Objectives: The main aim of this study was to systematically evaluate the expression patterns of the Nrf2 in the developing or damaged adult heart tissue, and probe into whether Nrf2 can be as a marker of cardiac stem cell.

Methods: Nrf2 expression was assessed in the embryonic 13.5 d and postnatal 1d, 7d, 1m, 3m old Nrf2-GFP transgenic mouse heart tissue by fluorescence microscopy, real-time quantitative PCR and RT-PCR. Myocardial infarction model was established by ligature of left anterior descending coronary in adult Nrf2-GFP mice and the Nrf2 expression was observed in the myocardium at 7d after injury. Then, the correlation between Nrf2 and other stem marker's expression in mouse heart tissue were determined by immunofluorescent assay.

Results: In Embryonic 13.5 d, the Nrf2 mainly expressed in the brain, spinal cord and the retina, and also can be observed in the heart tissue. After the mouse was born. Nrf2 expression is gradually reduced with growth, and that was also confirmed by the RT-PCR, Q-PCR analysis. Nrf2-positive cells increased significantly in myocardium compared to the normal tissue. Sca-1+, Kit+, Isl-1 and Nkx2.5 are widely expressed in heart tissue, but not co-expressed with Nrf2. However, in normal and injured tissue, Nrf2 was co-expressed with vimentin and musashi-1, neural cell marker.

Conclusions: These results indicate that nrf2 expression is highly correlated with cardiac development, and the Nrf2-positive myocardial cell might be arise from neural linage cells, which suggest that such cells play an important role in the growth and maintenance of the cardiogenesis and regeneration.

GW25-e0610

A Novel Model of Intimal Hyperplasia in the Bama Miniature Pig

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Objectives: To develop a bama miniature pig intimal hyperplasia model in superficial femoral artery.

Methods: Following 1 month of a 3% cholesterol diet, 4 pigs underwent surgical perfusion with distilled water (n=8). 3 pigs were subjected to sham-operation for control (n=6). After 3 months of the same diet, sonography and histologic sections of the vessels were analyzed.

Results: Intimal hyperplasia was confirmed in experimental group (8 of 8), whereas the control group remained intact. Lumen area was drastically decreased as assessed by sonography. Histologic sections showed that arteries of experimental group had a increased intimal areas (0.42±0.03 mm²), increased intimal area/ Media area ratios (0.5±0.12 mm²) and decreased lumen areas (0.35±0.05 mm² vs 0.62±0.03 mm²; P<0.05).

Conclusions: This novel intimal hyperplasia model may be a useful tool for evaluating drugs and therapeutic devices.

GW25-e0741

Calreticulin is localized in the mitochondria of rat cardiomyocytes and affected by furazolidone

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Objectives: Calreticulin is a calcium-buffering protein which is predominately located in endoplasmic reticulum. We have previously shown calreticulin is also localized in the myocardial mitochondria and up-regulated in a rat model of furazolidone-induced dilated cardiomyopathy. The aim of this study was to determine whether calreticulin is localized in the mitochondria of rat cardiomyocytes and whether mitochondrial calreticulin is affected by furazolidone.

Methods: The mitochondrial preparations were isolated from primary cultured neonatal rat cardiomyocytes and puriﬁed by differential centrifugation. The immunoreactivities of calreticulin and markers for cytosol, nucleus, endoplasmic reticulum and plasma membrane were detected by western blot. The distribution of calreticulin to mitochondria was further conﬁrmed by immuno-electron microscopy, flow cytometry and laser scanning confocal microscopy (double staining with Mitotracker Red and calreticulin). To study the effect of mitochondrial calreticulin affected by furazolidone, the rat cardiomyocytes were exposed to 100 μmol/L furazolidone for 48 h and then the mitochondrial calreticulin expression was analyzed using western blot.

Results: Western blot and immunoelectron microscopy showed that calreticulin was present in the mitochondria of rat cardiomyocytes; moreover, the co-localization of calreticulin and mitochondria was further confirmed by flow cytometry and laser scanning confocal microscopy. Furazolidone treatment signiﬁcantly increased the content of mitochondrial calreticulin by 3.7±0.7 fold (P<0.05) in the rat cardiomyocytes.

Conclusions: In summary, the present results suggest that calreticulin is localized in the mitochondria of rat cardiomyocytes and such localization is affected by furazolidone.

GW25-e0767

Bisphenol A can injure the heart via DNA damage

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Objectives: Bisphenol A (BPA) is a man-made high volume production chemical and human is widely-spread exposure to BPA. Previous studies have shown that the BPA exposure is associated with heart disease, but the mechanisms of BPA on the heart are still unclear. The purpose of this research is to investigate the relation between the concentrations of BPA and severity of the lesions in the heart and analyze the molecular mechanism of BPA harmful effect.

