Basic and Translational Medicine

Basic Research of Cardiovascular Disease

GW25-e1527
Cardioprotective Effects of Angiotensin-Converting Enzyme 2 in Apolipoprotein E-Deficient Mice in Response to Angiotensin II
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Objectives: Inhibition of the renin-angiotensin system (RAS) has lead to important pharmacological tools to treat atherosclerosis and other cardiovascular diseases. Angiotensin-converting enzyme 2 (ACE2) has emerged as a central negative regulator of RAS by degrading angiotensin (Ang) II to generate Ang-(1-7). We hypothesized that ACE2 would exert beneficial effects on myocardial injury in apolipoprotein E knockout (ApoEKO; ApoE−/−) mice.

Methods: In this work, we used 3-month-old wild-type, ApoEKO, and ApoE double-KO mice. We implanted subcutaneously mini-osmotic pumps with Ang II for cardiac disorders. Conversely, ACE2 overexpression alleviates Ang II-mediated in myocardial ultrastructure injury. Notably, treatment with hrACE2 strikingly alleviated oxidative stress and attenuated ultrastructure injuries in hearts of ApoE−/− mice with increased cardiac NADPH oxidase activity and elevated circulating Ang II levels. Notably, treatment with hrACE2 strikingly alleviated oxidative stress and attenuated ultrastructure injury in the hearts of Ang II-treated ApoE−/− mice linked with reduction of NADPH oxidase activity and abolishment of expression of CTGF, ERK1/2, CCL2, FKN, IL1β, IL17, and ICAM1 in the ApoE−/− mice with activation of the CTGF and ERK signaling (n=5-6; P=0.05 or P<0.01, respectively). Consistent with induction of inflammation, Ang II infusion led to elevation of superoxide production and severe ultrastructure injury in hearts of the ApoE−/− mice with increased cardiac NADPH oxidase activity and elevated circulating Ang II levels. Notably, treatment with hrACE2 strikingly alleviated oxidative stress and attenuated ultrastructure injury in the hearts of Ang II-treated ApoE−/− mice linked with reduction of NADPH oxidase activity and abolishment of expression of CTGF, P-ERK1/2, CCL2, FKN, IL1β, IL17, and ICAM1 in the ApoE−/− mice with activation of the CTGF and ERK signaling (n=5-6; P=0.05 or P<0.01, respectively). However, there were no changes in p38-MAPK and CXCR1 levels among groups (n=4-6; P=0.05; respectively).

Conclusions: Absence of ACE2 triggers greater increases in myocardial inflammation and oxidative stress in the ACE2/ApoE double-mutant mice with exacerbation of myocardial ultrastructure injury. Conversely, ACE2 overexpression alleviates Ang II-mediated inflammation and ultrastructure injury in hearts of the ApoE-deficient mice with suppression of superoxide generation and the CTGF-ERK signaling activation. Strategies aimed at enhancing ACE2 action may have important therapeutic potential for cardiac disorders.

GW25-e2289
Different Doses of Angiotensin Receptor Blocker Therapy for Early Diastolic Heart Failure on Myocardial Diastolic Stiffness: A Preliminary Diabetic Animal Study
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Objectives: Diastolic heart failure (DHF) patients had higher left ventricular (LV) stiffness, optimal therapy for DHF has not been determined. The efficiency of the angiotensin receptor blocker (ARB) on DHF treatment has been controversy. Accordingly, this preliminary study was to investigate the effect of the different doses of candesartan on the early DHF to prevent passive LV stiffness in diabetic rabbits.

Methods: Sixty rabbits were randomly divided into 4 groups (n=15 in each group). Group 1 (G1) and Group 2 (G2) were assigned to the following candesartan cetoxil (G1: 1.15 mg/kg/day) and candesartan (G2: 2.3 mg/kg/day) treatment for 4 days. Group 3 (G3) was the control group, while the fourth group (G4) received no treatment. The other rabbits were assigned to the diabetic group (GC, no medicine treatment of alloxan injection) and control group (GC, normal rabbits). LV function was measured by echocardiography. LV passive stiffness was evaluated by cardiac morphology, myocardial deposition of collagen and advanced glycation end products (AGEs) through electron microscopy, He staining method. Immunohistochemical method, and Quantitative histomorphometry. Cardiac total titin and titin-N2B/N2BA ratio were measured by gel electrophoresis, and total titin mRNA level expression was assessed by Quantitative real-time PCR.

