CONCLUSIONS Reduced expression of miR-98 may relate to excess LOX-1 expression and downstream signaling, foam cell formation and lipid accumulation. Plasma level of miR-98 may be a biomarker of atherosclerotic disease process and its modulation may offer a therapeutic strategy for atherosclerosis.

GW27-e0110

Effect of Combined Administration of Tanshinone IIA and △7-stigmastenol on Inflammatory Response in Chinese Mini Swine with Coronary Atherosclerosis

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OBJECTIVES To observe the effect of combined administration of tanshinone IIA and △7-stigmastenol on inflammatory response in Chinese mini swine with coronary atherosclerosis.

METHODS 48 Chinese mini swines were randomly divided into six groups, with eight in each group. Six groups included the normal control group, the model group, the simvastatin group with does of 1.0 mg/kg, combined administration groups at the same mass ratio with different does of 0.5, 1.0, 1.5mg/kg. Except for the normal control group, all of other groups were fed with high fat diet for 2 weeks. Swines were injured at their left anterior descending artery endothelium by interventional balloons, then were fed with high fat diet for 8 weeks to prepare the coronary atherosclerosis model. In the 8th week, the intravascular ultrasound was used to observe the total coronary artery plaque burden of each group. Inflammatory factors such as hs-CRP, TNF-α, sICAM-1 and IL-6 were detected by ELISA. The expression of NF-κB p65 nuclear translocation was observed by the immunohistochemical method.

RESULTS Compared with the normal control group, the model group showed significant increase in the total coronary artery plaque burden ($P<0.01$), and remarkable growth of hs-CRP, TNF-α, sICAM-1 and IL-6 levels ($P<0.01$). The immunohistochemical staining also showed the significant increase in the NF-κB p65 nuclear translocation of coronary artery of Chinese mini swines in the model group. Compared with the model group, combined administration of tanshinoneIIA and △7-stigmastenol could significantly attenuate atherosclerotic plaque burden ($P<0.01$), decrease such inflammatory cell factors as hs-CRP, TNF-α, sICAM-1 and IL-6 in serum, and inhibit the NF-κB p65 nuclear translocation of coronary artery ($P<0.01$). Compared with the simvastatin group, both the median and high dose combined administration group can better lower the level of inflammatory cell factors above ($P<0.05$ or $P<0.01$).

CONCLUSIONS Combined administration of tanshinone IIA and △7-stigmastenol can reduce the downstream inflammatory response by controlling NF-κB p65 nuclear translocation, further, inhibit the ocurrence and development of coronary atherosclerotic plaque in Chinese mini swine.

GW27-e0156

QSKL inhibits oxygen-glucose deprivation/recovery of oxygen glucose-induced H9C2 cells apoptosis through modulating p-Akt-p53/Mdm2 signaling pathway

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OBJECTIVES QSKL, a clinical effective compound, plays cardio-protective roles in heart failure. But its underlying mechanism needs to be further elaborated. The purpose of this study was to identify the protective effects of QSKL on oxygen-glucose deprivation/recovery of oxygen glucose (OGD/R) induced-apoptosis in H9C2 cells and to clarify the underlying mechanism.

METHODS A model of apoptosis induced by OGD/R was established in vitro. The changes in cell viability were examined with CCK-8 assay to determine the available concentration of QSKL. 2′, 7′-Dichloroflurescin diacetate (DCFH-DA) and Hoechst 33342 were used to detect the effect of QSKL on ROS level and apoptotic rate, respectively. JC-1 was used to detect the mitochondrial membrane potential. We also used western blot to examine the effect of QSKL on the regulation of proteins related to apoptosis: p53, Mdm2, Bcl-2, Bax, caspase-8 and cleaved-caspase-3. LY294002, an inhibitor of PI3K, was used to detect the effect of QSKL on PI3K/Akt signaling pathway.

