Gua Sha attenuates thermal hyperalgesia and decreases proinflammatory cytokine expression in serum in rats with lumbar disc herniation induced by autologous nucleus pulposus

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Abstract

OBJECTIVE: To investigate the analgesic effect of Gua Sha and its underlying mechanism in rats with noncompressive lumbar disk herniation induced by autologous nucleus pulposus.

METHODS: A rat model of noncompressive lumbar disk herniation was established and rats were randomly divided into model group, sham group, and Gua Sha group (24 in each group). Gua Sha was performed from the 5th day after the surgery, once every other day, 3 times for a course of treatment, and totally 3 courses. The thermal withdrawal latency was evaluated using the intelligent hot plate one day before the surgery, and on days 4 (the day before the treatment), 10 (the end of the first course), 16 (the end of the second course) and 22 (the end of the third course). On days 4, 10, 16 and 22, six rats in each group were picked randomly and their blood samples were drawn to assess the expression of interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α).

RESULTS: Compared to rats in the sham group, the application of nucleus pulposus to right L5 dorsal root ganglion induced prolonged thermal hyperalgesia, and up-regulated the expression of IL-1β, IL-6 and TNF-α in serum (P < 0.01). The therapy of Gua Sha attenuated thermal hyperalgesia potently, inhibited the expression of IL-1β, IL-6 and TNF-α in a time-dependent manner (P < 0.01). There were no significant differences in the thermal withdrawal latency and the expression of inflammatory cytokines between the sham and Gua Sha groups at the end of the treatment (P > 0.01).

CONCLUSION: The current study showed that Gua Sha might alleviate thermal hyperalgesia in rats with lumbar disc herniation induced by autologous nucleus pulposus via inhibiting the expression of proinflammatory cytokins.

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keywords: Gua Sha; Lumbar disc herniation; Nucleus pulposus; Hyperalgesia; Cytokines

INTRODUCTION

Lumbar disc herniation (LDH) has been proved to be the main reason of sciatica, femoral nerve, and low back pain,1–3 but the specific mechanism is not fully
known. The mechanism underlying the pains induced by LDH may involve two factors: nerve root or dorsal root ganglion (DRG) compression and contact with nucleus pulposus (NP). However, evidence increasingly supports the latter: it is the inflammatory milieu caused by the application of NP to never root or DRG that elicits symptoms of neural irritation. Furthermore, clinical and epidemiological research show that the outcome of disc surgery after one year is parallel to that of conservative therapy. That is to say, non-compression might underlie the pathogenesis of pain in LDH. Without compression, nerve root or DRG’s exposure to NP may be sufficient to induce symptoms. The inflammatory effects caused by the contact of NP are under investigation, and numerous mediators may be involved in varying degrees. It has been demonstrated that proinflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), play a vital role in the initiation or maintenance of neuropathic pain in LDH. Gua Sha (press-stroking), repeated and unidirectional scraping on the skin surface, with a smooth-edged tool, like coin, porcelain spoon and specially-made scraping plate made from cornu bubali, is an ancient healing technique popular in Asia. Normally, petechiae and ecchymosis appear at the scrapped area, which may fade completely within a few days. The theory of Traditional Chinese Medicine (TCM) believes Gua Sha is responsible for opening Couli (striae of the skin and muscles), promoting the flow of Qi and blood, dredging meridians and removing pathogenic toxin. Clinical trials and experimental studies have demonstrated that Gua Sha could alleviate pains, stimulate an unidentified pain-relieving biochemical pathway and modulate cytokines. However, the clinical significance and biomechanism of Gua Sha have not been empirically established. In Jiang et al’s study, compared with the model group, the level of IL-1 in serum of rats with LDH was significantly lower after Gua Sha. Yet, the effect of Gua Sha on hypersensitivity induced by LDH is a complex process, while the change of one single cytokine may not enough to explain it. We hypothesized that the mediation of inflammatory cytokines might involve in the pain-relieving procedure of Gua Sha. Therefore, in this study, the expression of IL-1β, IL-6 and TNF-α in serum of an animal model was quantified to further uncover the mechanism underpinning NP-induced thermal hyperalgesia.

