Catestatin protects oxidative-stress-induced apoptosis by activating the β2 adrenergic receptor and PKB/Akt pathway in ischemic-reperfused myocardium

Introduction: Apoptosis induced by oxidative stress is one of the most important cardiomyocytes losses during ischemia-reperfusion (I/R). Catestatin (CST) has been demonstrated to have the anti-oxidative capacity in vitro. We hypothesized that CST could reduce apoptosis of cardiomyocytes induced by oxidative stress in I/R.

Methods: In Langendorff-perfused rat heart global I/R model, cardiac function including LV developed pressure (LVDP), left ventricular end diastolic pressure (LVEDP), and maximal time derivative of left ventricular pressure (±dp/dtmax) were recorded. After I/R, MDA, SOD, and GSH-PX content in myocardial tissue were measured. TTC staining and TUNEL staining were used to evaluated the infarction area and apoptosis. Heart homogenates were used for LDH assay and Western blots. Primary cultured neonatal rat cardiomyocytes were exposed to H2O2. Apoptosis of the cardiomyocytes was assessed by Hoechst 33342 staining and DNA laddering.

Result: Global I/R induced infarction. Average infarct size of (33.66±3.61) % was found in I/R group. Treatment with CST led to reduced infarct size (20.25±3.23) % (p=0.011). LDH content in myocardium decreased significantly after reperfusion. CST intervention alleviated the LDH loss [(8994.4±963.8)U/g vs. (6843.5±1136.0)U/g, p<0.001]. After I/R, LVDP and ± dp/dtmax decreased while LVEDP increased significantly. CST improved LVDP and ±dp/dtmax (p=0.005), and reduced LVEDP(p=0.008). Reperfusion significantly increased apoptosis of cardiomyocytes and caspase-3 cleavage. CST intervention showed a significant decrease of apoptotic nuclei and caspase-3 cleavage. Ischemia-reperfusion induced elevation of MDA and depletion of anti-oxidative enzymes. CST intervention led to decrease of MDA level and restored activities of SOD and GSH-PX [SOD (222.8±26.6) vs. (175.3±20.6) in I/R group (U/mg) and GSH-Px (53.3±15.9) vs. (37.3±11.0) in I/R group (U/mg), all p<0.05]. Ischemia-reperfusion led to an increased expression of total ERK, phosphorylation activation of AMPK, and Akt. CST contributed to reduced AMPK activation but further Akt phosphorylation. CST did not influence the phosphorylation of ERK. In primary cultured neonatal cardiomyocytes, H2O2 stimulation induced apoptosis of the cells, activation of caspase-3 and phosphorylation of Akt. CST intervention further stimulated phosphorylation of Akt and alleviated the effect of H2O2 by decreasing apoptosis and inhibiting cleavage of caspase-3. PI3K inhibitor LY294002 partly abolished the protective effect of CST. Moreover, β2 inhibitor ICI118551 pretreatment suppressed the protective effect of CST toward Akt activation and caspase-3 cleavage.
Conclusion: Administration of CST might protect myocardial I/R injury by activation β2 receptor and reperfusion injury salvage kinase (RISK) pathway to reduce apoptosis of cardiomyocytes induced by oxidative stress.