**Effects of resveratrol on regulating apoptosis and autophagy in cerebral ischemia reperfusion in rats**

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### ABSTRACT

**Objective:** To study the effects of resveratrol (Res) on regulating apoptosis and autophagy in cerebral ischemia reperfusion (I/R) in rats. **Methods:** SD rats were selected as experimental animals and randomly divided into Sham group, I/R group and Res group. Sham group were given sham operation, I/R group were established into cerebral ischemia reperfusion models by suture method, and Res group were established into cerebral ischemia reperfusion models and then given resveratrol intervention. The protein levels of anti-apoptosis molecules, pro-apoptosis molecules and autophagy markers in brain tissues were measured 24 h after reperfusion. **Results:** Livin, Survivin, XIAP and p62 protein levels in brain tissue of I/R group were significantly lower than those of Sham group whereas CytC, AIF, Fas, FasL, Caspase-8, Caspase-9, LC3-II, Beclin1, Bnip-3 and Atg5 protein levels were significantly higher than those of Sham group; Livin, Survivin, XIAP and p62 protein levels in brain tissue of Res group were significantly higher than those of I/R group whereas CytC, AIF, Fas, FasL, Caspase-8, Caspase-9, LC3-II, Beclin1, Bnip-3 and Atg5 protein levels were significantly lower than those of I/R group. **Conclusion:** Resveratrol has a significant inhibitory effect on apoptosis and autophagy in cerebral ischemia reperfusion of rats.

### 1. Introduction

Acute cerebral infarction is a clinical common cerebrovascular disease with high morbidity and disability rates. With the continuous popularization of thrombolytic therapy in recent years, patients can receive reperfusion therapy as soon as possible after acute cerebral infarction, and the disease is improved greatly[1,2]. Nevertheless, some patients with acute cerebral infarction will develop obvious ischemia reperfusion injury after reperfusion therapy, which will affect the recovery of nerve function and increase the disability rate[3]. Resveratrol (Res) is a polyphenolic substance widespread in plants such as grape skin, peanut, veratrum nigrum and Cassia tora, and has biological activities such as anti-inflammation, anti-oxidation and antiplatelet. Existing animal experiments have shown that resveratrol has protective effect on the cerebral ischemia reperfusion injury[4], but the specific mechanism of action remains unclear. In the process of cerebral ischemia reperfusion injury, apoptosis and autophagy over-activation are the pathological links closely related to the impairment of nerve function. In order to define the mechanism of resveratrol to alleviate cerebral ischemia reperfusion injury, the regulating effect of resveratrol on the apoptosis and autophagy in the brain tissue of rats during ischemia reperfusion was specifically analyzed in the following study.

### 2. Materials and methods

#### 2.1. Experimental materials

Adult male SD rats with body mass of 220-300 g were selected as experimental animals and purchased in Beijing Vital River Laboratory Animal Technology Co., Ltd., and the license number was SKXX (Beijing) 2012-0001. The animal experiment was approved by the hospital ethics committee. Resveratrol was purchased from Sigma Company, protein lysis buffer and enzyme-linked immunosorbent assay kits were bought from Nanjing Jiancheng Biotechnology Company, and microplate reader was purchased from Bio-tek Company.
2.2. Experimental methods

2.2.1. Animal grouping and model establishing
SD rats were randomly divided into Sham group, I/R group and Res group, with 10 animals in each group. I/R group and Res group were established into cerebral ischemia reperfusion models as follows: intraperitoneal injection of 10% chloral hydrate was done for anesthesia, a median incision of the neck was made to separate common carotid artery as well as internal carotid artery and external carotid artery proximal, bulldog clamp was used to temporarily clip the internal carotid artery, an incision was made in the common carotid artery 1cm from the bifurcation, the suture was inserted into the beginning of the internal carotid artery, then the bulldog clamp was loosened, the suture was continuously inserted by about 18-20 cm, it had reached the middle cerebral artery when there was resistance, the suture was taken out after 1 h of blocking, the rats were put to death after 24 h of reperfusion, and the brain tissue with ischemia reperfusion injury was collected. Sham group were only given intraperitoneal anesthesia as well as the operations of separating carotid artery, internal carotid artery and external carotid artery.

2.2.2. Animal intervention
Res group were given intraperitoneal injection of 30 mg/kg resveratrol at 20 min before reperfusion, and Sham group and I/R group were given intraperitoneal injection of same dose of saline.

2.2.3. Molecule expression detection
The right amount of brain tissue with ischemia-reperfusion injury was collected and added in protein lysis buffer to extract the total protein, and enzyme-linked immunosorbent assay kits were used to determine the protein levels of Livin, Survivin, XIAP, CytC, AIF, Fas, FasL, Caspase-8, Caspase-9, LC3-II, Beclin1, p62, Bnip-3 and Atg5.

2.3. Statistical methods
SPSS 24.0 software was used to input and analyze data, the measurement data analysis among three groups was by variance analysis and there was statistical significance in differences if $P<0.05$.

