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ORIGINAL RESEARCH

Tuina Promotes Repair of Chronic Cervical Muscle Injury by Regulating Satellite Cell Proliferation and Differentiation and Inhibiting Myocyte Apoptosis

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Purpose: Chronic cervical muscle injury is the first common cause of the development of cervical spondylosis, and Tuina can effectively promote the repair of chronic cervical muscle injury and alleviate neck pain, but the mechanism behind its efficacy is still unknown. The proliferation and differentiation of muscle satellite cells and the apoptosis of cervical myocytes play important roles in the repair of chronic cervical muscle injuries; therefore, this study aimed to explore the potential mechanisms of Tuina to promote the repair of cervical muscle injuries in terms of the proliferation and differentiation of satellite cells and the apoptosis of myocytes.

Patients and Methods: Twenty-eight Wistar rats were randomly divided into control group, model group, Tuina group, and meloxicam group, with 7 rats in each group. Except for the control group, each group were establish a chronic cervical muscle injury model (CCMI). Meloxicam (0.79 mg/kg) was administered by gavage, and in the Tuina group, pressure was applied to the Fengchi acupoint on the affected side once a day. Morphological changes of cervical muscle tissues were detected by ultrasonic diagnostic instrument and HE staining, electrophysiological recordings of electromyographic changes, apoptosis rate was detected by TUNEL staining, and positive expression of Bax, Bcl-2, IGF-1, MyoD, and Pax-7 were detected by Immunohistochemistry and Western blot.

Results: In CCMI model rats, we observed that the cervical muscle fibers were disorganized, with irregular morphology, and the amplitude of electromyography was significantly weakened, while Tuina could significantly improve these symptoms and effectively promote the repair of chronic cervical muscle injury. Meanwhile, compared with the model group, Tuina could significantly increase the expression levels of IGF-1 ($P<0.01$) and MyoD ($P<0.05$) and decrease the expression level of Pax7 ($P<0.05$). In addition, we found that the number of apoptotic cells in cervical myocytes was reduced after Tuina intervention ($P<0.05$), and Tuina inhibited the expression of pro-apoptotic factor Bax ($P<0.01$) and promoted the expression of anti-apoptotic factor Bcl-2 ($P<0.05$).

Conclusion: Tuina can promote the proliferation and differentiation of satellite cells to repair chronic cervical muscle injury by regulating the expression of Pax7, MyoD, and IGF-1, as well as inhibiting the expression of Bax and promoting the expression of Bcl-2 to ameliorate the apoptosis of cervical myocytes in CCMI model rats.

Keywords: Tuina, cervical spondylosis, neck pain, satellite cell

Introduction

Cervical spondylosis is a common clinical degenerative condition that may present with mechanical neck pain, radicular pain, myelopathy, or a combination of these symptoms.[1] Among all the diseases threatening life expectancy, neck pain has ascended from the 12th in 1990, to the 4th in 2015.[2] With the increase in the use of electronic devices in daily life,

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there has been a year-on-year increase in the incidence rate of the condition, and a trend of affecting younger and younger generations. It is predicted that the number of people suffering from neck pain will increase by an additional 32.5% worldwide by the year 2050.[3] Studies have confirmed that abnormalities in cervical muscle morphology and function are closely related to the development of mechanical neck pain.[4,5] Chronic muscle injury, as the first common cause of the onset of cervical spondylosis, has an incidence rate as high as 50–70% during the onset of cervical spondylosis, especially among the long-term "bowed-head tribe", which has brought a heavy medical and economic burden to society.[6]

Skeletal muscles have been shown to possess a remarkable ability to regenerate following injury, which is largely dependent on muscle satellite cells.[7] Satellite cells can enter the cell cycle from quiescence after muscle injury. Activated satellite cells begin to generate new stem cells and a large quantity of proliferating myoblasts, which later differentiate into muscle cells (myocytes) to rebuild the muscle fibers, thus helping skeletal muscles to regenerate. Several transcription factors are required during the process of satellite cells regulation, such as the transcription factor paired box 7 (Pax7) and the myoblast determination protein (MyoD).[8,9] There is strong evidence showing that Pax7 is the classical marker for satellite cells during the quiescent/activated state, whereas MyoD is the marker for satellite cells under the proliferated/differentiated state.[10,11] In addition, the insulin-like growth factor (IGF-1) is a key regulator of satellite cell proliferation and terminal differentiation of muscle stem cells, and holds a key role in the healing of muscles after injury by promoting satellite cell proliferation and differentiation.[12] At the same time, there is a strong link between myocyte growth and apoptosis. Since large numbers of injury-induced apoptotic cells affect muscle growth, apoptosis inhibition is crucial to muscle repair.[13] The mitochondrial pathway is the most common apoptotic pathway, and the Bcl-2 family is the main regulator of apoptosis. Bcl-2 and Bax are key elements of the Bcl-2 family, belonging to the apoptosis suppressor and pro-apoptotic genes, respectively; together, they determine apoptosis in skeletal muscle cells.[14,15]

Currently, there are many ways to treat cervical spondylosis, and common methods include oral and topical medications, physical therapies, etc.[16,17] Tuina is a therapy through which diseases are treated by applying directed, deepened, and intensified pressure to the skin and muscles of the body through a variety of manipulations.[18] Tuina has been shown to be effective in the relief of chronic neck pain, with lasting long-term effects.[19,20] However, the underlying mechanisms of the treatment effects of Tuina still need to be investigated. Tuina has been shown to reduce the release of inflammatory factors from cervical intervertebral discs and alleviate pain symptoms by mediating the FAK-MAPK signaling pathway.[21] However, the mechanism of action of Tuina therapy for cervical spondylosis from the point of view of muscle repair is not known.

