第37回日本臨床薬理学会学術総会
The 37th Annual Scientific Meeting of
the Japanese Society of Clinical Pharmacology and Therapeutics

第6回 日中薬理学・臨床薬理学ジョイントミーティング
第六届 日中药理学与临床药理学交流会

“Perspective of academic collaboration between Japan and China in clinical and basic pharmacology”

- Program & Abstract Book -

Date  December 3 (Sat), 2016
Venue  BIG SHIP (Yonago Convention Center)
"Perspective of academic collaboration between Japan and China in clinical and basic pharmacology”

- Program -

Oral Presentation

Date & Time : 14:10 – 16:00, December 3 (Sat), 2016
Session Room : Room 2 (International Conference Room, 2F, BIG SHIP)
   Chairs : Kazutaka Shimoda (Department of Psychiatry, Dokkyo Medical University School of Medicine, Japan)
            Nai-hong Chen (Institute of Materia Medica, China Academy of Medical Sciences, China)

<Japan>
Oral-J1) Recent therapeutic progress in the management of pulmonary arterial hypertension
   Hiroshi Watanabe (Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, Japan/
   Center for Clinical Sciences, National Center for Global Health and Medicine, Japan)

Oral-J2) Uricosuric agents and renal tubular urate transporters
   Naohiko Anzai (Department of Pharmacology, Graduate School of Medicine, Chiba University, Japan)

<China>
Oral-C1) Salvianolic Acid A Alleviates Diaebtic Complicaitons by inhibition of Hyperglycemia and oxidantive stress
   Guanhua Du (National Center for Pharmaceutical Screening, Institute of Materia Medica, Chinese Academy of Medical Science & Pekin Union Medical College, Beijing, China)

Oral-C2) Urea Transporter UT-B as a Novel Anti-hypertansion Drug Target
   Baoxue Yang (Department of Medicine, Peking University, Beijing, China)

Oral-C3) Proteasome inhibitors reactivate latent HIV through recruiting HSP90/p-TEFb complex after the activation of HSF-1
   Shuwen Liu (School of Pharmaceutical Sciences, Southern Medical University, Guangzhou, China)

Presentation Time : 15 minutes for presentation
                  5 minutes for discussion
Poster Presentation

Date & Time: 13:20 – 14:00, December 3 (Sat), 2016
Session Room: Poster Room 1 (Conference Room 4, 5F, BIG SHIP)

Poster-C1) Parkin and Its Related diseases
Yi-na Jiang (Hunan University of Chinese Medicine, Changsha, China/
Institute of Material Medica, Chinese Academy of Medical Sciences & Peking
Union Medical College, Beijing, China)

Poster-C2) Variation Rules and Biological Activity of CKLF1 in Obstructive Jaundice
Piao Luo (College of Pharmacy, Hunan University of Chinese Medicine, Changsha, China/
Department of Pharmacology, Institute of Materia Medica, and Neuroscience
Center, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China)

Poster-C3) Ginsenoside Rg1, as multi-target drug candidate for liver diseases through multi-target
effects: Nrf2 induction and liver protection
Nai-hong Chen (State Key Laboratory of Bioactive Substances and Functions of Natural
Medicines, Institute of Materia Medica, Chinese Academy of Medical
Sciences and Peking Union Medical College, Beijing, China/
Hunan University of Chinese Medicine, Changsha, China)

Poster-C4) Antioxidant activities of ginsenoside Rg1 against cisplatin-induced hepatic injury through
Nrf2 signaling pathway in mice
Nai-hong Chen (State Key Laboratory of Bioactive Substances and Functions of Natural
Medicines, Institute of Materia Medica, Chinese Academy of Medical
Sciences and Peking Union Medical College, Beijing, China)

Poster-C5) A potential novel target for stroke treatment: selective modulation of microglia phenotype
Nai-hong Chen (State Key Laboratory of Bioactive Substances and Functions of Natural
Medicines, Institute of Materia Medica & Neuroscience Center, Chinese
Academy of Medical Sciences and Peking Union Medical College, Beijing,
China)

