Pharmacology of atorvastatin on myocardial ischemia-reperfusion in rats and drug effect analysis

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Abstract: Statins are the most important drugs in the treatment of atherosclerosis. In this paper, the authors analyze the protective effect of atorvastatin on myocardial ischemia-reperfusion in rats and its drug effect. The results showed that the ratio of myocardial infarction area to total ischemia area in atorvastatin group was smaller than that in ischemia reperfusion group (P<0.01). Compared with the ischemia-reperfusion group, the MDA (16.23±4.05), TNF alpha (41.84 ±5.61) and MPO (17.54±2.81) were decreased in atorvastatin group. The results showed that atorvastatin could improve many hemodynamic indexes including SBP, DBP, LVSP, LVEDP and so on. To sum up, atorvastatin can affect infarct size, improve hemodynamics and left ventricular function after myocardial ischemia-reperfusion injury.

Keywords: Atorvastatin, myocardial ischemia-reperfusion, apoptosis, oxidative stress, lipid regulating drugs.

INTRODUCTION

With the improvement of living standards, cardiovascular diseases have become the main killer of human health and life. Long-term myocardial ischemia can cause tissue damage and even cell death (Balasubramaniam et al., 2014). The key to solving ischemic injury is coronary artery recanalization. However, reperfusion after long-term myocardial ischemia often leads to more serious injury (Chang et al., 2016). This phenomenon is called ischemia/reperfusion (I/R). Ischemia reperfusion injury is an important complication after coronary artery recanalization, such as thrombolysis, PTCA or bypass. How to not only restore the blood perfusion of ischemic tissue, but also reduce the occurrence of I/R injury has become a hot issue of current research. In 1986, Murry proposed the concept of ischemic preconditioning (IPC), i.e. ischemic preconditioning (IPC), in which the myocardium undergoes several transient ischemia prior to long-term ischemia, and the myocardium can tolerate longer ischemia (Dinicolantonio et al., 2014). Statins are the most important drugs in the treatment of atherosclerosis. Studies have shown that long-term use of statins can not only improve long-term prognosis (Dalal et al., 2017), but also reduce the elevation of myocardial injury markers after percutaneous transluminal coronary intervention (PCI). Randomized controlled trials over the past two years have further shown that a higher dose of statins (whether statins are being used or not) 24 hours before PCI can significantly reduce the elevation of myocardial injury markers after PCI (Dan et al., 2015; Orivaldo et al., 2018).

Early recovery of myocardial perfusion through thrombolysis or percutaneous coronary intervention (PCI) after myocardial infarction is an important means to reduce the size of myocardial infarction and improve clinical prognosis (Fuu et al., 2017; Navaro, 2018). However, the recovery of ischemic myocardial blood flow may lead to ischemia reperfusion injury (IRI), which may reduce the efficacy of myocardial reperfusion. Statins drugs commonly used to regulate blood lipids and reduce plasma cholesterol and lipoprotein levels (Gaopeng et al., 2015; Shi et al., 2018). Recent studies have shown that these drugs have multiple cardiovascular protective functions (Ghoneum et al., 2015). In this study, the myocardial ischemia-reperfusion model of rats was used to study the protective effect of atorvastatin on myocardium. This research could be useful for further study and clinical drug use.

MATERIALS AND METHODS

Research object

60 clean SD rats aged 6-8 weeks were selected and provided by Animal studies department. They were 50% male and 50% female, weighing 180-250g. All 60 rats were randomly divided into 3 different groups as atorvastatin group (n=20), ischemia-reperfusion group (n =20) and control group (n=20): A torvastatin group: 1 week before operation, 15mg/kg of Atorvastatin and 2 ml of normal saline were administered orally; ischemia-reperfusion group and control group: 2ml of normal saline were administered orally once a day. All rats were fasted for 12h before operation. The control group was set up to eliminate the influence of feeding conditions, saline irrigation and routine operation on the experimental results.

The animal experiment program has been approved by the experimental animal ethics committee, No.DCWCHP/16ST.
Preparation of myocardial ischemia reperfusion model
Intraperitoneal injection of pentobarbital sodium 60 mg/kg anesthetized rats, and fixed the head and limbs on the operating table. Connect the electrocardiograph with standard lead. The left anterior descending coronary artery (LAD) was separated and ligated at the cone of pulmonary artery and 2-3 mm below the left atrial appendage between left ventricle and left ventricle in atorvastatin group and ischemia-reperfusion group. Bradycardia, dark red myocardium, elevation of ST segment and/or elevation of T wave in ECG were observed. The heart was homed and timed 30min, the heart was removed again, the ligature was removed and the reperfusion was 3 hours.

Measurement of infarct size
Three hours after reperfusion, 10 rats in each group were taken, 20 rats in the Atorvastatin group and the ischemia-reperfusion group were ligated in situ and 3mL Evans blue (10g/L) was injected into the apex of the heart for staining. Rats were sacrificed and their hearts were quickly removed. Atrial, right ventricle and large blood vessels were washed out with normal saline. Five thin slices of 2 mm were cut along the long axis and immersed in TTC phosphate buffer solution (0.5mg/mL, pH 7.4). 30 min was incubated at 37°C for dyeing. After TTC and Evans blue staining, myocardial tissue was divided into three parts: The blue staining part (Evans blue staining part) was normal myocardial tissue, the red staining part (TTC staining part) was ischemic myocardial tissue without infarction and the gray-white part was ischemic myocardial tissue. The photos were taken and calculated the area of each part with image analysis software.

