Resveratrol inhibits the P38MAPK pathway as well as downstream apoptosis, inflammation and oxidative stress molecule expression in secondary lung injury in intestinal ischemia-reperfusion

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ABSTRACT
Objective: To study the protective effect of resveratrol on secondary lung injury in intestinal ischemia reperfusion and its effect on P38MAPK pathway. Methods: Adult male SPF SD rats were selected and divided into control group, I/R group, Res-L group, Res-M group and Res-H group, the small intestinal ischemia reperfusion model was made, and Res-L group, Res-M group and Res-H group were given 5.0 mg/kg, 10.0 mg/kg and 15.0 mg/kg resveratrol for intervention. The contents of P38MAPK pathway molecules as well as downstream apoptotic molecules, inflammatory factors and oxidative stress products in the lung were detected. Results: P38MAPK, MAPKK, MAPKKK, Fas, FasL, caspase-8, caspase-9, NF-kB, TNF-α and IL-1β expression as well as ROS and MDA contents in lung tissue of I/R group were significantly higher than those of control group; P38MAPK, MAPKK, MAPKKK, Fas, FasL, caspase-8, caspase-9, NF-kB, TNF-α and IL-1β expression as well as ROS and MDA contents in lung tissue of Res-L group, Res-M group and Res-H group were significantly lower than those of I/R group. Conclusion: Resveratrol can inhibit the function of P38MAPK pathway to reduce apoptosis, inflammation and oxidative stress, and then protect the secondary lung injury induced by intestinal ischemia reperfusion.

1. Introduction

Intestinal ischemia-reperfusion injury can not only directly damage the intestinal mucosa barrier function and cause flora disorder, but can also cause the remote organ damage through the activation of systemic inflammatory response and oxidative stress reaction, and severe cases may lead to the occurrence of multiple organ dysfunction[1,2]. Acute lung damage is the common remote organ damage during intestinal ischemia reperfusion, and can cause acute respiratory distress syndrome and be life-threatening[3]. P38MAPK is the key signaling pathway regulating apoptosis, inflammation and oxidative stress in the body, the activation of the pathway is closely related to organ ischemia-reperfusion injury, and inhibiting P38MAPK activation is the key to treating intestinal ischemia-reperfusion injury and remote viscera damage[4]. Resveratrol is a kind of nonflavanoids polyphenol extracted from grape and peanut roots, which has regulating effect on cell apoptosis, oxidative stress, endoplasmic reticulum stress, inflammation and other pathological processes, and has been proven to have protective effect on neuronal, myocardial and vascular endothelial ischemia-reperfusion injury[5]. At present, it is not yet clear about the resveratrol value for treatment of secondary lung injury in the intestinal ischemia reperfusion injury. In the following studies, the protective effect of resveratrol on secondary lung injury in intestinal ischemia reperfusion and its effect on P38MAPK pathway were analyzed.

2. Materials and methods

2.1 Experimental materials

Resveratrol was purchased in MACKLIN Company, protein lysis...
buffer was bought in Shanghai Beyotime Company, enzyme-linked immunosorbent assay kits were bought in Shanghai Jining Industrial Company, and radioimmunoprecipitation kits were purchased from Shanghai Biotend Biotechnology Co., Ltd.

2.2 Experimental animals

Adult male SPF SD rats were selected as experimental animals and provided by Tongxing Biotechnology Co., Ltd., the animal license was SYXK (Guangdong) 2013-0002, the body mass was 200-250 g, they were free to eat and drink, and 12 h alternation of day and night was adopted. Animal experiments passed the hospital's ethical review and the procedures were followed for animal experiments and animal processing after death. There were a total of 40 rats, and they were randomly divided into control group, I/R group, Res-L group, Res-M group and Res-H group, 8 in each group.

2.3 Experimental methods

2.3.1 Small intestinal ischemia reperfusion model establishment

Rats were fasting for 12 h before model establishment, and I/R group, Res-L group, Res-M group and Res-H group were made into small intestinal ischemia reperfusion models according to the following methods: rats received intraperitoneal injection of 3.5 mL/kg 10% chloral hydrate for anesthesia, an abdominal median incision was made to enter into abdominal cavity, separate the superior mesenteric artery, clip it with bulldog clamp and then close the incision; after 45 min, the same incision was used to enter into the abdominal cavity and take out the bulldog clamp to establish the ischemia reperfusion model. For the control group, the same methods as those of I/R group, Res-L group, Res-M group and Res-H group were used for anesthesia and superior mesenteric membrane exposure, but the artery was not clipped by bulldog clamp.

2.3.2 Resveratrol intervention

After the bulldog clamp was taken out and the abdominal incision was closed, Res-L group received immediate intraperitoneal injection of 2.5 mg/kg resveratrol, Res-M group received immediate intraperitoneal injection of 5.0 mg/kg resveratrol, and the Res-H group received immediate intraperitoneal injection of 10.0 mg/kg resveratrol. The control group and I/R group received intraperitoneal injection of same dose of saline.

