METHODS A systematic search of studies on the association of SNPs with susceptibility to CAD was conducted in PubMed, Embase, Cochrane Library and CNKI. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to pool the effect size. A total of 6 case-control studies on rs28362491 in NFKB1-94ATTG were included.

RESULTS The significant association was found between rs28362491 polymorphism and CAD risk in four genetic models, (D versus I: OR = 1.10, 95% CI 1.04-1.18, $P_H = 0.509$; ID versus II: OR = 1.17, 95% CI 1.07-1.29, $P_H = 0.85$; DD versus II: OR = 1.18, 95% CI 1.04-1.34, $P_H = 0.465$; ID/DD versus II: OR = 1.18, 95% CI1.08-1.28, $P_H = 0.805$). A significant increased risk of CAD was observed in the rs28362491 polymorphism comparison, but there was insufficient data to fully confirm the association of CAD and rs28362491 in NFKB1-94ATTG.

CONCLUSIONS NFKB1-94ATTG ins/del rs28362491 polymorphism is correlated with CAD risk. However, the results of NFKB1-94ATTG rs28362491 should be interpreted with caution due to limited sample and heterogeneity. Large-scale and well designed studies are needed to validate our findings.

GW26-e2361

Association of APOB genetic polymorphisms and Aortic valve calcification in Han populations in Xinjiang, China

Yongtao Wang, Yong An, Yitong Ma
Department of Cardiology, First Affiliated Hospital of Xinjiang Medical University

OBJECTIVES Limited information is available when it comes to the impact of genetic on valvular calcification. Apolipoprotein B (apoB) is a key component in lipid metabolism and plays an important role in the dynamic equilibrium of cholesterol. The objective of this study was to investigate the association between aortic valve calcification and apoB genetic polymorphisms in the Han, Uygur and Kazak populations in China.

METHODS 583 participants, including 172 cases with aortic valve calcification and 411 controls were selected for the present study. Two SNPs (rs6725189 and rs693) of apoB were genotyped by using the polymerase chain reaction-restriction fragment length (PCR-RFLP) method. Independent-sample t-test, chi-square test and logistic regression were used to analyze.

RESULTS The rs6725189 was found to be associated with aortic valve calcification in the dominant model, and the difference remained statistically significant following multivariate adjustment ($p = 0.036$, $p = 0.004$, respectively). The rs693 was found to be associated with aortic valve calcification in the recessive model, and the difference remained statistically significant following multivariate adjustment ($p = 0.004$, $p = 0.028$, respectively).

CONCLUSIONS Both rs6725189 and rs693 of the apoB gene are associated with aortic valve calcification in the Han and Kazak populations of China.

GW26-e2444

MtDNA as a biomarkers in acute myocardial infarction and its effects on myocardial cell

Jing Liu, Liang Xie, Mingming Xi, Tingsong Liu, Jianbin Gong
Jinling Hospital, Nanjing University School of Medicine

OBJECTIVES An increasing studies have focused on the phenomenon that mitochondrial DNA (mtDNA) activates innate immunity responses. However, the specific role of mtDNA in acute myocardial infarction remains elusive. This study was designed to examine whether mtDNA can be served as a biomarker of acute myocardial infarction (AMI) patients and try to eliminate the damage effects of mtDNA on cardiomyocyte.

METHODS Plasma nuclear and mtDNA levels were measured by quantitative PCR in 50 AMI patients, 50 non-myocardial infarction (MI) (with MI risk) and 50 healthy control. Purified mtDNA or nuclear DNA was added to H9c2s cells, with or without pretreatment with chloroquine (an inhibitor of endosomal receptors like TLR9). The cell viability and apoptosis were tested by MTT and Flow cytometry, respectively. The levels of TLR9, p-p38 mitogen-activated protein kinase (MAPK) and caspase 3 were detected by western blot.

RESULTS The concentrations of mtDNA were significantly higher in the AMI group of hospital day 1 than that in the non-MI controls and healthy individuals (3.754±0.384 ng/μL vs. 1.851±0.3483 ng/μL, $P<0.05$; 3.754±0.384 ng/μL vs. 0.1517±0.0924 ng/μL, $p<0.05$) and decreased shortly after PCI. Exogenous mtDNA reduced the viability of H9c2 cells and induced TLR9 and p-p38 MAPK and caspase 3 activation. These effects were inhibited by chloroquine. Nuclear DNA did not induce TLR9, caspase3, p-p38 MAPK activation.

