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ORIGINAL RESEARCH

Effects of Xuefu Zhuyu Decoction on Inflammatory Factors and the NF-kappaB Signaling Pathway in Rats Model of Radicular Pain

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Objective: To investigate the therapeutic effects and mechanisms of Xuefu Zhuyu Decoction (XFZYD) on radicular pain in rats with lumbar disc herniation (LDH).

Methods: Rat models of radicular pain were established and randomly divided into five groups: a control group, a model group, and three groups receiving low, medium, and high doses of XFZYD, with 12 rats per group. The rats in the treatment groups received different doses of XFZYD via oral administration, while the control and model groups received an equal volume of normal saline once daily for 7 days. Pain thresholds were measured, including the paw withdrawal threshold (PWT) and thermal paw withdrawal latency (PWL). The levels of inflammatory factors in the spinal dorsal horn tissue, including tumor necrosis factor α (TNF-α), transforming growth factor-β (TGF-β), interleukin-1β (IL-1β), and interleukin-6 (IL-6), were assessed using enzyme-linked immunosorbent assay (ELISA). Additionally, the expression of phosphorylated NF-κB in the spinal dorsal horn tissue was analyzed by Western blotting.

Results: Compared to the control group, the PWT and PWL in the model group were significantly decreased ($p < 0.05$), while the levels of TNF-α, TGF-β1, IL-1β, IL-6, and phosphorylated NF-κB in the spinal dorsal horn were significantly elevated ($p < 0.05$). The PWT and PWL in the low, medium, and high dose groups receiving XFZYD showed significant increases ($p < 0.05$) compared to the model group, and the levels of TNF-α, TGF-β1, IL-1β, IL-6, and phosphorylated NF-κB were significantly reduced ($p < 0.05$) across all dose groups.

Conclusion: Xuefu Zhuyu Decoction effectively reduces the release of inflammatory factors in the spinal dorsal horn of rats, enhances pain thresholds, and alleviates radicular neuropathic pain. The underlying mechanism may involve the inhibition of the NF-κB signaling pathway.

Keywords: Xuefu Zhuyu Decoction, lumbar disc herniation, sciatica, inflammatory factors, NF-κB signaling pathway

Introduction

Lumbar disc herniation (LDH) is a condition characterized by the rupture of the annulus fibrosus of the lumbar intervertebral disc due to various factors. This rupture allows the nucleus pulposus to protrude, which can stimulate or compress adjacent spinal nerve roots, leading to pain.[1] The clinical incidence of LDH is notably high, with approximately 1–3% of the global population affected each year.[2] The primary clinical manifestation of LDH is radicular pain, which is characterized by segmental radiating pain in the lower limbs, often accompanied by hyperalgesia and abnormal sensations.[3] Currently, the clinical treatment options for radicular pain lack specificity, frequently resulting in prolonged illness.[4] This not only severely impacts patients' daily lives and work but also diminishes their quality of life and increases the social and economic burden. Therefore, addressing radicular pain in clinical practice remains an important issue that requires further research and improvement.

Due to its multiple targets and components, traditional Chinese medicine has emerged as a research hotspot for the treatment of radicular pain. Xuefu Zhuyu Decoction (XFZYD), a well-known formula for activating blood circulation

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and relieving pain, exhibits anti-inflammatory and analgesic effects, reduces edema, and accelerates tissue repair.[5,6] Numerous clinical studies have demonstrated that XFZYD can significantly alleviate radicular pain.[7,8] Recent studies have shown that the degree of degenerative processes in spinal structures, along with inflammation in the nerve roots, plays a critical role in the development and persistence of radicular pain.[9] Specifically, inflammation contributes to the sensitization of nociceptors, which exacerbates pain. This inflammatory process is thought to be one of the key factors that XFZYD targets in its therapeutic action.[10] However, its specific mechanisms of action remain unexplored. Therefore, this study aims to establish a rat model of radicular pain to investigate the therapeutic effects of XFZYD and its regulatory influence on the NF-κB signaling pathway, thereby providing a theoretical basis for understanding the mechanisms underlying its efficacy in treating radicular pain.

