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Effect of Yaobitong capsule on histomorphology of dorsal root ganglion and on expression of p38mark phosphorylation in autologous nucleus pulposus transplantation model of rats

Jian Xin^{1,2}, Feng-Shuang Jia³, Zhan-Wang Xu^{2*}¹Shandong University of Traditional Chinese Medicine, Ji'nan, China²Department of Orthopaedics, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Ji'nan 250011, Shandong, China³Department of Orthopaedics, Third Hospital of Ji'nan, Ji'nan 250011, Shandong, China

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ABSTRACT

Objective: To discuss the effect of Yaobitong capsule on histomorphology of dorsal root ganglion and on expression of p38MARK phosphorylation in autologous nucleus pulposus transplantation model of rats.

Methods: A total of 60 SD rats were randomly divided into the blank group, model group and Yaobitong capsule group, with 20 rats in each group. The animal model of autologous nucleus pulposus transplantation around the lumbar nerve root was built. Three days after the modeling, rats were given the drugs for the first time, while rats in the model group were given the equivalent normal saline. After 30 d of continuous administration, samples were collected from rats. HE staining was performed on the dorsal root ganglion of L4 and L5 spinal cord of rats in each group and the expression of p38MARK phosphorylation was measured. All data were treated with the statistical analysis.

Results: The histological examination showed that the histomorphology of dorsal root ganglion in the Yaobitong capsule group was more significantly improved than the one in the model group, while the results of western blot showed that Yaobitong capsule could significantly inhibit the level of p38MAPK phosphorylation of dorsal root ganglion cells.

Conclusions: Yaobitong capsule can improve the symptoms and nerve radiculopathy of autologous nucleus pulposus transplantation of rats and its mechanism may be associated with its inhibiting effect on the level of p38MAPK phosphorylation.

1. Introduction

With the degradation of human function, retrogression of the spinal structure, and abnormality in mechanics structure, the pathogenesis of lumbar disc herniation is complicated, and the basic pathological factor is the mechanical compression and inflammatory change [1]. The autoimmune inflammation induced by the autologous nucleus pulposus of intervertebral disc tissue and the post-operative inflammation caused by the remained nucleus pulposus are also the key factors to cause the pathological and physiological changes of dorsal root ganglion [2].

The mitogen-activated protein kinase (MAPK) is an important transmitter of signal from the cell surface to the nucleus, which can regulate the transcription expression of genes through the phosphorylated transcription factor. Where, after being activated by the physical and chemical stress and inflammatory cytokine, p38MARK plays an important role in the regulation of onset and development of inflammation [3]. According to previous researches, the activation of p38MARK signaling pathway is of critical importance for the onset and maintenance of neuropathic pain [4].

The original prescription of Yaobitong capsule originates from the summary of forty years of clinical experiences from Professor Sun. It consists of 8 traditional Chinese drugs, including pseudo-ginseng, *Ligusticum wallichii*, corydalis tuber, root of herbaceous peony, rhizoma cibotii, root of bidentate achyranthes, radix angelicae pubescentis, and stewed rheum officinale. Such prescription can promote blood circulation and remove blood stasis, promote circulation of Qi and relieve pain, and expel evil-wind and remove dampness, which has the good

*Corresponding author: Zhan-Wang Xu, Department of Orthopaedics, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Ji'nan 250011, Shandong, China.

Tel: +86 13793188097

E-mail: xzw6001@126.com

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clinical effect in the treatment of lumbar disc herniation [5]. However, there are limited researches about the effect of Yaobitong capsule on the degenerative intervertebral disc. In this study, the autologous nucleus pulposus of rats were transplanted at the epidural nerve root to build the animal model of local non-compressive inflammatory injury of rat dorsal root ganglion, in order to observe the histomorphology of dorsal root ganglion after the intervention of Yaobitong capsule and the expression of p38MARK phosphorylation and thus provide the theoretical support for the clinical effect.

2. Materials and methods

2.1. Laboratory animals, reagents and instruments

A total of 80 healthy SPF SD rats with the weight of about (120 ± 10) g were provided by the laboratory animal center of our hospital. The Yaobitong capsule, 0.42 g/pill, was provided by Lianyungang Kanion Pharmaceutical Co., Ltd. (Item No.: 121205). The HE staining reagent was purchased from Beijing Manhattan Bio-Medical Technology Co., Ltd., while IX71 optical microscope and RM2245 paraffin slicing machine from Leica, German.

