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Effect of Acupuncture at Points of the Bladder Meridian of Foot-Taiyang on Serum Inflammatory Factors in Rabbit Models of Cervical Spondylosis

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Abstract: *Objective*: Based on the theory of "Taiyang governs tendons", to explore the effect of acupuncture at points of the Bladder Meridian of Foot-Taiyang on the expression of serum inflammatory factors in rabbit models of cervical spondylosis. *Methods*: Thirty New Zealand white rabbits were randomly divided into a blank group, a model group, and a treatment group, with 10 rabbits in each group. The models of cervical spondylosis in the model group and treatment group were established by long-term head-down flexion combined with cold-damp stimulation. After modeling, the treatment group received acupuncture at three points (Kunlun, Weizhong, and Feishu) on the Bladder Meridian of Foot-Taiyang, once a day for 20 minutes each time, for 14 consecutive days; the model group and blank group received no therapeutic intervention. After the intervention, cardiac blood was collected from all rabbits to detect the expression levels of serum inflammatory factors IL-6, TNF-α, and IL-1β. Meanwhile, cervical muscle tissue was collected, stained with HE, and the morphological changes of the posterior cervical muscle tissue in each group were observed under an optical microscope. *Results*: After modeling, the levels of inflammatory factors in the serum of rabbits in the model group and treatment group were significantly increased compared with those before modeling, with a statistically significant difference ($p < 0.01$). After intervention, the serum inflammatory factors in the treatment group decreased significantly compared with those in the model group, and the difference was statistically significant ($p < 0.01$). *Conclusion*: Acupuncture at points of the Bladder Meridian of Foot-Taiyang in rabbit models of cervical spondylosis can reduce the serum levels of inflammatory factors TNF-α, IL-1β, and IL-6.

Keywords: Cervical spondylosis; Bladder meridian of foot-Taiyang; Acupuncture; Inflammatory factors

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1. Introduction

With the rapid development of the current network era and the popularization of various electronic digital products such as computers, mobile phones, and tablets, the incidence of cervical spondylosis has been increasing year by year, showing an increasingly younger trend [1]. According to statistics, the incidence of cervical spondylosis in China is close to 20%, among which nearly 40% of patients are under 30 years old. Therefore, exploring effective treatment methods for cervical spondylosis is of great significance. Cervical spondylosis is a disease governed by the Bladder Meridian of Foot-Taiyang. Huangdi Neijing (Yellow Emperor's Internal Classic), a famous ancient Chinese medical classic, established the basic theoretical system of traditional Chinese medicine (TCM) and has important guiding significance for the development of TCM [2].

Many theories proposed in it are well-known to TCM scholars and widely used in clinical practice, such as the classic theory of "the liver governs tendons and the kidney governs bones". It also records that "the Gallbladder Meridian of Foot-Shaoyang governs diseases related to bones" and "the Bladder Meridian of Foot-Taiyang governs diseases related to tendons".

Among them, the theory of "Shaoyang governs bones" has been extensively studied by later physicians and has become a research hotspot among TCM scholars in recent years. However, there are very few studies on the theory of "Taiyang governs tendons", and there are no basic research reports on the application of this theory in the treatment of cervical spondylosis to date. Based on the theory of "Taiyang governs tendons", this study conducted animal experiments to investigate the effect of acupuncture at points of the Bladder Meridian of Foot-Taiyang on the expression of serum inflammatory factors in rabbit models of cervical spondylosis, and to clarify its mechanism in treating cervical spondylosis. The results were reported as follows.

2. Experimental materials

2.1. Experimental animals

Thirty 6-month-old New Zealand white rabbits, weighing about 2 kg, half male and half female, were purchased from Guizhou Yikedada Biotechnology Co., Ltd., with the animal license number of SCXK (Qian) 2021-0001. The animal experiment was approved by the Animal Experiment Ethics Review Committee of Yunnan University of Traditional Chinese Medicine, with approval number: R-062021LH038. Animal feeding and experimental operations were carried out at Yunnan Luoyu Biotechnology Co., Ltd. Throughout the experiment, the animals were raised in accordance with the standard for experimental animal feeding, and all animal experimental operations were performed in compliance with the provisions of the Regulations on the Administration of Experimental Animals.

2.2. Reagents

Rabbit interleukin-1β (IL-1β) enzyme-linked immunosorbent assay (ELISA) kit (catalog number: ml027836-2), rabbit interleukin-6 (IL-6) ELISA kit (catalog number: ml027844-2), and rabbit tumor necrosis factor-α (TNF-α) ELISA kit (catalog number: ml028087-2) were all purchased from Shanghai Enzyme-Linked Biotechnology Co., Ltd.

