Experimental study that trimetazidine inhibits Fas/FasL pathway to relieve the myocardial ischemia reperfusion injury in rats

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Objective: To study the protective effect and molecular mechanism of trimetazidine on myocardial ischemia reperfusion injury in rats. Methods: Adult male SD rats were chosen as the experimental animals and randomly divided into control group, ischemia reperfusion group and trimetazidine group, and myocardial ischemia reperfusion models were established and then given intraperitoneal injection of trimetazidine hydrochloride for intervention. The expression levels of Fas/FasL pathway molecules as well as the contents of inflammatory and oxidative stress molecules in the myocardium, and the contents of myocardial enzymes in the blood circulation were measured 120 min after reperfusion. Results: Fas, FasL, Caspase-8 and Caspase-3 mRNA expression as well as TNF-α, ICAM-1, IL-17, IL-23, NOX2, NOX4, AOPP and MDA contents in myocardium, and LDH, CK and CK-MB contents in blood circulation of ischemia reperfusion group were significantly higher than those of control group, and Fas, FasL, Caspase-8 and Caspase-3 mRNA expression as well as TNF-α, ICAM-1, IL-17, IL-23, NOX2, NOX4, AOPP and MDA contents in myocardium, and LDH, CK and CK-MB contents in blood circulation of trimetazidine group were significantly lower than those of ischemia reperfusion group; LDH, CK and CK-MB contents in blood circulation as well as TNF-α, ICAM-1, IL-17, IL-23, NOX2, NOX4, AOPP and MDA contents in myocardium of trimetazidine group were positively correlated with Fas and FasL mRNA expression. Conclusion: Trimetazidine can inhibit Fas/FasL pathway to reduce the myocardial damage caused by inflammatory response and oxidative stress response during myocardial ischemia reperfusion in rats.

1. Introduction

Myocardial ischemia-reperfusion injury is an important pathological factor that influences the effect of reperfusion therapy, and the aggravation of myocardial cell damage in the process of reperfusion will increase the occurrence risk of heart failure, malignant arrhythmia, sudden cardiac death and other serious complications[1,2]. In clinical practice, taking early intervention to reduce the myocardial injury caused by ischemia reperfusion helps to ensure the effect of reperfusion therapy. Trimetazidine is a cardioprotective agent that is used in the treatment of coronary heart disease angina and can improve the energy metabolism of myocardial cells. In recent years, studies have reported that trimetazidine has protective effect on myocardial ischemia reperfusion injury, but the specific molecular mechanism has not been elucidated. Factor associated suicide (Fas) is a member of the tumor necrosis factor receptor superfamily, it can start the cascade activation of downstream signaling pathways and promote cell apoptosis after identifying ligand FasL, and Fas/FasL pathway activation is thought to be the key pathological link that causes myocardial ischemia reperfusion injury[3]. In the following studies, we specifically analyzed whether trimetazidine reduced myocardial ischemia reperfusion injury in rats by inhibiting the Fas/FasL pathway.
2. Experimental animals, materials and methods

2.1 Experimental animals and materials

Adult male SD rats with body mass of 180-220 g were selected as experimental animals and purchased from experimental animal center of Xi’an Jiaotong University Health Science Center; trimetazidine hydrochloride was bought from Sigma Company, electrochemiluminescence kits were from Univ-bio Company, the kits related to mRNA detection were purchased from CWBIO Company, and the enzyme-linked immunosorbent assay kits were purchased from Westang Biotechnology Company.

2.2 Experimental methods

2.2.1 Animal experiment methods

The experimental animals were randomly divided into control group, ischemia reperfusion group and trimetazidine group, 10 in each group. Ischemia reperfusion group and trimetazidine group were established into myocardial ischemia reperfusion injury models according to the following methods: after ether inhalation anesthesia, thoracotomy was done between the left 3-4 ribs to show the heart and separate left anterior descending coronary artery, No. 0 suture was used to ligature the coronary artery from 2-3 mm below left auricle for 30 min, and then the line knot was opened to restore myocardial reperfusion for 120 min; trimetazidine group were given intraperitoneal injection of 20 mg/kg trimetazidine before reperfusion. Control group only underwent ether inhalation anesthesia and thoracotomy between the left 3-4 ribs, and received no coronary separation and ligation.