Materials and Methods

Laboratory Animals

A total of 60 adult male Sprague Dawley rats (SPF grade), weighing 240 ± 20 g, were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Laboratory animals were housed in cages with 3 to 5 rats per cage and were kept under standardized conditions: including a room temperature of 25 °C and a 12 h/12 h light/dark cycle, with access to adequate food and water. All rats were allowed to acclimate to laboratory environment for 7 days. The animal experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Experimental Animal Ethics Committee of Chongqing University. We ensured that all procedures adhered to the principle of minimizing animal suffering.

Decoction Pieces and Reagents

The prescription of XFZYD comprised 12g of Persicae Semen; 9g each of Flos Carthami, Angelica sinensis, Radix Rehmanniae and Radix Achyranthis Bidentatae; 4.5g each of Rhizoma Chuanxiong and Radix Platycodonis; 6g each of Paeoniae Radix Rubra, Citrus aurantium, and Radix Glycyrrhizae; and 3g of Radix Bupleuri (Table 1). The decoction pieces were purchased from Beijing Tongrentang Co., Ltd. Tumor Necrosis Factor α (TNF-α) and Transforming Growth Factor β1 (TGF-β1) kits were purchased from Hangzhou Union Bio-Technology Co., Ltd. Interleukin 6 (IL-6) and Interleukin 1β (IL-1β) kits were purchased from Wuhan Huamei Bioengineering Co., Ltd. Anti-phospho NF-κB/p65 antibody and anti-NF-κB/p65 antibody were purchased from Cell Signaling Technology (CST). β-Actin antibody was purchased from Abcam, and HRP-labeled goat anti-rabbit secondary antibody was purchased from Hangzhou HuAn Biotechnology Co., Ltd.

Model Preparation

Anesthetized the rats by intraperitoneal injection of pentobarbital sodium solution (40 mg/kg). After hair removal at the lumbosacral region and the base of the tail, the area was disinfected with iodine and alcohol. Nucleus pulposus harvested

Table 1 Composition of XFZYD

Drug Names	Chinese Name	Family name	Dosage (g)	Part Used
Persicae Semen	*Tao Ren*	*Rosaceae*	12	Seed
Flos Carthami	*Hong Hua*	*Compositae*	9	Flower
Angelica sinensis	*Dang gui*	*Umbelliferae*	9	Root
Radix Rehmanniae	*Sheng di huang*	*Scrophulariaceae*	9	Root
Radix Achyranthis Bidentatae	*Niu xi*	*Amaranthaceae*	9	Root
Paeoniae Radix Rubra	*Chi shao*	*Buttercup*	6	Root
Citrus aurantium	*Zhi qiao*	*Rutaceae*	6	Fruit
Radix Glycyrrhizae	*Gan cao*	*Leguminosae*	6	Rhizome
Rhizoma Chuanxiong	*Chuan xiong*	*Umbelliferae*	4.5	Rhizome
Radix Platycodonis	*Jie geng*	*Campanulaceae*	4.5	Root
Radix Bupleuri	*Chai hu*	*Umbelliferae*	3	Root

