Effect of rosiglitazone on rabbit model of myocardial ischemia–reperfusion injury
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Abstract
Objective: To explore mechanism and protective effect of rosiglitazone on myocardial ischemia reperfusion (I/R) injury. Methods: A total of 48 male Japanese white big–ear rabbits were randomly divided into control group (A), I/R group (B), low dose of rosiglitazone group (C), high dose of rosiglitazone group (D). Plasma concentration of and also reduced the concentration of plasma serum creatine kinase (CK), CK–MB, high–sensitivity C–reactive protein (hsCRP), ultra–superoxide dismutase (SOD), malondialdehyde (MDA), lactic acid glutathione skin peroxidase (GSH–Px), nitric oxide (NO) and endothelin(ET) were measured 1 h later after I/R. Twenty–four hours after I/R the hearts were harvested for pathological and ultrastructural analysis. Area of myocardial infarction were tested. Results: Plasma concentration of CK, CK–MB, hsCRP, NO, MDA and ET were decreased in C, D group compared with group B. Plasma concentration of T–SOD and GSH–Px were increased significantly in C, D group compared with group B. Compared with group B, pathological and ultrastructural changes in C and D group were slightly. There was significant difference in myocardial infarction area between group C, D and group B (P<0.05). Myocardial infarction area and arrhythmia rate were lower in group C, D compare with group B. Conclusions: Rosiglitazone may protect myocardium from I/R injury by enhancing T–SOD and GSH–Px concentration, inhibit inflammatory reaction, and improve endothelial function.

1. Introduction
Myocardial ischemia–reperfusion injury often occurs after acute myocardial infarction thrombolysis, PTCA and coronary artery bypass grafting or other treatments. The mechanism of the injury is a patho–physiology phenomena caused by the expression of cytokines, especially the surface adhesion molecules[1], which can start and induce the aggregation and adhesion of inflammatory cells and cause immune injury of tissue cell. It seriously affects the treatment efficacy and prognosis of patients with myocardial infarction. Peroxisome proliferators–activated receptor (PPAR) is a member of nuclear receptor superfamily. Retinoic acid, degenerate prime, thyroid hormone, vitamin D, and orphan receptors are also members of the nuclear receptor superfamily[2]. PPAR γ is mainly expressed in adipose tissues and the immune system, which closely related to the adipocyte differentiation, insulin resistance and body immune[3].

Through activating nuclear receptors PPAR γ , rosiglitazone can regulate the production and transportation of the glucose, and also regulate the transcription of the insulin response genes. And rosiglitazone can relieve ischemia–reperfusion myocardial injury by some physiological effects such as inhibiting the inflammation, improving the endothelial function and reducing the oxidative stress[4,5]. In this study we investigated the protective effect of rosiglitazone on myocardial ischemia–reperfusion injury and its mechanism. We expect to provide new ideas for the clinical treatment of myocardial ischemia–reperfusion, thus more patients can be benefit from it.
2. Materials and methods

2.1. Animal

Male Japanese long–ear white rabbits, (2.50±0.25) kg, provided by medical experimental animal center of Liaoning Medical College. Permit number of experimental animals was SYXK (Liao) 20030019.

2.2. Medicines and reagent

Rosiglitazone hydrochloride tablets were from the Shanghai First Biochemical Pharmaceutical Co., Ltd., batch number 06080204. The tablets were temporary compound with saline, then stored for use after sterilization. Glucose injection was from Jinan Limin Pharmaceutical Co., Ltd., batch number 0512547. Sodium chloride injection was from Shenyang Zhiying Pharmaceutical Factory, batch number 06080204. ultra–superoxide dismutase (SOD), malondialdehyde (MDA), lactic acid glutathione skin peroxidase (GSH–PX), nitric oxide (NO) and endothelin (ET) kit were all purchased from Nanjing Jiancheng Bioengineering Institute.

2.3. Instruments

HITACHI 7170A automatic biochemical analyzer; 721 visible spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd.); Ultraviolet visible spectrophotometer type–UV300; K15–C low temperature ultracentrifuge (Beijing medical centrifuge factory); SHH. 7121.600 electric constant temperature tris–use water tank (Beijing Yong-Guangming medical equipment factory); Shimadzu LC-9A liquid chromatograph; Fluorescent detector type-RF535; IX–70 inverted fluorescence microscope; The OLYMPUS optical microscope type–CH20; OLYMPUS microscope photography type–C35A; Image analyzer CIAS–1000; Electronic balance; Enzyme mark instrument MC–ASCENT; Electrophoresis apparatus (DYY–6G); Low temperature refrigerator and ultra low temperature refrigerator (MDFU40865); Animal ventilator (HX–200).