In this study, we used ultrasound, electrophysiological techniques, and HE staining to determine whether Tuina is effective in alleviating chronic cervical muscle injury. The apoptosis of cervical muscle cells was detected by TUNEL assay, and the expression of MyoD, Pax7, IGF-1, Bax, and Bcl-2 in cervical muscle tissues was determined by immunohistochemistry and Western blotting, to determine the possible mechanisms by which Tuina repairs chronic cervical muscle injury.

Materials and Methodology

Animal Ethics

1-month-old male rats (SPF-grade Wistar), weighing 180–220 g, were used in this study. For one week, the animals were put through a process of adaptation to the conventional laboratory conditions that follows: environment temperature of 23 ± 1 °C, humidity of 45 ± 5%, 12/12 h light-dark cycles, with free access to food and water at all times. All processes found in this experiment were established in agreement with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals, with the consent of the Animal Ethics Committee of Fujian University of Traditional Chinese Medicine (protocol code, SYXK(min)2020–0007). The design of this experiment is revealed in Figure 1.

Chronic Cervical Muscle Injury Model (CCMI)

The CCMI model was established according to the method of Wang.[22] The rats were bound in the prone position, then anesthetized through injection with 10% chloral hydrate. The skin at the middle of the back of the neck was incised

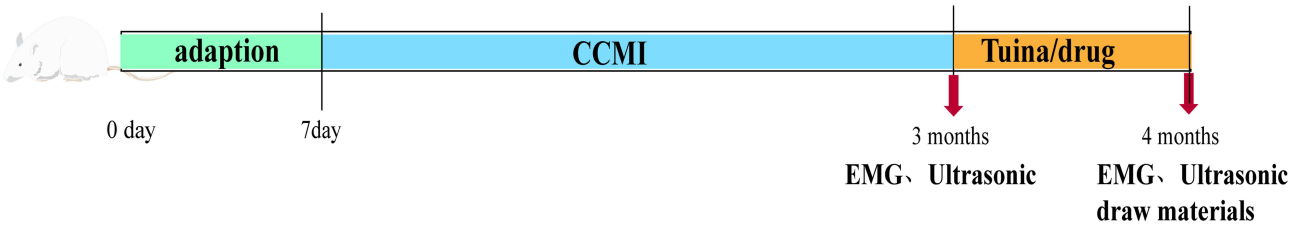

Figure 1 Experimental procedure in rat model of CCMI. Wistar rats were acclimatized for seven days to establish a CCMI rat model; the success of the CCMI model was evaluated after three months using EMG and ultrasonic; Tuina and drug interventions were performed on the first day of successful model preparation, and EMG and ultrasound tests and sampling were conducted and taken one month after the intervention.

longitudinally for 2 cm, and the layers of muscles were bluntly separated. The Splenius Cervicis, Longissimus Capitis and Longissimus Cervicis were cut transversely, the Musculus Semispinalis Capitis and Iliocostalis Cervicis were completely excised, and finally the supraspinal ligament and the interspinous ligament of C2-C7 were cut sequentially, which were then sutured one by one. Penicillin powder was spread after the operation to prevent infection, and the model was verified for success after 3 months.

Experimental Groups and Intervention

We numbered the rats according to body weight and randomized them according to the stratified randomization principle using the ProceedPlan procedure of the SPSS 23.0 statistical software. We established four animal experimental groups, with seven rats randomly assigned into each group: (i) Control group, in which no intervention was carried; (ii) Model group—exposition to CCMI only; (iii) Meloxicam group—exposition to CCMI and daily Meloxicam injections at a dosage of 0.79 mg/kg; (iv) Tuina group—daily exposition to CCMI and Tuina treatment.

The Tuina protocol used the homemade animal test Tuina strength apparatus (Figure 2), and the selected acupoint was the FengChi point (GB20) from the affected side of the rats. After the rats were fixed with a transparent fixation frame, a black cloth head cover was put on, and point pressing and Tuina were performed on the FengChi acupoint. The Tuina device is operated as follows (Supplementary Figure 1): the finger is pressed on the gray ring and the force of the manipulation is controlled at 0.4 kgf (≈4 N) by the force display on the computer.[23] "1s point press 1s interval" was performed at the FengChi acupoint. The treatment would last 20 min per intervention, and one intervention would be performed every day for 14 consecutive days, which is considered to be one treatment course; two intervention courses were carried out in total.

