GW26-e4718
Oleanolic Acid Alleviated Pressure-over Load Induced Cardiac remodeling
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OBJECTIVES Previous study has demonstrated that oleanolic acid (OA) possessing the anti-inflammatory and antioxidant properties, blunted high glucose induced diabetic cardiomyopathy, and ameliorated experimental autoimmune myocarditis in mice. However, little is known about its effects on pressure overload induced cardiac remodeling. Herein, we investigated the effect of OA on cardiac remodeling and underlying mechanism.

METHODS Mice, subjected to aortic banding (AB), were randomly assigned into control group and experimental group. OA premixed in diets was administered to mice after 3 days of AB. Echocardiography and catheter-based measurements of hemodynamic parameters were performed after 8 weeks’ treatment of OA. Histologic examination and molecular analyses was used to assess cardiac hypertrophy and tissue fibrosis. In addition, we investigated the inhibitory effects of OA on H9c2 cardiomyocytes and cardiac primary fibroblast in vitro.

RESULTS We demonstrated that OA alleviated cardiac remodeling induced by pressure-over load (AB), evidenced by heart weight/body weight and lung weight / body weight ratios, echocardiographic and hemodynamic parameters, gene expression of hypertrophic and fibroblastic markers in vivo and vitro. Pressure overload activated the phophorylation of Akt, mTOR, p70S6k, S6, GSK3β and Foxo3a, and treatment of OA attenuated the phosphorylation of these proteins.

CONCLUSIONS Our findings suggest that treatment of OA may have a benefit on retarding the progress of cardiac remodeling under long terms of pressure overload. The inhibitory effects of OA on cardiac remodeling may partly involve in the modulated of Akt/mTOR signaling pathway.

GW26-e4739
MaxiK Channel Responsible for Human Macrophage-derived Foam Cell Differentiation via Intracellular Ca2+ - Signaling Pathway
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OBJECTIVES The role of MaxiK channels in macrophage immunomodulation has been established. However, it remains unclear whether it is involved in the human macrophage-derived foam cell formation.

METHODS Human peripheral blood monocytes were separated from the platelet-free blood by cell density gradient centrifugation, and then cultured for 5d to differentiate into macrophages. The ratio of cholesterol ester (CE) in the macrophages following intake of oxidized low density lipoprotein (ox-LDL) was analyzed by an enzymatic fluorometric method. The expression level and function of MaxiK channels were investigated using real-time RT-PCR, western blotting, and patch clamp techniques, respectively. The membrane potential was analyzed with the optical mapping of the membrane potential with the voltage-sensitive dyes. Ca²⁺ fluorescence intensity of macrophages was measured by laser confocal microscopy.

RESULTS After the macrophages co-incubated with 30 mg/L ox-LDL at 37°C for 60h, the cellular volume obviously enlarged and many red lipid granules were deposited in cytoplasm. The total amount of cholesterol (TC), free cholesterol (FC) and cholesterol ester (CE) in cells markedly increased and the ratio of CE/TC rose from (29.1±2.4)% to (61.1±6.2)% (n=5, P<0.05). Meanwhile, MaxiK mRNA and protein expression were 2.4 and 7.27 times higher than those in 5d group (P<0.05). However, its current densities did not show a significant difference (31.6±1.4 pA/pF vs 32.4±4.0 pA/pF, P=0.05). MaxiK channel current (I_maxiK) in 5d and 10 μM/L, the specific blocker of MaxiK channel and inhibiting foam cells formation, at the two doses dose-dependently reduced the macrophage membrane potential over by 30% and 47%, respectively (P<0.05). Compared with ox-LDL group, calcium fluorescence intensity of the macrophages decreased by Pavilxin (4 and 10 μM/L) from (766.37±55.10) to (488.32±43.12) and (237.32±24.74), respectively (n=10-15, P<0.01).

CONCLUSIONS Although the expression of MaxiK channel is up-regulated after differentiating into foam cells, the current densities remains unchanged. It plays a key role in the lipid uptake of macrophage and formation of foam cells via intracellular Ca²⁺ signaling pathway.

GW26-e5327
Micro-calciﬁcation Regression in ApoE-/- Mice Spontaneous Atherosclerotic Plaque by Simvastatin on Inhibition of Endoplasmic Reticulum Mediated Apoptosis
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OBJECTIVES To investigate the effect of simvastatin on atherosclerotic micro-calcification and its endoplasmic reticulum mediated apoptosis pathway.