RESULTS Compared with control group, cell survival rate in model group was 54.3±6.6%. Compared with model group, 400μg/ml, 600μg/ml, 800μg/ml and 1000μg/ml QSKL elevated cell viability ($p < 0.01$) and cell survival rates were 73.7 ± 4.6%, 84.2 ± 2.9%, 87 ± 1.4%, 74.5 ± 3.7% respectively. In model group, ROS generation in cells increased, mitochondrial membrane potential and red/green fluorescence ratio decreased, apoptotic rate was 23.9 ± 5.8% ($p < 0.01$). Application of QSKL could make a reduction on intracellular ROS generation ($p < 0.01$) and increase mitochondrial membrane potential, which thus elevating the ratio of red/green fluorescence ($p < 0.01$). In addition, the apoptotic rate dropped to 8.9 ± 2.2%, which had a significant difference compared with the model group ($p < 0.01$). With the detection of western blot, Bcl-2 decreased slightly in model group, but had no difference compared with control group, while Bcl-2/Bax ratio was significantly lower ($p < 0.01$), caspase-8 and cleaved-caspase-3 was significantly increased ($p < 0.01$), Bax, p53 and Mdm2 slightly increased ($p < 0.05$). Compared with model group, QSKL significantly increased Bcl-2/Bax ratio ($p < 0.01$), reduced the expression of p53, Bax, caspase-8 and cleaved-caspase-3 ($p < 0.01$), and increased the expression of Mdm2 ($p < 0.05$). After application of LY294002, the protective effect of QSKL was inhibited, and there were no difference on the expression of cleaved-caspase-3 and p-Akt compared with model group ($p > 0.05$).

CONCLUSIONS QSKL protects H9C2 cells from OGD/R-induced apoptosis by reducing intracellular ROS level, elevating mitochondrial membrane potential and regulating apoptotic proteins. The protective and anti-apoptotic effects of QSKL could be mediated through modulating the p-Akt-P53/Mdm2 apoptotic pathway.

GW27-e0158

Tirofiban protects against myocardial ischemia and reperfusion injury in mice

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OBJECTIVES Myocardial ischemia-reperfusion (MI/R) can induce myocardial infarction and lethal ventricular arrhythmia. Tirofiban, a specific GP IIb/IIIa inhibitor, is one of the most frequently used agent in treating myocardial infarction after percutaneous coronary intervention. However, there is still no any published papers in vivo studying the direct effect of tirofiban on MI/R injury in mice up to now.The present study is aimed to assess the direct effect of tirofiban therapy for cardiac protection in a murine model of MI/R injury in vivo.

METHODS Tirofiban effects were evaluated in mouse heart preparation using 30-min coronary occlusion followed by 24-h reperfusion and compared among SHAM group (negative model control, n=7), I/R group (positive model control, n=8), VER (I/R with verapamil 20mg/kg pretreatment, positive drug control, n=6), and TIR (I/R with tirofiban 5mg/kg pretreatment, the aim group,n=8). The effects of DLT were characterized in infarction size (IS) compared with risk region (RR) and left ventricle using the Evans blue/triphenyltetrazolium chloride double dye staining method in vivo.

RESULTS There were no significantly differences in body weight among the four groups(SHAM: 25.6 ± 0.8g, I/R: 26.5 ± 0.7g, VER: 26.2 ± 0.8g, TIR: 28.1 ± 0.9g, $P>0.05$),respectively. The ratio of IS to RR was significantly smaller in the TIR and VER groups than the I/R group (I/R: 40.6±4.3%, VER: 15.1±2.6%, TIR:20.6± 3.7, $P<0.01$), the ratio of IS to left ventricle was also reduced in the TIR and VER groups(I/R: 18.3±2.8%, VER: 6.6±0.8%, TIR:7.0± 1.4, $P<0.01$), while there were no differences in RR among SHAM, I/R, VER, and TIR four groups ($P>0.05$). Experiments showed incidence of arrhythmias was reduced in the TIR group ($P<0.01$).

CONCLUSIONS In conclusion, tirofiban is effective in reducing the infarction size caused by MI/R injury. This is the first study directly studying the in-vivo effect of tirofiban on MI/R injury in mice, which results provided an experimental basis for clinical application of tirofiban in reducing I/R injury.

GW27-e0167

In silico studying the weak effect of lidocaine on acidosis

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OBJECTIVES Acidosis is a pathological process in myocardial ischemia, heart failure and sepsis. Although lidocaine appeared to

function exactly in arrhythmia, the Cardiac Arrhythmia Suppression Trial (CAST) paradoxically showed that it caused a two- to threefold increase in sudden cardiac death compared with placebo. It is still unclear why lidocaine is poor in acidosis up to now. The present study is to reveal the mechanism that lidocaine are weak in acidosis states.