Poster-C6) Association of ABCB1 genetic polymorphisms with susceptibility to colorectal cancer and
therapeutic prognosis
Min-Jie Wei (Department of Pharmacology, School of Pharmacy, Liaoning Key Laboratory
of Molecular Targeted Anti-Tumor Drug Development and Evaluation, China
Medical University, Shenyang, China)

Poster-C7) CUG-binding protein 1 regulates HSC activation and liver fibrogenesis
Xingxin Wu (State Key Laboratory of Pharmaceutical Biotechnology and Collaborative
Innovation Center of Chemistry for Life Sciences, School of Life Sciences,
Nanjing University, Nanjing, China)

* Presentation style: free discussion style
(There is no session chair.)
Banquet

Date & Time : 16:30~, December 3 (Sat), 2016
Place : Restaurant, 1F, Hotel Harvest in Yonago
(8-27 Yayoicho, Yonago, Tottori 683-0036, Japan)

* Participants are limited to the chairs and speakers of this Joint Meeting and Chinese delegates.
Recent therapeutic progress in the management of pulmonary arterial hypertension

Hiroshi Watanabe\textsuperscript{1,2}

Pulmonary arterial hypertension (PAH) is a disease characterized by extensive remodeling of the small pulmonary arteries resulting in increased elevated pulmonary arterial pressure and right ventricular heart failure. PAH is fatal if not treated, with only 34% survival rate after 5 years. Pathophysiologically, PAH is the result of a combination of many factors. Abnormal proliferation of endothelial cells is an important mechanism, which leads to increased arginase activity and down regulation of PGI\textsubscript{2} synthase in lung tissues, and subsequent reduction in the production of the vasodilators nitric oxide and prostacyclin. Endothelial hyperproliferation also increases the production of endothelin-1, one of the most potent vasoconstrictors. Endothelin-1 overexpression in turn reduces the production of NO and prostacyclin. These changes are associated with many other inter-related pathological changes, including vasoconstriction, hyperproliferation of vascular smooth muscle cells and fibroblasts, vascular wall hypertrophy, inflammation, platelet aggregation, and thrombosis, all contributing to the remodeling of the pulmonary vasculature in PAH. Based on these key pathophysiological features, currently approved therapies for PAH include phosphodiesterase 5 inhibitors (PDE5I), endothelin receptor antagonists (ERA) and prostacyclin derivatives. PDE5Is prevent cyclic GMP breakdown and therefore enhances the vasodilatory effect of NO, ameliorating the impact of reduced NO production. ERAs prevent ET-1 interaction with its receptors, thereby alleviating effects of excessive ET-1 in PAH patients. Prostacyclin derivatives increase PGI\textsubscript{2} levels, thereby alleviating the vasoconstriction caused by reduced concentrations of PGI\textsubscript{2} in PAH patients. PAH guidelines have proposed combination therapy (ERA and/or PDE5I and/or prostanoid) and upfront combination therapy targeting different pathologic process seems to be more effective rather than mono- or sequential-combination therapy. The aim of this presentation is to summarize recent advances in the therapeutic management of PAH with particular focus on the importance of NO-cGMP signaling.
Membrane transporters play important roles in various cellular functions. Renal tubular transporters function not only as pathways for nutrients uptake and metabolites efflux across cellular membranes but also as one of the specialized tissue functions such as transepithelial transport in the kidneys. Recent molecular cloning approaches successfully enable us to identify the molecular nature of various membrane transporters so that numerous inborn human diseases such as idiopathic renal hypouricemia are caused by the mutation of the transporter genes. In addition, several tubular transporters are clarified as drug targets such as NKCC2 (SLC12A1) for loop diuretics and NCC (SLC12A3) for thiazide diuretics. In this lecture, I will present following topics obtained from our laboratory to understand molecular physiology and pharmacology.

