(P<0.01) and lowered collagen I/ III ratio (P <0.01), suggesting that Tongxinluo improved myocardial collagen remodeling effectively.
Conclusions: Tongxinluo prevented NPY-induced cardiac microvascular spasm effectively, and the mechanism was due to that Tongxinluo relieved the myocardial inflammation, thinned the thickness of micrangium basement membrane and reversed myocardial collagen remodeling.

GW25-e3223

Urotensin II blockade with urantide attenuates cardiac fibrosis in spontaneous type 2 diabetic mice

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Objectives: The aim of this study was to investigate the changes of Urotensin II (UII) and its receptor UT during myocardial fibrosis in model of KK/Upj-Ay/J mice with spontaneous type 2 diabetes, and evaluate whether blockade of UII would attenuate cardiac fibrosis in the model.
Methods: Diabetic KK/upj-AY/J and control C57BL/6J mice were fed with high fat diet and normal chow diet respectively, for six weeks. Then, The KK/Upj - Ay/J mice were randomly divided into two groups, diabetes mellitus (DM) group and diabetes + urantide (DM + urantide) group, based on the level of fasting blood glucose. The DM + urantide mice were treated with urantide (30 mg $kg^{-1}day^{-1}$) by sc via an osmotic mini-pump for 4 weeks. The water and food intake, weight were recorded. Fasting blood glucose levels were assessed using blood collected from the tail vein. Myocardial morphology was observed by Hematoxylin-Eosin and Masson staining. The immunoreactivity of myocardial UII, UT, CD31, fibroblast -specific protein 1 (FSP-1) were determined by immunohistochemistry. Plasma UII contents were tested by radioimmunoassay. The protein expression of α-SMA, VE-cadherin and P-smad2/3, CTGF were detected by western blot.
Results: There was a significant increase in body weight, fasting blood glucose, water intake and food intake in the DM group compared to the control group (P<0.01). The DM group showed marked myocardial disarray and fibrosis. Both UII and UT immunoreactivity were increased in the DM group compared to the control group (P<0.05). The fibroblasts marker, FSP-1 immunoreactivity and α-SMA protein expression were increased, while the endothelial marker, CD31 immunoreactivity and VE-cadherin protein expression, were decreased, in the DM mice compared to control mice (P<0.05). The smad2/3 Phosphorylation and CTGF protein expression in the DM group were elevated than the controls (P<0.05). However, there is no significant difference in plasma UII levels between the DM and the controls (P>0.05). Importantly, treatment with Urantide significantly reduced the body weight, fasting blood glucose, water intake, and immunoreactivity of UII, UT, FSP-1, as well as protein expression of α-SMA, P-samd2/3, CTGF, and alleviated myocardial disarray and fibrosis significantly, while promoted CD31 immunoreactivity and VE-cadherin expression significantly (P<0.01 or P<0.05).
Conclusions: This study provided novel evidences that UII is involved in EndMT and cardiac fibrosis, and demonstrated that blockade of the UT receptor attenuated myocardial fibrosis in diabetes mellitus. It suggests that UII may represent a novel therapeutic target in the treatment of diabetes mellitus.

GW25-e3224

Omi/HtrA2 Cause Cardiomyocyte Apoptosis via Release of Cytochrome C in Aging Rats

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Objectives: Existing studies have shown that the expression of Omi/HtrA2, a pro-apoptosis protein, is increased in the aging heart, while the release of cytochrome c is involved in apoptosis during aging. The relationship between the increased expression of Omi/HtrA2 and the release of cytochrome c in apoptosis of the aging heart, however, has not been investigated. The present study was designed to determine whether or not the increased expression of Omi/HtrA2 causes cardiomyocyte apoptosis during aging via the release of cytochrome c.
Methods: Aging myocardial tissues were collected from aging rats (20-24 months), and expression of Omi/HtrA2 was determined by Western blot analysis. The activities of caspase-3 and -9 were chosen to reflect the level of apoptosis. H9C2 cells, a rat embryonic cardiomyocyte line with over-expressed mitochondrial Omi/HtrA2, were constructed. Both real-time PCR and Western blot technologies were used to identify stably transfected Omi/HtrA2 cells. Mitochondrial and cytosolic fractions were extracted by differential centrifugation, and the cytochrome c level in cytosolic fraction was detected by Western blot.
Results: The results showed that expression of Omi/HtrA2 and the activities of caspase-3 and -9 were significantly increased in aging myocardial issue. Moreover, the activities of caspase-3 and -9 in over-expressed Omi/HtrA2 cells were elevated. The release of cytochrome c was increased, which was reversed by the specific Omi/HtrA2 inhibitor, ucf-101.
Conclusions: These results demonstrate that increased expression of Omi/HtrA2 in aging cardiomyocytes promotes apoptosis via release of cytochrome c.

