

Methods: We established low-estrogen atherosclerotic animal model by feeding ovariectomized apoE^{-/-} mice a high-fat diet, and oxidative-injured cells model were induced by ox-LDL in HUVECs. Tanshinone IIA (mice: 30mg/kg, 60mg/kg, cells: 0.1μM, 1μM, 10μM) was given to the mice and cells, and estrogen (mice: 0.13mg/kg/d, cells: 0.01μM) and estrogen receptor antagonist (ICI182780, mice: 65mg/kg, cells: 0.1μM) were also designed to be given. The levels of NF-κB, sICAM-1, AP-1, E-selectin and 17β-estradiol (E₂) in serum and the levels of NF-κB, sICAM-1, AP-1 and E-selectin in supernatant were measured by ELISA. The expression of P-ERK1/2 in cells and mice aorta and expression of ERα in cells were assessed by Western blotting.

Results: Tanshinone IIA could not significantly increase the serum E₂ level of ovariectomized apoE^{-/-} mice. But it significantly inhibited the levels of NF-κB, AP-1, sICAM-1 and E-selectin in serum of ovariectomized ApoE^{-/-} mice and supernatant of ox-LDL injured HUVECs, and Tanshinone IIA significantly inhibited the expression of P-ERK1/2 in mice aorta and cells and inhibited the expression of ERα in cells, which was similar to the estradiol, and could be inhibited by ICI182780.

Conclusions: Tanshinone IIA has an anti-inflammatory effect on oxidative-injured cells, and the mechanism is that Tanshinone IIA could significantly attenuate the P-ERK1/2 to inhibit the mitogen-activated protein kinase (MAPK) signaling pathway by bonding with estrogen receptor, which is similar to the estrogen.

GW25-e1143

Alterations of the calreticulin-STAT3 pathway associates with mitochondria damage in selenium deficiency rat hearts

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Objectives: To study the changes of calreticulin-STAT3 signaling pathway and its effect on cardiac mitochondria damage in selenium deficiency rat hearts.

Methods: Twenty male Sprague-Dawley rats were randomized into normal control group (n=8) and selenium deficiency model group (n=12). When rats were fed for 20 weeks, the cardiac function was measured by hemodynamic studies. The signal molecules involved in the calreticulin-STAT3 pathway were investigated using real-time PCR and western-blot. The mitochondrial structure and function were assessed.

Results: Compared with the control group, the rats in the model group had reduced systolic and diastolic function. Calreticulin mRNA expression was 4.6-fold higher in the model group than that in the control group, and the protein level of calreticulin was 3.3-fold higher than that in the control group (P<0.05). The protein expression of STAT3 and P-STAT3 in the whole myocardium and cardiac mitochondria were both significantly down-regulated in the model group (P<0.05). The mRNA and protein levels of manganese superoxide dismutase (MnSOD), downstream to STAT3, were also significantly decreased in the model group (P<0.05). Under electron microscopic observation, the cardiac mitochondria in the model group were swelling with fractured or dissolved cristae. The mitochondrial membrane potential level of the isolated fresh cardiac mitochondria, and the enzyme activities of succinate dehydrogenase and cytochrome c oxidase in the model group were all significantly decreased as compared with the control group (P<0.05).

Conclusions: The development of selenium deficiency induced cardiomyopathy in rats, might be due to the up-regulated expression of calreticulin, which inhibits STAT3 phosphorylation in both the whole cell and mitochondrial fraction.

GW25-e1163

Ox-LDL induces endothelial cell apoptosis via the LOX-1-dependent endoplasmic reticulum stress pathway

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Objectives: To investigate the effect of lectin-like ox-LDL receptor-1 (LOX-1) on oxidized low-density lipoprotein (ox-LDL) -induced apoptosis and the involvement of the endoplasmic reticulum (ER) stress response pathway.

Methods: Human umbilical vein endothelial cells were treated with 50, 100, or 200 μg/ml ox-LDL and cultured for 12, 24, or 48 hr for concentration- and time-dependent studies. Cells were transfected with LOX-1 or Nox-4 shRNAs, and target proteins were inhibited with the corresponding antibodies for mechanistic studies. Active proteins and mRNAs were analysed by Western blotting and RT-PCR, respectively. Cell apoptosis was analysed by Annexin and Hoechst staining assays.

Results: Ox-LDL induced both apoptosis and protein expression of LOX-1 and Nox-4 through activation of ER stress sensors IRE1 and PERK, and nuclear translocation of ATF6 and their subsequent pathways were indicated by JNK, eukaryotic initiation factor 2 phosphorylation, XBP-1, and chaperone GRP78 expression; up-regulation of proapoptotic proteins CHOP and Bcl-2; and caspase-12 activity. LOX-1 gene silencing and treatment with an anti-LOX-1 antibody attenuated the effects of ox-LDL. Pretreatment with irastatin 9389, salubrinal, or AEBSF also blocked ox-LDL-induced expression of CHOP and Bcl-2 and activation of caspase-12 activity, leading to an attenuation of endothelial cell apoptosis. Furthermore, Nox-4 siRNA attenuated the up-regulated expression of GRP78, PERK, IRE1, and XBP-1 to reduce ox-LDL-induced endothelial cell apoptosis.

