

Doi: 10.1038/aps.2017.68

### S5.1

#### The metalloproteinase-dependent role of TIMPs in regulation of pulmonary microvascular endothelial cell barrier function during sepsis

Marcello G MASCANTONIO<sup>1,4</sup>, Sanjay MEHTA<sup>1,2,3</sup>, Lefeng WANG<sup>1,3</sup>, Marta ROHAN<sup>1</sup>, Cynthia PAPE<sup>1,3</sup>, Sean E GILL<sup>1,2,3,4</sup>

<sup>1</sup>Centre for Critical Illness Research, Lawson Health Research Institute; <sup>2</sup>Division of Respiriology, <sup>3</sup>Department of Medicine, <sup>4</sup>Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada  
\*To whom correspondence should be addressed.

E-mail: mmascian@uwo.ca

Sepsis, a systemic inflammatory response to infection, is a serious disease with significant mortality characterized by injury and dysfunction of pulmonary microvascular endothelial cells (PMVEC). Mechanisms protecting against septic PMVEC dysfunction are unclear; however, the tissue inhibitors of metalloproteinases (TIMPs), which regulate metalloproteinase activity, may be one such mechanism. Metalloproteinases, are associated with inflammation and tissue damage. My hypothesis is that murine septic PMVEC barrier dysfunction is due to disruption of the balance between metalloproteinases and TIMPs leading to increased metalloproteinase activity.

Analysis of metalloproteinase and TIMP expression by qRT-PCR revealed a significant alteration in TIMP and metalloproteinase expression *in vitro* following stimulation with cytomix and lipopolysaccharide. Additionally, metalloproteinase activity, specifically ADAM17 activity, was increased in PMVEC following septic stimulation and treatment with a global metalloproteinase inhibitor, Batimastat, reduced albumin flux across the PMVEC monolayer.

Further, while septic stimulation increased cell surface intracellular adhesion molecule (ICAM) 1 on wild type (WT) PMVEC, Timp3<sup>-/-</sup> PMVEC had significantly less cell surface ICAM1 *vs* WT PMVEC under basal and septic conditions. Additionally, this decrease in ICAM1 was associated with significantly impaired PMVEC-neutrophil (PMN) adhesion in Timp3<sup>-/-</sup> *vs* WT PMVEC.

My data demonstrates that the balance between metalloproteinases and TIMPs in PMVEC is altered under septic conditions leading to increased protein leak. Furthermore, disruption of this balance is also associated with impaired PMVEC-PMN interaction. Taken together, my studies highlight the critical role of metalloproteinases and TIMPs in septic PMVEC dysfunction, and understanding these mechanisms may provide insight into potential therapeutic interventions in human sepsis.

**Keywords:** TIMP; metalloproteinase; endothelial; sepsis

**Acknowledgements:** This work was supported by grants from the Canadian Institutes of Health Research, the Heart and Stroke Foundation of Ontario, the Lung Association, and the Lawson Health Research Institute

### S5.2

#### Salvianolic acid A, a component of *Salvia Miltiorrhiza* Bunge, attenuates endothelial-mesenchymal transition of HPAECs induced by hypoxia

Lian-hua FANG<sup>1</sup>, Tian-yi YUAN<sup>1</sup>, Yu-cai CHEN<sup>1</sup>, Hui-fang ZHANG<sup>1</sup>, Yang LYU<sup>2,\*</sup>, Guan-hua DU<sup>1,\*</sup>

<sup>1</sup>Beijing Key Laboratory of Drug Targets Identification and Drug Screening; <sup>2</sup>Beijing Key Laboratory of Polymorphic Drugs; Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

\*To whom correspondence should be addressed.

E-mail: luy@imm.ac.cn (Yang LYU);

dugh@imm.ac.cn (Guan-hua DU).

Salvianolic acid A (SAA), a polyphenols acid, is a bioactive ingredient from a traditional Chinese medicine named Dan shen (*Salvia Miltiorrhiza* Bunge). According to previous studies, it was shown to possess various effects such as anti-oxidative stress, anti-diabetic complications and anti-pulmonary hypertension. This study is aimed to investigate the effect of SAA on pulmonary arterial endothelial-mesenchymal transition (endoMT) induced by hypoxia and the underlying mechanisms. Primary cultured human pulmonary arterial endothelial cells (HPAECs) were exposed to 1% O<sub>2</sub> for 48 h with or without SAA treatment. SAA treatment improved the morphology of HPAECs and inhibited the cytoskeleton remodeling. SAA 3 μmol/L reduced migration distances from 262.2 μm to 198.4 μm at 24 h and 344.8 μm to 109.3 μm at 48 h. It was observed that the production of ROS in cells was significantly reduced by the treatment with 3 μmol/L SAA. Meanwhile, SAA alleviated the loss of CD31 and slightly inhibited the expression of α-SMA. The mechanisms study shows that SAA treatment increased the phosphorylation levels of Smad1/5, but inhibited that of Smad2/3. Furthermore,

SAA attenuated the phosphorylation levels of ERK and Cofilin, which were enhanced by hypoxia. According to these results, our study indicated that SAA treatment can protect HPAECs from endoMT-induced by hypoxia, which may perform via the downstream effectors of BMPRs or TGFβR including Smads, ERK, and ROCK/cofilin pathways.

