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[Original Article]

## A COMPARISON OF PAIN-RELATED BEHAVIOR FOLLOWING LOCAL APPLICATION OF NUCLEUS PULPOSUS AND/OR MECHANICAL COMPRESSION ON THE DORSAL ROOT GANGLION

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**Abstract :** Symptomatic induction of disc herniation involves both mechanical compression and chemical factors. Inhibitors of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) are known to reduce pain-related behavior in experimental models. Animals were divided into mechanical compression (MC) group ; a stainless steel rod was inserted on the dorsal root ganglion, nucleus pulposus (NP) group : NP was harvested from the coccygeal vertebral disc, MC and NP group ; rats were received stainless rod and NP, and sham group ; rats were received neither rod nor NP. Rats in the MC group received a TNF- $\alpha$  antibody (10 mg/kg) (antibody group) or were not treatment (untreated group). The withdrawal thresholds of the MC, NP and MC+NP groups decreased significantly compared with the sham group. In the antibody group, the threshold was significantly higher than that of the untreated group.

An anti-TNF- $\alpha$  antibody reduced allodynia caused by DRG compression.

**Key words :** lumbar disc herniation, nucleus pulposus, mechanical compression, dorsal root ganglia, anti-TNF- $\alpha$  antibody

### INTRODUCTION

Lumbar disc herniation and stenosis of the lumbar intervertebral foramen are major causes of low back pain and sciatica. Our knowledge of the pathological mechanisms of disc herniation has developed over recent years. Previous studies suggest that symptoms due to lumbar disc herniation may be induced by not only mechanical compression (MC) of the lumbar nerve root but also chemical factors from the nucleus pulposus (NP)<sup>1-9</sup>. It has also been reported that local application of the NP to spinal nerve roots or into the epidural space may lead to pain-related behavior<sup>4,7,10</sup>. 5-hydroxytryptamine and inflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) are considered chemical factors<sup>11-14</sup>. TNF- $\alpha$  has been suggested to play an important role

in the development or persistence of symptoms of disc herniation<sup>11,12,14-18</sup>. It has also been reported that inhibition of TNF- $\alpha$  can prevent NP-induced effects, such as reduction in nerve conduction velocity, histological changes in nerve tissue, and pain-related behavior in experimental animal models<sup>17,18</sup>. In clinical studies, it has been reported that the application of a TNF- $\alpha$  antibody reduced the pain associated with lumbar disc herniation<sup>15,16</sup>. On the other hand, chronic compression of the dorsal root ganglion (DRG) or the nerve root as a result of intervertebral foramen stenosis is also an important causative factor for low back pain and sciatica. In animal studies, chronic MC of the DRG without exogenous inflammatory factors can induce pain-related behavior and increase spontaneous electric activity in dorsal root fibers<sup>19-21</sup>. In addition, pain-related behavior caused by compression of the DRG

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is related to inflammation<sup>20–22</sup>). It has also been reported that the focal application of soluble TNF- $\alpha$  receptors on the DRG resulted in the temporary reduction of mimic-allodynia in the chronic DRG compression model<sup>22</sup>). Based on these findings, the mechanisms of symptomatic disc herniation appear to be related to both MC and chemical factors within the nerve root. It is necessary to compare the influence of each factor using different models that enable discriminative analysis of MC on nerve root from the chemical factors contained in NP tissues. The aims of the present study were to compare a pain-related behavior induced by MC and/or NP application to the DRG and clarify the effect of TNF- $\alpha$  inhibition in a chronic compression model.

### MATERIALS AND METHODS

The study was carried out under the control of the Animal Care and Use Committee in accordance with the Guidelines for Animal Experiments at our university and the Japanese Government Law Concerning the Protection and Control of Animals.

#### *Anesthesia and Surgical Procedure* *Series 1*

Adult male Sprague-Dawley rats ( $n=24$ , 220–290 g) were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The

lumbar section was surgically exposed by a midline incision, and the paraspinal muscles were separated from the L5–L6 laminae under a surgical microscope. A 2.5-mm laminectomy hole was made in the L5 vertebral arch using a 1-mm drill without destroying the L5/6 facet joint. The left L5 nerve root and DRG were exposed through the L5 partial laminectomy hole. The NP was harvested (ca. 0.1 mg) from a coccygeal vertebral disc. The rats were then divided into four groups: the NP group, the MC group, the MC plus NP group (MC+NP group), and the sham group. No other procedure was performed in the sham group ( $n=6$ ). In the NP group ( $n=6$ ), the harvested NP was applied proximal to the DRG through the laminectomy hole. In the MC group ( $n=6$ ), an L-shaped stainless steel rod (diameter=0.5 mm; 2×2 mm) was inserted from the partial laminectomy hole toward the intervertebral foramen along the nerve root. Special care was taken not to injure the nervous structures. The central edge of the rod was positioned at the proximal end of the DRG (Figure 1a). In the MC+NP group ( $n=6$ ), a stainless steel rod was inserted through the laminectomy hole, and the harvested NP was also applied to proximal to the DRG (Figure 1b). In all groups, the spinal muscles were sutured and the skin was closed with metal clips.

