the left and right coronary sinus. Along the wire, the rabbit coronary was isolated completely.
Results: Without the aid of a microscope, this separation method isolated rabbit coronary artery accurately.
Conclusions: This method provides a better way for the separation of coronary arteries. And it won't influence the results of further pathological observation.

GW25-e5261

Myocardial infarction accelerates the activation of systemic and local cellular immunity in STZ-induced type 1 diabetic rats

Dong Zhifeng, Penglong Wu, Wei Zhu, Meng Wei
Department of Cardiology, Shanghai Sixth Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200233, China

Objectives: Clinically, diabetes is very common in patients hospitalized for acute myocardial infarction (AMI). It is a strong and independent co-morbidity of all-cause mortality and readmission for post-myocardial infarction chronic heart failure (CHF). The central role for monocyte subset accumulation in the heart following AMI and the role of the spleen as monocyte reservoir were all recently demonstrated. However, whether the associated celluar immunity mechanism was involved in AMI with diabetes was unknown.
Methods: We performed the comparison in four separate groups: 1) rats with sham surgically induced myocardial infarction (Ctr, n=10); 2) rats with surgically induced myocardial infarction (MI, n=10); 3) STZ-induced type 1 diabetic rats (DB, n=10); 4) STZ-induced type 1 diabetic rats with surgically induced myocardial infarction (DB+MI, n=10). The parameters of cellular immunity in the heart, spleen and blood were evaluated by flow cytometry and immunohistochemistry etc. In addition, cardiac remodeling and function was also evaluated.
Results: Twelve weeks after the operation, compared with DB or MI rats, DB+MI rats exhibited the following: 1) significantly increased cardiac enlargement, fibrosis and deteriorated cardiac function; 2) significantly increased infiltration of CD_{4+} T cells and the expression of IFN-gamma, IL-17 and IL-4 in heart. 3) significantly increased proportion of CD_{4+} T cells and producing-IFN-γ, IL-17 and IL-4 CD_{4+} T cells and a decreased Treg/Th17 ratio in spleen; 4) significantly increased the proportion of producing IFN-gamma, IL-17 and IL-4 CD_{4+} T cells and Treg in blood. However the circulating immune complexes (CIC) and IgG did not showed the difference between them.
Conclusions: In this study, MI significantly accelerated cardiac infiltration of CD_{4+} T cell and the spleen and serum activation of CD_{4+} T cell especially its inflammation associated subgroup in STZ-induced type 1 diabetic rats. Systemtic and local celluar immunity probably involved in the post-MI CHF progression in diabetes.

GW25-e5274

Lycopene protects endoplasmic reticulum stress-induced apoptosis against neonatal mouse cardiomyocytes hypoxia/reoxygenation injury

Xu Jiqian[1,2], Chen Bin[2], Zhou Zhou[3], Xu Lei[1], Zhang Shuang[1], Yu Zhengping[3], Hu Houxiang[1]
[1]Department of Cardiology, North Sichuan Medical College First Affiliated Hospital, Nanchong, Sichuan, China, [2]Department of Anesthesiology, North Sichuan Medical College First Affiliated Hospital, Nanchong, Sichuan, China, [3]Department of Occupational Health, Third Military Medical University, Chongqing, China

