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Original Article

Therapeutic effect of electroacupuncture on rats with neuropathic pain

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Abstract: Objective: To study the therapeutic effect and possible mechanism of electroacupuncture on rats with neuropathic pain (NP). Methods: A total of 30 Sprague Dawley rats were randomized into three groups, group A (n=10), group B (n=10), and group C (n=10). In group A, a chronic constriction injury (CCI) model of NP was induced by loosely making four ligations at the mid-thigh level of the right sciatic nerve of rats with 4-0 chromic catgut, and electroacupuncture was used at day 1, day 4 and day 7 after surgery respectively as the treatment of NP. In group B, the CCI model was also induced and no treatment was given. In group C, the same surgery was made without tying the sciatic nerve. The thermal and mechanical pain thresholds of each rat before surgery and from day 1 to 7 after surgery were recorded and compared. The rats were sacrificed on day 8 after surgery and underwent perfusion-fixation for the removal of spinal cord. Western blot was used to detect the expression of CX3CR1 protein in the spinal cord. Enzyme-linked immunosorbent assay was used to measure the levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin 6 (IL-6) in the spinal cord. Results: In group A, mechanical withdrawal thresholds (MWT) and paw withdrawal latency (PWL) of right hind paw on day 1-3 after surgery were significantly lower than those before surgery ($P<0.05$); MWT and PWL were significantly higher on day 4-7 after surgery than those on day 3 after surgery ($P<0.05$), but were still lower than the preoperative levels ($P<0.05$). In group B, MWT and PWL of right hind paw on day 1-7 after surgery were significantly lower than those before surgery ($P<0.05$). There were no significant differences in MWT and PWL of right hind paw between group A and group B on day 1-3 after surgery ($P>0.05$). MWT and PWL in group A were significantly higher than those in group B on day 4-7 after surgery ($P<0.05$). Compared with the preoperative levels, there were no significant differences in MWT and PWL in group C after surgery ($P>0.05$). Compared with those in group C, MWT and PWL of right hind paw in group A and group B declined significantly after surgery (all $P<0.05$); In group A and group B, no significant differences were found in MWT and PWL of left hind paw before and after surgery (all $P>0.05$). The expression of CX3CR1 protein was significantly increased in group A and group B compared with that in group C (all $P<0.05$), and the expression of CX3CR1 protein in group A was significantly lower than that in group B ($P<0.05$). The levels of TNF-α, IL-1β and IL-6 in group A and group B were significantly higher compared with those in group C ($P<0.05$). The levels of TNF-α, IL-1β and IL-6 in group A were significantly lower than those in group B (all $P<0.05$). Conclusion: The thermal and mechanical pain thresholds of CCI rats decrease significantly. Electroacupuncture can significantly relieve pain and increase pain threshold. The development of NP may be associated with the increase of the expression of CX3CR1 protein and the levels of inflammatory factors in the spinal cord, including TNF-α, IL-1β and IL-6. The mechanism of electroacupuncture as a treatment for NP may be related to the reduction of the expression of CX3CR1 protein and the levels of inflammatory factors.

Keywords: Acupuncture, neuropathic pain, CX3CRI protein

Introduction

Pain is an early warning signal when an organ or tissue is injured [1]. The development of disease is often accompanied by pain, and patients suffer greatly from the chronic pain, physically and mentally. As a result, their quality of life has been significantly degraded. Neuropathic pain (NP) is a type of chronic pain caused by a lesion or disease of the somatosensory system, and may be derived from the direct damage or disorder of the peripheral and central nervous system [2, 3]. Its complex pathogenesis remains unclear. A previous study

suggested that the pathogenesis of NP involved the central and peripheral nervous system [4]. In recent years, central sensitization has been studied in detail. When nerve tissue lesions occur, glial cells are activated, and inflammatory factors such as IL-1 and IL-6 are produced, which act on postsynaptic neurons, causing central sensitization and eventually leading to NP [5, 6]. According to a previous study, activated microglia played a major role in the development of NP in the early stage of nerve lesions, and as it developed, astrocytes were also activated and participated in the development process [7]. Many studies have demonstrated that the expression levels of microglia-specific markers in the spinal cord increased when NP occurred, including Toll-like receptors, P2X4 receptors, CCR2, CX3CR1 [8, 9]. Therefore, cytokines and microglia have become the potential targets for therapeutic intervention in the treatment of NP.