3. Results

3.1. Anti-apoptosis molecule protein levels in brain tissue
Analysis of anti-apoptosis molecules Livin (ng/mL), Survivin (ng/mL) and XIAP (pg/mL) protein levels in brain tissue among three groups of rats was as follows: Livin, Survivin and XIAP protein levels in brain tissue of I/R group were significantly lower than those of Sham group, and Livin, Survivin and XIAP protein levels in brain tissue of Res group were significantly higher than those of I/R group, shown in Table 1.

3.2. Pro-apoptosis molecule protein levels in brain tissue
Analysis of pro-apoptosis molecules CytC, AIF, Fas, FasL, Caspase-8 and Caspase-9 protein levels in brain tissue among three groups of rats was as follows: CytC, AIF, Fas, FasL, Caspase-8 and Caspase-9 protein levels in brain tissue of I/R group were significantly higher than those of Sham group, and CytC, AIF, Fas, FasL, Caspase-8 and Caspase-9 protein levels in brain tissue of Res group were significantly lower than those of I/R group, shown in Table 2.

3.3. Autophagy marker molecule expression in brain tissue
Analysis of autophagy marker molecules LC3-II, Beclin1, p62, Bnip-3 and Atg5 protein levels in brain tissue among three groups of rats was as follows: LC3-II, Beclin1, Bnip-3 and Atg5 protein levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Livin</th>
<th>Survivin</th>
<th>XIAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>10</td>
<td>2.48±0.42</td>
<td>4.59±0.71</td>
<td>285.04±37.85</td>
</tr>
<tr>
<td>I/R</td>
<td>10</td>
<td>1.02±0.17*</td>
<td>1.93±0.26*</td>
<td>112.31±15.85*</td>
</tr>
<tr>
<td>Res</td>
<td>10</td>
<td>1.78±0.26*</td>
<td>3.36±0.36*</td>
<td>227.69±35.86*</td>
</tr>
</tbody>
</table>

*: compared with Sham group, $P<0.05$; #: compared with I/R group, $P<0.05$.

Table 2
Pro-apoptosis molecule expression in brain tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>CytC</th>
<th>AIF</th>
<th>Fas</th>
<th>FasL</th>
<th>Caspase-8</th>
<th>Caspase-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>10</td>
<td>0.73±0.11</td>
<td>127.56±16.85</td>
<td>0.92±0.12</td>
<td>1.03±0.16</td>
<td>92.51±11.28</td>
<td>158.64±22.05</td>
</tr>
<tr>
<td>I/R</td>
<td>10</td>
<td>2.94±0.52*</td>
<td>326.96±52.39*</td>
<td>4.21±0.62*</td>
<td>3.89±0.51*</td>
<td>305.96±11.28*</td>
<td>413.86±64.82*</td>
</tr>
<tr>
<td>Res</td>
<td>10</td>
<td>1.37±0.22*</td>
<td>217.58±29.27*</td>
<td>1.93±0.25*</td>
<td>2.05±0.36*</td>
<td>177.54±22.93*</td>
<td>277.54±31.94*</td>
</tr>
</tbody>
</table>

*: compared with Sham group, $P<0.05$; #: compared with I/R group, $P<0.05$. 
in brain tissue of I/R group were significantly higher than those of Sham group whereas p62 protein level was significantly lower than that of Sham group; LC3-II, Beclin1, Bnip-3 and Atg5 protein levels in brain tissue of Res group were significantly lower than those of I/R group whereas p62 protein level was significantly higher than that of I/R group, shown in Table 3.

4. Discussion

Ischemia reperfusion injury is an important factor that affects the reperfusion efficacy of cerebral infarction. It will further damage the nerve function on the basis of ischemic injury, and increase the disability rate and fatality rate of the disease[5]. Resveratrol is the non-flavonoid polyphenolic substance that has been used to treat myocardial and cerebral ischemia reperfusion injury in recent years. It is widespread in grape skin, peanut, veratrum nigrum, Cassia tora and other plants, and it has anti-inflammatory, antioxidant, antiplatelet and other biological activities[6,7]. Existing related animal experimental study has confirmed that resveratrol has protective effect on cerebral ischemia reperfusion injury, the neural function of animal models with cerebral ischemia reperfusion is improved after resveratrol intervention[8], but the neuroprotective mechanism of resveratrol is still not clarified. Excessive apoptosis is an important pathophysiological change in the cerebral ischemia reperfusion process, which is also closely related to the defect of nerve function. Livin, Survivin, XIAP and other anti-apoptosis molecules in local brain tissue have regulating effects on apoptosis, and these three molecules are able to inhibit the apoptosis mediated by the caspase family members and enhance the anti-apoptotic capacity of local tissue[9]. In order to define the molecular mechanisms for resveratrol to exert protective effect in the process of cerebral ischemia reperfusion, the above anti-apoptosis molecule levels in brain tissue were analyzed at first in the study, and the results showed that Livin, Survivin and XIAP protein levels in brain tissue of I/R group were significantly lower than those of Sham group, and Livin, Survivin and XIAP protein levels in brain tissue of Res group were significantly higher than those of I/R group. This indicates that ischemia reperfusion can inhibit the expression of anti-apoptosis molecules in brain tissue, and then weaken the anti-apoptotic ability and induce apoptosis through the changes in the expression of anti-apoptosis molecules; resveratrol intervention before reperfusion could increase the expression of anti-apoptosis molecules to enhance the anti-apoptotic ability of local tissue and inhibit apoptosis.

