Journal of Traditional Chinese Medical Sciences (2015) 2, 45–51

HOSTED BY

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/jtcms

Effect of electroacupuncture at distal–proximal acupoint combinations on spinal interleukin-1 beta in a rat model of neuropathic pain

Huili Jiang [a], Xue Yu [b], Xiujun Ren [a,*], Tingyu Fang [c], Ya Tu [a]

[a] School of Acupuncture-Moxibustion and Tuina, Beijing University of Chinese Medicine, Beijing 100029, China
[b] School of Basic Medical Science, Beijing University of Chinese Medicine, Beijing 100029, China
[c] Center for Research and Communication of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 100029, China

Received 15 October 2014; accepted 14 December 2014

Available online 15 December 2015

KEYWORDS
Electroacupuncture (EA);
Interleukin-1 beta (IL-1β);
Neuropathic pain;
Spinal cord;
Rats

Abstract *Objective:* Pain from herniated disc is a common type of neuropathic pain. This study investigated whether electroacupuncture (EA) stimulation at distal–proximal combinations of acupoints in the rat model of neuropathic pain modulates spinal interleukin-1 beta (IL-1β) to induce acupuncture analgesia and possibly serve as a pain-relief modality for herniated disc.

Methods: A rat model of neuropathic pain was established. Rats were randomly divided into normal, model, sham, EA 1, EA 2, and EA 3 groups. EA 1 rats were needled at bilateral Ex-B2, BL25, BL40, and BL60 acupoints. EA 2 rats were needled at bilateral BL40 and BL60. EA 3 rats were needled at bilateral L5 Ex-B2 and BL25. EA stimulation was administered once daily over 7 days. Mechanical withdrawal threshold from noxious mechanical stimulation was measured 1 day preoperatively and at 3, 5, and 7 days postoperatively. After 7 days of intervention, enzyme-linked immunosorbent assay (ELISA) was used to quantify IL-1β in the spinal cord.

Results: Mechanical withdrawal threshold of rats in the model group decreased at 3 days postoperatively when compared with the normal group ($P < 0.01$), lasting 7 days postoperatively. Mechanical withdrawal thresholds in the EA 1, EA 2, and EA 3 groups were elevated over the model group ($P < 0.05$; $P < 0.01$). No obvious differences were found between EA 1, EA 2,

* Corresponding author. School of Acupuncture-Moxibustion and Tuina, Beijing University of Chinese Medicine, Beijing 100029, China. Tel.: +86 10 64286271; fax: +86 01 64287525.
E-mail address: rxiujun@163.com (X. Ren).
Peer review under responsibility of Beijing University of Chinese Medicine.

http://dx.doi.org/10.1016/j.jtcms.2015.09.002

and EA 3 groups. ELISA demonstrated an increase in IL-1β in the spinal cord of rats in the model group compared with the normal group ($P < 0.01$). EA treatment attenuated the increase in spinal IL-1β in the model group. Expression of spinal IL-1β was significantly lower in EA 1, EA 2, and EA 3 groups.

Conclusion: EA at distal + proximal acupoints, distal points, as well as proximal points attenuated upregulation of spinal IL-1β, alleviated the extent of neuropathic pain hypersensitivity, and promoted mechanical withdrawal threshold, resulting in EA analgesia.

Introduction

Pain caused by herniated disc, or herniated nucleus pulposus (HNP), can be severe and may lead to irreversible nerve damage and disability. HNP is characterized by neuropathic pain hypersensitivity, including hyperalgesia and allodynia.[1–3] Lumbar disc herniation is a common disorder, causing low back pain, sciatica, and numbness.[4,5]

The pathologic mechanisms of neuropathic pain induced by HNP have been investigated showing that proinflammatory substances might be involved in the pain process.[6,7] Local production of these proinflammatory substances appear to be responsible for many of the pathologic and clinical manifestations of HNP pain. Studies have shown that inflammatory reactions can demyelinate the nerve root and lead to neural hypersensitivity and neuropathic pain.[8,9] Interleukin-1 beta (IL-1β), a proinflammatory cytokine, has been implicated in immune and inflammatory reactions,[10] with research suggesting that the level of IL-1β in the spinal cord is upregulated during the pain process and involved in pain hypersensitivity including hyperalgesia and allodynia.[11,12] Furthermore, intrathecal administration of IL-1β contributes to obvious hyperalgesic effect or mechanical allodynia.[13,14] Additional studies have indicated that activation of spinal glial cells and IL-1β lead to the maintenance of neuropathic pain hypersensitivity, that is, chronic pain.[15,16]

