concentration dependent manner. Ang II promoted fragmentation of nucleus and caspase-3 expression in SMCs, which was inhibited by Ang-(1-7). The utility of Ang II type 2 receptor antagonist effectively decreased Ang II-induced apoptosis. In addition, the inhibitory effects of Ang-(1-7) on Ang II-induced apoptosis were reversed by Ang-(1-7) antagonist A779.

CONCLUSIONS Our study suggests that Ang-(1-7) can negatively regulate Ang II-induced HSMC apoptosis, indicating that Ang-(1-7) may play a protective role in atherosclerosis via inhibiting SMC apoptosis.

GW27-e0227

Downregulation of the β1 adrenergic receptor in myocardium by AAV9-shRNA-ADRB1-ZsGreen injection reduce blood pressure in spontaneously hypertensive rats

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OBJECTIVES To explore the effect of downregulation of β1-AR in myocardium of SHRs on antihypertensive effect.

METHODS The recombinant plasmid pAAV-ZsGreen-ADRB1-shRNA was confirmed by enzyme digestion and gene sequencing. The plasmid was then packaged to recombinant adeno-associated virus named AAV9- shRNA-ADRB1-ZsGreen. The purity, titer and infection activity in vitro were detected. 18 SHRs were randomly divided into three groups as follows: AAV9-shRNA-ADRB1-ZsGreen injection group, AAV9-CMV-ADRB1-ZsGreen injection group and sham group. SHR myocardium was transfected by injecting virus vector to the pericardial cavity. Rats were killed after 4 weeks. The blood pressure was monitored weekly. The expression of β1-AR in myocardial tissue was assessed by Real-time PCR and Western blot.

RESULTS The pAAV-ZsGreen-ADRB1-shRNA plasmid was confirmed to be correct by enzyme digestion and gene sequencing. The purified AAV9- shRNA-ADRB1-ZsGreen titer was 1.5x1012vg/ml. In addition, the transfection efficiency of this viral vector to 293 cells reached up to 95%.

β1-AR mRNA was significantly decreased by AAV9-shRNA-ADRB1-ZsGreen injection compared with the negative control group (0.4142 ± 0.3399 vs. 1.0933 ± 0.5853, $P < 0.05$). The β1-AR protein expression was significantly lower than the negative control group (0.5156 ± 0.1199 vs. 0.8333 ± 0.1728, $P < 0.05$). Immunohistochemistry results showed that β1-AR protein expression in AAV9-shRNA-ADRB1-ZsGreen injection group was significantly decreased compared with the negative control group and sham group (50.02 ± 4.44 vs. 80.05 ± 3.72 vs. 79.30 ± 2.79, $P<0.05$).

CONCLUSIONS β1-AR expression in myocardium of SHRs was reduced by AAV9-shRNA-ADRB1-ZsGreen injection. Downregulation of β1-AR in myocardium of SHRs resulted in lower blood pressure.

GW27-e0241

Literature-based metabolomics study on myocardial infarction model of rats

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OBJECTIVES To analyse the metabolic pathways of myocardial infarction(MI) model of of rats by bioinformatics method.

METHODS The research literature of MI of rats were retrieved from China Knowledge Resource Integrated Database (CNKI), Wanfang Data Resource System (WANFANG DATA), VIP network (VIP), PubMed and Embase. The metabolic products described in the literature were collected and summarized. Molecular function annotation used HMDB database;Signaling pathways were analysised using KEGG database;Andmetabolites pathway visualization was performed with Metabolomics Pathway Analysis(MetPA).

RESULTS A total of 26 metabolic product were identified identified more than 2 times in the included literatures.Those metabolites related to 29 metabolic pathways.By using topology analysis, a total of 5 metabolic pathways that were selected and regarded as the metabolic pathways of MI in rats.They were Aminoacyl-tRNA biosynthesis; Glycine,serine and threonine metabolism; Valine, leucine and isoleucine biosynthesis; Biosynthesis of unsaturated fatty acids; Phenylalanine,tyrosine and tryptophan biosynthesis.

CONCLUSIONS The bioinformatics analysis of metabolites of MI in rats showed that the nosogenesis of MI related to the metabolism and metabolic pathways of carbohydrates metabolism, proteinmetabolism,fatmetabolism and RNA transport.The results of this study may have some implications for finding new diadynamic criterias and methods of MI.