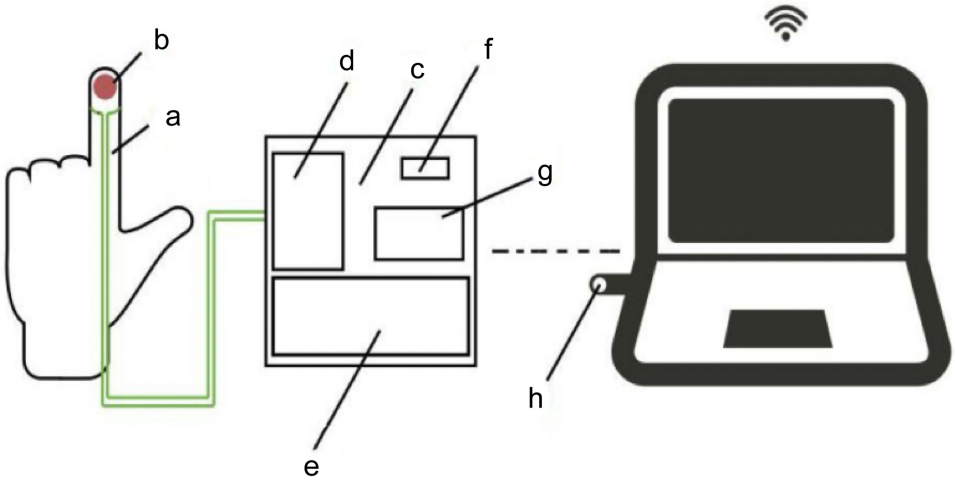

Figure 2 Design drawing for the animal testing Tuina strength apparatus. (a) Finger cot. (b) Pressure sensors. (c) Tuina strength signal acquisition device. (d) processing unit. (e) Power supply unit. (f) Switching devices. (g) communications unit. (h) Bluetooth module.

Electromyogram Testing (EMG)

EMG testing is widely used for the evaluation of muscle function.[24] Since the trapezius muscle is the largest muscle group in the neck, we examined the trapezius muscle on the affected side of the rat. After anesthesia, the rats' necks were dehaired, the trapezius muscle was fully exposed, positive, and negative electrodes were placed on the trapezius node with the two needles separated by 1–2 cm, and a ground electrode was placed on the tail of the rats. The rats were stimulated with a current of 5 hz to generate evoked potentials, and finally, the degree of attenuation of changes in myoelectric potentials and the amplitudes of the M-wave of the evoked potentials of the rats were recorded on electrophysiological recorders for 5 s consecutively. This procedure was conducted on the first day after successful modeling and on the first day after intervention completion.

Ultrasonic Diagnosis

Ultrasonic diagnosis provides a comprehensive view of the muscle structure and the group of soft tissue levels, clarifying the actual state of the damaged muscle.[25] We used a portable color Doppler ultrasound diagnostic 10 MHz linear array probe device (M-Turbo, Sonosite Inc., USA) to observe the trapezius muscle morphology. Isoflurane anesthesia of the rats and nuchal depilation were performed. Ultrasound coupling agent was applied to the ultrasound probe, which was then gently placed vertically above the location of the rat trapezius muscle, adjusting the probe's depth to obtain clear and bright images of the trapezius muscle tissue structure. This procedure was conducted on the first day after successful modeling and on the first day after intervention completion.

HE Staining

HE staining was used to observe the morphology of cervical muscle tissues of the rats in each group. Paraffin sections of rat cervical muscle tissues were routinely prepared. The paraffin sections were deparaffinized and stained with hematoxylin and eosin solution, and using a microscope, the morphology of the tissues was observed.

TUNEL Staining

TUNEL staining was used to observe myocyte apoptosis in rat trapezius muscle tissues. Paraffin sections of rat trapezius muscle tissues were routinely prepared. After they were deparaffinized with xylene and hydrated with gradient ethanol, the number of apoptotic cells was observed using a light microscope by following the instructions of the kit (Servicebio, G1501), and apoptotic cells were considered positive if the nuclei were brownish-yellow or brownish.

Immunohistochemical Staining

After dewaxing of the paraffin sections of trapezius muscle tissue microarrays, antigen repair was conducted on the sections utilizing citrate buffer. Following that, the sections were washed with 1 × PBS, blocked with 3% H2 O2 for 5 min, and then again with 5% serum for 15 min. Afterwards, the sections were incubated at 4 °C overnight by using the primary antibody, and then incubated again at 37 °C for 1 h using the second antibody. Subsequently, staining of the sections was performed by DAB in the dark for 5 min, followed by re-dying with hematoxylin for 15s. Finally, sealing of the sections was conducted with neutral gum, and photographs were taken using light microscopy. The primary and the second antibodies employed in this assay are shown in Table 1.