Urate is present at much higher levels in human blood (200-400 μM) than in other mammals (20 μM), because humans have acquired an effective renal urate reabsorption system in spite of the loss of hepatic uricase by mutational silencing during the course of their evolution. We successively identified renal urate transporters such as apical urate/anion exchanger URAT1 (SLC22A12) (Enomoto et al. Nature 417: 447, 2002) and basolateral voltage-driven urate efflux transporter URATv1/GLUT9 (SLC2A9) (Anzai et al. J Biol Chem, 283: 26834, 2008). Uricosuric agents such as benzbromarone inhibited URAT1- and URATv1-mediated urate uptake in a dose-dependent manner. In addition, we and others provide evidence that the defects both in URAT1 and URATv1 were found in patients with idiopathic renal hypouricemia. Thus, clarification of transcellular pathways for urate in human kidney should provide important insights into the nature of urate homeostasis, as well as lead to the development of better agents against hyperuricemia targeting renal tubular transporters (Anzai and Endou, Semin Nephrol, 2010).

Salvianolic acid A (SAA), extracted from the root of Salvia miltiorrhiza, is known to show a variety of pharmacological activities including antioxidant, anti-inflammatory. In present experiments, the effects of SAA on STZ-induced diabetic macrovascular and renal injury were investigated and also its mechanism was explored. The results shown that SAA (3mg/kg, po.) significantly improved impaired macrovascular structure and function, reduced loss of endothelial cells and lipid deposition. In cellular experiments, SAA (5 μM) significantly reduced expressions of VCAM-1 and the levels of ROS in 25mM glucose-treated in Human umbilical vein endothelial cell (HUVEC). SAA also decreased the kidney index, proteinuria, mesangial matrix expansion and MCP-1 in the diabetic mice. SAA reduced oxidative stress with increased express of antioxidant enzymes, such as HO-1, NQO-1 and GPx-1. As conclusion, these results suggest that SAA as a Nrf2 activators could alleviates diabetic complications by inhibition of hyperglycemia and oxidantive stress.
Oral-C2

Urea Transporter UT-B as a Novel Anti-hypertension Drug Target

Department of Medicine, Peking University, Beijing, China

Baoxue Yang

Aims: UT-B, a urea transporter, is expressed in renal and multiple extrarenal tissues, especially in vascular endothelial cells. The purpose of this study was to examine the role of UT-B in regulating blood pressure and to investigate the underlying mechanism.

Methods: UT-B null mice and spontaneous hypertensive rats (SHRs), were employed in this study. PU-14, a UT-B inhibitor, is used as a tool drug. The vasodilation capacity to acetylcholine or PU-14 was determined with isolated vascular perfusion assay. The expression levels of endothelial nitric oxide synthase (eNOS), phosphorylate eNOS (p-eNOS), arginase I and arginase II were measured in the thoracic aortas of mice and SHRs with Western blot. Bovine aortic endothelial cells (BAECs) were studied as an in vitro model to further clarify the mechanism.

Results: The deletion of UT-B caused lower blood pressure in mice. After administration of PU-14 for one week, blood pressure was lowered in both SHRs. Acetylcholine-induced endothelium-dependent relaxation is augmented in UT-B null mouse aortas. PU-14 caused endothelium-dependent relaxations in aortas and mesenteric arteries, and these relaxations were abolished by the presence of L-NAME (NOS inhibitor). Western blot analysis of thoracic aortas showed the up-regulated expression of eNOS, p-eNOS, and decreased expression of arginase I in UT-B null mice, as well as increased eNOS and p-eNOS showed in SHR model. PU-14 treatment increased NO level as well as the expression of eNOS, decreased arginase I in bovine aortic endothelial cells. And with increasing urea or PU-14 treatment, expression of arginase I was gradually decreased, while eNOS, p-eNOS were up-regulated in a dosage-dependent manner, meaning a mutual regulation between L-arginine-arginase-urea pathway and L-arginine-eNOS-NO pathway.