Because of its complex pathogenesis, NP is intractable, and drugs commonly used in its treatment including tricyclic antidepressants, antiepileptics, opioids and nonsteroidal anti-inflammatory drugs [10, 11]. But drugs are prone to cause adverse reactions. Moreover, the use of opioids is likely to cause addiction especially when the pain is intense. Therefore, it is necessary to find other treatments [12]. Electroacupuncture is the combination of the electrical nerve stimulation and the acupuncture of traditional Chinese medicine. It refers to the application of micro-current, similar to human bioelectricity, on filiform needle, which delivers sustained, stable, continuous and controllable pulses to acupoints, with electrophysiological effects [13]. A previous study showed that electroacupuncture had a therapeutic effect on rats with spinal cord injury, and it could promote the functional recovery of spinal cord, reduce pain and was instrumental in the treatment of acute spinal cord injury [14]. The spinal cord is an important gateway for transmitting pain signals to the brain, and the spinal dorsal horn can accept, transmit, and process the nociceptive information [10]. We therefore suspected that electroacupuncture could treat NP. At present, there has been no study that clearly demonstrated the therapeutic effect of electroacupuncture on NP. Therefore, this study mainly focused on the treatment of NP with electroacupuncture, and has also discussed its possible mechanism.

Materials and methods

Subjects

This study had been consented by the Animal Ethics Committee of Gansu Provincial Hospital. A total of 30 male Sprague Dawley rats (180-220 g body weight) were selected for the study. Under a lighting regime with 12 hours of light and 12 hours of dark per day, they were maintained in a quiet, sterile animal house where the room temperature was kept at 22.0±0.5°C, with free access to food and water [15]. The rats should be housed in this environment for at least 3 days before surgery. These rats were randomized into three groups, group A (n=10), group B (n=10), and group C (n=10). In group A, a chronic constriction injury (CCI) model of NP was induced and electroacupuncture was used at day 1, day 4 and day 7 after surgery respectively as a treatment of NP. In group B, the CCI model was also induced and no treatment was given. In group C, the same surgery was conducted without tying the sciatic nerve.

Establishment of rat models with CCI

After intraperitoneal injection (4.0 mL/kg) of 10% chloral hydrate (Sinopharm Chemical Reagent Co., Ltd.), the rat's right hind limb was prepared for ligation. After shaving and sterilization, an incision was made at its right hind limb, and the muscles were separated by blunt dissection to completely expose the sciatic nerve. Then, four ligatures (4-0 chromic catgut) were loosely tied at the mid-thigh level of the sciatic nerve to induce a CCI model of NP (each loop should be barely tight enough to slide on the nerve). Penicillin powder was administered postoperatively to prevent infection [16]. The thermal and mechanical pain thresholds were measured postoperatively to observe whether the model was successfully induced. If the model was not induced successfully, this rat should be replaced by another new rat to prepare the model to ensure that there were 10 rats per group.

Establishment of rat models without CCI

After intraperitoneal injection (4.0 mL/kg) of 10% chloral hydrate, the rat's right hind limb was prepared for ligation. After shaving and sterilization, an incision was made at the right hind limb, and the muscles were separated by blunt dissection to completely expose the sciatic nerve. Then the incision was sutured without the constriction of nerve. Penicillin powder

was administered postoperatively to prevent infection.

Treatment

In group A, electroacupuncture was used at day 1, day 4 and day 7 after surgery respectively as a treatment of NP. Before receiving treatment, the rat was fixed on a wooden frame, with its head and limbs free to move. After laying for 20 minutes, the acupuncture needles were inserted into the acupoints, "Weizhong Acupoint" and "Huantiao Acupoint", at the affected side of the rat [14]. The multi-function electroacupuncture treatment device was applied to give 2 Hz/100 Hz pulses for 30 minutes, the intensity of which should be strong enough to cause the muscles of the right hind limb shaking slightly, without causing the rat shrieking (≤ 1 mA) [14, 17].

Spinal cord removal

All rats were sacrificed on day 8 after surgery and underwent perfusion-fixation for the removal of spinal cord. After intraperitoneal injection (4.0 mL/kg) of 10% chloral hydrate, the rat was properly fixed, shaved and sterilized. A cut was made parallel to ribs and perpendicular to the xiphoid so as to expose the heart. After the heart was exposed, 200 mL of 4°C normal saline were perfused into the aorta. The clearing of the liver and the clear liquid flowing out from the auricula dextra were indicators of a good perfusion.