Objectives: Endoplasmic reticulum (ER) stress induced apoptosis has been implicated as a critical cause in the pathogenesis of myocardial ischemia reperfusion (I/R) injury. Our previous studies demonstrated that lycopene exhibits great pharmacological potential in protecting against the I/R-injury, but whether its effect is mediated through attenuation of ER stress-induced apoptosis remains unclear. The aim of this study was to investigate the effect of lycopene on hypoxia/reoxgenation (H/R) induced ER stress in primary cultured neonatal mouse cardiomyocytes.
Methods: Primary cardiomyocytes were isolated from neonatal C57BL/6 mice and divided into four groups: control, lycopene, H/R, lycopene + H/R. The cultured cardiomyocytes underwent 4h of hypoxia followed by 6h of reoxgenation to achieve H/R model. Cardiomyocytes were pretreated with lycopene (5 μM) prior to H/R treatment in lycopene + H/R. Cell viability was assessed using CCK-8 assay in each group. AnnexinV-FITC/PI assay was used to evaluate cardiomyocytes apoptosis in the different treatment groups. The expression of GRP78, a widely used marker of endoplasmic reticulum stress, was measured via western blot. The expression of ER-related apoptotic maker of CHOP/GADD153 and caspase-12 was measured by real-time PCR.
Results: Our results demonstrate that the cell viability significantly decreased to 66.30±4.84% of the control levels following H/R, the cell viability markedly improved in lycopene + H/R ($P<0.01$). The results from flow cytometer with Annexin V and PI double-staining illustrated that after exposure to H/R, apoptotic percentage significantly increased to 26.42±2.71% ($P<0.01$), while that of control and lycopene group were 4.96±1.51% and 4.69±1.42%, respectively. In contrast, lycopene + H/R markedly prevented the H/R-induced apoptosis (16.38±2.12%, $P<0.01$). Compared to control and lycopene, the expression of GRP78 protein increased more than two-fold in H/R treatment ($P<0.01$), while the expression of GRP78 protein only increased to 1.46-fold in lycopene + H/R ($P<0.01$). In addition, H/R treatment evoked a significant increase in GADD153/CHOP mRNA compared to control groups ($P<0.01$). However, the GADD153/CHOP mRNA expression was markedly down-regulated to 1.68-fold of control levels with lycopene pretreatment ($P<0.01$). Furthermore, the caspase-12 mRNA expression was also significantly increased in H/R treatment (1.82 folds of control group, $P<0.05$). However, pretreatment with lycopene efficiently reduced caspase-12 mRNA expression caused by H/R treatment.
Conclusions: These findings reveal that lycopene protects against H/R injury by attenuation of ER stress and ER stress induced apoptosis in primary cultured neonatal mouse cardiomyocytes; the protective effect of lycopene on cardiomyocytes highlights the therapeutic potential of plant-derived antioxidants against I/R-injury.

GW25-e5288

Non-antiplatelet effect of Clopidogrel: Improving endothelial function in Chinese healthy subjects with different CYP2C19 genotype

Yinzhuang Zhang, Chen Bilian
Xiangya Hospital, Central South University

Objectives: Clopidogrel is demonstated to improve endothelial function in vitro and in patients with coronary artery disease (CAD). But it remains unclear whether this effect of clopidogrel is associated with *CYP2C19* polymorphisms which determing antiplatelet effect of clopidogrel.
Methods: After genotyping, 12 healthy subjects were enrolled in our study. Among them, 6 subjects were *CYP2C19**1/*1 (extensive metabolisers, EMs) and the other 6 subjects were *CYP2C19**2/*2or*3 (poor metabolisers, PMs). All subjects received 300mg clopidogel orally. Endothelial function was assessed by measurement of flow-mediated dilation (FMD) of the brachial artery and ADP-induced platelet aggregation was determined using optical aggregometry before and 4h, 24 h after administration of 300mg clopidogrel.
Results: FMD was significantly higher at 4h and 24h after a loading-dose administration of clopidogrel in both CYP2C19 EMs and PMs groups, which showed no significant difference between the two groups. ADP-induced platelet aggregation was greatly inhibited at 4h and 24h after administration of clopidogrel in CYP2C19 EM group. However, there was no statistical correlation between the change in FMD and ADP-induced platelet aggregation in the two CYP2C19 groups.
Conclusions: It is the first time to report that clopidogrel improves endothelial function in healthy Chinese subjects, which is irrelated with *CYP2C19* genotype and independent of antiplatelet action.

GW25-e0839

Incidence of Acute Mountain Sickness in Young Adults at 3200 Meters-Comparison of the Lake Louise Scoring and Chinese Scoring Systems

Chenguo Zhu, Haunglan
Cardiovascular Department of Xinqiao Hospital, Third Military Medical University

