The apoptosis and autophagy in rats with spinal cord injury and the intervention effect of ferulic acid

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Objective: Too assess the apoptosis and autophagy in rats with spinal cord injury and study the intervention effect of ferulic acid. Methods: Adult male SD rats were selected and divided into sham operation group (Sham group), spinal cord injury group (SCI group) and ferulic acid intervention group (FA group), the models of spinal cord injury were made by bulldog clamp clamping and given intragastric administration of 100 mg/kg ferulic acid twice a day for intervention. 7 d and 14 d after intervention, the expression of apoptosis molecules, anti-apoptosis molecules and autophagy molecules in spinal cord were determined. Results: PARP-1, AIF, ASK-1, mTOR, PI3K, Akt, Beclin1 and LC3-II protein expression in spinal cord tissue of SCI group were significantly higher than those of Sham group while Survivin, NAIP and Bcl-2 protein expression were significantly lower than those of Sham group; PARP-1, AIF, ASK-1, mTOR, PI3K, Akt, Beclin1 and LC3-II protein expression in spinal cord tissue of FA group were significantly lower than those of SCI group while Survivin, NAIP and Bcl-2 protein expression were significantly higher than those of SCI group. Conclusion: Ferulic acid can inhibit cell apoptosis and autophagy in spinal cord injury.

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1. Introduction

Spinal cord injury is a type of injury with poor clinical prognosis and high morbidity, there are interrupted continuity and damaged integrity in spinal cord tissue under the action of external violence, and therefore, there will be irreversible damage to spinal cord function; the neuron regeneration is poorer, so the spinal cord function reconstruction is slow and the recovery is not ideal after injury[1,2]. The cell apoptosis and autophagy increase in local tissue after spinal cord injury are the important pathological links to cause neuronal damage, and inhibiting apoptosis and autophagy has become the important target to promote neural functional recovery after spinal cord injury. Ferulic acid (FA) is the polyphenol extracted from Chinese medicine angelica, and it has the biological activity such as scavenging oxygen free radicals, protecting cells and inhibiting apoptosis[3]. At present, there is little research about the effect of ferulic acid for treatment of spinal cord injury. In the following study, the assessment of apoptosis and autophagy levels in rats with spinal cord injury and the intervention effect of ferulic acid were analyzed.

2. Materials and methods

2.1 Experimental animals

A total of 60 adult male SD rats with body mass of 250-300 g were selected as experimental animals and bought in Hebei Medical University laboratory animal center. Animal experiments were upon the approval of the hospital animal ethics committee, and all animal experiments and the animal processing after execution were conducted according to the regulations.

2.2 Experimental reagents and instruments

The tweezers, vessel clamp, vessel clip and rongeur for animal experiments were provided by the operating room of Dingzhou People’s Hospital, the ferulic acid was bought in Sigma Company, enzyme-linked immunosorbent assay kits were purchased from Shanghai Westang Biotechnology Company, and microplate reader was bought from Bio-tek Company.
2.3 Experimental methods

2.3.1 Model establishment

Experimental animals were divided into sham operation group (Sham group), spinal cord injury group (SCI group) and ferulic acid intervention group (FA group), 20 in each group. SCI group and FA groups were made into models according to the following methods: they received intraperitoneal injection of 10% chloral hydrate for anesthesia, put in prone position after anesthesia and shaved on the back, a median incision was made to expose the 8th thoracic vertebra, then remove spinal process and vertebral plate with rongeur and expose the spinal cord, bulldog clamp was used to transversely clamp the spinal cord corresponding to the 8th thoracic vertebra, bulldog clamp force was 30 g, the clamping time was 60 s, then the bulldog clamp was loosened and the incision was closed to finish the establishment; for sham group, same methods as those of SCI group and FA group were followed to make a median incision and expose the spinal cord, but no clamping operation was conducted, and the incision was directly closed.

2.3.2 Intervention

FA group received ferulic acid intervention from the day of model establishment, and the method was as follows: sodium carboxymethyl cellulose solution was used to configure fresh ferulic acid solution and supply it through intragastric administration according to the dose of 100 mg/kg, 2 times a day. Sham group and SCL group were given intragastric administration of the same dose of sodium carboxymethyl cellulose solution, 2 times a day.