Pain caused by HNP is generally managed by medications, physical therapy, or surgery. There is insufficient evidence that one modality is superior to others.[17] Promoting axonal regeneration has been suggested for altering pain-related behavior from peripheral nerve neuropathy due to trauma.[18]

Acupuncture therapy has been researched for its ability to treat pain.[19–22] In the rat model, acupuncture appears to alleviate chronic neuropathic pain and inflammatory pain.[23,24] Efficacy of acupuncture is directly related to selection of appropriate acupoints, which is known as acupoint prescription, or acupoint combination.[25] To treat pain, 3 acupoint combinations are typically selected: proximal points near the affected area, distal points on the channels connected to the affected area, and distal points based on traditional Chinese syndrome differentiation. Electroacupuncture (EA) is a technique that integrates acupuncture needling and electrical stimulation. Studies have demonstrated that electroacupuncture applied after surgical trauma or neuropathic pain appears to have an immunomodulatory effect.[26–29] In the rat model of neuropathic pain, EA has been found to relieve hyperalgesia induced by traumatic nerve injury.[30]

As mentioned, IL-1β, the principal proinflammatory cytokine, is considered to be closely related to pain and inflammation. Research has shown that EA appears to attenuate expression of IL-1β in the nerve root region.[31] However, little is known about the effect acupoint combinations have on spinal IL-1β in acupuncture analgesia. Therefore, the present study investigated whether EA at different acupoint combinations modulate the expression level of IL-1β in the rat model of neuropathic pain, thus promoting mechanical withdrawal thresholds.

Materials and methods

Experimental animals

A total of 56 male Sprague-Dawley rats (300 ± 20 g), 5 weeks old, were obtained from the Animal Center of the Academy of Medical Sciences of the Chinese People's Liberation Army (Beijing, China). The rats were housed in a controlled environment of 23°C–26°C, 50% ± 10% humidity, and 12-h light/dark cycle. The animals had access *ad libitum* to standard rat chow and tap water. All experimental procedures were in full observance of the International Association for the Study of Pain Guidelines for the Use of Animals in Research, and approved by the Institute of Animal Care Committee of the Beijing University of Chinese Medicine.

Induction of neuropathic pain

A neuropathic pain model induced by herniated nucleus pulposus in the rats was established in this study as described previously.[32,33] Rats were anesthetized with 10% chloral hydrate (0.35 mL/100 g, i.p.). The tail was resected about 1 cm distal to the anus to harvest the nucleus pulposus followed by suturing of the incisions. Partial hemilaminectomy was performed to expose the left L5 nerve roots. The tail was amputated and the nucleus pulposus (weighing 4 mg) was harvested from the coccygeal intervertebral discs of the amputated tail and relocated to the juncture of the dural sac and the L5 nerve root. Mechanical compression was not made by autologous nucleus pulposus at the time of surgery. In the sham surgery group, the same

procedure was performed without relocation of the nucleus pulposus. The operative field was carefully washed with 0.9% stroke-physiological saline solution. The muscle and skin layers were closed with 4-0 nylon sutures.

Animal groups

Two rats were excluded from the study because of surgical site infection at 3 days postoperatively. Thus, a total of 54 rats were enrolled in the final analysis. Rats were randomly assigned to 6 groups using a random number table, with 9 rats in each group: control group, sham group, model group, and 3 electroacupuncture (EA) groups (Table 1). The model, EA 1, EA 2, and EA 3 groups were neuropathic pain model animals with nucleus pulposus relocation. EA 1, EA 2, and EA 3 groups received EA treatment once daily, for 7 days.

Electroacupuncture stimulation

EA commenced on Day 1 postoperatively, 20 minutes per session, 1 session daily for 7 days. After disinfection of the acupoint sites with 75% alcohol, acupuncture needles (0.3 mm in diameter and 25 mm long; Suzhou Medical Appliance Factory, Jiangsu, China) were inserted vertically into the following acupoints: Ex-B2 and BL25 were each needled to a depth of 5 mm, and BL40 and BL60 to a depth of 3 mm.[34] EA 1 rats were needled at bilateral Ex-B2, BL25, BL40, and BL60. EA 2 rats were needled at bilateral BL40 and BL60. EA 3 rats were needled at bilateral Ex-B2 and BL25 (Fig. 1). Bilateral acupoint needles were then connected to a HANS Acupoint Nerve Stimulator (model LH202H, Beijing, China). The frequency was adjusted to 2 Hz/100 Hz, while the intensity was automatically increased in a stepwise manner at 1 mA—2 mA—3 mA. EA stimuli were detected by slight trembling of the legs of the rats.