2.2.2 Detection of gene expression in myocardium

After 120 min of reperfusion, the myocardial tissues at ischemia reperfusion area were isolated, and the RNA extraction kit and reverse transcription kit were used to separate RNA from the tissue and synthesize it into cDNA by reverse transcription; cDNA samples were taken, PCR kits were used to configure the PCR reaction system and the mRNA expression levels of Fas, FasL, Caspase-8 and Caspase-3 were calculated after the amplification reaction.

2.2.3 Detection of myocardial enzymes in blood circulation

After 120 min of reperfusion, blood was taken from the heart, and then the contents of LDH, CK and CK-MB were determined according to the instructions of the electrochemiluminescence kit.

2.2.4 Detection of inflammatory and oxidative stress molecules in myocardium

After 120 min of reperfusion, the myocardial tissues at ischemia reperfusion area were isolated and joined by RIPA lysate to extract protein, and the instructions of enzyme-linked immunosorbent kit were referred to determine the contents of NK-κ B, TNF-α, ICAM-1, IL-17, IL-23, NOX2, NOX4, AOPP and MDA.

2.3 Statistical methods

Software SPSS 20.0 was used to input data, the data among three groups were analyzed by variance analysis and the correlation between data was analyzed by Pearson test. \( P<0.05 \) indicated statistical significance in differences.

3. Results

3.1 Fas/FasL pathway molecule expression in myocardium

Analysis of Fas/FasL pathway molecules Fas, FasL, Caspase-8 and Caspase-3 expression in myocardium among the three groups of rats was as follows: Fas, FasL, Caspase-8 and Caspase-3 mRNA expression in myocardium of ischemia reperfusion group were significantly higher than those of control group, and Fas, FasL, Caspase-8 and Caspase-3 mRNA expression in myocardium of trimetazidine group were significantly lower than those of ischemia reperfusion group.

3.2 Myocardial enzyme contents in blood circulation and their correlation with Fas/FasL

Analysis of myocardial enzymes LDH, CK and CK-MB contents in blood circulation among the three groups of rats was as follows: LDH, CK and CK-MB contents in blood circulation of ischemia reperfusion group were significantly higher than those of control group, and LDH, CK and CK-MB contents in blood circulation of ischemia reperfusion group were significantly higher than those of control group.

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Fas</th>
<th>FasL</th>
<th>Caspase-8</th>
<th>Caspase-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>10</td>
<td>1.05±0.12</td>
<td>0.98±0.13</td>
<td>1.01±0.14</td>
<td>1.06±0.18</td>
</tr>
<tr>
<td>Ischemia reperfusion</td>
<td>10</td>
<td>3.27±0.42</td>
<td>2.89±0.41</td>
<td>3.55±0.47</td>
<td>3.19±0.46</td>
</tr>
<tr>
<td>Trimetazidine group</td>
<td>10</td>
<td>1.89±0.24</td>
<td>1.74±0.20</td>
<td>2.03±0.32</td>
<td>1.93±0.27</td>
</tr>
</tbody>
</table>

\( ^* \): compared with control group, \( P<0.05 \); \( ^# \): compared with ischemia reperfusion group, \( P<0.05 \).

Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>LDH</th>
<th>CK</th>
<th>CK-MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>10</td>
<td>118.5±15.2</td>
<td>42.6±6.7</td>
<td>0.92±0.11</td>
</tr>
<tr>
<td>Ischemia reperfusion</td>
<td>10</td>
<td>626.5±76.1</td>
<td>298.5±37.1</td>
<td>4.29±0.54</td>
</tr>
<tr>
<td>Trimetazidine group</td>
<td>10</td>
<td>302.1±37.5</td>
<td>121.5±15.7</td>
<td>2.01±0.32</td>
</tr>
</tbody>
</table>

\( ^* \): compared with control group, \( P<0.05 \); \( ^# \): compared with ischemia reperfusion group, \( P<0.05 \).
group, and LDH, CK and CK-MB contents in blood circulation of trimetazidine group were significantly lower than those of ischemia reperfusion group. Pearson correlation analysis showed that LDH, CK and CK-MB contents in blood circulation of trimetazidine group were positively correlated with Fas and FasL mRNA expression.