2.3. Instruments

Pannoramic MIDI holographic scanner, HistoCore BloCu7 rotary microtome, HistoCore ArCadia H paraffin embedding machine, JT-12S automatic tissue dehydrator, JK-6 tissue flaking and baking machine, surgical instruments (Shanghai Jinzhong), rabbit fixator (zh-tgx-i), acupuncture needles (Wujiang Yunlong Medical Devices Co., Ltd.), Shanghai Senxin DGG-9140B electric thermostatic blast drying oven, Thermo Scientific high-speed refrigerated centrifuge, Eppendorf pipettes, Haier 4 °C/-20 °C refrigerators, and SPECTRA MAX190 microplate reader.

3. Methods

3.1. Grouping of experimental animals

Thirty 6-month-old New Zealand white rabbits were randomly divided into 3 groups using a random number table: blank group, model group, and treatment group, with 10 rabbits in each group.

3.2. Model establishment

For rabbits in the model group and treatment group, the non-invasive method for establishing a rabbit model of cervical spondylosis proposed by Zhang Xin et al. was adopted. The specific operation steps were as follows: First, the hair on the posterior cervical region of the rabbits was shaved with a hair clipper, and then the rabbits were fixed in a rabbit fixation box.

Next, the rabbit's neck was adjusted to a 45° head-down flexion position to keep the posterior cervical muscles in a stretched state [3,4]. Afterwards, a self-made ice pack was placed on the posterior cervical region of the rabbit, once a day for 5 hours each time, for 4 consecutive weeks. Rabbits in the blank group were fixed in the same fixator, allowing free movement of the neck for the same duration.

After modeling, the expression levels of serum IL-1β, TNF-α, and IL-6 in each group were detected and compared with those before modeling. Meanwhile, 2 rabbits were randomly selected from each group and sacrificed, and the posterior cervical muscle tissue was quickly collected, stained with HE, and the morphological changes of the posterior cervical muscle tissue were observed under an optical microscope to comprehensively evaluate the success of modeling.

3.3. Intervention

(1) Treatment group

After modeling, the acupuncture sites were disinfected. Referring to the acupoint localization methods in Experimental Acupuncture and Handbook of Experimental Animal Acupuncture, acupuncture was performed at three points (Kunlun, Weizhong, and Feishu) on the Bladder Meridian of Foot-Taiyang, once a day, with the needles retained for 20 minutes each time, for 14 days.

(2) Model group and blank group

Rabbits were fixed on a self-made rabbit platform for 20 minutes every day without any therapeutic intervention.

3.4. Sample collection and processing

(1) On the day before modeling, the 1st day after modeling, and the 1st day after intervention, cardiac blood was collected from all rabbits, centrifuged at 4 °C at 3000 r/min for 30 minutes in a timely manner, and the supernatant was aspirated to detect the expression levels of serum inflammatory factors IL-1β, TNF-α, and IL-6 by ELISA.

(2) After blood collection on the 1st day after modeling, 2 rabbits were randomly selected from each group, sacrificed by exsanguination via the heart after cardiac blood collection, and the posterior cervical muscle tissue was quickly collected, stained with HE, and the morphological changes of the posterior cervical

muscle tissue were observed under an optical microscope.

(3) After the intervention, the remaining rabbits were first subjected to cardiac blood collection, then sacrificed by exsanguination via the heart, and the posterior cervical muscle tissue was quickly collected, stained with HE, and the morphological changes of the posterior cervical muscle tissue were observed under an optical microscope.

3.5. Statistical analysis

The detection results of serum inflammatory factors TNF-α, IL-1β, and IL-6 were statistically analyzed. SPSS 21.0 statistical software was used for data analysis, and GraphPad Prism 9.0.0 software was used for graph plotting. One-way analysis of variance (ANOVA) was used for comparison between groups, and LSD-*t* test was further used for pairwise comparison if there were significant differences. $p < 0.05$ was considered statistically significant, and $p < 0.01$ was considered extremely statistically significant.

4. Results

4.1. Comparison of inflammatory factors among groups at the same time point

Before modeling, the levels of TNF-α, IL-1β, and IL-6 in each group were comparable, with no statistically significant differences. After modeling, the levels of TNF-α, IL-1β, and IL-6 in the model group and treatment group were significantly higher than those in the blank group, with extremely statistically significant differences ($p < 0.01$), indicating successful modeling; however, there was no statistically significant difference in the levels of inflammatory factors between the treatment group and the model group after modeling ($p > 0.05$) [5].

After treatment, the levels of TNF-α, IL-1β, and IL-6 in the treatment group were significantly lower than those in the model group, and the difference was statistically significant. See **Tables 1**, **2** and **3** and **Figures 1**, **2** and **3**.

