Objectives: Cardiovascular and renal remodeling is the major determinant of progress of chronic cardiovascular and renal diseases. Our previous study demonstrated that uncoupling protein 2 (UCP2) plays a protective role in salt-induced vascular dysfunction via an antioxidative action. The aim of this study was to investigate the effects of UCP2 on salt-induced cardiovascular and renal remodeling.
Methods: UCP2-knockout (KO) mice and wild-type (WT) littermates were fed with normal-salt diet (NS, 0.5%) and high-salt diet (HS, 8%) for 16 weeks. The histology, fibrosis, superoxide, expressions of matrix metalloproteinase (MMP) -2/-3/-9 and tissue inhibitor-1 of MMP (TIMP-1) in heart, aorta, mesenteric arterioles and kidneys were evaluated.
Results: In WT mice, salt had little effect on cardiomyocyte size, but, in KO mice, salt caused a significant increase in cardiomyocyte size compared with NS group (217±21 vs. 336±23 μm^2, P<0.01). As expected, salt-fed animals from both genotypes were found to have a significant fibrosis (both P<0.01). Interestingly, KO mice showed greater fibrosis after a salt intake compared with WT mice (20.8±2.3% vs. 8.4±1.6%, P<0.01). The wall thickness of both aorta and mesenteric arterioles were increased significantly due to salt-treated WT mice (both P<0.01) but were significantly higher in KO mice (aorta: 101±7 vs. 82±6 μm, P<0.05; mesenteric arterioles: 11.4±1 vs 8.6±0.8 μm, P<0.05). High-salt intake greatly increased aortic and mesenteric arteriolar fibrosis in both genotypes (both P<0.01), while had a significantly higher effect in KO mice (P<0.01 for aorta, P<0.05 for mesenteric arterioles). The high-salt diet resulted in no significant changes in glomerular and tubular size in WT mice, but a significant increase in KO mice (P<0.01 for tuft area, P<0.05 for tubular area). After 16 weeks of salt feeding, the glomerular tuft area in KO mice was significantly increased compared with WT mice (4.5±0.5 vs. 2.8±0.3 μm^2, P<0.01). The salt administration caused a remarkably renal fibrosis in both genotypic mice (both P<0.01), while the salt-induced renal fibrosis was exacerbated KO mice compared with WT mice (34.2±5.1 vs. 12.5±2.2%, P<0.01). Fluorescence assay showed that salt-induced superoxide production in heart, aorta and kidney tissues were elevated in UCP2 KO mice compared with WT littermates (all P<0.01). The cardiovascular and renal remodeling in both genotypes was accompanied by increased MMP-2/-3/-9 and decreased TIMP-1 protein expression compared with control groups (all P<0.01).
Conclusions: These data suggest that upregulation of UCP2 may attenuate the development of salt-induced cardiovascular and renal damage.

GW25-e2215

Angiotensin II Type 2 Receptor Re-expression After the Collar-induced Adventitia Injury in the Rat Carotid Artery

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Objectives: The present study was undertaken to observe the effects of the chronic adventitia injury on angiotensin II type 2 Receptor (AT_2R) expression in the rat carotid artery.
Methods: Adventitia injury was induced by positioning a silicone collar around the right carotid artery for one week in 20 Wistar Kyoto rats. Both side of carotid was harvested for analysis of AT_2R expression. The expression of AT_2R mRNA and protein was assessed by RT-PCR and western bloting respectively. The expression distribution of AT_2R protein was assessed by immunohistochemistry.
Results: The AT_2R mRNA expression increased 76% (P=0.0061) and the AT_2R protein expression increased 3.37 fold (P<0.0001) respectively in the collared right carotid artery when compared to non-collared left carotid artery. Immunohistochemical examination revealed that there was little AT_2R expression anywhere in the non-collared left carotid artery and there was highly AT_2R expression in intima and adventitia but not in media in the collared right carotid artery.
Conclusions: The expression of AT_2R mRNA and protein was upregulated in response to the collar placement. Collar-induced adventitia injury leaded to re-expression of AT_2R in intima and adventitia of the rat carotid artery.

GW25-e3177

β_2 adrenergic receptor selective agonist clenbuterol protects cardiac arrhythmia after reperfusion

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Objectives: Our Previous study has demonstrated that β_2 adrenergic receptor (β_2AR) activation reduces infarct size and myocardial apoptosis after myocardial ischemia/reperfusion. Cardiac arrhythmias are a major manifestation of reperfusion injury, which results in sudden death in acute myocardial infarction. The present study is designed to investigate whether β_2AR agonist clenbuterol will protect cardiac arrhythmias induced by reperfusion.
Methods: Reperfusion arrhythmias were induced by 10 min ligation of the left anterior descending coronary artery, followed by a 30 min reperfusion in anaesthetized rats. Electrocardiogram (ECG) in Lead II configuration was monitored throughout the I/R process. Cardiac damage was characterized by a higher incidence of reperfusion-induced ventricular tachycardia (VT) and ventricular fibrillation (VF).
Results: The incidence and severity of cardiac arrhythmias were significantly reduced by pretreatment with β_2AR agonist clenbuterol in anesthetized rats. The protective effect of clenbuterol was attenuated by a selective β_2AR antagonist ICI 118,551 or a gap-junction protein connexin (CX) inhibitor cabenoxolone. Clenbuterol also reduced the dephosphorylation and redistribution of Connexin 43 (CX43) induced by ischemia/reperfusion. Furthermore, pretreatment with clenbuterol significantly inhibited dephosphorylation of CX43 in isolated wild type not β_2AR-knock out mouse hearts after ischemia/reperfusion injury.
Conclusions: These data demonstrated that β_2AR agonist clenbuterol provides significant protection against arrhythmias induced by reperfusion, which is likely mediated at least in part by reducing dephosphorylation and redistribution of CX43, thereby enhancing the gating function of gap junctions in heart.

