Original Article
Effects of rosuvastatin on post-infarction cardiac function and its correlation with serum cytokine level

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Received February 20, 2017; Accepted March 17, 2017; Epub July 15, 2017; Published July 30, 2017

Abstract: Acute myocardial infarction is necrosis of cardiomyocytes caused by acute and persistent ischemia/hypoxia of coronary artery. Myocardial infarction has rapid onset and can induce heart failure, arrhythmia or even heart rupture. This study aimed to investigate the effect of Rosuvastatin on ventricular reconstruction and cardiac function after infarction, and to analyze its correlation with serum cytokines. C57BL6 mice were recruited to generate myocardial infarction model by coronary artery ligation. Experimental mice were divided into three groups and received saline, 2.5 mg/(kg/d) and 5 mg/(kg/d) Rosuvastatin by gavage. Cardiac function was evaluated by ultrasound examination to analyze LVDS, LVDD, LVFS and LVEF. Heart was collected by surgery and prepared in paraffin-based sections to examine infarction area. ELISA was used to test serum TNF-α, IL-6 and IL-10 expression levels. By analyzing cardiac function indexes including LVDD, LVDS, LVEF and LVFS, high dosage of Rosuvastatin treatment significantly lower LVDD or LVDS, plus higher LVEF and LVFS (P<0.05 compared to model group). No significant difference was found regarding cardiac infarction area among all groups. Serum cytokine assay showed that high dosage of Rosuvastatin remarkably decreased TNF-α level and increased IL-6 and IL-10 concentrations (P<0.05 compared to model group). Rosuvastatin treatment on myocardial infarction mice improved cardiac functions and alleviated ventricular reconstruction. Rosuvastatin can alleviate cardiomyocyte injury and protect cells via mediating expression of inflammatory cytokine expression.

Keywords: Myocardial infarction, rosuvastatin, cardiac function, ventricular reconstruction, TNF-α, IL-6, IL-10

Introduction

Myocardial infarction has rapid onset and can induce heart failure, arrhythmia or even heart rupture [1]. Myocardial infarction is acute and persistent necrosis of myocardial tissues with ischemia and hypoxia due to rupture of coronary artery atherosclerosis plaque, platelet aggregation and thrombosis formation to block artery cavity. Moreover, rapid increase of myocardial oxygen consumption or coronary artery spasm can also induce acute myocardial infarction [2]. After acute myocardial infarction, cardiomyocytes may develop changes of structure, function and phenotype of heart, forming myocardial reconstruction [3]. Such reconstruction and altered cardiac function post-infarction are closely correlated with heart failure. Therefore, the intervention of myocardial reconstruction after infarction is of critical importance for decreasing mortality of myocardial infarction patients and preventing heart failure [4].

Ventricular reconstruction process after infarction is due to altered gene expression in cardiomyocytes, further causing interaction of intracellular molecules and cell-to-cell interaction, thus manifesting myocardial hypertrophy, apoptosis and fibroblast proliferation, extracellular matrix (ECM) degradation and fiber aggregation at cellular level [5]. Meanwhile, other studies found significantly elevated cell inflammatory factors including TNF-α, IL-6 and IL-10 and matrix metalloproteinase (MMP) in myocardial infarction patients undergoing reconstruction [6]. Therefore, it is proposed that cellular inflammatory factor and MMP expression level might be related with myocardial reconstruction [7].

Rosuvastatin is one selective HMG-CoA reductase inhibitor [8]. Statin drugs have modulatory function for blood lipid level. Previous study found that Rosuvastatin could decrease the risk of myocardial infarction, stroke and coronary artery reconstruction [9]. Various clinical
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studies showed that Rosuvastatin effectively improved cardiac function and endothelial function in coronary heart disease and myocardial infarction patients, and modulated blood lipid to inhibit inflammatory response [10, 11]. This study aimed to investigate the effect of Rosuvastatin on cardiac function after infarction and its correlation with serum cytokines such as TNF-α, IL-6 and IL-10.

Materials and methods

Experimental animal grouping

C57BL6 mice (6 weeks old) were recruited as research objects. A total of 48 C57BL6 mice were purchased from Shandong University Laboratory Animal Center, including 24 males and 24 females, with average body weight at 31.6±2.5 g. All experimental mice were divided into myocardial infarction group (N=36) and control/Sham group (N=12). Experimental group received normal diet and food and water ad libitum, and were kept under 25°C with 60±10% relative humidity.

Mice were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Jinan Central Hospital Affiliated to Shandong University.

Generation of mouse myocardial infarction model

All mice were acclimated and weighted. 60 mg/kg hydrate chloral was used for anesthesia via intraperitoneal injection. EKG electrode was connected, and respiratory machine was connected to the tracheal intubation at 100 per min. Chest skin was cut between 3rd and 4th rib of left stenosis, left chest cavity was opened by blunt separation of chest muscle layer. The pericardial sac was opened, and nylon suture was sued to ligate 1/3 of mouse coronary artery. Significant elevation of ST segment on electrode II on EKG indicated successful ligation. In control (Sham) group, nylon suture was used to penetrate cardiac muscle on left descending branch but without ligation, leading to insignificant elevation of ST segment. After surgery, heart was relocated, followed by suture of chest cavity and skins. EKG monitor and respiratory machine were removed after obtaining stable vital signs. 20000 U penicillin was injected intramuscular to prevent infection [12].

