Cardiac troponin T (TNNT2) mutations in Chinese dilated cardiomyopathy patients

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Objectives: Dilated cardiomyopathy (DCM) is one of the leading causes of heart failure with high morbidity and mortality. Although more than 40 genes have been reported to cause DCM, the role of genetic testing in clinical practice is not well defined. Mutations in the troponin T (TNNT2) gene represent an important subset of known disease-causing mutations associated with DCM. Therefore, the aim of the present study was to determine the genetic variations in TNNT2 and the associations of these variations with DCM in Chinese patients.

Methods: An approximately 4 kb fragment of the TNNT2 gene was isolated from 103 DCM patients and 192 healthy controls and was analyzed by DNA sequence analysis for genetic variations.

Results: A total of 6 TNNT2 variants were identified in 99 patients, including a G321T missense mutation (Leu84Ph) and 5 novel intronic variants. Allele frequencies of two novel SNPs (c.192+353 C>A, OR=0.095; 95% CI: 0.013-0.714, P=0.02; c.192+463 C>A, OR=0.09, 95% CI: 0.012-0.675, P=0.019) and SNP rs3729845 (OR=4.77, 95% CI: 1.252-18.852; P=0.002) were significantly correlated with DCM.

Conclusions: These results suggest that variations in the TNNT2 gene might be associated with DCM in the Chinese population.

G2W5-e1128

Angiotensin II upregulates the Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel in Neonatal Rat Cardiomyocytes via a redox mechanism

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Objectives: To identify the effects of exogenous Angiotensin II (AngII) on the Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel (HCN) current and its regulation in neonatal rat cardiomyocytes (NRVM).

Methods: NRVM from 1- to 3-day-old Wistar rats were prepared by collagenase digestion, and incubated in 37°C, 5% CO2 for 1 hour by using patch-clamp recording. HCN channel protein expression was detected by western-blotting analysis.

Results: exposure (>20 min) of NRVM to AngII (100 ng/ml) markedly increased If density (4.7±0.6 pS/pF vs. 11.7±1.1 pS/pF) along increased conductance (Gmax: 48.7±5.6 pS/pF vs. 192.6±64.1 pS/pF), a shift in activation voltage (V0.5) to positive potentials (-81.2±1.6 mV vs. -64.7±2.0 mV) and increase of rate of activation (tact) (523.4±24.7 ms vs. 337.5±24.9 ms). Moreover, stimulation by AngII was largely inhibited by the non-specific tyrosine kinase blocker genistein (1µmol/L) or the c-Src-specific inhibitor PP2 (10 µmol/L). Aged cardiomyocyte phosphorylation of HCN2 channels with AngII treatment by determined by AngII Western blot using the c-Src inhibitor PP2 (10 µmol/L).

Conclusions: These results suggest that the c-Src family of tyrosine kinase mediate the activation of If current by exogenous oxidant agent AngII via a redox mechanism involving the Trx system. The studies will provide new insights into the relation of oxidative cell damage and ion channel remodeling.

G2W5-e1415

Anti-peroxynitrite Treatment Ameliorated Vasorelaxation of Resistance Arteries in Aging Rats: Involvement with NO-sGC-cGKs Pathway

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Objectives: Declined vasorelaxation function in aging resistance arteries is responsible for aging related multiple organ dysfunctions. The aim of this study is to explore the role of peroxynitrite (ONOO–) in aging resistance arterial vasorelaxation dysfunction and the possible mechanism.

Methods: In the present study, young (3-4 months olds) and aging (20 months olds) male Wistar rats were randomly randomized to receive vehicle (Saline) or FeTMPyP (ONOO– scavenger) for 2 weeks. The vasorelaxation of resistance arteries was determined in vitro; NOx level was tested by a colorimetric assay; the expression of nitrotyrosine (NT), soluble Guanylate Cyclase (sGC), vasodilator-stimulated phosphoprotein (VASP) and phosphorylated VASP (P-VASP) in resistance arteries was detected by immunohistochemical staining.

Results: In the present study, endothelium dependent dilation in aging resistance arteries was lower than younger ones (young vs. aging: 68.0±4.5% vs. 50.4±2.9%; P<0.01). And the endothelium independent dilation remained constant. Nitrative stress was increased in aging resistance arteries, evidenced by elevated NOx level in serum from aging rats (nmol/ml; young vs. aging: 3.3±1.4 vs. 5.3±1.0; P<0.05) and increased NT level (P<0.05). ONOO– was responsible for the vasorelaxation dysfunction evidenced by vasorelaxation was restored from after inhibition of ONOO– on its sources (P<0.05) and suppressed NT expression after FeTMPyP treatment (P<0.05). The expression of sGC was not significantly different between young and aging resistance arteries, but the P-VASP/VASP ratio (biochemical marker of NO-sGC-cGKs) signaling decreased, which was reversed by FeTMPyP treatment in vivo (P<0.05).