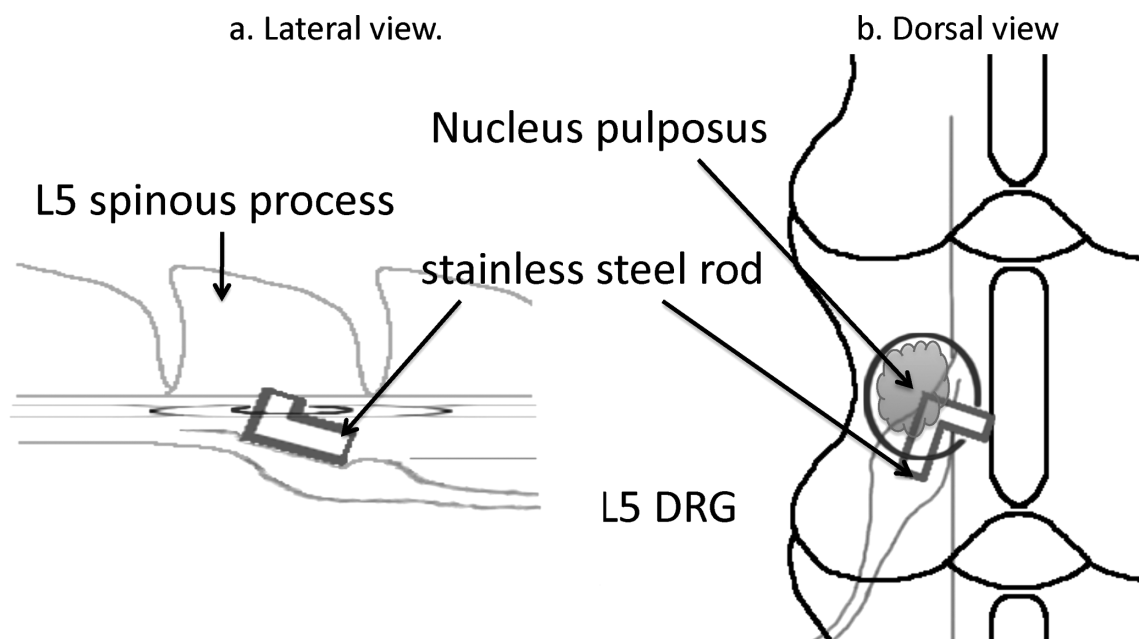


Fig. 1. Schematic diagram of the experimental model. (a) After making a partial laminectomy hole on the left L5 vertebral arch, a stainless steel rod was inserted via the fenestration and threaded along the nerve root through the intervertebral foramen. Nucleus pulposus harvested from the sacral vertebrate was applied in the laminectomy hole (a: lateral view, b: dorsal view).

### Series 2

Eighteen adult male Sprague-Dawley rats (220–290 g) were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The surgical procedures were the same as in series 1, and rats were divided into two groups: the sham group ( $n=6$ ) and the MC group ( $n=12$ ). Rats in the MC group were divided into two subgroups: the antibody group ( $n=6$ ) and the untreated group ( $n=6$ ). Rats in the antibody group received anti-rat TNF- $\alpha$  antibody (monoclonal anti-rat TNF- $\alpha$  antibody, TECHNE Corp., Minneapolis, MN, US) (10 mg/kg) in 0.3 ml phosphate-buffered saline into the coccygeal vein 6 days after surgery. Rats in the untreated group did not receive the anti-TNF- $\alpha$  antibody.

### Behavioral Tests

All behavioral testing was performed by a technician who was unaware of the experimental groupings. In series 1, responses to mechanical and thermal stimuli were separately monitored on days 1, 3, 5, 7, 10, 14, 21, 28, 35, 42, and 56, except for the thermal response which was assessed on days 1 and 5 after surgery. In series 2, mechanical stimuli were performed on days 1, 3, 5, 7, 14, 21, and 28 after surgery.