After the rat was sacrificed, its back side was exposed and the skin was incised in the median line. Muscles at both sides of the thoracolumbar spine were bluntly separated to remove the spine. The spine was then placed into the ice-cold normal saline. A syringe was used to eject the spinal cord from the caudal segment of the spine. The spinal pia mater was then removed and the spinal cord was frozen at-80°C.

Measurements

Measurement of mechanical pain thresholds: Von Frey test was conducted at the same time every day. Before measurement, rats were placed individually in small cages with a mesh floor for at least 30 minutes. Then both hind paws of a rat were assessed for mechanical withdrawal thresholds (MWT) using Von Frey filaments. Von Frey filaments of different forces were used to stimulate the center of the underside of paw. Filaments were presented in order of increasing force until a paw withdrawal was detected. MWT was defined as the least strength causing quick paw withdrawal and paw licking. MWT was measured for five times at 10-second intervals to obtain the mean value. The duration of each stimulation should last no more than 5 seconds. The measurement was carried out with a set of Von Frey filaments that spanned a range of forces from 0.4 g to 15.0 g (0.4 g, 0.6 g, 1.0 g, 1.4 g, 2.0 g, 4.0 g, 6.0 g, 8.0 g, 10.0 g, 15.0 g). If MWT was still higher than 4.0 g on day 3 after surgery, the establishment of the model was judged as failed. The lower the mechanical pain threshold, the more sensitive the rat was towards mechanical pain stimulation, indicating that the nerve was injured [18].

Measurement of thermal pain thresholds: The measurement of thermal pain thresholds should be conducted one hour after the measurement of MWT. Both hind paws of a rat were assessed. The unrestrained rat was placed individually in small enclosures with a glass floor. A pain threshold detector was used to shine lights on both of the rat's hind paws to measure the paw withdrawal latency (PWL). PWL was measured for three times at 10-minute intervals to obtain the mean value. To prevent the radiant heat from damaging the rat's tissue, the cut-off time of the measurement should be set at 20 seconds. If the pain threshold of the affected limb was significantly lower than the preoperative pain threshold and the threshold of the unaffected limbs, the establishment of the model was judged as successful. The lower the thermal pain threshold, the more sensitive the rat was towards thermal pain stimulation, indicating that the nerve was injured [19].

Measurement of cytokines in spinal cord: Enzyme-linked immunosorbent assay (ELISA) was conducted to determine the levels of TNF-α, IL-6 and IL-1β in the spinal cord. ELISA reagent kit was bought from Shanghai Xin Yu Biotech Co., Ltd. The measurement of cytokines in spinal cord was carried out according to the manufacturer's instructions.

Measurement of expression levels of CX3CR1 protein in spinal cord: Western blot (WB) was used to determine the expression levels of CX3CR1 protein in the spinal cord. The spinal cord was weighed before being transferred into the EP tube. The lysis buffer was then added into the tube and the mixture was homoge-

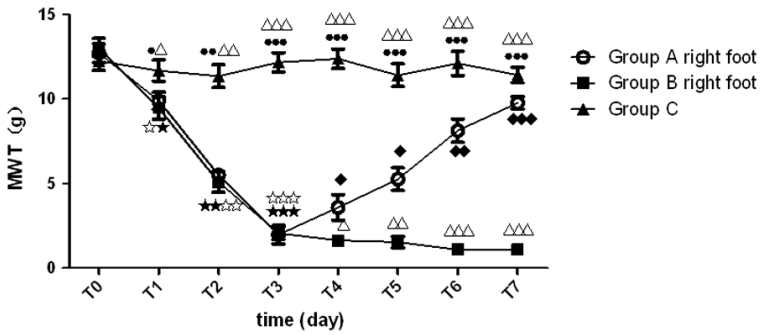

Figure 1. Comparisons of MWT among three groups. The x-ordinate represents MWT in rats. The y-ordinate represents days of surgery, of which T0 represents before surgery, and T1-T7 represents day 1-7 after surgery respectively. Compared with group A at T0, ★P<0.05, ★★P<0.01, and ★★★P<0.001. Compared with group A at T3, ◆P<0.05, ◆◆P<0.01, and ◆◆◆P<0.001. Compared with group A, △P<0.05, △△P<0.01, and △△△P<0.001. Compared with group B at T0, ☆P<0.05, ☆☆P<0.01, and ☆☆☆P<0.001. Compared with group B, •P<0.05, ••P<0.01, and •••P<0.001. MWT, mechanical withdrawal thresholds.

nized. Then the lysate was centrifuged. The supernatant was the protein, which was transferred to another EP tube. All procedures were completed in the ice bath. CX3CR1 antibody (1.0 mg/mL, 1:1,000) was then added into the EP tube according to the procedures of WB. CX3CR1 antibody was bought from Abcam (UK).