Objectives: The purpose of this study was to compare two scoring systems used for diagnosis of acute mountain sickness (AMS) : Lake Louise Scoring (AMS-LLS) and Chinese Scoring System (AMS-CSS).
Methods: 339 healthy young adult volunteers, resided at sea level (mean ± SD: age 24.59 ± 3.27 years; height 173.93 ± 5.18 cm; weight 68.21 ± 7.79 kg), ascended to 3200 m by train and bus, a total journey time of 48 hours, all the persons were ascend as same way, and were divided into three groups. Group 1 (n = 88), group 2 (n = 91) and group 3 (n = 160) were assessed after one, two and three nights, respectively, at altitude.
Results: The overall incidence of AMS was 17.11% (n = 58) and 29.79% (n = 101) according to AMS-LLS and AMS-CSS, respectively. Two participants (0.59%) experienced high altitude pulmonary edema. Both scoring systems showed the highest incidence of AMS after the second night at high altitude. There was a good correlation between AMS-CSS and AMS-LLS scores (Pearson = 0.820, P< 0.001). AMS-CSS identified all AMS subjects diagnosed by AMS-LLS, plus an additional 43 missed by AMS-LLS. The dominant symptoms were reduced exercise tolerance (61.7%), fatigue (49.05%), dizziness (28.9%), chest distress (28.3%) and headache (27.4%). Compared with AMS-LLS, the sensitivity, specificity, and positive and negative predictive values of AMS-CSS were 100%, 84.7%, 57.43% and 100%, respectively. There was no relationship between oxygen saturation (SpO_2) levels and AMS scores at 3200 m.
Conclusions: AMS-CSS is similar, but a little different details, with AMS-LLS. AMS positive diagnosis outnumbers the LLS standard, but there might be a false positive. Headache was not the dominant symptom at 3200 m high altitude in this study, and SpO2 levels did not correlate with AMS scores.

GW25-e1071

Interleukin-1 beta overexpression in hypothalamic paraventricular nucleus deteriorates heart failure

Liu Qiang, Wang Tao, Yu Huapeng, Liu Bin, Jia Ruyi
Department of Cardiology. The Fourth People's Hospital of Jinan, Medical School, Tai Shan Medical College

Objectives: To investigate whether interaction between interleukin-1β and angiotension II receptor 1 in the PVN contributes to progression of HF.
Methods: Rats were implanted with bilateral paraventricular nucleus (PVN) cannulae and subjected to coronary artery ligation or sham surgery (Sham). Subsequently, animals were treated for 4 weeks through PVN infusion with either vehicle, losartan (LOS, 200ug/d), IL-1β (IL, 1ug/d) or IL-1β together with losartan (LOS+IL).

Results: HF rats had higher levels of corticotropin-releasing hormone (CRH), norepinephrine (NE) and glutamate (Glu), lower levels of gamma-aminobutyric acid (GABA), and more positive fra-like activity in PVN when compared to Sham rats. HF rats also had higher level of NE, epinephrine (EPI) and IL-1β in plasma. PVN infusion of LOS attenuated the decreases in GABA and the increases in CRH, NE and Glu in the PVN of HF rats. PVN infusions of IL could further increase expression of CRH, NE, Glu, EPI and IL-1β and decrease GABA expression. Treatment with IL-1β together with losartan could eliminate the effects of IL-1β.
Conclusions: Interaction between AT1-R and IL-1β in the PVN contributes to deterioration of heart failure.

GW25-e1659

Nicotine induce mast cells degranulation to promotethe atherogenesis and reduce the atherosclerotic plaque stability

Chen Wang, Jian-an Wang
Cardiovascular Key Lab of Zhejiang Province, the Second Affiliated Hospital, School of Medicine, Zhejiang University

Objectives: Nicotine has been identified to promote atherosclerosis. But the mechanism of nicotine induced atherogenesis has not been well elucidated. Mast cell plays an important role in high-fat diet induced atherogenesis. This study focus on the role of mast cell in nicotine induced atherogenesis and plaque instability.
Methods: Peritoneal administration of 100mM disodium cromoglicate (DSCG) was introduced to inhibit mast cell degranulation. 45 ApoE deficient mice were divided into 3 groups: high-fat diet, high-fat diet + nicotine, and high-fat diet + nicotine + DSCG (n = 15 each). After 12 weeks of treatments, atherosclerotic lesion size of the aortas were quantified. Toluidine blue and tryptase staining identified mast cell count and activation at the lesion. Immuno-staining of CD68, CD45 were used to evaluate the inflammatory filtration.SMA, Ki-67 and sirus red staining were used to study smooth muscle cell proliferation and collagen content in the lesion. In vitro, bone marrow-derived mast cells (BMMCs) were harvested and divided into 5 groups, which PBS as a negative control, compound 48/80 as a positive control, 100μg/ml nicotine, 100μg/ml nicotine with 100mM DSCG pretreatment and nicotine 100μg/ml with 10μg/ml mecamylamine pretreatment. At 0.5hr, 1hr, 2hrs, supernatants were harvested to analyze the mast cell degranulation. Futhermore, conditioned medium were also used to induce the macrophage migration and foam cell formation.
Results: Nicotine increases plaque size, and macrophage infiltration, decreases smooth muscle collagen content along with the increases in mast cells count and activation ratio at the lesion, which could be inhibited by DSCG.Nicotine induced mast cell degranulation at 2 hours comparing to PBS (43.60% vs 2.3%), which could be inhibited by mast cell stablizer DSCG (23.7%) and nAChR blocker mecamylamine (20.35%).Macrophage migration ability in the compound 48/80 and nicotine conditional medium group were significantly higher comparing to PBS, DSCG and mecamylamine group. Foam cell formation ratio in the compound 48/80 and nicotine conditional group were significantly higher comparing to PBS, DSCG and mecamylamine group.
Conclusions: Nicotine might induce mast cell degranulation through nAChR and then activate mast cell to release a range of proinflammatory mediators to increase the migration ability of macrophages as well as the foam cell formation and destabilize the atherosclerostic plaque induced by the administration of ncotine. Administration of mast cell stabilizer revealed the potential of applying mast cell stabilizer in preventing nicotine induced atherogenesis.