Conclusion: UT-B may be a novel drug target of anti-hypertension medication, and UT-B inhibitor may be a potential drug for antihypertensive medication.

Oral-C3

Proteasome inhibitors reactivate latent HIV through recruiting HSP90/p-TEFb complex after the activation of HSF-1

1School of Pharmaceutical Sciences, Southern Medical University, Guangzhou, China
2Department of Biochemistry, Weizmann Science Institute, Rehovot, Israel

Shuwen Liu1, Xiaoyan Pan1, Wei Zhao1, Yechiel Shai2

The persistence of HIV in resting memory CD4+ T cells at a latent state is considered as the major barrier on the path to achieve a cure for HIV. Proteasome inhibitors (PIs) were previously reported as latency reversing agents (LRAs) but the mechanism underlying this function is yet unclear. Here we demonstrate that PIs, including MG-132, bortezomib (BTZ), and the second generation PI carfilzomib (CFZ), can reactivate latent HIV ex vivo without global T cell activation, and may facilitate host innate immune responses. Mechanistically, latent HIV reactivation induced by PIs is mediated by heat shock factor 1 (HSF1) via the recruitment of the heat shock protein (HSP) 90-positive transcriptional elongation factor b (p-TEFb) complex. Specifically, HSP90 downstream HSF1 gives positive feedback to the reactivation process through binding to cyclin-dependent kinase 9 (CDK9) and preventing it from undergoing degradation by the proteasome. Overall, these findings suggest proteasome inhibitors as potential latency reversing agents. In addition, HSF1/ HSP90 involved in HIV transcription elongation, may serve as therapeutic targets in HIV eradication.
Poster Presentation

December 3 (Sat)
Poster Room 1
(Conference Room 4, 5F, BIG SHIP)

13:20 – 14:00

Poster-C1
Parkin and Its Related diseases

1 Hunan University of Chinese Medicine, Changsha, China
2 Institute of Material Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

Yi-na Jiang1,2, Zhao Zhang2, Nai-hong Chen1,2

Since Parkin was confirmed by the Japanese scholar to be associated with juvenile Parkinson's disease, it has come to be the focus of the scholars and a lot of researches have been made on it. Besides Parkinson's disease, many other diseases have also been proved to be associated with the role of Parkin and its interaction with protein substrates, especially in various kinds of cancer diseases and leukemia. Focusing on the latest research on Parkin and its development in tumor diseases and leukemia, may be as a virtuous circle to understand the Parkin comprehensively, and further to promote the diagnosis and treatment disease.
Obstructive jaundice is a common clinical hepatobiliary system disease, and it is caused by main factors such as biliary obstruction and cholestasis. Its clinic symptoms and pathologic features include hepatobiliary metabolic disorder, hepatic fibrosis and hepatic tissue necrosis. Chemokines are important modulators of hepatobiliary system disease. Chemokine-like factor 1 (CKLF1) is a novel atypical chemokine, which has a broad spectrum of chemotactic activity. Whereas the biological activity of CKLF1 has been described in various diseases, but currently, there is no data of the mechanism of CKLF1 in obstructive jaundice. In this paper, we investigated the biological activity and the variation rules of CKLF1 by targeting CKLF1 in obstructive jaundice, and we found that, compared with control C57BL/6, CKLF1 is regularly changed with the prolongation of ligation time in but duct ligation (BDL) mouse model, reaching the peak at thirdly day, and mainly distributed around the necrotic cells or necrotic cells inside and outside through immunohistochemistry and Western blotting. Its receptor CCR-4 is also the similar variation. Moreover, we chose the liver necrosis area in the same position after BDL-3d by immunohistochemical staining for the CKLF1, IL-10 and IL-6. Immunohistochemical images indicate that CKLF1 is highly consistent with the expression of IL-10, but there is no obvious relationship with IL-6, and its biological effects need to be further studied.

