GG of rs2910164 (miR-146a) (P<0.01). Cox regression analysis showed that the miR-146aC>G polymorphism was associated with serious prognosis of CAD.
Conclusions: The miR-146aG allele is associated with pathogenesis and prognosis of CAD, especially for AMI patients.

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GW25-e5416

3-Bromopyruvate prevents monocrotaline-induced pulmonary arterial hypertension in rats

Zhang Rui, Sun Xiaoqing, Zhang Hongda, Liu Jinming
Department of Cardio-Pulmonary Circulation, Shanghai Pulmonary Hospital, Tongji University, School of Medicine, Shanghai, China

Objectives: Pulmonary arterial hypertension (PAH), which is characterized by a progressive elevation of pulmonary vascular resistance and leads to right heart failure and even death. Basis of the "Warburg effect", the metabolism shifts from oxidative mitochondria to glycolysis and the elevated enzyme hexokinase-2 (HK2) in PAH. An inhibitor of HK2, 3-bromopyruvate (3-BrPA), promptly and substantially suppresses ATP and lactic acid, however, it could prevents the development of PAH is not yet known.
Methods: Sprague-Dawley rats with monocrotaline (MCT, 60 mg/kg) -induced PAH were administered subcutaneous injection of 3-BrPA (7.5mg/kg/d, 15mg/kg/d and 30mg/kg/d, respectively) for 14 days. Hemodynamic parameters were acquired by right heart catheterization. Histopathology, immunohistochemisty, and assessments of relative enzyme expressions were performed in rat lung tissue.
Results: Compared with MCT rats, 3-BrPA significantly decreased mean pulmonary arterial pressure; pulmonary vascular resistance (PVR) and right heart hypertrophy (RVH), and increased cardiac output. In additional, 3-BrPA treatment markedly reduced MCT-induced increase in both the wall thickness and area. 3-BrPA significantly suppressed proliferation and enhanced apoptosis of pulmonary artery smooth muscle cells, attenuating small pulmonary artery remodeling. Furthermore, treatment with 3-BrPA significantly increased endothelial NO synthase expression, cytochrome c in cytoplasm and superoxide dismutase activity, and down-regulated HK2 expression and ATP production.
Conclusions: These investigations demonstrated the importance of glycolytic inhibition in PAH pathogenesis and highlight 3-BrPA may beneficial to the therapy of PAH.

GW25-e0008

Cortistatin protects myocardium from endoplasmic reticulum stress induced apoptosis during sepsis

Zhang Bo, Yin Xinhua
Department of Cardiology, First affiliated Hospital of Harbin Medical University

Objectives: Sepsis is generally viewed as a disease aggravated by a systemic inflammatory response to infection. Myocardial depression is a well-recognized manifestation of organ dysfunction in sepsis, and myocardial apoptosis is a key step for this progression. Cortistatin (CST), a cyclic neuropeptide related to somatostatin, prevents septic shock-associated lung and liver injury, and improves survival in experimental sepsis. However, the effect of CST on myocardium remains unclear. This study aimed to assess whether CST protects the myocardium from sepsis-induced apoptosis.
Methods: To test that, the murine model of cecal ligation and puncture (CLP) and LPS induced cardiomyocytes (CM) were used in vitro. To measure rat CST and its receptors mRNA levels, we used real-time PCR. Electron microscopy, TUNEL staining, caspase-3 activity and Bcl-2/Bax ratio were used to determine myocardial apoptosis. Western-blot was used to test endoplasmic reticulum stress markers, such as GRP94, caspase-12 and CHOP.
Results: Compared with the sepsis-alone group, rats under CST treatment showed higher MABP, by 37.8%. And CST elevated values of +LVdp/dtmax by 54.1%, and LVdp/dtmax by 48.3%, and LVSP by 27.5%. We further confirmed that by using TUNEL staining and caspase-3 activity assay, and showed there is a significant decrease of cardiomyocytes apoptosis after CST pretreatment. On Western blot analysis, compared with sepsis group the ratio of Bcl-2/Bax increased by 2.6-fold in the CST+sepsis group ($P<0.05$). We further investigated the expression of ERS marker: the protein levels of GRP94, caspase-12, CHOP were about 2.7-fold, 9.2-fold, 6.2-fold (all $P<0.05$) higher, respectively, in sepsis than control myocardium; however, the upregulated expression of these proteins was downregulated with CST treatment, by 52.8%, 29.5%, 36.6% (all $P<0.05$) respectively. In vitro, we also found that CST treatment significantly decrease LPS induced these molecules expression. To further investigate the direct inhibitory effect of CST on ERS in vitro, we used DTT to induce cardiomyocytes. The protein levels of ERS markers were significantly increased after chemicals incubation for 3h. Preincubation with cortistatin abolished the increased levels of ERS markers. We found that D-GHRP-6, GHS-R1a antagonist completely blocked cortistatin's inhibitory effect on cardiomyocyte ERS, indicating CST inhibit ERS through GHS-R1a.
Conclusions: In conclusion, CST pretreatment attenuated sepsis induced depressed cardiac function and apoptosis and inhibited the overexpression of ERS markers. In vitro, CST directly inhibited ERS, which might depend on GHS-R1a. For the first time, we show that CST protects heart against sepsis injury and apoptosis, at least partially through its inhibitory effect on myocardial ERS and through activation its receptor GHS-R1a, which may provide a new insight into the mechanism underlying the wide cardiovascular protective actions of CST atin.

