LVFS (left ventricular fraction shortening). Detection of plasma BNP (brain natriuretic peptide), and Masson staining determination of CVF (Collagen Volume Fraction), immunohistochemical detection of PKCα and PKCε in myocardial tissue, detection the PKCα and PKCε expression in myocardial cells by Western blot.
Results: In group Model + AS, the death number is less than the Model group, the cardiac function indexes have been improved and the myocardial fibrosis degree significantly reduced compared with the Model group. The expression of PKCα in the Model group is higher than in the Model+AS group; while the expression of PKCε in Model group is lower than the Model + AS group.
Conclusions: The pretreatment of astragalus in myocardial infarction model rats can inhibit the ventricular remodeling, and this effect may be caused by the up-regulation of PKCε and the inhibition of PKCα expression.

GW25-e2466

Knockdown of Neuregulin Receptor Degradation Protein-1 promotes angiotensin II-Induced Cardiomyocyte Hypertrophy

Yuan Zhang[1], Li Su[2], Li-sha Han[1], Hai Hu[1], Kun Zhang[1], Yan-guo Wang[1], Jia Liu[1]
[1]Department of Pathophysiology, BaoTou Medical College, BaoTou, Inner Mongolia 014060, China, [2]Department of Neurosurgery, the First Affiliated Hospital of BaoTou Medical College, BaoTou, Inner Mongolia 014010, China

Objectives: Recent studies indicate ubiquitin-proteasome system particularly the E3 ubiquitin ligase plays a key role in cardiac hypertrophy. Neuregulin receptor degradation protein-1 (Nrdp1) is a newly identified E3 ligase. In this study, we sought to investigate the probability of knockdown of Nrdp1 might exhibit prohypertrophic effects, and therefore we tested this hypothesis in vitro.
Methods: Neonatal cardiomyocytes were infected with adenovirus containing empty vector (Ad-siRNA -control) and siRNA-Nrdp1 (Ad-siRNA-Nrdp1), and treated with angiotensin II for 24 hours. Quantitative real-time PCR was used to determine the expression of hypertrophy marker genes. Immunofluorescence and were performed to measure the cell surface area. Western blotting analysis was used to detect the expression of phosphorylation of Akt and ERK1/2.
Results: After stimulated by angiotensinII, cell surface area of cardiomyocytes was increased by 1.3-fold in Ad-siRNA-Nrdp1 group compared with Ad-siRNA-control group ($P<0.05$). Furthermore, the mRNA levels of ANF,β-MHC and skeletal α-actin in Ad-siRNA-Nrdp1 group was 1.5-fold, 1.4-fold, 1.5-fold higher than that of Ad-siRNA-control group respectively ($P<0.05$). Knockdown of Nrdp1 augmented the phosphorylation level of Akt (0.75±0.04 arbitrary units vs 0.29±0.03 arbitrary units, $P<0.05$) and ERK1/2 (0.84±0.04 arbitrary units vs 0.27±0.04 arbitrary units, $P<0.05$) compared with control group**.**
Conclusions: Knockdown of Nrdp1 might accelerate pathology progress of cardiac hypertrophy, which may rely on promotion of the phosphorylation level of Akt and ERK1/2.

GW25-e2529

Comparative study of vitro transfection of different human vascular cells by Type 9 recombinant adeno-associated virus mediated R65 ribozyme gene

Zhai Hui[1,2], Yang Yi-ning[1,2], Ma Yi-tong[1,2]
[1]Department of Cardiology, First Affiliated Hospital of Xinjiang Medical University, Urumqi, People's Republic of China, [2]Xinjiang Key Laboratory of Cardiovascular Disease Research, Urumqi, People's Republic of China

