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Protective effects of sitagliptin on myocardial injury and cardiac function in an ischemia/reperfusion rat model

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ABSTRACT

The purpose of this study is to investigate the effects and the underlying mechanisms of sitagliptin pretreatment on myocardial injury and cardiac function in myocardial ischemia/reperfusion (I/R) rat model. The rat model of myocardial I/R was constructed by coronary occlusion. Rats were pretreated with sitagliptin (300 mg/kg/day) for 2 weeks, and then subjected to 30 min ischemia and 2 h reperfusion. The release of lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB), cardiac function and cardiomyocyte apoptosis were evaluated. The levels of malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in heart and glucagon-like peptide-1 (GLP-1) level in plasma were measured. Western blot analysis was performed to detect the target proteins of sitagliptin. Our results showed that sitagliptin pretreatment decreased LDH and CK-MB release, and MDA level in I/R rats. More importantly, we revealed for the first time that sitagliptin pretreatment decreased cardiomyocyte apoptosis while increased the levels of GSH-Px and SOD in heart. Sitagliptin also increased GLP-1 level and enhanced cardiac function in I/R rats. Furthermore, sitagliptin pretreatment up-regulated $Akt^{serine473}$ and $Bad^{serine136}$ phosphorylation, reduced the ratio of Bax/Bcl-2, and decreased expression levels of cleaved caspase-3 and caspase-3. Interestingly, the above observed effects of sitagliptin were all abolished when co-administered with GLP-1 receptor antagonist exendin-(9-39) or PI3K inhibitor LY294002. Taken together, our data indicate that sitagliptin pretreatment could reduce myocardial injury and improve cardiac function in I/R rats by reducing apoptosis and oxidative damage. The underlying mechanism might be the activation of PI3K/Akt signaling pathway by GLP-1/GLP-1 receptor.

1. Introduction

Myocardial infarction is a major cause of mortality and morbidity of patients with diabetes mellitus (Acar et al., 2011). In order to prevent the myocardium from further damage, the best therapeutic strategy for myocardial infarction is to reestablish the blood flow as earlier as possible. Nevertheless, ischemia/reperfusion (I/R) injury such as cardiomyocyte apoptosis is inevitable. Cardiomyocyte apoptosis induced by I/R plays an important role in causing a gradual decline of cardiac function (Gottlieb, 2011). Therefore, the exploration of new therapeutic agents that reduce I/R injury of myocardial infarction patients has become very important.

Glucagon-like peptide-1 (GLP-1) is secreted by the entero-endocrine L cells of the intestinal mucosa and released in response to nutrient ingestion (Nauck et al., 1993). It exerts insulinotropic and insulinomimetic effects via the G-protein-coupled GLP-1 receptor (Verge and Lopez, 2010). The therapy based on the functions of GLP-1 is currently used as a novel anti-diabetic approach (Doupis and Veves, 2008; Garber, 2012). However, GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP_4) enzyme in the blood (Green et al., 2006). The short half life time limited its clinical use. Thus, two classes of drugs, including GLP-1 analogs (Garber, 2012) (i.e. exenatide) and DPP4 inhibitors (Doupis and Veves, 2008) (i.e. sitagliptin), have been recently used for treating type 2 diabetes.

Recently, growing evidences have demonstrated the beneficial effects of GLP-1 analogs during I/R injury in both animal models and in clinical studies, such as limiting infarct, improving cardiac function and enhancing myocardial glucose uptake (Bhashyam et al., 2010; Chinda et al., 2012a; Lorber, 2012; Mundil et al., 2012). The mechanisms underlying the cardioprotective effects of GLP-1 analogs may be both GLP-1 receptor dependent and independent pathways (Ban et al., 2008; Chinda et al., 2012a). Unlike GLP-1 analogs, evidences regarding the cardioprotective effects of DPP4 inhibitors are scarce and controversial. Recently, more and more researchers have paid close attention to the cardioprotective effects of DPP4 inhibitors. Chinda, et al. (2012b) reported that DPP4 inhibitor could stabilize cardiac electrophysiology in a myocardial I/R pig model.

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In addition, DPP4 inhibitor has been shown to attenuate the infarct size and improve the left ventricular function during myocardial I/R injury (Chinda et al., 2012a; Jose and Inzucchi, 2012; Lenski et al., 2011; Scheen, 2012). However, the correlation between its cardioprotective effect and cardiomyocyte apoptosis during myocardial I/R is unclear.

Hereby, the purpose of this study is to investigate whether the cardioprotective effects of sitagliptin, a DPP4 inhibitor, is relative to its anti-apoptotic function and to explore the underlying mechanism. We hypothesized that sitagliptin played the role of cardioprotection in a myocardial I/R rat model by reducing cardiomyocyte apoptosis. To test this hypothesis, we pretreated rats with sitagliptin for 2 weeks before inducing myocardial I/R. Then the effects of sitagliptin on myocardial injury and cardiomyocyte apoptosis were determined. Finally, we used the GLP-1 receptor antagonist exendin-(9-39) to assess the role of GLP-1 receptor-dependent pathway in the cardioprotective effects of sitagliptin.

2. Materials and methods

2.1. Experimental animals and drugs

Male Sprague–Dawley rats aged between 6 and 8 weeks were purchased from the Laboratory Animal Center of Chongqing Medical University [certificate: SCXK (YU) 2007-0001]. Rats were housed under optimal conditions with standard hygiene, temperature, photoperiods (12L: 12D), standard rat chow and water ad libitum. All of these conditions were conformed to the Guidelines for Care and Use of Laboratory Animals. All procedures on animals were approved by the Ethical Committee of the Chongqing Medical University.

