The Anti-atherosclerotic Effect of Pseudolaric acid B in High-Fat Fed Apoe-Knockout Mice: Alterations in Circulating and Atherosclerotic Plaque Monocytes/Macrophages and The Underlying Mechanisms

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OBJECTIVES Atherosclerosis (AS) is the pathological basis of cardiovascular and cerebrovascular diseases. The chronic inflammation and lipid infiltration theory are thought to be the pathogenesis of AS. Mononuclear phagocytes (MNP) are key players involved in the lipid metabolism of plaque and chronic inflammation. Pseudolaric acid B (PB) is an extract of pseudolarics. Recently, we show that PB could activate peroxisome proliferator activated receptor γ, while is an important molecular target involved in the process of inflammation reaction and lipid metabolism. Accordingly we hypothesis that PB has therapeutic potential for AS.

METHODS In vivo study, Apoe−/− mice (8 weeks) were consumed high fat diet for 8 weeks, then randomly grouped into the baseline group, high fat diet group, PB treated with high fat diet group, normal diet group and PB treated with normal diet group (n=7 each group). We did express analysis, the proportion of monocyte subsets were analyzed. Confocal microscopy was used to investigate the density of the proliferation macrophages in plaque of aortic root. Oil Red O staining was used to explore the plaque size of aortic, common carotid artery and the aortic root. plasma triglycerides and total cholesterol were measured by real-time PCR. Western-blots assays were used to check relevant gene expression. Ectopic expression of miR-185 enhanced the apoptosis of primary cardiomyocytes induced by doxorubicin by directly targeting AKT1. Downregulation of AKT1 phenocopied the pro-apoptotic effect of miR-185. Furthermore, administration of constitutively active AKT1 promoted primary cardiomyocytes proliferation, and reversed pro-apoptotic effect of miR-185 overexpression.

RESULTS MiR-185 exacerbates CHF and reduces cardiomyocyte proliferation through downregulation of AKT1, suggesting that miR-185 might be a target to manage cardiac dysfunction.

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Apelin Ameliorates Myocardial Insulin Resistance and Improves Myocardial Injury in Diabetes

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OBJECTIVES Apelin as a beneficial adipokine has been linked to insulin resistance and cardiovascular protection in diabetes, however, the underlying molecular mechanisms of how apelin affects myocardial insulin resistance and myocardial injury remain poorly understood.

METHODS We collected 131 T2DM patients serum, myocardial tissues of 8 patients with T2DM and 6 accidental deaths without T2DM at Beijing Hospital. After the TNF-α administration, C57BL/6J male mice were randomized to receive either vehicle, apelin-13(10μg/kg), or F13A (a competitive antagonist for apelin receptor 20μg/kg) by i.p. injection.

RESULTS Plasma samples of 131 patients with type 2 diabetes mellitus (T2DM) and 54 healthy controls, and myocardial tissues of 8 T2DM patients and 6 accidental deaths without T2DM were collected. Increased levels of plasma apelin, ROS and TNF-α were accompanied by insulin resistance and myocardial injury in patients with T2DM, similar results were confirmed in diabetic ob/ob mice, suggesting that apelin, TNF-α and ROS were correlated with insulin resistance. In vitro, TNF-α could stimulate myocardial insulin resistance, ROS generation, and apelin expression in H9c2 cells. However, exposure apelin-13 to TNF-α-treated H9c2 cells and intraperitoneal injection of apelin-13 to TNF-α-induced insulin-resistant C57BL/6J mice rescued TNF-α-induced higher intracellular glucose content and impaired insulin signaling pathway. Moreover, the results demonstrated that an intraperitoneal injection of apelin-13 improved MI-induced increased glucose content and cardiac injury as evidenced by apelin, reduced myocardia inflammation and decreased myocardial apoptosis in both ob/ob mice and C57BL/6J mice. Further studies indicated that apelin-13 suppressed ROS generation through down-regulation of iNOS expression. Finally, F13A, a competitive antagonist for apelin receptor APJ, suppressed the effects of apelin-13 on insulin signaling pathway in both insulin-resistant H9c2 cells and insulin-resistant mice. Interestingly, F13A also impaired the effects of apelin-13 on iNOS expression, demonstrating that apelin ameliorated TNF-α-induced myocardial insulin resistance by its receptor APJ.

