

Neuroprotective effect and mechanism study of forsythia on acute spinal cord injury in rats

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Abstract: To investigate the effect of *Forsythia Forsythiae* on early inflammatory response in acute spinal cord injury in rats, the mechanism of neuroprotective effect was studied. 60 female adult healthy rats, weight 200-240g. Randomly divided into experimental control group (A group), spinal cord injury group (B group), forsythia fat group (C group). Each group was 20 rats. After anesthesia, A group performed laminectomy to remove the exposed dura mater. The acute rat spinal cord injury model was made by Allen's heavy blow method in B group and C group after exposure to dura mater. After one hour of successful modeling, saline was injected into B group, 10mg/kg, C group was given intraperitoneal injection of forsythia, 10mg/kg. After 24 hours, 10 rats were killed and spinal cord tissue was removed. ELISA method was used to detect TNF- α and IL-6 concentrations in spinal cord tissue. Western blot detection of protein expression in spinal cord tissue p38MAPK injury, HE staining to observe the tissue morphology after spinal cord injury in acute rats. BBB scores were used to evaluate the motor recovery of both lower limbs in each group at the 1st, 3rd, 7th, 14th, 21st and 28th days after operation. The score of 1st, 3rd, 7th, 14th, 21st and 28th days after operation BBB, A group was significantly higher than that of B, C group ($P < 0.05$). The score of BBB group was higher than that of B group ($P < 0.05$). ELISA tests, The TNF- α and IL-6 concentrations in A group were significantly lower than those in B, C group. C group was significantly lower than B group ($P < 0.05$). Western blot tests, the expression level of p38MAPK protein in A group was significantly lower than that in B, C group, and the expression level of p38MAPK protein in C group was significantly lower than that in B group ($P < 0.05$). HE results show, A normal nerve cells in the spinal cord, no bleeding, no edema, nerve cell necrosis and mitosis were not observed. Hemorrhage, infiltration of neutrophils and edema of nerve cells were found in B group. C neurons in the spinal cord, mild microglial proliferation, and the lesion was better than B group. *Forsythia forsythia* lipin inhibits the expression of p38MAPK signaling pathway, reducing inflammatory response and edema after acute spinal cord injury in rats, It has certain protective nerve function.

Keywords: Forsythiaside; spinal cord injury; inflammatory response; neuroprotection; p38MAPK signaling pathway

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1. Introduction

Spinal cord injury destroys the nerve conduction pathway, which can result in the loss of sensation and movement below the injury plane, the paralysis of limbs or lower limbs after injury, the loss of labor and self-care ability, the high rate of death and disability, and the heavy economic and spiritual burden on the family and society [1-2]. Inflammatory response is [3] an important part of secondary spinal cord injury. It not only induces neuronal apoptosis and stimulates glial scar formation, but also activates immune injury and eventually exacerbates neurological dysfunction. How to restore nerve function after spinal cord injury to make paralyzed patients stand up and return to society is still a problem. At present, the treatment methods of spinal reconstruction, surgical decompression and nutritional nerve can not fundamentally reshape nerve function. Therefore, it is necessary to find new drugs to treat spinal cord injury by improving nerve injury.

With the rapid development of Chinese medicine and the deepening of the research on traditional Chinese medicine, the role of traditional Chinese medicine components in spinal cord injury has been paid more and more attention [4-5]. forsythia adiponectin [6-9] is one of the most important active components

isolated from forsythia, which has been proved to have a wide range of pharmacological activities, including antioxidant, anti-inflammatory and hypolipidemic activities. However, there is no report on the effect of forsythia adiponectin on spinal cord injury.

By establishing a rat model of spinal cord injury, *Forsythia suspensa* was injected intraperitoneally into the experimental group to observe the morphological changes of the injured spinal cord tissue and the expression of p38MAPK proteins and inflammatory factors, and to explore the protective effect of *Forsythia suspensa* on spinal cord injury.