Methods: Mice were subcutaneously injected with normal saline or 0.1, 1 and 10mg/kg/day BPA for 1 month, and then were detected by Vero 770 ultrasonic diagnostic apparatus, respectively. The cardiac protein of isolated from normal rats and P21 were treated by PBS or 0.1, 1 and 10μM BPA. The protein of γH2AX was detected by western blot. The mRNA level and the protein level of P21 were tested by real-time PCR and western blot. The protein maps of the cardiomyocytes stimulated by PBS or BPA were measured by two-dimensional gel electrophoresis and the different protein spots were identified by mass spectrometry.

Results: EF value and FS value were significantly decreased in 1 and 10mg/kg/day BPA groups compared with normal saline group, and BPA produced a dose-dependent reduction in EF and FS value. The expression of γH2AX and P21 were obviously increased with the concentration of BPA in a dose-dependent manner. Some differentially expressed proteins were determined to be the signal transduction associated proteins of DNA damage.

Conclusions: This study mainly reveals that BPA is harmful to the heart and cardiomyocytes. Its mechanism may be that BPA causes DNA damage in cardiac muscle cell.

GW25-e0775

Hepatocyte Growth Factor Suppresses Hypoxia/Reoxygenation-induced XO Activation in Cardiac Microvascular Endothelial Cells

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Objectives: To detect the effect of hepatocyte growth factor (HGF) on xanthine oxidase (XO) under hypoxia/reoxygenation (H/R) conditions in rat cardiac microvascular endothelial cells (CMECs).

Methods: Primary cultured rat cardiac microvascular endothelia cells (CMECs) were exposed to 4h of hypoxia and followed by 1h of reoxygenation. Generation of ROS and cytosolic Ca²⁺ concentration was measured by flow cytometry qualification of DCFH-DA and fluo-3 AM staining cells, respectively. XDH mRNA was qualified by RT-PCR analysis. XO activity was determined by colorimetric assay and XO protein levels were determined by Western blot.

Results: After hypoxia/reoxygenation (H/R), cellular ROS production significantly increased. Both XO activity and XO protein increased after H/R. Cellular ROS elevation was inhibited by HGF (a potent XO inhibitor), indicating XO accounting for the generation of ROS after H/R. In addition, XDH mRNA increased after H/R, indicating a de novo XDH synthesise, which need to be converted to XO to become a source of superoxide. Pretreatment of HGF inhibited the elevation of XO activity and XO protein level after H/R; however, HGF has no effect on the increase of XDH mRNA. It has been reported that Ca²⁺ acts in regulating the post-transcriptional conversion from XDH to XO, and we also find an increase of the cytosolic Ca²⁺ in CMECs after H/R. BAPTA-AM, a cell-permeable Ca²⁺ chelator, prevented the increase of XO activity and XO protein levels, implicating the elevated cytosolic Ca²⁺ in CMECs after H/R. Thus, HGF inhibited XO production after H/R by blocking the post-transcriptional conversion of XDH to XO and, we also find an increase of the cytosolic Ca²⁺ in CMECs after H/R. PANTRA-AM, a cell-permeable Ca²⁺ chelator, prevented the increase of XO activity and XO protein levels, implicating the elevated cytosolic Ca²⁺ in CMECs after H/R. Thus, HGF inhibited XO production after H/R by blocking the elevation of cytosolic Ca²⁺ concentration in CMECs.

Conclusions: These findings suggest a novel mechanism whereby HGF inhibited XO-generated ROS production after H/R treatment. H/R induces a de novo synthesis of XDH, the XO precursor. In addition, H/R increases cytosolic Ca²⁺ concentration and promotes a Ca²⁺-involved XO conversion and XO activation. HGF has no effect on the increase of XDH mRNA, however HGF inhibited the elevation of XO protein level and XO activity after H/R in the post-transcriptional level primarily by inhibiting the increase of cytosolic Ca²⁺ concentration.

GW25-e0784

Knock-down of metallothionein exacerbates intermittent hypoxia induced oxidative and inflammatory injury in aorta

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Objectives: Obstructive sleep apnea (OSA) is an independent risk factor for cardiovascular diseases possibly via intermittent hypoxia (IH) - elicited oxidative stress and inflammation, while metallothionein (MT) has been recognized as an inducible anti-oxidant which may protect against damages from a variety of oxidant stimuli. The present study was to explore the effect of MT on IH-induced aortic pathogenic changes.

