential (ΔΨM) compared to the control conditions. Meanwhile, baicalin treatment reduced MG-induced reactive oxygen species (ROS), oxidative stress and alleviated the disruption in ΔΨM. The activities of inflammatory markers were significantly enhanced in the MG-infected HD11 cells. Increased protein expressions of TLR-2-NF-κB pathway, NLRP3-inflammasome and autophagy-related proteins were detected in the MG-infected HD11 cells. Besides, baicalin treatment significantly reduced the protein expressions of TLR-2-NF-κB pathway and NLRP3 inflammasome. While, the autophagy-related proteins were significantly enhanced with baicalin treatment in a dose-dependent manner in the MG-infected HD11 cells. The results showed that baicalin prevented HD11 cells from MG-induced oxidative stress and inflammation via the opposite modulation of TLR-2-NF - κB-mediated NLRP3-inflammasome pathway and autophagy, and baicalin could be a promising candidate for the prevention of inflammatory effects caused by MG-infection in macrophages.

Key words: Baicalin; NLRP3 inflammasome; Autophagy; *Mycoplasma gallisepticum*

Corresponding author: Muhammad Ishfaq, E-mail: muhammad@hgnu.edu.cn

T19-98-0045

Migration and toxicity of toltrazuil and its main metabolites in the environment

Huo Mei-xia[a,b], Ma Wen-jin[a,b], Xu Xiang-yue[a,b], Wang Lei[a,b], Lin Xu-dong[a,b], Huang Ling-li[a,b]

(a. College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China; b. National Laboratory for Veterinary Drug Safety Evaluation, Huazhong Agriculture University, Wuhan 430070, China)

Abstract: Veterinary drugs heavily used in livestock would be finally passed into the environment through manure application, resulting in the risks to terrestrial environments and humans. This work investigated the migration of toltrazuil and its main metabolites in compost, soil and vegetables, and then

evaluated the impacts of toltrazuil on the soil enzyme activities and phytotoxicity. Chicken manure and wheat straw were selected as the main raw materials for aerobic composting. After the composting, the compost products were applied to soil planted with lettuce and radish. The results showed that Toltrazuil sulfoxide was removed up to 76.69% during aerobic composting. Toltrazuil and ponazuril persisted in composting with the removal rate were 19.34% and 4.65%. Toltrazuil、Toltrazuil sulfoxide and ponazuril were detected in soil with the concentration of 9.92-196 $\mu g \cdot kg^{-1}$, 0.98-14.53 $\mu g \cdot kg^{-1}$, 37.21-107.31 $\mu g \cdot kg^{-1}$. However, only ponazuril were detected in lettuce and radish, and the concentrations of ponazuril varied greatly among the different types of vegetables. Lettuce had a relatively high bioaccumulation ability for ponazuril. The urease activities in the soil declined while catalase activities increased. In contrast, the alkaline phosphatase activities decreased at the beginning and then increased slightly. Toltrazuil and its metabolites could inhibit the germination of lettuce and radish seeds and root elongation. The inhibition rate of root elongation on lettuce and radish was 25.88% and 34.45%, respectively. These results suggested that the application of manure compost product containing toltrazuil would lead to great risk to the soil and plants in the environment. Thus, the potential ecotoxicity and danger to human and animal health via accumulation of toltrazuil and its main metabolites in the food chain need be further concerned.

Key words: Toltrazuil; Compost; Vegetables; Soil enzyme activity; Phytotoxicity

Corresponding author: Huang Ling-li, E-mail: huanglingli@mail.hzau.edu.cn

T19-98-0046

Fate and risk of florfenicol, and thiamphenicol during composting and land application of swine manure

Ma Wen-jin[a,b], Liu Hai-yan[a,b], Lin Xu-dong[a,b], Xu Xiang-yue[a,b], Huo Mei-xia[a,b], Huang Ling-li[a,b]*

(a. College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, 430070, China;
b. National Laboratory for Veterinary Drug Safety Evaluation, Huazhong Agriculture University, Wuhan 430070, China)

Abstract: Manure are commonly applied to soil, raising questions about whether antibiotic use in livestock could ultimately contribute to the migration of antibiotic to humans during food production. Here, we investigated the dissipation of florfenicol (FF) and thiamphenicol (TAP) during composting and land application, and evaluated their effects on physical and chemical properties of compost and risk of soils after fertilization. The results indicate that FF and TAP dissipated rapidly in compost, with half-life values of 5.1 and 1.6 d, respectively, composting could effectively remove FF and TAP from manure. The FF and TAP residues in the manure have a negative influence on the physical and chemical properties of compost, such as the temperature in FF treatments and the C: N ration in TAP treatments was low than control treatment, indicating that FF and TAP inhibited the microbial activity and have negatively impacts the microbes involved in nitrogen transformation. The electrical conductivity in the FF treatment was always higher than that in other two treatments, and slightly exceeded 4 mS/cm after the end of the composting, indicating that FF in manure may affect the maturity and safety of the composting products. During fertilization, FF in swine manure rapidly migrated to the soil, reaching 588 μg kg-1 on the 6th day and completely dissipated in the soil after 44 days. The TAP was not detected in fertilization soil because of it is degrade fast in manure and soil. The predicted environmental concentration of FF in soil was higher than the recommended risk trigger value (100 $\mu g \cdot kg^{-1}$), which indicated

that FF poses a great risk to the natural environment, and its ecotoxicity needs to be further studied. Longer manure storage periods and effective measures to reduce DT50 are suggested based on the use of compost as fertilizer and to reduce the risk of antibiotics entering the environment.

Key words: Antibiotic removal; Composting; Environmental risk; Soils fertilization

Corresponding author: Huang Ling-li, E-mail: huanglingli@mail.hzau.edu.cn

T19-98-0047

Migration, transformation and exposure risk assessment of doxycycline in the environment

Xu Xiang-yue[a,b], Wang Lei[a,b], LinXu-dong[a,b], Ma Wen-jin[a,b], Huo Mei-xia[a,b], Huang Ling-li[a,b]*.

(a. College of Veterinary Medicine, Huazhong Agriculture University, Wuhan 430070, China;

b. National Laboratory for Veterinary Drug Safety Evaluation, Huazhong Agriculture University, Wuhan 430070, China)

Abstract: Composting and fertilizationare widely used in agricultural practice as economic and effective technologies. Due to its better adsorption in the gastrointestinal tract, stronger tissue penetration and longer biological half-life, doxycycline (DOX) is commonly used to treat bacterial infections in swine and broiler. In current study, the fate of doxycycline (DOX) was studied duringaerobic composting and fertilizationunder natural environmental conditions. The predictedconcentration of DOX in soil was calculated by using the predicted environmental concentration (PEC) model to assess the exposure risk of DOX in soil. DOX was effectively removedfrom broiler manure and swine manure during aerobic composting. The removal rate of DOX was more than 97%, and the half-lives (DT50) of DOX in broiler manure and swine manure composting were 4.65 days and 5.64 days, respectively. In addition to moisture, there were significant differences in temperature, C/N and pH during composting. DOXslowlymigrated from animal manure to agricultural soil and the migration showed a trend of early accumulation and subsequent dissipation. The concentration of DOX in fertilized soil increased slowly from 0 to 40 days, and the highest concentration was 553.4 $\mu g \cdot kg^{-1}$ at 40 days.Subsequently, the concentration of DOX in fertilized soil decreased to 17.4 $\mu g \cdot kg^{-1}$ at 60 days.The PECsoil refined of DOXwas less than the risk trigger value (100 $\mu g \cdot kg^{-1}$), whichindicated that DOX showed low risk in soil. These results help to understand the migration and transformation of DOX in the environment, and to preliminarily assess the exposure risk of DOX after animal manure composting.

Key words: Doxycycline; Degradation; Migration; Aerobic composting; Fertilization; Predicted environmental concentration

T19-98-0048

Opposite regulation of JNK and p38 signaling pathways on copper sulfate-induced apoptosis and cell cycle arrest in HepG2 cells

Li Meng, Tang Shu-sheng*, Dai Chong-shan*,

(Department of Basic Veterinary Medicine, College of veterinary medicine, China Agricultural University 100193, P. R. China)

Abstract: Copper is an essential trace element in maintaining cell survival, proliferation, and activities of metabolic enzymes. However, overdose of copper exposure could induce toxic effects and underlying molecular mechanism is still unclear. This study was aimed to investigate the potential molecular mechanism of copper sulfate-induced liver toxicity *in vitro*. Our results showed that copper-sulfate induced cytotoxicity in dose-and time-dependent manners in HepG2 cells. Copper sulfate exposure significantly induced the production of ROS, followed by inducing cell apoptosis and G2 cell cycle arrest. RNA-seq data showed that copper sulfate-induced cell death involved ER stress, PI3K/AKT, MAPK, and NF-κB pathways. We further detected that copper sulfate increased the expression of p-JNK and p-p38 in time- and dose-dependent manners. Moreover, inhibition of p38 MAPK pathway significantly decreased copper sulfate-induced cell death and G2 cell cycle arrest. On the contrary, inhibition of JNK pathway significantly increased copper sulfate-induced cell death and G2 cell cycle arrest. In conclusions, our results reveal that the activation of MAPK pathway played a critical role in copper sulfate-induced apoptosis and G2 cell cycle arrest. For the first time, our results reveal the opposite regulation of JNK and p38 signaling pathways contributed to copper sulfate-induced apoptosis and cell cycle arrest in HepG2 cells. This study provides an insight on the understanding of copper sulfate-induced toxic effects.

Key words: MAPK pathway; Apoptosis; Cell cycle arrest; Cytotoxicity; HepG2 cells

Corresponding author: Dai Chong-shan, E-mail: daichongshan@cau.edu.cn; Tang Shu-sheng, E-mail: tssfj@163.com

T19-98-0049

A highly sensitive dual-readout immunochromatographic test strip for simultaneous detection of chloramphenicol and amantadine via the inner filter effect on fluorescence quenching of green-emitting gold nanoclusters

Jincheng Xiong, Haiyang Jiang

(Institution Name:Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, China Agricultural University, Beijing Key Laboratory of Detection Technology for Animal-Derived Food Safety, Beijing Laboratory for Food Quality and Safety, Beijing 100193, People's Republic of China)

Abstract: Immunochromatographic test strips have been used as a simple, rapid and sensitive tool for on-site monitoring in food safety. However, available ICTS for small molecule analytes were competitive and performed based on "turn-off" mode, which was not conducive to distinguish by naked eye and obtain high sensitivity. In this study, A new type fluorescence quenching immunochromatographic test strip (FQICTS) for simultaneous detection chloramphenicol (CAP) and amantadine (AMD) was developed based on the inner filter effect (IFE), with the combination of highly luminescent green-emitting gold nanoclusters and gold nanoparticles. The visual limits of detection of "turn-on" mode were 50 and 20 times better than that in "turn-off" mode for CAP, and AMD, respectively. The calculated limits of detection of dual-readout FQICTS for CAP and AMD were 0.01 and 0.4 ng · mL^{-1}, respectively. The "turn-on" mode of FQICTS showed high recovery for detection of CAP (82.5-94.5%) and AMD (81.9-110.7%) concentrations spiked into chicken samples. In addition, the performance and practicability of established dual-readout FQICTS were verified with commercial enzyme-immunoassay assay kits, and

good correlations were observed for CAP and AMD. Overall, the newly developed dual-readout FQ·ICTS exhibits great potential for rapid, sensitive, and efficient detecting of multiple food contaminants.

Key words: Immunochromatographic test strips; Gold nanoclusters; Inner filter effect; Chloramphenicol; Amantadine

Corresponding author: Haiyang Jiang, E-mail: xiongjincheng94@163.com

T19-98-0050

Maduramicin induces chicken hepatotoxicity via apoptosis and nonapoptotic cell death *in vitro* and *in vivo*

Junqi Wang, Xiuge Gao, Yuling Zheng, Xinhao Song, Shanxiang Jiang

(College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, Jiangsu)

Abstract: Drug-induced liver injury is a major cause of hepatic dysfunction in veterinary clinical practice. Maduramicin, an anticoccidial drug, frequently induces liver damage of broilers due to overdose. However, the underlying mechanism of maduramicin of maduramicin-caused liver damage in chicken remains unclear. Hence, in this study, primary chicken hepatocytes and broilers were used as in vitro and in vivo model to uncover the hepatotoxicity and its mechanism of maduramicin. Chicken primary hepatocytes were exposed to a series of concentrations of maduramicin (0.1-1 $\mu g \cdot mL^{-1}$) for 24 h to 72 h, and 21 day-old broiler chickens were orally administrated with different doses of maduramicin (5-15 $mg \cdot kg^{-1}$) for 21 days, followed by cell activity assay, microscopy, flow cytometry, RT-qPCR, immunofluorescence, hepatic enzymes test and histopathological examination. We found that chicken primary hepatocytes treated by maduramicin (0.1-1 $\mu g \cdot mL^{-1}$) for 24 h to 72 h showed severe cell damage and significant decrease in cell viability ($P<0.01$) in a concentration and time dependent manner, cells gradually shrunk and detached from the substrate of dish. Maduramicin exposure increased apoptosis rate significantly ($P<0.01$), compared with the control group, which were demonstrated by hoechst33342 and DAPI staining. Furthermore, the apoptotic genes caspase-3 / 8 / 9 were upregulated significantly ($P<0.01$) after maduramicin exposure. Histopathological examination and transmission electron microscopy observation exhibited maduramicin induced obvious liver damage, including the scattered liver cords, necrotic and inflammatory cell infiltration, the swollen mitochondria, the disorganized mitochondrial ridges, as well as cytoplasmic vacuolization. Taken together, apoptosis mediates maduramicin-induced hepatotoxicity of chicken *in vitro* and *in vivo*. The manipulation of caspases will provide effective way to attenuate maduramicin-triggered chicken hepatotoxicity.

Key words: Maduramicin; Hepatotoxicity; Chicken; Apoptosis.

Corresponding author: Shanxiang Jiang, E-mail: nauvy@sina.com

T19-98-0051

Residue depletion of diclofenac sodium injection in dairy cow

Ya-Jun YANG, Xi-Wang LIU, Shi-Hong LI, Zhe QIN, Jian-Yong LI*

(Key Laboratory of New Animal Drug Project of Gansu Province; Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture and Rural Affairs; Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Lanzhou 730050, China)

Abstract: Nowadays, diclofenac sodium is authorized as non-steroidal anti-inflammatory drug in swine and cattle for the related diseases in China, and the residual marker is diclofenac acid (DCF). The present study developed an LC-ESI$^+$-MS/MS method to determine the residues of DCF in cattle ed·ible tissues and milk. DCF-13C6 and organic solvents were used as the internal standard and extrac·tion solvent in all kinds of samples, respectively. In addition, n-hexane was employed to remove lipids of fat samples. The results showed the LOD and LOQ were 0.3 μg·kg^{-1} and 0.5 μg·kg^{-1} in beef, liver, kidney and fat, 0.03 μg·kg^{-1} and 0.05 μg·kg^{-1} in milk, respectively. The method showed good perfor·mance with accuracy (80%-120%), precision (RSD<20%) and linearity. So, the proposed method was used to evaluate residue depletion of diclofenac sodium injection in dairy cow. One group with 20 dairy cows were used to evaluate residue depletion in edible tissues, and another group with 20 lactating dairy cows were employed to evaluate in milk. Diclofenac sodium injection was i. m. administered at dose of 2.2 mg·kg^{-1} for 3 consecutive days. Every four cows' tissue samples were collected at five dif·ferent days after the last administration. All 20 lactating cows' milk samples were collected twice per day at morning and evening for 9 consecutive days after the last administration. The DCF residue in all samples were determined by the mentioned method. The MRLs of DCF in cattle muscle, liver, kidney, fat and milk are 5 μg·kg^{-1}, 5 μg·kg^{-1}, 10 μg·kg^{-1}, 1 μg·kg^{-1} and 0.1 μg·kg^{-1}, respectively. The residue da·ta were analyzed through WT 1.4 and WTM1.4 to calculate the withdrawal times of tissue and milk, re·spectively. The results showed that the tissues and milk withdrawal times of diclofenac sodium injection were 19 and 6 days, respectively.

Key words: diclofenac sodium; cow; residue; withdrawal time

Corresponding author: Jian-Yong LI, E-mail: lijy1971@163.com

T19-98-0052

Antioxidant activity and the potential mechanism of the fruit from *Ailanthus altissima* Swingle

Ya-nan Mo[a], Feng Cheng[a,c], Zhen Yang[a], Xiao-fei Shang[a], Jian-ping Liang[a], Ruo-feng Shang[a], Bao-cheng Hao[a], Xue-hong Wang[a], Hong-juan Zhang[a], Ahmidin Wali[c], Chun-fang Lu[c], Yu Liu[a,b]

(a. Key Laboratory of New Animal Drug Project, Gansu Province; Key Laboratory of Veterinary Pharma·ceutical Development, Ministry of Agriculture and Rural Affairs; Lanzhou Institute of Husbandry and Pharmaceutical Sciences of Chinese Academy of Agriculture Sciences, Lanzhou 730050, P. R. China; b. College of Veterinary Medicine, Gansu Agricultural University, Lanzhou 730070, P. R. China; c. Key Laboratory of Plant Resources and Chemistry in Arid Regions, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi 830011, P. R. China)

Abstract: The fruits of Ailanthus altissima Swingle (AS) possess a variety of pharmacological activ·ities. Its antioxidant activity and the potential mode of action have not yet been investigated. Antioxidant assay *in vitro* proved AS possessed fairly strong antioxidant capacity. Meanwhile, AS increased the ac·tivities of SOD, CAT, GSH-Px and decreased the MDA level compared with the H_2O_2 group, suggesting it relieved oxidative stress of RAW264.7 cells significantly. Network pharmacology analysis screened the core targets of AS like threonine kinase1 (AKT1), mitogen-activated protein kinase 1(MAPK1), sir·tuin-1(SIRT1), mechanistic target of rapamycin kinase (MTOR) and the key pathways involved PI3K- AKT and FoxO signaling pathway. Besides, qRT-PCR revealed AS preconditioning significantly up-reg·

ulated the expression level of AKT1, SIRT1, MAPK1, MTOR in model cells, and the effect was related to the regulation of FoxO and PI3K/AKT signaling pathway. In summary, AS revealed significant antioxi·dant activity and its potential mechanism was regulating FoxO and PI3K/AKT signaling pathway.

Key words: Ailanthus altissima Swingle; antioxidant activity; RAW264.7 cell; network pharmacology

Corresponding author: Yu Liu, E-mail: liuyu8108@163.com

T19-98-0053

Correlation analysis between virulence genes distribution and phylo-groups of escherichia coli in dairy breedings

TANG Min-jia, ZHANG Xue-jing, HE Zhuo-lin, PU Wan-xia*

(Key Laboratory of New Animal Drug Project, Gansu Province/Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture and Rural Affairs/ Lanzhou Institute of Husbandry and Pharmaceutical Sciences of Chinese Academy of Agriculture Sciences, Lanzhou 730050, China)

Abstract: To provide a reference for the research and prevention of *Escherichia coli* (*E. coli*), the phylogenetic grouping and virulence genes of E. coli which isolated from two large-scale dairy farms in Gansu Province were identified, and the correlation between phylo-groups and virulence genes of *E. coli* was analyzed. The phylogenetic grouping and virulence genes detecting of the E. coli strains were carried out by PCR. 281 E. coli strains were assigned to eight phylo-groups, and the phylo-groups B1, A, E, F, D, C, B2 and clades I or II were 51.96 %, 28.47 %, 11.39 %, 2.85 %, 1.78 %, 1.07 %, 0.71 % and 0.36 %, respectively. The detection rates of *ompA*, *ibeB*, *ompT*, *iroN*, *traT*, *iucD*, *fyuA* and *irp2* were 100%, 98.58%, 76.16%, 71.53%, 68.33%, 64.06%, 30.96% and 30.25%, respectively. There were significant differences in the distribution of some virulence genes in different years and dairy breedings. The strains carrying all identified virulence genes were mainly distributed in B1 and B2 phy·lo-groups and fecal-soil sourced samples. The distribution of traT in 2017 was significantly lower than that in 2018 and 2019. The distribution of *traT* and *iroN* in fecal-soil sourced isolates was higher than that of sewage sourced isolates. Phylo-group A was negatively correlated with *ompT* and *traT*, whereas phylo-group B1 was positively correlated with *ompT*, *irp2*, *fyuA*, and *iucD*. Phylo-group E had a nega·tive correlation with *irp2*, *fyuA*, and *iucD*, and a positive correlation with the *iroN*. Phylo-group B1 was more prevalent than other phylo-groups. There were significant differences in the distribution of some virulence genes in different years and breedings. The distribution of some virulence genes was associ·ated with phylo-groups.

Key words: *Escherichia coli*; dairy breedings; virulence genes; phylogenetic grouping

Corresponding author: PU Wan-xia, E-mail: puwanxia@caas.cn

T19-98-0054

Abnormal toxicity and pyrogen test of oxytetracycline injection

Zhe Qin, Ya-jun Yang, Xi-wang Liu, Shi-hong Li, Jian-yong Li *

(*Key Laboratory of New Animal Drug Project of Gansu Province; Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture and Rural Affairs; Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Lanzhou 730050, China)*

Abstract: OBJECTIVE To provide basis for drug safety evaluation, abnormal toxicity and pyrogen test of oxytetracycline injection were carried out in present study. **METHODS** Eighteen mice were devided into 3 groups randomly for 3 batches of oxytetracycline injection in the abnormal toxicity test. Oxytetracycline injection was i. v. administered at dose of 0.5 mL, and its toxicity was observed within 48 hours after administration. Experimental Japanese white rabbits were screened out by measuring body temperature 7 days before pyrogen test. Nine experimental rabbits were devided into 3 groups randomly for 3 batches of oxytetracycline injection, that were fasted 3 hours before pyrogen test. The experimental rabbits' body temperature was measured twice at an interval of 30 min, and the average was taken as the normal body temperature of the rabbit. Within 15 minutes after the normal body temperature was measured, the injection was slowly injected into the ear vein at dose of 0.56 mL · kg^{-1}, and body temperature of rabbits was measured once every 30 minutes for 6 times. The elevated temperature (°C) was calculated by subtracting the normal body temperature from the highest one of the 6 times. If one of the 3 experimental rabbits has a temperature increase of 0.6°C or higher, or the total temperature increase of the 3 experimental rabbits is 1.3°C or higher, another 5 experimental rabbits should be retested. **RESULTS** None of the mice died within 48 hours after administration of three batches of oxytetracycline injection in abnormal toxicity test. In pyrogen test of the injection, the temperature increment of 9 experimental rabbits was lower than 0.6°C, and the total temperature rise of 3 experimental rabbits in each batch of injection was lower than 1.3°C. **CONCLUSION** The abnormal toxicity and pyrogen of the oxytetracycline injection were in accordance with the provisions of Chinese Veterinary Pharmacopoeia.