CONCLUSIONS MtDNA level is increased after AMI and can used as a biomarker in AMI patients. MtDNA activates TLR9-P38MAPK and inducing cardiomyocyte cells death.

GW26-e2445

Protective effects and its mechanism of Helix B-surface peptide against cardiac microvascular endothelial cell injury induced by ischemia / reperfusion

Chen Wang, Rui Si, Mingming Zhang, Rongqing Zhang, Haichang Wang
Department of Cardiology, Xijing Hospital, Fourth Military Medical University

OBJECTIVES MI/R injury could paradoxically reduce the beneficial effects of myocardial reperfusion and cause contractile dysfunction and cellular damage, lacking of effective strategies of prevention cure. A helix-B surface peptide (HBSP), which is composed of 11 amino acids derived from the aqueous face of helix B of EPO, recently was developed and retained tissue-protective but avoiding erythropoietic property of EPO. Our previous experiment results have demonstrated that HBSP could reduce myocardial ischemia / reperfusion (MI/R) injury of Sprague-Dawley rats and increase the post ischemic myocardial functions via activating the phosphatidylinositol 3-kinase (PI3-K)-Akt cascade. However, it is still unclear whether HBSP can protect the cardiac microvascular endothelial cells (CMECs) when subjected to ischemia / reperfusion injury Therefore, the aims of the present study were to investigate the protective effect of HBSP against cardiac microvascular endothelial cells (CMECs) injury induced by ischemia / reperfusion and further explored the underlying mechanisms involved.

METHODS CMECs isolated from the adult hearts of Sprague-Dawley rats were exposed to hypoxia and ischemia buffer for 2h followed by 4h reoxygenation. Then CMECs were randomized to receive different concentrations of EPO-derived peptides HBSP, EPO, HBSP plus LY294002 (specific inhibitor of PI3K), HBSP plus rapamycin (specific inhibitor of mTOR) at the start of reperfusion. The cell viability of CEMCs was measured by MTT colorimetric assay and the apoptosis of CEMCs was detected by Tunel method. The wound scratch assay and transwell method were performed to detect the migration of CMECs. The expression of p-AKT, p-mTOR and p-p70S6K were analyzed by western blot analysis.

RESULTS Both cell viability and migration ability of CMECs were impaired after SI/R ($P<0.01$ vs.control), and the apoptotic index increased in comparison with control group ($P<0.01$). While administration of EPO and HBSP during reperfusion dramatically attenuated the dysfunction of CMECs. Compared with the SI/R group, HBSP treatment in CMECs exerted protective effects as evidenced by the increase of cell viability ($P<0.05$), inhibited CMECs apoptosis (25.5%± 0.43% vs. 41.1%±0.8%, $P<0.01$) and improved the migration ability of CMECs ($P<0.05$). Moreover, HBSP caused over Akt phosphorylation in the reperfusion CMECs, which was abrogated by the treatment of LY294002 ($P<0.05$), but not by rapamycin. Furthermore, mTOR phosphorylation following HBSP treatment was prevented by either LY294002 or rapamycin ($P<0.05$). Similarly, the phosphorylation of the mTOR downstream molecule p70S6K were up-regulated by HBSP treatment ($P<0.05$). While treating with LY294002 or rapamycin prevented HBSP-induced phosphorylation of p70S6K ($P<0.05$). Compared with the HBSP group, the apoptotic index increased while treating with LY294002 or rapamycin ($P<0.05$).

CONCLUSIONS HBSP might have protective effect of CMECs against ischemia/reperfusion injury, which may be related with activation of PI3K/AKT/mTOR signaling pathway.

GW26-e2449

The Role of Calpain in Myocardial Apoptosis Induced by Oxidative Stress in Mouse cardiomyocytes Hypoxia/Reoxygenation

Shuang Zhang, Houxiang Hu, Lei Xu, Rongchuan Yue, Chunyan Tan, Haiyan Chen, Rongyi Zhang
Department of Cardiology, Affiliated Hospital of North Sichuan Medical College

OBJECTIVES In the present study, we aimed to explore the effects of calpain and its inhibitor PD150606 (PD) on oxidative stress induced myocardial apoptosis in mouse hypoxia/reoxygenation (H/R) injury.

