expression of sh-CTSB or over-expressed CTSB by lentiviral vector to further confirm the in vivo results.
Results: Our data demonstrated that knockout of CTSB attenuated pressure overload-induced cardiac hypertrophy, fibrosis, and dysfunction. Further studies showed that pressure overload significantly induced the activation of c-Jun N-terminal kinase (JNK) signaling, which was blocked in CTSB-KO hearts. Mechanistically, we also discovered that the expression levels of apoptosis-related proteins, Bax, Bid and cleaved caspase3, cleaved caspase 9 being remarkably decreased in CTSB-KO hearts after aortic banding, compared with control hearts, while anti-apoptotic protein, Bcl-2 was significantly increased in CTSB-KO hearts after aortic banding. Similar results were observed in cultured CTSB-deficient H9c2 cardiomyocytes after the treatment with angiotensin II, whereas the CTSB-overexpressed H9c2 cardiomyocytes displayed the opposite phenotype.
Conclusions: These studies indicate that CTSB protein is a crucial component of the signaling pathway involved in cardiac remodeling, which may be associated with JNK/c-jun pathway and cell apoptosis.

GW25-e3243

Sanguinarine Inhibited Ang II Induced Apoptosis in H9c2 Cardiac Cells via Restoring ROS-decreased Mitochondrial Membrane Potential

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Objectives: Cell apoptosis induced by Angiotensin II (Ang II) plays a critical role in the development of cardiovascular diseases. The study was to investigate whether sanguinarine (SAN) can abolish Ang II induced cell apoptosis.
Methods: When H9c2 cells received different factor intervention for 24h; the toxicity of SAN on H9c2 cells was determined by Cell Counting Kit CCK-8 assay; the level of intracellular ROS generation was accessed using DCFH-DA; The change of mitochondrial membrane potential (MMP) was accessed using JC-1; the mRNA expression of NOX2 was test by RT-PCR; apoptosis was evaluated was determined using fluorescence-activated cell sorting (FACS); Western blot domenstrated the protein associated with apoptosis; The activity of caspase-3 and caspase-9 was accessed using the caspase-3 and caspase-9 activity kit.
Results: H9c2 cardiomyocytes were stimulated with 1μmol/L Ang II with or without sanguinarine/N-Acetyl-L-cysteine (NAC), respectively. Our results showed that sanguinarine inhibited the increase of NOX2 index and ROS generation, and ameliorated mitochondrial membrane potential (MMP) lose induced by Ang II in H9c2. It has also been shown that caspase-3 and caspase-9 proteins expression and activity were decreased, and Bcl-2/Bax ratio was enhanced accordingly.
Conclusions: Our findings indicate that sanguinarine could inhibite H9c2 cardiac cells apoptosis caused by Ang II and most likely is a result of restoring ROS-decreased mitochondrial membrane potential.

GW25-e3257

The Role of Mitochondrial KATP in High Glucose-Induced Myocytes Apoptosis

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Objectives: To explore the role of mitochondrial K_{ATP} in high glucose- induced apoptosis in cardiomyocytes.
Methods: Pretreatments of diazoxide (DZ) or 5-hydroxy- decanoic acid (5-HD) were administrated to primary rat myocytes before high-glucose incubation. Mitochondrial activity was assessed by MTT method; mitochondrial membrane potential (MMP) and cell apoptosis were evaluated by flow cytometry; Western-Blot was applied to detect the expressions of cytochrome C and cleaved caspases-3.
Results: Compared with normal control and DZ pretreatment, high-glucose incubation significantly increased cell apoptotic percentage, at the same time, mitochondrial activity was inhibited, MMP decreased, expressions of cytochrome C and caspase-3 increased significantly (all $P<0.05$). Compared with high-glucose incubation, pretreatment of 5-HD further increased apoptotic percentage, inhibited mitochondrial activity, decreased MMP and increased expressions of cytochrome C and caspase-3 increased significantly (all $P<0.05$).
Conclusions: Under high-glucose environment, mitoK_{ATP} in myocytes affects mitochondrial activity and integrity by impacting MMP, then induces apoptosis via mitochondria-dependent pathway.

GW25-e3454

CRP-induced apoptosis in human umbilical vein endothelial cells and the protection of atorvastatin

