stimulator, to create the tachycardia cardiomyopathy animal model, and then to pace the right ventricle.

RESULTS After one week of right ventricular pacing, cardiac ultrasound examination confirmed the left ventricular end diastolic diameter enlargement, reduction of left ventricular ejection fraction, increased serum BNP and MMP-9, and decreased TIMP-1. Pathology of HE staining showed myocardial structural derangement and interstitial inflammatory cell infiltration. Electron microscopy revealed mitochondrial swelling, dissolution, or even lysis and destruction, fuzzy Z line and unclear intercalated discs. Three weeks later, the heart color, serum BNP, MMP-9, TIMP-1, EF, FS in the rabbits recovered to former levels.

CONCLUSIONS Conclusions It is a simple and practical method to establish an animal model of tachycardiomyopathy by stimulating animal's right ventricle with mapping electrode line to create non-sustained ventricular tachycardia under minimally invasive, venous technique without X-ray radiation.

GW26-e2451

Correlation Between the BDNF Val66Met Polymorphisms and Metabolic Risks in Long-Lived Zhuang Population

Junhua Peng, Peng Sun, Chengwu Liu, Shangling Pan
Department of Pathophysiology, Guangxi Medical University, Nanning, Guangxi, China

OBJECTIVES We aim to evaluate the possible association between BDNF Val66Met polymorphism and common metabolic risks including BMI, fasting blood glucose (FBG) and lipid levels in a longevous population dwelling in Guangxi Hongshui River basin.

METHODS BDNF Val66Met was genotyped by ARMS-PCR technique for 487 Zhuang long-lived inhabitants (aged $\geq$ 90, long-lived group, LG), 593 offspring (age 60-77, offspring group, OG) and 582 matched healthy controls (aged 60-75, control group, CG) in Hongshui River Basin. Impacts of genotypes on metabolic risks were then evaluated.

RESULTS No significant difference was noted on the distribution of genotypic and allelic frequencies of BDNF Val66Met among the three groups (all $P > 0.05$), regardless of sex; however, AA genotype was dramatically higher in females than in males in the CG. The HDL-C level of A genotype (GA/AA) carriers was significantly lower than was non-A genotype (GG) carriers in the overall population and the CG ($P = 0.009$ and 0.006, respectively), which remained in females, hyperglycemic subgroup and normolipidemic subgroup of CG through stratification of sex, BMI, glucose and lipid status. In addition, A genotype carriers, with a higher systolic blood pressure, have 1.63 folds higher risk than non-A carriers to become overweight in CG (95% CI : 1.05 ~ 2.55). Multiple regression analysis showed that the TC level of LG was negatively correlated with BDNF Val66Met genotype.

CONCLUSIONS BDNF 66Met may lower the serum HDL-C level and lead to overweight in the general population in Hongshui River area but has less impact on the long-lived population and their offspring.

GW26-e2453

Beneficial Effect of Lycopene on Hypoxia/Reoxygenation-Induced Endoplasmic Reticulum Stress in Neonatal Mouse Cardiomyocytes

Jiqian Xu,[1,2] Houxiang Hu,[1] Bin Chen,[2] Rongchuan Yue,[1] Zhou Zhou,[3] Lei Xu,[1] Shuang Zhang,[1] Chunyan Tan,[1] Zhengping Yu[3]
[1]Department of Cardiology, North Sichuan Medical College Affiliated Hospital; [2]Department of Anesthesiology, North Sichuan Medical College Affiliated Hospital; [3]Department of Occupational Health, Third Military Medical University

OBJECTIVES Endoplasmic reticulum (ER) stress has become an important therapeutic target for myocardial ischemia reperfusion (I/R) injury. It has been reported that lycopene exhibits great pharmacological effect in alleviating myocardial I/R-injury, but whether it attenuates ER stress remains to be elucidated. The purpose of this study was to explore the potential protective benefits of lycopene against ER stress induced by hypoxia/reoxygenation in cultured neonatal mouse cardiomyocytes.

METHODS Primary cultured neonatal C57BL/6 cardiomyocytes were used to establish H/R model and divided to control, lycopene, H/R(4 h hypoxia followed by 6 h reoxgenation) and lycopene+H/R group (the cardiomyocytes were pretreatment with 5μmol/L lycopene for 4 h before H/R treatment). TUNEL assay was used to assess cardiomyocytes apoptosis. Western blotting was used to evaluate the expression of cleaved caspase-3, the level of phosphorylated eIF2α, a downstream target of the PERK pathway and the expression of CHOP, an ER stress-related apoptotic maker. The expression of GRP78 mRNA and level of the sliced Xbp-1 mRNA, a downstream target of the IRE1 pathway, were determined by Real-time PCR.