In a word, our data demonstrates that CKLF1 is regularly changed with the prolongation of ligation time in BDL mouse model and suggests that CKLF1 is related to the development of obstructive jaundice.

### Poster-C3

**Ginsenoside Rg1, as multi-target drug candidate for liver diseases through multi-target effects: Nrf2 induction and liver protection**

1. State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
2. Hunan University of Chinese Medicine, Changsha, China

◯ Nai-hong Chen1,2, Shi-feng Chu1,2, Yan Gao1

Multi-target drugs, such as cocktail therapy used for treating AIDS, usually show strong efficacy than single target drugs in treating complicated diseases. This review will mainly focus on the ginsenoside Rg1, a compound isolated from the traditional Chinese herbal medicine Panax ginseng C.A. Meyer. It is well known that ginseng has been used as a valuable tonic and for the treatment of various diseases including hepatic disorders. Ginseng saponins, commonly known as ginsenosides, are principal constituents and have believed to be responsible for multiple ginseng health benefits. To date, treatment options for common liver diseases such as hepatitis, cirrhosis, fatty liver, and chronic hepatitis remain problematic. In this regard, ginseng extracts and individual ginsenoside has shown a wide array of beneficial role in the regulation of regular liver functions and the treatment of liver disorders of acute/chronic hepatotoxicity, hepatitis, hepatic fibrosis/cirrhosis, hepatocellular carcinoma, and so on in various pathways and different mechanisms. The anti-oxidant liver protective function of ginsenoside Rg1 is mainly through the induction of Nrf2 signaling pathway. In summary, the nature of the multi-target actions of ginsenoside Rg1 substantiates it as a promising drug candidate for treating hepatic impairment in different liver diseases.
Antioxidant activities of ginsenoside Rg1 against cisplatin-induced hepatic injury through Nrf2 signaling pathway in mice

State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Nai-hong Chen, Shi-feng Chu, Yan Gao

Oxidative stress is mainly caused by reactive oxygen species (ROS). The damage causes a net stress on normal organs, leading to a gradual loss of vital physiological function. ROS, such as free radicals, represent a class of molecules which are derived from the metabolism of oxygen and exist inherently. However, excessive produced ROS can damage all aerobic organisms. Ginseng is one of the most commonly used alternative herbal medicines, also as a traditional Chinese medicine. The aim of this study is to investigate the antioxidant potential function of ginsenoside Rg1 against cisplatin-caused hepatic damage. Male mice were treated with cisplatin to induce oxidative stress to mimic the side effect of anti-cancer drug cisplatin. Ginsenoside Rg1 effectively prevented against cisplatin-induced hepatotoxicity, alleviating histological lesions. Antioxidant functions of Rg1 were restrained by the activation of p62-Keap1-Nrf2 signaling pathway, simultaneously accompanied with expression of protein products. Accumulative p62 and increased activation of JNK in hepatocytes promoted the activation of Nrf2. For the other, degradation of Nrf2 was guided by tyrosine phosphorylation, ubiquitin and Keap1. In summary, Rg1 prevented from hepatotoxicity mainly by inhibiting the binding of Keap1 and Nrf2, partly by p62 accumulation, and more important increasing the production of antioxidative proteins associated to Nrf2. Pharmacological activation of Nrf2 is an effective way in combating against liver injury.

A potential novel target for stroke treatment: selective modulation of microglia phenotype

State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica & Neuroscience Center, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Nai-Hong Chen, Cong-Yuan Xia

Resident microglia are the major immune cells in the brain, acting as the first defense of the central nervous system. Following cerebral ischemia, microglia respond to this injury rapidly and transform from surveying microglia to active state, playing a dual role in the ischemic injury due to distinct microglia phenotypes. Microglia show transient neuroprotective M2 phenotype followed by a shift to deleterious M1 phenotype, which is significantly related to ischemic injury. Our results proved that the dysfunction of autophagy-mediated imbalance between NF-κB and CREB participated in the regulation of microglia phenotype in oxygen glucose deprivation/reperfusion (OGD/R) model. These results indicated the potential role of autophagy in regulation of microglia phenotype, but whether the same phenomenon occurs in animal ischemia/reperfusion model or stroke patient need further study.
Association of \textit{ABCB1} genetic polymorphisms with susceptibility to colorectal cancer and therapeutic prognosis