Observation of rat behavior

All observations were conducted preoperatively on the morning of the surgery and at 3, 5, and 7 days post-operatively. All observations were performed by the same examiner who was blinded to the group allocations. Motor function of rats was detected. Rats were placed on the floor in an open field and gait patterns and behavior of rats were observed. Abnormal behaviors, such as lameness, poor appetite, licking the hind paws were recorded.

Mechanical withdrawal threshold

The Bennett and Xie scale was applied to assess neuropathic pain following nerve damage in the animal

Table 1 Summary of electroacupuncture intervention in rats with and without neuropathic pain induction.

	No electroacupuncture			Electroacupuncture		
	Control (n = 9)	Sham (n = 9)	Model (n = 9)	EA 1 (n = 9)	EA 2 (n = 9)	EA 3 (n = 9)
Treatment parameters	Untreated	Sham surgery without NP	NP	NP; EA at distal acupoints plus proximal acupoints	NP; EA at distal acupoints	NP; EA at proximal acupoints
Acupoints	—	—	—	Bilateral Ex-B2, BL25, BL40, BL60	Bilateral BL40, BL60	Bilateral Ex-B2, BL25

Abbreviations: EA, electroacupuncture; NP, neuropathic pain induction.

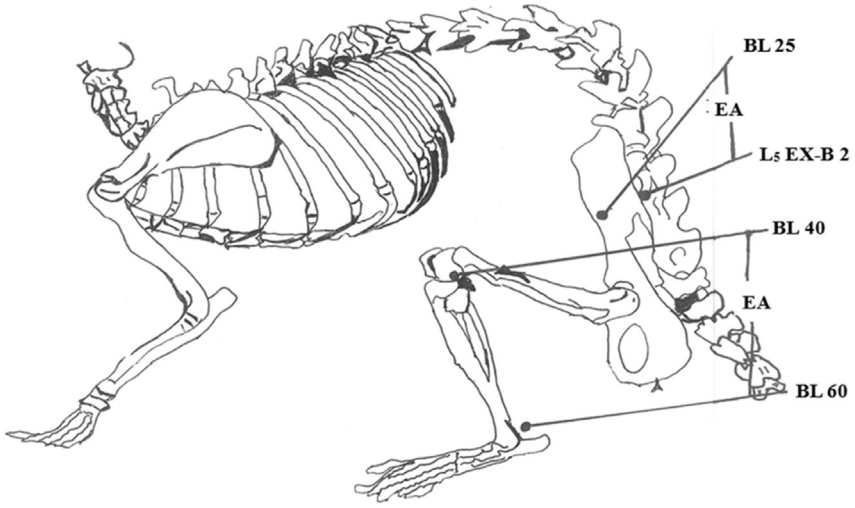

Figure 1 Location of acupoints on rats for EA stimulation.

model.[33,35] Mechanical withdrawal thresholds were detected using the Dynamic Plantar Aesthesiometer (model 37400; Ugo Basile, Milan, Italy). Before each test, all rats were placed in a glass cage with a nest floor and habituated to the environment for 20 minutes. After animals settled down, a metal needle was focused on the dorsal surface of the hind paw between the phalanx and metatarsal bones. Mechanical withdrawal thresholds were expressed in grams. Maximum stimulation was set at 50 g to avoid injuring rats. Three trials, 5 minutes apart, were conducted on each hind paw. Resulting data from the 3 trials were averaged. Mechanical stimulation was measured on Day 1 preoperatively and on Day 3, 5, and 7 postoperatively. The percentage difference in mechanical withdrawal threshold among these groups was calculated by the formula (immediate threshold − basal threshold)/basal threshold × 100%, with all values presented as percentage differences. Hyperalgesia and hypoalgesia were expressed by negative and positive percentages, respectively.