Western Blot

Using Western blotting, the alteration in expression of the MyoD and Pax7 proteins in the trapezius muscle was analyzed. Through utilizing a BCA Protein Assay Kit, the concentration of protein in the tissue lysis buffer was quantified for immunoblotting experiments. An SDS-PAGE gel electrophoresis separated the protein extracts and transferred them onto an NC membrane. This membrane was soaked in a blocking solution with 5% skim milk and incubated for two hours at room temperature. Primary antibodies were added and incubated under a temperature of 4°C overnight. On the subsequent day, the corresponding secondary antibody was added, then put under a temperature of 4°C for one hour. Following washing, in room deprived of light, the membrane was developed by ECL, exposed, and developed. With the

Table 1 Antibody Information of the Proteins Detected by Immunohistochemistry and Western Blot in CCMI Model Rats

Antibody	Company	Number	Dilution Ratio
Bax	Proteintech	50599-2-Ig	1:1000
Bcl-2	Proteintech	26593-1-AP	1:400
IGF-1	Proteintech	28530-1-AP	1:200
MyoD	Proteintech	18943-1-AP	1:3000
Pax7	Proteintech	20570-1-AP	1:600
GAPDH	Proteintech	10494-1-AP	1:20,000

help of the Image Lab software, statistical analysis was conducted. The primary and the second antibodies utilized in this assay are shown in Table 1.

Statistical Analysis

In order to conduct data analysis, the IBM SPSS (version 22.0, USA) statistical software was chosen for this study. Data are indicated as mean ± SD. When the data had a normal distribution with homogeneous variances, Comparisons between groups were made using one-way analysis of variance, followed by the least significant difference test for post-hoc comparisons. When the distribution was not expected, a nonparametric Kruskal–Wallis test was used. All reported tests were two-sided, and two-sided $P<0.05$ was considered to represent statistical significance.

Results

Tuina Effectively Promotes Morphological and Functional Repair of Cervical Muscle Tissues in CCMI Model Rats

To determine whether the CCMI model was successful or not and to clarify the effect of Tuina intervention on the repair of rat trapezius muscles, we first conducted EMG testing and ultrasound diagnosis. As illustrated in Figure 3A, in contrast with the control group, the myoelectric wave amplitude of the model group, the Tuina group and the meloxicam group was obviously attenuated, which might be due to the reduction in conduction function of muscle nerves of the CCMI rats. After the intervention, the EMG amplitude of the Tuina and meloxicam groups was significantly more elevated than that of the model group (Figure 4A).

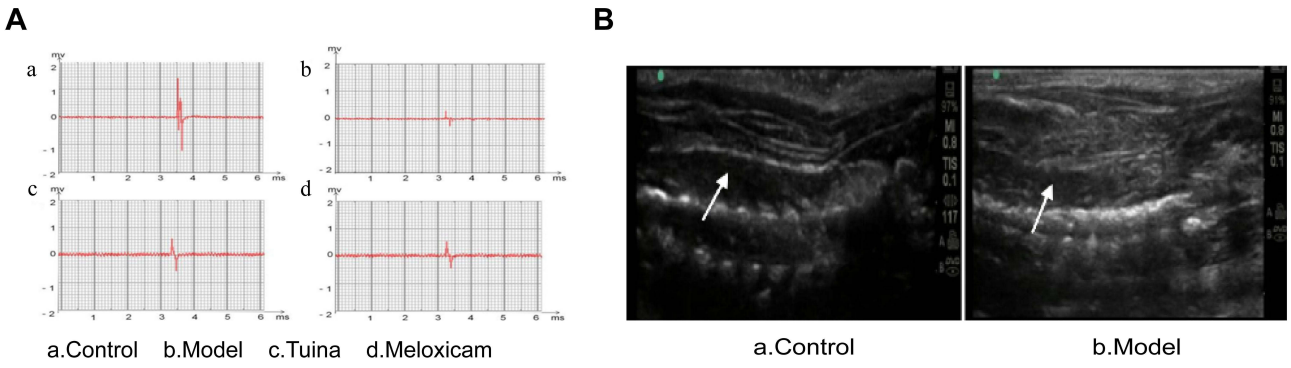

Figure 3 Detection of indicators of successful replication in the rat CCMI model. (**A**) EMG testing of trapezius muscle tissues before intervention after modeling, which showed a significant attenuation of the EMG wave amplitude compared with the control group (b-d); (**B**) Ultrasonic examination of trapezius muscle tissues before intervention after modeling.

Notes: The white arrows: (a) the normal cervical muscle tissue. (b) the cervical muscle tissue is damaged.