Table 1. Levels of inflammatory factors in each group before modeling

Group/Indicator (pg/mL)	TNF-a	IL-1β	IL-6
Blank group	41.6 ± 4.56	129.98 ± 21.73	38.61 ± 6.99
Model group	44.08 ± 3.01	151.9 ± 17.91	38.96 ± 8.13
Treatment group	41.46 ± 6.92	130.64 ± 20.1	33.09 ± 7.39

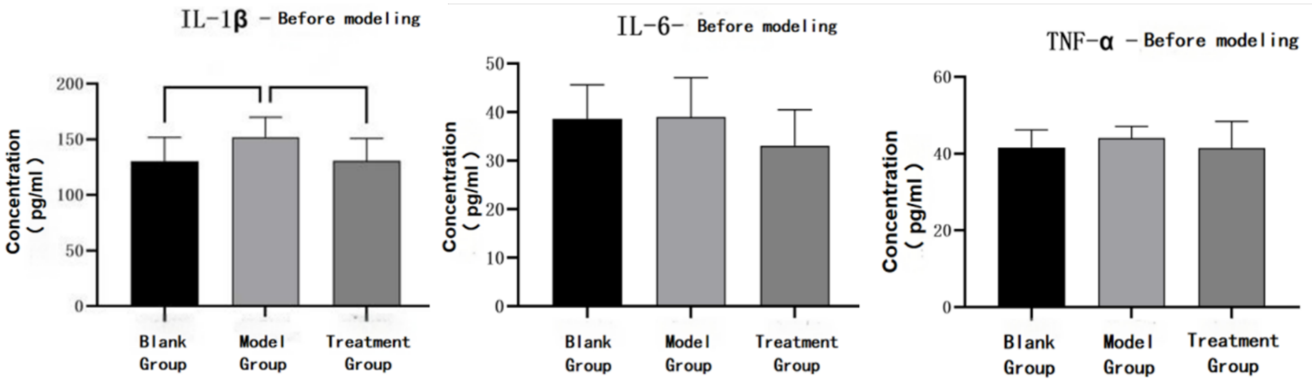


Figure 1. Levels of inflammatory factors in each group before modeling. A. Detection results of IL-1β in each group before modeling; B. Detection results of IL-6 in each group before modeling; C. Detection results of TNF-α in each group before modeling.

Table 2. Levels of inflammatory factors in each group after modeling

Group/Indicator (pg/mL)	TNF-a	IL-1β	IL-6
Blank group	38.67 ± 6.49	144.68 ± 31.59	37.1 ± 5.51
Model group	89.55 ± 6.68	340.22 ± 32.01	84.11 ± 7
Treatment group	86.82 ± 6.24	360.51 ± 28.65	90.26 ± 5.44

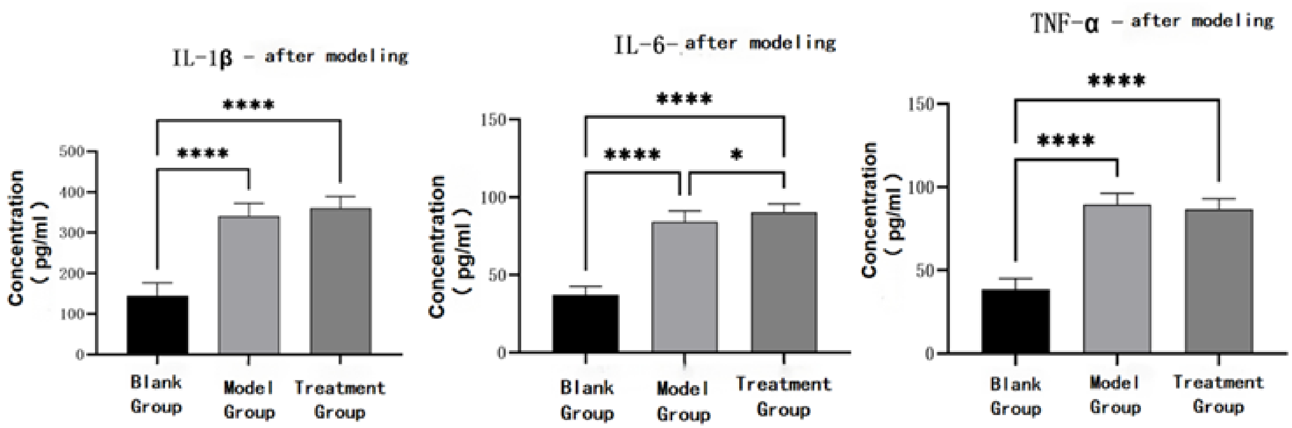


Figure 2. Levels of inflammatory factors in each group after modeling. A. Detection results of IL-1β in each group after modeling; B. Detection results of IL-6 in each group after modeling; C. Detection results of TNF-α in each group after modeling; *$p < 0.05$, ****$p < 0.0001$.