Rosuvastatin drug delivery

In those mice with successful surgery, 12 mice in Sham group were recruited as group A (control). The other 36 mice with completed myocardial infarction model generation were randomly divided into group B, C and D (N=12 each). Group C and group D received 2.5 mg/(kg.d) and 5 mg/(kg.d) Rosuvastatin by gavage for 4 week normal feeding. Group A and B mice received equal volume of saline by gavage, for 4-week feeding along with group C and D.

Cardiac function evaluation

Mice were anesthetized by 60 mg/kg hydrate chloral. Long-axial 2-D images of left ventricles adjacent to the stenosis were obtained. Using HPSONOS5500 ultrasound, probe frequency was set at 7.5 MHz and was placed in a vertical line against ventricle septum and posterior wall of left ventricle to obtain echocardiography. Interventricular septum (INS), left ventricular diastolic diameter (LVDD), left ventricular systolic diameter (LVDS) were measured. Left ventricular ejection fraction (EF) and left ventricular fractional shortening (LVFS) were measured by improved Simpson method [13].

Measurement of myocardial infarction area

All mice used in the experiment were fed for 4 weeks, and were sacrificed by 10% KCl infusion following blood collection (2 mL). The chest cavity was opened to harvest the heart, which was rinsed in pre-cold saline to remove residual blood. Water was drained by filter paper, and was separated for left ventricles using scalpel. All tissues were sectioned horizontally for frozen in liquid nitrogen and for fixation in 10% neutral buffered formalin for paraffin embedding. 5 μm slices were prepared for immunohistochemistry (IHC) and HE staining.

Paraffin-based sections were firstly dried at 30 min at 60°C, and were dewaxed by 15 min xylene immersion. Xylene was removed by gradient ethanol, followed by twice rinsing in distilled water. After drying out, hematoxylin was used for 15 min staining, followed by water rinsing, 1% HCl-ethanol differentiation for 30 s, and 15 min immersion in distilled water. After dry-
ing out, slices were stained in 1% eosin buffer for 3 min, and was carefully dehydrated in gradient ethanol following 70% ethanol. Slices were immersed in xylene-ethanol (1:1) for 5 min, followed by 15 min xylene immersion. And were mounted by resin for observation of infarction area under light field microscope to investigate cell/tissue morphology of infarction area and peripheral regions.

**ELISA assay**

Blood samples collected from experimental mice were centrifuged to separate serum, which was tested for TNF-α, IL-6 and IL-10 levels by ELISA test kits (Abcam). In brief, 100 μL mouse serum was added into 96-well plate, followed by incubation with 100 μL PBS (pH 7.4) for 4°C overnight incubation. After PBS rinsing, 2% H₂O₂-ethanol solution was used to block endogenous peroxidase activity. Non-specific binding site was blocked by adding 1% BSA solution. With PBS rinsing, rabbit anti-mouse TNF-α, IL-6 and IL-10 antibody (1:1000 dilution) was added for 37°C incubation for 2 h. Excess antibody was washed out, with the addition of biotin labelled goat anti-rabbit secondary antibody (1:200) was added for 37°C incubation for 1.5 h. Excess secondary antibody was washed out, followed by the addition of OPD development substrate for 6 min development at room temperature. 0.2 mM H₂SO₄ was added to stop the reaction. Contents of TNF-α, IL-6 and IL-10 were measured by a microplate reader (Biotek). Immunohistochemistry (IHC) staining was performed following previous methods [9].

**Statistical analysis**

All data were presented as mean ± standard deviation (SD). SPSS2.0 software was used for data analysis. One-way analysis of variance (ANOVA) was used for between-group comparison. A statistical significance was defined when P<0.05.

**Results**

**Generation of mouse myocardial infarction model**

Mouse myocardial infarction model was generated by ligation of coronary artery. EKG was used to observe dynamic change of mouse cardiac movement. As shown in **Figure 1**, ST segment on EKG electrode II was significantly elevated in infarction model, whilst Sham group showed no significant difference in EKG pattern compared to normal mice, indicating successful generation of mouse myocardial infarction model.

**Effects of rosuvastatin on cardiac function in infarction mice**

To evaluate the effect of Rosuvastatin on cardiac function in infraction model mice, ultrasound imaging was used to evaluate cardiac function indexes including LVDD, LVDS, LVEF and LVFS across different groups (**Table 1**). We found significantly higher survival rate of Sham group compared to model control or low dosage group (P<0.05) but without significant difference with high dosage group (P>0.05). Meanwhile, we found high Rosuvastatin experimental group had significantly lower LVDD and LVDS (P<0.05 compared to model control group), whilst LVEF and LVFS were higher (P<0.05). These results suggested that Rosuvastatin significantly improved cardiac function of myocardial infarction mice.