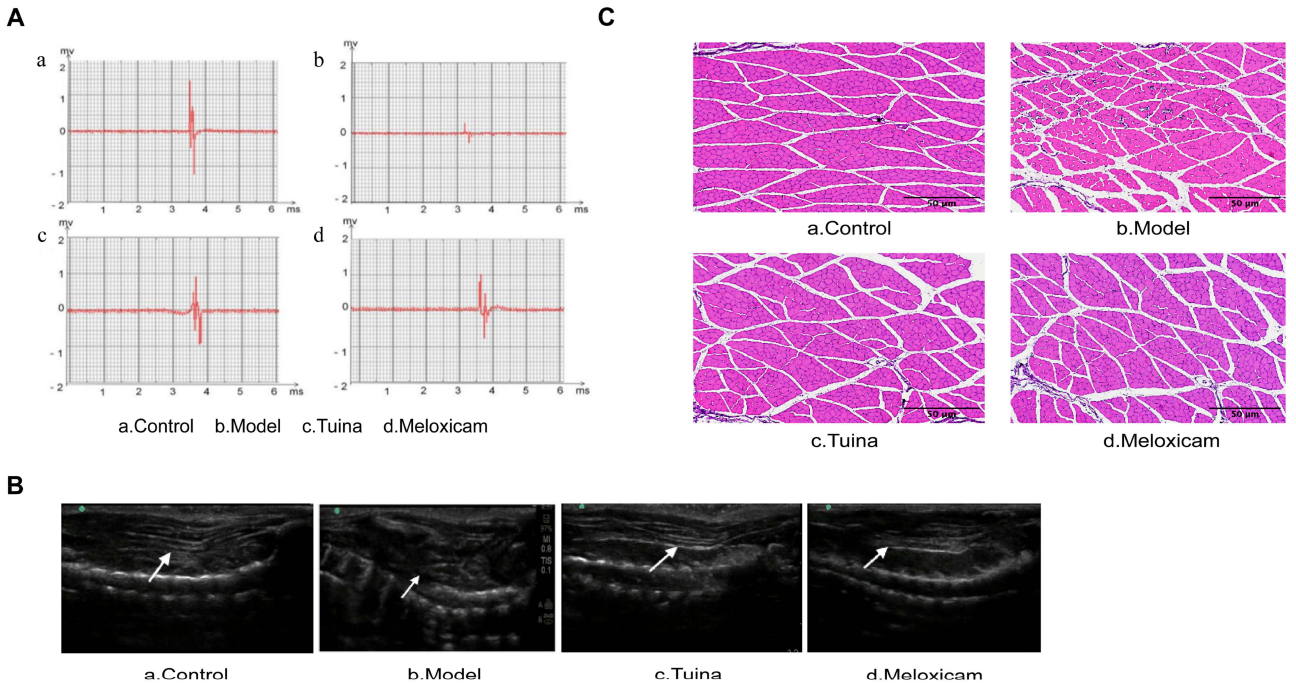

Figure 4 Tuina promotes morphological and functional repair of trapezius muscle tissue in CCMI model rats. (**A**) EMG test of the trapezius muscle tissue after the intervention, the amplitude of the EMG wave was significantly increased compared to the model group (c-d); (**B**) Ultrasonic examination of trapezius muscle tissues after the intervention. Note: The white arrows: (a) Normal cervical muscle tissue. (b) The damaged area of the cervical muscle tissue. (c-d) Recovery of cervical muscle tissue with aligned fibers; (**C**) Morphology of trapezius muscle tissue of rats in each group was observed by HE staining. (a) Normal cervical muscle morphology. (b) Irregular cervical muscle fibers with inflammatory infiltration. (c-d) Recovery of cervical muscle morphology after intervention.

Results from the ultrasonic diagnosis showed that the trapezius muscle tissues of rats in the control group had uniform echoes, regular morphology, clear boundaries, and an orderly and regular arrangement of the muscle fibers. The trapezius muscle tissues observed in the model group had uneven echoes, disorganized arrangement, fuzzy boundaries, and nodularity (Figure 3B). After the intervention, the trapezius muscle tissues observed in the Tuina and meloxicam groups had uniform echoes, clear boundaries, and the size of the nodules was reduced (Figure 4B).

Then, we performed HE staining to observe the morphological changes in trapezius muscle tissues, as shown in Figure 4C, the muscle fiber section of the control group was regular, the myogenic fibers were neatly arranged, and there was no inflammatory infiltration and abnormal morphological changes. The muscle fiber section of the model group was irregular in morphology, chaotic in structure, and the muscle gap was significantly widened, with obvious inflammatory infiltration and abnormal morphological changes. And finally, the muscle fiber section of the Tuina group and the meloxicam group were more regular in morphology, with intact structure, the muscle gap was reduced, and the inflammatory infiltration improved.

Taken together, these results indicate that Tuina can effectively ameliorate cervical trapezius muscle injury and promote morphological and functional repair of muscle tissues in CCMI model rats.

Tuina May Promote the Proliferation and Differentiation of Satellite Cells to Repair Chronic Cervical Muscle Injury by Regulating the Expression of Pax7, MyoD, and IGF-1

Since the activation, proliferation and differentiation of satellite cells are important factors in skeletal muscle regeneration,[26] the expression of the muscle activation factor Pax7 and the proliferation and differentiation factors MyoD and IGF-1 was detected by Western blot and immunohistochemistry in the trapezius muscle tissues of the animals in each group. As shown in Figure 5A–C, the level of expression of the MyoD and Pax7 proteins was decreased in the trapezius muscle tissues of the model group when compared with the control group ($P<0.01$). In contrast with the model