**MATERIALS AND METHODS**

**Experimental animals**

Seventy-two adult male Sprague-Dawley rats (300-400 g), three-month-old, obtained from Experimental Animal Center of Zhejiang Province (Zhejiang, China, Animal certificate: SCXK [Zhejiang] 2014-0001), were housed in the specific pathogen-free grade Experimental Animal Center at the Nanjing University of Chinese Medicine (Nanjing, China). They were fed ad libitum and were kept at a temperature of (23 ± 2) °C and 50% ± 10% humidity with an alternating 12 h light/dark cycle. Rats were randomly divided into 3 groups in accordance with the random digital tables: a model group, a sham operation group, and a Gua Sha group, with 24 rats in each. All animals treatments were in accordance with the Guidelines of Accommodation and Care for animals formulated by the Chinese Convention for the protection of vertebrate animals used for experimental and other scientific purposes. The study was approved by the ethic review committee of Nanjing University of Chinese Medicine.

**Instruments**

The microplate reader was purchased from PerkinElmer (Victor X3, Boston, MA, USA). YLS-6B Intelligent Hot Plate was purchased from Jinan Yiyi Technology Development Company (Jinan, China). The High-speed Refrigerated Centrifuge was purchased from Backman (Allegro 64R, Fullerton, CA, USA), and standard enzyme-linked immunosorbent assay (ELISA) kits were purchased from Nanjing Jin Yibai Biological Science And Technology Company (Nanjing, China).

**Surgical protocol**

As described previously, a model of non-compression LDH was established. Rats were anesthetized with 10% chloral hydrate. For the model and Gua Sha group, a midline dorsal and thoracolumbar fascia incision over the right L4-L5 spinous process were made, following which the paraspinal muscles were dissected. Then, partial unilateral laminotomy and medial facetectomy were conducted to expose the right L5 DRG. Afterwards, the first coccygeal intervertebral disc was exposed bilaterally and NP was harvested through a horizontal cut in the annulus fibrosus, and the gel-like NP was placed on the L5 DRG immediately. While for sham animals, their L5 DRGs were released and tails were cut off from the first coccygeal intervertebral disc, but the autologous NP was not implanted over the L5 DRG. At last, the surgical wound was sutured with 3-0 vicryl sutures for muscle and fascia and 3-0 nylon sutures for skin. For all animals, penicillin was injected intraperitoneally for continuous 3 d postoperatively to reduce the inflammation caused by the surgery.

**Gua Sha treatment**

In the Gua Sha group, Gua Sha was conducted on the 5th day after the surgery, once every other day, 3 times for a course of treatment, and totally 3 courses. It was applied on the back at the centerline and two sides of the centerline where DU Meridian and bilateral Bladder Meridian were located, and then the right hind leg. Scraping was repeated in one direction until the appearance of patterned ecchymosis. Plus, Shenshu
(BL 23), Weizhong (BL 40) and Huantiao (GB 30), Yanglingquan (GB 34) were scrapped heavily (Figure 1).

**Assessment of pain-related behavior**

As described in detail previously, the thermal hyperalgesia of rats was measured with the intelligent hot plate. The right hind paw of rat was exposed to a thermal stimulus (52.0 ± 0.2 °C), and the latency to withdrawal evoked by the stimulus was reported. Three tests, fifteen minutes apart, were conducted on each hind paw to get the mean. The assessment was performed one day before the surgery to get the baseline, and on days 4 (the day before the treatment), 10 (the end of the first course), 16 (the end of the second course) and 22 (the end of the third course). Rats were habituated to the testing environment for 1h before the behavioral test. Furthermore, investigators were blind to the group of animals.

**Measurement of serum cytokine levels**

On the 4th, 10th, 16th and 22nd day after surgery, 6 rats in each group were picked randomly and their blood samples were drawn from the orbit. After being placed quietly at room temperature for an hour, these blood samples were centrifuged (3 000 RPM/20 min) and the serum was collected for the quantification of IL-1β, IL-6 and TNF-α levels. The experimental procedure was presented in Figure 2.