The DPP4 inhibitor sitagliptin was purchased from Merck Sharp & Dohme Italia SPA. The PI3K inhibitor LY294002 was purchased from Santa Cruz Biotechnology, Inc. The GLP-1 receptor antagonist exendin-(9-39) was purchased from Sigma, St. Louis, MO, USA.

2.2. Establishment of myocardial I/R injury model

Forty Male Sprague–Dawley rats were randomly divided into the following five groups ($n=8$): the Sham group, the I/R group, the sitagliptin+I/R group (sitagliptin), the sitagliptin+exendin-(9-39)+I/R group (sitagliptin+E) and the sitagliptin+LY294002+I/R group (sitagliptin+L). Sitagliptin (300 mg/kg/day) was administrated by intraperitoneal injection for 2 weeks. Exendin-(9-39) (45 μg/kg/3 days) and LY294002 (0.3 mg/kg/3 days) were given by intraperitoneal injection 30 min before sitagliptin injection. Sitagliptin, exendin-(9-39) and LY294002 were all dissolved in dimethyl sulfoxide (DMSO). The Sham group and the I/R group received the same volume of DMSO for 2 weeks.

After pretreatment with sitagliptin for 2 weeks, all rats were anesthetized by chloral hydrate (concentration 3.5%, 10 ml/kg). Tracheotomy was carried out for ventilation by a respirator (ALC-V8B, Shanghai Alcott Biotech Co., Ltd.) with a stroke volume of 28 ml/kg, air pressure of 10 mmHg, respiration rate of 1:1 and at a rate of 86 strokes per minute. And the electrocardiogram of lead II was monitored. Thoracotomy was performed and the left anterior descending coronary artery was ligated by 6-0 silk. Then the left anterior descending coronary artery was subjected to 30 min of ischemia followed by reperfusion for 2 h. Rats in the Sham group were subjected to the same surgery process without coronary artery ligation.

Glucose levels were measured with a blood glucose monitor (Accu-Check®, Roche, Germany). Body weights of rats were weighted after the establishment of the I/R model. At the end of hemodynamic measurement, the blood plasma samples were collected from the heart using the anticoagulant tube. The hearts were rapidly excised and arrested in diastole in cold diethyl pyrocarbonate water after the rats were euthanized. Then the heart was transected parallel to the atrioventricular groove at the center of the ischemia area as previously described (Li et al., 2010). The right ventricle and atria were rapidly removed, and the left ventricle was weighed. The left ventricular weight index was expressed as the ratio of left ventricular weight to body weight. And the blood plasma samples and heart tissue were collected immediately and stored at −80 °C.

2.3. Hemodynamic measurements

During the entire I/R period, the right common carotid artery and left femoral artery were isolated. A polystyrene PE-20 catheter was inserted into the left ventricle via right common carotid artery, with one end connected to MPA-2000 multichannel physiologic recorder. The left ventricular end-systolic pressure (LVESP), left ventricular end-diastolic pressure (LVEDP) and the rates of maximum positive and negative left ventricular pressure development ($\pm$ LV$dp/dt$ max) were measured.

2.4. ELISA assay

Levels of active GLP-1 and creatine kinase-MB (CK-MB) in the plasma were detected using ELISA kits according to the instructions provided by the manufacturer (R&D Systems, Minneapolis, MN, USA). Briefly, plasma was centrifuged at 1600g for 10 min at 4 °C. The supernatants were collected for the detection of GLP-1 and CK-MB. Then the supernatants were incubated with the regents in kits. Finally, the absorbance values were measured using a microplate reader (Multiskan MK33, Thermolab systems, Helsinki, Finland). The GLP-1 level was expressed as pmol/l. The CK-MB level was expressed as U/l. The experiment of CK-MB was conducted for three times.

2.5. Colorimetry

The activity of lactate dehydrogenase (LDH) in plasma and the concentrations of malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in heart homogenate were determined by colorimetry. The experiment was performed using commercially available kits, according to the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, China). Briefly, plasma was collected as above described. Heart tissues were collected and lysed by cell lysis buffer. Then cell lysates were centrifuged at 1600g for 10 min at 4 °C. The supernatants of plasma and heart cell lysates were collected for the detection of LDH, MDA, GSH-Px and SOD. After incubation with the reagents in kits, the absorbance values at 340 nm, 450 nm, 412 nm and 532 nm were measured using a spectrophotometer (721D, Pudong Shanghai Physical Optical Instrument Factory, Shanghai, China). The LDH level was expressed as U/ml. The SOD and GSH-Px levels were expressed as U/mg protein. The MDA levels were expressed as nmol/mg protein. The experiment of LDH was performed for three times.

2.6. Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) staining

TUNEL staining was performed with the TUNEL staining assay kit according to the manufacturer's instructions (Boster Bioengineering Co., Ltd., Wuhan, China). Briefly, after deparaffinization, tissue sections were first treated with hydrogen peroxide (3%) and then digested with proteinase K (20 μg/ml; pH 7.4) at 25 °C. After digestion for 10 min, tissue sections were incubated with the labeling buffer (1:18) at 37 °C. After incubation for 120 min, tissue sections were incubated with biotinylated anti-digoxin antibody (1:100) for 30 min at 37 °C. Then incorporated fluorescein was detected with streptavidin–biotin-peroxidase and subsequently tissue sections were dyed with 3,3′-diaminobenzidine (DAB).

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