2.2. Grouping of laboratory animals and modeling of autologous nucleus pulposus transplantation

A total of 60 SD rats were randomly divided into three groups, namely blank group, model group, and Yaobitong capsule group, with 20 rats in each group. After 1 wk of adaptive feeding, except the blank group, rats in other groups were given the intraperitoneal injection of pentobarbital sodium (40 mg/kg) for the anesthesia. The coccyx nucleus pulposus was collected at 10 mg to expose the epidural space at L4–L6 nerve roots. The autologous nucleus pulposus was placed at the position of nerve root of rats in the Yaobitong capsule group and model group, with the embedded anesthetic tube. The standard for successful modeling of autologous nucleus pulposus transplantation was that rats appeared to be irritable, frequently wagged the tails, spontaneously lifted the hind limbs or feet, repeatedly licked the hind claws and bit the limbs. Compared with the blank group, rats in the Yaobitong capsule group and model group had shorter time of paretic hind limbs on the ground and the reduced capacity of paretic hind limbs to bear the weight when standing.

The first administration was given 3 d after the successful modeling. The dosage was calculated according to the body surface area ratio of rats. Rats in the Yaobitong capsule group were given the Yaobitong capsule, at 10 mL/kg, lasting for 30 d. Rats in the model group were given the equal dose of normal saline.

2.3. Histological examination

Rats were executed by cervical vertebra luxation. The lumbar spine at the operation site and the dorsal root ganglion tissue at the modeling site were collected and then put in the neutral formalin buffer for the fixation. Afterwards, they were dehydrated with ethanol at gradient degree, embedded with paraffin, cut into slices, and given the HE staining after being deparaffinized. The histomorphology of dorsal root ganglion was observed under the optical microscope.

2.4. Detection of expression of p38MARK phosphorylated protein by western blot

The dorsal root ganglion tissue was ground with the liquid nitrogen. The lysis buffer was added to mix it by pipetting. It was lysed at 4 °C for 2 h and centrifuged at 12 000 rpm for 18 min. The BCA protein assay was employed to detect the concentration of protein samples. The loading buffer was added in the tissue protein and it was treated in the water bath at 100 °C for 3–5 min. After the denaturation, samples were loaded for SDS-PAGE. After being transferred, the film was washed and mounted. It was then incubated with the rabbit anti-rat p38MARK phosphorylation monoclonal antibody and goat anti rabbit secondary antibody. It was exposed using the chemiluminescent method and then analyzed with the gel imaging system.

2.5. Statistical analysis

Statistical results were expressed by mean \pm standard deviation. The *t* test was employed for the comparison between groups. $P < 0.05$ was considered as significant difference.

3. Results

3.1. Histopathological change after successful modeling

The dorsal root ganglion tissue at the modeling site was collected for HE staining. Results showed that there was the obvious change in the histomorphology of dorsal root ganglion. As shown in Figure 1, the dorsal root ganglion of rats in the blank group showed that the nucleus was large and round, the distribution of Nissl bodies was evenly in the cytoplasm, with the clear nucleolus. The dorsal root ganglion of rats in the model group had the blurring boundary of nuclear membrane, the vacuole tissue in the cytoplasm, disordered distribution of Nissl bodies, and fading or disappearance of nucleolus staining. It indicated the successful modeling.

3.2. Histopathological change of laboratory animals

Similarly, the dorsal root ganglion of laboratory animals in the model group and Yaobitong capsule group was given HE staining. As shown in Figure 2, results showed that the dorsal root ganglion of rats in the model group still had the blurring boundary of nuclear membrane and fading or disappearance of nucleolus staining. But there was the significant improvement on the histopathological change of dorsal root ganglion of rats in the Yaobitong capsule group, with clearer boundary of nuclear membrane, relative even distribution of Nissl bodies in the cytoplasm and clearer nucleolus.