**Statistical analysis**

All the data were presented as the mean ± standard deviation ( ± s) and analyzed using SPSS 17.0 (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago, IL, USA). Data of animal thermal withdrawal latency and levels of cytokine expression were assessed with two-way analysis of variance (ANOVA) (group × time) with repeated measures, followed by the Bonferroni post hoc test. Two-tailed P value less than 0.01 was considered statistically different.

**RESULTS**

**Alleviation of thermal hyperalgesia after Gua Sha treatment**

As presented in Figure 3, no statistical difference in rats in each group was found before surgery (P > 0.01). The thermal withdrawal latency in the model group were decreased significantly after the application of NP to L5 DRG from day 4 (6.4 ± 1.0) to day 16 (6.9 ± 1.4, P < 0.01) post surgery compared to that on one day before the surgery, but there was no significant statistical difference on the 22th day (7.3 ± 1.4, P > 0.01). The thermal withdrawal latency in the sham group showed no statistical difference at different time (P > 0.01) and was always higher than that in the model group (P < 0.01). In the Gua Sha group, after the surgery, the thermal hyperalgesia was observed from day 4 (7.5 ± 1.0, P < 0.01) to day 10 (8.1 ± 0.9, P < 0.01) was considered statistically different.
Figure 3 Thermal withdrawal latency in the three groups
Seventy-two rats were randomly divided into three groups: the model group, the shame group and Gua Sha group. One day before the surgery, and on days 4, 10, 16 and 22, six rats in each group were picked randomly to assess the thermal withdrawal latency. All data are presented as mean ± standard deviation. Pre: preoperatively; Po: postoperatively. *P < 0.01, vs the model group; †P < 0.01, vs the shame group; ‡P < 0.01, vs 1 d Pre.

However, after the treatment of Gua Sha for two courses, the thermal hyperalgesia was alleviated significantly from day 16 (10.0 ± 1.5, *P < 0.01) to day 22 (10.7 ± 2.1, *P < 0.01) compared to that in the model group.

Gua Sha decreased the expression of IL-1β, IL-6 and TNF-α in serum
The NP application to the L5 DRG as well as the scraping changed expression of cytokine in a time-dependent manner.

In spite of the downward trend, compared to that in the sham group, the expression of IL-1β increased in the model group after the contact of NP with the L5 DRG (from 31 ± 5 to 20 ± 9, each *P < 0.01, except *P = 0.014 on day 10). The treatment of Gua Sha time-independently reduced the level of IL-1β (from 34 ± 13 to 9 ± 5). On days 16 and 22, there was no statistical difference for IL-1β expression between Gua Sha group and the sham group (*P > 0.01). Also, IL-1β expression in Gua Sha group was significantly lower than that on day 4 (the day before the treatment; *P < 0.01) (Figure 4).

IL-6 expression after NP application was also significantly greater in the model group than animals in sham operation group and lasted up to day 22 (from 129.3 ± 15.2 to 120.5 ± 14.5, *P < 0.01), being more greater on the 10th day (131.3 ± 21.4, *P < 0.01). Both started with the highest, IL-6 expression in sham and Gua Sha groups saw values fall from 45.6 ± 23.3 to 9.9 ± 3.8, 128.7 ± 11.1 to 17.1 ± 4.3 from day 4 to day 22, respectively. Though IL-6 expression in Gua Sha group was statistically significantly greater than the sham group in 16 days after surgery (53.4 ± 12.2 vs 17.1 ± 1.7, *P < 0.01), value of IL-6 became similar to the sham group after the third treatment course (*P > 0.01) (Figure 5).

The change trend of TNF-α in the model group resembled that of IL-6. It increased significantly after the surgery despite slight decline on day 22 (from 147 ± 44 to 135 ± 32), while there were statistical differences at all time points compared to the sham group (*P < 0.01). Gua Sha suppressed the increase of TNF-α (from 164 ± 26 to 48 ± 7), and on days 16 and 22, the value declined significantly compared to that in the model group (*P < 0.01) while there were no differences between Gua Sha and the sham group (*P > 0.01, Figure 6).