**Cardiac infarction area**

Different groups of mice were fed for four weeks and were sacrificed for harvesting heart tissues, which were horizontally sectioned in paraffin-based slices for HE staining to analyze change of myocardial infarction area, and histopathology manifestation in infarction area. We found that in non-infarction area of myocardial tissues, cells showed clear nuclear staining, complete structure of myocardial striatal, whilst infarction area showed destructed myocardial tissues with significant tissue breakage, complicated with condensed nucleus and platelet-like necrosis (**Figure 2A**). The statistics of infarction areas across all groups (**Figure 2B**).
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Table 1. Vascular collagen reconstruction of experimental mice

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Survival rate</th>
<th>LVDD (mm)</th>
<th>LVDS (mm)</th>
<th>LVEF (%)</th>
<th>LVFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>12</td>
<td>91.7%</td>
<td>3.75±0.99</td>
<td>2.39±0.61</td>
<td>81.02±3.61</td>
<td>42.39±3.61</td>
</tr>
<tr>
<td>Model</td>
<td>12</td>
<td>50.0%</td>
<td>6.05±1.13*</td>
<td>5.83±1.12*</td>
<td>45.83±4.12*</td>
<td>25.83±4.12*</td>
</tr>
<tr>
<td>Low dosage</td>
<td>12</td>
<td>66.7%</td>
<td>4.54±0.83*</td>
<td>4.65±0.96*</td>
<td>54.65±3.96*</td>
<td>29.65±2.96*</td>
</tr>
<tr>
<td>High dosage</td>
<td>12</td>
<td>83.3%</td>
<td>4.39±0.84*</td>
<td>3.12±0.85*</td>
<td>73.12±3.85*</td>
<td>38.12±2.85*</td>
</tr>
</tbody>
</table>

Note: *, P<0.05 compared to sham group; #, P<0.05 compared to model group; & P<0.05 compared to low dosage group.

Discussion

Myocardial infarction is one severe cardiovascular disease and can induce a series of symptoms including heart failure, arrhythmia or even heart rupture, thus severely threatening patient’s health [1]. Some studies believed that a series of gene expression regulation process and cytokine secretion or cellular interaction were accompanied with pathological process of myocardial infarction and heart failure, thus causing structural and functional change of hearts, forming myocardial reconstruction [14]. Study found that Statin drugs can protect cardiac function and reduce risk of myocardial infarction, stroke and coronary artery reconstruction via reducing serum or myocardial tissue inflammatory cytokine secretion, in addition to its blood lipid regulatory functions [15, 23].
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This study aimed to construct mouse myocardial infarction model, on which the correlation between Rosuvastatin and post-infarction cardiac function or serum inflammatory cytokines.

Mouse myocardial infarction model was successfully generated by coronary artery ligation surgery, and was divided into different groups following different gavage drugs delivery. Colored ultrasound was used to analyze cardiac function indexes including LVDD, LVDS, LVEF and LVFS. LVDD and LVDS parameters can be used to evaluate thickness of left ventricles, thus indirectly reflecting post-infarction reconstruction of left ventricles, whilst LVEF and LVFS can be used to evaluate cardiac function. Results showed that high dosage Rosuvastatin group mice had significantly lower LVDD or LVDS than model group, whilst LVEF and LVFS were higher. These results indicated that Rosuvastatin drug delivery can alleviate left ventricular reconstruction after infarction to improve cardiac function. Meanwhile, ELISA analysis was used to compare serum cytokine levels across different groups. High dosage Rosuvastatin group had significantly lower TNF-α and higher IL-6/IL-10 concentrations compared to model group.

Study found significantly elevated expression level of inflammatory cytokines including TNF-α, IL-6 and IL-10 and MMP in infarction patients undergoing myocardial reconstruction [17, 18]. It is thus proposed that inflammatory cytokine and MMP expression change might be related with myocardial reconstruction process. Up-regulation of pro-inflammatory factor may induce further damage of cardiomyocytes, causing myocardial fibrosis or even apoptosis [19]. IL-6 and IL-10 are important body protective cytokines. Previous studies showed decreased IL-6 or IL-10 expression in myocardial infarction patients, whilst TNF-α up-regulation could aggravate cell inflammation [20, 21]. After treatment using Rosuvastatin, IL-10 and IL-6 concentrations were increased, thus suppressing inflammation of myocardial tissues, and protecting cardiomyocytes.

Rosuvastatin is one selective HMG-CoA reductase inhibitor and can suppress reductase activity [8]. How does Rosuvastatin regulate inflammatory factor expression in injured myocardial tissues is still unclear yet. Some scholars found that Rosuvastatin treatment could increase SERCA protein expression, thus proposing that Rosuvastatin could change expression of cellular inflammatory factors via mediating calcium modulating proteins [22], although detailed mechanism still require further experiments.

Conclusion

Treatment using Rosuvastatin on myocardial infarction mice significantly improves cardiac function and alleviates ventricular reconstruction. Rosuvastatin can alleviate cardiomyocyte injury and protect myocardial tissues via modulating expression of cellular inflammatory factors.

Disclosure of conflict of interest

None.

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