Methods: To mimic hypoxia/reoxygenation events that occur in adult OSA patients, mice were exposed to IH for up to 8 weeks. The IH paradigm consisted of alternating cycles of 20.9% O₂ /8% O₂ FIO2( 30 episodes per hour) with 20 seconds at the nadir FIO2 for 12 hours a day during the light phase. Markers of oxidative damages, inflammation, and vascular remodeling were observed by immunohistochemical staining on aortic rings excised at 3 days, 1, 3 and 6 weeks after IH exposure.

Results: Endogenous MT was induced after 3 days of IH, but was significantly decreased after 8 weeks of IH. Compared with the wild-type mice, MT knock-out mice exhibited earlier and more severe pathogenic changes of oxidative damages, inflammatory responses and cellular apoptosis, as indicated by the significant
Conclusions: These findings suggested that chronic I H may lead to aortic damages characterized by oxidative stress and inflammation, and MT may play a pivotal role in the above pathogenesis process.

GW25-e0790
Effects of (P) RR and PLC-beta3 activation on cardiac hypertrophy in hypertensive rats
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Objectives: (Pro) renin receptor (P) RR, a newly identified member of the renin-angiotensin system, is a promising novel drug target because of its crucial involvement in renal and cardiac end-organ damage, but the mechanism of (P) RR on the end-organ damage remains unclear so far. Recently, some findings support the (pro) renin-(P) RR interaction at exceptionally high (pro) renin levels in vitro. However, the conflicting results obtained with an anti-renin-angiotensin (HRP) in vivo used to argue against the idea that this drug truly blocks the (pro) renin-(P) RR interaction in the intact animals. In this study, we investigated the role of cardiac (P) RR activation on the expression of PLC-beta3, PKC and ERK1/2 and on cardiac hypertrophy in hypertensive rats with abdominal aortic ligation.

Methods: Seventy-five rat SDs were divided into five groups (n=15 each group) as following: sham operated (SO), rats with the aortic ligation (AL), AL rats were given HRP (4ug kg⁻¹ d⁻¹, SC), AL rats given U73122 (40ug kg⁻¹ d⁻¹, SC) and AL rats given HRP + U73122. MAP was recorded using a tail-cuff method. After 4 weeks of treatment, levels of (P) RR, PLC-beta3, PKC-α and ERK1/2 in the heart were examined by RT-PCR and western blot. The surface area of cardiomyocytes was measured.

Results: The expression levels of (P) RR and PLC-beta3 significantly increased in the left ventricle in hypertensive rats (P<0.01, respectively). The surface area of cardiomyocytes and MAP rose markedly (P<0.01). HRP treatment significantly reduced the expression of (P) RR and U73122 suppressed the level of PLC-beta3. The combined treatment of HRP and U73122 significantly decreased levels of PKC-α and ERK1/2 in the heart (P<0.01). Meanwhile, the surface area of cardiomyocytes and MAP were decreased after the treatment (P<0.01).

Conclusions: This is the first report demonstrating that treatment of HRP and U73122 decreased levels of (P) RR, PLC-beta3, PKC-α and ERK1/2 in the heart. Meanwhile, the treatment reduced the surface area of cardiomyocytes and MAP. These findings indicate that cardiac (P) RR may activate PLC-beta3, PKC and ERK1/2 signals and result in hypertension and cardiac hypertrophy.

GW25-e0850
Effect of acute high altitude exposure on lung function and relationship between lung function and AMS
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Objectives: To investigate the effect of acute high altitude exposure on lung function and the relationship between lung function and AMS. Methods: We collected the lung function and Lewis Lake data of 73 subjects (age 18 to 26, male) at sea-level and jummachang after five days Exposure to 3000m, 3900m). Results: Compared with sea-level, lung function decreased in FVC, MMF, V50, V25 White FEVI, PEF, V75 did not change, MVC, FEVI, PEV, MMF changed in the relationship between lung function and AMS, there is No difference in LM function between AMS group and NON AMS group at sea-level, lung function of AMS group is statistically significant lower than NON AMS group in FVC, MMF at high altitude; there is differences between AMS group and NON AMS group in the rate of change of FVC, MMF; logistic regression analysis showed that the rate of change of FVC was independent risk factors, correlation analysis showed that the change of FVC and the change of oxygen saturation is relative.

Conclusions: Lung function showed restriction decreased after acute high altitude exposure, the changes of lung function will increase the hypoxia and susceptible AMS.