2.4. Establishment of myocardial ischemia–reperfusion injury models in rabbits

Healthy male Japanese big–ear rabbits were under anesthesia with urethane (20%, 1 g/kg). Animals were then bound to a plate with the back side down and chest shearing. A skin incision was made from the sternomandibular joint line to the point above the xiphoid process along the sternum midline. The 2nd, 3rd, 4th ribs were cut in the left sternal margin, stretched with a thoracic incision, then the pericardium was opened to expose the heart. A small round looper stab 2 mm below the left bundel of coronary artery was hold by needle holder with the 2T line for ligation.

2.5. Grouping and administration

A total of 48 rabbits were randomly divided into four groups: (1) Sham operation group: Coronary artery was only exposed but not ligated. Rabbits were fed with common pellet feedstuff for 3 days; (2) Ischemia–reperfusion group: ligated for 30 min, under reperfusion for 60 min. The rabbits were feed with common pellet feedstuff for 3 days; (3) Low dose rosiglitazone group: ligated for 30 min, under reperfusion for 60 min. Rabbits were fed with common pellet feedstuff for 3 days and rosiglitazone 0.5 mg/kg/day; (4) High dose rosiglitazone group: ligated for 30 min, under reperfusion for 60 min. Rabbits were fed with common pellet feedstuff for 3 days and rosiglitazone 3 mg/kg/day. Successful ligation was confirmed by significantly ascending ST–segment of ECG in the ischemia–reperfusion group, low dose rosiglitazone group and high dose rosiglitazone group after operation. After reperfusion for 60 min, common carotid artery blood was added with heparin anticoagulant tube for biochemical testing.

2.6. Indexes measurement

Plasmic level of myocardial enzymes plasma serum (CTn1, CK–MB), high–sensitivity C–reactive protein (hsCRP) were measured in carotid arterial blood under anticoagulation. The blood was centrifuged at 2 500 rpm for 10mins, and then the supernatant was collected to determine plasma SOD, MDA, GSH–PX, NO and ET.

The hearts were harvested, then were rinsed with PBS buffer. The ischemic area of the heart were cut into approximately 1 mm in parallel, and were placed in 1% TTC or 0.25% NBT phosphate buffer (pH=7.4), 37℃,under water bath for 30 min. The dyed and undyed areas were isolated under dissecting microscope. The infarcted myocardium was pale and the none–infarcted myocardium was blue. The BI2000 image analysis system was used to calculate the percentage of the infarcted myocardium area accounted for the total ventricular area.

Isolated hearts were washed with stationary liquid. The left ventricular anterior myocardial tissues were cut into strips, and then placed in stationary liquid for 2 h, followed by rinsed with 0.09 mmol KH2PO4 for 15 min, and stored in this solution. It was dehydrated in ascending series of ethanol, cleared in xylene, embedded in paraffin, and stained by HE staining. Then the structural c myocardial pathological change was observed under light microscope.

2.7. Statistical analysis

The experimental data were analyzed with SPSS17.0 statistical software. All measurement data were expressed with mean±SD. And single–factor analysis of variance (ANOVA) was applied in the comparison among many groups, q-test was used to compare between two groups. P <0.05 indicated statistical significance. Comparatively describing
3. Results

3.1. Effect on cardiac enzymes (CTn I, CK-MB) content and hsCRP content in plasma

The results showed that compared with the sham group, the CTn I, CK-MB, and hsCRP levels in plasma were significantly increased in the ischemia–reperfusion group. And compared with the ischemia–reperfusion group, the CTn I, CK-MB, hsCRP levels in plasma in the high dose rosiglitazone group and the low–dose rosiglitazone group were decreased significantly (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>CTn I (μg/mL)</th>
<th>CK-MB (μg/mL)</th>
<th>hsCRP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>110.35±25.96</td>
<td>134.75±35.12</td>
<td>47.10±2.82</td>
</tr>
<tr>
<td>B</td>
<td>182.37±30.46</td>
<td>214.52±43.78</td>
<td>77.35±1.32</td>
</tr>
<tr>
<td>C</td>
<td>136.42±28.37</td>
<td>157.21±45.91</td>
<td>53.38±1.56</td>
</tr>
<tr>
<td>D</td>
<td>120.53±31.03</td>
<td>142.36±40.13</td>
<td>50.24±1.47</td>
</tr>
</tbody>
</table>

Note: Compared with group B, P<0.01; compared with the sham group, P<0.05.