Key words: abnormal toxicity; pyrogen test; oxytetracycline injection

Corresponding author: Jian-yong Li, E-mail: lijy1971@163.com

T19-98-0055

Determination of twelve quinolones in honey by vortex assisted dispersive liquid liquid microextraction performed insyringe based on deep eutectic solventcombine with ultra performance liquid chromatography-mass spectrometry

Wang Yi-xia[a], Zhao Si-jun[b], YangLin-yan[a], Liu Chang[a], Wang Hong-yu[a], Li Dao-wen[a], Zhang Wei[a], LiLiuana, Song Cui-ping[b], Li Cun[a]

(a. Tianjin Key laboratory of Agricultural Animal Breeding and Healthy Husbandry, College of Animal Science and Veterinary Medicine, Tianjin Agricultural University, Tianjin, China 300392; b. China Animal Health and Epidemiology Center, 266032, Qingdao, Shandong China)

Abstract: A novel vortex assisted dispersive liquid-liquid microextraction method based on deep eutectic solvent (VA-DLLME-DES) completed in syringe has been established to extract twelve quinolones from honey. This method is an improvement of traditional DLLME. In this work, the green DES prepared with heptanoic acid and thymol was used for extractant rather than traditional organic extraction solvent. Without centrifugation, the phases were separated in the way of removing aqueous phase by pushing the syringe and extractant phase was collected for Ultra performance liquid chromatography-mass spectrometry (UPLC-MS) analysis. The stability of DES and some experimental parameters (type, molar ratios and volume of extraction solvent, type and volume of dispersant, vortex time and ef-

fect of pH and NaCl) were evaluated. In the range of 2-100 ng · mL^{-1}, the proposed method showed good linearities (r2 ranged from 0.996 to 0.999). The extraction recoveries were obtained in the range of 75.01 %-117.05 % and relative standard deviations were less than 13.83 % for inter- (n=6) and intra-day (n=3) precisions. The limits of detection and quantification were 3 ng g-1 and 10 ng·g^{-1}, respectively. The developed method with the advantages of simple operation, short extraction time and less consumption organic solvent provides a technical idea for green, simple and time-saving sample preparation methods.

Key words: Deep eutectic solvent; Dispersive liquid-liquid microextraction;Quinolones; Syringe; Ultra performance liquidchromatograph-massspectrometry

T19-98-0056

Fluvic acid ameliorates cadmium-induced hepatotoxicity through inhibiting oxidative stress and apoptosis in L02 cells

Li Hui, Dai Chong-shan, Tang Shu-sheng

(Department of Basic Veterinary Medicine, College of Veterinary Medicine, China Agricultural University, Beijing 100193)

Abstract: Fulvic acid has several nutraceutical properties, including antioxidant, anti-inflammatory and detoxification. It also could adsorb and chelate with metal ions. This study aims to use fulvic acid to exert the protective effect on hepatotoxicity against cadmium in vitro and illuminate the underlying mechanism. The effect of fulvic was examined by using LDH release assay, fluorescent staining, flow cytometry, spectrophotometer, and western blot. Our results revealed that fulvic acid can significantly decrease cadmium-induced cell death. And we found fulvic acid can decrease the production of ROS and MDA, it also decreased the activities of SOD and CAT. Moreover, fulvic acid treatment alleviated the decrease in mitochondrial membrane potential induced by cadmium. The apoptosis rates also showed a significant decrease after fulvic acid pretreatment. The protein levels of pro-caspase 3 and pro-caspase 9 were up-regulated, and cleaved-PARP-1, cleaved-caspase 3 and Nrf2 were down-regulated in the presence of fulvic acid. Taken together, our study demonstrates that fulvic acid ameliorates cadmium-induced hepatotoxicity in L02 cells through inhibiting oxidative stress and apoptosis.

Key words: Fluvic acid; Cadmium; Hepatotoxicity; Oxidative Stress; Apoptosis

Corresponding author: Dai Chong-shan, E-mail: daichongshan@cau.edu.com; Tang Shu-sheng, E-mail: tssfj@cau.edu.com

T19-98-0057

Mycoplasma gallisepticum induced inflammation-mediated Th1/Th2 immune imbalance via JAK/STAT signaling pathway in chicken trachea: Involvement of respiratory microbiota

Miao Yu-song, Wang Ji-an, Wu Zhi-yong, Muhammad Ishfaq, Bao Jia-xin, Li Ji-chang

(College of Veterinary Medicine, Northeast Agricultural University, 600 Changjiang Road, Xiangfang District, Harbin 150030, P. R. China)

Abstract: The respiratory microbiota plays a significant role in the host defence against Mycoplasma gallisepticum (MG) infection. The results showed that MG infection changed respiratory microbiota composition, which lead to the tracheal inflammation injury and oxidative stress. MG infection significantly induced immunosuppression in chicken at day 3 and 5 post-infection. In addition, MG infection increased the expression of pro-inflammatory cytokines in tracheal tissues and activated TLR4 mediated JAK/STAT signaling pathway at day 3 post-infection compared to the control group. Meanwhile, the expressions of pro-inflammatory cytokines were decreased and the expression of JAK / STAT signaling pathway was decreased at day 5 and day 7 post-infection. On the contrary, the expressions of anti-inflammatory cytokines were significantly decreased at day 3 post-infection and were increased at day 5 and day 7 post-infection in the MG infection group. The antibiotic cocktail group received the respiratory microbiota from the MG infected group, which induced inflammatory injury and oxidative stress, induced mucosal barrier damagevia down regulating tight junction-related genes and altered the expression of mucin, which could be the possible causes of dysregulated immune responses. Importantly, the expressions of pro-inflammatory cytokines were significantly decreased and TLR4 mediated JAK/STAT signaling pathway was downregulated at day 1 and 3 post-transplantation. While, respiratory microbiota transplanted from MG infection significantly increased the expression of pro-inflammatory cytokines and activated JAK/STAT signaling at day 7 post-transplantation. These results highlighted the role of respiratory microbiota in an MG-induced tracheal inflammation injury, and offered a new strategy for the preventive intervention of this disease.

Key words: Mycoplasma gallisepticum; Respiratory microbiota; Inflammatory cytokines; Respiratory microbiota transplantation; Immune function

Corresponding author: Li Ji-chang, E-mail: lijichang@neau.edu.cn

T19-98-0058

Study on the safety of diclofenac injection for target cows

Li Shi-hong, Qin Zhe, Kong Xiao-jun, Li Jian-yong

(Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS/Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture/Key Laboratory of New Animal Drug Project, Gansu Province, Lanzhou 730050, China)

Abstract: To evaluate the safety of Diclofenac Injection for target animal cows, 24 healthy lactating cows were randomly divided into 4 groups, including the control group (without any treatment), the commended dosage (2.2 mg/kg-bw) group, three times of recommend dosage (6.6 mg/kg-bw) group and five times of recommend dosage (11.0 mg/kg-bw) group.Intramuscularinjection were performed in the neck once a day for 3 days. During the experiment, the health condition, blood routine and serum biochemical indexes of cows were observed and measured. The results showed that no obvious adverse reactions were found in clinical observation during the whole experiment by using Diclofenac Injection at the recommended dose (2.2 mg/kg-bw) to 5 times the recommended dose (11.0 mg/kg-bw), and there was no significant difference in 18 indexes in blood routine examination compared with the control group. Serum biochemical tests showed there were significant differences in total protein, globulin, aspartate aminotransferase, creatine kinase and phosphorus after 3 days of administration compared with the control group, and all indexes returned to normal after 7 days of administration. The results show that Diclofenac Injection is safe to be used fortarget cows.

Key words: Diclofenac Injection; Safety; Target Cow
Corresponding author: Li Jian-yong, E-mail: lijy1971@163.com; Li Shi-hong, E-mail: lzlishihong@163.com

T19-98-0059

Establishment of LC-MS/MS method for detection of diflubenzuron in swine plasma

Yu-Zhen Xu, Zhe Qin, Xi-Wang Liu, Shi-Hong Li, Li-Xia Bai, Ya-Jun Yang, Jian-Yong Li
(Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Lanzhou 730050, China)

Abstract: Diflubenzuron (DFB) is an insect growth regulator that inhibits the synthesis of insect chitin synthase, preventing insect larvae from forming new epidermis during molting, which also results in stunted insect body development, deformity, and mortality, impacting an entire generation of insects. Domestically, the commonly used anti-fly and mosquito drugs in farms mainly include cyromazine, organophosphorus and pyrethroid drugs, as well as traditional Chinese medicine preparations such as Boluosan. DFB has been approved as an oral anti-fly maggot medicine for swine, cattle, sheep and other animals in United States, Germany and Brazil. To characterize the absorption, distribution and excretion of DFB after administration to swine, an LC-MS/MS method was developed to determine DFB concentration in swine plasma. 500 μL plasma was spiked with 10 μL DFB-13C6 internal standard working solution and mixed with a vortexer. 2 mL methanol was added into plasma, followed by vortex and centrifuge. The supernatant was dried. The residue was completely dissolved with 500 μL methanol-water (1 + 1), filtered and tested. The chromatographic conditions included with Agilent Zorbax Eclipse plus C18 column (3.0 × 50 mm, 1.8 μm); mobile phase was 5 mmol / L ammonium formate solution as the aqueous phase (containing 0.1% formic acid), methanol as the organic phase, gradient elution, with flow rate of 0.4 mL · min^{-1}. The injection volume was 10 μL, temperature of column was 35℃. Mass spectrometer with an electrospray ionization source interface operated in the positive ion of MRM mode was used for LC-MS / MS analysis. The product ions were 311.10→158.10*, 141.10 and 317.10→ 158.10*, 141.10 for DFB and internal standard, respectively. The LOD and LOQ for DFB using this method was 0.5 ng·mL^{-1} and the 1 ng·mL^{-1}, respectively. The method linearity range was 1-1000 ng· mL^{-1}, R^2=0.9959; the matrix effect was 97.57%~113.88%. The method′s accuracy was between 85% and 120%, the intra-assay coefficient of variation was 3.83% to 9.26%, and the inter-assay coefficient of variation was 1.95% to 10.07%. Good stability was observed in all tests. The above results met the Guidelines' requirements for the Verification of Quantitative Analysis Methods for Biological Samples in Appendix 9012 of the Chinese Pharmacopoeia and create a foundation for the pharmacokinetic study of diflubenzuron premix in swine.

Key words: Diflubenzuron; Swine; Plasma; LC-MS/MS; PK
Corresponding author: Ya-Jun Yang, E-mail: yangyue10224@163.com; Jian-Yong Li, E-mail: lijy1971@163.com

T19-98-0060

Method development for rifaximin and rifampicin residues in foodstuffs of animal origin

Li-Ping Fan, Zhe Qin, Xi-Wang Liu, Shi-Hong Li, Li-Xia Bai, Ya-Jun Yang*, Jian-Yong Li*

(Key Lab of New Animal Drug Project of Gansu Province, Key Lab of Veterinary Pharmaceutical Development of Ministry of Agriculture and Rural Affairs, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Lanzhou 730050, China)

Abstract: Both rifaximin and rifampicin are semi-synthetic rifamycin antibacterial drugs with strong antibacterial activity and broad antibacterial spectrum. In 2005, the Ministry of Agriculture Announcement No. 560 prohibits the use of rifamycins and their salts in veterinary clinic. Rifaximin perfusion preparations have been approved in veterinary clinic for dry period mastitis prevention, lactating mastitis and endometritis treatment. In 2010 (EU) No 37 / 2010, only the maximum residue limit of rifaximin in milk was 60μg/kg. No regulations on residue limits of rifampicin and rifaximin in other animal tissues. In China, only SN/T 2224-2008 was employed for detection and confirmation of rifaximin residues in pork, pig liver, pig kidney, and milk. There is no uniform standard method for rifampicin residue detection. The published papers mainly focused on the detection of aquatic product residues of rifampicin. At present, there is no simultaneous detection method for rifampicin and rifaximin residues in animal foods. Rifaximin and rifampicin with good antibacterial activity, so the possibility of illegal use is not ruled out. It is necessary to establish detection methods for rifaximin and rifampicin residues in animal foods and formulate corresponding standards to monitor the related residues in animal foods. This study aims to establish a liquid chromatography-tandem mass spectrometry method for detection of rifaximin and rifampicin residues in animal foods. The edible animal tissues were crushed, homogenized, and cryopreserved. The samples were thawed at room temperature before testing. The sample of 2.0±0.02 g was accurately weighed in a 50 ml plugged plastic centrifugal tube, then spiked with internal standard working solution of rifaxmin-d6 and rifampicin-d4. The extraction solution of 10ml ACN-DCM (6+4)(ACN for milk and fat) were added into tubes, and mixed with vortexer for 1 min. Then, 50 mg vitamin C and 2.0±0.05g anhydrous sodium sulfate were added, followed vortex mixing for 1 min. The mixture was extracted using an ultrasonic water bath at room temperature for 10 min, and mixed with a vortexer for 1 min. The system was centrifuged at 4500 rpm for 10 min at 4°C. The supernatant transferred into another centrifuge tube. The residue was re-extracted with 5 ml extraction solution, then combined the supernatants, and dried under nitrogen gas at 40°C water bath. The residue was dissolved with 2 ml of ACN, then 50 mg PSA and 50 mg C18 were added. The mixture was vortexed mixing for 1 min and centrifuged. The supernatant was determined by LC-MS/ MS after filtering through a syringe filter with pore size 0.22 μm. Mass spectrometer with an electrospray ionization source interface operated in the positive ion of MRM mode was used for LC-MS / MS analysis. The internal standard method was used for quantification by calibration curve of standard solutions. The method was suitable for muscle, liver, kidney, fat, milk and eggs of swine, cattle and chicken, as well as fish and shrimp. LOD and LOQ were 5 μg/kg and 10 μg/kg for both of analytes. The average recoveries of rifaxmin and rifampicin in different foodstuffs of animal origin were between 85% and 115%, the method precision were below 10% (RSD). The proposed method showed good performance for determination.

Key words: rifaxmin; rifampicin; residue

Corresponding author: Ya-Jun Yang, E-mail: yangyue10224@163.com; Jian-Yong Li, E-mail: lijy1971@163.com

T19-98-0061

Investigation of the uptake and transport of aspirin eugenol ester in Caco-2 cells model

Qi Tao, Zhen-Dong Zhang, Zhe Qin, Xi-Wang Liu, Shi-Hong Li, Li-Xia Bai, Ya-Jun Yang, Jian-Yong Li

(Key Lab of New Animal Drug Project of Gansu Province, Key Lab of Veterinary Pharmaceutical Development of Ministry of Agriculture and Rural Affairs, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Lanzhou 730050, China)

Abstract: Aspirin eugenol ester (AEE) is a new medicinal compound synthesized by esterification of aspirin with eugenol using the prodrug principle. AEE has anti-inflammatory, antipyretic, analgesic, anti-cardiovascular diseases, and anti-oxidative stress pharmacological activity. However, its oral bioavailability is poor, and its intestinal absorption and transport characteristics are still unknown. The purpose of this study is to investigate the uptake and transport mechanism of AEE in human intestinal Caco-2 cells. AEE was proved to be non-toxic to Caco-2 cells when its concentration is less than 256 $\mu mol \cdot L^{-1}$. Because higher concentrations of such AEE metabolite as salicylic acid were detected in the supernatant of the cell lysate and cell culture fluid while AEE could be not detected, the change in the content of salicylic acid was used to indicate the change in the content of AEE. The result showed the uptake and transport of AEE were related to time, concentration, and temperature. In the uptake experiment, the uptake of SA reached the maximum at 30 min when Caco-2 cells were incubated with 64 $\mu mol \cdot L^{-1}$ AEE from 0 to 120 min. Low temperature can significantly reduce the uptake of SA in Caco-2 cells. In the transport experiment, the concentration of AEE in the Caco-2 cell monolayer was constant and the transport volume increases with time within 120 minutes. The results showed that the transepithelial transport of salicylic acid from the AP-BL and BL-AP sides was time-dependent. Interestingly, the transport amount of salicylic acid in Caco-2 cells increases with the increase in concentration, but transmembrane was no certain correlation between transport rate and concentration. The apparent permeability coefficient (Papp) of low concentration was higher than that of high concentration Papp, which may be the saturation phenomenon of high concentration. The apparent permeability coefficient (Papp) of aspirin eugenol ester (64 $\mu mol \cdot L^{-1}$) from AP to BL was determined to be 0.034 ($\pm$0.012) $\times 10^{-6}$ cm/s, which was considered to have poor permeability and absorptive *in vivo* rate. Efflux ratio (ER)<1, indicating that their intestines transport mechanism was passive transport. In addition, the temperature had a significant effect on the transport of aspirin eugenol ester. In summary, the results indicated that the intestinal absorption of AEE through the Caco-2 cell monolayer involves passive transport. This experiment illustrates the absorption and transport properties of AEE. All these results may help to explore the mechanism of chemically synthesized drugs *in vitro* absorption and transport.

Key words: Aspirin eugenol ester; Metabolite; Caco-2 cells; Uptake; Transport

Corresponding author: Ya-Jun Yang, E-mail: yangyue10224@163.com; Jian-Yong Li, E-mail: lijy1971@163.com

T19-98-0062

Exploring the mechanism of curcumin on liver necroptosis in chickens induced by AFB1 based on ceRNA network

Sihong Li[a,b], Yixin Zhang[b], Ruimeng Liu[b], Gaoqiang Wei[b], Xiuying Zhang[a]

(a. Heilongjiang Key Laboratory for Animal Disease Control and Pharmaceutical Development. Faculty of Basic Veterinary Science, College of Veterinary Medicine, Northeast Agricultural University, Harbin 150000, China; b. Animal Genome Engineering Research Team, College of Animal Science and Technology, Zhejiang Agriculture &Forestry University, Hangzhou 311300, China)

Abstract: Aflatoxin B1 (AFB1) is a potent hepatotoxic and carcinogenic agent. Curcumin possesses potential anti-inflammatory, anti-oxidative and hepatoprotective effects. However, the role of LncRNAs in the protective mechanisms of curcumin against AFB1-induced liver damage is still elusive. Necroptosis is a newly discovered form of cell death in recent years. At present, the mechanism of curcumin on liver injury in chickens induced by AFB1 based on ceRNA regulatory network from the perspective of necroptosis and inflammation has not been reported. 1-day-old AA broilers were used as experimental animals. Based on the cases of established liver injury model induced by AFB1 exposure and curcumin intervention model both *in vivo* and *vitro*, as well as the model of LOC769044, miR-1679 and target STAT1 inhibition and overexpression LMH cell line in vitro. Transmission electron microscopy, RNA-seq, bioinformatics analysis tools, RT-PCR and western blot were performed. Dietary AFB1 caused hepatic morphological injury, and significantly increased the increased the expression levels of necroptosis related genes (RIPK1, RIPK3, MLKL), inflammatory pathways (TLR4, myd88, NF-κB) and cytokines (TNF-α, iNOS, IL-6, IL-1β). While, curcumin intervention significantly alleviated AFB1-induced broiler liver necroptosis and inflammation. Transcriptomic analysis showed that LOC769044 is the potential target LncRNA and located in the cytoplasm. ceRNA network prediction, dual luciferase reporter gene assay results showed that LOC769044 could bind to miR-1679, and miR-1679 could bind to STAT1. Inhibition and overexpression of LOC769044, miR-1679 and target STAT1 experiments indicated that LOC769044 / miR-1679 / STAT1 network plays an important role in regulating necroptosis in chicken hepatocytes, and curcumin can effectively alleviate AFB1-induced necroptosis in chicken hepatocytes by targeting LOC769044 / miR-1679 / STAT1 network. This study analyzed the possibility of LOC769044 as a new target for the prevention and treatment of AFB1-induced liver injury, and reveals the role of LOC769044 / miR-1679 / STAT1 regulatory network in curcumin against AFB1-induced liver necroptosis and its underlying molecular mechanism.

Key words: Aflatoxin B1; curcumin; broiler liver; ceRNA; necroptosis

Corresponding author: Xiuying Zhang, E-mail: lisihong@zafu.edu.cn

T19-98-0063

The exposure pathway and toxicity mechanism of microplastics

Sihong Li, Dong Niu, Xing Duan, Lu Liu, Xiang Ma

(Animal Genome Engineering Research Team, College of Animal Science and Technology, Zhejiang Agriculture &Forestry University, Hangzhou 311300, China)

Abstract: The environmental pollution problem of microplastics is becoming more and more serious with the widespread application of plastic products, which has attracted the attention of researchers. Plastic in the environment can be degraded to a particle size <5 mm under the action of physical erosion or biodegradation, known as microplastics (MPs). Oral administration, respiratory inhalation and contact with skin are the three important pathways of microplastics into the body. Microplastics can accumulate in humans body through the food chain, such as eating microplastics polluted fish, shrimp

and shellfish or drinking bottled water containing microplastics. Additionally, micro plastic particles floating in the air can enter the body through the respiratory tract. Although few microplastics enter the body through the skin, the microplastic particles in daily skin care products also have a potential impact on skin.