CONCLUSIONS We provide novel experimental evidence that apelin could ameliorate myocardial insulin resistance and improve myocardial injury through suppressing ROS generation via down-regulation of iNOS expression, in term, elevating insulin signaling transduction.

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confocal imaging. The cytokines in the supernatant of CD4+ T cells were determined by mouse CBA inflammation Kit (IL-6, IL-10, MCP-1, IFN-gamma, TNF and IL-12p70) and CBA Flex Set (IL-2, IL-4, IL-5, IL-13, IL-17A, MIP-1α and MIP-1β), the expression of cytokines of mice splenic CD4+ T cells were analyzed using quantitative RT-PCR.

**RESULTS** The supernatant of Necrosis group up-regulated DC maturation markers such as CD40, CD80 and CD86. After mice tail vein injection of DEXs, the average fluorescence efficiency in spleen was increased to peak on day 7 in negative and control groups but on day 2 in necrosis group, and the peak of fluorescence efficiency was significantly higher in necrosis group than in control and negative groups. But there was no significant difference between control and negative groups. The uptake of DEXs from necrosis group by CD4+ T cells, certified by confocal imaging, induced significant increases in the expression of chemokines and inflammatory cytokines by the cells both in vitro and vivo as compared with those from negative and control groups.

**CONCLUSIONS** Our results imply that CD4+ T cells are partly activated by DEXs through an endocrine pathway post-MI, our findings may provide a novel strategy for the treatment of MI through systemic delivery of DEXs after MI.

**GW26-e0478**

**Block of Human Cardiac Na+ Channels by Volatile Oil of Nardostachy Chinesis Batal**

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**OBJECTIVES** Nardostachys chinesis Batal (Gansong), one of the ingredients of herbal medicine, is clinically used in China for controlling cardiac arrhythmia. This study was aimed at elucidating the mechanism of block of cardiac Na+ channels by the volatile oil of Gansong.

**METHODS** cdNA encoding the human cardiac Na+ channel (hNa1.5) was expressed in human embryonic kidney (HEK293) cells, and channel activity was studied using the whole-cell patch clamp technique.

**RESULTS** The degree of block was reduced by hyperpolarization. Gansong shifted the voltage dependence of fast Na+ channel inactivation in the hyperpolarizing direction while shifting the voltage dependence of channel activation in the depolarizing direction. The voltage dependence of slow inactivation was not altered by Gansong. Analysis of the shift in the voltage dependence of fast inactivation by Gansong revealed that Gansong has a higher affinity for the fast inactivated Na+ channels than the resting channels.

**CONCLUSIONS** Gansong should be very effective in blocking Na+ channels in tissues where the membrane is depolarized. In addition, the opposite shift in the voltage dependence of activation and inactivation should reduce the window current, which could correct the delayed action potential repolarization seen in LQTS phenotype.

**GW26-e0714**

**MiR-139-3p Is Related to Left Ventricular Hypertrophy and Apoptosis in Hypertensive Rats**

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**OBJECTIVES** MicroRNAs (miRNAs) are key post-transcriptional regulators of gene expression. In previous studies have reported the role of miR-139-3p in cancer. However, its specific roles and functions in the heart undergoing hypertrophy and apoptotic processes have yet to be fully elucidated.

**METHODS** Male Wistar rats (weight, 120-150 g) were randomly divided into two groups; 2K1C group (n = 14) and sham group (n = 12). The systolic blood pressure (SBP) of the rats in each group was measured weekly using a non-invasive computerized tail-cuff system. Eight weeks after the initial surgery, the heart and kidneys of rats were immediately removed, blotted dry, weighed and stored at -80°C. Heart samples were stained with hematoxylin-eosin (HE) and van Gieson’s (VG). MicroRNAs expression profiling was performed with 821SK Agilent Rat miRNA Microarray V12.0 containing probes for 350 miRNAs. MicroRNAs with the highest fold change revealed by miRNA microarray experiments were selected for further validation by quantitative real-time PCR (qRT-PCR). All samples were run in triplicate. The target genes, biological functional of the differentially expressed rno-mir-139-3p were identified using IPA program. Meanwhile, the target genes of rno-mir-139-3p were subjected to GeneGo pathway annotation.

