Effect and mechanism of salvianolic acid B on the myocardial ischemia–reperfusion injury in rats

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1. Introduction

Acute myocardial infarction threatens human’s health seriously, and its incidence and mortality is increased year by year\textsuperscript{1}. The prevention and treatment are the most arduous task for medical staff. Currently the most direct and effective way to treat acute myocardial infarction is thrombolysis, PCI or CABG reperfusion myocardial\textsuperscript{2}. However, it is found in clinic that during acute myocardial ischemia reperfusion, the ischemia–reperfusion injury can seriously affect the therapeutic efficacy\textsuperscript{3}. Pretreatment and post–reperfusion can effectively reduce ischemia–reperfusion injury, which has been a research hotspot for cardiovascular research at home and abroad. Salvianolic acid B is the most abundant phenolic compound. Many experiments have certified that salvianolic acid B has the anti–ischemic effects\textsuperscript{4}, but the mechanism is still not very clear. This experiment observed the effect of the applications of salvianolic acid B on the myocardial ischemia–reperfusion injury in rats, and aims to provide a new theory and treatment for acute myocardial ischemia.

2. Materials and methods

2.1. Animals, drugs, reagents and instrument

A total of 50 male SD rats were provided by Liaoning Medical Experimental Animal Center, weighting (180±10) g.
Reagents included Salvianolic acid B injection, glucose injection, 0.9% saline, NO, SOD, MDA, ET kit and so on.

Instruments included Hitachi 7170A automatic biochemical analyzer; 721 visible spectrophotometer were manufactured by Shanghai Precision & Scientific Instrument Co., Ltd.; UV–Vis spectrophotometer UV300 type; K15–C–type low speed centrifuge were manufactured by Beijing Medical Centrifuge Plant; SHI. 7121.600 type electric thermostat tris–use water tank; Shimadzu LC–9A HPLC; OLYMPUS C–35A microscope camera; OLYMPUS CH20 optical microscope; CIAS–1000 image analyzer; Electronic balance; MC–ASCENT enzyme mark instrument; MDFU40865 low and ultra–low–temperature refrigerator; HX–200 animal respirator.

2.2. Methods

2.2.1. Myocardial ischemia–reperfusion injury model establishment

Experimental animals were given anesthesia with 20% urethane 1 g/kg. They were fixed at vertical presentation. Skin was shaved, and incised along the middle line. The incision was distracted with sternum retractor, the pericardium was opened to expose the rats’ heart. They were occluded at the 2 mm from the root of the left circumflex artery for the ligation of myocardial ischemia.

2.2.2. Grouping and administration.

The animals were randomly divided into 5 groups (n=10 in each). Rats in sham–operation group (A) were only occluded at the 2 mm from the root of the left circumflex artery, but without ligation. Rats in ischemia–reperfusion group (Group B) were only occluded at the 2 mm from the root of the left circumflex artery, after ligation for 30 min. They were reperfused for 60 min. Rats in salvianolic acid B low–dose group (group C) had ligation for 30 min, then reperfusion for 60 min. They received salvianolic acid B 20 mg /kg/d by intravenous administration. Rats in salvianolic acid B middle dose group (Group D) had ligation for 30 min, then reperfusion for 60 min. Salvianolic acid B 40 mg/kg/d was given by intravenous administration. Rats in salvianolic acid B high–dose group (E group) had ligation for 30 min, reperfusion for 60 min. Salvianolic acid B 60 mg/kg/d was given by intravenous administration. Significant ST–segments elevation indicates successful establishment of coronary artery ligation model. Then rats received reperfusion for 60 min, blood and heart tissue were obtained for detection.

2.2.3. Indexes detection

Plasma cardiac enzymes (CTn I , CK–MB) determination, plasma NO, ET determination, plasma T–SOD, MDA determination were carried out. The number of apoptotic was detected by Tunel assay, the infarct size was measured, and myocardial ultrastructure was observed by Electron microscopic.

2.2.4. Statistical analysis

The data were analyzed by SPSS 17.0 statistics software, and the measurement data were expressed as mean±SD. One–Way ANOVA was applied in the comparison between two groups, P<0.05 was considered as significant difference. Morphological data were analyzed and compared by comparative description.