The plastic particles less than 20 μm can enter the viscera, and the nano-plastic particles less than 0.1 μm can even penetrate the cell membrane, blood brain barrier, blood testosterone barrier, placental barrier, and so on, and accumulate and remain in the organ, resulting in varieties of toxicity. 1) genetic toxicity: juvenile fish exposed to polyethylene microplastics showed more significant external morphological changes and higher malformation rate, suggesting that polyethylene microplastics have teratogenicity to juvenile fish; 2) immunotoxicity: microplastics can stimulate the secretion of IL-6 and IL-8, induce the peroxidation of granulophils, and significantly increase the proportion of neutrophils and IgA concentration in blood; 3) hepatotoxicity: exposure to 1000 $\mu g \cdot L^{-1}$ PS for five weeks in the digestive tract of mice, the levels of triglyceride and total cholesterol in the liver decreased, suggesting that the lipid metabolism in the liver of mice was disturbed; 4) gastrointestinal toxicity: studies have shown that microplastics can change the composition of intestinal flora in adult male mice, and affect the main metabolic pathways of bacterial functional genes, resulting in changes in the diversity of intestinal flora, leading to metabolic disorders in mice; 5) Respiratory toxicity: CT examination of polypropylene flocking industry workers showed peribronchial thickening and diffuse frosted hyaline, suggesting lung interstitial lesions. The research on microplastics is in its initial stage, further research is needed for its absorption, distribution, metabolism and excretion, as well as the specific toxic mechanism of microplastics and the combined toxicity with other environmental pollutants, which can provide effective basis for protecting the environment and human health.

Key words: microplastics; exposure pathway; toxicity mechanism

Corresponding author: Sihong Li, E-mail: lisihong@zafu.edu.cn

T19-98-0064

Guideline for environmental risk assessment of veterinary drugs in China

Wu Yi-zhao, Deng Song-ge, Li Yin-sheng

(School of agriculture and biology, Shanghai Jiao Tong University)

Abstract: Ecotoxicity data is required during the declaration of some veterinary drugs, but there is no unified environmental risk assessment guideline for veterinary drugs in China. Meanwhile, due to the great differences in environment and species composition, the overseas standards may not be suitable for China.

In this situation, we published the group standard Guidelines for environmental risk assessment of veterinary drugs. Based on the EIA′ s for VMP′ s of VICH, some changes have been made in the guideline: (1) 19 questions of EIA′ s for VMP′ s PHASE I of VICH are concluded into two stages. In pre-evaluation phase, it is determined that whether the evaluation is necessary. Emission is assessed and PEC is calculated in Phase I. Besides, veterinary drugs only used in non-food animals are suggested to be evaluated since the PEC may exceed 100 $\mu g \cdot kg^{-1}$ in the soil in consideration of the large number of pets in urban agglomerations. (2) Chinese national standards are also added in this guide, such as GB/T 27856-2011 for the test of aerobic and anaerobic transformation in soil, to meet with the actual environment situation of our country. (3) For some tests that only have foreign standards, the local indicator species, which are also easy to culture in the laboratory, are suggested to be the test objects. For ex·

ample, in the acute toxicity test of marine crustaceans, Tigriopus japonicus is recommended, which is widely distributed in East Asia and has been treated as an indication of pollution in many researches.
(4) In the guidelines of VICH, there are a few tests without standards and methods, and some articles are listed as references in this guide. For example, in the grade B toxicity test of terrestrial organism, earthworm behavior tests, both traditional and 2D behavior tests included, are proposed to be an index of further toxicity evaluation on terrestrial organisms. According to numerous researches, these tests are sensitive and reliable to show the toxicity of veterinary drugs. Now, this group standard has been issued by Shanghai Toxicology Society.

Key words: veterinary drug; environmental risk assessment ; ecotoxicity ;

Corresponding author: Li Yin-sheng, E-mail: yinshengli@sjtu.edu.cn

T19-98-0065

The relationship between deltamethrin-induced behavioral changes in zebrafish Embryos/Larva and AchE

Liu Chun-yu, Niu Jie-yao, Yang Jing-feng, Dong Wu, Xue Jiang-dong*
(School of Animal Science and Technology, Inner Mongolia Minzu University, Key Laboratory of Toxicological Monitoring and Toxicology of Inner Mongolia Autonomous Region, InnerMongolia, Tongliao 028000)

Abstract: Deltamethrin (DM) is a type II synthetic pyrethroid. It is a broad-spectrum insecticide and is widely used for the prevention of agricultural pests. In this study, zebrafish embryos were used as experimental animals to explore the neurotoxicity of deltamethrin. Zebrafish embryos 4 hours (4 hpf) after fertilization were exposed to deltamethrin, the frequency of zebrafish curling was recorded at 24 hpf, the behavioral changes of zebrafish were detected at 120 hpf, and the activity of acetylcholinesterase was detected. The results of the study showed that DM caused pericardial edam of zebrafish, inhibit the development of swim bladder, curvature of the spine, convulsions and other morphological changes. DM below 50 $\mu g \cdot mL^{-1}$ did not cause the death of zebrafish embryos (the survival rate was 100%), At 24 hpf, 0.5 $\mu g \cdot mL^{-1}$ DM increased zebrafish coiling frequency by 62.32% compared with the control group. 5 and 50 $\mu g \cdot mL^{-1}$ DM reduced the coiling frequency of zebrafish. When the embryo develops to 120 hpf, 0.5 and 5 $\mu g \cdot mL^{-1}$ DM will increase the swimming distance of zebrafish. When the concentration reaches 50 $\mu g \cdot mL^{-1}$, the swimming distance will also decrease. The results of AchE activity showed that 0.5 and 5 $\mu g \cdot mL^{-1}$ DM increased the AchE activity of zebrafishlarva by 25.3% and 55.84%. The concentration of 50 $\mu g \cdot mL^{-1}$ also caused a decrease in AchE activity, indicating that deltamethrin causes behavioral changes in zebrafish larva, which may be closely related to the increase in acetylcholine induced by DM, and the experiment results also suggest the behavior of zebrafish embryos scientific changes may be used as biological monitoring of pesticides.

Key words: Deltamethrin; Zebrafish; Behavioral changes; Acetylcholinesterase activity

Corresponding author: Xue Jiang-dong, E-mail: xuejiangdong@hotmail.com

T19-98-0066

Methuosis: an excessive cytoplasm vacuolization related cell death mediatesmaduramicin-induced cardiotoxicity

Gao Xiu-ge, Ji Chun-lei, ZhengYu-ling, GuoDawei, Peng-Lin, Ji Hui, Jiang Shan-xiang
(College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, Jiangsu)

Abstract: Maduramicin frequently triggers severe cardiotoxicity in domestic animals as well as human accidentally. Apoptotic and non-apoptotic cell death contribute to the serious myocardial damage in clinic. The mechanisms underlying maduramicin-induced non-apoptotic cell death remain largely unknown. In present study, we aimed to elucidate the morphological characteristics and regulatory mechanisms of cytoplasmic vacuolization related cell death induced by maduramicin. H9c2 cells and primary chicken myocardial cells, as well as broiler chickens and rats were used as experimental models. We found that maduramicintriggered numerous phase-lucent vacuoles, which occupied large area of cytoplasm in primary and passage myocardial cells. The cytoplasmic vacuoles induced by maduramicin were generated in a concentration and time dependent manner, along with increased cell death rate. Furthermore, maduramicin-induced cytoplasmic vacuoles are generated from micropinocytosis, rather than the swelling of mitochondria, lysosome, endoplasmic reticulum and Golgi apparatus, demonstrating by the internalization of Dextran-AF 488 into myocardial cells. Intriguingly, the vacuoles acquired some characteristics of late endosomes and lysosomes rather than early endosomes and autophagosomes. Vacuolar H^+-ATPase inhibitor bafilomycin A1 efficiently prevented the generation of cytoplasmic vacuoles and decreased the cardiotoxicityinduced by maduramicin. Mechanism studying indicated that maduramicin activated H-Ras-Rac1 signaling pathway. However, the pharmacological inhibition and siRNA knockdown of Rac1 rescued maduramicin-induced cytotoxicity of H9c2 cells but did not alleviate cytoplasmic vacuolization. Moreover, maduramicin-induced methuosis was confirmed in rats and chickens with extensive vacuolar degenerationof myocardium. Proteomic analysis further indicated differentially expressed proteinswere significant enriched in SNARE interactions in vesicular transport, ECM-receptor interaction, PPAR signaling pathway, cell adhesion, regulation of actin cytoskeleton and Jak-STAT signaling pathway. In summary, methuosis, a novel non-apoptotic cell death, mediatesmaduramicin-induced cardiotoxicity in noncanonical programmed pathways.

Key words: Methuosis; Nonapoptosis; Cardiotoxicity; Cytoplasmic vacuolization; Cell death

Corresponding author: Jiang Shan-xiang, E-mail: nauvy@sina.com

T19-98-0067

Rac1-independent methuosis mediates maduramicin-induced cardiotoxicity

Zheng Yu-ling, Ji Chun-lei, Wang Jun-qi, Ji Hui, Guo-Dwei, Jiang Shan-xiang, Gao Xiu-ge
(College of Animal Medicine, Nanjing Agricultural University, Nanjing, Jiangsu 210095)

Abstract: Maduramicin often causes severe cardiotoxicity in target and nontarget animals. Apoptotic and non-apoptotic cell death mediate the cardiotoxicity induced by maduramicin. The effect and mechanism of non-apoptotic cell death are largely unknown. To understand the role of non-apoptotic cell death in maduramicin-induced cardiotoxicity, the rat cardiomyocytes (H9c2) were used as an in vitro model in present study. We found maduramicin induced a large number of cytoplasm vacuoles in

H9c2 cells, which were time-dependent and concentration-dependent. The vacuoles were phase-lucentcoated with monolayer membrane. In a certain time range, the vacuolation of H9c2 cells induced by maduramicin is reversible. Organelle staining showed that the cytoplasmic vacuoles induced by maduramicin did not originate from the swelling of mitochondria, endoplasmic reticulum and Golgi apparatus, but there was partial co-localization between vacuoles and lysosomes. The dextran uptake assay indicated that the cytoplasmic vacuoles mainlygenerated from macropinocytosis. However, there was no obvious co-localizationbetweenmaduramicin-induced cytoplasm vacuoles and early endosome marker protein EEA1, late endosome marker protein Rab7, lysosome marker protein LAMP1 as well as autophagy marker protein LC3B. H-Ras and Rac1 were significantly upregulated (P<0.05 or P<0.01) after maduramicin treatment for 24 h-72 h, along with the significant increased expression of activated Rac1. After silencing Rac1 gene, the death rate of H9c2 cells was significant reduced, but the number of cytoplasm vacuolesdid not decrease. These findings demonstrate a non-apoptotic cell death type methuosis mediates mduramicin-induced severe cardiotoxicity in Rac1-independent way.

Key words: Nonapoptotic cell death; Maduramicin; Methuosis; Cardiotoxicity

Corresponding author: Gao Xiu-ge, E-mail: vetgao@njau.edu.cn

T19-98-0068

MicroRNA-145-5p suppresses the progression of breast cancer via inhibiting SENP2-ERK2signaling

Xu Chen, Yuanyuan Qin, Hong Yuan, Qiqi Wang, Congcong Du, ZhenzhenZhang, Wenbin Lu, Yitao Qi, Hongmei Wu

(College of life science, Shaanxi Normal University, Shaanxi Xi'an 710119, China)

Abstract: Breast cancer remains a public-health issue on a global scale and a serious threat to human health due to the highly metastatic which result in remarkable short survival and poor prognosis. Therefore, the search for new therapeutic targets has always been the hot spot and leading-edged researchin breast cancer treatment. SENP2 is a SUMO-specific protease that is involved in the maturation of SUMO proteins and maintained the homeostasis of substrate proteins SUMOylation status. SENP2 has been demonstrated to regulate tumor progression in a variety of cancers, but its role in breast cancer has been rarely reported yet. In this report we aim to explore the molecular mechanism of SENP2 regulation role in breast cancer procession, hoping to provide new horizon for cancer treatment. **Methods and Results** We explored the public bioinformatic data combined withfurtherassay indicated that SENP2 is essential and highly expressed in breast cancer. The molecular and biochemistry assay showed that SENP2 acted as acarcinogenic factorpromoting the proliferation,migration and invasion of breast cancer.Further exploration identified that ERK2 activity was upregulated by SENP2 in breast cancer. Then we proved that ERK2 is SUMOylated by SUMO2 at K99 and K272 in vivo and vitro and is deSUMOylated by SENP2. Interestingly, further experimental evidences determined that ERK2 SUMOylation inhibited the proliferation and migration of breast cancer cells, indicating that SENP2-ERK2 signaling is essential for breast cancer procession. Furthermore, microRNA-145-5p is lowly expressed in breast cancer, it bound to 3′-untranslated region of SENP2 mRNA to regulate the SENP2 expression. MicroRNA-145-5p was predicted and confirmed as a direct upstream regulator of SENP2 in breast cancer cellsthrough inhibition of SENP2-ERK2 axis. **Conclusion and significance** In conclusion, we explored the molecular mechanism of SENP2 affecting the growth and metastasis of breast

cancer, and further confirmed its upstream microRNA-145-5p and downstream ERK2. We identified the oncogenic role of SENP2 in breast cancer and identified microRNA-145-5p as upstream regulator of SENP2.Moreover, we further confirmed the SUMOylation of ERK2 and its critical roles in promoting the growth and metastasis of breast cancer. In conclusion, the study showed the regulatory role of microR·NA-145-5p / SENP2 / ERK2 axis in breast cancer, providing a new horizon for the further treatment of breast cancer.

Key words: Breast cancer; SENP2; ERK2; microRNA-145-5p

Corresponding author: Hongmei Wu, E-mail: Hq8479@snnu.edu.cn

T19-98-0069

Levodopa inhibits *Salmonella enterica* serovar Typhimurium infection by targeting the type III secretion system

Jingyan Shu, Hongtao Liu, Qianghua Lv, Jianfeng Wang, Xuming Deng, Jiazhang Qiu*

(Key Laboratory of Zoonosis, Ministry of Education, College of Veterinary Medicine, Jilin University, Changchun, 130062, China)

Abstract: Salmonella enterica serovar Typhimurium (*S.* Typhimurium) is a zoonotic pathogen that can cause food poisoning and diarrhea in both humans and animals worldwide. The *Salmonella* pathogenicity island (SPI) genes encoded type III secretion system (T3SS) is important for S. Typhimurium invasion and replication in host cells. Due to the increasing problem of antibiotic resistance, antibiotic treatment for clinical *Salmonella* infection has gradually been limited. Anti-virulence inhibitors are a promising alternative to antibiotics because they do not easily induce bacterial antibiotic resistance. Here, we systematically evaluated the therapeutic effect of Levodopa on *Salmonella*-infected mice and elucidated its anti-infection mechanism. Levodopa treatment improved the survival rate of S. Typhimurium-infected mice and alleviated cecum pathological lesions. In addition, Levodopa inhibited S. Typhimurium invasion to HeLa cells without affecting their growth. Further studies showed that Levodopa could inhibit the expression of *sipA* and *sipB*. This inhibition may be implemented by inhibiting the transcription of key regulatory and structural genes of the T3SS. This study provides an alternative anti-virulence strategy for *Salmonella* infection treatment.

Key words: *Salmonella* typhimurium; Type III secretion system; Levodopa; Anti-virulence agent;

Corresponding author: Jiazhang Qiu, E-mail: qiujz@jlu.edu.cn

T19-98-0070

Effects of florfenicol combined with copper on biofilm formation and nitrogen regulatory gene expression in rhizobia

Mei Wang, Tong Zhou, Yi Liang, Fulin Li, Yongxue Sun*

(a. The Guangdong Provincial Key Laboratory of Veterinary Pharmaceutics Development and Safety Evaluation, South China Agricultural University, Guangzhou, China; b. National Laboratory of Safety Evaluation (Environmental Assessment) of Veterinary Drugs, South China Agricultural University, Guangzhou, China; c. National Risk Assessment Laboratory for Antimicrobial Resistance of

Animal Original Bacteria, South China Agricultural University, 483 Wushan Road, Guangzhou 510642, China)

Abstract: Nitrogen fixation mediated by nitrogen-fixing bacteria is the main nitrogen input pathway in soil ecosystem. Antibiotics and heavy metal residues may affect nitrogen-fixing bacteria. In this study, the rhizobia were isolated from the rhizosphere and identified as Rhizobium pusense strain RpEC2071 by morphological observation, carbon source utilization, 16S rDNA sequencing and whole genome sequencing. Florfenicol and copper were selected respectively to evaluate their effects on rhizobia. Blank group (0 $\mu g \cdot mL^{-1}$), florfenicol group (40 $\mu g \cdot mL^{-1}$), copper group (200 $\mu g \cdot mL^{-1}$) and florfenicol mixed with copper sulfate group (40 $\mu g \cdot mL^{-1}$+200 $\mu g \cdot mL^{-1}$) were set respectively. After treatment, samples were collected at 120 h, 132 h, 144 h, 156 h, 168 h to determine the contents of extracellular polysaccharide and biofilm, and the mRNA expression levels of nitrogenase structure gene (*nifH*), nitrogen metabolism regulation genes (*ntrY*, *ntrX*, *glnK*, *nnrR*) and biofilm-related genes (*flaF*, *fliL*, *flhA*, *fliQ*) in each group. The results showed that florfenicol and copper alone promoted the formation of biofilms while mixed treatment inhibited that. The change of extracellular polysaccharide content was consistent with biofilm formation ability. Correlation analysis showed that biofilm-related genes (*flaF*, *fliL*, *flhA*, *fliQ*) were significantly positively correlated with nitrogen metabolism regulation gene (*ntrX*) (r=0.691~0.775, $P<0.01$). Copper treatment significantly increased the expression of *nifH*, while florfenicol treatment promoted the expression of *fliQ*. In addition, mixed treatment had significant antagonistic effect on the expression of *fliQ* gene ($P<0.05$), but there was a significant synergistic effect on the expression of nnrR gene ($P<0.05$).

Key words: Rhizobia; Florfenicol; Copper sulfate; Soil

Corresponding author: Yongxue Sun, E-mail: sunyx@scau.edu.cn

T19-98-0071

A proposed "steric-like effect" for the slowdown of enrofloxacin antibiotic metabolism by ciprofloxacin, and its mechanism

Xu Xiao-qing, Lu Qirong Yang Ya-qin, Wang Xu*

(National Reference Laboratory of Veterinary Drug Residues, Huazhong Agricultural University, Wuhan, Hubei 430070, China)

Abstract: The results of monitoring over the years have shown that the mixing and coexistence of various low-level antibiotic residual pollutants has increased significantly, among which, the problems of enrofloxacin (ENR) and ciprofloxacin (CIP) were more prominent. At present, research studies on the metabolism of ENR or CIP are focused on the individual drugs, and there is no relevant research reporting on the effect of the combination of the two antibiotics on the metabolism of ENR. This research study evaluated the effect of CIP on ENR metabolism in pigs and its mechanism *in vivo* and *in vitro*. The resultsshowed that CIP changed the pharmacokinetics of ENR through the inhibition of CYP3A29 and the "steric-like effect" of ENR binding to CYP3A29, which increased the residual concentration of ENR in pigs, a result that requires an extension of the withdrawal period. In order to ensure human health, the combined use of these two drugs, CIP and ENR, must be avoided in veterinary medicine in food producing animals.

Key words: ENR; CIP; CYPA29; Steric-like effect; Withdrawal time

Corresponding author: Wang Xu, E-mail: wangxu@mail.hzau.edu.cn

T19-98-0072

The effect of endotoxinon the mucus layer on the intestinal in no- and post-pregnancy mice

Liyan An, Mingming Zhang, Baoyu Zhao, Chenchen Wu

(College of Veterinary Medicine, Northwest A & F University, Yangling, Shaanxi, People's Republic of China 712100)

Abstract: The intestine is the most extensive storage of bacteria and endotoxins, and the mucosal immune system is the first barrier of intestinal endotoxin invasion. The MUC2 (mucin2) is the major component of the mucus layers. In this study, we explored that MUC2 plays a role in how LPS invade the fetus from the gut to the uterus in pregnant mice. The results showed that the LPS level of ileal, colonic and uterine was significantly increased, and the content of sIgA in the ileum, colon and uterus tissues was significantly decreased in the LPS(+) group on the 35th day after LPS treatment. On the 16th day of pregnancy, compared with the LPS(-) group, the level of ileal LPS was significantly decreased, and the content of LPS in the fetus was significantly increased in the LPS(+) group. There was no significant difference in the levels of LPS in the uterus and placenta, and the sIgA content in the fetus was significantly decreased. The expression of MUC2 in the uterus, ileum and colon was increased significantly in the LPS(+) group, especially in the uterus. It is suggested that the endotoxins accumulated in the uterus during non-pregnancy. The high expression of MUC2 in the uterus can prevent LPS from translocating into uterus tissue. It revealed that, after pregnancy, MUC2 still performs the function of protecting the uterine tissue, allowing a large number of LPS to enter the fetal body through blood circulation. Therefore, the level of sIgA significantly decreased, resulting in a decline of the fetal innate immune function.