Table 3. Levels of inflammatory factors in each group after treatment

Group/Indicator (pg/mL)	TNF-a	IL-1β	IL-6
Blank group	43.21 ± 7.04	135.07 ± 22.75	37.31 ± 6.39
Model group	81.79 ± 3.68	338.56 ± 27.94	82.69 ± 8.65
Treatment group	59.97 ± 4.21	239.24 ± 18.34	59.05 ± 7.53

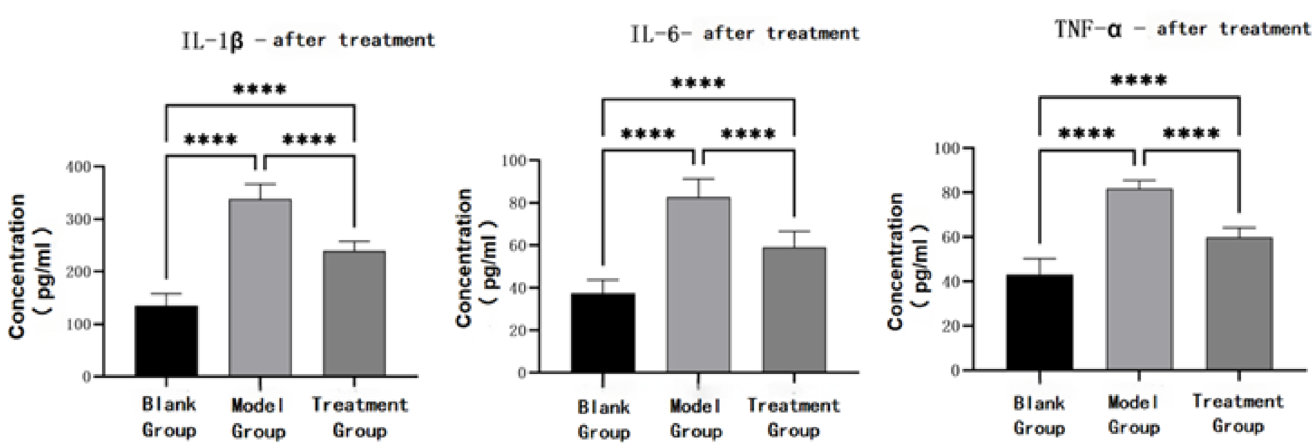


Figure 3. Levels of inflammatory factors in each group after treatment. A. Detection results of IL-1β in each group after treatment; B. Detection results of IL-6 in each group after treatment; C. Detection results of TNF-α in each group after treatment; *$p < 0.05$, ****$p < 0.0001$.

4.2. Comparison of inflammatory factors in the same group at three time points

In the blank group, the levels of TNF-α, IL-1β, and IL-6 before modeling, after modeling, and after treatment were comparable, with no statistically significant differences ($p > 0.05$).

In the model group, the levels of TNF-α, IL-1β, and IL-6 after modeling and after treatment were significantly higher than those before modeling, with extremely statistically significant differences ($p < 0.01$); there was no statistically significant difference in the levels of IL-1β and IL-6 between after modeling and after treatment, but the level of TNF-α was statistically different ($p < 0.05$).

In the treatment group, the levels of TNF-α, IL-1β, and IL-6 after modeling and after treatment were significantly higher than those before modeling, with extremely statistically significant differences ($p < 0.01$); after treatment, the levels of TNF-α, IL-1β, and IL-6 were significantly lower than those after modeling, with extremely statistically significant differences ($p < 0.01$). See **Tables 4**, **5** and **6** and **Figures 4**, **5** and **6** .

Table 4. Levels of inflammatory factors in the blank group across three time points

Group/Indicator (pg/mL)	TNF-a	IL-1β	IL-6
Before modeling	41.6 ± 4.56	129.98 ± 21.73	38.61 ± 6.99
After modeling	38.67 ± 6.49	144.68 ± 31.59	37.1 ± 5.51
After treatment	43.21 ± 7.04	135.07 ± 22.75	37.31 ± 6.39