Enzyme-linked immunosorbent assay (ELISA)

Tissue processing for ELISA

After 7 days of EA intervention, rats in the 6 groups were sacrificed on Day 8. The animals were anesthetized with an injection of 10% chloral hydrate (0.35 mL/100 g, i.p.) followed by decapitation. The spinal cords were ejected from the vertebral column using a syringe filled with cold saline. Segments of the spinal cord were serially sectioned between T_{13} and L_5 (weighing 100 mg—120 mg) and snap frozen in liquid nitrogen. Samples were then placed in a freezer at −80°C for the next experiments.

Quantitative determination of spinal IL-1β

Segments of the spinal cord stored at −80°C were used for quantitative determination of spinal IL-1β. ELISA assay was performed in accord with the manufacturer's protocol (Boster Biological Technology, Wuhan, China). ELISA was used to minimize usage of radioactive materials included in the samples. Both ELISA and radioimmunoassay (RIA) were used to quantify IL-1β.[36] The microplate reader was set to 450 nm to determine the optical densities (ODs) of the 96-well microplates. Results of duplicate wells were averaged. Peptide value of each sample was expressed as picogram/100 μg (IL-1β content of spine/the protein content of spinal cord).

Statistical analysis

SPSS 17.0 (IBM, Armonk, NY, USA) software was used to analyze the experimental data. All data were expressed as mean ± SEM. Within each group, mechanical withdrawal threshold data at different time points were analyzed by two-way analysis of variance (ANOVA) with Tukey's post-hoc test. Levels of expression of spinal IL-1β were analyzed using one-way ANOVA. Differences between individual means were tested for significance using Fisher's least significant difference (LSD) procedure. Probability values less than 0.05 were considered highly significant.

Results

Motor function and behavioral observations

All rats in each group exhibited normal gait. No rat exhibited lameness, poor appetite, or licking the hind paws. Thus, the neuropathic pain induction procedure did not affect motor function or behavior of the rats.

Mechanical withdrawal threshold

Compared with the control group, rats in the model group exhibited significant mechanical hyperalgesia in the hind paws at 3, 5, and 7 days postoperatively ($P < 0.01$). However, the difference between the control and sham groups was not significant ($P > 0.05$). In comparison with the model group, mechanical hypoalgesia was observed in rats in the EA 1, EA 2, and EA 3 groups at 5 and 7 days postoperatively ($P < 0.05$). There were no significant differences in responses to noxious mechanical stimuli among the EA 1, EA 2, and EA 3 groups postoperatively ($P > 0.05$) (Fig. 2, Table 2).

Changes in spinal IL-1β level following EA treatment

ELISA assay results showed that there was a significant increase in IL-1β level in the spinal cord in rats in the model group when compared with the control group ($P < 0.01$). EA reversed the increase in IL-1β in the model group ($P < 0.05$). Expression of IL-1â was significantly lower in the EA 1, EA 2, and EA 3 groups. However, there were no significant differences in the expression of IL-1β among EA 1, EA 2, and EA 3 groups ($P > 0.05$) (Fig. 3, Table 3).

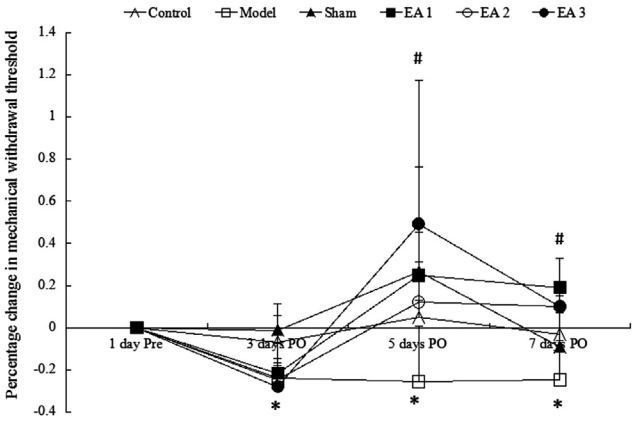

Figure 2 Mechanical withdrawal threshold.
Mechanical hyperalgesia was pronounced in the hind paws of model group rats at 3, 5, and 7 days postoperatively, compared with the control group. Rats in the EA 1, EA 2, and EA 3 groups presented mechanical hypoalgesia in the hind paws at 5 and 7 days postoperatively, compared with the model group. No significant differences were observed in sensitivities to noxious stimuli in the EA 1, EA 2, and EA 3 groups. All data were expressed as mean ± SEM. EA 1 = distal acupoints + proximal acupoints; EA 2 = distal acupoints; EA 3 = proximal acupoints. Pre = preoperative, PO = postoperative. $*P < 0.01$ versus control group; $^{\#}P < 0.05$ versus model group.