GW25-e0051

Suppression of cardiac TFEB sumoylation promotes age-associated reduction in autophagy

Heng Ma, Yan Li, Le Zhang, Lu Yu
Fourth Military Medical University

Objectives: Aging-dependent decline of autophagy contributes to cardiac dysfunction and ischemic intolerance. Transcription factor EB (TFEB) is a master transcriptional regulator of the autophagy-lysosome pathway. The present study aimed to characterize the role of TFEB in the autophagic decrease with aging.
Methods: We analyzed age-associated autophagic changes in male C57BL/6 young (4-6 mo) and aged (22-24 mo) mice.
Results: The results demonstrated that TFEB expressed predominantly as a SUMOylated form in cardiomyocyte nuclei and this SUMOylation of TFEB declined in aged heart associated with autophagy reduction. Interestingly, SUMOylation of TFEB was unaffected by rapamycin. Rapamycin induced translocation of TFEB into nucleus but lower level of nuclear TFEB in aged hearts than that seen in young hearts ($P<0.05$). SUMO1 downregulation by adeno-associated-virus-mediated small hairpin RNA (rAAV9-shSUMO1) significantly reduced nuclear TFEB levels ($P<0.05$), depressed cardiac autophagy and accelerated cardiomyocyte contractile dysfunction with worse hypoxia/reoxygenation (H/R) injury (all $P<0.05$). Therefore, impaired SUMOylation decreased nuclear TFEB during aging. By contrast, SUMO1 restitution significantly augmented nuclear SUMOylated TFEB with enhanced autophagy and ultimately reduced infarct size in aged heart. However, knockdown of cardiac TFEB blocked the protective effect of upregulation of SUMO1 in aged hearts, resulted in decline of autophagy and worse *in vivo* I/R injury.
Conclusions: The present study newly demonstrates that SUMOylation is a critical post-translational modification that regulates cardiac TFEB. Impaired SUMOylation of TFEB aggravates decline of autophagy in the senescent heart. Targeting SUMO1 may provide a novel therapeutic strategy for the treatment of aging-related loss of cardioprotection.

GW25-e0306

Effect of Xiongdan on Blood Pressure, Mesenteric Vascular Structure and Function in Spontaneously Hypertensive Rats