**RESULTS** The 2K1C rats exhibited an increase in SBP (P < 0.05) and a significant increase in the weight of the heart, the cardiac index and the right renal index after 8 weeks when compared with the sham surgery group (P < 0.05). 2K1C rats promoted cardiomyocyte hypertrophy and increased interstitial collagen deposition and infiltration of the myocardium. Furthermore, miRNAs were differentially expressed between two groups. The analysis identified that 11 miRNAs were upregulated and 18 were downregulated in 2K1C group compared with sham-operated group (P < 0.05). The highest upregulated expression miRNA was rno-mir-139-3p, the results were confirmed by using Q-RT-PCR. The target genes of the differentially expressed rno-mir-139-3p were analyzed using IPA tool. One target gene MAPK1 was identified after analysis of published data, which has been validated by biological experiments. Of relevant diseases and biological functions of rno-mir-139-3p, Cell Death and Survival was the highest rated function. Among the Cell Death and Survival, cell death and apoptosis were identified as the top 2 significant functions. GeneGo analysis revealed that target gene MAPK1 mainly participate in pathways linked to the apoptosis.

**CONCLUSIONS** The present study suggests that miR-139-3p may play a key role in the left ventricular remodeling process. Analysis of its target genes and signaling pathways may add new mechanism to its roles.

**GW26-e0722**

**Repression of miR-1 Is Required for Recovery of Hsp90aa1 Against Cardiac Ischemia/Reperfusion Injury in Rats**

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**OBJECTIVES** MicroRNA-1 (miR-1) is one of the abundant microRNAs in heart, but its expression and potential targets in myocardial ischemia/reperfusion (I/R) injury is not well known. This study investigated the role of miR-1 in I/R-induced apoptosis of cardiomyocytes.

**METHODS** I/R was induced by 45-min occlusion, followed by reperfusion of the left anterior descending coronary artery (LAD). The rat ischemic myocardium was harvested for investigation on day 1, 3 and day 7 post-I/R, respectively. The ischemic injury on neonatal rat ventricular cells (NRVCs) was induced by hypoxia in a serum- and glucose-free medium and reoxygenation.

**RESULTS** Significant apoptosis of cardiomyocytes was observed in the ischemic rat myocardium on day 1 post-I/R, and the cardiac apoptosis was shown decreased on day 3 and day 7 post-I/R. Consistently, protein expression of heat shock protein 90 (Hsp90) αα1, but not Hsp90β1, markedly reversed on day 3 and day 7 post-I/R. Quantitative reverse transcription-PCR (qRT-PCR) showed that miR-1 was significantly reduced on day 3 and day 7 post-I/R. Repression of miR-1 in cultured NRVCs led to increase of Bcl-2 and decreases of Bax and active caspase-3. The dual luciferase reporter assay revealed that miR-1 interacted with the site of 310-315 nt at the 3’UTR of Hsp90αα1, and miR-1 was verified to inhibit Hsp90αα1 expression at the posttranscriptional level. Additionally, miR-1 mimic, in parallel to Hsp90αα1 siRNA, could enhance oxygen-glucose deprivation (OGD)-induced apoptosis of NRVCs, with decrease of Bcl-2 and increases of Bax and active caspase-3.

**CONCLUSIONS** MiR-1 was demonstrated decreased in rat myocardium post-I/R, the repression of miR-1 contributed to recovery of Hsp90αα1 against myocardial I/R injury.

**GW26-e0766**

**Effects of Mesenchymal Stem Cell Transplantation on the Ventricular Fibillation Threshold in Rats with Myocardial Infarction in Short-Term, Medium-Term and Long-Term Period**

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**OBJECTIVES** Arrhythmia is of concern after mesenchymal stem cell (MSC) transplantation in repairing infarcted myocardium. However, whether transplanted MSCs improved ventricular fibrillation threshold (VFT) in the myocardial infarction model is still unclear. We sought to investigate the VFT in rats with myocardial infarction treated with MSCs.