Table 2 Comparison of percentage change of mechanical withdrawal threshold among the 6 groups (n = 9).

Group	1 day Pre	3 days PO	5 days PO	7 days PO
Control	0 ± 0	−0.068 ± 0.126	0.050 ± 0.080	−0.031 ± 0.104
Model	0 ± 0	−0.239 ± 0.071[a]	−0.255 ± 0.264[a]	−0.247 ± 0.187[a]
Sham	0 ± 0	−0.013 ± 0.126	0.266 ± 0.907	−0.092 ± 0.242
EA 1	0 ± 0	−0.216 ± 0.143[a,b]	0.249 ± 0.204[b]	0.191 ± 0.140[b]
EA 2	0 ± 0	−0.246 ± 0.101[a,b]	0.122 ± 0.191[b]	0.099 ± 0.099[b]
EA 3	0 ± 0	−0.280 ± 0.099[a,b]	0.492 ± 0.269[b]	0.101 ± 0.086[b]

Abbreviations: PO, postoperative; Pre, preoperative.
All data were expressed as mean ± SEM.
Note: Two-way ANOVA: F = 3.882, $P = 0.000$; group: F = 1.404, $P = 0.005$; time: F = 27.915, $P = 0.000$; group * time: F = 2.341, $P = 0.004$.
[a] $P < 0.01$ versus control group.
[b] $P < 0.05$ versus model group.

Discussion

Neuropathic pain from HNP, especially of the lumbar region, is characterized by low back pain and lower extremity symptoms. Most patients with HNP exhibit pain-related behaviors. Studies have shown that HNP is involved in the occurrence and persistence of nerve root damage and associated neuropathies without its mechanical compression.[32,37] For this reason, the animal model we used in this study is likely adequate for investigating neuropathic pain induced by HNP. In the present study, we evaluated neuropathic pain by measuring mechanical withdrawal threshold in rats with experimentally induced traumatic nerve injury following the research by Bennett and Xie, which showed that mechanical pain states in the rat model are approximately consistent with symptoms in humans with peripheral neuropathic pain.[35]

Acupuncture exerts its therapeutic effect by regulating a multi-dimensional network comprised of the nervous, endocrine, and immune systems, all of which are important in maintaining the body's normal physiology.[31] Since ancient times, the beneficial effect of acupuncture has been based on the concept of point prescriptions, that is, specific combinations of acupoints used to treat specific diseases. The distal—proximal point prescription is a standard combination that uses points distal to the elbows and knees to treat disorders that are located on the course of the channel away from the acupoints.[25] Research has shown that sustained acupuncture stimulation, such as with electroacupuncture, produces analgesia.[38] Neuropathic pain involves an increase (upregulation) in proinflammatory substances such as IL-β,[39] and in the case of HNP, spinal IL-1β. The upregulation of IL-1β in this study is in agreement with previous studies in which spinal IL-1β was found to be increased during inflammatory and neuropathic pain.[11,40] Reports have documented that electroacupuncture appears to attenuate cytokine expression.[41,42] However, because little is known about the effect and mechanism of distal—proximal acupoint treatment on neuropathic pain and regulation of spinal IL-1β, we undertook this current study.

During our investigation, EA was applied to the distal acupoints of BL 40 and BL 60, and to the distal + proximal acupoints of Ex-B2, BL 25, BL 40, and BL60. These points were selected because they are standard point prescriptions for low back pain.[43] We found that mechanical