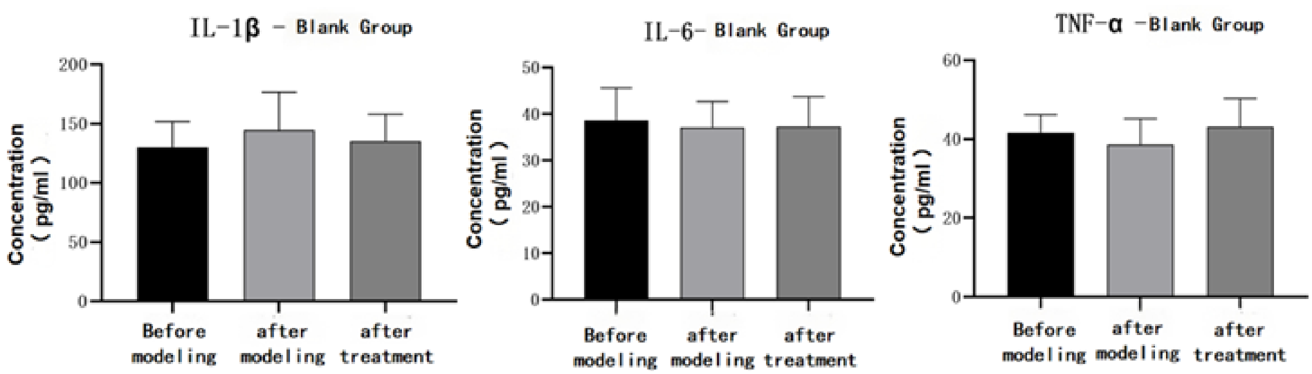


Figure 4. Levels of inflammatory factors in the blank group. A. Detection results of IL-1β in the blank group at three time points; B. Detection results of IL-6 in the blank group at three time points; C. Detection results of TNF-α in the blank group at three time points.

Table 5. Levels of inflammatory factors in the model group across three time points

Group/Indicator (pg/mL)	TNF-a	IL-1β	IL-6
Before modeling	44.08 ± 3.01	151.9 ± 17.91	38.96 ± 8.13
After modeling	89.55 ± 6.68	340.22 ± 32.01	84.11 ± 7
After treatment	81.79 ± 3.68	338.56 ± 27.94	82.69 ± 8.65

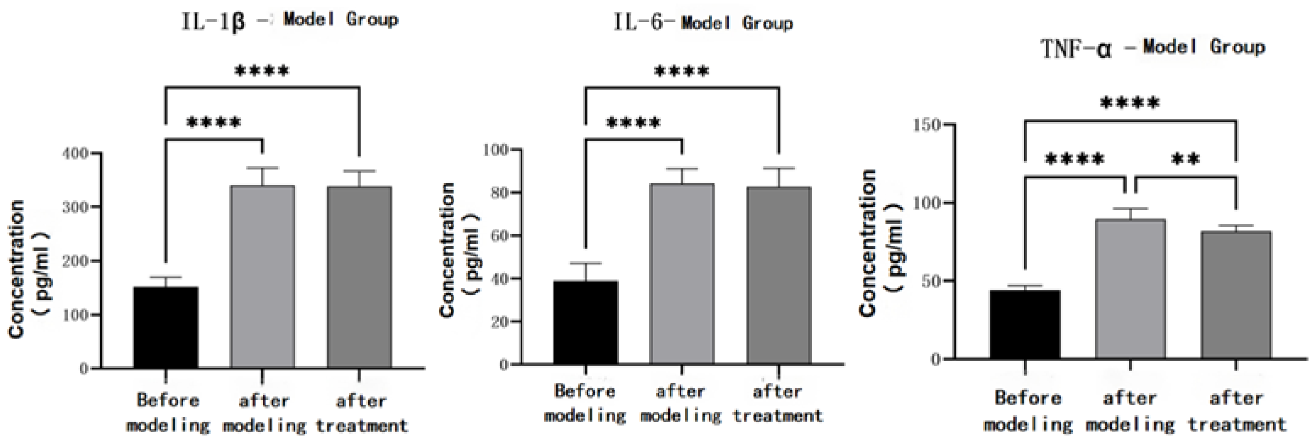


Figure 5. Levels of inflammatory factors in the model group. A. Detection results of IL-1β in the model group at three time points; B. Detection results of IL-6 in the model group at three time points; C. Detection results of TNF-α in the model group at three time points; ***$p < 0.005$, *****$p < 0.0001$.

Table 6. Levels of inflammatory factors in the treatment group across three time points

Group/Indicator (pg/mL)	TNF-a	IL-1β	IL-6
Before modeling	41.46 ± 6.92	130.64 ± 20.1	33.09 ± 7.39
After modeling	86.82 ± 6.24	360.51 ± 28.65	90.26 ± 5.44
After treatment	59.97 ± 4.21	239.24 ± 18.34	59.05 ± 7.53