Statistical analysis

SPSS 19.0 software was used for data analysis. Graphpad Prism 5 was used for graphing. Measurement data were expressed as mean ± standard deviation ($\bar{x}$ ± sd). Comparisons between the two groups were based on independent t-test or repeated measures analysis of variance (ANOVA). Bonferroni posttest was used to compare the differences of measurement data between two groups at different time points. Comparisons among the three groups were based on ANOVA. The target bands from WB were analyzed using Image J software and the results were analyzed with one-way ANOVA. P<0.05 indicated statistical significance.

Results

General information

All included rats were free of infection after surgery, and their wound completely healed 7 days after surgery. All rats were well developed during the perioperative period, with normal food intake and no significant weight gain or loss. The CCI model rats exhibited significant deformity at the right hind paw 1 day after surgery (toes held together and plantar-flexed and paw everted), and presented symptoms like occasionally licking of hind paw, fear of touching ground. There was neither autotomy behavior nor abnormality that was observed in the left hind paw. In group C, no abnormality was identified in both hind paws.

Comparisons of MWT of right hind paw among three groups

In group A, MWT on day 1-3 after surgery were significantly lower than those before surgery (P=0.042, F=15.345; P=0.003, F=20.345; P<0.001, F=24.345); MWT on day 4-7 after surgery were significantly higher than those on day 3 after surgery (P=0.038, F=14.392; P=0.024, F=16.955; P=0.002, F=21.457; P<0.001, F=26.467), but were still lower than the preoperative levels (P=0.028, F=16.245). In group B, MWT on day 1-7 after surgery were significantly lower than those before surgery (P<0.001, F=27.345). There were no significant differences in MWT between group A and group B on day 1-3 after surgery (P=0.754, F=3.284). MWT in group A were significantly higher than those in group B on day 4-7 after surgery (P<0.001, F=26.875). Compared with the preoperative levels, there were no significant differences in MWT in group C after surgery (P=3.675, F=5.136). Compared with those in group C, MWT of right hind paw in group A and group B declined significantly after surgery (P<0.001, F=27.376; P<0.001, F=25.975). See **Figure 1**.

Comparisons of PWL of right hind paw among three groups

In group A, PWL on day 1-3 after surgery were significantly lower than those before surgery (P=0.045, F=13.367; P=0.004, F=19.356; P<0.001, F=25.732); PWL were significantly higher on day 4-7 after surgery than those on day 3 after surgery (P=0.026, F=17.342; P=0.002, F=22.012; P=0.002, F=20.421; P<0.001, F=26.357), but were still lower than the preoperative levels (P=0.019, F=18.267). In group B,

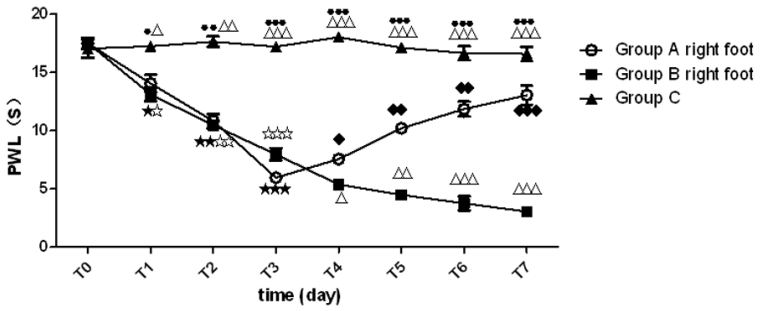

Figure 2. Comparisons of PWL among three groups. The x-ordinate represents PWL in rats. The y-ordinate represents days of surgery, of which T0 represents before surgery, and T1-T7 represents day 1-7 after surgery respectively. Compared with group A at T0, ★P<0.05, ★★P<0.01, and ★★★P<0.001. Compared with group A at T3, ◆P<0.05, ◆◆P<0.01, and ◆◆◆P<0.001. Compared with group A, △P<0.05, △△P<0.01, and △△△P<0.001. Compared with group B at T0, ☆P<0.05, ☆☆P<0.01, and ☆☆☆P<0.001. Compared with group B, •P<0.05, ••P<0.01, and •••P<0.001. PWL, paw withdrawal latency.