RESULTS The rate of TUNEL-positive cells significantly increased to 25.62±2.61% and that of control and lycopene group were respectively 4.87±1.54% and 4.91±1.63% ($P < 0.01$). After pretreatment with lycopene, the rate decreased to 18.18±2.18% ($P < 0.05$). Representative western blotting analysis and Quantitation of the Western blotting by densitometric scanning showed approximately a 1.89-fold increase in cleaved caspase-3 level ($P < 0.01$), 1.84-fold increase in p-eIF2a ($P < 0.05$) and 1.98-fold increase in CHOP expression ($P < 0.01$) compared with the control group after H/R treatment. The cleaved caspase-3 level decreased to 1.36-fold of normal levels ($P < 0.05$), the level of phosphorylated eIF2α reduced to 1.47-fold of normal levels ($P < 0.05$) and the CHOP expression decreased to 1.54-fold of control levels ($P < 0.05$) with lycopene pretreatment. Data from real-time PCR displayed that H/R treatment induced a more than 4-fold increase in the expression of GRP78 mRNA ($P < 0.01$). Conversely, the mRNA expression of GRP78 was significantly attenuated to approximately 2-fold of normal levels with 5 μM lycopene pretreatment ($P < 0.01$), but not completely come back the control levels. The level of the sliced Xbp-1 mRNA had a 2.23-fold increase in H/R-treated cardiomyocytes compared to control groups ($P < 0.01$), in contrast, the level of the sliced Xbp-1 mRNA was markedly downregulated to 1.20-fold of control levels with 5 μM lycopene pretreatment ($P < 0.01$), but not completely come back the normal levels.

CONCLUSIONS The present study demonstrated that lycopene protects primary cultured neonatal mouse cardiomyocytes against H/R injury by inhibiting the activation of the PERK and the IRE1 pathway of the ER stress, the protective effect of lycopene on cardiomyocytes highlights the therapeutic potential of plant-derived antioxidants against I/R-injury.

GW26-e2455

Vagal Nerve Stimulation Reverses Cardiac Dysfunction and Subcellular Calcium Handling in Heart Failure Rats After Myocardial Infarction

Yunhe Zhang, Ao Chen, Lei Song, Min Li, Ben He, Yingmin Chen
Department of Cardiology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

OBJECTIVES Vagal nerve stimulation (VNS) as a non-pharmacological approach to retrieve the autonomic balance, has showed beneficial therapeutic effects for chronic heart failure (CHF). Moreover, calcium cycling is critical in cardiac excitation-contraction coupling (ECC) and participates in the antiarrhythmic effects of VNS. Taken together, we hypothesized that VNS could improve the calcium handling properties which might be underlying mechanisms of VNS for heart failure treatment.

METHODS In this study, 32 SD rats were divided into three groups as Control (sham operated), CHF-SS (CHF rats with sham stimulation) and CHF-VNS (CHF rats with VNS). Cardiac function was evaluated by echocardiography while the structural remodeling was assessed by HE and Masson staining. ELISA was used to detect the plasma BNP, norepinephrine and angiotensin II concentrations. The proteins and mRNAs expression of sarcoplasmic reticulum Ca2+ ATPase (SERCA2a), phospholamban (PLB), ryanodine receptor 2 (RyR2), Na^+-Ca^{2+} exchanger 1 (NCX1) were analyzed by Western blot and RT-PCR.

RESULTS Compared with CHF-SS rats, rats from CHF-VNS group received 8 weeks of VNS showed significantly improved left ventricular ejection fraction (LVEF, P=0.001) and less myocardial interstitial collagen. The elevated plasma concentrations of BNP, norepinephrine and Ang II in CHF rats were partially restored by VNS. After VNS, the rats in CHF-VNS group exhibited statistically significant higher cardiac SERCA2a protein and mRNA contents than CHF-SS group. RyR2 and depressed PLB expressions were unaffected between rats with or without VNS, whereas NCX1 expression was significant lower in CHF-VNS group.

CONCLUSIONS The results suggest that the improvement of cardiac performance by VNS is accompanied with reversal of changes in calcium handling properties including SERCA2a, PLB and NCX1 which may be underlying mechanisms of VNS for heart failure therapy.