referring to the methods reported by Shoji Yabuki:[11] the rat was placed in a supine position, and a left skin incision was made along the L4-L6 region. Muscles were carefully removed to expose the transverse processes, followed by L4-L5 laminectomy, during which the intervertebral joints and associated structures were excised using rongeurs. The spinal cord, dorsal root ganglia, and nerve roots on the left side of L5 were then exposed, while saline-soaked cotton balls were used to maintain moisture on the exposed spinal cord tissue. The rat was subsequently turned into a prone position; the base of the tail was disinfected and incised to expose the caudal artery and caudal vein. The artery was ligated with silk sutures, and the ligament between tail vertebrae 2 and 3 was cut to expose the intervertebral disc. The outer fibrous ring was carefully separated, and the jelly-like nucleus pulposus (approximately 10 mg), which had been previously disinfected, was extracted using ophthalmic forceps. The rat was then repositioned, and the nucleus pulposus was meticulously placed between the exposed nerve roots and dorsal root ganglia. Finally, the muscles and tissues in the lumbar and tail regions were gradually sutured. For the control group, rats underwent the same exposure of the spinal cord, dorsal root ganglia, and nerve roots, and the autologous nucleus pulposus was removed, but they did not receive nucleus pulposus transplantation surgery.

Group and Drug Administration

Rats were randomly divided into five groups using a random number method: a physiological saline control group (control group), a radicular pain model group (model group), a low-dose XFZYD group (low-dose group), a medium-dose XFZYD group (medium-dose group), and a high-dose XFZYD group (high-dose group), with 12 rats in each group. The control and model groups received physiological saline via gavage for intervention. The doses of XFZYD for the treatment groups were calculated based on the "equivalent dose ratio table for humans and animals converted according to body surface area", and were set at 1, 2, and 4 times the normal dose, after establishing the model, the rats were treated with the respective concentrations of the test drug by gavage once daily for 7 days.

Behavioral Test

To eliminate the influence of environmental and psychological factors on behavioral test results, for the first 3 days before testing, rats were placed in the testing box for 20 minutes each day.

Mechanical allodynia thresholds were determined using paw withdrawal thresholds (PWT). On day 8, using the "up-down" method described by Chaplan[12] and other scholars, 50% of the PWT in rats was tested using eight logarithmically increasing strength von Frey filaments (Stoelting company). Rats were placed in an organic glass box with a metal grid bottom for 10 minutes, and the von Frey filament was applied vertically to the sole of the rat's foot. The filament was slightly curved, maintained at the sole for 4–5 seconds. If there was a rapid withdrawal or licking response, it was considered a positive reaction. Starting at 2.04g, if the withdrawal response was positive, the next adjacent, decreasing strength was tested; if the withdrawal response was negative, the next adjacent, increasing strength was tested, and so on. Each rat was tested 5 times, with at least a 5 minutes pause between each test.

Pain sensitivity in response to heat stimuli was assessed using thermal paw withdrawal latencies (PWL). On day 8, the PWL of the operated hind limbs of rats was measured using the BME-410C automatic thermal pain stimulator. To begin, the rats were placed in an acrylic glass box with a smooth glass bottom for a 20 minutes adaptation period. Once the rats were calm, a strong heat beam was directed at the center of the paw skin, and timing commenced. Timing was stopped immediately when the animal began to withdraw or lick its paw, and the withdrawal time was recorded. To avoid burns, the maximum testing duration was limited to 20 seconds. Each test was repeated three times with a 10 minutes interval between repetitions.

ELISA

On the eighth day post-surgery, rats from each group were sacrificed, and the lumbar enlargement segment of the spinal cord dorsal horn tissue was collected and immediately frozen. The spinal cord dorsal horn specimens were subsequently retrieved from the freezer and thawed in an icebox. An appropriate volume of pre-cooled physiological saline was added, and the mixture was processed in a 1mL homogenizer to prepare a 10% tissue homogenate. This homogenate was then transferred to a centrifuge and centrifuged, 4°C, 10000 g/min, 30 minutes. The supernatant was carefully aliquoted and stored at −80°C.

Following the manufacturer's instructions, the supernatant was tested using rat TNF-α, TGF-β1, IL-6, IL1β assay kits. After diluting the samples, the ELISA procedure was strictly followed. In the sample wells, the inflammatory factor samples to be tested were added, and the plate was sealed with a plate seal and incubated at 4°C overnight. After incubation, the plate was washed at room temperature for 2 hours; the primary antibody was added and incubated at 37°C. After washing to remove any residue, the secondary antibody was added and incubated at 37°C for 2 hours, followed by washing. The reaction was stopped after color development in the dark, and the samples were read in a microplate reader to calculate the content of TNF-α, TGF-β1, IL-6, and IL1β in each group.