Key words: Lipopolysaccharide; MUC2; Intestines; Uterus; Fetus

Corresponding author: Chenchen Wu, E-mail:wucen95888@163.com

T19-98-0073

Safety evaluation ofrelieve cough and asthma extract

Zhang Aoxue, Chen Zhen, Xie shuyu*

(National Reference Laboratory of Veterinary Drug Residues (HZAU) and MAO Key Laboratory for Detection of Veterinary Drug Residues, Wuhan, Hubei 430070, China)

Abstract: OBJECTIVE To evaluate the acute toxicity, subchronic toxicity and safety pharmacolo·gyof relieve cough and asthma extract, and provide reliablepharmacological and toxicological basis for further clinical trials and clinical application.MethodsThe acute toxicity of relieve cough and asthma ex·tract to Kunming mice was studied by one-time oral gavage;Then, according to LD50, SD rats were giv·en high, medium and low doses of relieve cough and asthma extract by gavage continuously for 30

days. By observing its clinical manifestations, blood physiological and biochemical indicators and histological structure, the toxic and side effects of the extracts for relieving cough and asthma are studied. High, medium and low dose were given to SD rats by gavage for 5 days, and observe its effects on the central nervous system, cardiovascular system and respiratory system to study its potential adverse reactions. Results The LD_{50} of relieve cough and asthma extract was 132.13 g·kg^{-1}, and the 95% confidence interval was 116.95 g·kg^{-1}–169.04 g·kg^{-1}. According to the acute toxicity classification standard, relieve cough and asthma extract is a non-toxic drug. After 30 days of continuous intragastric administration at the dose of 1.80 g·kg^{-1}–7.22 g·kg^{-1}, it was found that the intake of food, drinking water and weight gain of the experimental group were normal, and it contributed to the weight gain of the rats. Blood routine examination showed that relieve cough and asthma extract had no effect on the blood system and bone marrow hematopoietic function of SD rats. The observation of blood biochemical parameters and pathological sections showed that relieve cough and asthma extract had certain effects on the kidney of rats, but the damage to its cells was reversible, and it could return to normal after stopping administration.The dose of 7.22 g·kg^{-1}.bw and below would not produce obvious toxic reactions to the body of animals. At the dose of 3.31 g/kg ~ 13.21 g·kg^{-1}, there was no significant difference in respiratory rate and heart rate between different experimental groups (P>0.05), and compared with the control group, the difference was not significant (P>0.05), which indicated that relieve cough and asthma extract had no significant effect on the central nervous system, respiratory system and cardiovascular system of SD rats. Conclusionrelieve cough and asthma extract is a safe and effective oral treatment for cough and asthma in animals.

Key words: relieve cough and asthma extract; acute toxicity; safety pharmacology

T19-98-0074

The relationship between deltamethrin-induced behavioral changes in zebrafish Embryos/Larva and AchE

Liu Chun-yu, Niu Jie-yao, Yang Jing-feng, Dong Wu, Xue Jiang-dong*

(School of Animal Science and Technology, Inner Mongolia Minzu University, Key Laboratory of Toxicological Monitoring and Toxicology of Inner Mongolia Autonomous Region, InnerMongolia, Tongliao 028000)

Abstract: Deltamethrin (DM) is a type II synthetic pyrethroid. It is a broad-spectrum insecticide and is widely used for the prevention of agricultural pests. In this study, zebrafish embryos were used as experimental animals to explore the neurotoxicity of deltamethrin. Zebrafish embryos 4 hours (4 hpf) after fertilization were exposed to deltamethrin, the frequency of zebrafish curling was recorded at 24 hpf, the behavioral changes of zebrafish were detected at 120 hpf, and the activity of acetylcholinesterase was detected. The results of the study showed that DM caused pericardial edam of zebrafish, inhibit the development of swim bladder, curvature of the spine, convulsions and other morphological changes. DM below 50 μg·mL^{-1} did not cause the death of zebrafish embryos (the survival rate was 100%), At 24 hpf, 0.5 μg·mL^{-1} DM increased zebrafish coiling frequency by 62.32% compared with the control group. 5 and 50 μg·mL^{-1} DM reduced the coiling frequency of zebrafish. When the embryo develops to 120 hpf, 0.5 and 5 μg·mL^{-1} DM will increase the swimming distance of zebrafish. When the concentration reaches 50 μg·mL^{-1}, the swimming distance will also decrease. The results of AchE activity

showed that 0.5 and 5 μg·mL^{-1} DM increased the AchE activity of zebrafishlarva by 25.3% and 55.84%. The concentration of 50 μg·mL^{-1} also caused a decrease in AchE activity, indicating that deltamethrin causes behavioral changes in zebrafish larva, which may be closely related to the increase in acetylcholine induced by DM, and the experiment results also suggest the behavior of zebrafish embryos scientific changes may be used as biological monitoring of pesticides.

Key words: Deltamethrin; Zebrafish; Behavioral changes; Acetylcholinesterase activity

Corresponding author: Xue Jiang-dong, E-mail: xuejiangdong@hotmail.com

T19-98-0075

Safety evaluation of ponazuril suspension in target animal sucklingpiglets

WANG Zhen[a], XU Qinse[b], ZHANG Wei[b], GAO Yanjun[a], NIE Ya[a], HUO Haoyuan[a]
XIAO Wenhua[b], PU Shijin[a]

(a. College of Veterinary Medicine,Yangzhou University, Yangzhou 225000, China;
b. Jiangsu Agri-animal Husbandry Vocational College, Taizhou 225300, China)

Abstract: purpose To study the safety of ponazuril suspension on target piglets, and to provide basis for clinical safe drug use. METHOD 32 sucking piglets aged 3 ~ 5 day-old were divided into 4 groups with 8 piglets (half male and half female) in this experiment. Swine safety studies were performed with multiple dose levels of the drug given. The dose levels of 5% ponazuril suspension included 0 (for the control group), 1×(15 mg·kg^{-1} for test group Ⅰ), 3×(45 mg·kg^{-1} for test group Ⅱ), and 5× times (75 mg·kg^{-1} for test group Ⅲ). The tested drug wasorally administered 3 consecutive times with each interval of 3 days. The control group was given 5 ×, the maximum recommended dose of normal saline. The experiment lasted for 17days; during which periodic weighting, clinical observation, and physical examinations were performed; hematology, blood and serum chemistries, as well as gross autopsy and histopathological examination were carried out in predetermined periods of time. RESULT The results showed that, until the end of the trial, there had been no death of the piglets, all of which were clinically normal. No pathologic lesions were found at autopsy for all the test animals. Thehistopathological examinations in the 5 × recommended dose group and the control group did not identify anyabnormal tissue lesions. CONCLUSION The oral administration of 5% ponazuril suspension was of greater safety for piglets according to the recommended dosing schedule.

Key words: ponazuril suspension; target animal safety; suckling piglets

Corresponding author: PU Shijin, E-mail:sjbo@yzu.edu.cn

T19-98-0076

The effect of endotoxinon the mucus layer on the intestinal in no- and post-pregnancy mice

Liyan An, Mingming Zhang, Baoyu Zhao, Chenchen Wu

(College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, People's Republic of China, 712100)

Abstract: The intestine is the most extensive storage of bacteria and endotoxins, and the mucosal

immune system is the first barrier of intestinal endotoxin invasion. The MUC2 (mucin2) is the major component of the mucus layers. In this study, we explored that MUC2 plays a role in how LPS invade the fetus from the gut to the uterus in pregnant mice. The results showed that the LPS level of ileal, colonic and uterine was significantly increased, and the content of sIgA in the ileum, colon and uterus tissues was significantly decreased in the LPS (+) group on the 35th day after LPS treatment. On the 16th day of pregnancy, compared with the LPS (-) group, the level of ileal LPS was significantly decreased, and the content of LPS in the fetus was significantly increased in the LPS (+) group. There was no significant difference in the levels of LPS in the uterus and placenta, and the sIgA content in the fetus was significantly decreased. The expression of MUC2 in the uterus, ileum and colon was increased significantly in the LPS (+) group, especially in the uterus. It is suggested that the endotoxins accumulated in the uterus during non-pregnancy. The high expression of MUC2 in the uterus can prevent LPS from translocating into uterus tissue. It revealed that, after pregnancy, MUC2 still performs the function of protecting the uterine tissue, allowing a large number of LPS to enter the fetal body through blood circulation. Therefore, the level of sIgA significantly decreased, resulting in a decline of the fetal innate immune function.

Key words: Lipopolysaccharide; MUC2; Intestines; Uterus; Fetus

Corresponding author: Chenchen Wu, E-mail:wucen95888@163.com

T19-98-0077

Effect of sodium dehydroacetate on cytochrome P450 of rats

Wang Cun-kai, Xiao Jin-zha, Xiao Yi-rong, Zhang Yuan, Zhang Yu-mei

(Department of veterinary pharmacology and toxicology, College of Veterinary Medicine, Yangzhou University, Yangzhou Jiangsu 225009, China)

Abstract: Sodium Dehydroacetate (Na-DHA) with CAS number 4418-26-2 appeared broad-spectrum antibacterial and antifungal effect. It is widely used in food or feed, personal care products industries used as a preservative or fungicide. It is still involved in some pharmaceutical drugs such as oral liquid and soft medicinal extract. There is more potential chance of Na-DHA to coexist with other drugs. No information about the interaction of it and other drug up to now. The study was to determine whether Na-DHA influences the cytochrome P450 (CYP450) system involved in drug metabolism or drug- drug interaction. **METHODS** Rats were randomly divided into two groups (Na-DHA treated and control). They were administered 200 $mg \cdot kg^{-1}$ or physiological saline for consecutive five days. On day 6^{th}, rats were administrated cocktail drugs as probe substrates of the four CYP or liver samples were collected for determination of CYP450 content. The four-drug cocktail was composed of phenacetin (PHE), omeprazole (OME), chlorzoxazone (CHL) and dapsone (DAP) (all administered concomitantly) to phenotype for rat CYP1A2, -2D1/2, -2E1 and -3A1/2, respectively. The expression of CYP isoforms were detected by RT-PCR and ELISA methods. **RESULTS** Na-DHA displayed significantly decreased the metabolism of PHE, OME and DAP in female than in male rats and no clear change in metabolism of CHL, indicating Na-DHA remarkably inhibited the activity of CYP1A2, -2D1/2 and -3A1/2 of rats in female and no significantly effect on activity of CYP2E1. The level of CYP2D1/2 and CYP3A1 mRNA in male treated by Na-DHA were both significantly higher than that in male of control, or than that in female in Na-DHA group ($P<0.05$) in RT-PCR analysis. There were no significant effect of Na-DHA on CYP450 enzyme contents of rats by ELISA analysis. **CONCLUSION** Na-DHA was found significantly

inhibited the activity of CYP1A2, -2D1/2 and -3A1/2 of rats in female than in male, may induce the ex·pression of CYP2D1/2 and CYP3A1 mRNA in male.

Key words: Sodium Dehydroacetate; Cytochrome P450; Cocktail probes; Rats

Corresponding author: Zhang Yu-mei, E-mail: zym@yzu.edu.cn

T19-98-0078

Analysis of the genetic environment of the plasmid habouring bla_{CTX-M} in Escherichia coli and its fitness study

Wang Wei-wei[a,b,c], Zhang Ji-yu[a,b,c]

(a. Key Laboratory of New Animal Drug Project of Gansu Province, Lanzhou, Gansu Province 730050, People's Republic of China; b. Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture, Lanzhou, Gansu Province 730050, People's Republic of China; c. Lanzhou Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu Province 730050, People's Republic of China)

Abstract: Detailed abstract or brief article CTX-M is the fastest growing ultra-broad-spectrum β -lactamase, known as cefotaxime. In view of the current situation of drug resistance caused by the mis·use and irrational use of clinical cephalosporin antibiotics, further in-depth study on the transmission pathway of blaCTX-M gene in E. coli of bovine origin, resolution of the role played by mobile elements in the horizontal transfer of bla_{CTX-M} gene, analysis of the adaptive surrogate level of plasmid-carrying re·sistance gene, and provision of strategies to block the transmission of bovine-derived E. coli pathogen, so as to enhance the monitoring and control of drug resistance. In this experiment, the complete ge·nomic data were obtained after sequencing using Pacbio triple sequencing platform, splicing by SMRT Link v5.0.1 software and correction by Illumina data platform, and the annotated results were submitted to Genebank database to obtain the Accession Number. The genetic environment of the plasmid carry·ing the resistance gene bla_{CTX-M} was mapped according to BRIG software, and the plasmid genome was mapped for comparison using Easyfig software. The level of adaptation substitution was analyzed by growth curve assay, combined with transfer assay, plasmid stability, and competitive growth. To eluci·date the genetic environment of the blaCTX-M gene in bovine-derived E. coli, this study explored the genetic environment of nine strains of E. coli carrying blaCTX-M by bioinformatics. Nine strains of E. co·li carrying CTX-M genes located on plasmids, among which IncHI2 (7 strains), IncFIB (1 strain), and In·cI1 (1 strain) carried different types of blaCTX-M resistance genes. The donor bacteria carrying the blaCTX-M gene were all able to transfer horizontally in the recipient (J53), but with different transfer fre·quencies, and the transconjuants remained stably after a 30-day stability test.

Key words: bla_{CTX-M}; plasmid; E.coli; genetic environment; fitness

T19-98-0079

The effects of two coccidiostats on the microbial flora in the cecum of chickens and soil

Xiaolei cheng[1], Chenzhong Fei[1], Wen Zhou[1], Mi Wang[1], Xiaoyang Wang[1], Feiqun Xue[1], Juan Li[2], Keyu Zhang[1]

(1. Key Laboratory of Veterinary Chemical Drugs and Pharmaceutics, Ministry of Agriculture and Rural Affairs, Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, 518 Ziyue RD, Minhang District, Shanghai 200241, China; 2. College of Chemistry, Xiangtan University, Yuhu District, Xiangtan 411105, Hunan, China)

Abstract: In order to systematically study the effects of Salinomycin and Ethanamizuril on microbial diversity and function, the high-throughput sequencing technology and biolog-ECO detection method were used to study the effects of these two coccidiostats on microorganisms in the process of manure composting and the effect of adding two coccidiostats on the functional activity of soil microbial communities respectively. The results showed that the dominant species at the phylum level in fresh feces were also Proteobacteria, Firmicutes, and Bacteroides. With the extension of composting time, Firmicutes in the three groups of samples had an absolute advantage. Clostridium, as the dominant strain in the composting process, had no significant difference in relative abundance among the three groups of samples. The use of these two coccidiostats will not significantly change the dominant species in manure. The addition of Ethanamizuril did not change the diversity of soil microbial community within a certain period of time. However, adding salinomycin can increase soil microbial activity in a certain period.

Key words: Salinomycin; Ethanamizuril; microbial diversity and function; manure composting; soil

T19-98-0080

Effects of ethanamizuril and sulfachloropyridazine sodium on the microbial flora in the cecum of chickens

Xin Li[1,2], Chenzhong Fei[1], Wen Zhou[1], Mi Wang[1], Xiaoyang Wang[1], Feiqun Xue[1], Juan Li[2], Keyu Zhang[1]

(1. Key Laboratory of Veterinary Chemical Drugs and Pharmaceutics, Ministry of Agriculture and Rural Affairs, Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, 518 Ziyue RD, Minhang District, Shanghai 200241, China; 2. College of Chemistry, Xiangtan University, Yuhu District, Xiangtan 411105, Hunan, China)

Abstract: Compared with coccidiosis, it is well known that dysbacteriosis could cause some health problems. As two coccidiostats with excellent anticoccidial property, the effects of ethanamizuril (EZL) and Sulfachloropyridazine sodium (SLD) on intestinal microecology are still unknown. In the present study, we investigated the microbial communities of chickens cecum by 16S rDNA high-throughput sequencing method, after the infected coccidia oocysts animals were treated with EZL, SLD and EZL + SLD. The results showed there were significant differences ($P<0.05$) in species between each groups at genus level after SLD and EZL treated. The abundance of Bacteroidesin the 2.0 g·L^{-1} SLD treated group was significantly higher than that in the infection model group ($P<0.05$), and the abundance of the infection model group significantly higher than thatin healthy control group ($P<0.05$). Furthermore, compared with healthy control group, the abundance of Butyricicoccus in 2.0 g·L^{-1} SLD group and the infection model group were significantly increased. In addition, the abundance of Lampropedia and Neamegalonema in the 10 mg·L^{-1} EZL group and healthy control group was significantly lower than that in the infection model group ($P<0.05$). Taken together, our results suggested that the treatment of SLD exacerbated the imbalance of the chicken cecum microecology, EZL exerts maintaining effects on intestinal microecological homeostasis.

Key words: intestinal microflora; homeostasis; ethanamizuril; sulfachloropyridazine

T19-98-0081

Discovery and verification of a Novel NDM-1 inhibitor T3289

Li Xiao-ting, Zhao Xin-rong, Zhao Dong-mei, Li Wei-na, Zhang Xiu-ying

(Department of Basic Veterinary Science, College of Veterinary Medicine, Northeast Agricultural University, Harbin, 150036)

Abstract: OBJECTIVE Antimicrobial resistance poses a huge threat to public health worldwide. β-lactam antibiotics have become an important weapon to fight against pathogens infections due to its broad spectrum, high efficiency of antibacterial and low toxicity. Unfortunately, the emergence ofmetallo-β-lactamase (MBLs) severely astricted the application of β-lactam antibiotics. Among MBLs, New Delhi metallo-β-lactamase-1 (NDM-1) is extremely efficient in inactivating nearly all-available antibiotics including last resort carbapenems, andno inhibitors for NDM-1 are currently available in therapy. Therefore, the discovery of the novel drug-resistant inhibitors targeting NDM-1 to be particularly urgent. **METHOD** In this study, we performed a structure-based in molecular docking screening of a commercially Bioactive Compound Library using glide, and identified several lowest docking energies as promisingcandidates active against NDM-1. These compounds were tested by enzyme inhibition screening to find potential inhibitors. For the best inhibitors, checkerboard microdilution assays and time-killingassays were conducted to evaluate their synergistic effect in combination with the carbapenem meropenem in vitro. A mice infection model was used to evaluate the efficacy of combined therapyin vivo, and the inhibitor mechanism was explored based on molecular dynamics simulation. **RESULTS** As results, T3289 was screenedas a potential inhibitor of NDM-1, and its binding affinities docking score was -12.183 Kcal/mol. The enzyme inhibition assay showed that T3289 exerted a significant dose-dependent inhibition of NDM-1 enzyme activity, the value of IC50 was 25.4±0.6 μmol-L^{-1} and the maximum inhibition rate was 82.3%.Antibacterial activity assaysindicated that T3289 effectively restored the effectiveness of meropenem *in vitro* with NDM-expressing isolates. Therapeutic assays showed that received a combination therapy were significantly decrease mice in the bacterial burden in the organ and inflammatory factor content in plasma,suggested that T3289 in combination with meropenem was effective in vivo. Molecular dynamics simulation observed that T3289 exactly located in the catalytic activity pocket of NDM-1 andits inhibitor mechanism was the competitive inhibition. **CONCLUSION** This study demonstrates a high potential of T3289 combined with meropenem to treat NDM-1-positive bacterial infection, and provide an available approach to research and develop new drug against NDM-1 and treatment for bacterial resistance.

Key words: NDM-1; Drug-resistant inhibitor; Virtual Screening; Antibacterial activity

Corresponding author: Zhang Xiu-ying, E-mail: zhangxiuying@neau.edu.cn

T19-98-0082

Ivermectin induces BCRP/ABCG2 expression and function by regulation of CXR and mRNA stability in chicken primary hepatocytes

Xu ziyong, Li mei, Zhang yujuan

(School of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang 212100, China)

Abstract: Breast cancer resistance protein (BCRP, Abcg2) are known to influence the pharmacoki·

netics and toxicity of substrate drugs. Considerable evidence has accumulated to indicate that BCRP expression can be rapidly induced following acute exposure to some drugs, thus causing pharmacoki·netic drug-drug interactions. Ivermectin is widely used in poultry for the control of helminth infections; however, the influence of ivermectin on the expression of chicken BCRP has not been investigated. In this study, the role of ivermectin in modulating chicken BCRP expression and the molecular mecha·nisms were investigated in cultured chicken primary hepatocytes. BCRP mRNA and protein levels were assessed by RT-PCR and western blot, respectively. Ivermectin induced a clear time- and concentra·tion-dependent up-regulation of Abcg2 mRNA levels (as early as a 12-h exposure and up to 3.3-fold at 10 $\mu mol \cdot L^{-1}$) and protein levels (as early as a 12-h exposure and up to 2.4-fold at 10 $\mu mol \cdot L^{-1}$). More·over, drug accumulation analysis showed that ivermectin-treated cells displayed enhanced cellular ef·flux of the BCRP substrate mitoxantrone. After chicken xenobiotic receptor (CXR) was knocked down using siRNA, BCRP induction was dramatically suppressed, indicating the involvement of CXR in the regulation of BCRP expression in chicken primary hepatocytes when exposed to ivermectin. However, ivermectin did not displayed CXR ligand activity in CXR reporter gene assay, but up-regulate CXR ex·pression in a concentration-and time-dependent manner. Moreover, studies with actinomycin D re·vealed that the half-life of Abcg2 mRNA was significantly prolonged from 6.0 h to 9.1 h in ivermectin-treated cells, suggesting a post-transcriptional mode of ivermectin regulation. This study demonstrates that ivermectin induces BCRP expression and function by regulation of CXR and mRNA stability, thus should be helpful in guiding the rational use of drugs in the poultry industry to increase drug bioavailabil·ity or avoid possible adverse effects.

Key words: Ivermectin; BCRP; CXR; mRNA stability; chicken primary hepatocytes

Corresponding author: Zhang yujuan, E-mail: zhangyujuan907@163.com

T19-98-0083

Effect of sodium dehydroacetate on cytochrome P450 of rats

Wang Cun-kai, Xiao Jin-zha, Xiao Yi-rong, Zhang Yuan, Zhang Yu-mei

(Department of veterinary pharmacology and toxicology, College of Veterinary Medicine, Yangzhou University, Yangzhou Jiangsu 225009, China)

Abstract: OBJECTIVE Sodium Dehydroacetate (Na-DHA) with CAS number 4418-26-2 appeared broad-spectrum antibacterial and antifungal effect. It is widely used in food or feed, personal care prod·ucts industries used as a preservative or fungicide. It is still involved in some pharmaceutical drugs such as oral liquid and soft medicinal extract. There is more potential chance of Na-DHA to coexist with other drugs. No information about the interaction of it and other drug up to now. The study was to de·termine whether Na-DHA influences the cytochrome P450 (CYP450) system involved in drug metabo·lism or drug-drug interaction. **METHODS** Rats were randomly divided into two groups (Na-DHA treated and control). They were administered 200 $mg \cdot kg^{-1}$ or physiological saline for consecutive five days. On day 6^{th}, rats were administrated cocktail drugs as probe substrates of the four CYP or liver samples were collected for determination of CYP450 content. The four-drug cocktail was composed of phenace·tin (PHE), omeprazole (OME), chlorzoxazone (CHL) and dapsone (DAP) (all administered concomitant·ly) to phenotype for rat CYP1A2, -2D1/2, -2E1 and -3A1/2, respectively. The expression of CYP iso·forms were detected by RT-PCR and ELISA methods. **RESULTS** Na-DHA displayed significantly de·creased the metabolism of PHE, OME and DAP in female than in male rats and no clear change in me·

tabolism of CHL, indicating Na-DHA remarkably inhibited the activity of CYP1A2, -2D1/2 and -3A1/2 of rats in female and no significantly effect on activity of CYP2E1. The level of CYP2D1 / 2 and CYP3A1 mRNA in male treated by Na-DHA were both significantly higher than that in male of control, or than that in female in Na-DHA group (P<0.05) in RT-PCR analysis. There were no significant effect of Na-DHA on CYP450 enzyme contents of rats by ELISA analysis. **CONCLUSION** Na-DHA was found significantly inhibited the activity of CYP1A2, -2D1/2 and -3A1/2 of rats in female than in male, may induce the expression of CYP2D1/2 and CYP3A1 mRNA in male.