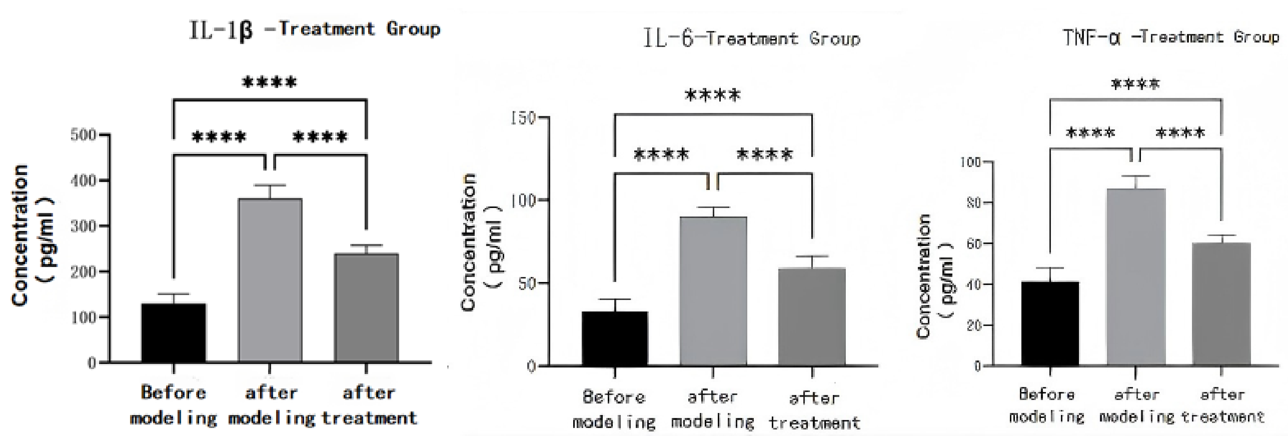


Figure 6. Levels of inflammatory factors in the treatment group. A. Detection results of IL-1β in the treatment group at three time points; B. Detection results of IL-6 in the treatment group at three time points; C. Detection results of TNF-α in the treatment group at three time points; *****$p < 0.0001$.

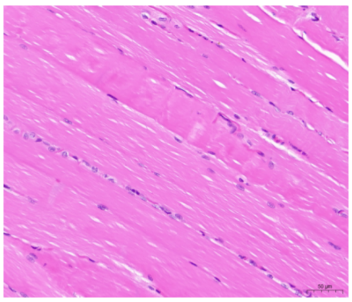
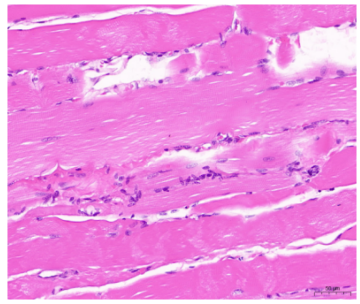
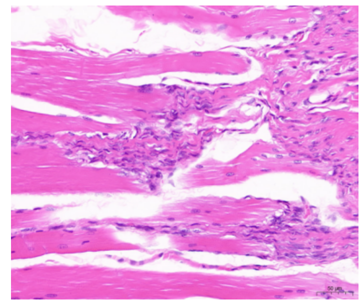
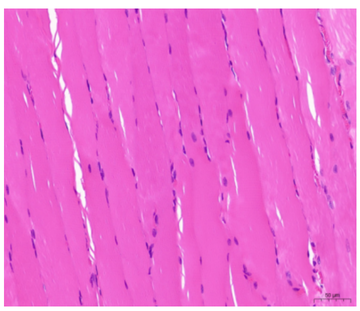
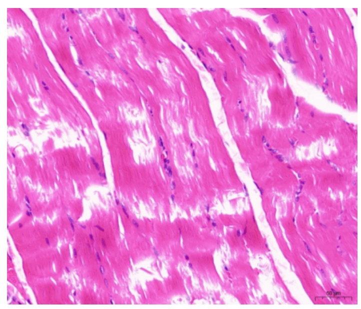
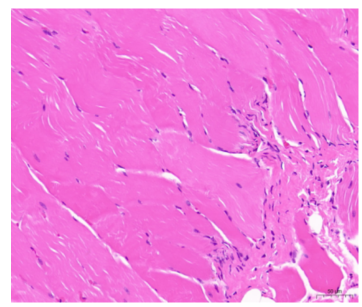
4.3. Morphological changes of posterior cervical muscle tissue

After the rabbits were sacrificed, the posterior cervical muscle tissue was collected, stained with HE, and the morphological changes were observed under an optical microscope.

In the blank group, the posterior cervical muscle fibers had a clear structure, neat arrangement, similar thickness, no inflammatory cell infiltration, and no myocyte degeneration or edema. After modeling, in the model group and treatment group, uneven staining of muscle fibers, disordered arrangement, muscle fiber rupture and torsion, accompanied by inflammatory cell infiltration, and disordered arrangement of collagen fibers were observed [6].

After treatment, the treatment group showed mild proliferation of connective tissue, relatively uniform distribution of myonuclei, mild torsion of myocytes, and no swelling. See **Table 7**.