Fang Zhoufei, Liangdi Xie
First Affiliated Hospital of Fujian Medical University

Objectives: To observe the effect of Xiongdan on blood pressure, vascular structure and dilatation function of 3rd grade branch mesenteric arteries in spontaneously hypertensive rats (SHRs).
Methods: Sixteen male SHRs at 12 wks old were randomly divided into 2 groups: Xiongdan (SHR-X, n=8, a traditional Chinese herbal compound, 800mg·kg^{-1}·d^{-1}) and untreated controls (SHR, n=8). Age- and weight-matched WKY rats served as controls (WKY, n=8). Systolic blood pressure (SBP) was measured by tail-cuff method before treatment, 4 and 8 wks after treatment. The wall-to-lumen area ratios (W/L), the ratios of wall thickness (WT) to lumen radius (LR) of 3rd grade branch mesenteric arteries were assessed morphometrically. Endothelium-dependent relaxation (EDdR), endothelium-independent relaxation (EDiR) was measured by PowerLab biological signal analytical system.
Results: SBP in SHRs were higher than that in WKY from 12 to 20 wks. SBP in X treated rats was significantly lower than that in untreated rats [SBP/mmHg, 4wks: SHR-X 176.45±11.44 vs SHR 200.27±13.94; 8wks: SHR-X 169.43±11.97 vs SHR 189.88±10.06, both P<0.01]. W/L and WT/LR of 3rd grade branch mesenteric arteries in X treated rats were markedly lower than that of untreated SHR [W/L: SHR-X 0.53±0.09 vs SHR 1.82±0.96; WT/LR: SHR-X 0.24±0.08 vs SHR 0.53±0.29, both P<0.01], similar to the level as that of WKY (P>0.05). Compared with SHR, EDdR [Emax/%: SHR-X 59.29±15.15 vs SHR: 20.69±6.31, pD_2 : SHR-X 8.24±0.13 vs SHR 5.82±0.23; both P <0.01] and EDiR [Emax/%: SHR-X 96.37±1.87 vs SHR 29.04±4.56, pD_2: SHR-X 7.79±0.15 vs SHR 5.31±0. 14; both P<0.01] of 3rd grade branch mesenteric arteries were increased in X treated after 8wks treatment.
Conclusions: The treatment of Xiongdan may lower blood pressure, ameliorate the vascular structure and dilatation function of 3rd grade branch mesenteric arteries in SHRs.

GW25-e0419

Prenatal Lipopolysaccharide Exposure Results in Dysfunction of Renal Dopamine D1 Receptor in Offspring Rats

Wang Xinquan[1,2], Zeng Chunyu[1,2]
[1]Department of Cardiology, Daping Hospital, The Third Military Medical University, P. R. China, [2]Chongqing Institute of Cardiology, Chongqing, P. R. China

Objectives: Adverse environmental exposure in utero predisposes to adult disease, including hypertension. Exposure to lipopolysaccharide (LPS) results in increased blood pressure in offspring, but the exact mechanisms are not clear. Our previous study shows dysfunction of renal D1 receptor (D1R) is ascribed to the pathogenesis of hypertension, which is associated with reactive oxidative stress (ROS). In this study, we test whether dysfunction of renal D1R is involved in fetal programmed hypertension, and whether oxidative stress contributes to this process.

Methods: Pregnant Sprague-Dawley (SD) rats were intraperitoneally injected with LPS (0.79mg/kg) or saline (0.5ml) at gestation day 8, 10 and 12. After birth, the blood pressure is measured, and treated with or without antioxidant tempol in tap water for 3 weeks at postnatal 12 week.
Results: As compared with control rats, the LPS-treated offspring rats showed higher blood pressure, decreased renal sodium excretion with increased plasma ROS activity. After treatment with tempol for 3 week, the increased blood pressure, decreased sodium excretion were reversed to normal levels in LPS rats. Our further study found LPS rats had lower renal D1R expression, higher D1R phosphorylation, and D1R-mediated natriuresis and diuresis were lost. As an important kinase of D1R phosphorylation, G coupled receptor protein kinase 4 (GRK4) expression was increased in LPS rats. Tempol treatment reversed the decreased D1R expression, increased D1R phosphorylation and GRK4 expression. Moreover, the impaired D1R-mediated natriuresis and diuresis were restored to the control levels in LPS rats after tempol treatment.
Conclusions: Pprenatal LPS exposure, via impairment of ROS on renal D1R function, leads to hypertension in offspring. Reversion of renal D1R function by alleviation of ROS might be a target for therapy of fetal programming hypertension.

GW25-e0547

Down-regulation of the Small-Conductance Calcium-Activated Potassium Channels in Diabetic Mouse Atria

Yi Fu[1,2], Ling Tian-You[3], Lu Tong[2], Wang Xiao-Li[2], William C. Claycomb[4], Shen Wen-Kuang[5], Lee Hon-Chi[2]
[1]Department of Cardiovascular Diseases, Xijing Hospital, Fourth Military Medical University, Xi'an, [2]Department of Internal Medicine, Division of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota, USA, [3]Department of Cardiology, Ruijin Hospital, Shanghai JiaoTong University School of Medicine, Shanghai, [4]Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, New Orleans, LA, USA, [5]Department of Internal Medicine, Division of Cardiovascular Diseases, Mayo Clinic, Phoenix, Arizona USA