Key words: Sodium Dehydroacetate; Cytochrome P450; Cocktail probes; Rats

Corresponding author: Zhang Yu-mei, Email: zym@yzu.edu.cn

T19-98-0084

Swainsonine induces cell death, involving apoptosis and autophagy, and paraptosis in rat primary renal tubular epithelial cells

Shuai Wang[a,b], Chen Yang[a,b], Ruijie Huang[a,b], Yuting Wen[a,b], Chunyan Zhang[a,b], Baoyu Zhao[a,b]

(a. College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, 712100, China; b. Institute of Poisonous Plants in Western China, Northwest A&F University, Yangling, Shaanxi 712100, China)

Abstract: Swainsonine (SW), an indolizidine alkaloid, is the principal toxic component of locoweeds, and is produced by fungi living within locoweeds. The research shows that swainsonine can cause injury of multiple tissues/organs of grazing livestock, and the extensive vacuolar degeneration is major pathological manifestation. Locoweed poisoning causes huge economical loss annually which severely hampered the development of the grassland. However, the underlying mechanisms involved in SW-induced animal poisoning remain poorly understood. It is known that programmed cell death (PCD) includes apoptosis, autophagy, paraptosis, oncosis, swelling, etc., and recent studies indicate that SW induces a variety of cell apoptosis. Nevertheless, a drug can cause more than one kind of programmed cell death at the same time. Here, we evaluated the toxicity of SW in rat primary renal tubular epithelial cells (RTECs) and the related mechanisms of action. We examined the effect of SW on cytotoxicity using western blotting, transmission electron microscopy, fluorescent microscopy, and flow cytometry. We found that SW induced RTECs death accompanied by vacuolation in vitro. The results showed that SW treatment significantly enhanced the expression of the apoptosis proteins cleaved caspase-3, Cyt C, AIF, FasL, and increased the number of GFP-LC3 puncta per cell, LC3-I/II conversion, and the accumulation of autophagosomes in RTECs, stating that SW induced both autophagy and apoptosis. Activating autophagy using rapamycin (Rapa) inhibited apoptosis, while suppressing autophagy using bafilomycin A1 (Baf A1) greatly enhanced SW-induced apoptosis, indicating that autophagy protected RTECs from cellular damage. Furthermore, the level of ER stress markers, such as Bip, CHOP and cytoplasmic concentration of Ca2+ have drastically increased, suggesting the presence of ER stress. SW induces an intense vacuolation prevented by the ER stress inhibitor 4-PBA and the protein synthesis inhibitor cycloheximide, supporting the induction of paraptosis by SW. In summary, these results suggest that SW causes toxicity by inducing apoptosis and autophagy, and ER stress-dependent paraptosis, thus further laying a theoretical foundation for the study of SW toxicity mechanism.

Key words: Swainsonine; apoptosis; autophagy; paraptosis; ERs

Corresponding author: Baoyu Zhao, E-mail: zhaobaoyu12005@nwafu.edu.cn

T19-98-0085

Research progress on distribution, toxicity, control and utilization of poisonous grass species in natural grassland of China

ChenYang[a], ShuaiWang[a], Chonghui Mo[b], ChenchenWu[a], HaoLu[a], Baoyu Zhao[a]

(a. College of Veterinary Medicine, Northwest A&F University,Yangling,Shaanxi province 712100, China; b. College of Agriculture and Animal Husbandry, Xining, Qinghaiprovince 810016, China)

Abstract: China is a major country in grassland resources, ranking first in the world, with 3.93mil·lion hm2 of natural grassland, accounting for 12% of the world's grassland area. The natural grassland is the material basis for herdsmen to survive, and it is also the natural green barrier to maintain ecologi·cal balance, which is of great significance to ecological protection, economic development and social stability. But for a long time, the global climate change, overload, overgrazing, excessive use of natural and man-made factors, such as results in the decrease of grassland degradation, vegetation coverage, biodiversity destruction, poison grass spread harmful organisms such as ecological problems, such as, poison grass and even become a "green killer" of natural grassland, prairie pastoral animal husbandry development poses a serious economic loss. So far, natural grassland in our country about poison grass 52 families, 168 genera and 316 species, distributed in the five grassland pastoral areas of north·east Steppe, Mengninggan steppe, Xinjiang steppe, Qinghai-Tibet steppe and southern grassy hills and slopes, mainly related to Tibet, xinjiang, qinghai, Inner Mongolia, gansu, sichuan, yunnan, ningxia, shanxi, hebei and shaanxi 11 provinces and autonomous regions. Except Shaanxi, the proportion of toxic grass to available grassland area in the above provinces and regions was 20.56%, 17.82%, 10.46%, 7.97%, 10.93%, 37.31%, 24.71%, 38.76%, 15.16% and 10.28%, respectively. In this paper, the species distribution, dominant population and toxicity of toxic grass in natural steppe of 11 provinc·es and regions were reviewed, and the damage status, toxicity and harm of five major toxic grass spe·cies, including locoweed, Aconitum, Stellariachamaejasme, Achnatheruminebrians and Eupatorium ad· enophorum, were highlighted. On this basis, it is suggested to strengthen the basic research on toxic grass, establish monitoring and early warning system, give full play to various ecological functions of toxic grass, scientifically locate the benefits and harms of toxic grass, and establish the idea of "turning harm into benefit", which will help people understand the ecological effects of toxic grass scientifically. To improve the comprehensive prevention and control level of toxic grass disaster and provide theoreti·cal basis and technical guidance for scientific utilization of toxic grass resources.

Key words: Natural grassland; Poisonous and injurious plant; Species and distribution; Toxicity; Control and utilization

Corresponding author: Baoyu Zhao, E-mail: zhaobaoyu12005@163.com

T19-98-0086

Zinc oxide nanoparticles induce hepatotoxicity by pyroptosis

Xingyao Pei[a], Liuxing Yu[b], Lidao Wen[b], Tangshusheng[a]

(a. Department of Pharmacology and Toxicology, College of Veterinary Medicine, China Agricultural University, Yuanmingyuan West Road No.2, Haidian District, Beijing, 100193, China; b. Tianjin Key Laboratory of Agricultural Animal Breeding and Healthy Husbandry, College of Animal Science and

Veterinary Medicine, Tianjin Agricultural University, Jinjing Road No.22, Xiqing District, Tianjin 300384, China)

Abstract: In recent years, with the rapid development of nanotechnology, ZnO nanoparticles (ZnO NPs) have been widely used in the fields of daily necessities, medicine, food and animal feed. Previous studies have shown that misuse or improper use of ZnO NPs could pose a health risk to humans and animals. Liver has been known as a main toxic target organ of ZnO NPs, which have been shown to mediate hepatocyte death. However, the question whether zinc oxide nanoparticles lead to hepatocyte death through cell pyroptosis has not been answered yet. Based on our studies in vitro and in vivo, we revealed that ZnO NPs disrupted zinc homeostasis in rat liver, leading to massive zinc accumulation and liver impairment. Meanwhile, ZnO NPs induced the assembly of the NLRP3-ASC-Caspase-1 inflammatory complex in hepatocyte, and further induced the cleavage of GSDMD, promoting the overexpression and leakage of inflammatory cytokines, such as IL-1β and IL-18. These evidences confirmed the activation of Caspase-1-dependent classical cell pyroptosis pathway induced by ZnO NPs and provide a basis for application supervision and risk assessment of ZnO NPs.

Key words: ZnO NPs; pyroptosis; liver; inflammasome; GSDMD

Corresponding author: Xingyao Pei, E-mail: xingyaopei@163.com

T19-98-0087

Environmental carbon tetrachloride exposure disrupts the liver structure and metabolic detoxification function in mice via p38MAPK/NF-κB/NLRP3 pathway

Yuan-yuan Wei[a], Yi-meng Fan[a], Chen Gao[a], Qing-tao Wang[b], Yan-yan Yuan[c], Yan-nan Zhang[a], Jun-cheng Han[b], Zhi-hui Hao[a]

(a. Innovation Centre of Chinese veterinary medicine, College of Veterinary Medicine, China Agricultural University, Beijing 100086, China; b. Xinjiang Agricultural University, Urumqi, Xinjiang 830052, China; c. Agricultural Bio-pharmaceutical Laboratory, College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, Qingdao 266109, China)

Abstract: To investigate the effects of environmental exposure to different concentrations of carbon tetrachloride (CCl_4) on liver structure and detoxification function in mice.Detection of CCl_4 liver toxicological pathway and functional enrichment were using CTD database. Twenty-five mice were randomly segregated into five groups. They were given CCl_4 solution with concentrations of 0, 0.1%, 0.2%, 0.4% and 0.8% respectively. After intraperitoneal injection for 7 days, blood and liver were collected after anesthesia. The activity of serum GST, the content of TP in liver tissue, the activities of CarE, LDH and Ache, and the levels of T-SOD, CAT and MDA were measured. Histopathological evaluation was performed by H&E staining, Masson and AB-PAS staining. The expression of NLRP3, NF-κBp65 and IKK were detected by IHC. The protein expression of NF-κBp65, IKK, p38MAPK, IL-1β and TNF-α were detected by WB. The mRNA relative expression levels of IL-1β, TNF-α, NLRP3, caspase-1 and GSDMD were detected by qPCR. It found that CCl_4 may affect the protein metabolism, gene expression and signal transduction by REACT pathway enrichment. Besides, the NF-κB pathway, MAPK pathway and cell death process could play a role in CCl_4 exposure model assayed by GO analysis. All dose

of CCl_4 exposure could damage the liver structure and prompted the fibrosis. 0.2%-0.8% CCl_4 decreased the liver CarE, LDH activity and TP level, 0.2% and 0.8% CCl_4 increased the liver AchE activity (P<0.05). While 0.4%-0.8% CCl_4 increased serum GST activity, 0.1%-0.8% CCl_4 significantly increased the level of liver MDA, 0.2% CCl_4 increased the level of liver T-SOD and 0.4%-0.8% CCl_4 increased the level of liver CAT (P<0.05). The protein and mRNA expression of NLRP3, IL-1β and TNF-α were significant elevated in 0.2%-0.8% CCl_4 exposure (P<0.05). Whereas the expression of p38MAPK and GSDMD were decreased in 0.1%-0.8% CCl4 exposure and the apoptosis were no statistical difference in all groups. The expression of IKK were also decreased in liver in all doses (P<0.05). It indicates that even 0.1% CCl_4 exposure could damage the liver structure and detoxification function in mice via p38MAPK/NF-κB/NLRP3 pathway.

Key words: carbon tetrachloride; liver injury; antioxidant; inflammation; network toxicology

T19-98-0088

Protective effect of aqueous extract of Yinshanlian on acute liver injury induced by carbon tetrachloride in mice

Yuanyuan Wei[a], Yimeng Fan[a], Yanyan Yuan[b], Yu Ga[a], Yannan Zhang[a], Juncheng Han[c], Zhihui Hao[a]

(a. Innovation Centre of Chinese veterinary medicine, College of Veterinary Medicine, China Agricultural University, Beijing 100086, China; b. Agricultural Bio-pharmaceutical Laboratory, College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, Qingdao 266109, China; c. Xinjiang Agricultural University, Urumqi, Xinjiang 830052, China)

Abstract: In order to explore the protective effect and possible mechanism of aqueous extract of Yinshanlian (YSL) on acute liver injury induced by carbon tetrachloride (CCl_4) in mice, so as to provide theoretical guidance for further research and clinical transformation of YSL. Sixty ICR mice were randomly divided into 6 groups (n=10): control group, CCl_4 group, low (1.17 mg·g^{-1}), medium (2.34 mg·g^{-1}) and high (4.68 mg·g^{-1}) dosage groups. The drugs were administered by gavage once a day for 7 days. One hour after the last administration, except the control group, the mice in other groups were injected intraperitoneally with 0.2% carbon tetrachloride to establish the model of acute liver injury. After 12 hours, blood was collected from mouse eyeballs, serum was isolated, and liver tissue was collected. The levels of serum total protein (TP) and glutathione s transferase (GSH), glutathione s transferase, succinate dehydrogenase (SDH), catalase (CAT) and total superoxide dismutase (T-SOD) in liver tissue were detected by biochemical analysis. The liver pathology and liver fibrosis were observed by H&E staining and Masson staining. Expression and localization of IL-1β, tumor necrosis factor alpha (TNF-α), angiogenesis factor A (VEGFA), matrix metalloproteinase 9 (MMP9) and transcription factor NF-κBp65, protein kinase IKK, and p38MAPK in liver tissue were detected by Western blot and immunohistochemistry. The results showed that 2.34 mg·g^{-1} YSL aqueous extract could significantly reduce the level of serum GST and significantly increase the level of intrahepatic CAT compared with the model group. There was no significant difference in T-SOD between these groups. There was no significant difference in the levels of liver GST, SDH and serum TP between 2.34 mg·g^{-1} YSL aqueous extract group and the control group. Besides, 2.34 mg·g^{-1} YSL aqueous extract could alleviate liver pathological injury and fibrosis, and decrease the expression of liver IL-1β, TNF-α, VEGFA, NF-κBp65 and IKK. It is suggested that the aqueous extract of YSL inhibits liver oxidative stress and inflammation, through

NF-κB and p38MAPK pathways so as to alleviate acute liver injury in mice.

Key words: aqueous extract of yinshanshen; acute liver injury; CCl4; mechanism

T19-98-0089

Studies on the role of *IS1216E* in the formation and dissemination of poxtA-carrying plasmids in an Enterococcus faecium clade A1 isolate

Shan Xin-xin[a], Li Xin-Sheng[a], Wang Nan-nan[a], Stefan Schwarz[b], Zhang Su-Mei[a],
Li De-xi[a], Du Xiang-dang[a]
(a. College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450046, P. R. China; b. Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, FreieUniversität Berlin, Berlin, Germany)

Abstract: Linezolid is a last-resort antimicrobial agent that is used in human medicine to treat infections caused by MRSA, VRE and MDR *Streptococcus pneumoniae*. Florfenicol is exclusively approved for use in farm animals and fish. The poxtA gene encodes a ribosomal protection protein of the ABC－F family, which in addition to phenicol and oxazolidinone resistance also confers decreased susceptibility to tetracycline, and has recently been identified in MRSA and enterococci. In this study, we aimed to investigate the role of *IS1216E* in the dissemination of the phenicol-oxazolidinone-tetracycline resistance gene poxtA in an *Enterococcus faecium* clade A1 isolateby conjugation experiments, PCR, S1-PFGE, Southern hybridization, and WGS analysis. Two plasmids in*Enterococcus faecium*E1077 were identified, designated as pE1077-217 and pE1077-23, respectively. pE1077-217 was 217661 bp conjugative plasmid harbourederm(B), *aac*(A) - *aph*(D), *aadE*, *spw*, *lsa*(E), *lnu*(B), *aphA3* and *dfrG*, whereas pE1077-23 was pE1077-23 and carried a Tn6657-like transposon containingpoxtA and *fexB*. pE1077-23 was apparently formed by an IS1216E-mediated composite transposon － plasmid fusion event, involving a replicative transposition process. Conjugation experiments showed that pE1077-23 is mobilizable by pE1077-217. Moreover, a novel 31742 bp plasmid, pT-E1077-31, was found in a transconjugant. WGS analysis indicated that pT-E1077-31 was formed by the integration of a Tn*6657*-derived, *IS1216E*-based translocatable unit, which carried *fexB* and *poxtA*, into a copy of pE1077-23.This study showed the presence of two cointegrate formation events in the formation and spread of *apoxtA*/*fexB*-carrying plasmid in *E. faecium*. One was the integration of a transposon into a plasmid while the other was the integration of a TU into a different site of the same type of plasmid-borne transposon from which it originated. In both events, *IS1216E* played a major role, suggesting tha *IS1216E*-mediated transposition and translocation processes

Key words: *Enterococcus*; linezolid resistance; *poxtA*; plasmid integration

Corresponding author: Du Xiang-dang, E-mail: xddu@henau.edu.cn

T19-98-0090

Investigation of the uptake and transport of aspirin eugenol ester in Caco-2 cells model

Qi Tao, Zhen-Dong Zhang, Zhe Qin, Xi-Wang Liu, Shi-Hong Li, Li-Xia Bai, Ya-Jun Yang*, Jian-Yong Li*

(Key Lab of New Animal Drug Project of Gansu Province, Key Lab of Veterinary Pharmaceutical Development of Ministry of Agriculture and Rural Affairs, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Lanzhou 730050, China)

Abstract: Aspirin eugenol ester (AEE) is a new medicinal compound synthesized by esterification of aspirin with eugenol using the prodrug principle. AEE has anti-inflammatory, antipyretic, analgesic, anti-cardiovascular diseases, and anti-oxidative stress pharmacological activity. However, its oral bioavailability is poor, and its intestinal absorption and transport characteristics are still unknown. The purpose of this study is to investigate the uptake and transport mechanism of AEE in human intestinal Caco-2 cells. AEE was proved to be non-toxic to Caco-2 cells when its concentration is less than 256μM. Because higher concentrations of such AEE metabolite as salicylic acid were detected in the supernatant of the cell lysate and cell culture fluid while AEE could be not detected, the change in the content of salicylic acid was used to indicate the change in the content of AEE. The result showed the uptake and transport of AEE were related to time, concentration, and temperature. In the uptake experiment, the uptake of SA reached the maximum at 30 min when Caco-2 cells were incubated with 64μM AEE from 0 to 120min. Low temperature can significantly reduce the uptake of SA in Caco-2 cells. In the transport experiment, the concentration of AEE in the Caco-2 cell monolayer was constant and the transport volume increases with time within 120 minutes. The results showed that the transepithelial transport of salicylic acid from the AP-BL and BL-AP sides was time-dependent. Interestingly, the transport amount of salicylic acid in Caco-2 cells increases with the increase in concentration, but transmembrane was no certain correlation between transport rate and concentration. The apparent permeability coefficient (Papp) of low concentration was higher than that of high concentration Papp, which may be the saturation phenomenon of high concentration. The apparent permeability coefficient (Papp) of aspirin eugenol ester (64 μM) from AP to BL was determined to be 0.034 (± 0.012) × 10^{-6} cm/ s, which was considered to have poor permeability and absorptive in vivo rate. Efflux ratio (ER) <1, indicating that their intestines transport mechanism was passive transport. In addition, the temperature had a significant effect on the transport of aspirin eugenol ester. In summary, the results indicated that the intestinal absorption of AEE through the Caco-2 cell monolayer involves passive transport. This experiment illustrates the absorption and transport properties of AEE. All these results may help to explore the mechanism of chemically synthesized drugs in vitro absorption and transport.

Key words: Aspirin eugenol ester; Metabolite; Caco-2 cells; Uptake; Transport

Corresponding author: Ya-Jun Yang, E-mail: yangyue10224@163.com; Jian-Yong Li, E-mail: lijy1971@163.com

T19-98-0091

Safety test of liaobo powder on target animal in chickens

Yannan Zhang, Yimeng Fan, Yuanyuan Wei, Yu Ga, Juncheng Han, Zhihui Hao

(Department of Basic Veterinary Medicine, College of veterinary medicine, China Agricultural University, 100193, P. R. China)

Abstract: In order to evaluate the safety of liaobo powder on target animal (chickens), In the experiment, 60 healthy chickens (15 days of age) were randomly divided into four groups (n=15), the 5 times group (62.5 g·kg^{-1}), 3 times (37.5 g·kg^{-1}), and 1 times group (12.5 g·kg^{-1}) dose group of liaobo

powder, and control group (0.0 g·kg^{-1}), all the chickens was administered with mixed feed for 5 consec·utive days, and then observed continuously up to the 15th days. The results showed that the test chick·ens in each administration group were normal in the clinical manifestations. There was no significant difference on the average feed intake, average daily weight gain, feed-to-weight ratio and the blood rou·tine and serum biochemical indexes of the test chickens compared with control group (P>0.05). The in·dexes of organs such as liver, spleen, stomach, intestine, bursa of fabricius had no adverse effects. There were no obvious pathological changes in chickens in each group. The results suggested that liao·bo powder had high clinical safety for chickens at 62.5 g·kg^{-1} for 5 days. The study provided a scientific basis for clinical rational use of liaobo powder.