2.3.3 Lung injury evaluation

6 h after reperfusion, the rats were put to death and anatomized to get the lung tissue and add it into protein lysis buffer to extract total protein, enzyme-linked immunosorbent assay kits were used to detect P38MAPK, MAPKK, MAPKKK, Fas, FasL, caspase-8, caspase-9, NF-κB, TNF-α and IL-1β expression, and the contents of ROS and MDA were determined by radioimmunoprecipitation kits.

2.4 Statistical methods

SPSS 17.0 software was used to input and analyze data, data comparison among groups was by variance analysis and \( P<0.05 \) indicated statistical significance in differences.

3. Results

3.1 P38MAPK signaling pathway molecule expression in lung tissue

Analysis of P38MAPK signaling pathway molecules P38MAPK, MAPKK and MAPKKK expression in lung tissue among groups was as follows: P38MAPK, MAPKK and MAPKKK expression in lung tissue of I/R group were significantly higher than those of control group; P38MAPK, MAPKK and MAPKKK expression in lung tissue of Res-L group, Res-M group and Res-H group were significantly lower than those of I/R group. Differences in pair-wise comparison of P38MAPK, MAPKK and MAPKKK expression in lung tissue were statistically significant among groups \( (P<0.05) \).

3.2 P38MAPK downstream apoptosis molecule expression in lung tissue

Analysis of P38MAPK downstream apoptosis molecules Fas, FasL, caspase-8 and caspase-9 expression in lung tissue among groups was as follows: Fas, FasL, caspase-8 and caspase-9 expression in lung tissue of I/R group were significantly higher than those of control group; P38MAPK, MAPKKK expression in lung tissue of Res-L group, Res-M group and Res-H group were significantly lower than those of I/R group. Differences in pair-wise comparison of P38MAPK, MAPKKK expression in lung tissue were statistically significant among groups \( (P<0.05) \).

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>P38MAPK</th>
<th>MAPKK</th>
<th>MAPKKK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>8</td>
<td>1.86±0.24</td>
<td>0.84±0.11</td>
<td>0.59±0.08</td>
</tr>
<tr>
<td>I/R group</td>
<td>8</td>
<td>6.58±0.92</td>
<td>4.25±0.79</td>
<td>2.98±0.42</td>
</tr>
<tr>
<td>Res-L group</td>
<td>8</td>
<td>4.57±0.62</td>
<td>2.89±0.42</td>
<td>1.83±0.22</td>
</tr>
<tr>
<td>Res-M group</td>
<td>8</td>
<td>3.77±0.52</td>
<td>2.15±0.36</td>
<td>1.32±0.17</td>
</tr>
<tr>
<td>Res-H group</td>
<td>8</td>
<td>2.42±0.35</td>
<td>1.42±0.18</td>
<td>0.92±0.12</td>
</tr>
</tbody>
</table>

\(^{1}\) compared with control group, \( P<0.05 \); \(^{2}\) compared with I/R group, \( P<0.05 \); \(^{3}\) compared with Res-L group, \( P<0.05 \); \(^{4}\) compared with Res-M group, \( P<0.05 \).
Table 2.
P38MAPK downstream apoptosis molecule expression in lung tissue of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Fas</th>
<th>FasL</th>
<th>Caspase-8</th>
<th>Caspase-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>3.26±0.52</td>
<td>1.47±0.22</td>
<td>105.69±11.58</td>
<td>93.21±10.26</td>
</tr>
<tr>
<td>I/R</td>
<td>8</td>
<td>12.52±1.98</td>
<td>6.23±0.87</td>
<td>379.41±52.66</td>
<td>325.28±51.39</td>
</tr>
<tr>
<td>Res-L</td>
<td>8</td>
<td>7.28±0.93</td>
<td>4.03±0.54</td>
<td>224.63±31.49</td>
<td>203.41±25.68</td>
</tr>
<tr>
<td>Res-M</td>
<td>8</td>
<td>5.42±0.78</td>
<td>3.26±0.42</td>
<td>179.42±20.34</td>
<td>183.31±23.15</td>
</tr>
<tr>
<td>Res-H</td>
<td>8</td>
<td>4.29±0.55</td>
<td>2.59±0.35</td>
<td>148.54±18.93</td>
<td>139.55±22.18</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; #: compared with I/R group, P<0.05; &: compared with Res-L group, P<0.05; b: compared with Res-M group, P<0.05.

3.3 P38MAPK downstream inflammatory factor expression and oxidative stress product content in lung tissue

Analysis of P38MAPK downstream inflammatory factors NF-κB (μg/L), TNF-α (ng/L) and IL-1β (ng/L) expression as well as oxidative stress products ROS (nmol/L) and MDA (nmol/L) content in lung tissue among groups was as follows: NF-κB, TNF-α and IL-1β expression as well as ROS and MDA contents in lung tissue of I/R group were significantly higher than those of control group; NF-κB, TNF-α and IL-1β expression as well as ROS and MDA contents in lung tissue of Res-L group, Res-M group and Res-H group were significantly lower than those of I/R group. Differences in pair-wise comparison of Fas, FasL, caspase-8 and caspase-9 expression in lung tissue were statistically significant among groups (P<0.05).