3. Results

3.1. Plasma cardiac enzymes (CTn I , CK–MB) concentrations

Experimental results showed that compared with group A, plasma CTn I and CK–MB levels were significantly increased in group B, C, D and E (P<0.01). Compared with group B, plasma CTn I, CK–MB levels were all decreased in group C, D and E (P<0.01). Compared with group E, the plasma CTn I, CK–MB levels were significantly increased in group C, D (P<0.01) (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>CTn I (μg/mL)</th>
<th>CK–MB (μg/mL)</th>
</tr>
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<tbody>
<tr>
<td>Group A</td>
<td>4.35±2.96</td>
<td>80.75±35.12</td>
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<tr>
<td>Group B</td>
<td>38.37±9.45</td>
<td>224.52±43.78</td>
</tr>
<tr>
<td>Group C</td>
<td>23.42±8.36</td>
<td>177.21±45.91</td>
</tr>
<tr>
<td>Group D</td>
<td>15.33±6.33</td>
<td>141.36±40.13</td>
</tr>
<tr>
<td>Group E</td>
<td>8.52±5.06</td>
<td>112.36±39.76</td>
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</tbody>
</table>

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

3.2. Plasma NO, ET concentration in each group

Experimental results showed that compared with group A, plasma NO level was significantly decreased, ET level was increased in group B, C, D, E (P<0.01). Compared with group B, plasma NO levels were significantly increased, ET levels were significantly decreased in group C, D, E (P<0.01). Compared with group E, plasma CTn I, CK–MB levels were
significantly increased in group C, D (P<0.01) (Table 2).

Table 2
plasma NO ( μmol/L), ET (pg/mL) content in each group (mean±sd).

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO</th>
<th>ET</th>
</tr>
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<tbody>
<tr>
<td>Group A</td>
<td>71.28±4.32</td>
<td>40.23±6.35</td>
</tr>
<tr>
<td>Group B</td>
<td>22.51±5.25</td>
<td>297.57±7.58</td>
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<tr>
<td>Group C</td>
<td>37.32±6.34</td>
<td>143.56±6.54</td>
</tr>
<tr>
<td>Group D</td>
<td>45.84±5.05</td>
<td>101.2±8.82</td>
</tr>
<tr>
<td>Group E</td>
<td>59.47±5.13</td>
<td>79.73±7.53</td>
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Note: Compared with ischemia–reperfusion group, ★ ★ P<0.05, ★ P<0.01; compared with the sham group, △ P<0.01; compared with the salvianolic acid B low-dose group, ▲ P>0.05.

3.3. Effect on plasma T–SOD and MDA contents

Experimental results showed that compared with group A, plasma MDA levels were significantly increased, plasma T–SOD levels were significantly decreased in B, C, D, E group (P<0.01). Compared with group E, plasma MDA levels were significantly increased, plasma T–SOD levels were significantly decreased in group C, B (P<0.01).

Table 3
Effect on plasma T–SOD and MDA contents (mean±sd).

<table>
<thead>
<tr>
<th>Groups</th>
<th>T–SOD(U/L)</th>
<th>MDA(nmol/ml)</th>
</tr>
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<tbody>
<tr>
<td>Group A</td>
<td>521.38±34.42</td>
<td>6.14±0.42</td>
</tr>
<tr>
<td>Group B</td>
<td>220.54±33.86</td>
<td>12.84±0.79</td>
</tr>
<tr>
<td>Group C</td>
<td>300.58±25.43</td>
<td>9.10±0.54</td>
</tr>
<tr>
<td>Group D</td>
<td>399.27±15.62</td>
<td>8.46±0.37</td>
</tr>
<tr>
<td>Group E</td>
<td>432.46±14.98</td>
<td>7.13±0.41</td>
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</tbody>
</table>

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

3.4. Apoptosis index

Myocardial apoptosis index of the sham group, model group and salvianolic acid B treatment groups were as follows: group A (1.12±0.02%), group B (30.12±4.37%), group C (20.02±3.56%), group D (12.57±2.93%), group E (4.12±1.03%). Apoptotic index of the treatment group was significantly lower than that in the model group, there were significant differences between groups (P<0.05), which shows dose-dependence.

3.5. Myocardial infarct size

The myocardial infarct size [(43.3±8.6)%] of ischemia–reperfusion was significantly higher than that in the sham group [(15.2±5.3)%] (P<0.05). Compare with the ischemic reperfusion group, the myocardial infarct size in salvianolic acid B low–dose group [(33.5±7.1)%], salvianolic acid B middle dose group [(29.4±6.9)%], salvianolic acid B high–dose group [(20.4±6.5)%] were significantly decreased (P<0.05).