GW26-e3532

miR-433 Controls Cardiac Fibrosis

Lichan Tao, Xinli Li
Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

OBJECTIVES Cardiac fibrosis, a result of multiple injurious insults in heart, is a final common manifestation of chronic heart diseases and

can lead to end-stage cardiac failure. MicroRNAs (miRNAs, miRs) participate in many essential biological processes and their dysfunction has been implicated in a variety of cardiovascular diseases including fibrosis. miR-433 has recently been implicated in renal fibrosis, however, its role in cardiac fibrosis is unclear.

METHODS Cardiac fibrosis were induced by TGF-β (10ng/ml, 24h) and AngII (100nmol, 48h) on neonatal cardiac fibroblasts. Quantitative reverse transcription polymerase chain reactions (RT-PCRs), immunofluorescence and western blotting were used to determine the expression levels of cardiac fibrosis, includingα-SMA and collagen. EdU and Ki-67 staining were used to evaluate cell proliferation.

RESULTS miR-433 was increased in heart samples from dilated cardiomyopathy patients. In addition, miR-433 was also consistently upregulated in mice model of cardiac fibrosis after myocardial infarction or heart failure. Additionally, miR-433 was found to be enriched in fibroblasts compared to cardiomyocytes. In neonatal cardiac fibroblasts, forced expression of miR-433 promoted cell proliferation as indicated by EdU and Ki-67 staining. Moreover, miR-433 overexpression promoted the transdifferentiation of fibroblasts into myofibroblasts as determined for α-SMA and collagen whether in the presence of TGF-β, AngII or not, indicating that miR-433 is sufficient to induce fibrosis. In addition, knockdown of miR-433 inhibited proliferation and the transdifferentiation into myofibroblasts, indicating that miR-433 is required for cardiac fibrosis. Interestingly, miR-433 did not affect the migration of cardiac fibroblast. Importantly, miR-433 antagomir could partially attenuate cardiac fibrosis induced by myocardial infarction in mice.

CONCLUSIONS miR-433 controls cardiac fibrosis both in vitro and in vivo. Inhibition of miR-433 represents a novel therapeutic strategy for cardiac fibrosis.

GW26-e3832

Rosuvastatin Improves Atherosclerotic Plaques Stability: An Intravascular Ultrasonic Elastography Study

Zhaohuan Li,[1,2] Lin Wang,[1] Xiaobo Hu,[1] Mingjun Xu,[1] Yi Wang,[1,2] Yun Zhang,[1] Mei Zhang[1]
[1]The Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Health, Qilu Hospital; [2]Cardiovascular Ultrasound and Non-invasive Cardiology Department, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital

OBJECTIVES The present study aimed to investigate the association between potent rosuvastatin therapy and plaque mechanical stabilization imaged by IVUSE.

METHODS 14 purebred New Zealand rabbits underwent a high-cholesterol diet, then balloon-induced abdominal aorta endothelium injury after 2 weeks. Seven rabbits received potent rosuvastatin treatment for 8 weeks since the 13th week, while the seven other rabbits took in saline. Intravascular ultrasound (IVUS) images of abdominal aortas were acquired before and after the administration. Each rabbit were chosen 2 obvious atherosclerotic plaques and 2 consecutive frames near the end-diastole images in situ were used to construct an IVUS elastogram.

RESULTS Plaques in the rosuvastatin group demonstrated a stable magnitude of sheer strain (SS) and area strain (AS) in the total plaque (2.23±2.62% *vs.* 1.58±1.67%, p = NS; 3.84±4.63% *vs.* 2.93±2.36%, p = NS; respectively), but the untreated rabbits displayed a significant increase in the SS and AS index (1.91±1.05% *vs.* 3.54±1.73%, p =0.005; 3.46±2.10% *vs.*5.95±2.59%, p =0.008; respectively). Differences of the changes in SS and AS between the rosuvastatin and control groups on serial follow-up were significant (-0.99±2.83% *vs.* 1.15±1.96%, p = 0.013; -1.67±5.05% *vs.* 1.25±2.29%, p = 0.022; respectively). SS and AS were negatively correlated with collagen (r^2 = 0.341, p =0.001 and r^2 = 0.322, p =0.002, respectively) and smooth muscle cell content (r^2 = 0.308, p = 0.002 and r^2 = 0.277, p = 0.004, respectively) and positively with macrophage (r^2 = 0.266, p = 0.005 and r^2 = 0.198, p = 0.018, respectively), lipid content(r^2 = 0.388, p < 0.001 and r^2 = 0.357, p = 0.001, respectively) and vulnerability index (r^2 = 0.557, p < 0.001 and r^2 = 0.483, p < 0.001, respectively).