Objectives: To evaluate the transfection efficiency using recombinant adeno-associatedvirus serotype 9 (rAAV9) mediated anti-nuclear factor-κB (NF-κB) ribozyme and enhanced green fluorescent protein (rAAV9-EGFP-R65) in Human Umbilical Vein Endothelial Cells (HUVECs) and in Human Aortic Smooth Muscle Cells (HASMCs), and to assess the influence of cell proliferation from the virus vector and the P65 expressionof the NF-κB pathway.
Methods: HUVECs and HASMCs were respectively transfected with AAV9-eGFP-R65at different Multiplicity of Infection (MOI= 1×10^5, 1×10^6 and 1×10^7 v.g./cel1), and the two kinds of cells were observed and analyzed with fluorescence microscopy and flow cytometry to assess the transfection efficiency. Alamar Blue assay was used to assess the proliferation of the transfected cells in HUVECsand HASMCs.Western blot was used in detection P65 protein expression of NF-κB signaling pathway in HUVECs and HASMCs.
Results: The fluorescence intensity was enhanced with the increaseing of MOI and time of transfection.HUVECs and HASMCs transfected with AAV9-eGFP-R65 were begin to express after transfected 24 hours, and the expression peak appeared in the sixth day in HUVECs, and the fifth day in HASMCs. Multiplicity of infection of 1×10^5, 1×10^6 and 1×10^7in the sixth day after transfection in HUVECs was (1.40±1.20)%, (12.30±1.35)% and (52.80±2.05)%.And the fifth day in HASMCs was (5.30±1.04)%, (18.30±2.24)% and (52.40±3.21)%. Cell growth was not affected and cell form was normal inthe whole observation period. Alamar Blue assay did notreveal significant diference in the absorbance between the transfected cells and the control cells in the two kinds of cells. Western blotting showed that the expression of P65 of NF-κB signaling pathway was decreased by the AAV9-eGFP-R65 in two cells.
Conclusions: HUVECs and HASMCs can be efficiently transfected by AAV9-eGFP-R65, and there was no proliferation inhibition between the two kinds of cells. And P65 expression was restrained by the rAAV9-eGFP-R65 in both HUVECs and HASMCs.

GW25-e3134

Polymorphisms of the CYP2C9 Gene are not Associated with Essential hypertension in Chinese Population

Zhu Qing[1,2], Zhenyan Fu[1,2], Yitong Ma[1,2]
[1]Department of Cardiology, First Affiliated Hospital of Xinjiang Medical University, Urumqi, People's Republic of China, [2]Xinjiang Key Laboratory of Cardiovascular Disease Research, Urumqi, People's Republic of China

Objectives: Cytochrome P450 (CYP) 2C9 catalyzes a wide spectrum of drugs and is also responsible for the metabolism of arachidonic acid to biologically active epoxyeicosatrienoic acids (EETs). EETs are known to be a vasoactive substance and play an important role in a hypertensive episode. The aim of the present study was to assess the association between the human CYP2C9 gene and essential hypertension (EH) in Chinese Population.
Methods: We use two independent case-control studies: a Han population (267 EH patients and 253 control subjects) and a Uygur population (220 EH patients and 166 control subjects). All EH patients and controls were genotyped for the same four single nucleotide polymorphisms (SNPs) (rs4086116, rs2475376, rs1057910 and rs1934967) of CYP2C9 gene by a Real-time PCR instrument. The data was assessed for 3 groups: total, men, and women via Haplotype-based case-control studies.
Results: It was not found that the statistically significant differences in the distribution of the allele and genotype frequencies of CYP2C9 between the EH patients and control participants both in Han population and in Uygur population ($P>0.05$, all). When using Haplotype-based case-control studies, we still did not find all the haplotypes in CYP2C9 gene were significant different between EH patients and control subjects ($P>0.05$, all).
Conclusions: These results suggest that these SNPs of CYP2C9 gene are not associated with increased/decreased risk of hypertension in the Chinese population.

GW25-e3139

A Novel Polymorphism of the GP78 Gene is Associated with Coronary Artery Disease in Han Population in China

Cha Erdenbat[1,2], Zhenyan Fu[1,2], Yitong Ma[1,2]
[1]Department of Cardiology, First Affiliated Hospital of Xinjiang Medical University, Urumqi, People's Republic of China, [2]Xinjiang Key Laboratory of Cardiovascular Disease Research, Urumqi, People's Republic of China

Objectives: GP78 is a membrane-anchored ubiquitin ligase mediating the degradation of HMG-CoA reductase (HMGCR) and Insig-1, which was very essential for the synthesis of cholesterol process. The cholesterol has a causal role in the development of cardiovascular disease. The aim of the present study was to assess the association between the human gp78 gene polymorphism and coronary artery disease (CAD) in a Han and Uygur population of China.
Methods: We use two independent case-control studies: a Han population (602 CAD patients and 572control subjects) and a Uygur population (374 CAD patients and 376control subjects). All CAD patients and controls were genotyped for the same three single nucleotide polymorphisms (SNPs) (rs731119, rs2617849and rs2440472) of gp78 gene by a Real-time PCR instrument.
Results: the Han population, for total and men, the distribution of SNP3 (rs2440472) genotypes showed a significant difference between CAD and control participants (for total: P=0.008, for men: P=0.007), the distribution of SNP3 (rs2440472) alleles and the dominant model (AA vs AG+GG) and recessive model (GG vs AG+AA) showed a significant difference between CAD and control participants (for allele: P=0.003 and P=0.002, respectively; for dominant model: P=0.041 and P=0.026, respectively; for recessive model: P=0.004 and P=0.004, respectively). The significant difference in both the two models was retained after adjustment for covariates (for dominant model P=0.042 and P=0.022, respectively; for recessive model P=0.018 and P=0.000, respectively).
Conclusions: The GG genotype and G allele of rs2440472 in gp78 gene could be a risk genetic marker of CAD in Han population in China.