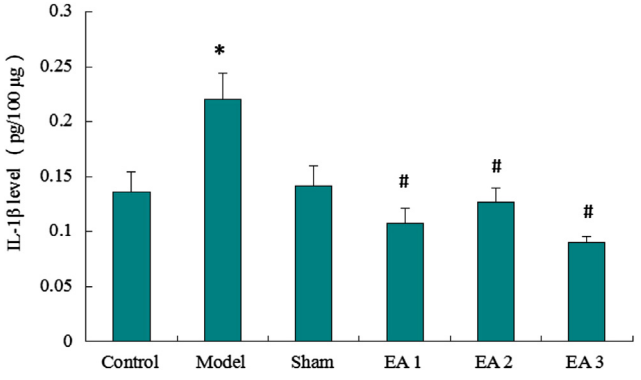

Figure 3 Changes in spinal IL-1β level in rats following EA treatment.
ELISA showed that electroacupuncture (EA 1, EA 2, EA 3) reversed NP-induced IL-1β upregulation in the local spine after surgery ($P < 0.01$). However, there were no significant differences in the expression level of spinal IL-1β among EA 1, EA 2, and EA 3 groups ($P > 0.05$). All data presented as mean (SEM). EA 1 = distal acupoints + proximal acupoints; EA 2 = distal acupoints; EA 3 = proximal acupoints. $*P < 0.01$ versus control group; $^{\#}P < 0.05$ versus model group.

Table 3 Comparison of changes in spinal IL-1β among 6 groups (n = 9) of rats following EA.

Group	IL-1β level in the spinal cord (pg/100 μg)
Control	0.136 ± 0.018
Model	0.220 ± 0.024[a]
Sham	0.141 ± 0.019
EA 1	0.108 ± 0.013[a,b]
EA 2	0.127 ± 0.013[a,b]
EA 3	0.090 ± 0.006[a,b]
F	66.576
P	0.000

Abbreviations: Pre, preoperative; PO, postoperative.
All data were expressed as mean ± SEM
[a] $P < 0.01$ versus control group.
[b] $P < 0.05$ versus model group.

withdrawal threshold of rats in the model group was decreased in the hind paws at 3 days postoperatively when compared with the normal group, with the effect lasting 7 days postoperatively. However, there was no significant difference between the control and sham groups. After EA stimulation, mechanical withdrawal threshold levels in the EA 1, EA 2, and EA 3 groups were higher than in the model group. No differences were found between the EA 1, EA 2, and EA 3 groups. However, the up-regulation of mechanical withdrawal threshold levels in the EA 1 and EA 3 groups tended to be more obvious than the EA 2 group at 7 days postoperatively. It was noteworthy that rats in the model and sham groups exhibited a trend toward recovery from mechanical pain state at 5 and 7 days respectively, which may be attributed to self-healing. The precise mechanism remains to be further clarified.

ELISA assay showed an increase in IL-1β in the spinal cords of rats in the model group as compared with the normal group at 7 days postoperatively. EA stimulation attenuated the increase in spinal IL-1β in the model group. Expression of spinal IL-1β was significantly lower in the EA 1, EA 2, and EA 3 groups. However, expression of spinal IL-1β was significantly downregulated in the EA 1 and EA 3 groups compared with the EA 2 group. Therefore, we believe that EA at distal + proximal acupoints as well as proximal acupoints raises the mechanical withdrawal threshold. It is possible that stimulation at distal—proximal point combinations for neuropathic pain inhibits release of spinal IL-1β, thus raising the mechanical withdrawal threshold, which in turn limits damage to the spinal nerve root.

In conclusion, electroacupuncture at distal—proximal point combinations appears to mitigate spinal IL-1β level in the rat model of neuropathic pain to achieve an analgesic effect. This may further our understanding of the mechanism by which stimulation at certain acupoint combinations can alleviate neuropathic pain such as from herniated nucleus pulposus.

Conflicts of interest

The authors declare no financial interests or other conflicts of interest.

Funding

This study was supported by grants from the Project of Beijing University of Chinese Medicine in China (No. JYB22 — JS022).

Acknowledgments

The authors are grateful to the Beijing University of Chinese Medicine for funding this study. We would like to thank Professor Qian Hua, School of Basic Medicine, Beijing University of Chinese Medicine, for kindly providing research support.