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Objectives: The present study examined the effect of atorvastatin on CRP-induced apoptosis in human umbilical vein endothelial cells.
Methods: The HUVECs were cultured in vitro. Apoptosis of HUVECs were observed by acridine orange/ethidium bromide fluorescence staining. After HUVECs were incubated with different concentrations of CRP (5ug/mL, 10ug/mL, 20ug/mL) and atorvastatin (0.1μmol/L, 1μmol/L, 10μmol/L) for 24 hours. The early apoptosis rates were detected respectively by flow cytometry. The expression of Bcl-2/Bax and P53 were detected respectively by RT-PCR and western blotting.
Results: Treated with different concentrations of CRP after 24 hours, the early apoptosis rates of HUVECs gradually increased with concentration-dependted. Compared with the control group (0.64±0.06%), the early apoptosis rates of 5 ug / mL CRP group, 10 ug / mL CRP group and 20ug/mL CRP respectively were (2.50±0.44%, 8.07±0.7%, 12.84±0.77%, respectively), there were a statistically significant difference ($P<0.01$). Compared with 10mg/L CRP group, the early apoptosis rates of 20mg/L CRP group also increased significantly ($P<0.01$). In different concentrations of CRP for 24 hours, the expression of Bcl-2 protein decreased gradually. However, the protein expression of Bax and P53 were increased. The ratios of Bcl-2/Bax and P53/ Bax were gradually decreased. There were statistically significant differences between experimental group and control group ($P<0.01$). Treated with 20ug/mL CRP and different concentrations of atorvastatin 24 hours later, compared with 20mg/L CRP group, the early apoptosis rates of HUVECs of atorvastatin intervention group gradually decreased with concentration-dependted. The early apoptosis rate of HUVECs of 0.1μmol/L atorvastatin group, 1μmol/L atorvastatin group and 10μmol/L atorvastatin group respectively were 10.45±0.74%, 8.38±0.6% and 7.35±0.44%. Compared with 20mg/L CRP group, the early apoptosis rate of the high and middle concentrations of atorvastatin group were significantly decreased ($P<0.01$). There were also statistically significant difference between middle concentration of atorvastatin group and high concentrations of atorvastatin group ($P<0.01$). With concentration-dependted of atorvastatin, the expression of Bcl-2 protein gradually increased, the protein expression of Bax and P53 gradually decreased; the ratio of Bcl -2/Bax gradually decreased, the ratio of P53/Bax also decreased gradually. Compared with the 20ug/mL CRP, there were statistically significant difference in the expression of Bcl-2 protein, Bax protein and P53 protein of the middle and high concentrations of atorvastatin group ($P<0.01$). The ratio of P53/Bax of high concentrations of atorvastatin group was statistically significant compared with the 20ug/mL CRP ($P<0.01$).
Conclusions: The CRP can induce apoptosis in HUVECs, which is connected with up-regulating the expression of P53 protein and Bax protein, down-regulating the expression of Bcl-2 protein. Middle and high concentrations of atorvastatin probably inhibit the apoptosis in human umbilical vein endothelial cells induced by CRP though up-regulating expression of Bcl-2 protein, down-regulating expression of P53 protein and Bax protein. Expression of Bcl-2 protein maybe negativly modulates expression of Bax protein. P53 protein maybe regulates the activation of Bax protein.

GW25-e3467

Sphingosine 1-phosphate signaling contributes to cardiac inflammation, remodeling and dysfunction following myocardial infarction

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Objectives: Sphingosine 1-phosphate (S1P) generated by sphingosine kinases (SphK) regulates multiple pathophysiological processes in cardiovascular system. However, the role of SphK/S1P axis involved in heart failure remains unclear. The study was designed to investigate the role of S1P signaling in myocardial infarction (MI) induced heart failure and the underlying mechanisms.
Methods: Male C57BL/6J mice were subjected to permanent left coronary artery occlusion and were randomized to receive vehicle, FTY720 (3mg/kg, i.P. daily) and ABC294640 (5mg/kg, i.P. daily) treatment for 4 weeks. Cardiac function was evaluated by echocardiography and myocardial fibrosis was detected by masson-trichrome staining 4 weeks after MI. In vitro experiments were conducted on cultured neonatal rat ventricular cardiomyocytes. Real-time PCR and western blot were performed to determine the expression levels of the molecules involved in the study.
Results: The infarcted heart showed increased SphK1 expression and increased S1P content. S1PR1, the predominant type of S1P receptors expressed in left ventricle, was upregulated by 3-fold in cardiac tissue following MI (n=6, all $P<0.05$). Further, we observed that S1P significantly activated NF-κB/STAT3 signaling and upregulated pro-inflammatory cytokines (TNF-α and IL-6) expression in a dose-dependent manner in cultured cardiomyocytes (n=3, all $P<0.01$), all of which were almost blocked by pretreatment with S1PR1 siRNA or FTY720, a functional S1PR1 inhibitor (n=3, all $P<0.01$). In vivo, administration of FTY720 decreased SphK1/S1P/S1PR1 axis, inhibited persistent NF-κB/STAT3 signaling activation and blocked pro-inflammatory cytokines production in post-MI heart. Accordingly, FTY720 remarkably alleviated cardiac fibrosis and dysfunction (ejection fraction: 35.5±3.0% vs. 29.4±2.7%, $P<0.05$). Intriguingly, administration of SphK2 inhibitor ABC294640 amplified cardiac SphK1/S1P/S1PR1 axis, augmented NF-κB/STAT3 signaling activation and increased inflammatory cytokines expression. Consequently, SphK2 inhibition reduced survival rate (68.4% vs. 83.3%, $P<0.05$) and exacerbated cardiac maladaptive structural changes and ventricular dysfunction induced by MI (ejection fraction: 25.4±2.3% vs. 30.4±3.2%, $P<0.05$).
Conclusions: Our results provide the evidence that upregulated SphK1/S1P/S1PR1 signaling axis links persistent NF-κB/STAT3 activation, chronic cardiac inflammation and heart failure progression following MI, whereas SphK2 serves as an endogenous suppressor of pathological S1P signaling. Targeting SphK1/S1P/S1PR1 axis by inhibiting SphK1 activity or enhancing SphK2 activity might be a potential therapeutic strategy for ischemic heart failure.