Results: All the diabetic rabbits were identified that LV diastolic dysfunction with preserved systolic function measured by echocardiography through the different stages of cardiac dysfunction (angiography stage), normalized percentage (%): G1, 46.1±3.05; G2, 48.8±3.04; GD, 40.3±2.75; P<0.05; GC, 50.22±2.99; P<0.05). Immunohistochemistry CML scoring (score/mm2) (G1, 29.5±4.4; 22, 24.8±2.97; GD, 40.8±3.01 vs. GC, 15.59±1.88; P<0.05) and the expression of titin mRNA (G1, 4.5±0.05; G2, 40.5±0.3; GC, 3.5±0.58 vs. GC, 1±0.2; P<0.05) were differ significantly between G1 and G2 (P=0.05), however, differ significantly among these four groups, but there were not differ significantly between ARB treatment groups and diabetic group in the Immunohistochemistry CML scoring. In all diabetic rabbits, there was not investigated of interstitial fibrosis by Masson's trichrome stain. Total titin amount and N2B/N2BA ratio by method of Quantitative protein analysis were no differences between all the groups.

Conclusions: ARB therapy which is independent of its dosage tailored reducing the deleterious effects of the CML-AGEs deposited in diabetic cardiomyopathy of early stage was being experiment and providing evidence of possible underlying mechanisms of DHF.

GW25-e2291
A Novel Haplotype in the GOSR2 Gene is Associated With Coronary Artery Disease in Chinese Han population
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Objectives: The Golgi SNAP Receptor Complex Member 2 (GOSR2) gene is Golgi-associated soluble factor attachment receptor (SNARE) protein. Some SNPs in GOSR2 gene are found to be associated with coronary artery disease (CAD) and myocardial infarction (MI). The aim of the present study was to detect the association between the human GOSR2 gene and coronary artery disease using a haplotype-based case-control study in Chinese Han population.

Methods: A total of 283 CAD patients and 280 controls were genotyped for the five single-nucleotide polymorphisms (SNPs) used as genetic markers for the human GOSR2 gene (rs197932, rs3785889, rs197922, rs17608766, and rs16941382). Data were analyzed for three separate groups: the total subjects, men, and women.

Results: In the recessive model of rs197932 in men, there was statistical difference in CC genotype and TT+CT genotypes distribution between CAD and control group. In women, the overall distribution of the haplotypes established by rs3785889, rs197922, rs17608766, and rs16941382 were significantly different between the CAD patients and the control subjects. For the total subjects, the frequency of the G-T haplotype established by rs3785889/rs16941382 was significantly higher for the CAD patients as compared to the control subjects (P=0.009). Multiple logistic regression analysis also revealed that the people with G-T haplotype established by rs3785889/rs16941382 (homozogote) were found to have significantly higher chance of suffering from CAD than the ones without this haplotype after adjustment for the major risk factors (OR=1.87, 95% CI: 1.16-3.02).

Conclusions: The results of this study indicate that the G-T haplotype established by rs3785889/rs16941382 may be a useful genetic marker for CAD patients in Chinese Han population.

GW25-e2292
The effects of Angiotensin-1(-7) (Mas/PJ3K/Akt/NO axis in acute atrial tachycardia canine model and the possible role of atrial natriuretic peptide
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Objectives: To investigate the effects of Angiotensin-1(-7) [Ang(1-7)] signaling pathway on atrial electrical remodeling in rapid atrial pacing canine model and the possible role of atrial natriuretic peptide (ANP).

Methods: Thirty-five dogs were assigned to seven groups randomly. There are sham group, paced control group, paced + Ang-(1-7) group, paced + Ang-(1-7) + A-779 group, paced + Ang-(1-7) + API-2 group, paced + Ang-(1-7) + wortmannin group, and paced + Ang-(1-7) + L-NAME group. 5 dogs in each group. Rapid atrial pacing at 600 bpm was maintained for 2 hours except the animals from the sham group. During the pacing, Ang-(1-7) (6ig kg−1 h−1), A-779 (Mas inhibitor, 5.83 μg kg−1 h−1), API-2 (Akt inhibitor, 2.14 μg kg−1 h−1), wortmannin (PI3K inhibitor, 2.86ig kg−1 h−1) or L-NAME (NO synthase inhibitor, 180ig kg−1 h−1) were given intravenously, respectively. Electrophysiological parameters including atrial effective refractory periods (ERPs), inducibility and duration of atrial fibrillation (AF) under different basic pacing cycle length (300, 250, and 200 ms) were measured. Following electrophysiological study, ANP concentration of the left atrium was detected.