References

1. Zhang JJ, Song W, Luo WY, et al. Autologous nucleus pulposus transplantation to lumbar 5 dorsal root ganglion after epineurium dissection in rats: a modified model of non-compressive lumbar herniated intervertebral disc. *Chin Med J (Engl)*. 2011;124:2009—2014.
2. Hunt SP, Mantyh PW. The molecular dynamics of paincontrol. *Nat Rev Neurosci*. 2001;2:83—91.
3. Ji RR, Woolf CJ. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. *Neurobiol Dis*. 2001;8:1—10.
4. Suzuki M, Inoue G, Gemba T, et al. Nuclear factor-kappa B decoy suppresses nerve injury and improves mechanical allodynia and thermal hyperalgesia in a rat lumbar disc herniation model. *Eur Spine J*. 2009;18:1001—1007.
5. Shamji MF, Allen KD, So S, et al. Gait abnormalities and inflammatory cytokines in an autologous nucleus pulposus model of radiculopathy. *Spine (Phila Pa 1976)*. 2009;34:648—654.
6. Kayama S, Olmarker K, Larsson K, Sjögren-Jansson E, Lindahl A, Rydevik B. Cultured, autologous nucleus pulposus cells induce functional changes in spinal nerve roots. *Spine*. 1998;23:2155—2158.
7. Olmarker K, Rydevik B, Nordborg C. Autologous nucleus pulposus induces neurophysiologic and histologic changes in porcine caudaequina nerve roots. *Spine (Phila PA 1976)*. 1993;18:1425—1432.
8. Ozaktay AC, Kallakuri S, Takebayashi T, et al. Effects of interleukin-1 beta, interleukin-6, and tumor necrosis on sensitivity of dorsal root ganglion and peripheral receptive fields in rats. *Eur Spine J*. 2006;15:1529—1537.
9. Igarashi A, Kikuchi S, Konno S. Correlation between inflammatory cytokines released from the lumbar facet joint tissue and symptoms in degenerative lumbar spinal disorders. *J Orthop Sci*. 2007;12:154—160.
10. Habtematiam A, Virri J, Gronblad M, Seitsalo S, Karaharju E. The role of mast cells in disc herniation inflammation. *Spine (Phila PA 1976)*. 1999;24:1516—1520.
11. Sweitzer SM, Colburn RW, Rutkowski M, DeLeo JA. Acute peripheral inflammation induces moderate glial activation and spinal IL-1 beta expression that correlates with pain behavior in the rat. *Brain Res*. 1999;829:209—221.
12. Boddeke EW. Involvement of chemokines in pain. *Eur J Pharmacol*. 2001;429:115—119.
13. Choi HS, Lee HJ, Jung CY, Ju JS, Park JS, Ahn DK. Central cyclooxygenase-2 participates in interleukin-1 beta-induced hyperalgesia in the orofacial formalin test of freely moving rats. *Neurosci Lett*. 2003;352:187—190.
14. Reeve AJ, Patel S, Fox A, Walker K, Urban L. Intrathecally administered endotoxin or cytokines produce allodynia, hyperalgesia and changes in spinal cord neuronal responses to nociceptive stimuli in the rat. *Eur J Pain*. 2000;4:247—257.
15. DeLeo JA, Yezierski RP. The role of neuroinflammation and neuroimmune activation in persistent pain. *Pain*. 2001;90:1—6.
16. Watkins LR, Maier SF. Beyond neurons: evidence that immune and glial cells contribute to pathological pain states. *Physiol Rev*. 2002;82:981—1011.
17. Kreiner DS, Shaffer WO, Baisden JL, et al. An evidence-based clinical guideline for the diagnosis and treatment of degenerative lumbar spinal stenosis (update). *Spine J*. 2013;13:734—743.
18. Hoang NS, Sar C, Valmier J, Sieso V, Scamps F. Electroacupuncture on functional peripheral nerve regeneration in mice: a behavioural study. *BMC Complement Altern Med*. 2012;12:141—151.
19. Campbell A. Point specificity of acupuncture in the light of recent clinical and imaging studies. *Acupunct Med*. 2006;24:118—122.