GW25-e3240

Puerarin reduces inflammatory responses and apoptosis in LPS-stimulated cardiomyoblasts

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Objectives: To investigate the effect of puerarin, a kind of Chinese herbal medicine, on cardiomyoblast inflammatory response in a sepsis model using LPS.
Methods: H9c2 cardiomyoblasts were incubated with different concentrations of puerarin (1μM, 5μM, 10μM, 20μM, 40μM) in the presence of LPS (1μg/mL) or not. The mRNA expression of inflammatory cytokine including IL-1, IL-6 and TNFα were detected using real-time PCR. Immunofluorescent staining was used to evaluate NFκB p65 nuclear translocation. LPS-induced apoptosis was measured by TUNEL staining and immunoblotting of Bcl-2 and Bax expression. The activation state of NFκB signaling was investigated using Western Blotting.
Results: Puerarin blunted proinflammatory cytokine production in LPS-stimulated H9c2 cardiomyoblasts in a concentration dependent manner. In addition, treatment of H9c2 cardiomyoblasts with puerarin (40μM) inhibited LPS-induced apoptosis. Moreover, the NFκB p65 nuclear translocation and activation of NFκB signaling after LPS stimulation were suppressed by puerarin (40μM).
Conclusions: Puerarin may serve as a valuable protective agent in cardiovascular inflammatory diseases.

GW25-e3401

Exogenous Administration Of Adiponectin Could Effectively Alleviate Coronary No Reflow Injury In Type 2 Diabetic Rats

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Objectives: Coronary no reflow (NR) phenomenon of varying degrees widely exists in patients undergoing coronary artery recanalization therapy with type 2 diabetes mellitus (T2DM) significantly increasing the severity of it. Meanwhile, plasma adiponectin (APN) level is significantly reduced in patients with T2DM. To demonstrate whether hypoadiponectinemia could lead to aggravated coronary NR injury in T2DM and the protective effect of APN on coronary microcirculation, we conducted the following experiments.
Methods: Healthy male SD rats of 6-week-old were fed with normal diet (10kcal %, ND) or high-fat and high-sugar diet (60kcal%, HD). All rats were fed continuously for 32 weeks. Body weight, fasting glucose, fasting insulin and serum APN levels were monitored regularly. 8 weeks later, intraperitoneal injections of glucose tolerance test (IPGTT) were performed every 4 weeks. And abnormal IPGTT was selected as a measurement for successful model of type 2 diabetes. Successfully fed type 2 diabetic rats and normal rats were divided into 4 groups: Sham group; normal control group (ND); diabetic rats group (DM); diabetic rats+globular adiponectin (gAd) intervention group (DM+gAd); gAd of 20μg/kg or 0.5ml of saline solution were injected into rats via tail vein at the 31st weekend. In each group, ischemia was produced via left anterior descending coronary artery slip-knot ligation for 1.5hours and then reperfusion for 12 hours. No reflow injury areas were measured using Evan's Blue and Thioflavin S staining methods. The reperfusion areas of myocardial microcirculation were showed with fluorescent microspheres. Left ventricular pressure was measured to evaluate cardiac function of rats. Serum adiponectin (APN), endothelin-1 (ET-1), intercellular adhesion molecule-1 (ICAM-1) and vascular endothelial adhesion molecule-1 (VCAM-1) levels were measured simultaneously to assess vascular endothelial injury.
Results: (1) Serum adiponectin levels of diabetic rats increased firstly and then continued to reduce in the process of HD feeding. ET-1, ICAM-1, VCAM-1 in both DM group and DM+gAd group were significantly increased in the 32th weekend. After administration of gAd, ET-1 was significantly reduced (108.6.13±8.428 vs.132.10±7.213, P<0.05). (2) NR injury in DM group was increased after I/R (48.64±2.93% vs. 35.48±4.31%, P< 0.05). Cardiac function showed obviously impaired (+dp/dt: 2514.36±188.93 vs.3714.73±118.90, P<0.01) with increased serum levels of ICAM-1 and VCAM-1.3) NR injury (28.48±1.441% vs 48.64±1.928%, P<0.01) and cardiac function after I/R injury in DM+gAd group improved significantly (+dp/dt: 3280.18±192.84 vs.2514.36±188.93, P<0.01) with decreased serum ICAM-1 and VCAM-1 levels.