METHODS 24 male ApoE-/- mice on a C57BL/6J genetic background and 12 male C57BL/6J mice were selected when they were 8-week-old. 24 male ApoE-/- mice were randomly divided into model group and simvastatin group (n=12 per group). 12 male C57BL/6J mice were regarded as control group. After receiving adaptive feeding in the animal center for two weeks, all mice were treated with intragastric administration, and model group and simvastatin group were feed with a high-fat diet. Control group and model group received 0.2ml PBS at every per day for 4 weeks. Simvastatin group received simvastatin (20mg/kg dissolved in 0.2ml PBS buffer for intragastric administration) every day for 8 weeks. The body weight of mice were recorded every day during the whole experiment. They were sacrified at the end of the study. And the serum of the three groups were processed with HE, Von kossa, TUNEL and immunohistochemical staining to observe plaque size, atherosclerotic micro-calcification, apoptosis and expression of related endoplasmic reticulum pathway proteins.

RESULTS Compared to model group, simvastatin group was not statistically significant (P<0.05). Serum lipid parameters of TG, TC, LDL-C, HDL-C in the model group were signiﬁcantly higher, compared with control group and model group (P<0.01). HDL-C/LDL-C value of simvastatin group was 0.17 ± 0.005, which represented a slight increase compared with model group (0.15 ± 0.003), but the difference was not statistically signiﬁcant (P = 0.09). Percentage of aortic plaque area of model group and simvastatin group were (54.50 ± 15.41)% and (33.69 ± 9.72)% which simvastatin group signiﬁcantly reduced (P<0.05). And the mean plaque area of aortic sinus of simvastatin group was signiﬁcantly less than model group (P<0.05). No calcification was found in control group. In addition, percentage of micro-calcification area of simvastatin group (2.33 ± 0.73)% was lower than model group (10.87 ± 2.14)% (P<0.05). A certain level of apoptosis was observed in each group. Apoptosis rate of control group was (30.90 ± 1.75)%, model group (66.43 ± 4.05)% and simvastatin group (47.01 ± 5.94) %, respectively. Apoptosis rate of model group was signiﬁcantly higher than control group (P<0.01). And it was significantly reduced in simvastatin group compared with the model group (P<0.05). Protein expressions in aortic sinus of the three groups of GRP78, CHOP and Caspase12 were observed in varying degrees. The highest expression levels of the three proteins were observed in model group, which were signiﬁcantly decreased in simvastatin group.

CONCLUSIONS Simvastatin may reduce endoplasmic reticulum stress-mediated apoptosis involved regression of atherosclerotic micro-calcification.

GW26-e0405
The applied research of C-phycocyanin from spirulina platensis on the targeted therapy of CD59 gene in atherosclerotic mice
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OBJECTIVES To study the effects of C-phycocyanin on occurrence and development of atherosclerosis, and research the regulatory effects of CD59 gene on anti-atherosclerosis of C-phycocyanin(C-PC).

METHODS 80 mice with ApoE gene deletion (ApoE(-/-)) were randomly divided into five groups: control group, CD59siRNA
treated group, CDS9 treated group, C-PC treated group and C-PC+CD59 treated group. The high-fat diet fed mice were treated with drug intervention. At the end of the 12th week, CD59 mRNA levels in whole blood were determined by RT-PCR and CD59 protein expressions in tissue were detected by western blot. The biochemical indexes such as TG, TC, LDL, HDL and ApoB in blood serum were detected. The paraffin sections of aortic root of mice were made and the degrees of atherosclerotic plaques formation were observed by HE staining. The expressions of cell apoptosis related proteins (Bcl-2 and Fas) and plaque stability related protein (MMP-2) were detected by immunohistochemistry. Then the cell apoptosis levels were detected by TUNEL, the expression of cell cycle protein Di (Cyclin D1) were detected by immunofluorescence and the mRNA level of cyclin dependent protein kinase 4 (CDK4) were detected by qRT-PCR.

RESULTS Atherosclerotic mouse model was successfully established. CD59 gene was overexpressed in blood cells and tissue cells by liposome transfection. Results showed that both CD59 and C-PC could reduce blood lipid levels, positively regulate cell cycle, maintain the stability of cell proliferation and apoptosis of aorta cells, and finally slow down the development of atherosclerotic vulnerable plaque. In addition, C-PC was proved to be able to promote expression of CD59 gene in mice.

CONCLUSIONS Both CD59 gene and C-PC can significantly inhibit the progression of atherosclerosis in ApoE (−/−) mice, and the anti-atherosclerotic effects of C-PC may be fulfilled by promoting the CD59 gene expression and smooth muscle cell proliferation and preventing the apoptosis of endothelial cells, reducing blood fat levels, and at last inhibit the development of atherosclerosis.

GW26-e0440
CamKII is involved in the cardiotoxicity of Doxorubicin
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OBJECTIVES To explore the roles of CamKII in Doxorubicin (DOX) induced cardiomyopathy.