Western Blotting

Frozen rat spinal cord dorsal horn tissue samples (three animals per group, randomly selected) were thawed and ground into a powder using liquid nitrogen. To this powdered tissue, 400 μL of lysis buffer containing phenylmethanesulfonyl fluoride (PMSF) was added. The mixture was homogenized and incubated on ice for 30 minutes. Following this, the homogenate was transferred to a centrifuge tube and centrifuged at 15,000r/min for 30 minutes. The protein concentration of the supernatant was then measured using the BCA method.

Protein samples were denatured by heating in boiling water for 5 minutes, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, the proteins were transferred to a membrane and blocked with 5% skim milk at room temperature for 2 hours. The membrane was washed three times with TBST washing buffer, then incubated overnight on a shaker at 4°C with a 1:1000 dilution of phosphor NF-κB/p65 (p-p65) antibody, NF-κB/p65 antibody, and β-Actin antibody. After washing the membrane three times with TBST, it was incubated overnight on a shaker at 4°C with a 1:1000 dilution of HRP-conjugated goat anti-rabbit IgG secondary antibody, followed by another three washes with TBST.

Finally, the membrane was developed using ECL chemiluminescence reagent and exposed with a gel imaging system. Band grayscale values were measured using ImageJ software, and relevant statistical analyses were performed.

Statistical Analysis

Statistical analysis was performed using SPSS 26.0 and GraphPad Prism 9.0 software. All data were presented as the mean ± standard deviation. One-way analysis of variance was performed for comparison between groups, Paired *t*-test was used for statistical analysis within a group. A p value < 0.05 was considered statistically significant.

Result

Xuefu Zhuyu Decoction Increased the Pain Threshold in Rat Model of Radicular Pain

Compared to the control group, the PWT and PWL of rats in the model group were significantly decreased ($p < 0.05$, Figure 1). In contrast, the PWT and PWL in the low, medium, and high dose groups of Xuefu Zhuyu Decoction were significantly increased compared to the model group ($p < 0.05$, Figure 1).

Xuefu Zhuyu Decoction Reduces Inflammatory Factors in the Spinal Cord Dorsal Horn Tissue of Rats

Compared to the control group, levels of TNF-α, TGF-β1, IL-1β, and IL-6 were significantly elevated in the spinal cord dorsal horn of rats in the model group ($p < 0.05$, Figure 2). In contrast, levels of TNF-α, TGF-β1, IL-1β, and IL-6 were significantly reduced in the spinal cord dorsal horn of rats in all dose groups of XFZYD compared to the model group ($p < 0.05$, Figure 2).

Xuefu Zhuyu Decoction Inhibited the NF-κB Signaling Pathway in Rat Model of Radicular Pain

Compared to the control group, the expression levels of phosphorylated NF-κB/p65 in the spinal dorsal horn tissues of the model group rats were significantly elevated ($p < 0.05$, Figure 3). In contrast, the expression levels of phosphorylated