METHODS Markov models of voltage dependent sodium current(INa), rapid delayed rectified potassium current (IKr) and L-type calcium current(ICaL) were formulated via numerical optimization from experimentally derived rate constants that formed the basis action potential(AP) in acidosis. The drug-channel model was incorporated into Roberts-Christini AP model of the guinea pig. 2D Spiral waves and 3D magnetic resonance imaging (MRI)-based Spiral scrolls were constructed in cardiac ventricles. Simulations were encoded in C++ and run on 3 nodes' server based on Pros 3.6 GHz 20-Core using OpenMP and MPI.

RESULTS Firstly, INa, IKr, and ICaL were simulated with published data in acidosis(extracellular pH and intracellular pH both 6.5) and control(extracellular pH 7.4 and intracellular pH 7.2). Peak INa reduced 50%, peak IKr reduced 60%, and peak ICaL reduced 50% with AP duration delayed 20ms. Secondly, we studied the effects of lidocaine on single-cell upstroke velocity in acidosis. With 0, 5 and 20mM lidocaine, the upstroke velocity were ~380, 315, and 285 V/s in control and 300, 285, and 255 V/s in acidosis, respectively. Pacing in 60, 80,120,160 bpm with 20 mM lidocaine, the upstroke velocity is ~270, 255, 235,200 V/s in control, and 250, 235, 215, 195 V/s in acidosis respectively. Therefore, both dose-dependent and frequency-dependent effects of lidocaine on upstroke velocity were reduced in acidosis. Fourthly, we expanded to one-dimensional (1D) 100 cells cardiac tissue of 20 mM lidocaine at 120 BPM on conduction velocity (CV). CV was 50 cm/s in control and reduced to 40 cm/s in acidosis. Fifthly, we used the 1D predictions to guide 2D simulations with 20 mM lidocaine at 120 BPM in a 2D homogeneous 500 by 500 cells. the spiral wave of reentry was 1.1s in acidosis and 0.5s in control. Finally, we tested in a MRI-based, anatomically detailed 3D model of the rabbit ventricles and paced from the apex at a rate of 120 BPM with high-dose lidocaine (20 mM). An ectopic stimulus initiated a >2.2 s persistent reentrant wave in acidosis while <0.6 s in normal tissue.

CONCLUSIONS This is the most detailed acidosis action potential model up to now as we know, it included INa,IKr markov model, dynamic intracellular and extracellular pH changes. Furthermore, our study interpreted why lidocaine takes weak effects on acidosis, which provide a novel thinking of anti-arrhythmia drugs on heart diseases.

GW27-e0181

Secreted frizzled related protein 1 protects H9C2 cells from hypoxia/re-oxygenation injury by blocking the Wnt signaling pathway

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OBJECTIVES The present study was designed to investigate the possible protective actions of Sfrp1 on cardiac muscle cells using an in vitro model of ischemia/reperfusion, and to evaluate the possible involvement of the Wnt signaling pathway.

METHODS We used a recombinant AAV9 vector to deliver the Sfrp1 gene into H9C2 rat cardiomyoblasts and adopted an in vitro model of ischemia/reperfusion. Cell vitality was measured by CKK-8 and the trypan blue exclusion assay. Western blot was used to evaluate the expression of Dvl-1, -catenin, c-Myc, Bax, and Bcl-2. Flow cytometry analysis of cardiomyocyte apoptosis was performed.

RESULTS We confirmed that Sfrp1 significantly increased cell viability (assayed by trypan blue and CKK-8) and decreased apoptosis (assayed by flow cytometry analysis and the Bax/Bcl-2 ratio). These effects were partly attributable to the ability of Sfrp1 to down-regulate Wnt signaling pathway (assayed by Western blot to evaluate the expression of Dvl-1, -catenin, and c-Myc). Indeed, reactivation of the Wnt signaling pathway activity with the specific activator, Licl, reduced Sfrp1-induced cardioprotection during hypoxia and reoxygenation.