2.3.3 Sampling and index detection

7 d and 14 d after model establishment, 10 rats were taken from each group, put to death and anatomized to get the injured spinal cord tissue and add it in tissue protein lysis buffer to extract total protein, and enzyme-linked immunosorbent assay kits were used to determine the content of PARP-1, AIF, ASK-1, Survivin, NAIP, Bcl-2, mTOR, PI3K, Akt, Beclin1 and LC3-II in spinal cord tissue protein.

2.4 Statistical processing

SPSS 18.0 software was used to input the apoptosis and autophagy molecule levels, the above data among three groups was by variance analysis and $P<0.05$ was the standard of statistical significance in difference.

3. Results

3.1 Comparison of apoptosis in spinal cord of three groups of rats

7 d and 14 d after model establishment, analysis of apoptosis molecules PARP-1 (ng/L), AIF (ng/L) and ASK-1 (μg/L) expression in spinal cord tissue among three groups of rats was as follows: PARP-1, AIF and ASK-1 protein expression in spinal cord tissue of SCI group were significantly higher than those of Sham group, and PARP-1, AIF and ASK-1 protein expression in spinal cord tissue of FA group were significantly lower than those of SCI group. Differences in pair-wise comparison of PARP-1, AIF and ASK-1 expression in spinal cord tissue were statistically significant among three groups of rats ($P<0.05$).

| Table 1. Apoptosis molecule expression in spinal cord tissue of three groups of rats. |
|---------------------|------|------|------|------|
| Groups              | Time (d) | PARP-1 | AIF   | ASK-1 |
| Sham group          | 7     | 42.37±6.59 | 64.19±8.76 | 1.58±0.22 |
|                     | 14    | 48.51±7.76 | 70.11±8.15 | 1.71±0.25 |
| SCI group           | 7     | 127.69±20.25 | 154.59±18.71 | 4.49±0.64 |
|                     | 14    | 154.12±18.95 | 189.33±24.62 | 5.32±0.78 |
| FA group            | 7     | 89.87±11.25 | 103.58±11.27 | 3.01±0.55 |
|                     | 14    | 71.35±8.95 | 79.87±9.35 | 2.42±0.46 |

*: compared with Sham group, $P<0.05$; **: compared with SCI group, $P<0.05$.

| Table 2. Anti-apoptosis molecule expression in spinal cord tissue of three groups of rats. |
|---------------------|------|------|------|------|
| Groups              | Time (d) | Survivin | NAIP  | Bcl-2 |
| Sham group          | 7     | 0.89±0.12 | 2.42±0.46 | 1.89±0.31 |
|                     | 14    | 1.56±0.21 | 168.76±22.15 | 2.03±0.36 |
| SCI group           | 7     | 0.75±0.09 | 177.14±23.68 | 2.21±0.39 |
|                     | 14    | 0.56±0.07 | 75.79±9.35 | 1.14±0.17 |
| FA group            | 7     | 1.03±0.15 | 60.25±7.84 | 0.89±0.12 |
|                     | 14    | 1.28±0.21 | 114.27±16.78 | 1.65±0.23 |

*: compared with Sham group, $P<0.05$; **: compared with SCI group, $P<0.05$. 
accumulation of oxygen free radicals, inflammatory factors and caused by the apoptosis, autophagy and other processes after the improved by surgical intervention; secondary injury is the damage mechanical damage caused by violence, and it can be effectively includes primary injury and secondary injury, primary injury is the difficult after injury. The spinal cord injury caused by violence is interrupted spinal cord tissue continuity and the damaged integrity caused by external violence will cause irreversible damage to the spinal cord function, the reconstruction of the spinal cord function and apoptosis molecules mTOR (μg/L), PI3K (μg/L), Akt (μg/L), Beclin1 (ng/L) and LC3-II (ng/L) expression in spinal cord tissue of SCI group were significantly higher than those of Sham group, and mTOR, PI3K, Akt, Beclin1 and LC3-II protein expression in spinal cord tissue of SCI group were significantly lower than those of SCI group. Differences in pair-wise comparison of mTOR, PI3K, Akt, Beclin1 and LC3-II expression in spinal cord tissue of SCI group were significantly lower than those of SCI group. This means that ferulic acid can inhibit expression in spinal cord tissue of SCI group were significantly lower than those of SCI group. Differences in pair-wise comparison of mTOR, PI3K, Akt, Beclin1 and LC3-II expression in spinal cord tissue of SCI group were significantly lower than those of SCI group.