Results: Atrial ERP, AF inducibility and duration were altered after 2 hours, the atrial ERP of 6 sites were shortened with the increase in inducibility and duration of AF in paced control group (P<0.05, vs sham). ANP concentration of the left atrium also increased in paced control group (P<0.05, vs sham). The shortening of atrial ERPs, and the increasing of inducibility and duration of AF induced by atrial pacing were attenuated by Ang-(1-7) treatment.
The increasing of left atrial ANP concentration was promoted by Ang-(1-7). A-779, Ang-(1-7) did not affect NADE. NME treatment blocked the effects of Ang-(1-7) on electrophysiological parameters and ANP secretion.

**Conclusions:** Ang-(1-7) prevented the acute electrical remodeling in rapid atrial pacing canine model via Ang-(1-7)/Mas/PI3K/Akt/NO signaling pathway. Furthermore, ANP was related to the anti-arrhythmic effects of Ang-(1-7) and may play a potential role in Ang-(1-7)/Mas/PI3K/Akt/NO signaling pathway.

**GW25215**

Sodium chemerin levels and risk of coronary atherosclerosis in early-onset coronary artery disease of Chinese population

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**Objectives:** Increasing evidence indicates that adipokines may play an important role in the pathogenesis of coronary artery disease (CAD). However, it remains uncertain whether chemerin, a novel adipokine, is associated with coronary atherosclerosis and could predict for CAD. In the present study, we sought to determine: 1) the relationship between chemerin and the severity of coronary atherosclerosis, and 2) whether chemerin is an independent risk factor for CAD in Chinese population.

**Methods:** 382 early-onset CAD (EOCAD) patients and 305 matched controls undergoing coronary angiography were included into this study. Their serum levels of chemerin were measured by enzyme-linked immunosorbent assay (ELISA). The severity of coronary stenosis was evaluated by Gensini score. Chemerin was the independent risk factor for CAD (OR=1.218; 95% CI, 1.197-1.340; P<0.005) after adjusting for conventional cardiovascular risk factors.ROC analysis indicated that chemerin had greater area than adiponectin with no significant difference (P=0.05).

**Conclusions:** Chemerin is negatively associated with the severity of coronary atherosclerosis, as well as an independent risk factor for CAD. It may be a promising target for further elucidating the role of metabolic factors in pathogenesis of CAD.

**GW254350**

The Possibility of Rapid Assessment of Cardiac Contractility Using Electromyography

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**Objectives:** Myocardial contractility can describe the function of the heart and is an effective indicator to evaluate whether the heart is health or not. The most effective way to measure the contractility is using the catheter. There also exists a noninvasive method, the time interval in which the contraction takes place (time to peak filling). The volumetric analysis by echocardiogram (BCG) records the gravity variation caused by the reaction force during the ejection of blood in heart which highly represents the mechanical activity of heart. In this paper, we proved the hypothesis that the time interval between the J wave of BCG and the R wave of electrocardiogram (ECG) which is referred as RJ interval is correlated to PEP.

**Methods:** Four healthy male subjects and one male subject with premature ventricular beats participated in our study. The Doppler echocardiogram (ECHO), ECG and BCG were recorded synchronously. The PEP is the time interval between the Q wave of ECG and the beginning of ventricular filling which is a bright line in ECHO. The BCG measurement system is self-developed by the Institute of Medical Physics and Engineering of Tsinghua University, in which the BCG sensor is imbedded on a wheelchair. It can record the BCG and ECG signals simultaneously. Finally the RJ intervals were measured by the corresponding squared RR intervals, which were referred as RR interval correction, and its corre- lation with PEP was also investigated.