GW27-e0242

Effect of TRPV4 on renal injury induced by angiotensin II in mice

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OBJECTIVES It is well established that transient receptor potential vanilloid type 4 channel (TRPV4) is primarily expressed in endothelial cells, and activation of TRPV4 enhances inflammatory response characterized by monocyte/macrophage infiltration. Hypertension is one of the most key risk factors contributing to renal injury. Angiotensin II (Ang II) is the most important pathological factor in the development of hypertension. Recent studies have shown that monocyte/macrophage mediates Ang II-induced renal injury. Therefore, this study was designed to determine whether deletion of TRPV4 attenuates Ang II-induced renal injury.

METHODS Ang II was infused systemically into wild type (WT) and TRPV4-null mutant (TRPV4-/-) mice for 4 weeks.

RESULTS The increased systolic blood pressure determined by tail-cuff was found in Ang II-treated WT and TRPV4-/- mice with the similar degree ($P>0.05$). Ang II treatment increased urinary excretion of albumin and 8-isoprostane, and decreased creatinine clearance in both WT and TRPV4-/- mice with a greater magnitude in the former strain (albumin: 32.2±3.7 vs. 15.6±4.5 μg/24h; 8-isoprostane: 3.12±0.98 vs. 1.39±0.62 ng/24h; creatinine clearance: 197±21 vs. 257±28 ml/24 h, $P<0.05$). Periodic acid-Schiff and Masson's trichrome staining showed that kidneys of Ang II-treated WT mice exhibited more severe glomerulosclerosis and tubulointerstitial injury compared with Ang II-treated TRPV4-/- mice (glomerulosclerosis index: 1.78±0.30 vs. 1.01±0.22; tubulointerstitial injury score: 3.20±0.83 vs. 1.96±0.71, $P<0.05$). Hydroxyproline assay and F4/80-staining showed that renal collagen levels and monocyte/macrophage infiltration were greater in Ang II-treated WT mice compared with TRPV4-/- mice (collagen content: 26.8±3.5 vs. 10.3±3.8 μg/mg dry tissue; monocyte/macrophage infiltration: 69±10 vs. 39±8 cells/mm^2, $P<0.05$).

CONCLUSIONS Thus, our data show that deletion of TRPV4 alleviates renal injury in the absence of alteration in blood pressure in Ang II-hypertensive mice. The results suggest that TRPV4 could serve as a novel target for treating renal injury associated with hypertension. [This work was supported by grants from the Henan Provincial Science and Technique foundation for International Cooperation Program (No. 162102410048) and National Natural Science Foundation of China (No. 81170243). Corresponding author: Youping Wang].

GW27-e0244

Isoproterenol Stress Testing in the Evaluation of Electromechanical-Window in Long QT Rabbits Induced by Hypokalemia

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OBJECTIVES Electrical and mechanical abnormalities play an important role in symptomatic individuals with LQTS or LQT subjects under the condition of low-dose isoproterenol, despite the paradoxic increase in the uncorrected QT interval. Profound electromechanical window(EMW) negativity correlates significantly with major arrhythmic events. However, how the relationship of electrical and mechanical is regulated in LQTS induced by cell surface ion channel expression levels degradation in hypokalemia is uncertain. This study is to determine the Electro-mechanical window(EMW; duration of LV-mechanical systole minus QT interval) in addition of isoproterenol infusion mimicking sympathetic stimuli, to find out whether it is responsible for the development of life-threatening arrhythmias either in vivo or in vitro under in hypokalemia or low K+ conditions. Moreover, it helps to provide further evidence to prove that EMW is superior in detecting arrhythmias than QT interval alone.

METHODS We used hypokalemia rabbit model induced by low potassium diet and performed electrocardiogram in in vivo. We also maked wedge of rabbit left ventricle and perfused with low K+ Tyrode's solution, contraction and a transmural electrocardiogram were simultaneously recorded in vitro. QT interval was subtracted from the duration of Q-onset to aortic-valve closure (QAoC) midline assessed non-invasively by continuous-wave echocardiography, measured in the same beat in hypokalemia rabbits. The effect of beta-adrenergic agonists isoproterenol on corrected QT interval(QTc) and Electro-mechanical window(EMW) were examined in vivo and in vitro. Western blot was carried out to observe the IKr protein and explore the molecular basis underlying the mechanism of isoproterenol in the process.