### Mechanical stimulus

Rats were placed individually into an acrylic cage ( $20 \times 11 \times 13$  cm<sup>3</sup>) with a mesh floor, and were allowed to acclimate for 10 min. The plantar surface of the operated hind limb was stimulated with eight von Frey filaments (0.6, 1.2, 2.0, 3.6, 5.5, 8.5, 15.1, and 28.8 g) threaded under the mesh floor. Stimulation was initiated with the 3.6-g filament. Stimulation was delivered perpendicularly to the plantar surface via von Frey filaments for 8 sec. The response was taken as positive if the hind limb indicated an escape response. Stimulation was executed by the up-down method<sup>23)</sup>, according to the procedure described previously<sup>24)</sup>. The 50% withdrawal threshold was determined. The statistical significance of differences between the groups was assessed using analysis of variance (Fisher's PLSD). A  $p$ -value less than 0.05 was considered statistically significant.

### Thermal stimulus

Responses to thermal stimuli were monitored according to the method described by Hargreaves *et al.*<sup>25)</sup>. Rats were placed in a cage with a glass floor

installed with Plantar Test (Ugo Basile Co. Ltd., Comerio, Italy). After a 10-min period of acclimatization, a non-contact infrared thermal source embedded under the glass floor was switched on to irradiate the plantar skin of rats. The interval from stimulus switch-on to induction of escape latency was measured. Monitoring of two trials of thermal responses was repeated at intervals of more than 10 min, and the mean value was taken as the thermal response. These mean response values were measured on both operated and non-operated hind limbs, and differential scores were derived by subtracting the latter from the former. The statistical significance of differences of differential scores between the groups was assessed using ANOVA (Fisher's PLSD). A  $p$ -value of less than 0.05 was considered statistically significant.

## RESULTS

Rats in all groups showed stable condition before surgery in response to mechanical and thermal stimulations.

### Mechanical stimulus

#### Series 1

The mechanical threshold in the NP and MC+NP groups significantly decreased compared with the sham group from post-operative day 1 to 28 ( $p < 0.01$ ). In the MC group, the threshold decreased gradually from post-operative day 1. In addition, the thresholds in the MC group significantly decreased compared with the sham group from day 3 to 28 ( $p < 0.01$ ). The thresholds in the MC group were higher than those in the NP and MC+NP groups on day 1 and 3 ( $p < 0.05$ ). There were no significant differences of the thresholds among the NP, MC, and MC+NP groups from day 5 to 28 (Figure 2). The reduction in threshold induced by MC appeared later than that induced by NP application.

### Thermal stimulus

Although a reduction was found in the differential score between the NP, MC, and MC+NP groups on post-operative day 3 and 7, there were no significant differences of the score among three groups (Figure 3).

#### Series 2

In the mechanical stimuli test, there were no significant differences in the withdrawal threshold among the three groups on day 1 post-surgery. On