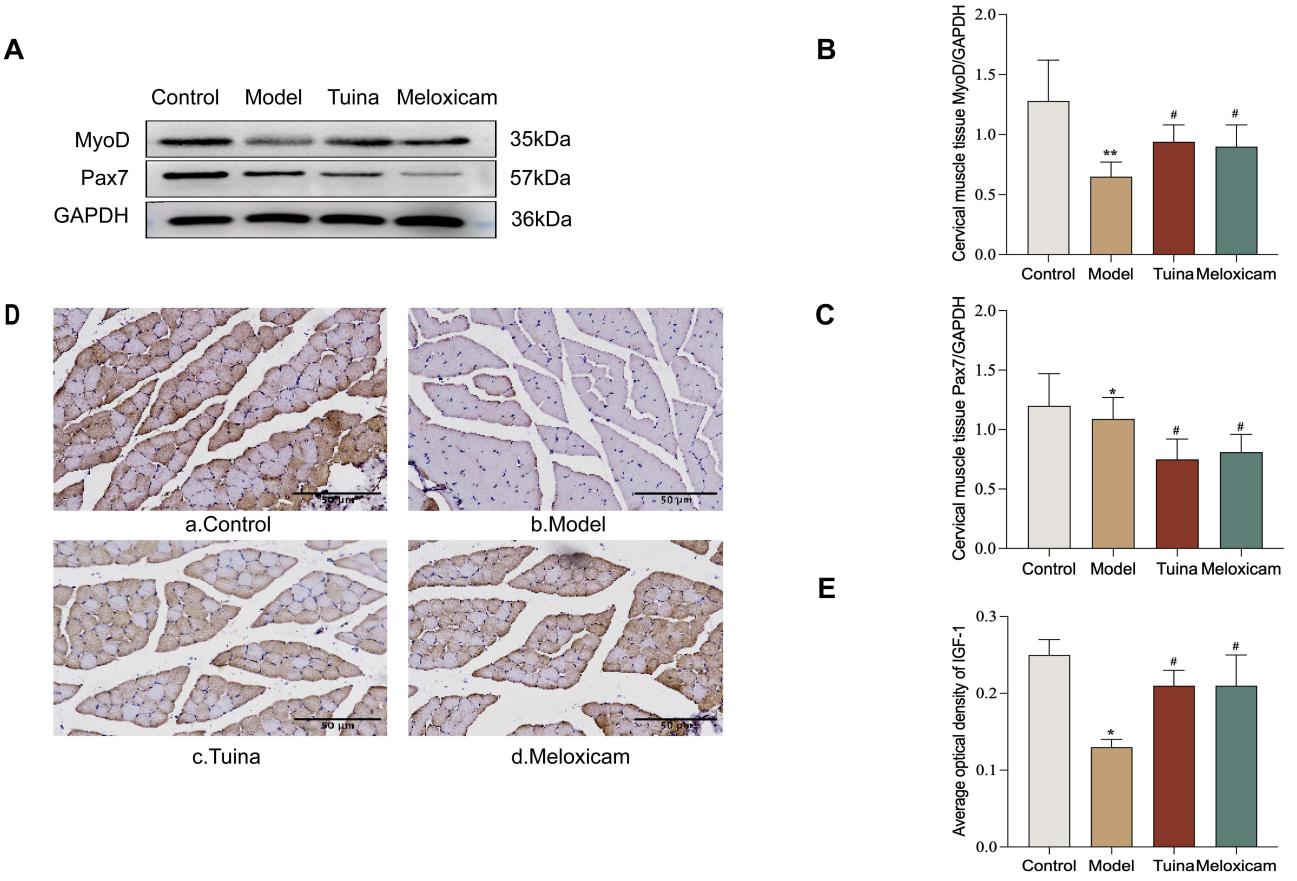

Figure 5 Tuina promoted the proliferation and differentiation of satellite cells to repair chronic cervical muscle injury by regulating the expression of Pax7, MyoD, and IGF-1. Protein levels of Pax7 and MyoD in trapezius muscle tissues of rats were analyzed by Western blot (**A**–**C**). The expression of IGF-1 in the trapezius muscle tissues of the rats in each group was detected by immunohistochemical staining (**D** and **E**). Data are indicated as mean ± SD. *P < 0.05, **P < 0.01 when contrasted with the control group. #P < 0.05 when contrasted with the Model group.

group, the expression of the MyoD protein was increased and the expression of the Pax7 protein was decreased in the Tuina and meloxicam groups (P<0.05). As shown in Figure 5D and E, the expression of IGF-1 was decreased in the trapezius muscle tissues of the model group in comparison with the control group (P<0.01). By comparing with the model group, an increase in IGF-1 expression could be observed in the Tuina and meloxicam groups (P<0.01). These results indicate that Tuina may promote the proliferation and differentiation of satellite cells to repair cervical muscle injury by regulating the expression of Pax7, MyoD, and IGF-1.

Tuina May Ameliorate the Apoptosis of Cervical Myocytes in the CCMI Model Rats by Inhibiting the Expression of Bax and Promoting the Expression of Bcl-2

Since skeletal muscle cell apoptosis is an important manifestation found in chronic cervical muscle injury,[27] we first performed TUNEL staining to determine the number of apoptotic cells in each group. We observed that brownish apoptotic cells were seen in trapezius muscle fibers in all groups. By comparing with the control group, an increase in the number of apoptotic cells could be noted in the model group (P<0.01), which was significantly improved after intervention in the Tuina and meloxicam groups (P<0.05) (Figure 6A and B).