3.3. Expression of phosphorylated 38MAPK protein in dorsal root ganglion

As shown in Figure 3, the Western blot was employed to detect the expression of phosphorylated 38MAPK in the blank group, model group and Yaobitong capsule group. Results showed that, compared with the blank group, the expression of phosphorylated 38MAPK in the model group was significantly

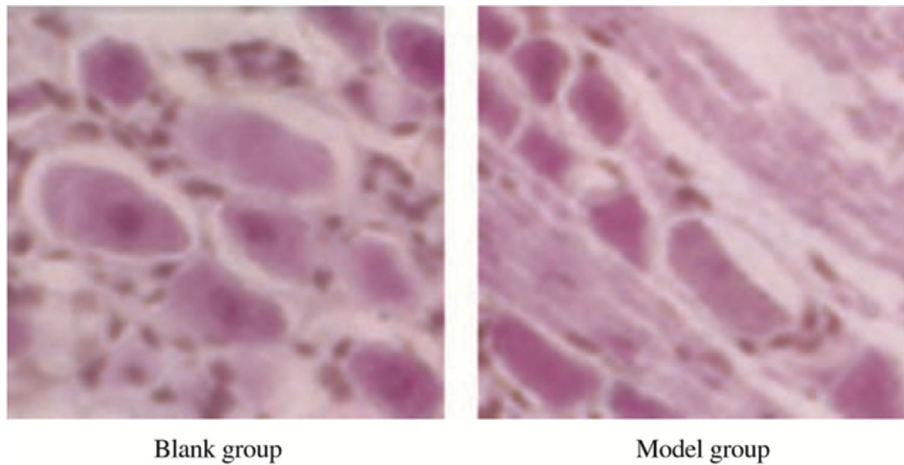


Figure 1. HE staining of dorsal root ganglion at the modeling site of rats in blank group and model group.

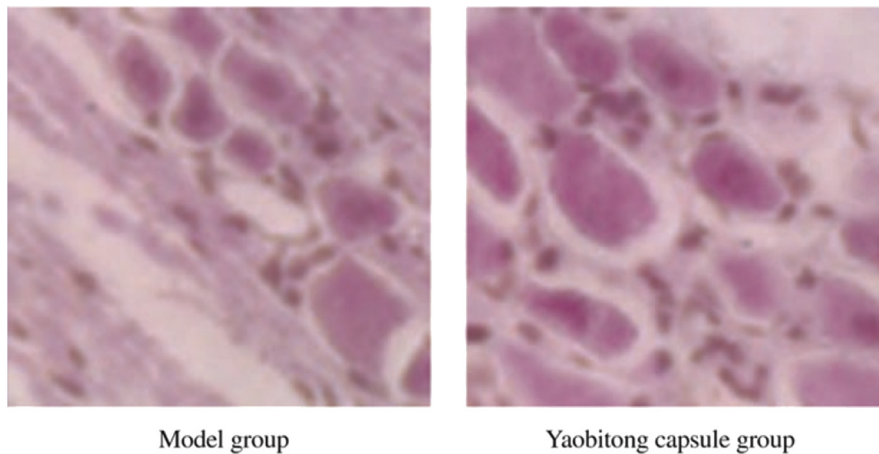


Figure 2. HE staining for dorsal root ganglion tissue at the modeling site of rats in model group and Yaobitong capsule group.

increased; however, compared with the model group, the expression of phosphorylated 38MAPK in the Yaobitong capsule group was significantly decreased, which indicated that Yaobitong capsule might affect the expression of phosphorylated 38MAPK to play its role.

3.4. Expression of inflammatory factors in dorsal root ganglion

The inflammatory factors of IL-1 and TNF- α are classic downstream target genes of phosphorylated 38MAPK. Accordingly, in this study, the Western blot was used to detect the

expression of IL-1 and TNF- α in the blank group, model group and Yaobitong capsule group. As shown in Figure 4, results showed that, compared with the blank group, the expression of IL-1 and TNF- α in the model group was significantly increased, while the expression was significantly decreased after the treatment in Yaobitong capsule group. It indicated that Yaobitong capsule might affect the expression of phosphorylated p38MAPK to inhibit the expression of inflammatory factors and thus improve the symptoms.