GW25-e1582

Impact of SOD mimetic tempol and exercise training on NOS in spontaneously hypertensive rats

Li Nan, Liu Min
The First Hospital of Jilin University, Changchun, China

Objectives: The exercise training (Ex) and superoxide dismutase (SOD) mimetic tempol have antihypertensive effects in spontaneously hypertensive rats (SHR). However, the effects of the combination with Ex and tempol on NOS expression in SHR remain to be elucidated. To clarify the mechanism of antihypertensive and renal-protective effect of the Ex, the present study tested the effects of the Ex and tempol on the NOS expression in the kidney of SHR.
Methods: 5-week-old, male SHRs were randomly divided into four groups; a control group, an Ex group, a tempol-treated (Tmp) group and an Ex+Tmp group. The treadmill running (20 m/min, 60 min/day, 6 times/week) was performed to the Ex and the Ex+Tmp groups, and tempol in drinking water (1 mmol/l) was given to the Tmp and the Ex+Tmp groups for 8 weeks. The systolic BP (SBP) were monitored each week by the tail-cuff method (UR-5000;Ueda). After 8 weeks, NADPH oxidase activity was measured by lucigenin luminescence. H_2O_2 and NO_2/NO_3 (NOx) in plasma and urine were measured by Amplex Red kit or Nitrate/Nitrite Colorimetric Assay Kit (Cayman chemical). The NOS activity and expression were examined in the kidney cortex, the outer medulla, the inner medulla and thoracic aorta. Date are presented as the means±SEM. The significance of differences in mean values was evaluated using ANOVA and values of $P<0.05$ were considered to indicate statistical significance.
Results: Ex and tempol significantly lowed SBP (by 17% and 16%), reduced the renal NADPH oxidase activity (by 29% and 38%) and improved GFR which reflected by increasing Ccr (by 81% and 37%) in SHR. Ex and tempol also upregulated the eNOS and nNOS expressions in the kidney cortex (eNOS:25% and 31%, nNOS:24% and 35%), the outer medulla (eNOS:24% and 40%, nNOS:23% and 33%), the inner medulla (eNOS:21% and 43%, nNOS:22% and 25%) and thoracic aorta (eNOS:20% and 37%, nNOS:18% and 32%) of SHR with the increased plasma and urinary H_2O_2 (plasma: by 22% and 23%; urinary: by 26% and 22%) and NOx (plasma: by 14% and 14%; urinary: by 23% and 24%) significantly. Furthermore, the effects of the combination therapy with Ex and tempol on these factors were cumulate in SHR.
Conclusions: These results indicate that tempol enhances the Ex-induced antihypertensive and renal-protective effects through the upregulation of NOS expression and NO production in SHR. H_2O_2 may mediate these effects of the Ex and tempol in SHR.