Objectives: Small-conductance calcium-activated potassium (SK) channels have recently been found to be expressed in the heart, and genome-wide association studies have shown that they are implicated in atrial fibrillation (AF). Diabetes mellitus (DM) is an independent risk factor of AF, but the ionic mechanism underlying this relationship remains unclear. We hypothesized that SK channel function is abnormal in DM, leading to altered cardiac electrophysiology.
Methods: DM was induced in mice by streptozotocin injection and animals were used after 8 weeks. Expressions of SK channel isoforms were measured by real-time PCR and Western blot analysis. Standard microelectrode techniques were used to measure action potentials in isolated atrial tissues and the effects of apamin (100 pM), an SK channel inhibitor, on action potential duration (APD) and on the development of arrhythmias were determined. Whole-cell SK currents were determined as the apamin-sensitive K^+ currents in isolated atrial myocytes.
Results: We found that in streptozotocin-induced diabetic mice, the expression of SK2 and SK3 isoforms were down-regulated by 85% and 92% respectively, while that of SK1 was not changed. SK currents from isolated diabetic mouse atrial myocytes were significantly reduced compared to controls. The resting potentials of isolated atrial preparations were similar between control and diabetic mice, but action potential durations (APDs) were significantly prolonged in the diabetic atria. Exposure to apamin significantly prolonged APDs in control but not in diabetic atria. Production of reactive oxygen species was significantly increased in diabetic atria and in high-glucose cultured HL-1 cells, while exposure of HL-1 cells in normal glucose culture to H_2O_2 reduced the expression of SK2 and SK3.
Conclusions: Our results suggest that increased oxidative stress in diabetes results in SK channel-associated electrical remodeling in diabetic atria and may promote arrhythmogenesis.

GW25-e0612

Angiotensin-(1-7) prevent atrial tachycardia-induced sodium channel remodeling

Wang Xuewen[1,2], Li Guangping[1,2]
[1]Second Hospital of Tianjin Medical University, [2]Tianjin Institute of Cardiology

Objectives: Activation of the renin-angiotensin system (RAS) plays an important role in atrial electrical remodeling (AER); Angiotensin-(1-7) [Ang-(1-7)] counter-balances the actions of angiotensin II (Ang II). The aim of the present study was to determine the effects of Ang-(1-7) on cardiac sodium current (I_{Na}) in a canine model of atrial tachycardia.
Methods: Eighteen dogs were randomly assigned to sham, pacing or pacing+Ang-(1-7) groups (n=6 in each group). Rapid atrial pacing (500 bpm) was maintained for two weeks, while the dogs in the sham group were not paced. Ang-(1-7) ($6\ \mu g \cdot Kg^{-1} \cdot h^{-1}$) was administered intravenously during pacing. Whole-cell patch clamp techniques were utilized to record I_{Na} from canine atrial myocytes. Reverse transcription-polymerase chain reaction (RT-PCR) was used to assess possible underlying changes in cardiac Na^+ channels (Nav1.5).
Results: Our results showed that I_{Na} density and expression of the Nav1.5 mRNA, significantly decreased following pacing ($P<0.05$ vs sham), however the half-activation voltage ($V_{1/2act}$) and half-inactivation voltage ($V_{1/2inact}$) of I_{Na} were not significantly altered ($P>0.05$ vs. sham). Ang-(1-7) treatment significantly increased I_{Na} densities and hyperpolarized $V_{1/2act}$ without concomitant changes in $V_{1/2inact}$ but have no effect on the expression of the Nav1.5 gene.
Conclusions: Ang-(1-7) significantly increased I_{Na} densities, which contributed to improving intra-atrial conduction and decreasing the likelihood of atrial fibrillation maintenance.