Mitochondrial pathway and death receptor pathway in cells are the important mechanisms for regulating apoptosis. The release of CytC from mitochondria to cytoplasm is the key regulating link in the mitochondrial pathway of apoptosis, the ischemia and ischemia reperfusion stimuli can change the mitochondrial membrane permeability to CytC and cause a large amount of CytC to enter into the cytoplasm; the CytC in the cytoplasm can form multimer with Apaf-1 and recruit Caspase-9 to activate apoptosis cascade activation[10]. In death receptor pathway of apoptosis, the extracellular receptor Fas can identify the corresponding ligand FasL, to recruit FADD, then activate Caspase-8 through the signal transduction of FADD, and ultimately result in the apoptosis cascade mediated by Caspase[11,12]. In the study, analysis of above pro-apoptosis molecule expression during cerebral ischemia reperfusion indicated that CytC, AIF, Fas, FasL, Caspase-8 and Caspase-9 protein levels in brain tissue of I/R group were significantly higher than those of Sham group. This indicates that the ischemia reperfusion process can significantly activate the apoptosis of mitochondrial pathways and death receptor pathways in brain tissues, and the related molecules of both pathways of apoptosis are highly expressed. Further analysis of the effect of resveratrol on pro-apoptosis molecule expression during cerebral ischemia reperfusion showed that CytC, AIF, Fas, FasL, Caspase-8 and Caspase-9 protein levels in brain tissue of Res group were significantly lower than those of I/R group. This shows that the resveratrol intervention before reperfusion can inhibit the apoptosis of mitochondrial pathways and death receptor pathways in brain tissues.

Cell autophagy is the newly discovered mechanism of apoptosis in recent years, which is also known as type II apoptosis. In the process of cell autophagy, the autophagosome is combined with lysosome to become autolysosome and degrade the damaged proteins and organelles in cells, which helps to maintain the cellular homeostasis and provide energy for cell metabolism. However, during the pathological process of ischemia hypoxia or ischemia reperfusion, excessive cell autophagy can lead to cell damage[13,14]. LC3-II is directly involved in the formation of autophagosome and directly related to the level of autophagy[15]. Beclin1 and Atg5 are involved in regulating the is directly related to the level of autophagy and autophagy precursor formation[16], Bnip3 is the molecule that promotes both autophagy and apoptosis, and the

Table 3.

Autophagy marker molecule expression in brain tissue (ng/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>LC3-II</th>
<th>Beclin1</th>
<th>p62</th>
<th>Bnip3</th>
<th>Atg5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham group</td>
<td>10</td>
<td>1.73±0.24*</td>
<td>1.02±0.17</td>
<td>3.26±0.52</td>
<td>1.81±0.26</td>
<td>1.35±0.17</td>
</tr>
<tr>
<td>I/R group</td>
<td>10</td>
<td>5.28±0.79*</td>
<td>3.89±0.62*</td>
<td>0.78±0.10*</td>
<td>5.23±0.62*</td>
<td>6.52±0.71*</td>
</tr>
<tr>
<td>Res group</td>
<td>10</td>
<td>2.51±0.41*</td>
<td>1.87±0.25*</td>
<td>1.45±0.18*</td>
<td>2.44±0.36*</td>
<td>3.02±0.52*</td>
</tr>
</tbody>
</table>

* compared with Sham group, P<0.05; # compared with I/R group, P<0.05.

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expression of these three kinds of molecules increases in the process of autophagy.\(^{[17]}\); p62 is an autophagy degradation substrate and its expression decreases during autophagy. In order to define the change of cell autophagy during cerebral ischemia reperfusion, the contents of above autophagy marker molecule were analyzed in the study, and the results showed that LC3-II, Beclin1, Bnip-3 and Atg5 protein levels in brain tissue of I/R group were significantly higher than those of Sham group whereas p62 protein level was significantly lower than that of Sham group. This means that the cell autophagy is significantly intensified in the process of cerebral ischemia reperfusion, and on the one hand autophagy can scavenge the organelles and proteins to enhance the tolerance of local tissue to ischemia reperfusion, and on the other hand, it can damage the nerve function through the cell damage. Further analysis of the influence of resveratrol on cell autophagy in brain tissue during ischemia reperfusion showed that LC3-II, Beclin1, Bnip-3 and Atg5 protein levels in brain tissue of Res group were significantly lower than those of I/R group whereas p62 protein level was significantly higher than that of I/R group. This shows that the resveratrol intervention before reperfusion can inhibit the degree of cell autophagy in brain tissue.

The apoptosis and autophagy in brain tissue are significantly intensified in the process of ischemia reperfusion, and the intervention resveratrol can inhibit the apoptosis and autophagy in brain tissue during ischemia reperfusion.

References