GW25-e3151

Changes in levels of angiotensin II and its receptors in a model of stress-induced cardiomyopathy

Guo Ruiwei, Yang Lixia
Department of Cardiology, Kunming General Hospital of Chengdu Military Command, Yunnan

Objectives: Stress-induced cardiomyopathy (SIC) is characterized by extensive ventricular akinesis involving apical segments with preserved function in basal segments. Beta-blockers and angiotensin-converting enzyme inhibitors (ACEIs) are the main treatments for SIC. We assessed changes in levels of angiotensin II, angiotension-II receptors and ACE responses to SIC.
Methods: A model of SIC was established in rabbits by vagal electrical stimulation. The serum concentration of angiotensin II and angiotensin (1-7) was detected by enzyme-linked immunosorbent assay. Expression of angiotensin-II receptors was measured by western blotting and real-time RT-PCR, with localization detected by immunofluorescent staining. ACE-II expression in the myocardium was measured by western blotting.

Results: From 1 day after vagal stimulation, concentrations of angiotensin II were significantly higher in the experimental group than those in the control group ($P<0.05$). Stress induced a time-dependent decrease in AT1 expression and a time-dependent increase in AT2 expression only in the apical portion of the myocardium. Immunofluorescent staining showed clear immunoreactivity for AT1 and AT2 in scattered cells in the myocardium of basal and apical portions. From 3 days after vagal stimulation, angiotensin (1-7) levels were significantly lower in the experimental group compared with the control group ($P<0.05$). Expression of ACE2 protein was significantly downregulated in the experimental group compared with the control group from 3 days after vagal stimulation ($P<0.05$).
Conclusions: Expression of angiotensin II, its receptors, ACE-II and angiotensin (1-7) was altered in response to SIC. The rennin-angiotensin system could represent a therapeutic target in the prevention of SIC.

GW25-e3166

Inhibition of dipeptyl peptidase-4 attenuates intimal hyperplasia following arterial injury in rats

Xu Changwu, Chen Jing, Zhang Jing, Hu Qi, Hu Xiaorong, Lu Zhibing, Jiang Hong
Department of Cardiology, Renmin Hospital of Wuhan University

Objectives: Intimal hyperplasia is a major process of proliferative vascular disease, including restenosis after angioplasty. Dipeptyl peptidase-4 (DPP-4) is a membrane-bound exopeptidase which rapidly degrades glucagon-like peptide, and DPP-4 inhibitors are used for the treatment for type 2 diabetes mellitus. Recent studies showed that soluble DPP-4 augmented cultured VSMCs proliferation and inhibition of DPP-4 suppressed the proliferation. This study was to explore the effect of a DPP-4 inhibitor, sitagliptin, in the development of intimal hyperplasia after vascular injury.
Methods: Forty SD rats were randomly assigned into three groups, Sham group (n=10), Control group (n=15) and Sitagliptin group (n=15). Carotid artery balloon injury model was performed in the Control and Sitagliptin groups. Rats in Sitagliptin group were treated with sitagliptin (300 mg/kg/day) 3 days before the surgery and until the day of sacrifice. The carotid arteries of rats were used for immunohistochemical staining and morphometric analysis at 7 days and 28 days after balloon injury.
Results: Compared to the Control group, treatment of sitagliptin could significantly decrease the ratio of neointimal area to media area ratio by 15.7 % and 36.1 % at 7 days and 28 days after balloon injury, respectively. Immunohistochemisty analyses showed that there was less expression of proliferating cell nuclear antigen and NF-κB p65 in the neointima 7 days after surgery in sitagliptin-treated rats. Moreover, the serum level of 8-iso-prostaglandin F2α in sitagliptin-treated rats was significantly decreased.
Conclusions: Inhibition of DPP-4 attenuated neointimal formation after artery injury. Therefore, inhibition of DPP-4 might represent a novel therapeutic strategy for vascular injury.