Determination of malondialde Hyde (MDA), tumor necrosis factor alpha (TNF alpha) and myeloperoxidase (MPO) in myocardium of ischemic non-infarct area
The myocardial tissue of rats with left ventricular ischemia and non-infarction was taken from 0.7 to 1.0g (the same position as the control group), mixed with lystate and normal saline. The 10% tissue homogenate was prepared by Omni Prep auto-tissue homogenizer. The supernatant was centrifuged for 10 minutes with a low-temperature and low-speed centrifuge for 3000 r/min. The content of MDA was determined by thiobarbituric acid (TBA) and the contents of TNF-α and MPO were determined by enzyme linked immuno sorbent assay (ELISA). All operations were carried out strictly according to the instructions of the kit.

Detection of related indicators
The indexes of ventricular remodeling were measured. The rats were sacrificed after measuring body mass and the hearts were separated by thoracotomy. The relative mass of left and right ventricles (relative mass of left and right ventricles = mass / mass of left and right ventricles) was measured and the thickness of ventricular septum was measured.

STATISTICAL ANALYSIS
SPSS 17 statistical software was used, and all data has carried on the statistical tests. The measurement data were expressed by X± s. The comparison between the 2 groups was conducted by two independent sample t-test. Chi square test was used to compare the difference between counting data and constituent ratios. And P<0.05 means statistically significant.

RESULTS

Myocardial infarction area measurement results
The area of myocardial infarction in the Atorvastatin group was smaller than that in the ischemia-reperfusion group and the area of ischemia-reperfusion group was larger than that in the ischemia-reperfusion group (P<0.05). The total area of ischemia in the Atorvastatin group was smaller than that in the ischemia-reperfusion group (P>0.05). The ratio of myocardial infarction area to total ischemia area in atorvastatin group was smaller than that in ischemia reperfusion group (P<0.01), as shown in table 1. Myocardial HE staining in each group was shown in fig. 1.

![HE staining of myocardium in rats of each group](image)

Fig. 1: HE staining of myocardium in rats of each group

Determination results of MDA, TNF-α and MPO contents
Compared with the control group, MDA, TNF-α and MPO were increased in the ischemia-reperfusion group and atorvastatin group (P<0.05). Compared with ischemia-reperfusion group, MDA, TNF-α and MPO in atorvastatin group were decreased (P<0.05) (table 2).

Hemodynamic measurements
Compared with the control group, HR increased, SBP, DBP and LVSP decreased, LVEDP increased in the ischemia reperfusion group (P<0.05). Compared with ischemia-reperfusion group, HR decreased, SBP, DBP, LVSP increased, LVEDP and LVEDP decreased in atorvastatin group (P<0.05) (table 3).

Measurement results of ventricular remodeling
There was no significant difference in body mass between the two groups (P>0.05). Compared with the control group, the relative weight of left and right ventricles and the thickness of interventricular septum in the ischemia-reperfusion group were larger (P<0.05). Compared with...
the ischemia-reperfusion group, the relative mass of left and right ventricles in atorvastatin group was smaller and the thickness of ventricular septum was narrower (P< 0.05), as shown in table 4.

**DISCUSSION**

Statins are widely used lipid-lowering drugs. Studies have shown that statins can reduce the level of myocardial injury after myocardial ischemia reperfusion and improve long-term prognosis (Hausenloy et al., 2015; Herrera et al., 2018). The results showed that there was no difference in total ischemic area between the two groups, but the myocardial infarction area was smaller than that of the ischemia-reperfusion group, the area without ischemia-reperfusion group was larger than that of the ischemia-reperfusion group and the proportion of myocardial infarction area to total ischemia-reperfusion group was smaller than that of the ischemia-reperfusion group (Hsuan et al., 2016; Samano et al., 2018). The significance of research is consistent with previous studies. These results suggest that atorvastatin may reduce the area of myocardial infarction, thereby reducing the injury caused by reperfusion, but not by reducing the area of ischemia to play a protective role (Li et al., 2014; Alejandrina et al., 2018).

The significant increase of free radicals is closely related to the injury after myocardial ischemia and reperfusion (Wang, 2016; Zhu et al., 2015). MDA as the end product of lipid peroxide, its content can reflect the degree of oxygen free radical damage (Luo, 2015). The results of this study showed that compared with the control group, MDA in the ischemia-reperfusion group and atorvastatin group increased, but the increase in atorvastatin group was less than that in the ischemia-reperfusion group (Meybohm et al., 2015). TNF-a is an important inflammatory cytokine, which can aggravate IRI by stimulating the production of oxygen free radicals and activating cardiomyocyte apoptosis (Xiong et al., 2015; Peterzan et al., 2017). It was found that the content of TNF-a increased significantly after myocardial ischemia-reperfusion in rats, which was consistent with the results of this study, but also showed that the effect of atorvastatin on TNF-a increased slightly in rats (Oka et al., 2014). Activation of neutrophils plays an important role in IRI. MPO, as a marker, is closely related to oxidative stress during inflammatory reaction and tissue damage leading to IRI (Sheng et al., 2015).