Conclusions: The present study suggested that ONOO– is responsible for the decline of endothelial dependent vasorelaxation in aging resistance arteries, and this effect likely involves the dysfunction of the NO-sG-cGKs pathway.

G2W5-e1453

Silk fibroin/chitosan nanofibers based adipose tissue-derived mesenchymal stem cell patches prevent myocardial remodeling after myocardial infarction in rat

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Objectives: To fabricate novel biocompatible silk fibroin/chitosan (SF/CS) nanofibers seeded with adipose tissue-derived mesenchymal stem cells (AD-MSCs) for cardiac tissue regeneration.

Methods: Silk fibroin with good elasticity and chitosan as hydrophil were assembled onto the cellulose electrospun mat via layer-by-layer method. The micro-structure of the scaffold was characterized by X-ray photoelectron spectroscopy (XPS) and scanning electron microscope (SEM). AD-MSCs were isolated from Flac-EGFP transgenic mice constitutively expressing both firefly luciferase (Fluc) and enhanced green fluorescent protein (EGFP). Rat model of acute myocardial infarction (AMI) was induced by ligation of the left anterior descending coronary artery. The SF/CS nanofibrous scaffolds (patches that 9x9mm2) seeded with or without ADMSCs (seeding density: 2x10^5 cells/patch) were adhered onto the epicardium of the injured area. Whole type Sprague Dawley rats (male, 120-140g) were randomized into four groups (n=10): Sham group, MI, MI/SF/CS group and MI/ADMSC/CSF/CS patches respectively. Three days post-operation, cytokolate cytomodocytes apoptosis and the paracrine factors in the peri-infarct area was determined by TUNEL staining and by ELISA assay respectively. The viability of engrafted AD-MSCs was tracked using longitudinal bioluminescence imaging (Luciferase) and cardiac function was measured by transthoracic echocardiography (TTE) 1, 7, 14 and 28 days post-operation. Four weeks after AMI operation, H&E, Masson’s Trichrome, Tropinin I, CD68 and CD31 immunofluorescence stainings were performed to evaluate myocardium fibrosis, tissue regeneration, neovascularization, and inflammatory reaction.

Results: BLI showed AD-MSCs were detectable until four weeks after transplantation. By TTE, ADMSC/CSF patches improved left ventricular ejection fraction (LVEF) (sham: 73.1±2.2%, MI: 24.3±5%, MI/ADMSC/CSF: 33.4±4%; n=10 per group; P<0.05, four weeks post-operation). These results were in line with the implantations of SF/CS/ADMSC/SCF/CS patches respectively. Three days post-operation, cytokolate cytomodocytes apoptosis and the paracrine factors (P<0.05). Four weeks after operation, the patches in both groups were intact anchored on the MI areas with minor inflammatory as compared with MI group (P<0.05), while angiogenesis was improved (P<0.05) and fibrosis size was reduced (sham: 0, MI: 23.3±4%, MI/ADMSC/CSF: 15.2±3%, MI/ADMSC/SCF: 9±3%; n=10 per group; P<0.05). Furthermore, EGFP+ cardiomyocytes (cTnI-positive) and endothelial cells (CD31-positive) could be identified in MI/ADMSC/CSF group.

Conclusions: This study demonstrated that the SF/CS nanofibers provide three-dimensional microenvironments and scaffold to support the retention and viability of engrafted AD-MSCs, synergistically promote the therapeutic efficacy to alleviate cardiac fibrosis, attenuate ventricular remodeling and induce angiogenesis and cardiomyocyte regeneration.
Conclusions: These results suggested an impact of Western blot analysis showed that metoprolol restored expression of AT2R in SHR, apoptosis by inhibiting oxidative stress.

GW25-e4186
Dipeptidyl peptidase (DPP) -4 inhibitor exhibits anti-apoptosis effects in isoproterenol-induced myocardial infarcted rats by inhibiting oxidative stress
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Objectives: Cardiac apoptosis plays an important role in the pathology of myocardial infarction. The protective effects of Dipeptidyl peptidase (DPP) -4 inhibitor (vildagliptin) on cardiac apoptosis were evaluated in isoproterenol induced myocardial infarcted rats.

Methods: Male Wistar rats are treated intravenously with Vildagliptin (2 mg/kg/day) daily for a period of 21 days. After 21 days of pretreatment, isoproterenol (100 mg/kg) was injected subcutaneously to rats at an interval of 24 h for 2 days (on 22th and 23th day) to induce myocardial infarction. Cardiac diagnostic markers, heart lipid peroxidation, antioxidant system, histopathological changes of the heart and apoptosis were evaluated in isoproterenol induced myocardial infarcted rats.