GW25-e3174

Genetic mutation of klotho induces salt-sensitive hypertension by CCR2 pathway

Zhou Xiaoli[1], Lei Han[1], Luo Suxin[1], Sun Zhongjie[2]
[1]The First Affiliated Hospital of Chongqing Medical University, [2]University of Oklahoma Health Sciences Center

Objectives: Klotho (KL) is identified as an anti-aging gene. Genetic mutation of KL in mice results in accelerated aging syndrome. Hypertension is a common aging-related disorder. An increase in salt sensitivity has also been noted with advancing age, especially in hypertensive individuals. The aim of the present study is to investigate if KL gene deficiency causes salt-sensitive hypertension and its mechanism.
Methods: One group of KL mutant heterozygous (+/-) mice and one group of wild type (WT) mice were used to measure BP by tail-cuff method for 15 weeks. Then each stain was divided into two sub-groups receiving high salt (HS) drinking or regular tap water for 4 weeks. After that, each sub-group of each strain was further divided into two mini-groups receiving INCB3284 (CCR2 antagonist) or vehicle for 10 days. BP was continued to measure during treatment of HS and INCB3284. Blood, urine and kidneys of all the 8 mini-groups were collected after the mice were sacrificed. Plasma urea and urine creatinine level were detected with a quantichromTM assay kits. Urine albumin concentration was measured with a microalbuminuria ELISA kit. HE and Trichrome staining were performed in kidney sections for morphological examination. Immunohistochemical staining was used to detect inflammatory cells (CD4, CD8, and CD68) infiltration in kidneys. Western blotting was done to investigate the expression of MCP-1, TNF-α, CCR2, MR, SGK1, NCC and ATP synthase β in kidneys.
Results: Systolic BP in KL (+/-) mice increased gradually and reached significance after the age of 16 weeks, showing KL gene deficiency caused spontaneous hypertension. High salt (HS) intake further increased BP and exacerbated hypertension in KL (+/-) mice, but did not affect BP in WT mice, indicating that KL deficiency elicited salt-sensitive hypertension. Notably, the expression of MCP-1 and TNF-α, as well as the infiltration of macrophages and T cells in kidneys was increased in KL (+/-) mice, which were exacerbated by HS. Blockade of CC chemokine receptor 2 (CCR2), a receptor of MCP-1, by a specific CCR2 antagonist (INCB3284) abolished the HS-induced further increase of BP in KL (+/-) mice and the infiltration of macrophages and T cells in kidneys of both KL (+/-) and KL (+/-) -HS mice. The results implied that MCP-1/CCR2-mediated inflammation activation in kidneys induced by KL gene deficiency contributed to the development of salt-sensitive hypertension. Moreover, the expression of Sgk1, NCC and ATP synthase β were upregulated in kidneys of KL (+/-) mice and further enhanced after HS loading, which also can be eliminated by INCB3284. It suggested that CCR2-mediated inflammation activation in kidneys resulted in Sgk1/NCC upregulation, even after HS, which may be one cause for salt sensitivity. Additionally, blockade of CCR2 attenuated HS-induced renal structural damage and functional impairment in KL (+/-) mice.
Conclusions: KL gene deficiency contributes to salt-sensitive hypertension via MCP-1/CCR2-mediated inflammation activation in kidneys.

GW25-e3198

The combination of transforming growth factor β1 and 5-azacytidine improve the differentiation effects of rat Bone marrow mesenchymal stem cells into cardiomyocytes

Shi Shutian, Nie Shaoping, Wu Xingxin, Wang Xiao, Miao Huangtai, Ren Hongmei, Zhen Lei
Emergency & Critical Care Center, Beijing Anzhen Hospital, Capital Medical University, Beijing, China