RESULTS Hypokalemia prolonged corrected QT interval (0.32 ± 0.01 sec, versus 0.26 ± 0.03 sec before feeding; n = 3; P < 0.05) in rabbits. Low [K+]o (2 mmol/L) Tyrode's solution perfusion significantly prolonged QT interval in rabbit left ventricular arterially perfused wedges(370.33 ± 20.14ms, versus 347.33 ± 14.87ms before low K+ perfusion; n = 6; P < 0.05). Addition of isoproterenol(0.03ug/kg) decreased the QTc interval (0.24 ± 0.02 sec, versus 0.31 ± 0.00 sec before injection; n = 3; P < 0.05)in hypokalemia rabbits. However, in vitro ISO(100nM/L) decreased the negativity of EMW(154.67 ± 19.64ms, versus 170.00 ± 27.06ms before ISO perfusion; n = 3; P=0.437). The expression level of the IKr protein (110-kDa band) in rabbits on the low-K+ diet was much lower than that in control rabbits.

CONCLUSIONS Beta-adrenergic stimulation decrease the negativity of EMW in low K+ solution induced LQT2 in vitro, probably because of potassium fluctuation during ISO infusion in wedges, in contrast with hypokalemia rabbits in vivo which IKrprotein expression levels was degraded, presented as permanently IKr blockage.

GW27-e0247

Impaired the effects of nitric oxide on BKCa in human Mesenteric Artery Smooth Muscle Cells during Hypertension

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OBJECTIVES The large conductance calcium-activated potassium channels (BKCa) dysfunction occurs in several vascular diseases including hypertension. Nitric Oxide (NO) is an endothelium-independent relaxant agent and also one of the important cellular messengers to regulate the function of cardiovascular system. The effect of NO on BKCa in human artery SMCs during hypertension is rarely studied. The aim of present study was to investigate whether the change of NO on BKCa channels during hypertension.

METHODS The use of sodium nitroprusside (SNP) as NO donor in solutions was utilized. The freshly isolated SMCs were used to record BKCa currents using the patch-clamp technique.

RESULTS (1) In cell-attached patch (Vm = + 40 mV), 100μM SNP stimulates BKCa activity significantly in the normotensive patients (NT group): NO enhanced BKCa open probability(NPo), from 0.007±0.002 to 0.018±0.006, the mean open time (To) of BKCa-channels was increased from (6.128 ± 1.003) ms to (12.532±2.031) ms, and the mean close time (Tc) of BKCa channels was reduced from (1486.862±246.189) ms to (806.327 ± 193.261) ms (n=8, P<0.05). In the hypertensive patients (HT group), NOhad no significant effect on BKCa. (2) In inside-out patch (Vm=+40 mV), application of SNP, NPo of BKCa channel was increased from 0.006 ± 0.001 to 0.015±0.003, To of BKCa channels was increased from (5.963±0.821) ms to (11.972±1.782) ms, andTc of BKCa channels was reduced from (1648.634±367.326) ms to (753.017±258.148) ms (n=7, P <0.05). In the HT group, NO had no significant effect on BKCa.(3) In the amphotericin-perforated whole-cell patch-clamp configuration, the current densityof BKCa in the NT group at the voltage of -60～ +20 mV had no significant change after adding 100μM SNP, but the current density of BKCa at the voltage of + 30 mV, + 40 mV, + 50 mV and + 60 mV was increased significantly, from 11.352±1.256 pA/pF, 16.633 ±1.841 pA/pF, 22.4227±1.287 pA/pF and 29.434±1.346 pA/pF to 15.530±1.339 pA/pF, 22.517±1.463 pA/pF, 31.672±1.438 pA/pF and 45.657±2.981 pA/pF (n=7, P<0.05). In the HT group, AngII had no significant effect on macroscopic current of BKCa channels.

CONCLUSIONS Our results suggested that NO can activate BKCa channels of human mesenteric artery SMCs. Furthermore, the sensitivity of BKCa channels to NO is significantly lowered during hypertension.