### Table 3.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time (d)</th>
<th>n</th>
<th>mTOR</th>
<th>PI3K</th>
<th>Akt</th>
<th>Beclin1</th>
<th>LC3-II</th>
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<tr>
<td>Sham</td>
<td>7</td>
<td>10</td>
<td>1.03±0.15</td>
<td>0.78±0.11</td>
<td>0.54±0.08</td>
<td>75.58±9.35</td>
<td>89.44±11.25</td>
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<td></td>
<td>14</td>
<td>10</td>
<td>1.12±0.17</td>
<td>0.84±0.09</td>
<td>0.62±0.09</td>
<td>80.12±11.24</td>
<td>94.51±11.95</td>
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<tr>
<td>SCI</td>
<td>7</td>
<td>10</td>
<td>2.89±0.41</td>
<td>2.21±0.35</td>
<td>1.79±0.24</td>
<td>179.55±22.15</td>
<td>203.56±27.85</td>
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<tr>
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<td>14</td>
<td>10</td>
<td>3.24±0.55</td>
<td>2.54±0.52</td>
<td>2.18±0.32</td>
<td>201.24±26.85</td>
<td>242.54±32.17</td>
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<tr>
<td>FA</td>
<td>7</td>
<td>10</td>
<td>1.76±0.25</td>
<td>1.32±0.18</td>
<td>1.12±0.15</td>
<td>124.42±15.75</td>
<td>148.76±19.27</td>
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<tr>
<td></td>
<td>14</td>
<td>10</td>
<td>1.59±0.22</td>
<td>1.01±0.15</td>
<td>0.99±0.12</td>
<td>101.34±13.57</td>
<td>121.39±16.86</td>
</tr>
</tbody>
</table>

\( ^{\circledast} \): compared with Sham group, \( P<0.05 \); \( ^{\circledast} \): compared with SCI group, \( P<0.05 \).

### 3.2 Comparison of cell autophagy in spinal cord of three groups of rats

7 d and 14 d after model establishment, analysis of cell autophagy 7 d and 14 d after model establishment, analysis of cell autophagy molecules mTOR (μg/L), PI3K (μg/L), Akt (μg/L), Beclin1 (ng/L) and LC3-II (ng/L) expression in spinal cord tissue of three groups of rats was as follows: mTOR, PI3K, Akt, Beclin1 and LC3-II protein expression in spinal cord tissue of SCI group were significantly higher than those of Sham group, and mTOR, PI3K, Akt, Beclin1 and LC3-II protein expression in spinal cord tissue of FA group were significantly lower than those of SCI group. Differences in pair-wise comparison of mTOR, PI3K, Akt, Beclin1 and LC3-II expression in spinal cord tissue were statistically significant among three groups of rats \( (P<0.05) \).

### 4. Discussion

Spinal cord injury is a key problem for clinical treatment of trauma surgery, surgical intervention can relieve the continuous spinal cord tissue damage caused by oppression and other factors, but the interrupted spinal cord tissue continuity and the damaged integrity caused by external violence will cause irreversible damage to the spinal cord function, the reconstruction of the spinal cord function is difficult after injury. The spinal cord injury caused by violence includes primary injury and secondary injury, primary injury is the mechanical damage caused by violence, and it can be effectively improved by surgical intervention; secondary injury is the damage caused by the apoptosis, autophagy and other processes after the accumulation of oxygen free radicals, inflammatory factors and other metabolites in the local tissue\(^{4,5}\), it is difficult to be improved through surgical intervention, and it needs to be improved by drug intervention and exercise during rehabilitation. Ferulic acid is the effective component in Chinese medicine angelica, and it has extensive biological effects such as scavenging oxygen free radicals, protecting cells and inhibiting apoptosis. A large number of studies have shown that ferulic acid can protect the pancreas cells, liver cells and vascular endothelial cells\(^{6-8}\), and ferulic acid also plays a promoting role in regeneration of peripheral nerve myelin\(^{9}\). However, there is no clear report whether ferulic acid has protective effect on spinal cord injury.