3.2. Effect on NO, ET and GSH-Px content in plasma

The results showed that compared with the sham group, the NO, ET, GSH-Px levels in plasma were significantly increased in the ischemia–reperfusion group, while ET levels decreased significantly, compared with the sham group. Compared with the high-dose rosiglitazone group and the low-dose rosiglitazone group, the NO, ET, GSH-Px levels in plasma were decreased significantly (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>CTn I (μg/mL)</th>
<th>CK-MB (μg/mL)</th>
<th>hsCRP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>521.38±34.42</td>
<td>7.14±0.42</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>390.54±33.86</td>
<td>12.84±0.79</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>454.58±25.43</td>
<td>8.10±0.54</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>489.27±15.62</td>
<td>7.46±0.37</td>
<td></td>
</tr>
</tbody>
</table>

Note: Compared with ischemia–reperfusion group, P<0.05, P<0.01; compared with the sham group, P<0.01.

3.3. Myocardial infarct size

As compared with sham–operated group [19.2±5.5%], the myocardial infarct size of the ischemia–reperfusion group [41.3±8.5%] was significantly increased (P<0.05). Compared with the ischemic reperfusion group, the myocardial infarct size of the low–dose rosiglitazone group [26.5±7.1%] and the high–dose rosiglitazone group [23.4±6.6%] were significantly decreased (P<0.05). Compared with the sham group, the rosiglitazone low–dose group and the rosiglitazone high–dose group showed no significant difference (P>0.05).

3.4. Pathological structures under light microscopy

Myocardial fibers arranged in neat rows in the sham group under the light microscope, without inflammatory cell infiltration of the myocardial interstitium. Group I/R were of swelling and disorder myocardial fibers, inflammatory cells, and even focal necrosis in the myocardial interstitial. Myocardial cell nuclei in high and low dose rosiglitazone groups were in uniform size, myocardial fibers were mild swollen, and there were fibroblasts and inflammatory cell infiltration at different degrees.

4. Discussion

Acute myocardial infarction is the leading disease threatening the human life and health at present, while myocardial ischemia–reperfusion can cause irreversible damage, death and apoptosis to the damaged myocardial cells, thus aggregate myocardial damage and increase mortality[6]. Myocardial ischemia reperfusion injury is a process which was participated by a variety of inflammatory cell nucleus inflammatory cytokines. Neutrophils, monococytes, mast cells, and macrophages all play important roles in this process, while TNF–α, IL–1, IL–6, IL–8 were also involved in the reperfusion injury[7].

PPAR–γ ligand 15d–PGJ2 can regulate many immune responses. With the presence of PPAR–γ, 15d–PGJ2 at very low concentrations can inhibit the transcriptional effects which is mediated by LPS, active protein 21, NF–κB, and the signal TRANSDUCER and activator of transcription 1[8]. With
the interactions with NF-κB protein, PPAR-γ can prevent the binding of NF-κB with the inflammatory cytokines gene promoter region homologous cis element. PPAR-γ plays an important role in regulating the differentiation of immune cells, such as mononuclear P macrophages, T cells and NK cells. By PPAR-γ–dependent pathway, PPAR-γ ligands can inhibit T cells and NK cells produce IFN2γ. Gene chip technology showed that the PPAR γ expression in type 2 T cells is significantly stronger than in the 1–type T cells (approximately 5 to 8 times). In culture condition, type 2 immune cells (added with IL-4 and IFN-γ antibody) can induce the expression of PPAR-γ in NK cells. The activated PPAR-γ can induce the production of NF-α, IL-1, IL-2 and IL-6, which can inhibit monocyte inflammatory cytokines TNF-α and produce the anti-inflammatory effect. The key to the activation of T lymphocyte is to control the early differentiation of IL-2 gene expression of the lymphocyte reaction. The activation of PPAR-γ can inhibit the gene expression of IL-2, thus can inhibit the early activation of T lymphocytes and shows anti inflammatory effect.

The experiment showed that the pretreatment of PPAR-γ receptor rosiglitazone on myocardial ischemia–reperfusion injury can significantly reduce the incidence of arrhythmia and improve the function of heart. Rosiglitazone can improve myocardial mitochondrial function of rats and significantly reduce the size of myocardial infarction. Rosiglitazone can significantly increase the concentration of oxygen free radicals. The specific mechanism is the cross talk between RAGE and nitrative thioredoxin inactivation during diabetic myocardial ischemia–reperfusion injury. Am J Physiol Endocrinol Metab 2012; 303(7): E841–E852.