**Keywords:** Salvianolic acid A; endothelial-mesenchymal transition; HPAEC; hypoxia; Smads pathway

**Acknowledgements:** This work was supported by grants from the National Natural Science Foundation of China (No 81573645 and 81603101) and the Natural Science Foundation of Beijing (No 7174322) and CAMS Innovation Fund for Medical Sciences (No 2016-I2M-1-010).

### S5.3

#### Effects of melatonin on apoptosis of myocardium in renal ischemia reperfusion rats

Xiao-hong XIA<sup>1</sup>, Hao RONG, Yan-xia TIAN, Jiao JIN, Zhi-hui MIAO

Hebei Academy of Medical Sciences, Shijiazhuang 050021, China

\*To whom correspondence should be addressed.

E-mail: xiaonmc@163.com

The present study aimed to investigate the effects of melatonin (MT) on renal ischemia reperfusion (RIR) induced myocardium injury in rats. The model of RIR was induced by bilateral clamping the renal artery and vein for 45 min followed by reperfusion. SD rats were randomly divided into sham, RIR, vitamin C (VC) and MT groups. MT and VC group rats were given MT (10 mg/kg) and vitamin c (125 mg/kg), respectively. After 24 h of reperfusion, the blood samples were taken for detecting contents of serum creatinine (Scr), blood urea nitrogen (BUN), activities of lactate dehydrogenase (LDH) and creatine kinase (CK). After blood was taken, the heart was excised for detection of the activity of superoxide dismutase (SOD), the content of malondialdehyde (MDA), expression of Bcl-2, Bax, and apoptosis rate. The result showed that Scr, BUN, LDH and CK in serum and MDA in the myocardium were significantly increased and SOD was significantly decreased in RIR group compared to sham group ( $P < 0.01$ ); while Scr, BUN, LDH and CK in serum were significantly decreased ( $P < 0.05$ ), SOD were significantly increased in MT groups ( $P < 0.05$ ). The apoptotic rate of myocardium in RIR group was significantly higher than that of sham group ( $20.76\% \pm 2.02\%$  *vs*  $2.66\% \pm 0.53\%$ ,  $P < 0.01$ ). The percentage of apoptosis of myocardium cells in both of MT ( $13.40\% \pm 0.96\%$ ) and VC ( $13.49\% \pm 1.51\%$ ) group were significantly lower than that of RIR group ( $P < 0.01$ ). The expression of Bcl-2 was markedly increased but those of Bax were significantly decreased in MT group. These results suggest that MT could be diminished by RIR-induced myocardial injury in rats. The mechanism is partly related to the enhancement of anti-oxidation, up-regulation of the expression of anti-apoptotic protein Bcl-2 and down-regulation of the expression of pro-apoptotic protein Bax.

**Keywords:** melatonin; renal ischemia reperfusion; myocardium; antioxdaition; apoptosis

### S5.4

#### Carbon monoxide enhances glucose-stimulated insulin secretion by activating PLC signal pathway in *db/db* pancreatic β-cells

Yu-chun GU\*

Molecular Pharmacology, Institute of Molecular Medicine, Peking University, Beijing 100871, China

\*To whom correspondence should be addressed.

E-mail: 1006172508@pku.edu.cn

Pancreatic β-cells played an important role in glucose homeostatic control. In diabetes, insulin secretion from pancreatic β-cells was increased to deal with the elevated blood glucose. However, this compensatory mechanism had not been clarified. Here, we found that CO elevated intracellular calcium ([Ca<sup>2+</sup>]<sub>i</sub>) and enhanced glucose-stimulated insulin secretion (GSIS) of pancreatic β-cells in *db/db* but not in C57 mice. Inhibition of PLC signal pathway blocked the stimulatory effects of CO on β-cell function in *db/db* mice. Consistent with the stimulatory effects of CO observed in *db/db* mice, *db/db* islets possessed higher expression of PLCα1 and PLCα1 isoforms compared with C57 islets. When overexpressing PLCα1 and PLCα1 isoforms in C57 islets, exogenous CO obviously elevated [Ca<sup>2+</sup>]<sub>i</sub> and enhanced GSIS. After interfering PLCα1 and PLCα1 expression in *db/db* islets, the stimulatory effects of CO were dramatically attenuated.