CONCLUSIONS The present study demonstrated that Sfrp1 directly protected H9C2 cells from hypoxia and reoxygenation induced reperfusion injury and apoptosis through inhibition of the Wnt signaling pathway, and added new mechanistic insight regarding the cardioprotective role of Sfrp1 on ischemic damage.

GW27-e0195

Interleukin 37 impact on the NF-kappa B-ICAM-1 in human coronary artery endothelial cells by TLRs mediated

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OBJECTIVES Testing under the treatment of TLRs ligand peptidoglycan, HCAECs respectively in normal state, IL - 37 interference and IL - 37 b expression condition, the expression of ICAM - 1 mRNA in 0, 30 min, 60 min and 120 min.And the protein expression of ICAM - 1in 24 hours. We want to explore IL - 37 in the role of conscious and peptidoglycan lead to HCAECs inflammation. Research state, three kinds of cells under the stimulus peptidoglycan, the nf-kappa B mRNA expression in 0, 30 min, 60 min and 120 min.And we detect the phosphorylation of nf-kappa B protein expression levels, discusses the possible mechanisms of IL -37 that down-regulation the expression of ICAM-1.After that we do and the results we got,we can and provides theory basis about atherosclerosis by metabolic target therapy.

METHODS 1.Treatment with PGN in 0, 30 min, 60 min, 120 min,we use RT-PCR to detect the mRNA expression of ICAM-1 and nf-kappa B and we use western blot to test the phosphorylation protein of nf-kappa B.Treatment with PGN in 24 hours, we test the ICAM 1 protein by western blot.

2.Using RNA interference suppression HCAECs to get the expression of IL - 37, treatment with PGN in 0, 30 min, 60 min, 120 min,we use RT-PCR to detect the mRNA expression of ICAM-1 and nf-kappa B and we use western blot to test the phosphorylation protein of nf-kappa B.Treatment with PGN in 24 hours, we test the ICAM-1 protein by western blot.

3.Using plasmid expression vector in HCAECs to get the high expression of IL - 37, treatment with PGN in 0, 30 min, 60 min, 120 min,we use RT-PCR to detect the mRNA expression of ICAM-1 and nf-kappa B and we use western blot to test the phosphorylation protein of nf-kappa B.Treatment with PGN in 24 hours, we test the ICAM-1 protein by western blot.

RESULTS 1.With PGN stimulation, HCAECs inflammatory factor ICAM - 1 mRNA and protein levels are rising in the untreated group, nf-kappa mRNA level and protein phosphorylation also rise.

2.With PGN stimulation,after interference HCAECs expression of IL - 37,ICAM - 1 mRNA and protein levels are increased significantly compared with normal cells, nf-kappa mRNA level and protein phosphorylation also increased significantly compared with normal endothelial cells.

3.With PGN stimulation,after transfection HCAECs expression of IL - 37, after testing ICAM - 1 mRNA level and protein level are significantly lower than normal cells, nf-kappa mRNA level and protein phosphorylation also are significantly lower than normal cells.

CONCLUSIONS IL - 37 can inhibit peptidoglycan to stimulate human coronary artery endothelial cells the secretion of ICAM-1.And the nf-kappa mRNA and protein phosphorylation level were decreased at the same time.

GW27-e0223

Angiotensin-(1-7) regulates Angiotensin II-induced apoptosis in smooth muscle cells

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OBJECTIVES Angiotensin-(1-7) (Ang-(1-7)) is discovered as a new peptide in the renin-angiotensin system. Ang-(1-7) has been shown to counteract many bioactivities of Angiotensin II (Ang II) and protect against cardiovascular disease. Smooth muscle cell apoptosis plays an important role in the progression of atherosclerotic plaque. Therefore, we aim to evaluate the effect of Ang-(1-7) on Ang II-induced apoptosis in human smooth muscle cells (SMCs).

METHODS MTT was used to determine cellular viability. Hoechst 33342 staining was used to analyze the apoptosis of SMC. Western Blot was performed to observe the expression of cleaved caspase-3.

RESULTS The results showed that Ang II decreased SMC viability, while Ang-(1-7) alone showed no effects. However, Ang-(1-7) negatively modulated the inhibitory effect of Ang II on SMC viability in a