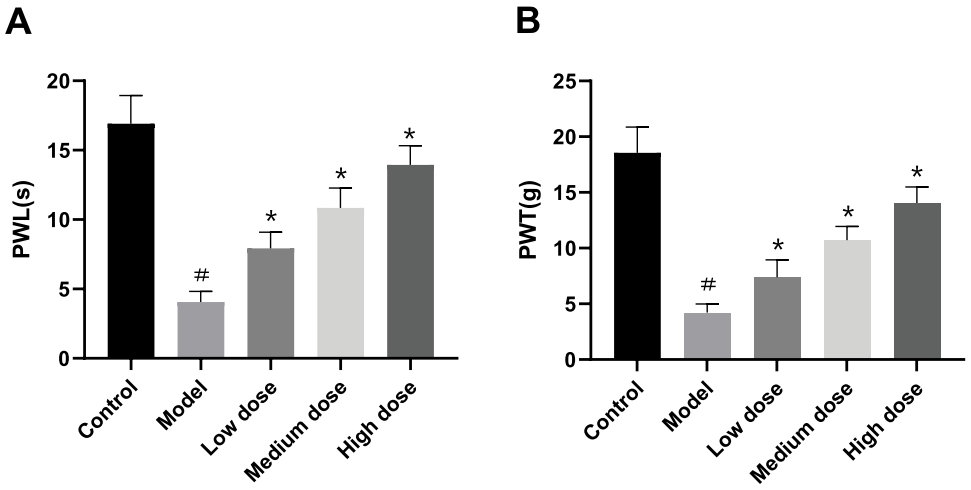

Figure 1 Comparison of pain thresholds in each group of rats ($\bar{x} \pm s$, n=12). #p < 0.05 Compared with the control group; *p < 0.05 Compared with the model group.

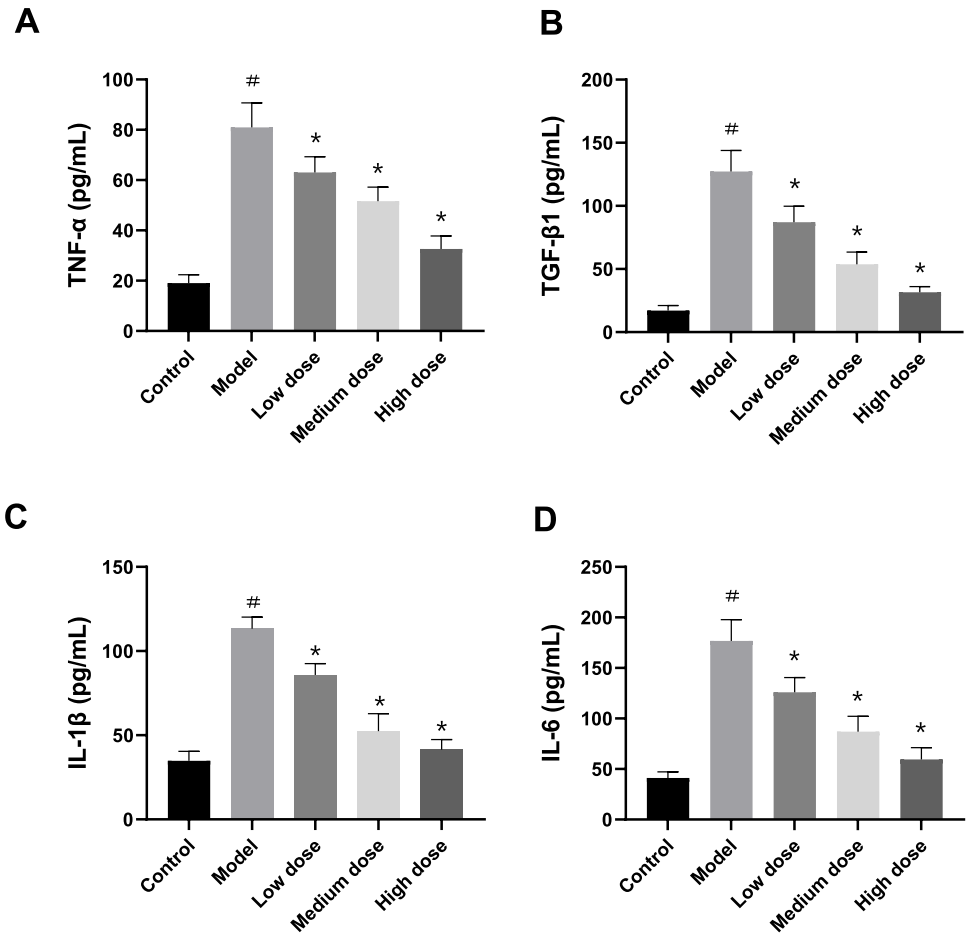

Figure 2 Comparison of inflammatory factors in each group of rats ($\bar{x} \pm s$, n=12). #p < 0.05 Compared with the control group; *p < 0.05 Compared with the model group.