CONCLUSIONS These findings prove rosuvastatin therapy is associated with less vulnerable plaque features. IVUSE provides a technique that may develop into a clinically available tool for detecting vulnerable plaques and monitoring treatments.

GW26-e3859

Atorvastatin Calcium Inhibits Phenotypic Modulation of PDGF-BB-Induced VSMCs Via Down-Regulation the Akt Signaling Pathway

Shuang Chen,[1] Baoqin Liu,[2] Dehui Kong,[2] Si Li,[2] Chao Li,[2] Huaqin Wang,[2] Yingxian Sun[1]
[1]Department of Cardiology, the First Hospital of China Medical University, Shenyang, Liaoning, China; [2]Department of Biochemistry and Molecular Biology, China Medical University, Shenyang Liaoning, China

OBJECTIVES Plasticity of vascular smooth muscle cells (VSMCs) plays a central role in the onset and progression of proliferative vascular diseases. In adult tissue, VSMCs exist in a physiological contractile-quiescent phenotype, which is defined by lack of the ability of proliferation and migration, while high expression of contractile marker proteins. After injury to the vessel, VSMC shifts from a contractile phenotype to a pathological synthetic phenotype, associated with increased proliferation, migration and matrix secretion. It has been demonstrated that PDGF-BB is a critical mediator of VSMCs phenotypic switch. Atorvastatin calcium, a selective inhibitor of 3-hydroxy-3-methyl-glutaryl l coenzyme A (HMG-CoA) reductase, exhibits various protective effects against VSMCs. In this study, we investigated the effects of atorvastatin calcium on phenotype modulation of PDGF-BB-induced VSMCs and the related intracellular signal transduction pathways.

METHODS VSMCs were grown to 70-80% confluence and precultured in serum-free medium for 24h before treatment. Atorvastatin calcium was dissolved in methanol for a stock solution of 100mM and then diluted to desired concentrations with media prior to cell treatment. Cells were treated with various concentrations of atorvastatin calcium from 1 to 50M in cell proliferation assay, 10M in cell morphology and western blotting on quiescent cells with or without 20ng/mL PDGF-BB for designated times.

RESULTS Treatment of VSMCs with atorvastatin calcium showed dose-dependent inhibition of PDGF-BB-induced proliferation. Atorvastatin calcium co-treatment inhibited the phenotype modulation and cytoskeleton rearrangements and improved the expression of contractile phenotype marker proteins such as α-SM actin, SM22α and calponin in comparison with PDGF-BB alone stimulated VSMCs. Although Akt phosphorylation was strongly elicited by PDGF-BB, Akt activation was attenuated when PDGF-BB was co-administrated with atorvastatin calcium.

CONCLUSIONS In conclusion, atorvastatin calcium inhibits phenotype modulation of PDGF-BB-induced VSMCs and activation of the Akt signaling pathway, indicating that Akt might play a vital role in the modulation of phenotype.

GW26-e4374

Tanshinone IIA Protects H9c2 Cells From Oxidative Stress-Induced Cell Death Via Up-Regulation of MicroRNA-133 and Akt Activation

Yunfei Gu, Jun Jin, Shouyan Zhang
Luoyang Central Hospital Affiliated to Zhengzhou University

OBJECTIVES In this study the cardioprotective effect and molecular mechanisms of tanshinone IIA were investigated.

METHODS Cardiac H9c2 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. For transfection experiments, cells were transfected with 50 nM miR-133 mimic or inhibitor; 8 hours later transfected cells were treated for 24 hours with various combination of H2O2 and tanshinone IIA as indicated in figure legends. For inhibitor experiments, cells were pre-incubated with selective PI3K inhibitor for 30 min, and then treated with H2O2 and/or tanshinone IIA. Cell viability was determined by CCK-8 kit. Data were presented as percentage of control. MiR-133 expression levels were relatively quantified by Bulge-Loop™ miRNA qRT-PCR Primer Set in conjunction with real-time PCR with SYBR. The relative expression of miR-133 was calculated and normalized to U6 using the comparative Ct method. Relative expression intensity values were calculated as $2^{-\Delta\Delta Ct}$. After treatment H9c2 cells were harvested and lysed in RIPA lysis buffer (Applygen Technologies Inc. Beijing, China). Then the whole cell lysates were resolved by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a 0.4 um-PVDF membrane. After blocking in 5% nonfat milk for 2 h at room temperature, the PVDF membranes were probed with primary antibody overnight. Following a 30 minute wash, the membranes were incubated with secondary antibody conjugated to HRP for 1 hour at room temperature. The membranes were then washed for 30 minutes and