Our findings showed that HO-1, PLCα1 and PLCα1 isoforms were up-regulated in *db/db* islets. CO, the product of HO-1, elevated [Ca<sup>2+</sup>]<sub>i</sub> and enhanced GSIS by activating PLCα1 and PLCα1 in *db/db* pancreatic β-cells. This is an important compensatory mechanism by which CO promotes pancreatic β-cell function to

prevent hyperglycemia, resulting in providing insights into the therapy for type II diabetic patients and offering a potential target for pharmacological and therapeutic application of CO.

### S5.5

#### Study on pharmacodynamics of QiHu preparation for diabetes

Xin-tong YAO<sup>1</sup>, Xiao-li LI<sup>2</sup>, Ning WANG<sup>3</sup>, Xuan ZHANG<sup>2</sup>, Wei-ying ZHOU<sup>1\*</sup>

<sup>1</sup>School of Pharmacy, Chongqing Medical University, Chongqing 400016, China

<sup>2</sup>Department of Pharmacology, College of Pharmacy; <sup>3</sup>First Affiliated Hospital's Central Laboratory, Third Military Medical University, Chongqing 400038, China.

\*To whom correspondence should be addressed.

E-mail: wyzhou0118@163.com

To examine effects of the QiHu preparation for diabetes (QiHu) on reduction of blood glucose levels and protection of organ injuries caused by diabetes. Fifty 6-week-old female KK-Ay mice were randomly divided into 5 groups: model control (MC) group (given with adjuvant 0.3% CMCNa), QiHu low-, middle- and high dose groups (0.75, 1.5, and 3.0 g crude drug/kg), and positive control group (metformin, 0.13 g/kg). Food intake was significantly decreased in low-, middle- and high dose QiHu groups and metformin group compared with MC group. Body weight were significantly lower in high dose QiHu group and metformin group than that in MC group. Random blood glucose levels of low-(18.47±8.41 mmol/L), middle-(17.93±5.90 mmol/L), high-dose QiHu groups (15.10±2.60 mmol/L) and metformin group (17.58±7.72 mmol/L) were significantly lower than that of MC group (26.95±4.62 mmol/L) at day 29. Levels of TG and CHOL were moderately decreased in low-, middle- and high dose groups and metformin group compared with the MC group. Less liver damage and pancreatic atrophy were observed in all the QiHu groups and metformin group compared with MC group. There was no difference in above indexes between high dose QiHu groups and metformin group. QiHu can significantly reduce blood glucose levels and attenuate injuries of liver and pancreas in the KK-Ay mice.

**Keywords:** diabetes; QiHu preparation for diabetes; hyperglycemia; hyperlipidemia

### S5.6

#### DEC1 promotes osteogenesis: an involvement of PI3K/Akt/GSK3β/β-catenin signaling pathway

Jin-hua HU<sup>1</sup>, Yu-wen WANG<sup>1</sup>, Zhao MAO<sup>2</sup>, Rui NING<sup>1</sup>, Wei LIU<sup>1</sup>, Jian YANG<sup>1\*</sup>

<sup>1</sup>Nanjing Medical University, Nanjing 210029, China; <sup>2</sup>Jinling Hospital, Nanjing Medical University, Nanjing 210029, China

\*To whom correspondence should be addressed.

E-mail: jianyang@njmu.edu.cn

In this study, we confirm that glucocorticoid disturbs osteogenic differentiation characterized by the declined alkaline phosphatase activity, the decreased alkaline phosphatase staining intensities, the reduced Runx2 protein expression and the diminished extracellular matrix mineralization in SaoS-2 cells. In contrast, icariin increases osteogenic capacity and reverses the decreased osteoblastic phenotypes induced by glucocorticoid. Dexamethasone and icariin regulate osteogenic differentiation and DEC1 participates in this process. Our data *in vivo* authenticate the low DEC1 expression in glucocorticoid-induced osteoporosis mice. In addition, the inactivation of DEC1 in SaoS-2 cells results in the absence of matrix mineralization and the decrease of other osteogenesis-related phenotype markers. DEC1 knockout mice also present a typical osteoporosis phenotype. The continued expression of DEC1 is necessary for the osteogenic differentiation and bone mass formation, and the expression of osteoblastic phenotypes is associated with the increased β-catenin expression in the nuclei. When the molecular mechanism of DEC1 induced β-catenin nuclear translocation is examined, we find that the expression of PI3K subunit PIK3CA is enhanced by DEC1, which results in its downstream signaling activation. Taken together, DEC1 promotes the osteogenesis through activating the PI3K/Akt/GSK3β/β-catenin signaling pathway.

**Keywords:** differentiated embryonic chondrocyte expressed gene 1 (DEC1); glucocorticoid; icariin; osteogenesis

**Acknowledgements:** This study was supported by the National Natural Science Foundation of China (No 81573503, 81373443, 81173128, 81102457, 81302855), the

Major Project of Jiangsu Provincial Department of Education (No 13KJA310003).