To further verify the apoptotic mechanism, we used Immunohistochemistry to detect the levels of expression of the pro-apoptotic factor Bax and the anti-apoptotic factor Bcl-2. As illustrated in Figure 6, in contrast with the control group, an increase in the positive expression of Bax (Figure 6C and D) and a decrease in the expression of Bcl-2 could be

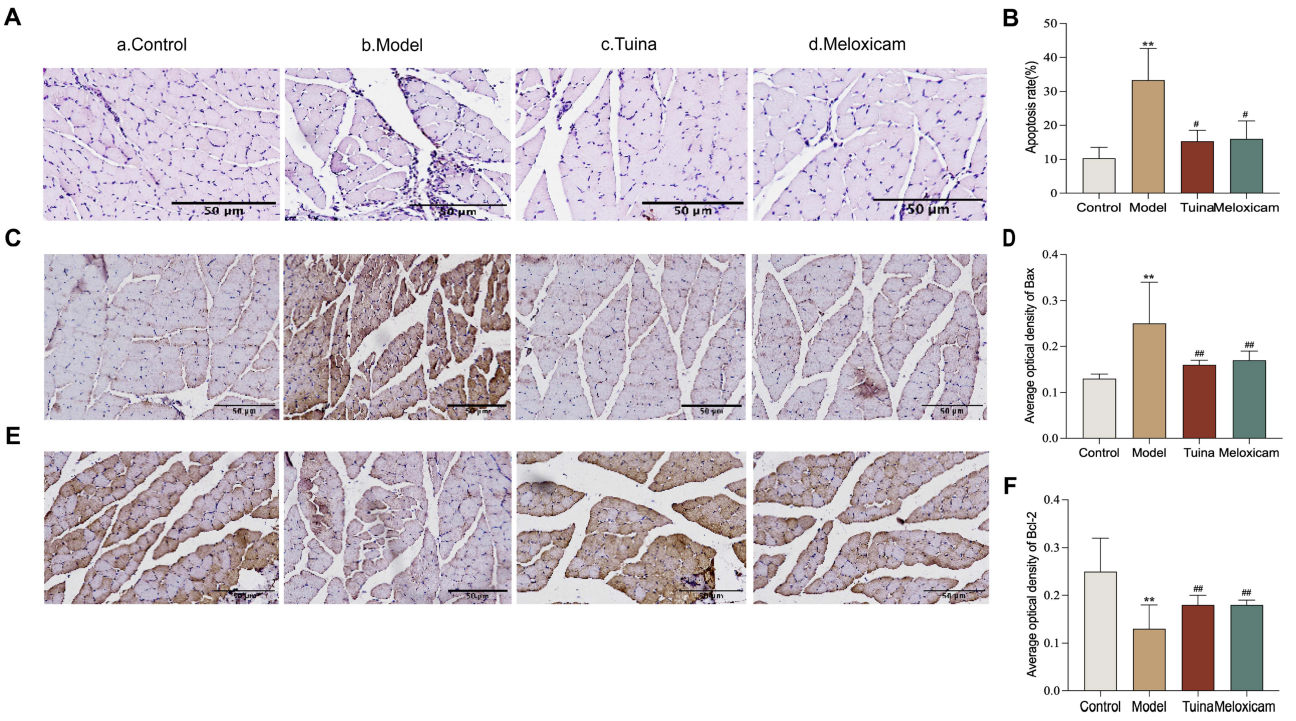

Figure 6 Tuina ameliorated the apoptosis of cervical myocytes in the CCMI model rats by inhibiting the expression of Bax and promoting the expression of Bcl-2. Detection of apoptosis in rat trapezius muscle tissues by TUNEL staining (**A** and **B**). The expression of Bax in the rats' trapezius muscle tissues of each group was detected by immunohistochemical staining (**C** and **D**). The expression of Bcl-2 in the rats' trapezius muscle tissues of each group was detected by immunohistochemical staining (**E** and **F**). Data are indicated as mean ± SD. **P < 0.01 when contrasted with the control group. #P < 0.05, ##P < 0.01 when contrasted with the Model group.

observed in the model group (P<0.01) (Figure 6E and F); in contrast with the model group, the positive expression of Bax was decreased (P<0.01) (Figure 6C and D) while the expression of Bcl-2 was increased (P<0.05)(Figure 6E and F) in the Tuina and meloxicam groups.

In brief, the above results indicated that Tuina was able to improve the apoptosis of cervical myocytes in CCMI model rats, which might be related to the ability of Tuina to inhibit the expression of Bax and promote the expression of Bcl-2.

Discussion

Our study of the effects of Tuina on the repair of chronic cervical muscle injuries has demonstrated the therapeutic potential of this intervention. Our study confirmed that Tuina could effectively promote the repair of trapezius muscle tissues in a rat model of CCMI. Meanwhile, we found that the mechanism of Tuina in promoting the repair of CCMI may involve the following two aspects: First, Tuina may promote the proliferation and differentiation of muscle satellite cells by regulating the expression of Pax7, MyoD, and IGF-1. Second, Tuina may inhibit Bax expression and promote Bcl-2 expression to inhibit the apoptosis of cervical myocytes. A conceptual diagram of the relevant mechanisms is shown in Supplementary Figure 2.