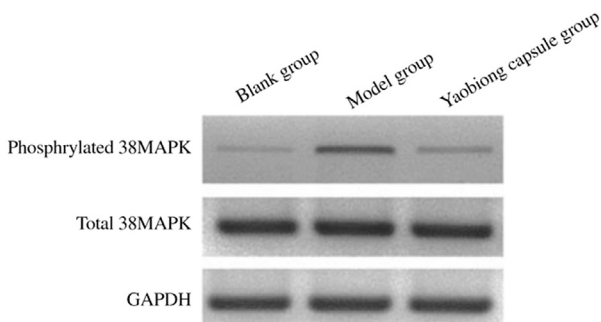


Figure 3. Expression change of phosphorylated 38MAPK in dorsal root ganglion.

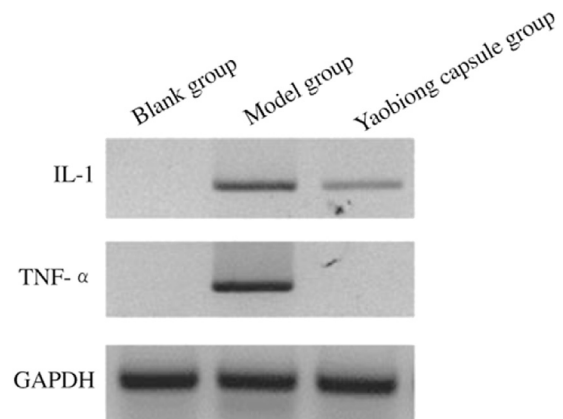


Figure 4. Expression change of IL-1 and TNF- α in the dorsal root ganglion.

4. Discussion

The lumbar disc herniation is one of common causes for lumbocrural pain. The main factor is the inflammatory response and degeneration of intervertebral disc and nucleus protrusion caused by the release of chemical substances to stimulate or compress the cauda equina at the nerve root and then result in the mechanical compression [6]. Most of patients can be relieved or cured through the non-surgical treatment clinically and the non-surgical treatment is the first choice for such disease. There has been the well understanding and sophisticated treatment of lumbar disc herniation in traditional Chinese medicine, with the good clinical effect. The different prescriptions are adopted aiming at the different symptoms that follow the principle of dialectical treatment, which possesses the unique advantages in the clinical practice.

In the study on the pain mechanism of lumbar disc herniation, as the primary afferent neuron of sensory information into the central nervous system, the dorsal root ganglion is the gathering place of primary afferent neuron somas and one of important links in the peripheral mechanism of neuropathic pain [7,8]. The noxious stimulation at the affected area is transduced in the peripheral primary neurons. The conversion into the electrical signal is one of important links, while the electrical signal is transmitted through the spinal cord, brain stem and thalamus to finally cause the sense of pain in the cerebral cortex [9,10].

p38MAPK is an important member of MAPK family. The p38MAPK pathway can be activated by the inflammatory factors (IL-1, TNF- α and EGF), stressors (Uv, H₂O₂, heat shock and hypoxia) and cell wall constituent of gram positive bacteria, which can thus affect the cell transcription, protein synthesis and expression of cell surface receptor [11,12]. The activation of p38MAPK signaling pathway in the dorsal root ganglion is a key process for the inflammatory response and neuropathic pain of dorsal root ganglion, as one of mechanism to cause the neuropathic pain in the herniated disc [13–15].

Yaobitong capsule is the experienced prescription for the treatment of lumbar disc herniation. According to the long-term clinical practices, Yaobitong capsule can remove obstruction in channels to relieve pain and promote the blood circulation and regulate Qi, which is mainly employed in the treatment of lumbar disc herniation with the blood stasis and Qi stagnation and obstruction in channels. Results of histological examination showed that the histomorphology of dorsal root ganglion in the Yaobitong capsule group was improved; while the results of protein assay showed that Yaobitong capsule could significantly inhibit the phosphorylation of p38MAPK in dorsal root ganglion cells. The study can fully prove that the early application of Yaobitong capsule can improve the symptoms and nerve root diseases of autologous nucleus pulposus transplantation model of rats and it can inhibit the phosphorylation of p38MAPK to play its role, which can provide the theoretical support for the clinical application.

Conflict of interest statement

We declare that we have no interest of conflict.

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