Key words: Liaobo Powder; safety test; chickens; clinical observation; serum biochemical index

Corresponding author: Zhihui Hao, E-mail: haozhihui@cau.edu.com

T19-98-0092

Preliminary safety test of amoxicillin &scutellaria baicalensis extract suspension

Yi DD, Wen XM, Xiang YF, Wan BJ, Deng ZY, Xu YF, Yan GQ, He JK

(College of animal science and technology, Guangxi University, Nanning, Guangxi 530005)

Abstract: OBJECTIVE Amoxicillin &Scutellariabaicalensis extract suspension (AS) is a veterinary suspension. This study is aimed to evaluates thepreliminarily safety of the preparation by the acute tox·icity test and muscle irritation testunder the requirements of technical guiding principles for veterinary drug research. METHODS Acute toxicity test: Thirty ICR mice were randomly divided into 3 groups, with 10 mice in each group (half male and half female). One group was taken each time and repeated 3 times. ASwas injected intraperitoneally to ICR mice. After exposure, the general manifestations, poison·ing symptoms, and death of mice were observed, and the dead mice were dissected and recorded. The observation time was 1 week. Local irritation test: Tworabbitswastaken for muscle (vascular)stimulation test 48 hours after administration; Another 2 rabbits were taken for muscle (vascular) stimulation test (recovery test) 7 days after administration. RESULTS Acute toxicity test: ASwas administered intraperi·toneally to mice at the dose of 5000 mg·kg^{-1}. After three repeated tests, ICR mice showed no clinical poisoning symptoms and death during the experimental observation period, and no abnormalities were found in dissection. During the observation period of the three repeated tests, the mortality of the tested animals was 0% (0/10, /10, 0/10). It can be determined that the LD50 of AS in the acute toxicity test of ICR mice is greater than 5000 mg·kg^{-1} body weight, which is non-toxic. Local irritation test: in the mus·cle (vascular) stimulation response test 48 hours after administration, the quadriceps femoris of each rabbit in the drug injection group had slight hyperemia, ranging from 0.5 cm×Below 1.0 cm, the stimula·tion response is classified as grade 2, belonging to moderate stimulation; In the muscle (vascular) stim·ulation reaction test 7 days after administration, the rabbits' dietary behavior was normal. Dissecting the quadriceps femoris of rabbits, it was found that there were small gray-white class lines under the fascia at the injection site, and there were no red spots around the class lines. The muscle samples at the injection site were taken and made into sections. In the drug group, a small part of muscle cells shrank seriously and the cytoplasm was deeply stained. Inflammatory cells infiltrated between muscle cells. However, the surrounding tissues recovered well, and the morphology was no different from that

of the control group, indicating that the recovery was excellent and the damage was reversible. **CONCLUSION** The results of the acute toxicity test showed that AS was safe; The results of the muscle lo·cal irritation test showed that AS had certain irritation and injury to the animal muscle, but its injury was reversible and could be used for intramuscular injection.

Key words: Amoxicillin& Scutellaria baicalensis extract suspension; Acute toxicity; Local irritation

Corresponding author: He JK, E-mail:ahong18@vip.sina.com

T19-98-0093

Safety evaluation, antibacterial and anti-inflammatory effect *in vitro* of toad venom lactobitis spray

Deng ZY, Yang L, Mo YH, Huang L, Qin LQ, Wen XM, Xu YF, Yan GQ, He JK

(College of animal science and technology, Guangxi University, Nanning, Guangxi 530005)

Abstract: OBJECTIVE Toad Venom Lactobitis Spray is a Chinese medicine compound liniment composed of toad venom, clove, and borneol based on the theory of syndrome differentiation and treat·ment by traditional Chinese veterinarians. The aim of this study is that evaluates the safety and antibac·terial and anti-inflammatory effect in vitro of the Chinese herbal compound preparation. **METHOD** (1) Skin acute toxicity test: ten New Zealand rabbits were randomly divided into two groups, and their hairs about 10×10 cm2 on both sides of the rabbit′ s back spine were removed by depilatory. The rabbits of the damaged skin group have nicked the skin and draw blood, but the intact skin group kept skin com·plete and no scar. Six milliliters of the tested drug was evenly coated on the hair removal area of each rabbit. After 24 hours of drug administration, removing the residual drug, each animal was observed continuously for 14 days. The changes in body weight, skin hair, eyes and mucosa, respiration, behav·ior, and neurologic symptoms, and other toxic manifestations of animals as well were recorded. (2) Lo·cal skin irritant test: the test employed self-comparison between the left and right sides of the hairless area in the ribbit's back. The left side was equally coated on 6 mL of the tested drug, the right side was 75 % alcohol as control. The degree of local skin irritation was evaluated by the recorded score based on the skin irritation scoring standard and the skin reaction observed at different time points. Mean·while, the irritation test of the Chinese medicine preparation to the local skin was evaluated utilizing dif·ferent stimulation meanings: single administration and repeated administration on complete or dam·aged skin. (3) Antibacterial test *in vitro*: the minimum inhibitory concentration (MIC) of the tested drug against Escherichia coli, Pasteurella multocida, Streptococcus, Salmonella, Bacillus cereus was deter·mined by the tube double dilution method to study its antibacterial activity *in vitro*. (4) *In vitro* anti-inflam·matory effect: using the MTT method to confirm the safety concentration of Toad Venom Lactobitis Spray, and analyzing the changes in secretion of pro-inflammatory cytokines of RAW 264.7 cells in·duced by LPS to investigate in vitro anti-inflammatory effect at different safety concentrations. **RESULT** During two weeks of the observation period, in all animals, bodyweight was no decrease, the skin was complete and no swelling, eyes, mucosa, respiration, intelligence, and activity were normal, other toxic manifestations and died animal not observed as well, which indicate that the Chinese formulation is safety, no toxicity or little toxicity. After single administration, the total score of skin irritation of the test·ed drug was zero and that of repeated administration was 0.5, the rabbits were no abnormal reaction and their skin color in the area coating drug was natural, demonstrating no skin irritation generated

from the Chinese preparation. The tested drug had antibacterial effects on all five strains, especially against Escherichia coli. The safe concentration of Toad Venom Lactobitis Spray on RAW 264.7 cells was 64×10^{-4}. In the safe concentration range, high-dose tested drug can inhibit the secretion of pro-inflammatory cytokines IL-6, IL-1β and anti-inflammatory cytokines IL-2 from LPS-induced RAW 264.7 cells, thereby regulating cytokine balance to contribute anti-inflammatory effect. **CONCLUSION** Toad Venom Lactobitis Spray is safe and has no toxicity or little toxicity, and possesses anti-inflammatory and antibacterial effects *in vitro*.

Key words: Toad Venom Lactobitis Spray; skin acute toxicity; local skin irritant; antibacterial; anti-inflammatory; RAW 264.7 cells

Corresponding author: He JK, E-mail: ahong18@vip.sina.com

T19-98-0094

Ecotoxicological effects of zearalenone on artificial rumen

Huangfu he-ping, Li zhi-qiang, Ye xin, Song chao, Li xin-feng, Dong qing, Zhang ai-guo, Shi dong-mei*

(College of Veterinary Medicine, Henan University of Animal Husbandry and Economy, Zhengzhou 450046, China)

Abstract: To study the effects of zearalenone (ZEN) on rumen environment and microbial population structure, we used double outflow artificial rumen simulation system. ZEN group was supplemented with 2.5 mg·kg^{-1} ZEN, and the control group was fed normally, with 3 horizontal controls respectively. The system fermented continuously for 7 days, then collected the fermentation broth on the 5th, 6th and 7th days, and determined the pH value, ammonia nitrogen and protozoa number. Finally, DNA was extracted after mixing samples of the same group of every day, and 16S rRNA gene and 18S rRNA gene were amplified for macrogenomic sequencing analysis. Results There was no significant difference in pH value between the two groups, but it decreased with the extension of culture time (P<0.05). The concentration of ammonia nitrogen in the two groups increased first, then decreased and then increased, with an overall upward trend; the concentration of ammonia nitrogen in Zen group was lower than that in the control group (P<0.05). The number of protozoa in the two groups was lower than that in the original rumen juice,showing a downward trend with the extension of time, including Zen group's P<0.05 and control group's P>0.05. Metagenomics analysis of 16S rRNA gene showed that the Shannon value of the two groups was higher than that of the original rumen juice, and the Simpson value was lower than that of the original rumen juice. However, metagenomics analysis of 18S rRNA gene showed that the Shannon value of the two groups was lower than that of the original rumen juice, and the Simpson value was higher than that of the original rumen juice. The relative abundance of unclassified Bacteroidales in ZEN group was lower than that in control group (P<0.05), and the abundance of Piromyces and Litostomatea in eukaryotes was also lower than that in control group; With the extension of fermentation time, the relative abundance of unclassified Bacteroidales increased significantly (P<0.05), the relative abundance of Butyrivibrio decreased first and then increased, but there was no significant difference among eukaryotes (P>0.05). The results showed that in vitro culture and addition of ZEN could inhibit the growth of protozoa and promote the diversity of prokaryotes; ZEN decreased the abundance of Bacteroidales, resulting in the weakening of rumen protein hydrolysis ability, but the extension of fermentation time significantly increased the relative abundance of Butyrivibrio and Bacteroidales, improving the ability to hydrolyze proteins and produce acid; ZEN can inhibit the growth of Piro·

myces, thus,we speculate that it will also affect the degradation of plant fiber in rumen; the sequencing results showed that there were many non annotated fungi and protozoa, indicating that the current research on rumen eukaryotes is not comprehensive enough, and further research and excavation are needed to fully reveal the truth of rumen ecology and physiological function.

Key words: Artificial rumen; Zearalenone; High throughput sequencing; Microbial population structure

T19-98-0095

MS4A3-HSP27 target pathway reveals potential for haematopoietic disorder treatment in alimentary toxic aleukia

Qirong Lu[a], Pu Guo[a], Xiaohui Wang[a], Irma Ares[c], Marta Martínez[c], María-Rosa Martínez-Larrañaga[c], Tingting Li[a], Yuanyuan Zhang[a], Xu Wang[a,b], Arturo Anadón[c], María-Aránzazu Martínez[c],

(a. National Reference Laboratory of Veterinary Drug Residues (HZAU) and MAO Key Laboratory for Detection of Veterinary Drug Residues, Huazhong Agricultural University, Wuhan, Hubei 430070, China; b. MOA Laboratory for Risk Assessment of Quality and Safety of Livestock and Poultry Products, Hubei 430070, China; c. Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Madrid 28040, Spain)

Abstract: Alimentary toxic aleukia (ATA) is correlated with consuming grains contaminated by Fusarium species, particularly T-2 toxin, which causes serious hurt to human and animal health, chiefly in disorders of the haematopoietic system. However, the mechanism of haematopoietic dysfunction induced by T-2 toxin and the possible target pathway for the treatment of T-2 toxin-induced haematopoietic disorder of ATA remains unclear. In this study, genomes and proteomics were used for the first time to investigate the key differential genes and proteins that inhibit erythroid differentiation of K562 cells caused by T-2 toxin, and it was found that heat shock protein 27 (HSP27) and membrane-spanning 4-domains, subfamily A, member 3 (MS4A3) may play an important role in erythroid differentiation. Meanwhile, MS4A3 interference can inhibit the occurrence of erythroid differentiation of K562 cells and promote the phosphorylation of HSP27. Moreover, the binding of HSP27 to MS4A3 in natural state can activate the phosphorylation site of HSP27 (Ser-83), while T-2 toxin can abolish the activation of phosphorylation site by inhibiting the expression of MS4A3. These findings for the first time demonstrated that the MS4A3-HSP27 pathway may function an efficient therapeutic target pathway for treating T-2 toxin elicited haematopoietic disorders of ATA.

Key words: T-2 toxin; ATA; MS4A3; HSP27; hematopoietic disorder

T19-98-0096

Genistin inhibits the activity of SrtA and LLO in *Listeria monocytogenes*

Yonglin Zhou, Minda Liu, Xuming Deng*

(Key Laboratory of Zoonosis Research, Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun 130062, China)

Abstract: As a common intracellular facultative gram-positive bacteria, *Listeria monocytogenes* (*L. monocytogenes*) has strong resistance to extreme environments such as low temperature and a wide

range of pH values, and is highly susceptible to pollution in food production and processing. Sortase A (SrtA) and listeriolysin O (LLO), as two important virulence factors related to successful Listeria infection, are important potential targets for the development of anti-*L. monocytogenes* infection drugs]. In this study, genistin can simultaneously inhibited the enzyme activity of SrtA and the expression of LLO, without affecting the growth of *L. monocytogenes*, reducing the fear of drug resistance. Furthermore, genistin reduced the formation of *L. monocytogenes* biofilm, and *L. monocytogenes* in cells incubated with genistin showed a significant reduction in vacuolar escape and slow intracellular growth. Fortunately, genistin also reduced the mortality and pathological damage of mice infected with L. monocytogenes. The results show that genistin has a good therapeutic effect on *L. monocytogenes* infection and is a promising anti-infective therapeutic agent.

Key words: Listeria monocytogene; Sortase A; listeriolysin O; genistin

Corresponding author: Xuming Deng, E-mail: dengxm@jlu.edu.cn

T19-98-0097

Hesperetin as an alternative strategy for the gas gangrene of Clostridium perfringens infection by targeting Type IV pili

Qianghua Lv, Xuming Deng*

(Key Laboratory of Zoonosis Research, Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun 130062, China)

Abstract: Clostridium perfringens (*C. perfringens*) is a Gram-positive, anaerobic, oxygen-tolerant, rodshaped bacterium, that is one of the most common pathogens in natural environment. The Type IV pili (TFP) is one of the most important virulence factor of *C. perfringens*, which mainly mediates its adhesion to host cells, invasion, gliding motility, and biofilm formation. Therefore, TFP may be an ideal target for the treatment of *C. perfringens* disease. In this study, the inhibitory effects of hesperetin, extracted from the Chinese medicinal Humulus lupulus Linn, on the gliding motility, biofilm formation, cell adhesion and antibacterial activity of *C. perfringens* were determined. In addition, we have also proved that the inhibition of hesperetin on TFP function is related to the down-regulation of TFP-related coding genes and two-component regulatory genes. Most importantly, the protective effect of hesperetin was confirmed in a *C. perfringens* mouse model of gas gangrene. This study demonstrates hesperetin as a potential drug to treat infections caused by *C. perfringens*.

Key words: C. perfringens; gas gangrene; isoxanthohumol; TFP; inhibitor

Corresponding author: Xuming Deng, E-mail: dengxm@jlu.edu.cn

T19-98-0098

Inhibitory effect of xanthohumol against pneumolysin activity *in vitro*

Jianfeng Wang, Xuming Deng*

(Key Laboratory of Zoonosis, Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun, China)

Abstract: Streptococcus pneumoniae could cause a variety of invasive diseases such as pneumo·

nia, meningitis, bacteremia and other invasive diseases. Pneumolysin (PLY) is an important virulence factor secreted by Streptococcus pneumoniae, which can penetrate cell membranes and lead to cell ly·sis and inflammation. Therefore, rendering PLY a potential therapeutic target to develop a new inhibitor is attractive. This study analyzed and confirmed the inhibitory effect of the natural compound xanthohu·mol on PLY. In addition, xanthohumol exhibited excellent biofilm inhibition and eradication activity. Safe·ty and stability experiments showed that xanthohumol exhibited low toxicity in A549 cells and Hela cells. Collectively, these results showed that xanthohumol has great potential as a new strategy for the treatment of pneumococcal diseases.

Key words: Pneumolysin; Streptococcus pneumoniae; biofilm; anti-infective

Corresponding author: Xuming Deng, E-mail: dengxm@jlu.edu.cn

T19-98-0099

Transmission characteristics of enterococcal optrA genes from different sources in swinefarms

Xingyun Li, Nannan Wang, Bo Zhang, Chunyan Xu, Dexi Li, Xiangdang Du

(College of Veterinary Medicine, Henan Agricultural University, Zhengzhou, P. R. China)

Abstract: The use of the animal-specific antimicrobial drug florfenicol in pig farming has accelerat·ed the presence and dissemination of the cross-resistance gene *optrA*. In this study, enterococci strains of different origins (sows, piglets, sick pigs, drinking water, flies, spiders, birds, and sewage) in a pig farm in Henan Province were analyzed to investigate the role of various factors (environmental me·dia, insect vectors, and birds) on the dissemination of *optrA* gene in the pig breeding environment. The enterococci strains were isolated from the samples collected from sows, piglets, sick pigs, tap water, spiders, flies, birds, and sewage sources in a pig farm. The *optrA* gene and the associated Tn*6674* were detected in these enterococci strains. Whole-genome sequencing and MLST typing of optrA-posi·tive enterococci were performed. A total of 160 enterococci strains were isolated, with 127 *optrA* posi·tive. Genetic environment analysis of the *optrA* gene showed that a total of 60 enterococci strains car·ried Tn*6674* based on whole genome sequencing results. All Tn*6674* -positive strains were Enterococ·cus feacalis and the Tn*6674*-positive strains were distributed among 13 ST types (including 2 new ST types). Of these, ST691 (*n*=16, originated from sows, piglets, drinking water, sewage and birds) was the most frequently, followed byST256 (*n*=7, originated from sows, piglets, sick pigs, and birds), ST16 (n=6, originated from sows, piglets, spiders) and ST476 (*n*=6, originated from sows, piglets, and birds). Of the total optrA-positive strains, Tn*6674*-positive ones accounted for 47.2%, which belonged to multi·ple ST types with complex genetic relationships, suggesting that interactive transmission can occur among these strains from sows, piglets, drinking water, sewage, spiders, birds, and sick pigs. Environ·mental media (such as drinking water, sewage), insect vectors (such as spiders), and birds play impor·tant roles in the transmission of the *optrA* genes in pig breeding environment.

Key words: *optrA*; MLST; environmental factors

Corresponding author: Dexi Li, E-mail: lidexi2006@126.com; Xiangdang Du, E-mail: xddu@henau.edu.cn

T19-98-0100

Residue and elimination of ciprofloxacin in eggs of hyline gray laying hens

Yafei Li *, Qiuquan Su, Ying Wang, Chenhong Ding, Zefeng Cui, Weili Wang

(Institution Name: Institute of Quality Standard and Monitoring Technology for Agro-Products of Guangdong Academy of Agricultural Sciences, Guangzhou 510640)

Abstract: Ciprofloxacin is a third-generation quinolone drug, with a bactericidal broad spectrum, suitable for the treatment of various infectious diseases of livestock, poultry and small animals caused by sensitive bacteria and mycoplasma. In veterinary clinic, it is mainly used to treat chicken chronic respiratory disease, colibacillosis, pasteurellosis, avian typhoid fever, as well as yellow and white scour of piglets. Recently, the residue of antibacterials in eggs has attracted great attention. Whether medications in the early stage before laying may cause drug residues in eggs, there are currently few studies. Therefore, this study was carried out to investigate the ciprofloxacin residue behavior in Hyline gray hens. This will provide scientific and technological support for the use of veterinary drugs in clinic. 0.5 g of ciprofloxacin hydrochloride soluble powder (specification: 10%) was mixed in 1 kg feed and administered for 5 days. Sampling time points were set according to the scheduled laying time of hens (calculated based on 50% laying hens) as follows: 32 days, 29 days, 26 days, 23 days, 20 days, 18 days, 16 days, 14 days, 12 days, 10 days, 7 days, 4 days, 1 day before laying. 3 replicate groups at each time point, each with 10 chickens were used to calculate from the time when the first egg was produced, and egg samples were continuously collected after laying for 20 days. The egg samples were collected and freezed at -20° C for later use. The internal standard method was used to pre-process the samples, and HPLC-MS/ MS equipment was used to detect the drug concentrations in the eggs. It was at least 16 days for ciprofloxacin to be undetectable in the newborn eggs of different groups for laying hens (LOQ: 2 $\mu g \cdot kg^{-1}$). The withdrawal period is suggested 16 days when ciprofloxacin is used before the laying eggs produce eggs. Quinolones has been prohibited during the laying period of laying hens and the withdrawal period of ciprofloxacin soluble powder administered through drinking water is tentatively set at 28 days in China. However, the residue of ciprofloxacin in eggs still occurs. This study investigated the elimination characteristics of ciprofloxacin, and suggested that the withdrawal period of ciprofloxacin soluble powder in the newborn eggs of hyline gray hens is 16 days when administered by mixed feeding.

Key words: Ciprofloxacin; egg; residue

Corresponding author: Yafei Li, E-mail: yafei.lee@163.com

T19-98-0101

Lipopolysaccharide induces oxidative damage of chicken hepatocytes by inhibiting Nrf2 signal pathway

Yuan Liang, Liu Fangping

(College of Veterinary Medicine, Northeast Agricultural University, Harbin, China, 150036)

Abstract: Bacterial diseases, especially Gram-negative bacterial infections, are one of the significant problems that have plagued the healthy development of the poultry breeding industry for a long

time, which lead to a decline in the productivity of poultry or death. Lipopolysaccharide (LPS) is a central component of the outer membrane in Gram-negative bacteria and plays a crucial role in pathogenesis. Nuclear factor erythroid-2 related factor 2 (Nrf2) activation can regulate inflammation and oxidative stress. The purpose of this study is to make clear whether LPS could induce chicken liver injury by inhibiting Nrf2 signaling pathway, and provides a data basis for study of the mechanisms of liver injury. The primary chicken hepatocytes were obtained by isolation, and identified by glycogen staining method to get high abundance and active cells; then the cells were divided into control group and LPS group. The results showed that after LPS treatment, cell viability significantly reduced (P<0.01), while supernatant LDH activity markedly increased (P<0.05). LPS could markedly reduce the activities of cell GSH-PX, CAT and SOD (P<0.05), and increase the cell ROS content. Additionally, LPS could significantly reduce the expression of Nrf2, NQO1 and HO-1 mRNA and protein, and increase the expression of Keap1 mRNA and protein (P<0.01). On this basis, Nrf2 activator TBHQ was used, and the optimal concentration of TBHQ was 5 μmol · L^{-1} by screening. Then the cells were divided into control group, LPS group, LPS+TBHQ group, and TBHQ group. Compared with control group, the expression of Nrf2 nucleoprotein in LPS group was markedly decreased (P<0.05), while the expression of Nrf2 nucleoprotein in TBHQ group was markedly increased (P<0.05). Compared with LPS group, the expression of Nrf2 nucleoprotein in LPS+ TBHQ group was significantly increased (P<0.01). Compared with control group, supernatant LDH activity increased significantly in LPS group (P<0.01), while supernatant LDH activity in TBHQ group and LPS+ TBHQ group decreased significantly compared with LPS group (P< 0.01), indicating that the activation of Nrf2 signal pathway could reduce hepatocyte injury. In summary, LPS induces oxidative damage of chicken hepatocytes by inhibiting the Nrf2 signal pathway, and the activation of Nrf2 signal pathway could alleviate LPS-induced hepatocyte injury.