METHODS After treated with DOX, the cell viability, as well as the expression level and activity of CamKII of cardiomyocytes H9c2 were monitored. The CamKII gene was knocked out using CRISPR method. The responses to DOX treatment, including the variation of viability, NF-κb activity and miR-146a expression, were compared between paracrine and CamKII KO cardiomyocytes.

RESULTS DOX inhibited the proliferation of cardiomyocytes, in which the activity of CamKII was increased, with little changes in expression level. The CamKII expression was successfully knocked out using CRISPR. The sensitivity to DOX was decreased after CamKII knockout. Meanwhile, deletion of CamKIIB enhanced the DOX induced NF-κb activation and miR-146a upregulation.

CONCLUSIONS CamKII participates in the cardiotoxicity of DOX, which involve NF-κb and miR-146a.

GW26-e0495
Impact of Myocardial Remodeling in a Rat Model of Heart Failure Transfected with Recombinant Adenovirus Vector-mediated Klotho Gene
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OBJECTIVES To assess the impact of Transfection with recombinant adenovirus vector-mediated Klotho gene on myocardial remodeling in a rat model of heart failure (HF) by intraperitoneal injection of isoproterenol. In addition, we approach to the possible mechanisms how Klotho gene repair the myocardial injury.

METHODS Rats were divided into 5 groups (n=5 each) by table of exponential random numbers. All groups were tested left ventricular ejection fraction (LVEF) with echocardiography; hemodynamic parameters obtained by multi-channel physiological recorder; myocardial tissue underwent pathohistological examination. Additionally, the green fluorescein expression was observed on frozen heart section. Myocardial fibrosis correlated gene expression including Klotho gene, Collagen I and III was detected by RealTime-PCR. Moreover, plasma levels of B-type natriuretic peptide (BNP) were measured with ELISA.

RESULTS In contrast to saline control group, LVEF, LVSP and ±dp/dt max (all P<0.01) were significantly decreased myocardial fibrosis and myocardial remodeling were significantly attenuated in the AD. Klotho group. And there was green fluorescein distribution in the AD. Klotho group. However, LVEDP was increased in groups of heart failure (P<0.01). In addition, Klotho expression was down-regulated and Collagen I and III expression was up-regulated in HF rats compared to normal control group (all P<0.05). And these changes could be significantly reversed in AD. Klotho group (all P<0.05). Plasma BNP level was also significantly lower in AD. Klotho group than in others (P<0.05).

CONCLUSIONS Models of rat heart failure can be constructed effectively by isoproterenol-induced intraperitoneal injection. Klotho gene transfection could improve cardiac function and attenuate cardiac remodeling and reducing myocardial fibrosis.

GW26-e0776
Regulation of NOD1/RIP2 Signal Pathway in Macrophage Inflammatory Activation
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OBJECTIVES To explore the role of NOD1/RIP2 signaling pathway in the initiation and progression of atherosclerosis, we observed the effects of NOD1/RIP2 signaling pathway on macrophage inflammatory activation and polarity switch by the human mononuclear cell line THP-1.

METHODS Human THP-1 cells were differentiated into macrophages by the addition of 160nml/l phorbol 12-myristate 13-acetate(PMA) for 24h. Macrophages were incubated with different concentrations of OX-LDL(10, 25, 50 mg/L) for 24h. The expression of NOD1, RIP2 was detected by semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) and Western blotting. Enzyme-linked immunosorbent assay (ELISA) was used to detect the secretion of monocyte chemotactic protein 1(MCP-1) and macrophage migration inhibition factor (MIF); fluorescence activated cell sorting (FACS) was used to detect membrane molecule CD66,CD68.

RESULTS 1. OX-LDL could up-regulate the expression of NOD1/RIP2 signal pathway as a dose-dependent manner in THP-1 derived macrophages. With the increasing concentration of OX-LDL stimulation, the expression of NOD1 mRNA up-regulated (control: 0.291±0.011; 10 mg/L: 0.52±0.015; 25 mg/L: 0.738±0.034; 50 mg/L: 0.877±0.02). OX-LDL could also up-regulate the expression of NOD1 protein (control: 0.176±0.053; 10 mg/L: 0.323±0.062; 25 mg/L: 0.757±0.125; 50 mg/L: 1.08±0.123). 2. The activation of NOD1/RIP2 signaling pathway could increase the expression of MIF and MIF from the cell culture supernatants. 3. The activation of NOD1/RIP2 signaling pathway could change the expression of membrane molecule CD68, CD66. With different OX-LDL concentration, the mean fluorescence intensity(MFI) of CD68, CD66 varied.50mg/L group could significantly reduce the expression of CD68, CD66 (CD68: 29.3±2.5; CD66: 25.2±2.3).