Objectives: Bone marrow mesenchymal stem cells (BMMSCs) are used in cardiac tissue engineering for regeneration of diseased hearts because they are relatively easy to obtain from bone marrow tissue and can be differentiate into cardiomyocytes. In this study, we examined the differentiation effects of rat BMMSCs into cardiomyocyte-like cells by inducing with combination of transforming growth factor β1 (TGFβ1) and 5-azacytidine (5-AZA).
Methods: BMMSCs were collected from the femur and tibial bone marrow of 4-week-old male Sprague-Dawley rats by density gradient centrifugation. The third passage BMMSCs were divided into follow groups: the TGFβ1 group, the 5-aza group, the TGFβ1 combined with 5-aza group, and the untreated group as control. The effect of TGFβ1 and 5-aza on BMMSC proliferation was measured by Non-Radioactive Cell Proliferation Assay (MTS) at day 1, 3, 5, and 7 in vitro. The differentiation rates and the apoptosis of induced BMMSCs were obtained by flow cytometry. The expressions of cardiac Troponin I (cTnI) and Connexin 43 (CX-43) in the BMMSCs after four weeks of induction were detected by immunofluorescence.
Results: The MTS assay showed that the TGFβ1 combined with 5-aza group exhibit a higher cell proliferation rate than the TGFβ1 group and the 5-aza group. Flow cytometry showed that the differentiation rate in TGFβ1 combined with 5-aza group was higher than that in TGFβ1 group and 5-aza group. In addition, TGFβ1 combined with 5-aza ameliorated the apoptosis of induced BMMSCs. The expression levels of cTnI and CX-43 in TGFβ1 combined with 5-aza group were notably higher than those in the TGFβ1 group and the 5-aza group.
Conclusions: The combination of TGFβ1 and 5-aza can improve the differentiation effects of rat BMMSCs into cardiomyocyte-like cells and alleviate the cell damage effects in vitro.

GW25-e3208

The Mechanism of Tongxinluo Capsule on Coronary Micrangium Spasm: inflammation, micrangium basement membrane and collagen remodeling

Xiao Li[1], Ping Jiang[2], Xiang-dong Xu[1], Hai-qing Lin[1], Yong Zhang[1], Xue Wang[2], Yue-hua Jiang[1]
[1]Affiliated Hospital of Shandong University of Traditional Chinese Medicine, [2]Shandong University of Traditional Chinese Medicine

Objectives: To observe the preventive effect of Tongxinluo capsule, a commonly used angina-relief Chinese patent medicine, on micrangium spasm by porcine model induced by neuropeptide Y (NPY). And to explore the mechanism of Tongxinluo Capsule further in streptozotocin-induced diabetic rats (STZ-rats) and type 2 diabetic Goto-Kakizaki (GK) rats.
Methods: (1) 14 miniature pigs were divided into Tongxinluo group and model group randomly. After intragastric administration of Tongxinluo for 15d, NPY was injected into the bloodstream in the middle of anterior descending artery through microcatheter. Micrangium volume (α), filling rate (β) and microcirculation blood flow (MBF) before injection, 10min and 30min after injection was determined by myocardial contrast echocardiography. The content of endothelin (ET-l) in serum in coronary artery was determined by radioimmunoassay; (2) STZ-rats and GK rats were divided into Tongxinluo group, insulin group, diabetic model group and normal control group, and intervened with corresponding drugs for 8 weeks respectively. Left ventricle was stained by HE, the basement membrane was stained by Hexamine silver and the density of nuclear factor-κB (NF-κB) and collagen I, III and IV in left ventricle was assayed by immunohistochemistry. The content of NF-κB, intercellular adhesion molecule-1 (ICAM-1), tumor necrosis factor-α (TNF-α), ET-1, angiotensin-II (Ang-II) and transforming growth factor (TGF-β1) in myocardial homogenates was determined by ELISA.
Results: (1) NPY induced porcine cardiac microvascular spasm model successfully, which demonstrated decrease of α and MBF and increase of ET-1; Tongxinluo maintained α, β and MBF at normal levels effectively, increased MBF significantly at 30min after NPY injection, and decreased ET-1 levels, $P<0.05$, suggesting effectively preventive efficacy of Tongxinluo on NPY-induced porcine cardiac microvascular spasm. (2) In both STZ-rats and GK rats, Tongxinluo relieved the myocardial inflammation and diminished the content of vasoconstrictor substances, demonstrating the decreased density of NF-κB in left ventricle ($P<0.01$) and the lowered content of NF-κB, ICAM-1, TNF-α, ET-1 and Ang-II in myocardial homogenates ($P<0.01$-0.05). (3) Tongxinluo thinned the thickness of myocardial basement membrane ($P<0.05$), decreased the density of collagen IV ($P<0.01$) and improved the myocardial morphology in left ventricle. (4) Tongxinluo decreased the density of collagen I and improved that of collagen III ($P<0.01$-0.05), increased the content of TGF-β1