Key words: LPS; Nrf2 signal pathway; Oxidative Stress; Chicken

Corresponding author: Yuan Liang, E-mail:2650502676@qq.com; Liu Fangping, E-mail: fangping·liu@126.com

T19-98-0102

Non-targeted screening for chemicals in food

Bing Shao

(Beijing Center for Disease Prevention and Control)

Abstract: The emergence of massive chemical substances has facilitated and changed people's life, but their irrational use has also threatened food safety and human health. In order to cope with the situation of food safety, the 13th Five-Year National Food Safety Plan proposes to "establish a comprehensive, combined, non-targeted testing system". The US FDA has implemented scientific strategic plan during 2012-2015 and 2015-2018 for enhancement capacity of non-targeted screening of unknown food substances in foods, and the screening program based on chromatographic separation coupled to high-resolution mass spectrometry (LC-HRMS) is becoming one prevailing way.

With financial supported by the National Key R&D Program of China and the National Natural Science Foundation of China, our team has prepared efficient purification materials and constructed a non-targeted screening database platform, throughout sample pre-processing and data post-processing. Hierarchically porous COFs and MOFs materials with controlled porosity were firstly prepared for specific removing of the interference of endogenous substances such as phytochrome and fat in foods. We fab·

ricate a hierarchical micro- and mesoporous metal–organic framework-based magnetic nanosphere by the self-assembly of framework-building blocks in thepresence of surfactant micelles on the surface of Fe_3O_4 nanoparticles (H-MOF@Fe_3O_4). The nanospheres showed preferable stabilities in both organic solvents and water with excellent reusability. The HMOF6@Fe_3O_4 nanospheres offered excellent phyto·chromes removal capacity and excellent recoveries for chemical hazards. We synthesized a 3D amino-functionalized imine-linked covalent organic framework (DTBD-NH2) from 2D materials by the facile building block exchange strategy. The composite showed high surface area, good stability and excel·lent reusability. In animal origin foods, the DTBD-NH2 nanohybrid exhibited higher adsorption efficiency to triglyceride and fatty acid, meanwhile, it had excellent recoveries for chemical hazards.

We developed a web-based non-targeted screening platform for chemicals in food (massFOCUS). The platform include data-conversion system, mass spectrometry libraries, toxicological database, non-targeted screening system, omics data system. The Data-Conversion System conquered the compati·bility of different mass spectrum data and libraries from different manufacturers. The system can be compatible with data from Waters, Angilent, AB Scienx, Shimadz, Thermo Fishers Co. The mass spec·tral library contains more than 8,000 compounds with information including retention time, full scan MS, secondary MS, toxicological data, etc. The platform can also be used for other fields such as environ·ment, public security, and clinic.

Key words: Non-targeted screening; chemcials; foods

Corresponding author: Bing Shao, E-mail: shaobingch@sina.com

T19-98-0103

Novel Tet(L) efflux pump variants conferring resistance to tigecycline and eravacycline in *Staphylococcus* spp

Wang Nan-nan[a], Li Xing-yun[a], Chen Zheng[a], Li De-xi[a], Qin Shang-shang[b], Yao Hong[a], Du Xiang-dang[a]

(a. College of Veterinary Medicine, Henan Agricultural University, Zhengzhou, 450046, People's Republic of China; b. School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou, 450001, People's Republic of China)

Abstract: Tigecycline is regarded as one of the few important last-resort antibiotics to treat compli·cated skin and intra-abdominal infections. Members of the genus ***Staphylococcus*** are zoonotic patho·gens and pose a serious threat to public health. Tigecycline resistance in this species appears to be a rare phenomenon, and the mechanisms underlying tigecycline resistance have not been fully elucidat·ed. Here, we report two novel variants of the *tet*(L) gene in ***Staphylococcus*** spp. from swine in China, designed as *tet*(L)F58L and *tet*$(L)_{A117V}$. The *tet*(L)F58L was located within a 18, 720 bp chromosomal multidrug resistance gene cluster flanked by two copies of IS257 in ***Staphylococcus cohnii*** 11-B-312, while the *tet*$(L)_{A117V}$ was located on a 6, 292 bp plasmid in S. haemolyticus 11-B-93, which could be transferred to S. aureus by electrotransformation. Cloning of each of the two *tet*(L) variants into ***S. aureus*** RN4220 showed 16- or 8-fold increases in the MICs, which can fully confer the resistance to tigecy·cline and eravacycline, but no increase in the MICs of omadacycline, compared with the MICs of the re·cipient strain ***S. aureus*** RN4220. In the ***in vivo*** murine sepsis and in the murine pneumonia models, an increase in colony forming units of ***S. aureus*** 29213_pT93 carrying the *tet*$(L)_{A117V}$ was seen despite tige·cycline treatment. This observation suggests that the *tet*$(L)_{A117V}$ and its associated gene product compro·

mise the efficacy of tigecycline treatment *in-vivo* and may lead to clinical treatment failure. Our finding, that novel Tet(L) efflux pump variants which confer tigecycline and eravacycline resistance have been identified in *Staphylococcus* spp., requires urgent attention.

Key words: Tet(L); efflux pump; variant; tigecycline; Staphylococcus spp.

Corresponding author: Yao Hong, E-mail: yaoh0913@henau.edu.cn; Du Xiang-dang, E-mail: xddu@henau.edu.cn

T19-98-0104

Emergence of a *tet*(M) variant conferring resistance to tigecycline in *Streptococcus suis*

Yu Rui[a], Zhang Yue[a], Xu Yin-di[b], Stefan Schwarz[c], Li Xin-Sheng[a], Shang Yan-Hong[a], Du Xiang-Dang[a]

(a. College of Veterinary Medicine, Henan Agricultural University, Zhengzhou, P. R. China; b. Institute for Animal Husbandry and Veterinary Research, Henan Academy of Agricultural Sciences, Zhengzhou, P. R. China; c. Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany)

Abstract: The aim of this study was to gain insight into the resistance determinants conferring resistance to tigecycline in *Streptococcus* (*S.*) *suis* and to investigate the genetic elements involved in their horizontal transfer. A total of 31 tetracycline-resistant S. suis isolates were screened for tigecycline resistance by broth microdilution. S. suis isolate SC128 was subjected to whole genome sequencing with particular reference to resistance determinants involved in tigecycline resistance. Transferability of genomic island (GI) GISsuSC128 was investigated by transformation. The roles of *tet*(L) or *tet*(M) in contributing to tigecycline resistance in *S. suis* was confirmed by transformation using different *tet*(L)- or *tet*(M)-carrying constructs. Only *S. suis* SC128 showed a tigecycline resistance phenotype. A *tet*(L)- *tet*(M) and catA8 co-carrying GISsuSC128 was identified in this isolate. After transfer of the novel GI into a susceptible recipient, this recipient showed the same tigecycline resistance phenotype. Further transfer experiments with specific *tet*(L) - or *tet*(M) - carrying constructs confirmed that only *tet*(M), but not *tet*(L), contributes to resistance to tigecycline. Protein sequence analysis identified a Tet(M) variant, which is responsible for tigecycline resistance in *S. suis* SC128. It displayed 94.8% amino acid identity with the reference Tet(M) of Enterococcus faecium DO plasmid 1. To the best of our knowledge, this is the first time that a *tet*(M) variant conferring resistance to tigecycline was identified in *S. suis*. Its location on a GI will accelerate its transmission among the *S. suis* population.

Key words: Streptococcus suis; genomic island; tigecycline; resistance

Corresponding author: Xiang-Dang Du, E-mail: xddu@henau. edu. cn; Yan-Hong Shang, E-mail: shangyanhong@henau.edu.cn

T19-98-0105

Mobilization of *tet*(X4) by IS1 family elements in porcine *Escherichia coli* isolates

Yu Runhao[a], Chen Zheng[a], Stefan Schwarz[b], Yao Hong[a], Du Xiang-Dang[a]

(a. College of Veterinary Medicine, Henan Agricultural University, Zhengzhou, 450046, P. R. China; b. Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany)

Abstract: In this study, we investigated the dissemination of the high-level tigecycline resistance gene *tet*(X4) in porcine *Escherichia coli*. The corresponding isolates were subjected to PCR analysis, conjugation, S1-PFGE, Southern blot hybridization, transposition experiments and WGS analysis. *tet*(X4) and other antimicrobial resistance genes were located on the plasmids p1919D3-1 and p1919D62-1 and flanked by two or three copies of *IS1* family elements, which can form one to three translocatable units (TUs). Using a reduced transposition model, *IS1A* was experimentally demonstrated to mediate the transposition of *tet*(X4) from a suicide plasmid into the *E. coli* chromosome. To the best of our knowledge, this study reported for the first time that *IS1* family elements, in addition to the previously described *IS91*-like elements, can mobilize *tet*(X4) in porcine *E. coli*. The mobilization of *tet*(X4) by an increasing number of IS elements and its co-selection by multiple antimicrobial agents in *E. coli* isolates requires continuing monitoring and surveillance in both human and veterinary medicine.

Key words: *tet*(X4); *IS1* family elements; *Escherichia coli*; transposition

Corresponding author: Du Xiang-Dang, E-mail: xddu@henau.edu.cn

T19-98-0106

Global distribution, dissemination and overexpression of potent multidrug efflux pump RE-CmeABC in *Campylobacter jejuni*

Yao Hong[a], Zhao Wenbo[a], Jiao Dian[a], Stefan Schwarz[b], Zhang Rongmin[c], Li Xin-Sheng[a], Du Xiang-Dang[a]

(a. College of Veterinary Medicine, Henan Agricultural University, Zhengzhou, 450046, P. R. China; b. Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, FreieUniversität Berlin, Berlin, Germany; c. College of Veterinary Medicine, National Risk Assessment Laboratory for Antimicrobial Resistance of Microorganisms in Animals, South China Agricultural University, Guangzhou 510642, P. R. China)

Abstract: *Campylobacter*, especially Campylobacter *jejuni* and Campylobacter coli, are important foodborne pathogen that can be transmitted to humans through food chain, causing gastroenteritis as well as other disease. Compared with the wild-type CmeABC efflux pump, RE-CmeABC has stronger efflux function and can make the multidrug resistance level of *Campylobacter* higher. In this study, the global distribution, dissemination and overexpression of RE-CmeABC in *C. jejuni* were investigated. WGS information for 433 RE-cmeABC-positive *C. jejuni* isolates (including 18 isolates sequenced in thisstudy and 415 isolates from GenBank) was used for the generation of minimum-spanning trees with STs. WGS analysis revealed the global distribution of RE-*cmeABC* among *C. jejuni* from more than 10 countries. MLST results indicated that various STs were involved in the dissemination of RE-*cmeABC*, with ST2109being the most predominant. Phylogenetic analysis revealed the close relationship between RE-*cmeABC*-carrying *C. jejuni* isolates from poultry and humans. The IR polymorphism in the RE-CmeABC promoter region isassociated with the overexpression of RE-*cmeABC*, which was demonstrated experimentally by RT-PCR. Our analysis represents the first view of the global distribution of RE-Cme·

ABC, which is horizontally transferable anddiffused regionally in a clonalmanner. The close relationshipof RE-*cmeABC*-positive *C. jejuni* from poultry and humans supports the potential of these isolates for zoonotictransmission. Overexpressed RE-CmeABC in *C. jejuni* will increase the fitness of the corresponding bacteria andbe of advantage under antimicrobial selection.

Key words: *Camylobacter jejuni*; RE-CmeABC; genetic diversity; poultry; overexpression

Corresponding author: Du Xiang-Dang, E-mail: xddu@henau.edu.cn

T19-98-0107

Aggregation-Induced electrochemiluminescence sensor for the rapid detection of chloramphenicol

Zhang Ziyi[a,b], Chen Lifen[b], He Jiakang[a], Chen Hailan[a], Guo Longhua[b]

(a. School of Animal Science and Technology, Guangxi University, Nanning 530004, China; b. School of Biochemistry and Chemical Engineering, Jiaxing University, Jiaxing 314001, China)

Abstract: Chloramphenicol (CAP), a broad-spectrum antibiotic, is widely used in veterinary medicine. However, the use of CAP is associated with side effects, such as aplastic anemia, leukemia and bone marrow suppression, in humans, suggesting that residues present in the food chain could potentially create a food safety issue. In this work, an aggregation-induced electrochemiluminescence sensor was proposed for chloramphenicol (CAP) detection. In this sensing system, the ligand 1,1,2,2-tetra(4-carboxylbiphenyl)ethylene (H4TCBPE) was cross-linked with gold ion, leading to a stronger electrochemiluminescence (ECL) emission in comparison to H4TCBPE monomers, showing similar effect to previous reports. Then a CAP specific aptamer was modified with ferrocene at both ends, which can automatically bind to H4TCBPE, resulting in the effective quenching of H4TCBPE/Au ECL emission. However, when CAP is present, they are recognized and caught by the modified aptamer, causing the reverse of the H4TCBPE/Au structure switch and thus the recovery of ECL emission. This work reveals a new direction for detecting CAP or other target compounds

Key words: Aggregation-Induced electrochemiluminescence; Chloramphenicol detection; aptamer

Corresponding author: Zhang Ziyi, E-mail: 626232853@qq. com; Chen Hailan, E-mail: hlchen319@163.com; Guo Longhua, E-mail: glonghua@163.com

T19-98-0108

Zinc oxide nanoparticles induce hepatotoxicity by pyroptosis

Xingyao Pei[a], Xingyu Liu[b], Daowen Li[b], Shusheng Tang[a]

(a Department of Pharmacology and Toxicology, College of Veterinary Medicine, China Agricultural University, Yuanmingyuan West Road No.2, Haidian District, Beijing 100193, China; b. Tianjin Key Laboratory of Agricultural Animal Breeding and Healthy Husbandry, College of Animal Science and Veterinary Medicine, Tianjin Agricultural University, Jinjing Road No.22, Xiqing District, Tianjin 300384, China)

Abstract: In recent years, with the rapid development of nanotechnology, ZnO nanoparticles (ZnO NPs) have been widely used in the fields of daily necessities, medicine, food and animal feed. Previous

studies have shown that misuse or improper use of ZnO NPs could pose a health risk to humans and animals. Liver has been known as a main toxic target organ of ZnO NPs, which have been shown to mediate hepatocyte death. However, the question whether zinc oxide nanoparticles lead to hepatocyte death through cell pyroptosis has not been answered yet. Based on our studies in vitro and in vivo, we revealed that ZnO NPs disrupted zinc homeostasis in rat liver, leading to massive zinc accumulation and liver impairment. Meanwhile, ZnO NPs induced the assembly of the NLRP3-ASC-Caspase-1 inflammatory complex in hepatocyte, and further induced the cleavage of GSDMD, promoting the over·expression and leakage of inflammatory cytokines, such as IL-1β and IL-18. These evidences confirmed the activation of Caspase-1-dependent classical pyroptosis pathway induced by ZnO NPs and provide a basis for application supervision and risk assessment of ZnO NPs.

Key words: ZnO NPs; pyroptosis; liver; inflammasome; GSDMD

Corresponding author: Xingyao Pei, E-mail: xingyaopei@163.com

T19-98-0109

ROS/NLRP3 inflammasome mediates LPS-induced chicken primary intestinal epithelial cell injury

Chang Yi-cong, Liu Fang-ping

(College of Veterinary Medicine, Northeast Agricultural University, Harbin, Heilongjiang, 150030)

Abstract: Lipopolysaccharide (LPS), the main component of the cell wall of Gram-negative bacteria, inducesintestine inflammation.ROSoverproduction induces oxidative stress and closely relates to apop·tosis and NLRP3 inflammasomeactivation.Therefore, this study aims to explore the mediating effect of ROS/NLRP3 inflammasomes on LPS-induced chicken primary intestinal epithelial cell (IEC) injury, and provides a scientific basis for study and prevention of LPS toxicology. The cells were randomly divided into four groups, namely control group, LPS group, ROS inhibitor/NLRP3 inhibitor+LPS group and ROS inhibitor/NLRP3 inhibitorgroup.After being treated with 2.5 mmol·L^{-1} NAC (ROS inhibitor)or 10 μmol/L MCC950 (NLRP3 inhibitor) for 2 h, the cells were co-treated with 100 μg·mL^{-1} LPS for 12 h.ROS con·tent, oxidative stress, barrier function, apoptosis, inflammation, and NLRP3 inflammasomes have been detected. The results showed that IECs were isolated, cultured and identified by using 14-day-old chick·en embryo, and 100 μg·mL^{-1} LPS treated cells for 12 h establishedthe injury model. LPS inducedchang·es in levels of allindicators (P<0.01) compared to control group. Compared to LPS group, NACreduced ROS generation, decreased MDA content, and increased GSH content, T-SOD and GSH-Px activities (P<0.01). NAC enhanced of ZO-1, occludin and claudin-1 expression (P<0.01). Moreover, NAC in·creased bcl-2expression, and reduced bax and caspase-3 expression, decreased IL-6, IL-8 and TNF-αexpression, and enhanced IL-10expression (P<0.01). Additionally, NAC inhibited NLRP3 and cas·pase-1expression and activation, and IL-1β and IL-18expression and content (P<0.01). Consistently, MCC950inhibitedNLRP3 inflammasomes activation and IL-1β and IL-18secretion (P<0.01). This study found that inhibiting ROS alleviatesLPS-induced IECs injury and maintains barrier function.ROS inhibi·tion reduces oxidative stress, apoptosis and inflammation. Importantly, ROS and NLRP3 inflamma·somes suppression reduces IL-1β and IL-18release by inhibiting NLRP3 inflammasomesactivation, indi·cating that ROS/NLRP3 inflammasomes are involved in mediating LPS-induced chicken primary IECinjury.

Key words: ROS; NLRP3 inflammasome; LPS; primary intestinal epithelial cells; chicken

Corresponding author: Chang Yi-cong, E-mail: 476587664@qq.com, Liu Fang-ping, E-mail: fangpingliu@126.com

T19-98-0110

Safety evaluation of chlorogenic acid colistin sulfate injection in pigs

Li Kai-yuan, Tang Shu-sheng

(Department of Basic Veterinary Medicine, College of veterinary medicine, China Agricultural University 100193, P. R. China)

Abstract: Colistin sulfate chlorogenic acid injection was a self-made product in this study. In order to evaluate the safety of this injection, a safety experiment was conducted in pigs. Four experimental groups were set up, colistin sulfate injection 5 times dose group, dose 12.5 mg/ kg/ d; Colistin sulfate chlorogenic acid injection was given in one(2.5 mg/ kg/ d), three(7.5 mg/ kg/ d) and five(12.5 mg/ kg/ d) times dosage groups. The drug was administered twice a day with an interval of 12 h, and a blank control group was set up.

The results showed that 1 day after administration, the creatinine and urea nitrogen levels in colistin sulfate injection and colistin sulfate chlorogenic acid injection 5 times group were increased compared with the blank group ($P<0.05$), the other dose groups had no significant difference; The creatinine and urea nitrogen levels in the compound group were significantly lower than those in the single dose group ($P<0.05$). So chlorogenic acid can significantly inhibit the rise of serum creatinine and urea nitrogen induced by colistin. Histopathological section results showed that the renal tubules in the 5 times dose colistin sulfate injection group were severely damaged, the epithelial cells were denaturated and necrotic, separated from the basement membrane, and the protein tubules appeared in the lumen. However, the damage of colistin sulfate chlorogenic acid group was significantly reduced, and compared with the control group, there were no obvious abnormalities in renal tubules in other compound dose groups.

This study showed that the preparation has good safety when used at 1-3 times of the recommended dose, providing data support for the use of the preparation in clinical treatment.

Key words: Colistin sulfate, chlorogenic acid, safety, dose,

Corresponding author: Tang Shu-sheng, E-mail: tssfj@cau.edu.com

T19-98-0111

Stimulation of quadriceps femoris with amoxicillin and baicalein breast injection in New Zealand white rabbits

Li Kai-yuan, Tang Shu-sheng

(Department of Basic Veterinary Medicine, College of veterinary medicine, China Agricultural University 100193, P. R. China)

Abstract: In this study, the stimulation test of Amoxicillin Baicalein Intramammary Suspension on quadriceps femoris of New Zealand white rabbits was conducted. In this study, the stimulation test of amoxicillin and baicalein breast injection on quadriceps femoris of New Zealand white rabbits was con·

ducted. Six healthy adult New Zealand white rabbits were randomly divided into 3 treatment groups with 2 rabbits in each group and half male and half female (female rabbits should not be pregnant).

A drug for three batches of Amoxicillin Baicalein Intramammary Suspension, each batch 2 drugs take healthy New Zealand white rabbit, cut to the front of the hind legs rabbit hair, the skin with alcohol after disinfection, one side in the hind leg quadriceps injection of 1 ml of the test, in the other side of the corresponding parts of the hind limbs injected with sterilized saline as a control volume. The symptoms of loose hair, lethargy, anorexia and movement difficulty were observed carefully.

After 24 hours of administration, the rabbits were killed by injecting air into the ear vein. The quadriceps femoris muscle was dissected and dissected longitudinally to observe the local stimulus response and record the corresponding stimulus intensity (response level). The quadriceps femoris muscle at the injection site was fixed in 4% formaldehyde solution and prepared for section and microscopic examination.

The results showed that after 3 batches of Amoxicillin Baicalein Intramammary Suspension, only a small amount of nuclear increase was observed in one group, and no obvious abnormality was observed in other groups. Naked eye observation of the reaction order of 0.5, 0, 0.5, less than 1.4, indicating that the product is small irritation, perfusion safety.