4. Discussion

The secondary remote organ damage induced by the small intestinal ischemia-reperfusion is closely related to apoptosis, inflammatory response and oxidative stress response[6,7]. P38MAPK is an important signaling pathway to regulate apoptosis, inflammation and oxidative stress response[8,9], and in order to define the role that P38MAPK signaling pathway played in the secondary lung injury of small intestinal ischemia-reperfusion, P38MAPK signaling pathway molecule expression in lung tissue of ischemia reperfusion model rats were analyzed in the study, and the results showed that P38MAPK, MAPKK and MAPKKK expression in lung tissue of I/R group were significantly higher than those of control group. This indicates that the abnormal activation of P38MAPK signaling pathway is closely related to the occurrence of secondary lung injury of the small intestinal ischemia reperfusion. Resveratrol is the nonflavonoids polyphenol with anti-apoptotic, anti-inflammatory and antioxidant effects, and can exert therapeutic effect on the cell apoptosis, inflammation and oxidative stress mediated by P38MAPK signaling pathway. In order to further clarify the resveratrol effect on P38MAPK signaling pathway in the secondary lung injury of intestinal ischemia reperfusion, different doses of resveratrol were used to intervene the small intestinal ischemia-reperfusion model rats, the expression of P38MAPK signaling pathway molecules in lung tissue were analyzed in the study, and the results showed that different doses of resveratrol could inhibit the P38MAPK, MAPKK and MAPKKK expression in lung tissue, and the larger the resveratrol dosage, the more obvious the inhibitory effect. This shows that resveratrol is effective in inhibiting the activation of P38MAPK signaling pathway in the secondary lung injury of small intestinal ischemia reperfusion.

The activation of apoptosis pathway is an important link of tissue viscosa injury caused by ischemia-reperfusion, and the death receptor apoptosis pathway mediated by Fas/Fasl is the important apoptosis pathway regulated by P38MAPK signaling pathway. Fas is a member of the tumor necrosis factor receptor superfamily, which can be combined with FasL to activate the cascade amplification mediated by caspase-8 and caspase-9 and promote apoptosis[10,11]. In the case of over-activation of P38MAPK signaling pathways, the Fas/Fasl cell apoptosis pathway is also abnormally activated and exacerbates the apoptosis of cells in the tissue organ[12]. In the study, the analysis of above apoptosis molecule expression in lung...
tissue of ischemia reperfusion model rats showed that Fas, FasL, caspase-8 and caspase-9 expression in lung tissue of I/R group were significantly higher than those of control group. This indicates that the small intestinal ischemia-reperfusion injury can significantly activate the cell apoptosis mediated by Fas/FasL in lung tissue, and then cause secondary lung damage. Resveratrol has anti-apoptotic activity, and further analysis of resveratrol effect on Fas/FasL apoptosis pathway in lung tissue of ischemia-reperfusion model rats in the study showed that different doses of resveratrol could inhibit the expression of Fas, FasL, caspase-8 and caspase-9 in lung tissue, and the larger the resveratrol dosage, the more obvious the inhibitory effect. This shows that resveratrol is effective in inhibiting the cell apoptosis during secondary lung injury of small intestinal ischemia reperfusion.

The remote organ damage caused by ischemia reperfusion is not only related to the activation of apoptosis, but also associated with the activation of systemic inflammatory response and oxidative stress response. The inflammatory mediators secreted by inflammatory response activation and the oxidation products produced by oxidative stress reaction activation can reach the remote organs through the blood circulation, and then result in remote viscera damage[13,14]. NK-kB is an important transcription factor of P38MAPK signaling pathway downstream, and it can initiate the expression of inflammatory mediators TNF-α and IL-1β after transferring into the nucleus[15,16]; ROS is the important oxidation product of the P38MAPK signaling pathway downstream, which can directly cause tissue viscera damage, also cause lipid oxidation and produce MDA[17,18]. In the study, analysis of inflammatory factor expression and oxidation product content in lung tissue of ischemia-reperfusion model rats showed that NF-kB, TNF-α and IL-1β expression as well as ROS and MDA contents in lung tissue of I/R group were significantly higher than those of control group. It means that small intestinal ischemia-reperfusion injury can significantly activate the inflammation and oxidative stress in the lung tissue, and then cause secondary lung injury through inflammatory mediators and oxidation products. Resveratrol has anti-inflammatory and antioxidant activity, and further analysis of resveratrol effect on inflammation and oxidative stress in lung tissue of ischemia-reperfusion model rats in the study showed that different doses of resveratrol could inhibit the NF-kB, TNF-α and IL-1β expression as well as ROS and MDA generation in lung tissue, and the larger the resveratrol dose, the more obvious the inhibitory effect. This shows that resveratrol is effective in inhibiting the inflammatory response and oxidative stress response during secondary lung injury of intestinal ischemia reperfusion.

To sum up, it is believed that resveratrol can protect the secondary lung damage of small intestinal ischemia-reperfusion; the molecular mechanism of the protective effect of resveratrol is to inhibit the P38MAPK pathway function as well as the downstream cell apoptosis, inflammatory response and oxidative stress response mediated by it.