GW25-e3429

Intramyocardial relaxin-2 gene delivery improves diastolic function of pressure-overloaded rats via increasing phospholamban phosphorylation by activating nuclear-targeted Akt

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Objectives: Relaxin is a peptide hormone with potent cardiovascular effects, which has been demonstrated to be safe and effective in acute heart failure in clinic trials. However, effect of relaxin on heart failure with preserved ejection fraction (HFpEF) is unknown. The aims of the study were to determine whether relaxin could improve the diastolic function of HFpEF and to investigate the underling mechanisms.
Methods: In the present study, adenoviral vector expression relaxin-2 (Ad-RLN-2) and GFP (Ad-GFP) were constructed. Pressure-overloaded rat model was established by performing trans-aortic constriction (TAC) on SD rats, and 4 weeks later, echocardiography and cardiac hemodynamics were performed. These TAC rats were randomly assigned into 3 groups (TAC control group, without intramyocardial injection; TAC+Ad-RLN-2, with intramyocardial injection of Ad-RLN-2; TAC+Ad-GFP, with intramyocardial injection of Ad-GFP). And 12 days after intramyocardial injection, diastolic and systolic function were determined by echocardiography and cardiac hemodynamics. And then the rats were sacrificed, proteins and RNA were extracted from left ventricles; Western Blotting and quantitative real-time PCR were performed. Neonatal cardiomyocytes were isolated and were cultured with Ad-RLN-2 and Ad-GFP. And then laser confocal was used to detect the intracellular location of phosphorylation Akt and PLB in neonatal cardiomyocytes.
Results: RLN-2 gene therapy was demonstrated to improve diastolic function by echocardiographic and hemodynamic parameters. E/A ratio significantly increased in rats with RLN-2 gene delivery (1.86±0.26 vs 1.54±0.32 and 1.52±0.21, P<0.05), and meanwhile, tissue Doppler image early (e') and late (a') velocity ratio at septal part (e'/a') was almost normalized to 1.20±0.49 (P<0.001), IVRT was significantly reduced (37.78±5.80ms vs 47.01±7.07ms and 45.85±7.44ms, P=0.008 and 0.027, respectively), dP/dt_{min} was lowered (-5956±1104 vs -4864±1171 and -4583±1123, P<0.05 for both comparison), and Tau was reduced (17.4±2.4 vs 26.1±2.7 and 24.8±3.1, P<0.05 for both comparison) in rats with RLN-2 gene delivery when compared with the TAC control and TAC+Ad-GFP groups. In rats injected with Ad-RLN-2, phosphorylation level of nuclear-targeted Akt and phospholamban were elevated, and SERCA2 activity was increased without changing SERCA2 level.
Conclusions: RLN-2 gene therapy was demonstrated to improve diastolic function. The potential mechanism may be that RLN-2 gene transfer increased SERCA2 activity by activating phospholamban phosphorylaiton at both Ser16 and Thr17 sites via activating nuclear-targeted Akt phosphorylation without changing the SERCA2 level.

GW25-e3441

Analysis of SCN5A Mutation in Patients with Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia

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Objectives: Arrhythmogenic right ventricular cardiomyopathy/Dysplasia (ARVC/D) is a genetically determined disorder, characterized by the two components: cardiomyopathy and arrhythmia. To date, the molecular pathogenesis underly this phenomenon is poorly understood. Whether the ion channel defect involved in the ARVC/D is unknown. The aim of this study was systematically evaluate the sodium channel variants in ARVC/D.
Methods: The patients according to the diagnostic guideline of ARVC/D revised in 2010 were collected. Genomic DNA was extracted from peripheral blood lymphocytes. All the exons and exon-intron boundaries of the SCN5A gene and desmosomal genes known to be associated with ARVC/D, including DSC2, DSG2, DSP, JUP and PKP2 were sequenced through the direct DNA sequencing.
Results: A total of 13 unrelated index patients were collected. A new missense heterozygote mutation I137M in SCN5A gene was found in one proband 5. The mutation sited at the exon 4 of the SCN5A and the S1 segment in Domain I of Nav1.5, consisted of an C-to-G substitution at nucleotide site 411 (c.411C>G), which predicted a substitution of isoleucine for methionine at codon site 137 (P. Ile137Met, I137M). I137M was not detected in the 400 healthy control chromosomes from individuals of the same ethnic background, which indicated that this mutation was a conservative site in SCN5A gene and the encoding protein - Nav1.5 may have a functional defect.
Conclusions: Our study for the first time systematically evaluates the sodium channel variants in patients with ARVC/D and finds a new SCN5A mutation - I137M. The result increases the insight of genetic pathogenesis in ARVC/D. The mutational sodium channel may destroy the "desmosomal-related complex" and cause the genesis of ARVC/D.