METHODS The ventricular myocytes of adult C57BL/6 mice were isolated and cultured. The cardiomyocytes were randomly divided

into three groups, control group, H/R group and PD150606 plus H/R group (PD150606 group). To achieve a suitable simulated H/R, cardiomyocytes were incubated with serum-free and glucose-free medium under conditions of hypoxia for 20 min, then they were returned to a normoxic environment with the normal culture medium at 37°C for 2 hours. The viability of cultured cardiomyocytes was examined using trypan blue exclusion test. The cytosolic cytochrome c levels were determined using an ELISA kit. The fluorimetric caspase-3 kit was assayed to measure its activity in the cardiomyocytes. The Suc-LLVY-AMC was used as a fluorescence substrate to measure of calpain activity of cardiomyocytes. Formation of 2-hydroxyethidium (2-EOH) in cardiomyocytes was measured by HPLC to quantify superoxide production.

RESULTS After subjected to I/R, the viable rod-shaped cells were decreased to 37.8% in I/R group when compared with the control group (75.6%), while the calpain inhibitor PD150606 prevented 15% of the rod-shaped cells against H/R-induced cell death. Similarly, compared to I/R group, PD150606 inhibited myocardial apoptosis by decreasing caspase-3 activity 65% and cytochrome c release 47%. Calpain activity was increased 58% in the H/R-cultured cardiomyocytes, yet blunted to 23% by PD150606. Compared to control group, the superoxide (2-EOH) was overproduced 5.43-fold and 4.22-fold, respectively, in I/R and PD150606 group. 2-EOH was partially prevented by PD150606.

CONCLUSIONS Calpain inhibitor PD150606 can effectively reduce H/R injury by reducing oxidative stress mediated myocardial apoptosis. It is plausible that calpain inhibition would be an effective method for protecting the cardiomyocytes from H/R injury.

GW26-e2452

A Study in Construction of Short Hairpin Small Interfering RNA Expression Vector Target Lectin Like Oxidized Low Density Lipoprotein Receptor-1 Gene and Its Effect on foam cells

Huiyu Yang, Yunfei Bian, Bin Liang, Rui Bai, Zhiming Yang
Department of Cardiology, The Second Hospital of Shanxi Medical University

OBJECTIVES To construct the short hairpin small interfering RNA(shRNA) eukaryotic expression vector specific to mouse lectin like oxidized low density lipoprotein receptor 1(LOX-1) Gene and to observe its silencing effect on LOX-1 in RAW264.7 cells.

METHODS (1) The pLOX-1-shRNA expression vector was constructed by gene recombination, Then transfected into the cultured RAW264.7 cells. At 48 h after Transfection, the expression of LOX-1 mRNA in RAW264.7 cells were determined by semi-quantitative RT-PCR, the expression of LOX-1 proteins examined by Western blot.

(2) Oil Red O Dyeing experiment was used to show the cellular lipid droplets in lipid-loaded cells. The method of cholesterol oxidase analysis was performed to determine the content of cellular cholesterol. the ability of uptake Dil-ox-LDL in RAW264.7 cells was assayed by fluorescence microscopy.

RESULTS pLOX-1-shRNA expression vector was successfully constructed. Transfection of pLOX-1-shRNA expression vector into RAW264.7 cells down regulated the expression level of LOX-1 gene, as compared with the control Group, transfection of the RAW264.7 cells with LOX 1-shRNA led to a remarkable reduction of the number macrophages transformed into foam cell, and could suppress the uptake of ox-LDL.

CONCLUSIONS The pLOX-1-shRNA expression vector can inhibit The expression of LOX 1 in RAW264.7 cells and the transformation of the macrophages into foam, which may he beneficial in searching new gene therapy of atherosclerosis.

GW26-e2469

Study on Anti-atherosclerosis Mechanism of Yang Xin Shi

Liang Hou, Yanchun Ding
Department of Cardiology V, the Second Affiliated Hospital of Dalian Medical University, Dalian 1160027, China

OBJECTIVES To provide experimental basis for revealing the mechanism of yang xin shi in prevention and treatment of coronary heart disease ,we observe its effects on macrophage inflammatory activation and polarity switch by culturing the human monocytic cell line THP-1 with Yang Xin Shi.

METHODS Human THP-1 cells were differentiated into macrophages by the addition of 160 nmol/l phorbol 12-myristate 13-acetate(PMA) for 24 h. We treated macrophages with different concentrations of yang xin shi (10 mg/L, 50 mg/L, 100 mg/L, 200 mg/L) for 12 h, then we stimulated these cells by OX-LDL and detected the secretion of monocyte chemotactic protein 1 (MCP-1) and macrophage migration inhibition factor (MIF) by Enzyme-linked immunosorbent assay (ELISA), and the membrane molecule CD16, CD68 were tested by flow cytometry.