### 3.3 Inflammatory cytokine contents in myocardium and their correlation with Fas/FasL

Analysis of inflammatory cytokines NK-κB (ng/mL), TNF-α (ng/mL), ICAM-1 (ng/mL), IL-17 (pg/mL) and IL-23 (pg/mL) contents in myocardium among the three groups of rats was as follows: NK-κB, TNF-α, ICAM-1, IL-17 and IL-23 contents in myocardium of ischemia reperfusion group were significantly higher than those of control group, and NK-κB, TNF-α, ICAM-1, IL-17 and IL-23 contents in myocardium of trimetazidine group were significantly lower than those of ischemia reperfusion group. Pearson correlation analysis showed that NK-κB, TNF-α, ICAM-1, IL-17 and IL-23 contents in myocardium of trimetazidine group were positively correlated with Fas and FasL mRNA expression.

### 3.4 Oxidative stress molecule contents in myocardium and their correlation with Fas/FasL

Analysis of oxidative stress molecules NOX2 (ng/mL), NOX4 (ng/mL), AOPP (nmol/mL) and MDA (nmol/mL) contents in myocardium among the three groups of rats was as follows: NOX2, NOX4, AOPP and MDA contents in myocardium of ischemia reperfusion group were significantly higher than those of control group, and NOX2, NOX4, AOPP and MDA contents in myocardium of trimetazidine group were significantly lower than those of ischemia reperfusion group. Pearson correlation analysis showed that NOX2, NOX4, AOPP and MDA contents in myocardium of trimetazidine group were positively correlated with Fas and FasL mRNA expression.

### 4. Discussion

Myocardial ischemia-reperfusion injury is the pathological phenomenon of myocardial injury aggravation after reperfusion therapy. Fas/FasL pathway is the mechanism to regulate the death receptor pathway apoptosis, and it is also thought to be associated with the occurrence of myocardial ischemia reperfusion injury. Fas can be combined with FasL to recruit FADD through the intracellular area of Fas, then activate Caspase-8 and start a series of cascade activation reactions, and finally activate Caspase-3 and mediate apoptosis[4,5]. In the study, analysis of the changing trends of Fas/FasL pathway molecules in myocardial tissues of ischemia reperfusion showed that Fas, FasL, Caspase-8 and Caspase-3 mRNA expression in myocardium of ischemia reperfusion group were significantly higher than those of control group. It indicates that ischemia reperfusion can excessively activate the Fas/FasL pathway in myocardial tissue to activate the apoptosis and aggravate the myocardial cell injury. Trimetazidine is a drug with myocardial protective effect, which can selectively inhibit the 3-ketoacyl CoA thiolase to block the β oxidation of fatty acid, increase the oxygenolysis of glucose and the energy supply of myocardial cells and help protect the myocardial cells[6]. Studies have confirmed that trimetazidine has protective effect on myocardial cell ischemia reperfusion injury[7,8], but the specific molecular mechanism has not been elucidated yet. In order to define whether the trimetazidine alleviated myocardial ischemia-reperfusion injury through the Fas/FasL pathway, the changes of Fas/FasL pathway molecules in ischemia-reperfusion myocardium after trimetazidine intervention, and the results showed that Fas, FasL, Caspase-8 and Caspase-3 mRNA expression in myocardium of trimetazidine group were significantly lower than those of ischemia reperfusion group. This means that trimetazidine can inhibit the activation of Fas/FasL way in the process of myocardial ischemia reperfusion, and it indicates that trimetazidine might inhibit Fas/FasL pathway to alleviate the ischemia reperfusion injury of myocardial cells.