The results of this study showed that MPO increased in both ischemia reperfusion group and atorvastatin group, but the increase in MPO in atorvastatin group was less

### Table 1: Comparison of myocardial infarction area

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Myocardial infarct ratio (%)</th>
<th>Ischemic not myocardial infarct ratio (%)</th>
<th>Ischemic myocardium ratio (%)</th>
<th>Myocardial infarct size/ischemic myocardium size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia reperfusion</td>
<td>14.27±1.58</td>
<td>32.16±5.14</td>
<td>55.21±8.47</td>
<td>28.14±6.51</td>
</tr>
<tr>
<td>Atorvastatin group</td>
<td>22.65±1.76</td>
<td>21.08±3.48</td>
<td>42.74±5.12</td>
<td>55.18±12.08</td>
</tr>
</tbody>
</table>

### Table 2: Comparison of the results of MDA, TNF-α and MPO in rats of each group

<table>
<thead>
<tr>
<th>Grouping</th>
<th>MDA(μmol/L)</th>
<th>TNF-α(μg/L)</th>
<th>MPO(μ g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>4.29±1.56</td>
<td>15.27±1.89</td>
<td>4.23±0.78</td>
</tr>
<tr>
<td>Ischemia reperfusion</td>
<td>16.23±4.05</td>
<td>41.84±5.61</td>
<td>17.54±2.81</td>
</tr>
<tr>
<td>Atorvastatin group</td>
<td>9.54±2.78</td>
<td>31.54±3.48</td>
<td>11.04±1.24</td>
</tr>
</tbody>
</table>

### Table 3: Comparison of hemodynamic results in rats of each group

<table>
<thead>
<tr>
<th>Grouping</th>
<th>HR (BPM)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>LVSP (mmHg)</th>
<th>LVEDP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>315.18±32.05</td>
<td>175.12±7.45</td>
<td>159.46±5.87</td>
<td>164.20±5.84</td>
<td>26.2±1.94</td>
</tr>
<tr>
<td>Ischemia reperfusion</td>
<td>465.72±43.21</td>
<td>134.49±11.28</td>
<td>126.91±5.13</td>
<td>127.56±12.74</td>
<td>31.25±8.49</td>
</tr>
<tr>
<td>Atorvastatin group</td>
<td>412.64±31.48</td>
<td>158.26±6.54</td>
<td>138.29±8.36</td>
<td>152.18±12.40</td>
<td>17.42±6.18</td>
</tr>
</tbody>
</table>

### Table 4: Comparison of ventricular remodeling in rats of each group

<table>
<thead>
<tr>
<th>Grouping</th>
<th>BW(g)</th>
<th>LVRW(mg/g)</th>
<th>RVRW(mg/g)</th>
<th>IVST(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>367.31±38.12</td>
<td>1.59±0.35</td>
<td>0.57±0.08</td>
<td>1.46±0.34</td>
</tr>
<tr>
<td>Ischemia reperfusion</td>
<td>334.27±59.26</td>
<td>2.48±0.41</td>
<td>0.71±0.25</td>
<td>2.81±0.46</td>
</tr>
<tr>
<td>Atorvastatin group</td>
<td>342.59±62.47</td>
<td>1.84±0.24</td>
<td>0.49±0.12</td>
<td>2.16±0.49</td>
</tr>
</tbody>
</table>

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than that in ischemia reperfusion group (Pistevou et al., 2015). The results showed that atorvastatin could improve many hemodynamic parameters including SBP, DBP, LVSP, LVEDP, the maximum rate of left ventricular pressure rise and the maximum rate of left ventricular pressure drop (Reber et al., 2013; Wei et al., 2017). The results of measurement of ventricular remodeling showed that atorvastatin could effectively inhibit left ventricular dilatation after ischemia-reperfusion, improve left ventricular function and alleviate myocardial injury caused by reperfusion (Stephen et al., 2016). Other animal studies have shown that the protective effect of atorvastatin may be related to the repair of damaged endothelial cells, the promotion of mitochondrial ATP-sensitive potassium channel opening and the regulation of nitric oxide production, but the mechanism remains to be further studied (Schmidt et al., 2013).

CONCLUSION

Reperfusion therapy of ischemic myocardium is one of the main treatments for ischemic heart disease, and the accompanying ischemic reperfusion injury has become an important factor affecting the curative effect. Statins can reduce myocardial ischemia reperfusion injury and play a protective role in cardiovascular system. To sum up, atorvastatin can affect the infarct size, improve hemodynamics and left ventricular function after myocardial ischemia reperfusion injury, which provides an important theoretical basis for further study of statins in the treatment of myocardial IRI.

REFERENCES