GW25-e1654

Comparison of Transplantation of bone marrow-derived stem cells, adipose-derived stem cells and endometrium- derived stem cells in the Infarcted Heart

Kan Wang, Jian-an Wang
Cardiovascular Key Lab of Zhejiang Province, the Second Affiliated Hospital, School of Medicine, Zhejiang University

Objectives: A variety of adult stem cells have been used to transplant into the infarcted heart to cure myocardial infarction (MI), however, comparative studies are lacking to show more suitable source of cells for transplantation. Mesenchymal stem cells hold promise for myocardial regeneration therapy. Derivation of these cells from the endometrium tissue might be easier compared to bone marrow and adipose tissue. However, the in vivo fate and function of endometrium stem cells (EnSCs) in the infarcted heart has never been compared directly to mesenchymal cells derived from bone marrow (BMMSCs) and adipose tissue (AdMSCs).
Methods: EnSCs, AdMSCs and BMMSCs were isolated from healthy donors were characterized using flow cytometry for surface markers identification and microscopy for cell morphology. They were characterized with β-actin promoter driving firefly luciferase and green fluorescent protein (Fluc-GFP) double fusion reporter gene, and were characterized using flow cytometry, bioluminescence imaging (BLI) and luminometry. Cell proliferation was tested by CCK-8 kit, colony forming unit (CFU) was stained by crystal violet staining and apoptosis ratio were detected by TUNEL assay. Rat (n=8/group) underwent myocardial infarction followed by intramyocardial injection of 5×105 EnSCs, AdMSCs and BMMSCs, or saline (negative control). Cell survival was measured using BLI for 6 weeks and cardiac function was monitored by echocardiography and hemodynamics analysis. Ventricular morphology was assessed using histology.
Results: EnSCs, AdMSCs and BMMSCs were CD29+, CD90+, CD105+, shared similar morphology and cell surface markers, but EnSCs had best proliferation, colony-forming and anti-apoptosis activity of 3 types of MSCs. Cells expressed Fluc reporter genes in a number-dependent fashion, as confirmed by luminometry. After cardiac transplantation, transplantation of EnSCs was better capable of preserving ventricular function and dimensions than others, as confirmed by echo test, PV-loops and histology.
Conclusions: This is the first study comparing the in in vitro results and vivo behavior of 3 types of MSCs in the infarcted heart. AdMSCs and BMMSCs do not tolerate well in the cardiac environment, resulting in more cell death andworse cardiac function than EnSCs groups.

GW25-e3339

Exhaustive Swimming Induces Cardiac Lesion in Rats

Xu Peng, Haiyan Liu, Xiaohui Zhao, Chenyang Wang, Xuebin Cao
Department of Cardiology, Geriatric Cardiovascular Disease Research and Treatment Center, No. 252 Hospital of People's Liberation Army

Objectives: The purpose of present study was to investigate the heart injury caused by exhaustive swimming in detail in rats.
Methods: Adult male Sprague-Dawley rats randomly were divided into two groups: the control group and acute exhaustive group. The acute exhaustive group rats were exposed to exhaustive swimming. Refering to Thomas exhaustive standards, single bout of exhaustive swimming were trained to exhaustion. The control animals lived in the same environment as the exhaustive animals with free access to food and water excepting exhaustive swimming. The content in serum of myocardial damage markers, troponin I (TnI) troponin T (TnT) and Brain natriuretic peptide (BNP) were detected by ELISA method, as well as the stress hormone epinephrine (E) and norepinephrine (NE) levels. Cardiac function of rat was measured by pressure-volume conductance catheter technique. The pressure and volumes curves of the left ventricle were recorded continuously in anaesthetized rats. Pressure-volume loop (P-V loop) was set up and ventricular energy indexes embodied by the P-V loop were measured and calculated.
Results: (1). The content of BNP, TnT, TnI in acute exhaustive rats in serum (86.80±4.33 ng/L, 90.40±19.26 pg/mL, 132.81±26.11 pg/mL) was markly increased ($P<0.05$) compared with the values of control group (71.87±16.59 ng/L, 58.82±21.65 pg/mL, 85.20±20.57 pg/mL) caused by exhaustive swimming. (2). Compared with the levels of E, NE in the control group (137.45±18.22 ng/L, 305.95±19.90 ng/L), acute exhaustive group (158.74±23.69 ng/L, 330.35±14.90 ng/L) was obviously increased, and there were significantly differences ($P<0.05$). (3). After exhaustive swimming, stroke volume became larger while end-diastolic volume increased (143.54±25.43 μL vs 109.97±19.54 μL, 217.37±37.84 μL vs 165.80±33.58 μL, respectively, $P<0.05$). The left ventricular end-systolic pressure (LVESP) obviously decreased ($P<0.05$). The heart rate (HR) 258.47±52.69 bpm markedly decreased ($P<0.05$) compared with 399.94±67.34 bpm in Control group.