Results: Isoproterenol induced myocardial infarcted rats showed a significant increase in the levels of serum cardiac diagnostic markers, heart lipid peroxidation products and a significant decrease in the activity/levels of heart antioxidants, compared with normal rats. Additionally, Histopathological findings of myocardial infarcted rats revealed marked necrosis of myocardial tissue. Polymerase Chain Reaction study revealed an increase in the myocardial expression of Bax, caspase-8, caspase-9 and Fas genes and a decrease in the myocardial expression of Bcl-2 and Bcl-xL genes.Vildagliptin (2 mg/kg/day) pre-treatment decreased the levels of serum cardiac marker enzymes, reduced heart lipid peroxidation and minimized the alterations the activities/levels of heart antioxidants of isoproterenol-induced myocardial infarcted rats. Histopathological study evidenced that the pretreatment with Vildagliptin inhibited myocardial damage. Vildagliptin pre-treatment also showed protective effects on apoptosis. In vitro study also revealed the free radical scavenging and anti-apoptosis activity of Vildagliptin.

Conclusions: Thus, Vildagliptin protected the myocardial infarcted rat’s heart against apoptosis by inhibiting oxidative stress.

GW25-e4228
Effect of metoprolol on expression and vasodilatation function of AT2R in SHR
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Objectives: The interactional relationship between the sympathetic nervous systems (SNS) and the renin-angiotensienaldosterone system (RAS) has been revealed but poorly investigated. The present work was designed to explore the effect of metoprolol (MET) treatment on the RAS, especially the expression and vasomotor function of AT2R, in spontaneously hypertensive rats (SHR).

Methods: SHR was treated with metoprolol for 4 weeks. The dynamic changes of the blood pressure, renin activity, Ang II concentration and AT2R expression were investigated. Supernumerary resistance arteries were isolated for determining the protein expression of AT1R and AT2R. Perfusion experiment was carried out to explore the vasomotor function of AT2R.

Results: The results showed that up-regulated renin activity and Ang II concentration of plasma in SHR were inhibited by metoprolol treatment. In isolated superior mesenteric arteries from both WKY and SHR, Ang II perfusion induced vasodilatation after AT1R inhibition by telmisartan, although the vasodilatation was harmed in SHR. Furthermore, AT2R inhibitor PD123319 arrested the vasodilatation induced by Ang II. SHR received metoprolol exerted improved vasodilatation mediated by AT2R (p<0.05 vs. 1% MET and SHR, respectively, P<0.05). Western blot analysis showed that metoprolol restored expression of AT2R in SHR, which may contribute to metoprolol’s antihypertensive effect.

Conclusions: These results suggested an impact of β-adrenergic blocker on RAS and supported an important role of AT2R in antihypertensive treatment.

GW25-e4266
Action and Mechanism of Secreted Frizzled-Related Protein 5 in Cardiomyocyte Hypertrophy
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Objectives: Secreted frizzled-related protein 5 (Sfrp5) has been described as novel adipokine with anti-inflammatory properties at present. However, it’s expression and protection mechanisms in the cardiomyocyte hypertrophy at the cellular level are not thoroughly understood. Here, the authors report for the first time by means of angiogenin II induced cardiomyocyte hypertrophy in vitro that the expression and mechanisms of Sfrp5.

Methods: Cardiomyocytes were preincubated with Ang II through primary culture of cardiac cells of neonatal SD rats. Telmisartan and PD123319 were used to block Ang II type-1 and type-1 receptors respectively. Rho proteins have been suggested as major contributors to cardiac hypertrophy. Here, inhibition of the Rho/ROCK pathway by Y-27632, a selective inhibitor of ROCK, we determined the hypothesis that Rho and Rho-associated kinase (Rho/ROCK) as mediators of the expression of β1AR in Ang II (Ang II) -induced cardiomyocyte hypertrophy. Using transcriptional polymerase chain reaction and Western blot were used to estimate the expression of β1AR, brain natriuretic peptide (BNP) and phosphorylation of MYPT-1.

Results: β1AR was discovered expressed in cardiomyocytes. Ang II treatment induced an increased expression of β1AR and BNP in a dose- and time-dependent manner in cardiomyocytes, after preincubating with AT1-R antagonist telmisartan completely blocked Ang II-induced β1AR expression increment. The recession of phosphorylation of MYPT-1 (1-β1AR) and β1AR were expressed after inhibiting of ROCK by Y-27632.

Conclusions: The increased β1AR expression was mainly through the AT1-R-Rho/ ROCK signaling pathway in the process of Ang IInduced cardiomyocyte hypertrophy.