GW25-e1164

Asymmetric dimethylarginine triggers macrophages apoptosis via endoplasmic reticulum stress pathway

Fu Qiong Mei[1], Bai Yong Ping[3], Hong Dan[2], Zhang Guogang[2]
[1]Department of Health Management Center, Xiangya Hospital, Central South University, [2]Department of Cardiovascular Medicine, Xiangya Hospital, Central South University, [3]Department of Geriatric Medicine, Xiangya Hospital, Central South University

Objectives: ADMA, an endogenous inhibitor of NOS, is emerging as a key contributor for atherogenesis. Macrophage apoptosis plays important roles in atherosclerosis. The goal herein was to determine the effect of ADMA on macrophage apoptosis and test the possible involvement of the endoplasmic reticulum stress (ER stress) pathway in vivo and vitro.
Methods: Macrophage apoptosis was evaluated by Annexin V-PI double staining assay. CHOP was evaluated by western blot and reverse transcription PCR. Caspase-4 activity was measured using a colorimetric protease assay kit. Activations of ERS sensors were characterized by Western blot and Immunohistochemistry.
Results: Our results showed that four-week oral ADMA treatment (5 mg/Kg/day) significantly increase atherosclerotic lesion size and induce ER stress marker-GRP78 expression in ApoE -/- mice, which was attenuated by pre-administration with L-arginine. ADMA (1-30 μM) induced apoptosis of macrophages as well as GRP78 expression in a dose- and time-dependent manner. Moreover, ADMA dramatically increased pro-apoptotic CHOP expression, activated intracellular caspase-4 activity and triggered apoptosis in macrophages, which was markedly inhibited by PERK siRNA or IRE1 siRNA. In addition, to determine further the role of ER stress in ADMA-induced macrophages apoptosis, pretreatment with L-arginine, the specific eIF2α inhibitor salubrinal, the specific IRE1 inhibitors irestatin 9389 or the specific JNK inhibitors SP600125 also significantly inhibited ADMA-induced macrophage apoptosis, expression of CHOP and activation of caspase-4 activity.
Conclusions: In summary, our present study demonstrated that ADMA triggered macrophage apoptosis via endoplasmic reticulum stress pathway.

GW25-e1390

Localization of genetic susceptibility loci for essential hypertension on chromosome 2 and 12 in Chinese population.

Luan Meng, Chen Gang, Zhou Peng, Chen Xing, Wang Bo
Institute of Basic Medicine, Shandong Academy of Medical Sciences, Jinan, 250062, China

Objectives: Essential hypertension (EH) is a multifactorial disease with a complex genetic component. Blood pressure levels and susceptibility to development of essential hypertension are partially determined by genetic factors that are poorly understood. In the study, we aim to identify these genetic factors by scanning the human genome for susceptibility genes for essential hypertension. We used microsatellite markers on chromosome 2 and 12, and then we performed an association study between EH and control subjects. Here we report a susceptibility locus for essential hypertension mapped by a genome microsatellite scan in a Chinese population.
Methods: The case group consisted of 450 patients with essential hypertension. A total of 450 healthy control subjects were consecutively selected from the Blood Center of Shandong province. The cases and controls were all from Shandong peninsula, with no blood relationship between individuals. Genomic DNA was extracted from peripheral venous blood by a modified phenol-chloroform method, and then DNA samples were diluted to 20ng/μl as working concentrations. Two DNA pools from cases and controls were constructed respectively by adding up 10μl of each DNA sample. DNA was amplified with fluorescently labelled primers from the Applied Biosystems PRISM Linkage Mapping Set MD-10 comprising 49 highly polymorphic microsatellite markers with an average spacing of 10 cM on chromosome 2 and 12. Genotyping was performed with GeneMapper 3.5 software, and the statistical significance was calculated with CLUMP software which can analyze multi-allelic markers.
Results: The genotypes of all 49 microsatellite markers on chromosome 2 and 12 were verified at the end of analysis. All the genetic loci were polymorphic in cases and controls, and the numbers of alleles on these loci ranged from 4 to 15. We used CLUMP software for comparing the difference in allele frequency of each locus between the two groups. Significant statistical differences were found between allele frequencies in cases and controls at marker D2S2211 (X^2=33.04483, P=0.001). Allele frequencies at the remaining 48 loci on chromosome 2 and 12 showed no significant statistical difference between cases and controls.
Conclusions: There was no evidence for a susceptibility locus for essential hypertension on chromosome 12 in this study. The data from case-control association study suggest that microsatellite marker D2S2211 locus (2p25.1) might be associated with the genetic susceptibility to essential hypertension in a Chinese population in Shandong peninsula, and this finding implies that a region on chromosome 2 at or near the locus of D2S2211 may harbor genes responsible for the development of essential hypertension. Future studies should focus on the fine mapping of this region and assessment of candidate genes.