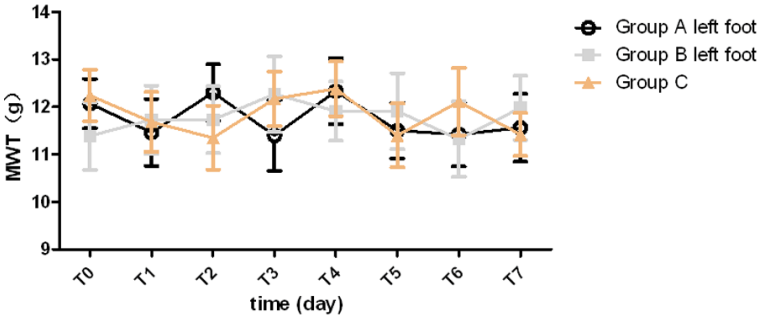

Figure 3. Comparisons of MWT of left hind paw among three groups. The x-ordinate represents MWT in rats. The y-ordinate represents days of surgery, of which T0 represents before surgery, and T1-T7 represents day 1-7 after surgery respectively. MWT, mechanical withdrawal thresholds.

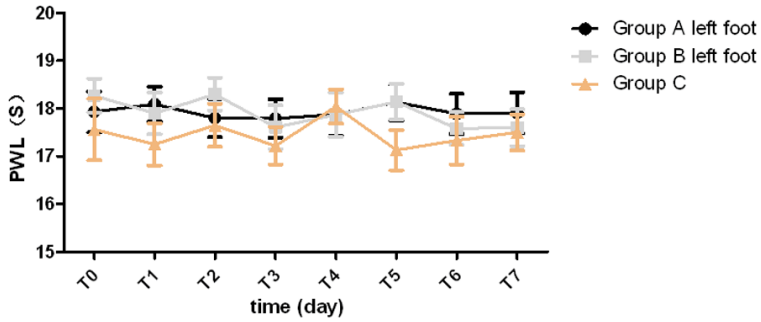

Figure 4. Comparisons of PWL of left hind paw among three groups. The x-ordinate represents PWL in rats. The y-ordinate represents days of surgery, of which T0 represents before surgery, and T1-T7 represents day 1-7 after surgery respectively. PWL, paw withdrawal latency.

PWL on day 1-7 after surgery were significantly lower than that before surgery (P<0.001, F=27.335). There were no significant differences in PWL between group A and group B on day 1-3 after surgery (P=0.568, F=4.675). PWL in group A were significantly higher than those in group B on day 4-7 after surgery (P<0.001, F=25.686). Compared with the preoperative levels, there were no significant differences in PWL in group C after surgery (P=5.468, F=3.579). Compared with those in group C, PWL of right hind paw in group A and group B declined significantly after surgery (P<0.001, F=5.688; P<0.001, F=27.478). See **Figure 2**.

Comparisons of MWT of left hind paw among three groups

No significant difference was observed in the MWT of left hind paw in group A and group B compared with that in group C (P=0.687, F=3.865, P=0.478, F=6.553). See **Figure 3**.

Comparisons of PWL of left hind paw among three groups

No significant difference was observed in the PWL of left hind paw in group A and group B compared with that in group C (P=0.466, F=6.356, P=0.723, F=3.854). See **Figure 4**.

Comparisons of levels of cytokines in spinal cord among three groups

The levels of TNF-α, IL-1β and IL-6 were significantly increased in group A and group B compared with that in group C (all P<0.05), and the levels of TNF-α, IL-1β and IL-6 in group A were significantly lower than that in group B (all P<0.05). See **Table 1**.

Comparisons of expression levels of CX3CR1 protein in spinal cord among three groups

The expression levels of CX3CR1 were significantly increased in group A and group B compared with that in group C (P=0.035, F=5.674;

Table 1. Comparisons of levels of cytokines in spinal cord among three groups

Group	Group A	Group B	Group C	F	P
TNF-α (pg/mL)	19.41±5.72★,☆☆	27.24±5.19★★★	14.73±3.85	16.646	0.004
IL-1β (pg/mL)	24.56±6.46★★,☆☆☆	35.46±5.94★★★	15.42±4.28	25.864	<0.001
IL-6 (pg/mL)	19.45±5.63★,☆☆☆	32.72±5.17★★★	11.57±4.46	19.578	0.002

Note: TNF-α: tumor necrosis factor alpha; IL-1β: interleukin 1 beta; IL-6: interleukin 6. Compared with group C, ★P<0.05, ★★P<0.01, and ★★★P<0.001. Compared with group B, ☆☆P<0.01, ☆☆☆P<0.001.