2. Materials and methods

2.1 laboratory animal and main reagent instruments

60 healthy adult female Sprague-Dawley rats, weight, 200-240g were provided by the Animal Research Center of Shandong University. *Forsythia forsythia* was from Shanghai Yuanye Biotechnology Co., Ltd). TNF- α ELISA、IL-6ELISA antibodies and related kits were from Wuhan Liuhe Biotechnology Co., Ltd. 4°C cryogenic centrifuge (Eppendorf, Germany), enzyme scale (Thermo Fisher Scientific, Finland), CO2 cell incubator (Forma Scientific, United States (USA)); high pressure sterilization box (Aianm Technology Co., Ltd., Germany), rapid tissue cell crusher (Wuxi Waxin Instrument Manufacturing Co., Ltd) and an inverted phase contrast

microscope (Olympus/ Olympus, Japan) were used in this work.

2.2 experimental subgroup and methods

SD rats were randomly divided into three groups: experimental control group (A group), spinal cord injury group (B group) and forsythia fat group (C group), Each group of 20. The rats were anesthetized with pentobarbital sodium, ketamine, atropine and placed on the operating table, fixed limbs. After routine disinfection, along the dorsal side of the rat, cut the skin, skin, fascia, revealed, blunt stripping of back muscles to locate 8 segments of the chest, surgical microscopically assisted thoracotomy, exposing the dura mater. Only the dura mater was exposed in the control group, without any damage, close the incision layer by layer. B、C group had a Allen's strike after exposure to dura, adjust the standard Allen's strike moulding device. The 10 g hammer falls vertically at 5 cm and strikes the spinal cord. The standard of successful construction of spinal cord injury model in rats is hyperemia and edema. Spastic convulsions of the lower limbs, then suture the dorsal tissue of the rat. After one hour of successful modeling, saline was injected into B group, 10mg/kg, C group was given intraperitoneal injection of forsythia, 10mg/kg. After operation, rats were kept in clean cages, daily postoperative massage of the bladder to assist urination, prevent infection. The spinal cord tissue of rats was selected on the 3rd day after successful modeling. BBB score was used to evaluate the recovery of lower extremity movement in the remaining 10 rats in each group.

2.3 Observation indicators

2.3.1 BBB score

Postoperative 1, 3, 7, 14, 21, 28 d using the BBB score to evaluate the hindlimb motor function of each group of rats, in the conscious state of the rats, the lower limb load bearing and motor ability were observed. The lower limb muscle strength of the rats was evaluated according to the scoring criteria, and the mean value of the hindlimb score on both sides of each rat was taken as the final score.

2.3.2 HE staining

24h of post-operation ,40 g/L of paraformaldehyde were perfused, the spinal cord tissue of each group was removed ,4% paraformaldehyde was fixed for 48 h, paraffin embedding, 5 μ m sections were cut by slicer, and HE staining was performed. The morphological changes and neutrophil infiltration of gray matter were observed with microscope.

2.3.3 ELISA detection

Take out the preserved spinal cord tissue, add the PBS solution, centrifuge the supernatant under the condition of homogenizing 6 min, 4°C in the tissue cell crusher for 20 min, operate according to the specification of the

kit, add the final solution and mix well. The expression concentration TNF- α 、IL-6 inflammatory factors in spinal cord was measured.

2.3.4 Western blot

After weighing, appropriate amount of protein lysate was added to the preserved spinal cord tissue, then ground in the tissue cell crusher and cracked fully. The supernatant was collected and the protein concentration was determined by BCA kit. After adding sample, electrophoresis, film transfer, sealing, incubation, adding chromogenic agent color. The developer and measured the gray value of p38MAPK protein by ImageJ software.

2.4 Statistical methods

SPSS22.0 statistical software is used for analysis. The data were expressed by $\bar{x} \pm s$. Single factor ANOVA, LSD test were applied and test level was $\alpha=0.05$.

3. Results

3.1 BBB score

All the remaining rats survived successfully until the end of the experiment, the BBB score of the control group was significantly higher than that of the spinal cord injury group and the forsythia adiponectin group ($P<0.05$), and the score of the C group was higher than that of the B group ($P<0.05$).