Table 7. Morphological changes of posterior cervical muscle tissue in each group observed under an optical microscope

	Blank group	Model group	Treatment group
Before treatment			
After treatment			

5. Discussion

"Cervical spondylosis" is not clearly recorded in TCM monographs. According to its pathogenesis and related symptoms, the disease belongs to the categories of "neck bi" and "bi syndrome" in TCM [7]. The occurrence of neck bi is closely related to the Bladder Meridian of Foot-Taiyang. Lingshu·Jingmai (Spiritual Pivot·Meridians) states: "The Bladder Meridian of Foot-Taiyang... Diseases related to tendons governed by it include hemorrhoids, malaria, mania, epilepsy, pain in the head, fontanelle, and neck, yellow eyes, lacrimation, epistaxis, pain in the neck, back, waist, buttocks, popliteal fossa, calves, and feet, and inability to use the little toe", which puts forward the theory of "Taiyang governs tendons" [8]. It describes that the symptoms of diseases of the Bladder Meridian of Foot-Taiyang include pain and dysfunction from the head and neck along the meridian to the waist and buttocks, especially pain and discomfort in the head, neck, and nape, as well as dysfunction, which is consistent with the clinical symptoms of cervical spondylosis. The Twelve Muscle Meridians are the peripheral accessory parts of the Twelve Meridians, and muscles and joints are nourished by the Qi and blood of the Twelve Meridians [9]. Therefore, the meridians and muscle meridians of the Foot-Taiyang Meridian are interdependent in physiology and interact in pathology [10]. Zhang Jiebin also believed that "the muscles and tendons all over the body are mostly and largely distributed along the Foot-Taiyang Meridian. Its lower parts connect to the heels, calves, popliteal fossae, and buttocks; its upper parts cling to the waist and spine, connect to the shoulders and neck, and extend to the head as the superior orbital ridge [11]. Therefore, all conditions such as spasm, flaccidity, and opisthotonos are caused by insufficient fluid of the Foot-Taiyang Meridian, which governs diseases related to tendons" [12]. Therefore, cervical spondylosis is also a disease governed by the Bladder Meridian of Foot-Taiyang and is closely related to this meridian. Cai Gengxi et al. conducted data analysis on the acupoint selection rules of acupuncture for treating cervical spondylosis at home and abroad and found that the acupoints of the Bladder Meridian of Foot-Taiyang accounted for 16.62% of the total therapeutic acupoints, with the highest proportion. This indicates that the Bladder Meridian of Foot-Taiyang is the most commonly used meridian in the treatment of cervical spondylosis. Huang Hongxi's research found that the muscle meridian lesions of patients with cervical spondylosis are divided into three categories: point-like, linear, and planar lesions, with point-like muscle meridian lesions being the main type, and various muscle meridian lesions are mainly concentrated in the muscle meridian of the Foot-Taiyang Meridian. Therefore, the treatment should also start with the Bladder Meridian of Foot-Taiyang. Acupuncture has a long history in the treatment of cervical spondylosis. As early as Lingshu·Zabing (Spiritual Pivot·Miscellaneous Diseases), it was recorded that "for neck pain with inability to bend forward and backward, puncture the Foot-Taiyang Meridian; for inability to turn the head, puncture the Hand-Taiyang Meridian" [13]. Acupuncture in the treatment of cervical spondylosis has the characteristics of obvious, long-lasting, and stable curative effect, with few adverse reactions. As a traditional external therapy of TCM, acupuncture regulates Qi and blood, dredges meridians, and balances Yin and Yang by stimulating meridians and acupoints [14].