Myocardial enzymes are the common biochemical indicators to reflect myocardial damage degree, LDH, CK and CK-MB are the catalytic enzymes in myocardial cells involved in the regulation of energy metabolism, but when hypoxia ischemia, ischemia reperfusion and other pathological conditions causes myocardial cell injury and damage, the catalytic enzymes in the cells are released into the extracellular area, and the contents of corresponding catalytic enzymes in blood circulation increase. In the study, analysis of the changes of myocardial enzyme levels in blood circulation of rats with myocardial ischemia reperfusion showed that LDH, CK and CK-MB contents in blood circulation of ischemia reperfusion group were significantly higher than those of control group, and LDH, CK and CK-MB contents in blood circulation of trimetazidine group were significantly lower than those of ischemia reperfusion group. It means that ischemia reperfusion can cause myocardial cell damage and increase the myocardial enzyme contents in blood.
circulation, and trimetazidine intervention can alleviate the damage of ischemia reperfusion to the myocardial cells and reduce the release of myocardial enzymes. Further analysis of the correlation between myocardial enzyme contents in the blood circulation and Fas/FasL pathway after trimetazidine intervention showed that LDH, CK and CK-MB contents in blood circulation of trimetazidine group were positively correlated with Fas and FasL mRNA expression in myocardial tissues. It indicates that trimetazidine can relieve the damage of myocardial cells and reduce the release of myocardial enzymes by inhibiting the activation of Fas/FasL pathway in ischemia reperfusion myocardium.

In the process of myocardial ischemia reperfusion, the blood reperfusion in ischemic myocardium will cause a large number of inflammatory cells to infiltrate in the local tissues and then start inflammatory and stress response. NF-κB is a key regulatory factor of inflammatory response activation, it starts the expression of TNF-α and ICAM-1 to mediate the activation of inflammatory response[9,10] and it can also increase the secretion of IL-17, IL-23 and other pro-inflammatory cytokines in local area to facilitate the cascade amplification of inflammatory response[11,12]. When the inflammatory reaction is activated, the barrier of electron transfer will increase the production of reactive oxygen species. NOX2 and NOX4 are the key catalytic enzymes to catalyze electron transfer to oxygen molecules and generate reactive oxygen species[13,14]; however, the massively produced reactive oxygen species can oxidize the proteins and lipids in local tissues to cause tissue damage and generate MDA and AOPP[15,16]. In the study, analysis of the changes in inflammation and oxidative stress molecules in myocardial tissues of rats with myocardial ischemia reperfusion showed the regulatory role of NF-κB, TNF-α, ICAM-1, IL-17, IL-23, NOX2, NOX4, AOPP and MDA contents in myocardium of ischemia reperfusion group were significantly higher than those of control group, and NF-κB, TNF-α, ICAM-1, IL-17, IL-23, NOX2, NOX4, AOPP and MDA contents in myocardium of trimetazidine group were significantly lower than those of ischemia reperfusion group. This means that there is the excessive activation of inflammation and oxidative stress in local tissue in the process of myocardial ischemia reperfusion, inflammation and oxidative stress can cause myocardial cell damage, and trimetazidine can inhibit the inflammatory and oxidative stress response in the process of myocardial ischemia reperfusion. Further analysis of the correlation of myocardial inflammatory and stress response activation with Fas/FasL pathway after trimetazidine intervention showed that NF-κB, TNF-α, ICAM-1, IL-17, IL-23, NOX2, NOX4, AOPP and MDA contents in myocardium of trimetazidine group were positively correlated with Fas and FasL mRNA expression. This indicates that trimetazidine can inhibit the activation of Fas/FasL pathway in myocardial ischemia reperfusion tissues to reduce the myocardial cell damage caused by inflammatory and oxidative stress response.

Based on the analysis of the above animal experiment data, it can be concluded that trimetazidine can reduce the myocardial injury as well as inflammatory and oxidative stress response during myocardial ischemia reperfusion in rats; inhibiting the over-activation of Fas/FasL pathway is the molecular pathway for trimetazidine to plays the myocardial protective role.

References