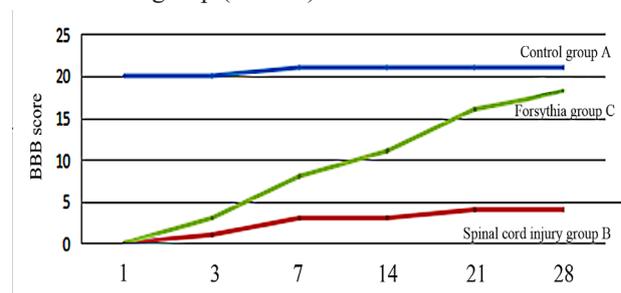


Figure 1 Three group BBB score with the days increase

3.2 HE stain result

In the control group, spinal cord nerve cells were normal, no necrosis and degeneration, hemorrhage, neutrophil infiltration, neuronal and glial cell enlargement, local liquefaction, necrosis and scar formation were found in the spinal cord injury group. Neutrophil infiltration, slight enlargement of neurons and glial cells, and no obvious necrotic area were found in the forsythia adipocyte group.

3.3 ELISA test result

The TNF- α , IL-6 concentration in the control group was significantly lower than that in the spinal cord injury group, the forsythia fat group, and the forsythia fat group was significantly lower than that in the spinal

Table 1 TNF- α , IL-6 concentrations in three groups of spinal cord tissue (unit pg/mL)

	Control group (A)	Spinal cord injury group(B)	Forsythia group(C)
TNF- α concentration	118.90 \pm 14.06	510.30 \pm 19.21	290.30 \pm 15.21
IL-6concentration	65.30 \pm 14.21	189.50 \pm 6.50	116.10 \pm 9.36
P		0.0124	0.0011

cord injury group (P<0.05).

3.4 Western blot result

The relative expression of p38MAPK protein in the control group was significantly lower than that in the spinal cord injury group, the forsythia group and the spinal cord injury group (P<0.05).

4. Discussion

Spinal cord injury (SCI) is a catastrophic disease. Severe neurological dysfunction caused by injury brings great pain to patients and families [1-2]. Spinal cord injury mainly includes primary spinal cord injury and secondary injury. Inflammatory reaction is an important part of spinal cord secondary injury. It not only induces neuronal apoptosis and stimulates glial scar formation, but also activates immune injury. Eventually aggravate neurological dysfunction. After primary injury, the blood-cerebrospinal fluid barrier is damaged due to the rupture of spinal cord vessels. The exudation and migration [14] of inflammatory cells are rapidly caused. After exudation of neutrophils, inflammatory cells mainly release TNF- α , IL-6 and other inflammatory mediators to mediate neurotoxic reactions and aggravate spinal cord injury [12-13]. This study found that the TNF- α , IL-6 of spinal cord injury group was significantly higher than that of control group, indicating that inflammatory reaction occurred after spinal cord injury, but after the treatment of forsythia adiponectin, compared with spinal cord injury group, the TNF- α , IL-6 was significantly decreased.

Spinal cord injury, after the inflammatory response, the homeostasis of glial cell regulation is broken, the activation of astrocytes, and release glial scar [15-16]. The production of glial scar originally limited the inflammatory response, neuroprotective functions such as wrapping the damaged spinal cord. But as inflammation intensifies, excessive secretion of glial scar, his initial neuroprotective function gradually evolved into a barrier [17] that limited nerve regeneration. P38 mitogen-activated protein kinase (p38MAPK) pathway is the most important member of the MAPK family to control inflammation. The activated p38MAPK can induce the expression of iNOS and COX enzymes. Control inflammatory response [18],

after intervention with forsythia and spinal cord injury, p38MAPK expression decreased in animal models. It shows that after the intervention of forsythia, P38MAPK expression reduced. So it reduces the inflammatory response in the spinal cord injury area. Inhibition of scar formation related pathways after spinal cord injury, to some extent protect the spinal cord, promote nerve recovery.

5. Conclusion

According to this study, forsythia has a certain neuroprotective effect on spinal cord injury. The mechanism is to reduce the level of inflammatory factors in the early stage of spinal cord injury by inhibiting the inflammatory response mediated by p38MAPK pathway, thus reducing spinal cord injury and then promote the recovery of nerve function. The results of this experiment can provide a new way of thinking and treatment for the control of inflammatory response and neuroprotection in acute spinal cord injury.

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