20. Leibing E, Leonhardt U, Koster G, et al. Acupuncture treatment of chronic low-back pain-a randomized, blinded, placebo-controlled trial with 9-month follow-up. *Pain*. 2002;96: 189—196.
21. Carlsson CP, Sjolund BH. Acupuncture for chronic low back pain: a randomized placebo-controlled study with long-term follow-up. *Clin J Pain*. 2001;17:296—305.
22. Shen YF, Goddard G. The short-term effects of acupuncture on myofascial pain patients after clenching. *Pain Pract*. 2007;7: 256—264.
23. Kim HN, Park JH, Kim SK, et al. Electroacupuncture potentiates the antiallodynic effect of intrathecal neostigmine in a rat model of neuropathic pain. *J Physiol Sci*. 2008;58:357—360.
24. Yim YK, Lee H, Hong KE, et al. Electroacupuncture at acupoint ST36 reduces inflammation and regulates immune activity in collagen-induced arthritic mice. *Evid Based Complement Altern Med*. 2007;4:51—57.
25. Shen XY, Wang H, Zhao BX. *Acupuncture and Moxibustion*. vol. 1. Beijing, China: People's Medical Publishing House; 2007: 277—282 [Chinese].
26. Quispe-Cabanillas JG, Damasceno A, Von Glehn F, et al. Impact of electroacupuncture on quality of life for patients with relapsing-remitting multiple sclerosis under treatment with immunomodulators: a randomized study. *BMC Complement Altern Med*. 2012;12:209—235.
27. Lin JG, Chen WL. Acupuncture analgesia: a review of its mechanisms of actions. *Am J Chin Med*. 2008;36:635—645.
28. Cabyoglu MT, Ergene N, Tan U. The mechanism of acupuncture and clinical applications. *Int J Neurosci*. 2006;116:115—125.
29. Kim CK, Choi GS, Oh SD, et al. Electroacupuncture up-regulates natural killer cell activity identification of genes altering their expressions in electroacupuncture induced up-regulation of natural killer cell activity. *J Neuroimmunol*. 2005;168:144—153.
30. Kim JH, Min BI, Na HS, Park DS. Relieving effects of electro-acupuncture on mechanical allodynia in neuropathic pain model of inferior caudal trunk injury in rat: mediation by spinal opioid receptors. *Brain Res*. 2004;998:230—236.
31. Wu YC, Zhang JF, Wang CM, Xie YY, Zhou JH. Acupuncture at the "Huatuojiaji" point affects nerve root regional interleukin-1 level in a rat model of lumbar nerve root compression. *Neural Regen Res*. 2008;3:881—884.
32. Kawakami M, Tamaki T, Hayashi N, Hashizume H, Nishi H. Possible mechanism of painful radiculopathy in lumbar disc herniation. *Clin J Orthop*. 1998;351:241—251.
33. Zhao LY, Jiang HL, Ren XJ, Tu Y. Influences of electro-acupuncture on pain-related behaviors and histomorphological changes of spinal nerve root in rat model of lumbar disc herniation. *J Beijing Univ Traditi Chin Med*. 2014;37:553—556 [Chinese].
34. Yu SG, Guo Y. *Experimental Acupuncture*. Shanghai, China: Shanghai Science and Technology Press; 2009:150—152 [Chinese].
35. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain*. 1988;33:87—107.
36. Habtematiam A, Virri J, Gronblad M, Seitsalo S, Karaharju E. The role of mast cells in disc herniation inflammation. *Spine (Phila Pa 1976)*. 1999;24:1516—1520.
37. Otani K, Arai I, Mao GP, Konno S, Olmarker K, Kikuchi S. Experimental disc herniation: evaluation of the natural course. *Spine (Phila Pa 1976)*. 1997;22:2894—2899.
38. Leung L. Neurophysiological basis of acupuncture-induced analgesia-an updated review. *J Acupunct Meridian Stud*. 2012;5:261—270.
39. Ren K, Torres R. Role of interleukin-1β during pain and inflammation. *Brain Res Rev*. 2009;60:57—64.
40. Takahashi H, Suguro T, Okazima Y, Motegi M, Okada Y, Kakiuchi T. Inflammatory cytokines in the herniated disc of the lumbar spine. *Spine (Phila Pa 1976)*. 1996;21: 218—224.
41. Cha MH, Nam TS, Kwak Y, Lee H, Lee BH. Changes in cytokine expression after electroacupuncture in neuropathic rats. *Evid Based Complement Altern Med*. 2012;2012: 792765.
42. Tu W, Wang W, Xi H, He R, Gao L, Jiang S. Regulation of neurotrophin-3 and interleukin-1β and inhibition of spinal glial activation contribute to the analgesic effect of electro-acupuncture in chronic neuropathic pain states of rats. *Evid Based Complement Altern Med*. 2015;2015: 642081.
43. Jiang HL, Ji LL, Ren XJ, Long XQ, Tu Y. Exploration of acupuncture prescriptions for chronic low back pain: a review of ancient and modern literature. *J Beijing Univ Traditi Chin Med*. 2015;38:280—283 [Chinese].