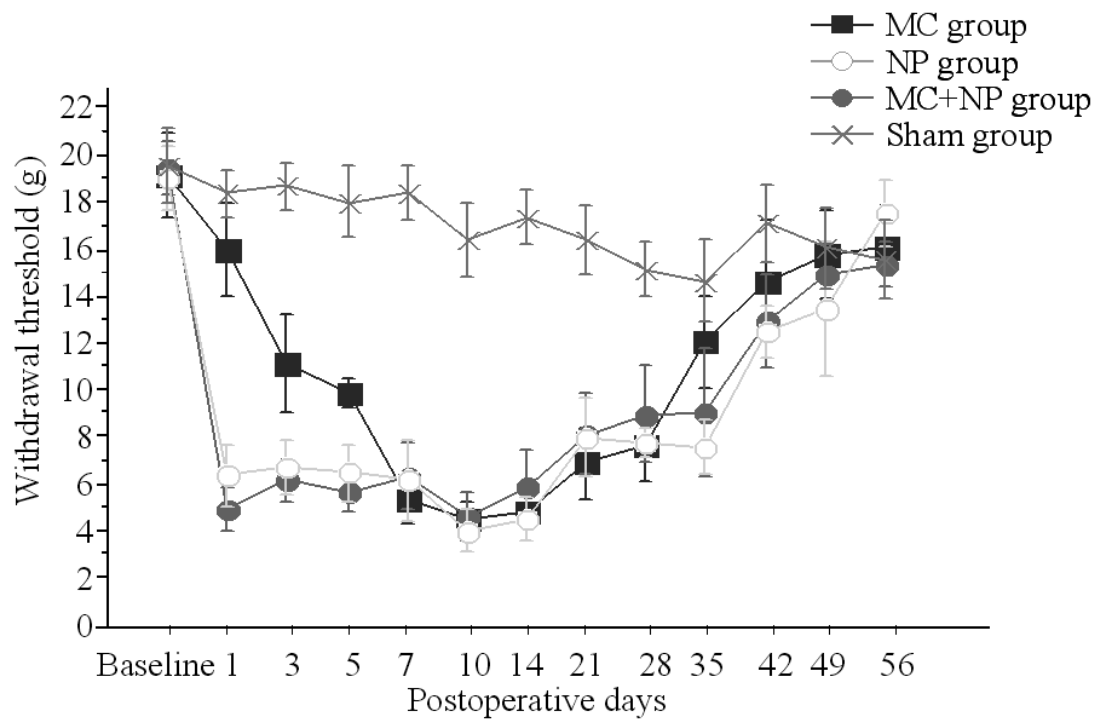


Fig. 2. Changes in mechanical withdrawal threshold.

The mechanical thresholds in the NP and MC+NP groups decreased compared with the sham group ( $p < 0.01$ ). In the MC group, the thresholds decreased gradually, and there was significant difference in thresholds from day 3 to 28 day compared with the sham group ( $p < 0.01$ ).

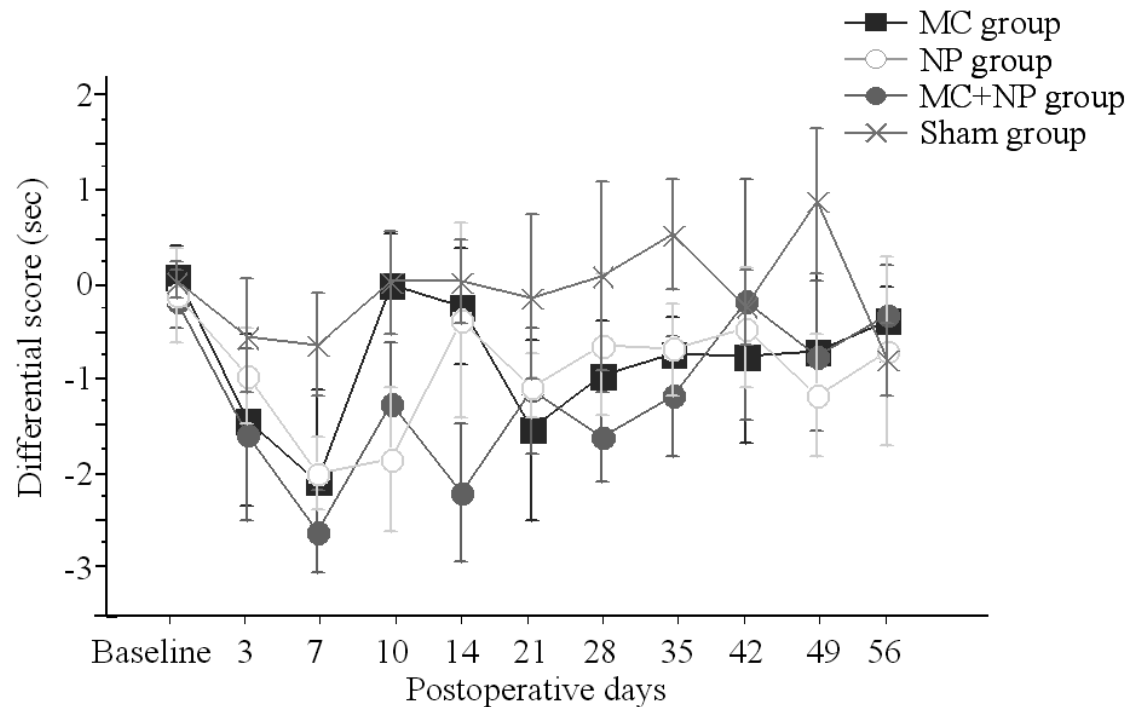


Fig. 3. Response time of thermal stimulation.

Values monitored in rats during the experimental period were not statistically different.

day 7, the day immediately following administration of the TNF- $\alpha$  antibody, the withdrawal threshold of the antibody group was significantly higher than the untreated group ( $p < 0.01$ ). However, there were no significant differences between the antibody and the untreated groups at any of the observation points except for day 7 (Figure 4).