3.6. Ultrastructural changes of rat cardiomyocytes

Compared with the sham group (Figure A), ischemia–reperfusion rat showed more severe cardiomyocytes damage, sarcolemma breakage, subsarcolemic cytoplasmic swelling and the shrinking of nucleus, nuclear membrane; Myocardial mitochondrial showed apparent damage and vacuole–shape, or even dissolve; Also showed disordered arrangement of the myocardial fiber, a large number of collagen fibers between intercellular substance, different size vacuole were among the myofibrils, sarcoplasmic reticulum dilation. Sarcomere periodic structure was destroyed, with unclear bright–dim zones, Z line broke, twisted, with myofibril breakage; local expansion of the intercalated disk, both sides of sarcoplasmic become filamentous low electron density region.

Compared with the ischemia–reperfusion group, most of the nuclear in the salvianolic acid B high dose group and middle dose group were clear and complete, only a small part of nuclear membrane was incomplete, normal mitochondrial structure and neatly arranged, part of the mitochondria showed fracture and fragmentation, with neatly arranged myocardial fibers, less collagenous fibers among intercellular substance.

Rat cardiomyocytes of the salvianolic acid B low dose group showed: slight swelling, neatly arranged and dense myocardial fibers, significantly more collagen fibers, mainly distributed in the stromal cells, myocardial mitochondrial also with many swelling and damage and integration. Part of them were in a more disordered arrangement, but were improved slightly compared with the control group.
4. Discussion

Myocardial ischemia–reperfusion injury occurs when blood supply returns to the tissue after a period of ischemia or lack of oxygen, sometimes the injury is even unchangeable. In recent years, studies showed that its mechanism is related to oxygen free radicals and calcium overload cell apoptosis and so on[5-9].

Under normal physiological conditions, endothelial plays an important role. By a series of signal stimulation, endothelial cells can synthesis and release the relaxant and contraction signal factors to regulate normal vascular tension[9-11]. If endothelial function is in disorder, the incidence of hypertension, atherosclerosis and heart failure and other cardiovascular diseases is high[12-15].

So far, the endothelin peptide has showed a strong and long–lasting effect on the cardiovascular system. It can contract the blood vessels and regulate the cardiovascular system[16-19]. During myocardial experience ischemia and reperfusion, ET levels are significantly increased in the coronary and the ischemic area tissues. NO exists in the vascular endothelium, which is responsible for vascular relaxing. It can maintain vascular tension with endothelin. It can also participate in the signal transmission, inhibit the aggregation and adhesion of the neutrophil and platelet[9].

Researches showed that during myocardial ischemia and reperfusion, the synthesis of nitric oxide synthase began to decline, thereby the NO levels in the coronary arteries were reduced[20-22]. The study also confirmed that salvianolic acid can not only effectively inhibit the release of endothelin from vascular endothelial, but also effectively increase NO content, reduce the ischemia–reperfusion injury of myocardial. Its efficacy is positively related with the dose.

During ischemia, myocardial oxygen free radicals increased, which induces decreased SOD activity. A large amounts of oxygen free radicals can form MDA by a series of complex roles, and disrupt subcellular membranes and membrane structure system with various mechanisms, resulting in varying degrees of ischemia–reperfusion injury[23–25]. In addition to these direct mode of action, the oxygen free radicals can also promote calcium overload and induce reperfusion injury indirectly[26].This study showed that salvianolic acid B can improve the superoxide dismutase (SOD) activity of myocardial tissue and reduced malondialdehyde levels, then reduce the oxidative stress, which can play a protective role on myocardial ischemia–reperfusion injury.

Myocardial apoptosis is the common pathological physiological basis of the occurrence and development of cardiovascular disease, which is active and orderly programmed cell death of myocardial caused by pathological factors. It is an important mechanism of a variety of cardiovascular injury[27]. This experiments suggest that salvianolic acid B can inhibit apoptosis of myocardial cells, thereby reduce myocardial cell damage and protecte myocardial cells, then reduce ischemia–reperfusion injury. Traditional Chinese drug Danshen can promote blood circulation and remove blood stasis, which is widely used in clinical treatment of cardiovascular disease[28,29]. Salvianolic acid B is a major water–soluble components of Danshen[30]. It is reported that salvianolic acid B can lower lipids, antioxidant and protecte vascular endothelial cells. This study showed that salvianolic acid B can reduce myocardial infarct size and effectively reduce myocardial ischemia–reperfusion injury. Its mechanism may be related to the regulation of plasma endothelin and NO levels, reduction of oxidative stress and myocardial apoptosis and so on.

Conflict of interest statement

We declare that we have no conflict of interest.

References