GW25-e0876
Role of GRK4 variant 142V in the regulation of renal ETB receptor in hypertension
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Objectives: the endothelin receptor B (ETBR) regulates blood pressure and water and electrolyte balance by engendering natriuresis. In hypertensive states, the ETBR mediated diuresis and natriuresis is impaired. However, the underlying mechanisms are not clear. G protein-coupled receptor kinase 4 (GRK4), whose gene locus 4p16.3 is linked to essential hypertension, causes sodium retention and increase blood pressure via impairment of renal dopamine receptor and enhancement of renin-angiotensin system functions. Due to the higher activity of GRK4 in kidney from spontaneously hypertensive rats (SHRs) and hypertensive patients, we hypothesize that GRK4 might be the cause of ETRB impairment in hypertension.

Methods: Experiments were carried out in male anaesthetized spontaneously hypertensive rats (SHR) and in normotensive Wistar-Kyoto (WKY) rats. The ETBR agonist, BQ-3020 (0.1,0.5,1.0ug/kg/min) were infused via supra-retinal artery at a rate of 0.04ml/min for 40 minutes. The same experiments were conducted in GRK4 A142V and GRK4 Wild Type transgenic mice. The ETBR function were also checked in the wild-type and A142V transfected renal proximal tubule (RPT) cells from mice.

Results: We found that diuresis and natriuresis of ETBR agonist, BQ3020, in Wistar-Kyoto (WKY) rats, which was impaired in SHRs. The GRK4 expression was higher in renal cortex from SHRs as compared with WKY rats. In GRK4 A142V transgenic mice, it resulted that ETBR-mediated diuresis and natriuresis was impaired compared with Wild type. In wild-type transfected cells, activation of ETBR inhibited Na+-K+-ATPase activity; while in A142V transfected cells, the inhibitory effect was lost. There are co-localization and co-immunoprecipitation between ETBR and GRK4 in RPT cells. The linkage of ETBR/GRK4 was higher in wild-type cells than in A142V cells. Similar phenomenon was found in the kidney from WKY and SHRs, SHRs had higher ETBR/GRK4 linkage, accompanied with higher ETBR phosphorylation, which might account for the impaired ETBR function in hypertension.

Conclusions: This study provides a mechanism by which GRK4, via regulation of renal ETBR function, participates in the pathogenesis of hypertension.

GW25-e1095
Cardiac Electrical Activity Improved by Overexpression of the Sarcolemmal Retinol Cuc1-ATPase in Rat Myocardial Failure After Myocardial Infarction Evaluated by Microelectrode Arrays Technology
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Objectives: To explore overexpression recombinant adenovirus (rAd)-mediated sarcoplasmic reticulum Ca²⁺-ATPase (SERCA2a) for cardiac rhythmicity and conductivity in rat heart failure after myocardial infarction and its possibly electrical mechanisms.

Methods: 26 adult male SD rats were randomly divided into three groups: sham group (n = 10), rAd.β-gal group (n = 8) and rAd.SERCA2a group (n = 8). Shm operation consisted of thoracotomy and cardiac exposure but without coronary artery ligation. RAd.β-gal group and rAd.SERCA2a group were ligated the left anterior descending coronary artery for rat heart failure animal model after myocardial infarctions, while the transfecting β-gal and SERCA2a gene into heart respectively. We used ultrasound electrocardiogram for evaluating cardiac diastolic and systolic function, ECG monitoring and microelectrode arrays (MEA) technology for myocardium electrical activity intracellular.

Results: rAd carrying SERCA2a and β-gal gene were successfully transfected in heart failure rats. rAd.SERCA2a group could improve failing heart function, the ventricular end diastolic volume, left ventricular end-systolic volume, left ventricular ejection fraction and fractional shortening. Compared with the sham group, ECG could be found that QT interval prolonged (94.7 ms vs. 71.7 ms, P<0.05) the incidence of premature ventricular contractions (PVC) was 71.5% in rAd.β-gal group, but in rAd.SERCA2a group QT interval shortened and the incidence rate of PVC was 14.3%. No significant difference in the heart rate of rAd.SERCA2a group. The field potential duration were statistically different between the infarct zone and the contralateral normal zone (71.8±7.35 ms, n=5, P<0.05) in rAd.β-gal group, and field potential duration dispersion in infarct zone with 60 channels record was larger than rAd.SERCA2a group. The conduction time was simultaneous in rAd.SERCA2a group, and the cardiac electro-conduction activity could keep consistency and improve in myocardial infarction tissue.

Conclusions: Overexpression of SERCA2a may significantly improve left ventricular systolic and diastolic function, as well as it may be reduced incidence of arrhythmias by line heart model after acute myocardial infarction and improve uniform conduction of cardiac electrical activity. MEA technology is an ideal technology for observing rhythm, frequency and conduction activities in cardiovascular disease animal models.