Conclusions: LOX-1 plays a critical role in ox-LDL-induced endothelial cell apoptosis via the ER stress pathway.

GW25-e1371

Establishment and evaluation of reparative myocardial fibrosis model in rat

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Objectives: Establish the reparative myocardial fibrosis rat model and evaluate it.

Methods: Mix the pig cardiac myosin (the concentration is 6.4 mg/ml) with equal volume proportion of complete Freund's adjuvant (CFA) (with 1mg mycobacterium tuberculosis per milliliter by thermal inactivation), then push them in 4°C refrigerator repeatedly to achieve the aim of sufficient emulsification, form a kind of emulsion which is sticky and water-in-oil (the final concentration of the pig cardiac myosin is 3.2mg/ml). Each Lewis rat was immunized with 1mg/0.3ml of an emulsion containing cardiac myosin with an equal volume of CFA by subcutaneous injection on days 0 and 7. Both the left and right hind leg foot pad of the rat was injected. Negative control rat were immunized with PBS/CFA. On days 28 after the first injection, take the material and weigh the body weight, the quality of heart and left ventricle. Hematoxylin-eosin (HE) staining and Masson staining was performed on paraffin-embedded heart sections. The sample alkali hydrolysis method was used to detect myocardial tissue hydroxyproline (HYP) content. Enzyme linked immunosorbent assay (ELISA) was conducted to detect the level of Carboxyterminal propeptide of procollagen type I (PICP), N-terminal peptide of procollagen type III (PIIINT), C-telopeptide of type I collagen (CTX-I) in serum.

Results: Compared with negative control rats, cardiac index and left ventricular mass index increased significantly in model rats. Myocardium of HE staining showed inflammatory cell infiltrate and cardiac myocyte hypertrophy with myocardium decreased and necrosis in model rats. That of Masson staining showed the reparative myocardial fibrosis transformation that large bundled quantity of blue myofibril, deposited and disordered arrangement of collagen in myocardial matrix and fibre replaced necrotic myocardium. Content of HYP in myocardium of model rats increased significantly comparing with negative control rats. There was a higher level of PICP and PIIINT indicating collagen synthesis and CTX-I indicating collagen degradation in model rats serum than negative control rats.

Conclusions: Pig cardiac myosin could induce the typical reparative myocardial fibrosis in Lewis rat.

GW25-e1400

Curcumin inhibits cardiac fibrosis in vitro and in vivo by inhibiting myofibroblast differentiation

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Objectives: Cardiac fibrosis is a hallmark of heart disease and plays a vital role in cardiac remodeling during most types of cardiac injury. Curcumin has been demonstrated to exhibit a variety of potent beneficial effects such as antioxidant, anti-inflammatory and cardioprotective potential. However, the effect of curcumin on fibroblasts in cardiac fibrosis has not yet been investigated.

Methods: Isoproterenol (ISO) -induced cardiac fibrosis rats were treated with curcumin (150 or 300 mg/kg/d) for 28d. In addition, cardiac fibroblasts were treated with Ang II for 24 h alone or pretreated with curcumin in different concentrations (5, 10, and 20 mmol) for 1 h before treated with Ang II.

Results: Curcumin significantly reduced cardiac fibrosis in rats by decreasing interstitial and perivascular myocardial collagen deposition and cardiac weight index (2.695±0.025 in control vs. 3.414±0.038 in ISO group, 3.165±0.031 in ISO+Cur-L group, P<0.05 vs. ISO group alone; 2.956±0.026 in ISO+Cur-H group, P<0.05 vs. ISO group alone) as well as reducing protein expression of collagen I (1.376±0.169 in ISO+Cur-H group, P<0.05 vs. 2.624±0.215 in ISO group) and III (0.831±0.096 in ISO+Cur-H group, P<0.05 vs. 1.214±0.158 in ISO group) in hearts. Curcumin directly inhibited angiotensin II (Ang II) -induced fibroblast proliferation and collagen type I/III expression in cardiac fibroblasts. We also found that curcumin inhibited fibrosis by inhibiting myofibroblast differentiation. Curcumin also decreased transforming growth factor (TGF) -β1, matrix metalloproteinase (MMP) -9 and tissue inhibitor of metalloproteinase (TIMP) -1 expression but had no effects on Smad3 in Ang II incubated cardiac fibroblasts.

Conclusions: Curcumin reduces cardiac fibrosis in rats and Ang II-induced collagen proliferation, by inhibiting myofibroblast differentiation and decreasing collagen synthesis and accelerating collagen degradation via regulation of TGF-β1, MMPs/TIMPs. The present findings provided novel insights and underscore the therapeutic potential of curcumin as an anti-fibrotic agent for the treatment of cardiac fibrosis.

GW25-e1409

CKIP-1 can regulate physiological cardiac hypertrophy through inhibition of HDAC4 phosphorylation

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