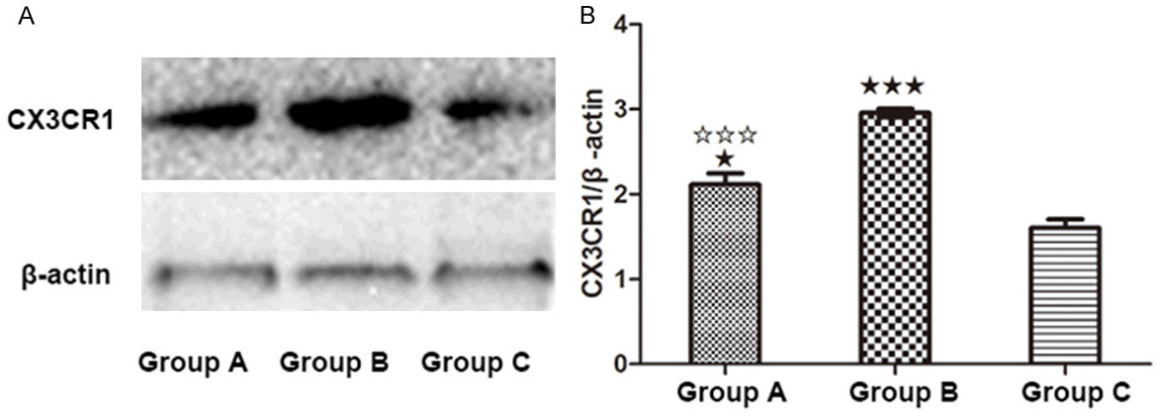

Figure 5. Comparisons of expression levels of CX3CR1 protein in spinal cord among three groups. A. Comparisons of CX3CR1 protein in spinal cord among three groups; B. Comparisons of gray values of CX3CR1 protein in spinal cord among three groups. TNF-α: tumor necrosis factor alpha; IL-1β: interleukin 1 beta; IL-6: interleukin 6. Compared with group C, ★P<0.05, and ★★★P<0.001. Compared with group B, ☆☆☆P<0.001.

P<0.001, F=26.533), and the expression levels of CX3CR1 in group A were significantly lower than that in group B (P<0.001, F=25.257). See **Figure 5**.

Discussion

In recent years, a growing number of studies have focused on NP, and models of NP are also increasing, three of which commonly used are chronic constriction injury of sciatic nerve (CCI) model, spinal nerve ligation (SNL) model and spared nerve nerve injury (SNI) model. CCI and SNI models are relatively simple to prepare, with low risks of death, disability, infection and other complications in rats. The CCI model is sensitive to thermal radiation as its thermal pain threshold is significantly decreased. This may be attributed to the fact that the CCI model is formed by the ligation of gut suture, which causes aseptic inflammation on the sciatic nerve. That produces not only NP in rats, but also inflammatory pain. This is also the case with many patients as they suffer not only from NP, but also from inflammatory pain due to inflammation of the nerves. Therefore, the CCI model adopted in this study would closer to the clinical scenario.

The pathogenesis of NP is complex and may be caused by the activation of microglia producing inflammatory cytokines. CX3CR1 is a chemokine receptor expressed primarily in spinal microglia. CX3CR1-expressing microglia are macrophages of the central nervous system and can be distributed throughout the central nervous system [20, 21]. A previous study showed that the SNL model in rats could cause the activation of spinal microglia, with the expression of surface markers increased, which indicated that the increase in the expression of CX3CR1 represented the activation and proliferation of microglia [22]. The activation of microglia can also increase the release of inflammatory cytokines. Cytokines can be divided into two types, anti-inflammatory cytokines and proinflammatory cytokines. According to a previous study, proinflammatory cytokines played an important role in the development of pain [5]. Therefore, three of the most studied proinflammatory cytokines related to pain were included in the study. TNF-α is an inflammatory