RESULTS 1. Yang xin shi could inhibit the expression of MCP-1 and MIF from the cell culture supernatants as a dose-dependent manner. With the increasing concentrations of Yang xin shi, the secretion of MCP-1 (control: 33.30±2.37 pg/mL; 10 mg/mL: 26.78±1.48 pg/mL; 50 mg/L:25.73±2.25pg/mL;100 mg/L:9.95±2.09pg/mL; 200mg/L: 8.53±1.37pg/mL) and MIF (control: 18.65±0.15 ng/mL; 10 mg/L: 15.50±0.27ng/mL; 50 mg/L: 9.07±0.26ng/mL; 100 mg/L: 4.85±0.12ng/mL; 200mg/L: 4.58±0.36ng/mL) decreased. The difference was statistically significant.

2. Yang xin shi could change the expression of CD16 and CD68 as a dose-dependent manner, the mean fluorescence intensity (MFI) of 100mg/L group could significantly up-regulated the expression of CD16, CD68 (CD16: 96 vs 71.2; CD68: 91.6 vs 54.7). As the concentration increased, the expression of CD16, CD68 were down-regulated in 200mg/L group (CD16:80.6,CD68:72.1).

CONCLUSIONS Yang xin shi may execute its anti-atherosclerotic effect by inhibiting macrophage inflammatory activation and affecting polarity switch.

GW26-e2492

Dynamic research on the heart failure model caused by transverse aortic constriction in Kunming mice

Hongjian Lian, Wei Wang
Beijing University of Chinese Medicine

OBJECTIVES Transverse aortic constriction is widely used to induce heart failure by increasing afterload. It was believed that different pathological and functional changes may appear during a long period after TAC surgery. But comprehensive and dynamic researches about TAC model were still limited. In this study, we try to explore these dynamic pathological and functional changes in TAC mice by multi-measurement during 16 weeks.

METHODS 150 healthy KM mice were divided into two groups randomly: TAC (operated with TAC) group (n=90) and Sham-operation (SH) group (n=60). Pathological, functional detection was performed at 6 different time-points, including 2 weeks, 4 weeks, 6 weeks, 8 weeks, 12 weeks and 16 weeks after surgery. Symptoms and signs of animals were observed and recorded. Echocardiography was used in evaluation of cardiac function and structure by detecting left ventricular mass (LVM) and left ventricular mass index (LVMI), measuring the thickness of anterior or posterior wall, the diameter and volume of left ventricular at both systolic and diastolic stage and analyzing left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS). After gross anatomy and general observation of heart, the heart was weighed and heart/body index was calculated. Haematoxylin-eosin stain and Masson stain were performed to show the histological of heart.

RESULTS (1) Compared with SH, LVM, LVMI and heart/body index of TAC group increased significantly ($P < 0.05$, $P < 0.01$) from 2 to 16 weeks after surgery. LVEF started to reduce after 6 weeks ($P < 0.05$, $P < 0.01$). LVFS started to reduce significantly after 4 weeks ($P < 0.05$, $P < 0.01$). The thickness of left ventricular wall started to thicken significantly after 2 weeks ($P < 0.05$, $P < 0.01$). Left ventricular end-systolic volume and left ventricular internal diameter (systolic) started to increase significantly after 6 weeks ($P < 0.05$, $P < 0.01$). Left ventricular internal diameter (diastolic) and left ventricular end-diastolic volume started to reduce significantly after 12 weeks ($P < 0.05$, $P < 0.01$).

(2) Heart tissue morphology showed vessel wall thickening after 2 weeks. Myocardial hypertrophy appeared after 4 weeks. Lesions aggravated with widened myocardial fiber gap after 12 weeks.

(3) Manifestations of pulmonary congestion were visually observed at each time point. Animals of TAC group were observed with locomotor activity decrease after 4 weeks, swollen soles after 8 weeks, and respiratory distress followed by sudden death after 12 weeks.

CONCLUSIONS Transverse aortic constriction could successfully induce elevated afterload of KM mice and cause Myocardial Hypertrophy/Heart failure in mice. From 2 to 8 weeks after surgery, left ventricular hypertrophy appeared in the TAC mice. According to clinical judgment in the diagnosis of congestive heart failure, this period belonged to pre-clinical stage of heart failure. Similarly,