Acupuncture treatment has the effects of improving Qi and blood circulation, restoring patients' functions, and relieving spasm, while Tuina (massage) also has the effects of dredging meridians, nourishing tendons, and relieving pain by regulating tendons [15]. Modern studies have found that acupuncture in the treatment of cervical spondylosis can not only reduce inflammation and pain, dredge meridians and tendons, but also relieve cervical muscle spasm. Acupuncture can effectively promote and enhance cervical blood circulation and metabolism, help patients absorb local inflammation, thereby better eliminating existing edema, and exert a good advantage in relieving neck and shoulder pain [16]. Zhang Wanyu et al. found that the eight-confluent points compatibility therapy can effectively improve the Visual Analogue Scale (VAS) score, self-made symptom and sign score, and Neck Pain Questionnaire (NPQ) score of patients with cervical spondylosis. Wu Zhongliang et al. adopted the quick puncture method, which had a better effect in reducing neck pain, alleviating inflammatory response, and improving hemorheology in patients with cervical spondylosis. Chen Youyi established a model of cervical spondylosis and found that after electroacupuncture at the "Dazhui" point, the contents of SOD, GSH-Px, and Glu were significantly higher than those in the model group, while the content of MDA was significantly lower. He believed that the mechanism of acupuncture in the treatment of cervical spondylosis may be to reduce muscle damage by improving the free radical scavenging ability of tissue cells and the content of Glu, thereby achieving the effect of relieving neck and shoulder pain. He Jian et al. used the same modeling method and found that the contents of nitric oxide synthase (NOS) and NO in the serum and muscle tissue homogenate were significantly decreased, suggesting that long-term head-down flexion of the cervical spine can inhibit information transmission [17]. Guided by the theory of "Taiyang governs tendons", this study selected three points (Kunlun, Weizhong, and Feishu) on the Bladder Meridian of Foot-Taiyang for acupuncture in the treatment of cervical spondylosis. Among them, Feishu is located locally in the neck and nape, following the principle of "acupoints correspond to local disorders", guiding the local Qi to "reach the affected area", dredging local meridian Qi and blood, improving blood microcirculation, inhibiting the release of pain mediators, and stimulating peripheral nerves, thereby reducing neck and shoulder pain in patients [18]. Nanjing·Liushiba Nan (Classic of Difficult Issues·Sixty-Eighth Difficulty) states: "Jing (Well) points treat stuffiness in the chest and hypochondrium; Ying (Spring) points treat body heat; Shu (Stream) points treat heaviness and joint pain; Jing (River) points treat cough, asthma, cold, and heat; He (Sea) points treat adverse Qi and diarrhea" [19]. Kunlun and Weizhong points are acupoints of the Bladder Meridian of Foot-Taiyang. According to Huangdi Neijing·Lingshu·Jingmai (Yellow Emperor's Internal Classic·Spiritual Pivot·Meridians): "The Bladder Meridian of Foot-Taiyang originates from the inner canthus of the eye. Its straight branch enters the brain from the top of the head, exits and descends to the neck". The Bladder Meridian of Foot-Taiyang runs from the head to the back and then to the feet, and the entire neck and back of the human body are mainly traversed by the Bladder Meridian. According to the principle of "meridians govern disorders along their pathways", it can treat diseases along the Foot-Taiyang Meridian [20].

6. Conclusion

In summary, since meridians transport Qi and blood to nourish muscle meridians, enabling muscle meridians to bind bones and regulate joints, the occurrence of cervical spondylosis is related to meridians, and most closely related to the muscle meridians of the Hand-Taiyang and Foot-Taiyang Meridians; from the perspective of the distribution of the muscle meridian of the Foot-Taiyang Meridian, it highly overlaps with the lesion site of cervical spondylosis [21]. Secondly, the Foot-Taiyang Meridian is the one with the most abundant defensive Yang among the Twelve Meridians. Defensive Yang originates from the Foot-Taiyang Meridian. Abundant defensive Yang warms and nourishes the muscle meridians; if defensive Yang is insufficient, the cervical muscle meridians lose warmth, resulting in symptoms such as pain and stiffness of cervical spondylosis. Therefore, cervical spondylosis is also a disease governed by the Bladder Meridian of Foot-Taiyang and is closely related to this meridian [22]. Modern medical studies have shown that the imbalance of cervical biomechanical balance and inflammatory damage of cervical muscle tissue are closely related to the occurrence of cervical spondylosis [4]. People who work at their desks for a long time or have bad living habits are prone to imbalance of cervical biomechanical balance and inflammatory damage of cervical muscle tissue. In the occurrence and development of cervical spondylosis, inflammatory factors such as IL-1β, IL-6, and TNF-α play key roles. Relevant studies have shown that the expression level of TNF-α is significantly increased after cervical disc degeneration. This increase not only promotes the contact between macrophages and degenerated disc tissue, thereby triggering an inflammatory response, but also is the initiating factor leading to the inflammatory response of degenerated discs. As a pro-inflammatory factor, the expression and release of IL-6 can be regulated by muscle contraction. At the same time, muscle contraction can also promote the expression and release of inflammatory factors such as IL-1β, and promote the release and expression of other inflammatory cytokines such as TNF-α, ultimately aggravating the degree of inflammatory damage. In this experiment, the expression levels of IL-1β, IL-6, and TNF-α in rabbits were significantly increased after modeling, and after treatment with acupuncture at points of the Bladder Meridian of Foot-Taiyang, the expression levels of the above inflammatory factors were lower than those in the model group. In conclusion, acupuncture at points of the Bladder Meridian of Foot-Taiyang may exert a therapeutic effect by reducing the release of inflammatory factors such as IL-1β, IL-6, and TNF-α and inhibiting the inflammatory damage of cervical muscles. Therefore, in the treatment of cervical spondylosis, acupuncture can be used under the guidance of the theory of "Taiyang governs tendons".

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Disclosure statement

The authors declare no conflict of interest.

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