mediator in the peripheral and central nervous system that can trigger the inflammatory cascade [23]. It has been demonstrated that intrathecal injection of TNF-α in rats could increase pain and lead to mechanical and thermal allodynia [24]. IL-1β can release proteases such as calmodulin and MMPs, increasing the excitability of neurons to cause pain hypersensitivity, and resulting in a cascade reaction by inducing the release of other inflammatory factors [25]. Normally, the spinal cord has low levels of IL-1β, but its expression would significantly increase following peripheral nerve injury [26]. A previous study demonstrated that IL-1β functioned in two ways in NP, increasing the excitability of neurons indirectly by activating the signaling pathways of immune cells and directly by acting on neurons to increase sodium or calcium ions [26]. It has been proved that in NP model rats injected with IL-1 receptor antagonist, the pain response caused by nerve injury was significantly reduced [27]. The mechanism of IL-6 in the development of NP remains unclear. Studies have shown that IL-6 could cause pain hypersensitivity through JAK/STAT3 signaling pathway, but in some cases, it could produce anti-inflammatory effects, regulate the activity of microglia, and induce the expression of the CX3CR1 [23, 28, 29]. Our results showed that the levels of CX3CR1, TNF-α, IL-1β and IL-6 in the spinal cord of rats after electroacupuncture were significantly lower than those in the spinal cord of untreated rats, and their pain thresholds also decreased, indicating that CX3CR1, TNF-α, IL-1β and IL-6 in the spinal cord were indeed related to the development and treatment of NP. The mechanism of electroacupuncture in the treatment of NP may be associated with the reduction of activated microglia by lowering the expression of CX3CR1 in the spinal cord and the decreased levels of TNF-α, IL-1β and IL-6. However, the expression of CX3CR1 in group A was still higher than that in group C. This means that while electroacupuncture can treat NP and ease pain by reducing the levels of CX3CR1, TNF-α, IL-1β and IL-6, it cannot restore their levels. This study also revealed that there was no significant adverse reaction in rats after electroacupuncture.

Our results showed that the CCI model was a sound and ideal model of NP. On day 1 after surgery, mechanical and thermal pain thresholds were reduced, and pain thresholds declined in a steady manner 5 days after surgery. On day 1-3 after surgery, pain thresholds in group A were still decreasing, and there was no significant difference between group A and group B in pain thresholds, indicating that it took a certain period of time and more than one time of electroacupuncture to relieve the pain. On day 4-7 after surgery, pain thresholds in group A increased significantly, and was significantly higher than that in group B and group C. This demonstrated that electroacupuncture had significant effect on pain relief and could effectively treat NP. However, pain thresholds could not be restored to the preoperative levels, and the deformity of paw caused by the ligation of sciatic nerve did not improved. This suggested that the nerve injury was irreversible, and electroacupuncture could only relieve the pain without reversing the damage. According to the results, there was no significant change in pain thresholds in group C, indicating that NP was caused directly by nerve damage instead of skin and muscle damages. The model in this study was based on the right hind paw. Compared with that of right hind paw, there was no significant change in the MWT and PWL of left hind paw, which showed that the nerve injury did not affect the contralateral nerve through nerve or spinal cord conduction, and did not cause mirror-image pain. This indicated that the nerve injury was unilateral and nerve injury alone could not cause bilateral pain.

This study revealed that electroacupuncture could treat rats with NP with no significant adverse reaction. But given to the great differences between human and rats, the model prepared using rats cannot completely simulate the complexity of human diseases. Moreover, a human disease is often complicated by a variety of diseases, which cannot be simulated by the rat model. Therefore, more experiments are required before applying the treatment to the human body. Due to time limit, there are still many shortcomings in this study. This study only focused on the first 7 days after surgery, while NP symptoms can last long enough for even more than 10 years. Therefore, this study only proved the effectiveness of electroacupuncture in the short term without giving results of its long-term effect, which warrants more experiments.

In conclusion, the CCI model is an ideal NP model. In a CCI model, thermal and mechanical

pain thresholds significantly decline. Electroacupuncture can significantly relieve pain and increase pain thresholds. The development of NP may be associated with the increased expression of CX3CR1 protein in the spinal cord, which activates microglia and upregulates the expression of inflammatory cytokines including TNF-α, IL-1β and IL-6. The mechanism of electroacupuncture in the treatment of rats with NP may be related to the reduction of CX3CR1 protein expression and levels of inflammatory cytokines.

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Disclosure of conflict of interest

None.

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