**Results:** In our study, across all the five subjects, 1974 beats were correlated. The correlation coefficient between PEP and RJ intervals was 0.79. As for the best linear fitting, the slope was 1.12, and the intercept was 99.56. The correlation coefficient r between PEP and the corrected RJ interval was improved to 0.88. It is summarized the correction coefficient of correlation of original data and corrected data. In consideration of all the RJ intervals and PEP, the minimum of correlation coefficient was 0.68 and the maximum was 0.99, except the subject with premature ventricular beats. After correction, the minimum became 0.88, and the maximum became 0.98. As for the subject who had premature ventricular beat, the correlation coefficient of RJ interval and PEP was 0.63, which was much lower than the average level of other normal subjects. But after RJ interval correction, it was improved to 0.88.

**Conclusions:** The results support the hypothesis that the PEP is correlated to the RJ. Consequently, to some extent-at least for healthy people, the corrected RJ intervals can be suggested as a better indicator for assessment of myocardial contractility. However, the validity of this correction for those whose cardiac function is abnormal should be studied in the future. In our study, BCG, objectively the corrected RJ interval, can be suggested as an effective method for assessment of myocardial contractility. Moreover, the BCG measurement system allows family health care and long-term monitoring of changes in cardiac contractility.

**GW254345**

Prenatal nicotine exposure induces gender-related left ventricular uncoupling in adult offspring

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**Objectives:** Changes in vascular and myocardial structure and function have been demonstrated in prenatal nicotine exposure offspring, but limited data are available on how they changes interact in maturity, with our study, BCG, objectively the corrected RJ interval, can be suggested as an effective method for assessment of myocardial contractility. Moreover, the BCG measurement system allows family health care and long-term monitoring of changes in cardiac contractility.

**GW254355**

Prevention of Angiotensin II-induced Cardiomyopathy by Sulforaphane-activated Nrf2 Partially via AKT/GSK-3β/Fyn Pathway

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**Objectives:** Angiotensin II (Ang II) is an important causative of diabetic cardiomyopathy. In the diabetes, hyperglycemia induces the generation of Ang II in the heart. Mechanistically the pathogenesis of cardiac Ang II in the development of diabetic cardiomyopathy was considered its induction of nitrosative and oxidative stress via Ang II interaction with its receptor. Recently, oxidative stress modulation of superoxide and peroxynitrite. Nuclear factor-related factor 2 (Nrf2) is a master regulator of cellular detoxification responses and redox status. Sulforaphane (SFN) is an anti-oxidative supplement via up-regulating Nrf2-mediated multiple endogenous antioxidant enzymes. Therefore, the present study examined whether SFN could protect from Ang II-induced cardiomyopathy and the underlying mechanism.

**Methods:** FVB mice were given subcutaneous injection of Ang II (0.5 mg/kg) for 2 months with or without SFN treatment (0.5 mg/kg) for 3 months and then kept until 6 months. At 3 and 6 months, blood pressure and cardiac function were measured. Cardiac fibrosis, inflammation, and oxidative damage were detected by Western blotting, real-time PCR, and immunohistochemical staining. Furthermore, cardiac overexpressing Nrf2 gene (Nrf2-TG) and wild-type (WT) mice were treated with Ang II (0.5 mg/kg) for 2 months to confirm the critical role of cardiac Nrf2 on prevention of Ang II-induced cardiomyopathy. In vitro, H9c2 cells were treated with SFN and Ang II with or without Nrf2 siRNA or Akt inhibitor (LY294002) to detect the mechanism of SFN’s protective effect.

**Results:** SFN significantly prevented Ang II-induced high blood pressure at 6 months and cardiac dysfunction at both 3 and 6 months. Ang II caused remarkable pathological changes, including myocardial hypertrophy and collagen accumulation, along with increases in cardiac oxidative damage (3-NT and 4-HNE), inflammation (TNF-α and PAI-1), and fibrotic response (TGFB-1 and CTGF). Those damages were almost significantly reduced by Nrf2 overexpression, which was protected from Ang II-mediated oxidative damage reflected by increased Nrf2 phosphorylation and downstream antioxidants. To determine the direct role of SFN-activated Nrf2 in preventing Ang II-induced cardiomyopathy, in vitro H9c2 cells were treated with Ang II in the absence or presence of Nrf2 siRNA to