We used an experimental animal model of CCMI, a modeling method that does not injure the intervertebral discs and gradually causes cervical disc degeneration due to the destruction of the soft-tissue structure at the back of the neck, which gives the animal a tendency to lower its head. This modeling method imitates the cause of cervical spondylosis in modern people who suffer from long-term head-down strain.[22] It is important to note that the normal physiological movement and stability of the neck is based on static equilibrium (bones and ligaments maintain joint stability and balance) and relies on muscle movement to achieve dynamic equilibrium.[28] Our results showed that in the rat model of CCMI, muscle morphology was significantly altered, nodules were formed, muscle fibers were disorganized, and muscle thickness was thinned with obvious inflammatory infiltration; in addition, trapezius myoelectric amplitude was

significantly attenuated, suggesting that the model was successfully reproduced. In contrast, Tuina intervention effectively promoted the repair of trapezius muscle tissues in rats with CCMI, as evidenced by the neat arrangement of the trapezius muscle fibers, thickening of muscle thickness compared with the model group, and significant increase in trapezius myoelectric amplitude, suggesting that Tuina has a strong potential for repairing chronic cervical muscle injuries, which is consistent with previous literature.[29]

Previous researches have indicated that the proliferation and differentiation of satellite cells are regulated by a variety of factors such as Pax7, MyoD, and IGF-1.[30–32] In particular, Pax7 is an activator of myosatellite cells, and a decrease in Pax7 leads to an arrest of the myosatellite cell cycle.[33] When satellite cells are activated, they enter the proliferation phase and begin to differentiate into adult myoblasts, during which MyoD and IGF-1 play very important roles. As satellite cells begin to differentiate, the expression level of Pax7 is downregulated. This is because Pax7 is no longer an essential transcription factor during myoblast differentiation, and other transcription factors such as MyoD take over the role of Pax7.[34] We found a reduction in Pax7, MyoD and IGF-1 expressions in the model group when compared with the control group. Although previous studies have suggested that satellite cells can be actively activated to initiate the repair process after muscle injury, this process has a distinctly temporal nature, and satellite cells gradually return to their resting state in about 2 weeks.[35,36] However, in the case of CCMI, the natural recovery time is usually more than 1 month. By consequence, the satellite cells have already entered in their resting state by that time, and their active repair process has also been terminated. Therefore, CCMI may inhibit the proliferation and differentiation of cervical muscle satellite cells, resulting in the stagnation of their cycles. Notably, the expression of Pax7 was reduced and the expression of IGF-1 and MyoD were increased after Tuina intervention, suggesting that Tuina's effect on CCMI may be associated with the promotion of proliferation and differentiation of satellite cells in cervical muscle tissues.

The apoptosis of myocytes is just as important in the repair of skeletal muscle injuries.[27] We further investigated the mechanism by which Tuina inhibits myocyte apoptosis in the rat model of CCMI. Bcl-2 is an anti-apoptotic factor that contributes to cell survival and inhibits apoptosis, whereas Bax is a pro-apoptotic factor that promotes apoptosis and inhibits cell survival. Both Bax and Bcl-2 together regulate myocyte apoptosis.[14,15,37] Our study found that the quantity of apoptotic cells in cervical muscle tissue tan was significantly increased in all groups under the pathological condition of CCMI. Meanwhile, the expression of Bax raised, while that of Bcl-2 decreased. Interestingly, in comparison with the model group, the number of apoptotic cells was remarkably lower in the Tuina group. In addition, Tuina downregulated the expression of Bax and upregulated the expression of Bcl-2, indicating that Tuina effectively inhibited the process of myocyte apoptosis in rats with CCMI.

In conclusion, the results of this study provide strong evidence to support the role of Tuina in repairing chronic cervical muscle injuries. In the present study, it was first suggested that Tuina could promote the proliferation and differentiation of satellite cells to promote the repair of chronic cervical muscle injuries. Also, the assumption that the proliferation and differentiation process might be accomplished by the action of related transcription factors was explored. At the same time, the inhibition of muscle cell apoptosis by Tuina in the CCMI model rats was observed. This study also has shortcomings. First, the CCMI model is not a widely used model for cervical spondylosis. This is the first time we explored the pathogenesis of cervical spondylosis from the perspective of muscle injury. Second, the mechanism by which Tuina promotes CCMI repair is very complex. Therefore, in follow-up, we would like to further explore the potential pathways and delve into the mechanism of myo-satellite cell's role in the repair of CCMI by Tuina.

Conclusion

Our data suggest that Tuina can promote the healing of chronic cervical muscle injuries. Although the intrinsic mechanisms need to be further investigated, our study proposes that Tuina can promote the proliferation and differentiation of muscle satellite cells to repair cervical muscle injury by regulating the expression of Pax7, MyoD, and IGF-1, as well as inhibiting the expression of Bax and promoting the expression of Bcl-2 to ameliorate the apoptosis of cervical myocytes in CCMI model rats. This study provides some theoretical foundation for the practice of Tuina in the repair of chronic cervical muscle injuries.

Institutional Review Board Statement

The study was executed strictly in accordance with the Declaration of Helsinki, and received the approval of the Animal Ethics Committee of the Fujian University of Traditional Chinese Medicine (protocol code, SYXK(min)2020-0007).

Data Sharing Statement

Upon proper request, data encouraging the results from this study are obtainable from the corresponding author.

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Disclosure

The author(s) report no conflicts of interest in this work.

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