DISCUSSION
In this study, with the assistance of rat model, the pain-relieving efficacy and mechanism of Gua Sha therapy was carried out in relation to its effect on inflammatory cytokines. In the process, Du Meridian and Bladder Meridian were selected to be scraped. Both of them are the most treated areas for LDH in TCM techniques, for the main symptoms due to lesion in Du Meridian and Bladder Meridian were consistent with low back pain. Furthermore, four acupoints were chosen to be scraped heavily in the study. Shen shu (BL 23), the acupoint of Du meridian, where channel Qi of...
the kidney infuses in the low back, has the function of regulating Qi of the kidney and dispelling cold and dampness. Weizhong (BL 40) is located on the mid-point of the popliteal transverse crease and between the tendons of biceps femoris and semitendinosus. The classic TCM theory 'treating lumbar back problems by resorting to Weizhong (BL 40)' is a typical example that highlights the importance of remote acupoint for therapy. Xu et al.'s study demonstrated that scratching Weizhong (BL 40) can promote cutaneous microcirculation and mitigate pain.24 Huantiao (GB 30) and Yanglingguan (GB 34) are acupoints in Gallbladder meridian of foot-shaoyang. Stimulation on them could re-

Ding the acceleration and activation of inflammatory cells as well as the release of inflammatory transmitter, thus facilitates the inflammatory responses of tissues. TNF-α is a vital cytokine in the inflammatory response initiation. It can promote the macrophages to synthesize and release IL-1β and IL-6, and amplify their biological effects.25 Therefore, the specific expression and synergistic effects of above proinflammatory cytokines lead to the intense neurogenic pain induced by NP. By placing NP on the right L5 DRG, we reproduced the LDH model successfully. Animals with NP implementation performed an obvious hyperalgesia responding to the thermal stimulation. Meanwhile, levels of IL-1β, IL-6, TNF-α were all significantly higher than the sham operation group especially during the acute phase. While over time, levels of IL-1β in the model group was not further enhanced, which may suggest the existence of a physiologic ceiling effect or the divergent inflammatory cascade.33 In the present study, we demonstrated that Gua Sha could attenuate the hyperalgesia in a time-dependent manner. Of note, in line with the previous study,32 we also observed a tendency toward recovery of hyperalgesia in the model group, which was probably attributed to the natural resorption of NP. Noteworthily, the reduction in the intensity of the hyperalgesia by Gua Sha was more intense in the second treatment course. However, the rate of decrease of the inflammatory cytokines in serum should be considered for interpretation of it. The different time points at which Gua Sha regulated expression of cytokine remarkably suggest that every cytokine might exert its maximum influence at a diverse time after the surgery. After three treatment courses, levels of proinflammatory cytokines of animals in the scratching group decreased dramatically and were similar to those in the sham group. Therefore, we deduce that the reduction of proinflammatory cytokines after scratching has a key influence on the significant alleviation of thermal hyperalgesia in LDH.

However, which signal pathway is involved during the process of Gua Sha for LDH remains as a gap in our knowledge. It was found p38 mitogen-activated protein kinase (p38 MAPK) plays a critical role in the development of intervertebral disc degeneration through regulating the expression of IL-1β, IL-6 and TNF-α.35 In addition, p38 MAPK pathway might be associated with the development of neuropathic pain after the application of NP to the DRG.36 Possibly, the inhibition of p38 MAPK pathway might be involved in the mechanism of antinflammation and pain-relieving of Gua Sha, and further studies are needed to investigate the underlying mechanism.

In conclusion, this study showed that Gua Sha exhibits a remarkable effect on reducing pains in an autologous NP model of LDH. The down-regulation of proinflammatory cytokines might be, at least in part, responsible to the potential mechanisms. Thus, the findings may bring new evidence for the effects of Gua Sha therapy on the neuropathic pain induced by LDH.

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