NF-κB/p65 in the spinal dorsal horn tissues of rats treated with different doses of XFZYD were significantly reduced compared to the model group ($p < 0.05$, Figure 3). However, no significant differences were observed in the levels of non-phosphorylated NF-κB/p65 protein among the various treatment groups ($p > 0.05$, Figure 3).

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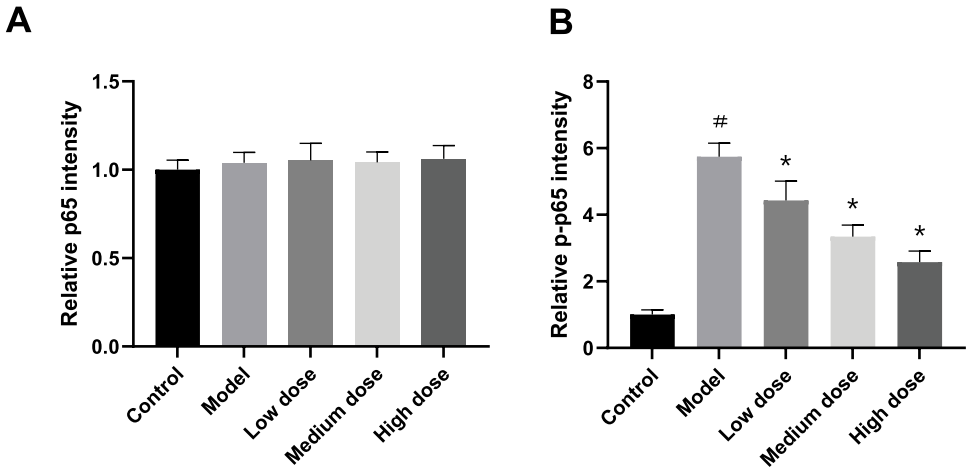

Figure 3 Comparison of NF-κB signaling pathway-related proteins in each group of rats ($\bar{x} \pm s$, n=3). #$p < 0.05$ Compared with the control group; * $p < 0.05$ Compared with the model group.

Discussion

Radicular pain results from harmful stimulation of the spinal nerve roots due to a variety of factors and is the most common clinical symptom associated with lumbar disc herniation.[13] This chronic pain significantly impacts quality of life and contributes to the economic burden on society. The pathogenesis and clinical management of radicular pain have become critical areas of research. The traditional mechanical compression hypothesis posits that herniated nucleus pulposus compresses the nerve roots, leading to obstructed venous return, edema, and degeneration, which ultimately trigger radicular pain. However, this theory fails to explain the presence of asymptomatic patients with lumbar disc herniation, thereby prompting increased interest in the autoimmune response theory of radicular pain.[14] Recent studies suggest that neuroimmune inflammation induced by nucleus pulposus tissue is a significant contributor to radicular pain, with the synthesis and secretion of various inflammatory cytokines playing a central role in its pathogenesis.[15,16] Consequently, targeting and inhibiting the production of these cytokines may offer a promising strategy for the treatment of radicular pain.