Cell apoptosis is an important part of the secondary spinal cord injury, mitochondrial pathway, death receptor pathway and endoplasmic reticulum pathway are the three common pathways regulating apoptosis in the body, and the relationship is the closest between mitochondrial apoptosis and spinal cord injury. PARP-1 is a DNA repair enzyme in cells, and severe tissue damage can activate oxidative stress, lead to DNA single-strand fracture and excessive PARP-1 activation, and massively consume NAD+ and ATP, which result in the energy depletion, mitochondrial injury and apoptosis\(^{10}\); AIF is the apoptosis-regulating molecule in mitochondrial intermembrane space, and can open the mitochondrial membrane transition pore and be released into cytoplasm to activate apoptosis\(^{11}\); ASK1 is an apoptosis-regulating molecule with protein kinase activity, and it causes apoptosis through c-fos, c-jun and other pathways\(^{12}\). In the study, analysis of the expression of apoptosis molecules after spinal cord injury showed that PARP-1, AIF and ASK1 protein expression in spinal cord tissue of SCI group were significantly higher than those of Sham group. This means that the enhanced mitochondrial apoptosis mediated by PARP-1, AIF and ASK1 is closely associated with spinal cord injury. Further analysis of ferulic acid effect on the apoptosis molecule expression in the spinal cord tissue showed that PARP-1, AIF and ASK1 protein expression in spinal cord tissue of FA group were significantly lower than those of SCI group. This means that ferulic acid can inhibit mitochondrial apoptosis in the process of spinal cord injury, and is beneficial to relieving spinal cord injury and improving the spinal cord function.

At the same time of apoptosis in local spinal cord injury, the content and function of a variety of anti-apoptosis molecules change correspondingly. Survivin, NAIP and Bcl-2 are the important anti-apoptosis molecules in the body. Survivin is the most powerful anti-apoptosis molecule at present, and can antagonize the biological effect of many kinds of molecules in caspase family, then inhibit cascade activation reaction mediated by caspase molecules, and hinder apoptosis; NAIP is an anti-apoptosis molecule specifically existing in neurons and glial cells, and it can inhibit the nerve cells apoptosis caused by ischemia hypoxia, mechanical compression and other factors\(^{13}\); Bcl-2 is the inhibitor of mitochondrial apoptosis pathway, and it can cause the closing of the mitochondrial membrane transition pore and block the mitochondrial apoptosis\(^{14}\).
In the study, analysis of the expression of above anti-apoptosis molecules after spinal cord injury showed that Survivin, NAIP and Bcl-2 protein expression in spinal cord tissue of SCI group were significantly lower than those of Sham group. This means that the blocked anti-apoptosis mediated by Survivin, NAIP and Bcl-2 is closely associated with spinal cord injury. Further analysis of ferulic acid effect on the anti-apoptosis molecule expression in spinal cord tissue showed that Survivin, NAIP and Bcl-2 protein expression in spinal cord tissue of FA group were significantly higher than those of SCI group. This means that ferulic acid can enhance the anti-apoptosis in the process of spinal cord injury, and is beneficial to inhibiting the spinal cord injury caused by apoptosis.

Cell autophagy is the new cell death process discovered in recent years, and also known as type II programmed cell death. mTOR and PI3K/Akt pathways are the important mechanisms that are now known to regulate cell autophagy[15]. Autophagosome formation is the symbol of the cell autophagy, Beclin1 is the key molecule involved in autophagosome formation, and LC3-I transition to LC3-II is also the important step to mediate autophagosome formation[16,17]. In the study, analysis of the expression of above cell autophagy molecules after spinal cord injury showed that mTOR, PI3K, Akt, Beclin1 and LC3-II protein expression in spinal cord tissue of SCI group were significantly higher than those of Sham group. This means that the cell autophagy mediated by mTOR and PI3K/Akt pathway is closely associated with spinal cord injury. Further analysis of the ferulic acid effect on above cell autophagy molecule expression in spinal cord tissue showed that mTOR, PI3K, Akt, Beclin1 and LC3-II protein expression in spinal cord tissue of SCI group were significantly lower than those of FA group. This means that ferulic acid can inhibit the cell autophagy mediated by mTOR and PI3K/Akt pathway in the process of spinal cord injury, which is helpful to reduce the spinal cord injury and improve the spinal cord function.

Based on the discussion of above animal experimental results, it is believed that cell apoptosis and autophagy enhancement is closely related to the spinal cord injury; ferulic acid can inhibit the cell apoptosis and autophagy in the process of spinal cord injury so as to reduce the spinal cord injury and improve the spinal cord function.

References