Key words: amoxicillin; Baicalein; Suspension; stimulation test

Corresponding author: Tang Shu-sheng, E-mail: tssfj@cau.edu.com

T19-98-0112

Evaluation of mucosal irritation of amoxicillin baicalein intramammary injection in rats

Chen Ting-ting, Tang Shu-sheng

(Department of Basic Veterinary Medicine, College of veterinary medicine, China Agricultural University 100193, P. R. China)

Abstract: Amoxicillin Baicalein Intramammary Injection was a self-developed preparation for the treatment of clinical dairy cow mastitis. To assess the safety to the skin after injection, twelve SPF-grade female rats (90-100 days old) were randomly divided into two groups (n=6). The rats in test group were infused with 0.5 mL of the test drug in the vagina, and the same amount of soybean oil was given to the animals in the control group. All rats were administrated twice a day for 7 consecutive days. After each infusion, observe the clinical manifestations within 1 hour and 4 hours. The results showed that all the animals had no symptoms of poisoning and no deaths. Gross autopsy revealed that there was no abnormal changes on the hearts, livers, spleens, lungs, kidneys, gastrointestinal tracts and other tissues and organs in the tested rats. Compared to the control group, the vaginal tissue slic·es in the treatment group did not show any pathological changes, indicating that the injection did not have irritation to the vaginal mucosa of rats. This study preliminarily provided guidance for Amoxicillin Baicalein Intramammary Injection safely used in the treatment of mastitis.

Key words: amoxicillin; baicalein; mastitis; mucosal irritation

Corresponding author: Tang Shu-sheng, E-mail: tssfj@cau.edu.com

T19-98-0113

TGF-β/NOX4/mtROS pathway contributes to colistin-induced pulmonary toxicity

Dai Chong-shan*, Tang Shu-sheng*, Shen Jian-zhon*
(College of Veterinary Medicine, China Agricultural University, Beijing 100193, P. R. China)

Abstract: Colistin therapy can cause pulmonary toxicity, however, our understanding of the underlying molecular mechanism remains incomplete. This study aimed to investigate the molecular mechanism of colistin-induced pulmonary toxicity in vitro and in vivo. Our results showed that intraperitoneal colistin treatment significantly increased the expression of TGF - β and NOX4 protein and mRNA, then triggers oxidative stress and apoptosis in the lung tissue of mice and A549 cells. Moreover, colistin treatment significantly increased levels of mitochondrial ROS (mtROS) and autophagy flux in A549 cells. Inhibition of NOX4 protected A549 cells against colistin-induced mtROS and apoptosis. Colistin treatment also down-regulated the expression of p-Akt and p-mTOR proteins (all $P<0.05$ or $P<0.01$) in lung tissues of mice or A549 cells. Inhibition of autophagy or Akt pathways by chloroquine (CQ), 3-Methyladenine (3-MA) or LY294002 promoted colistin-induced mitochondrial damage, and caspase-dependent cellular apoptosis. Whereas, activation of autophagy by rapamycin pretreatment of A549 cells partly abolished colistin-induced cytotoxicity, mitochondrial dysfunction, and apoptosis. This is first study to show that colistin-induced pulmonary toxicity involves the activation of TGF - β/NOX4 / mtROS pathway and the inhibition of Akt/mTOR pathway in lung tissues of mice and highlights the key protective role of autophagy activation.

Key words: Colistin; Mitochondrial ROS; Akt/TSC2/mTOR pathway; NOX4/TGF-β pathway

Corresponding author: Shen Jian-zhon, E-mail: daichongshan@cau.edu.cn; Tang Shu-sheng, E-mail: tssfj@cau.edu.cn

T19-98-0114

Antibacterial properties of curcumin and its nano-formulations: molecular mechanisms and clinical implications

Dai Chong-shan, Tang Shu-sheng
(College of Veterinary Medicine, China Agricultural University, Beijing 100193, P. R. China)

Abstract: Currently, the rapid spread of antibiotic resistance and lack of effective drugs for treating infections caused by multi-drug resistant bacteria force us to find new antibacterial candidates or strategies. Natural products have served as powerful therapeutics against bacterial infection and are still the source for the discovery of novel antibacterial drugs. Curcumin, an important constituent of turmeric, exhibited the great activities against Gram-negative and Gram-positive bacteria through inhibiting the production of bacterial virulence factors, biofilm formation, metabolism and inducing oxidative stress, programmed cell death, and phototoxicity. *In vitro* and animal experiments showed that curcumin in combination with clinical antibiotic drugs (e. g., polymyxin B, colistin, ciprofloxacin, and tetracycline) exhibited the marked synergistic anti-bacterial activity. Nano-formulations of curcumin are prepared and characterized to increase its water solubility and bioavailability. This review summarizes the anti·

bacterial properties and current underlying molecular mechanism of curcumin per se, curcumin-based combination, and its Nano-formulations and clinical trials and provides prospect into the further application as a promise antimicrobial candidate in clinic.

Key words: Antibacterial resistance; Curcumin; Bacterial infection; Molecular mechanism; Nano-formulations

Corresponding author: Dai Chong-shan, E-mail: daichongshan@cau.edu.cn; Tang Shu-sheng, E-mail: tssfj@cau.edu.cn

T19-98-0115

p21 governs the protective effect of berberine on colistin-induced nephrotoxicity through modulating AMPK/Akt/Nrf2/HO-1 axis

Dai Chong-shan, Tang Shu-sheng, Shen Jian-zhong

(College of Veterinary Medicine, China Agricultural University, Beijing 100193, P. R. China)

Abstract: Nephrotoxicity is the major adverse effect patients experience during colistin therapy. The development of effective nephroprotective agents that can be co-administered during polymyxin therapy remains a priority area in antimicrobial chemotherapy. In this study, we performed a high-throughput screening via a small molecule compound library and the results showed that berberine exhibited the most obviously protective effect on colistin-induced cytotoxicity in HEK293T cells. In a mouse model, our results showed that berberine supplementation at 100 mg · kg^{-1} significantly improved colistin treatment-induced oxidative stress, mitochondrial dysfunction and renal pathology damage. In HEK293T cells, our results showed thar berberine treatment significantly inhibited colistin-induced production of mitochondrial ROS, loss of mitochondrial membrane potential, and apoptotic cell death. Drug structure-activity network interaction analysis showed that cyclin-dependent protein serine /threonine kinase regulator activity pathway is ranked in the first and its targets contain p21, CCND1 and HO-1 proteins. Then, we found that berberine treatment significantly upregulated the expression of p21, HO-1, Nrf2, phosphorylation (p)-AMPK (Thr172), and p-Akt (Ser473) proteins, and downregulated the expression of CCND1 protein in a dose-dependent manner. Compared to colistin alone treatment, berberine co-treatment significantly increased the expression of p21, p-AMPK (Thr172), p-Akt (Ser473), Nrf2 and HO-1 proteins, but did not rescue the decrease of CCND1 protein caused by colistin treatment. Gene knockout of p21 by CRISPR partly improved colistin-induced cell death, but completely abolished the regulated effect of berberine on the expression of p- AMPK (Thr172), p-Akt (Ser473), and Nrf2 proteins. Pharmacology inhibitions of Akt, Nrf2, and HO-1 protein expression significantly promoted colistin-induced cytotoxicity in HEK293T cells. In conclusions, our study for the fist time reveals that p21 plays a critical role in the protective effect of berberine on colistin-induced nephrotoxicity, which may involve the modulation of AMPK/Akt/Nrf2/HO-1 axis. Our study highlights that oral berberine could potentially ameliorate nephrotoxicity in patients undergoing polymyxin therapy.

Key words: Colistin; AMPK/Akt/Nrf2/HO-1 axis; Nephrotoxicity; Berberine

Corresponding author: Dai Chong-shan, E-mail: daichongshan@cau.edu.cn; Shen Jian-zhong, E- mail: sjz@cau.edu.cn

T19-98-0116

Qicao decoction improves dairy herd of subclinical mastitis cows and the mechanism of prediction

Yi-meng Fan[a], Qingyu Wang[a], Yuan-yuan Wei[a], Pingping Wang[a], Fengting Lang[b], Xiaoqin Xu[c], Qihe Tang[b], Zhihui Hao[a]

(a. Innovation Centre of Chinese veterinary medicine, College of Veterinary Medicine, China Agricultural University, Beijing 100086, China; b. Agricultural Bio-pharmaceutical Laboratory, College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, Qingdao 266109, China; c. Center for Comparative Medicine, Yangzhou University, Yangzhou 225009, China)

Abstract: **OBJECTIVE** Mastitis is one of the most serious diseases affecting dairy cattle, and it leads to substantial socioeconomic losses. Recently, subclinical mastitis has developed resistance to antibiotics, and therefore, new effective anti-mastitis treatments without antibiotics use should be introduced. Qicao decoction (QCD) is a Chinese formula used to treat cow mastitis, and its curative effect and mechanism of action on cows with subclinical mastitis were investigated in this study. **METHODS** We first tested the safety of QCD by routine blood biochemistry tests, and then evaluated the dairy herd improvement (DHI) of cows with subclinical mastitis after treatment with QCD. In addition, network pharmacology and molecular docking were used to explore the potential ingredients and mechanisms of action of QCD. **RESULTS** After treatment with QCD, milk somatic cell count (SCC) significantly decreased, and dairy herd improvement (DHI) was observed. QCD application also improved the milk nutritional value by increasing its lactose, fat, and protein contents. Further network pharmacology studies demonstrated that the therapeutic effects of QCD are likely attributable to its antioxidant and anti-inflammatory effects by regulating MAPK / PI3K-Akt/ NF - κB. **CONCLUSION** QCD has a significant effect in cows with mastitis through a "multi-component-target pathway". Our findings also provide a reference for the study of the mechanism of traditional Chinese medicine in the treatment of complex diseases.

Key words: Qicao Decoction; Subclinical Mastitis; Pharmacodynamic Evaluation; Network Pharmacology

T19-98-0117

Toxicological assessment of pesticide metabolites

Anja Hueser
Bayer AG

Abstract: Pesticide application can lead to residues of metabolites in plant and livestock. To assure consumer (dietary) safety the toxicological properties of the metabolites need to be assessed to derive Health based guidance values (HBGV) for risk assessment. Depending on the pesticidal active substance large numbers of metabolites can be formed. However, most of the potential metabolites have only low exposure contribution. Toxicological testing of all metabolites is not possible due to animal welfare considerations, synthesis feasibility, capacity, and timelines. Metabolites with higher exposure contribution need to be prioritized for toxicological testing, but new approaches (in silico, (Q)SAR, grouping and read-across) can be used for the assessment of the toxicological properties of metabo·

lites. Although in silico tools ((Q)SAR) for the prediction of higher tier endpoints (e.g. carcinogenicity, re·productive toxicity) are currently not reliable, they can be used as supporting information. The grouping can be done on the basis of structural similarity, same toxicophore or mode of action, or metabolic path·way considerations. For building groups and the read-across all available information need to evaluat·ed in a weight of evidence assessment.

T19-98-0118

Long-term low-dose multiple exposures to α particles increase the malignancy rather than the risk of lung carcinoma in comparison with a single exposure to the same dose

Weiwei Peia, Wenying Yanb, Wanshi Lia, Jing Niea, Wentao Hua, Tom K. Heic, Guangming Zhoua*

(a. State Key Laboratory of Radiation Medicine and Protection, School of Radiation Medicine and Protection, Collaborative Innovation Center of Radiological Medicine of Jiangsu Higher Education Institutions, Soochow University, Suzhou 215123, China; b. Center for Systems Biology, School of Biology and Basic Medical Sciences, Soochow University, Suzhou 215123, China; c. Center for Radiological Research, College of Physician and Surgeons, Columbia University, New York, NY 10032, USA)

Abstract: Space radiation is the primary concern for manned space exploration into deep space. As such, space radiation risk assessment and effective countermeasures must be well established pri·or to long-term manned space exploration missions including stationing on the Moon and traveling to the Mars. Carcinogenesis is one of the health risks of space radiation exposures, however, systematic studies on the carcinogenic potential of low dose rate space radiation are still far from enough to estab·lish a reliable risk assessment system. Since alpha particles are the second most abundant types of space ionizing radiation and contribute much to effective dose, we simulated the space radiation envi·ronment by multiple exposures of cells *in vitro* to 0.02 Gy alpha particles in every three days. The po·tential of the low dose rate alpha particles in inducing malignant transformation was investigated using human lung bronchial epithelial BEAS-2B cells. Dose effect curves for malignant transformation were established in a total dose range less than 0.5 Gy. Tumor formation in NOD/SCID mice and changes in gene expression, occurrence of cancer stem-like cells, and epithelial-mesenchymal transition (EMT), were examined at various passages during post-irradiation subculture. Meanwhile, the differentially ex·pressed genes and related signaling pathways between single and multiple exposure groups were ana·lyzed by employing deep sequencing together with functional enrichment analysis and biological net·work modeling.

In vivo experiments and pathological analysis demonstrated that both multiple and single expo·sures induced malignant transformation at the 50th passages in a dose-dependent manner and there was no significant difference between two kinds of exposures. Both morphological changes and the ex·pression of EMT-related genes indicated the induction of EMT by the exposure to alpha particles. Cell proliferation, migration and invasion of the irradiated cells increased significantly at the 50th passage. Furthermore, these neoplastic characteristics were significantly more pronounced in the multiple expo·sure group than the single exposure group. The proportion of lung cancer stem-like cells in irradiated cells at 30th passage increased significantly when compared with controls. Besides, the bioinformatics

analysis revealed some established cancer-related pathways (such as PI3K-Akt signaling) and genes (such as WNT5A and HER2) were activated by low dose rate alpha particles irradiation. Moreover, protein-protein interaction (PPI) network analysis suggests that long-term low-dose multiple exposures induced a significantly higher network degree, in which the top three hub genes were CDKN2A, CCND1, and BMP4.

Our results suggest that alpha particle irradiation is tumorigenic to human bronchial epithelial cells and, moreover, long-term low-dose multiple exposures to α particles do not increase the risk but the malignancy of lung carcinoma in comparison with a single exposure to the same dose.

Key words: Tumorigenesis; Human lung epithelial cells; Alpha particles; Low dose/Low dose rate

Corresponding author: E-mail: gmzhou@suda.edu.cn

T19-98-0119

Postnatal exposure to tetrabromobisphenol A retarded testis development in mice

Li Yuanyuan[a,b], Dong Mengqi[a,b], Qin Zhanfen[a,b]*

(a. State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China; b. University of Chinese Academy of Sciences, Beijing 100049, China)

Abstract: Whether or not tetrabromobisphenol A (TBBPA), a ubiquitous pollutant frequently detected in humans, has reproductive developmental toxicity remains controversial. Here, we evaluated the effects of TBBPA exposure from postnatal day (PND) 0 to 56 through drinking water (15, 150, 1500 ng/mL) on testis development in mice. On PND 7, a dose-dependent retardation of testes development was observed in TBBPA-treated pups, which was characterized by thinned seminiferous tubules and disordered Sertoli cell arrangement, coupled with decreases in the Sertoli cell number and the germ cell number per seminiferous tubule as well as the downregulation of their marker gene expression. Up to puberty (PND 35), most of these TBBPA-caused alternations were still observable, indicating that the effects of TBBPA on testis development was persistent. In adulthood (PND 56), the seminiferous tubule area and the Sertoli cell number were still smaller in the TBBPA-treated testes than controls, along with downregulated expression of germ cell marker genes, despite no alternations in testis weight, sperm parameters, the seminiferous epithelium cycle, and the testosterone level. All the findings convincingly demonstrate that postnatal exposure to TBBPA retarded testis development in early life and ultimately caused adverse outcomes in adult testes, highlighting its health risk for humans.

Key words: Tetrabromobisphenol A; testis development; seminiferous tubule; Sertoli cell; germ cell

T19-98-0120

Bacterial whole-cell biosensors for visual monitoring of toxic heavy metals in the environment

Chang-ye Hui*, Xue-qin Yang, Chao-xian Gao, Li-mei Li, Yu-ting Chen, Zheng-yu Liu, Yan Sha, Juan Yi, Yan Guo, Naixing Zhang

(Department of Pathology & Toxicology, Shenzhen Prevention and Treatment Center for Occupational Diseases, Shenzhen 518020, China)

Abstract: During the last few decades, whole-cell biosensors have attracted increasing attention for their enormous potential in monitoring bioavailable heavy metal contaminations in the ecosystem. Visual and measurable output signals by employing natural pigments have been demonstrated to offer another potential choice to indicate the existence of bioavailable heavy metals in recent years. The biosynthesis of the purple violacein and the blue indigoidine has been achieved in *E. coli* following heterologous expression of pigment biosynthetic enzymes, which are triggered by the heavy metal-responsive transcription regulators. The resulting visual pigment-based biosensors presented a good selectivity and high sensitivity to target metal ions including Cd(II), Pb(II), and Hg(II). High concentration of target metal exposure could be clearly recognized by the naked eye due to the color change by the secretion of pigment, and quantified by measuring the absorbance of the culture supernatants within the visible wavelength range. Although fairly good linear relationships were obtained in a relatively limited concentration range of the concentrations of heavy metal ions, these findings suggest that genetically controlled pigment biosynthesis triggered by the MerR family transcriptional regulator can enable a sensitive, visual, and qualitative whole-cell biosensor for bioindicating the presence of bioaccessible heavy metal in environmental water samples.

Key words: Whole-cell biosensor, Heavy metal, pigment, Environmetal contamination

Corresponding author: Chang-ye Hui, E-mail: hcy_sypu@163.com

T19-98-0121

Antifungal agent Terbinafine restrains tumor growth in preclinical models of hepatocellular carcinoma via AMPK-mTOR axis

Er-Bin Zhang[1,8], Xiuping Zhang[2,3,8], Kang Wang[2], Fengkun Zhang[1], Tian-Wei Chen[1], Ning Ma[1], Qian-Zhi Ni[1], Yi-Kang Wang[1], Qian-Wen Zheng[1,4], Hui-Jun Cao[1], Ji Xia[1], Bing Zhu[1], Sheng Xu[1], Xufen Ding[1], Xiang Wang[5], Zhigang Li[6], Shuqun Cheng[2], Dong Xie[1,4,7], Jing-Jing Li

(1. CAS Key Laboratory of Nutrition, Metabolism and Food Safety, Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences 200031 Shanghai, China; 2. Department of Hepatic Surgery VI, Eastern Hepatobiliary Surgery Hospital, Naval Medical University, 2000438 Shanghai, China; 3. Faculty ofHepato-Pancreato-Biliary Surgery, Chinese People Liberation Army (PLA) General Hospital; Institute of Hepatobiliary Surgery of Chinese PLA, 100853 Beijing, China; 4. School of LifeScience and Technology, ShanghaiTech University 201210 Shanghai, China. 5First People's Hospital of Huzhou, First Affiliated Hospital of Huzhou University 313000 Huzhou, Zhejiang, China; 6. Department of Thoracic Surgery, Section of Esophageal Surgery, Shanghai Chest

Hospital, Shanghai Jiao Tong University 200030 Shanghai, China; 7. NHC KeyLaboratory of Food Safety Risk Assessment, China National Center for Food Safety Risk Assessment 100022 Beijing, China)

Abstract: Backgroud Liver cancer is one of the leading causes of cancer-related death worldwide and is especially prevalent in China. According to the global cancer statistics, liver cancer ranks the sixth most common malignancy and the fourth leading cause of cancer-related deaths globally. Hepatocellular carcinoma (HCC) is the major form of liver cancer that threatens human health. Despite recent improvement in the diagnosis and treatment approaches, most HCC patients diagnosed at advanced stages with unfavorable patient conditions suffer from limited treatment options. The two FDA-approved first-line drugs, sorafenib and lenvatinib, can only extend patients' survival by less than 4 months. Therefore, there is a strong urge to develop more effective therapeutic approaches to improve the outcome of HCC patients. Compared to traditional drug development, drug repurposing offers a shorter approval process and straightforward path to clinical translation. Terbinafine, an allylamine antifungal agent, is well-established in the treatment of onychomycosis. Here, we have provided a comprehensive investigation of Terbinafine in HCC.

METHODS The influence of Terbinafine on cell growth, 3D spheroid formation, clonogenic survival and protein synthesis was investigated in human HCC cell lines. Co-immunoprecipitation, immunofluorescence and other techniques were employed to explore how Terbinafine exerts its anticancer effect. Subcutaneous tumorigenicity assay, orthotopic and patient-derived xenograft (PDX) HCC models were used to evaluate the anticancer effect of Terbinafine monotherapy and the combinatorial treatment with Terbinafine and sorafenib against HCC.

RESULTS Terbinafine treatment dramatically suppressed the proliferation of HCC cells. The Edu incorporation assay revealed that Terbinafine inhibited DNA synthesis and reduced S-phase population. In addition, the in vitro 3D culture also demonstrated the anti-proliferative activity of Terbinafine against HCC cells.

Terbinafine exerts its fungicidal properties by inhibiting fungal squalene epoxidase (SQLE), which is a key enzyme in the synthesis of ergosterol, an essential component of fungal cell membranes. However, knockdown of SQLE did not influence the influence of Terbinafine, indicating the anticancer activity of Terbinafine was SQLE-independent. Further mechanistic study disclosed that AMPK-mTOR signaling was responsible for the anticancer effect of Terbinafine.

The strong inhibitory effect of Terbinafine on mTORC1 signaling prompted us to evaluate whether the combinatorial treatment with Terbinafine and the first-line drug sorafenib would be an effective therapeutic approach. Interestingly, Terbinafine alone modestly inhibited mTORC1 signaling at low concentration, and sorafenib exhibited similar suppression of mTORC1 signaling. It was worthy to note that combined treatment with Terbinafine and sorafenib almost completely blocked mTORC1 signaling.

Consistently, Terbinafine or sorafenib single treatment inhibited tumor growth in tumorigenicity assay. However, combination of sorafenib and Terbinafine led to a stronger reduction in tumor growth, and decreased tumor burden more effectively than either single agent. Similar effect was also observed in Patient-derived xenografts model. In addition to suppression of mTOR signaling, Terbinafine in combination with sorafenib caused severe DNA damage, which contributed to the strong anticancer activity of their combination.

CONCLUSIONS In conclusion, our study revealed that Terbinafine exerted anticancer activity independent of the antifungal target SQLE. Mechanistically, Terbinafine suppressed mTORC1 activity to inhibit hepatocellular carcinoma growth by activation of AMPK, concurrent treatment with sorafenib po-

tentiated Terbinafine-mediated growth inhibition and caused a defect in homologous recombination (HR)- pathway, leading to cell death and ultimate repression of HCC. Therefore, our study provides a safe and potentially effective strategy to enhance the efficacy of sorafenib by combining treatment with Terbinafine.