### S5.7

#### Three decades of GLP-1 action—how does it work?

Daniel J DRUCKER

Lunenfeld-Tanenbaum research Institute, Department of Medicine, Mt. Sinai Hospital, University of Toronto, Toronto ON M5G 1X5 Canada

Gut hormones circulate at very low levels in the fasting and state, and secretion for many gut hormones rises transiently following meal ingestion. Renal clearance and enzymatic inactivation represent two dominant mechanisms for terminating gut hormone bioactivity. The prototype L cell peptide GLP-1 functions as an incretin hormone to augment glucose-dependent insulin secretion enabling meal-related glucose disposal. GLP-1 also exerts multiple actions beyond the beta cell, many of them conducive to the treatment of type 2 diabetes. GLP-1 exhibits a short circulating half-life and is cleaved at the N-terminus by dipeptidyl peptidase-4 (DPP-4). Remarkably, levels of tissue and plasma DPP4 activity rise in the context of experimental or clinical obesity, further highlighting the challenge in sustaining gut hormone activity in the obese dysglycemic state. Despite several decades of study, the mechanism(s) through which GLP-1, and in turn, DPP4, exert their metabolic effects, remain poorly understood. Here we summarize studies using mouse genetics, as well as human physiological analyses, to identify key tissues and substrates underlying GLP-1 and DPP4-mediated control of metabolism.

**Acknowledgements:** Funded by the Canadian Institutes of Health Research.

### S5.8

#### Neuroprotective K<sub>ATP</sub> channels and the cerebrovascular risk of sulfonylureas in the treatment of type 2 diabetes

Vivian Ying SZETO<sup>1</sup>, Rui LIU<sup>1</sup>, Hai-tao WANG<sup>1,2</sup>, Bao-feng XU<sup>1,2</sup>, Tianru JIN<sup>1,3</sup>, Edoardo MANNUCCI<sup>4</sup>, Zhong-ping FENG<sup>1\*</sup>, Hong-shuo SUN<sup>1,2,3,5,\*</sup>

Departments of <sup>1</sup>Physiology, <sup>2</sup>Surgery, and <sup>3</sup>Pharmacology, <sup>4</sup>Institute of Medical Science, Faculty of Medicine, University of Toronto, Canada, <sup>5</sup>Diabetology, Careggi Hospital, University of Florence, Italy

Diabetes mellitus (DM) and stroke are leading causes of death and disability. Diabetes and hyperglycemia increase stroke risk significantly, therefore strict glycemic control is a top priority. Sulfonylureas are the oldest and one of the most commonly used oral anti-diabetics. Sulfonylureas block pancreatic K<sub>ATP</sub> channels, depolarizing the β-cell and triggering insulin granule release. This may be problematic as K<sub>ATP</sub> channels are involved in ischemic preconditioning which is neuroprotection against ischemia. Many studies focus on the cardiovascular safety of sulfonylureas while few address the associated stroke risk. Thus, we investigated the role of K<sub>ATP</sub> channels in neuroprotection against ischemic insult and the stroke risk associated with treatment of diabetes, particularly with K<sub>ATP</sub> blocker sulfonylurea. To investigate the effect of DM on stroke outcomes, DM was established in male C57BL/6J mice by streptozotocin (STZ) injection and MCAO was performed. To investigate the role of K<sub>ATP</sub> channels in neuroprotection, K<sub>ATP</sub> agonist (diazoxide) and antagonist (tolbutamide) were applied in *in vitro* (oxygen glucose deprivation, neuron cultures) and *in vivo* (middle cerebral artery occlusion (MCAO)) models of ischemic stroke. To evaluate stroke risk associated with DM treatments, a systematic meta-analysis was performed in accordance with PRISMA guidelines and multivariate logistic regression was used to evaluate the stroke risk associated with sulfonylureas in literature. STZ-induced diabetic mice displayed larger infarct areas and greater behavioral deficit post-MCAO and expressed higher levels of NMDAR-2B, PSD-95 and less phosphorylated GSK-3β proteins. K<sub>ATP</sub> blockade by tolbutamide increased OGD-induced cell death, MCAO-induced infarct. K<sub>ATP</sub> activation by diazoxide conferred neuroprotection. Meta-analysis showed higher stroke morbidity ratio in T2DM patients using sulfonylureas as either monotherapy or in conjunction with other anti-diabetics. In conclusion, K<sub>ATP</sub> channels have a neuroprotective role in ischemia and sulfonylurea use reduces neuroprotection by K<sub>ATP</sub> channels in ischemic events and increases stroke risk in diabetic patients as compared to other anti-diabetic drugs.