GW27-e0256

Panax quinquefolium saponin combined with dual antiplatelet drugs inhibits platelet adhesion to injured HUVECs via PI3K/AKT and COX pathways

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OBJECTIVES To compare PQS combined with DA and DA alone in inhibiting platelet adhesion to injured human umbilical vein endothelial cells (HUVECs) and to explore the possible mechanisms focusing on PI3K/AKT, COX-2/6-keto-PGF1α, and COX-1/TXB2 pathways.

METHODS HUVECs injured by oxidized low-density lipoprotein(ox-LDL) were randomly allocated into control, model, DA, PQS+DA (P+DA), LY294002 (a PI3K inhibitor)+DA (L+DA), and LY294002+PQS+DA (LP+DA) groups. HUVEC apoptosis, platelet adhesion to injured HUVECs, and platelet CD62p expression were assayed by fluorescence activated cell sorting (FACS). The concentrations of 6-keto-PGF1α and TXB2 in the supernatant were measured by radioimmunoassay. Protein expression of phosphorylated-PI3K, PI3K, phosphorylated-AKT, AKT, COX-1, and COX-2 in both platelets and HUVECs was evaluated by western blot.

RESULTS Compared to DA alone, PQS combined with DA reduced platelet adhesion to HUVECs and HUVEC apoptosis more potently, increased the concentration of supernatant 6-keto-PGF1α and up-regulated phospho-AKT protein in HUVECs. LY294002 mitigated the effects of PQS on HUVEC apoptosis and platelet adhesion.

CONCLUSIONS These findings show that PQS as a powerful supplement to DA, attenuated HUVEC apoptosis and improved the DA-mediated reduction of platelet adhesion to injured HUVECs and the underlying mechanisms may be associated with PI3K/AKT and COX pathways in HUVECs and platelets. PQS might provide a new complementary approach to improve the prognosis of thrombotic diseases in future.

GW27-e0258

Regulation of AT1-Calcineurin Signaling Pathway on Ion Channels Expression of Hypertrophic Ventricular Myocytes

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OBJECTIVES To explore the role of angiotensin receptor I type (AT1)-calcinerurin signaling pathway on ion channels expression in hypertrophic neonatal rat ventricular myocytes.

METHODS Ventricular myocytes were isolated from the ventricles of 1 day-old neonatal Sprague-Dawley rats and cultured for 48 hours before any treatment. Drug interventions were as follows, 1) Control group. 2) Phenylephrine (PE) group, incubation with PE (100μM) for 24 hours. 3) Losartan (Los) + PE group, incubation with Los (10μM) for 2 hours then treated with PE (100μM). 4) CyclosporinA (CsA) + PE group, incubation with CsA 10μM for 2 hours then treated with PE (100μM). The MOI=50 corresponding to the amount of adenovirus was choosen, and adenoviral RNA interference (RNAi) vector administration was as follows, 1) Ad-Null group, transfection with the adenovirus containing the empty expression vector for 48 hours. 2) Ad-Null+PE group, transfection with the adenovirus containing the empty expression vector for 24 hours then treated with PE (100μM) for 24 hours. 3) Ad-CnAβshRNA1, transfection with the adenovirus containing the CnAβshRNA1 for 48 hours. 4) Ad-CnAβshRNA1+PE group, transfection with the adenovirus containing the CnAβshRNA1 for 24 hours then treated with PE (100μM) for 24 hours. Gene expressions of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), myosin heavy chain beta(β-MHC), Nav1.5 and Kv4.2 were detected by real-time reverse transcriptase-polymerase chain reaction(real-time RT-PCR). CnAβ, Nav1.5, Kv4.2 proteins in whole-cell extracts were assayed by western blot analysis.

RESULTS PE treatment increased the protein-to-DNA ratio and the gene expressions of ANP, BNP and β-MHC. The size of cell surface was also increased after PE treatment. Treatment of neonatal rat ventricular myocytes with PE increased the protein expression of CnAβ, and reduced the protein expression of subunit Nav1.5, but didn't significantly altered its gene expression. The gene and protein expressions of Kv4.2 were reduced by PE treatment. Los and CsA both prevented those effects of PE.Siliencing of CnAβ in neonatal rat Ventricular myocytes using Ad-CnAβshRNA inhibited the ability of PE to increase the gene expression of BNP, and diminished the ability of PE to reduce