Radicular pain lacks a specific treatment protocol. Current management strategies are categorized into conservative and surgical approaches. However, due to the significant trauma, high risk, and stringent surgical indications associated with surgical interventions, only 10% to 20% of patients are deemed suitable for such procedures, leaving the majority to rely on conservative treatment.[17] Unfortunately, the efficacy of existing pharmacological treatments for radicular pain in clinical practice is often limited, and these treatments frequently produce numerous adverse reactions. In recent years, traditional Chinese medicine has gained attention as a promising alternative for radicular pain management due to its multi-target and multi-component characteristics, making it an important focus of research in this area.[18]

In traditional Chinese medicine theory, radicular pain belongs to the category of "Bi syndrome", with the main pathogenesis being Qi stagnation, blood stasis, and poor blood circulation. The treatment mainly focuses on promoting blood circulation, removing blood stasis, and relieving pain. XFZYD was first found in "Medicine Forest Correction", written by Wang Qingren, a famous doctor in the Qing dynasty. It consists of 11 Chinese herbal medicines including Persicae Semen, Flos Carthami, Angelica sinensis, Radix Rehmanniae, Radix Achyranthis Bidentatae, Paeoniae Radix Rubra, Citrus aurantium, Radix Glycyrrhizae, Rhizoma Chuanxiong, Radix Platycodonis, Radix Bupleuri. The overall formula promotes blood circulation and Qi movement, removes stasis and generates new blood. When the herbs are combined, they can eliminate stasis, unblock the meridians, and achieve the effect of relieving pain.

This study established a rat model of radicular pain through autologous nucleus pulposus transplantation. The results indicated that, compared to the control group, both the PWT and PWL of the model group rats were significantly decreased, suggesting a lower pain threshold and confirming the validity of the model. Furthermore, levels of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and TGF-β1 in the spinal dorsal horn tissue of the model group

were significantly elevated relative to the control group, indicating the presence of inflammatory responses associated with radicular pain. Notably, TNF-α is one of the most extensively studied inflammatory cytokines in relation to nerve inflammation,[19] playing a critical role in both the initiation and progression of nerve inflammation, as well as in the central and peripheral sensitization associated with chronic pain.[20] IL-6 is a key regulator of inflammatory cell differentiation, produced by various cell types, including fibroblasts, monocytes/macrophages, and T-lymphocytes. It facilitates the activation of macrophage differentiation and infiltration, enhances the expression of other cytokines, and thereby amplifies the inflammatory response.[21] IL-1β, a non-secreted pro-inflammatory cytokine, is produced by activated monocytes and macrophages. As a classical pro-inflammatory cytokine, the signaling pathways activated by IL-1β play a crucial role in the development, persistence, and propagation of pain.[22] TGF-β1 is generally recognized for its anti-inflammatory effects in immune responses; however, under certain pathological conditions, TGF-β1 may also exhibit pro-inflammatory properties.[23] Following the administration of XFZYD, both the PWT and PWL in the treated rats significantly increased, indicating an elevated pain threshold. This finding suggests that XFZYD effectively alleviates radicular pain. Additionally, levels of TNF-α, IL-1β, IL-6 and TGF-β1 in the treatment groups were significantly reduced compared to the model group. These results imply that XFZYD can decrease the release of inflammatory mediators, further contributing to the observed increase in pain threshold and the alleviation of radicular pain.

The results of this study indicate that the levels of phosphorylated NF-κB/p65 protein in the dorsal horn of the spinal cord in the model rats were significantly elevated, suggesting that NF-κB plays an important role in the development and progression of radicular pain. NF-κB is a crucial transcriptional regulator that plays a significant role in the induction and maintenance of acute and chronic pain following neuroinflammation or injury.[24] Upon phosphorylation, NF-κB can translocate to the nucleus, further promoting the release of inflammatory mediators such as TNF-α and IL-1β.[25] After the intervention with XFZYD, the levels of phosphorylated NF-κB/p65 protein in the dorsal horn of the spinal cord in rats were significantly reduced, indicating that XFZYD may inhibit the NF-κB signaling pathway.

In conclusion, the Xuefu Zhuyu Decoction can reduce the release of inflammatory factors, increase pain threshold, thereby improving radicular pain, and its mechanism of action may be related to the inhibition of the NF-κB signaling pathway.

Disclosure

The authors report no conflicts of interest in this work.

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