Department of Pharmacology, School of Pharmacy, Liaoning Key Laboratory of Molecular Targeted Anti-Tumor Drug Development and Evaluation, China Medical University, Shenyang, China

Min-Jie Wei, Hui-Zhe Wu

We evaluate the relevance between \textit{ABCB1} gene polymorphisms and susceptibility to colorectal cancer (CRC) and clinical outcomes of CRC patients with chemotherapy. A case-control study was performed on the C3435T, C1236T, and G2677T/A polymorphisms in \textit{ABCB1} gene in 1028 CRC patients and 1230 controls. The 3 SNPs were genotyped by PCR-restriction fragment length polymorphism assays. We observed that the \textit{ABCB1} C3435T TT genotype and T allele significantly decreased the risk of CRC (P=0.0023 and P=0.0370, respectively). The distribution frequency of \textit{ABCB1} G2677T/A GT/GA, GT+GA+TT+AA genotypes, and haplotype 3435T-1236T-2677T were significantly higher in CRC patients (OR (95% CI): 1.271 (1.060-1.523), 1.237 (1.040-1.472), and 1.261(1.097-1.451), respectively). Furthermore, the heterozygous genotype \textit{ABCB1} 3435CT had significant effect on time to recurrence [adjusted HR(95%CI)=0.560(0.355-0.882); \(P=0.012\)], and \textit{ABCB1} 1236 CT or TT variants displayed a longer overall survival in CRCs after postoperative oxaliplatin-based chemotherapy [adjusted HR(95%CI): 0.354(0.182-0.692), 0.464 (0.458-0.910); \(P=0.002, 0.013\); respectively]. Moreover, the \textit{ABCB1} G2677T/A genotype and the haplotype 3435TT-1236TT-2677TT were associated with a longer PFS or OS in postoperative oxaliplatin-treated patients with increased B-mg, CA12-5, or CA15-3 levels. The \textit{ABCB1} polymorphisms might be a candidate pharmacogenomic factor to assess susceptibility and prognosis after oxaliplatin-based chemotherapy for CRC patients.

CUG-binding protein 1 regulates HSC activation and liver fibrogenesis

State Key Laboratory of Pharmaceutical Biotechnology and Collaborative Innovation Center of Chemistry for Life Sciences, School of Life Sciences, Nanjing University, Nanjing, China.

Xingxin Wu, Qiang Xu

Excessive activation of hepatic stellate cells (HSCs) is a key step in liver fibrogenesis. Here we report that CUG-binding protein 1 (CUGBP1) expression is elevated in HSCs and positively correlates with liver fibrosis severity in human liver biopsies. Transforming growth factor-beta (TGF-\(\beta\)) selectively increases CUGBP1 expression in cultured HSCs in a p38 mitogen activated protein kinase (MAPK)-dependent manner. Knockdown of CUGBP1 inhibits alpha smooth muscle actin (\(\alpha\)-SMA) expression and promotes interferon gamma (IFN-\(\gamma\)) production in HSCs in vitro. We further show that CUGBP1 specifically binds to the 3’ untranslated region (UTR) of human IFN-\(\gamma\) mRNA and promotes its decay. In mice, knockdown of CUGBP1 alleviates, whereas its overexpression exacerbates, bile duct ligation (BDL)-induced hepatic fibrosis. Therefore, CUGBP1-mediated IFN-\(\gamma\) mRNA decay is a key event for profibrotic TGF-\(\beta\)-dependent activation of HSCs, and inhibiting CUGBP1 to promote IFN-\(\gamma\) signalling in activated HSCs could be a novel strategy to treat liver fibrosis.