### DISCUSSION

We developed three different models to compare MC and NP application associated with nerve conditions in lumbar disc herniation. The withdrawal threshold in the MC group was reduced gradually within 7 days after surgery, although a recovery tendency was apparent from post-operative day 28 to 56. According to Macnab<sup>26)</sup>, sensory abnormalities and numbness, but not pain, are induced when acute compression is exerted on a normal nerve root in humans. However, allodynia in the hind limb is inflicted on application of chronic MC on the lumbar nerve root and DRG in experimental models<sup>19,21,22,27)</sup>. Based on these findings, short-term compression of the nerve root would induce sensory abnormalities but not pain, whereas similar long-term stimuli would induce hyperalgesia. On the other hand, it has been reported that a rat

chronic cauda equina compression model, which does not include a compressed DRG, did not induce allodynia<sup>28,29)</sup>. In our previous study using the MC model, 200-kDa neurofilament (RT97) afferents increased in the superficial dorsal horn of the spinal cord, and the increase of RT97 afferents was suggested to be related to the pain-related behavior induced by DRG compression<sup>30)</sup>. These findings suggest that direct mechanical stimuli to the DRG may be closely related to the development of allodynia.

NP application without compression of the nerve root and DRG produces morphologic/functional changes and blood flow impairment<sup>2,6,9,31)</sup>, response of secondary neurons to noxious stimuli is enhanced<sup>1)</sup>, and allodynia in the hind limb has been demonstrated<sup>32)</sup>. The present study showed that allodynia was induced from post-operative day 1 in the NP and MC+NP groups. In other word, NP or cytokines containing NP may influence nerve tissue rapidly, whereas the change to nerve tissue caused by DRG compression may develop gradually. Our present study did not find any differences in the change of withdrawal threshold between the NP and MC+NP groups. This result might suggest that NP exerts a more potent effect than MC. Changes in the withdrawal threshold are more closely associ-

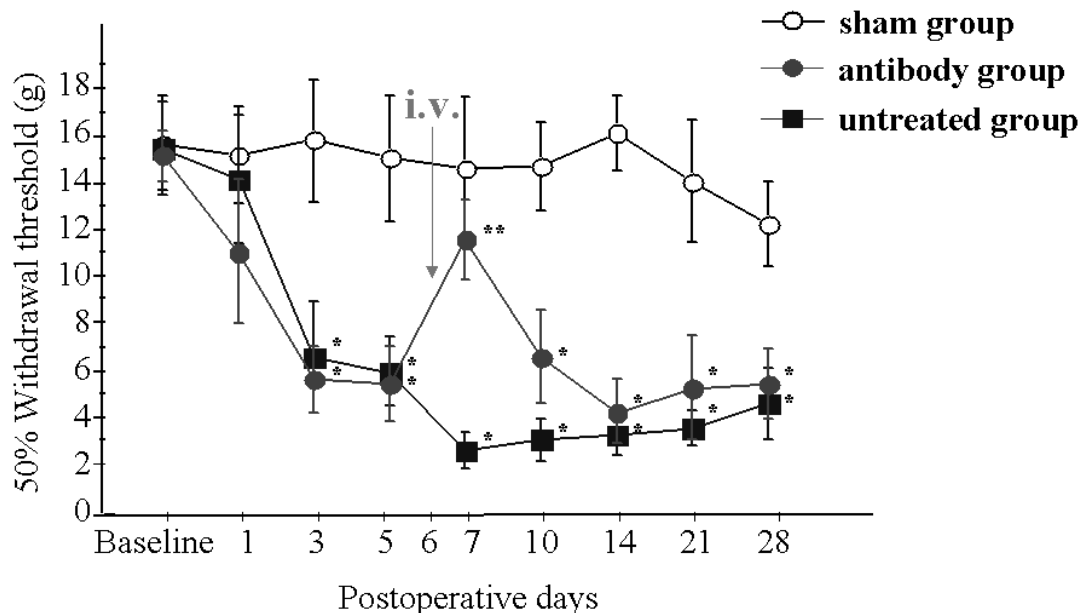


Fig. 4. Changes in the mechanical withdrawal threshold.

Compared with the sham group, the withdrawal threshold of the untreated group was significantly lower from post-operative day 3 to 28 ( $p < 0.01$ ). On day 7, the day immediately following the administration of TNF- $\alpha$  antibody, the threshold of the antibody group was significantly higher than that of the untreated group ( $p < 0.01$ ). \* Statistically significant differences versus the sham group ( $p < 0.01$ ). \*\* Statistically significant differences versus the untreated group ( $p < 0.01$ ).



ated with the presence of chemical factors prevailing in NP more than the effect elicited by compression-derived mechanical factors. When compression and NP application were concurrently applied to the nerve root, a decrease in the nociceptive threshold of the hind limb<sup>27,33</sup> and a delay of conduction velocity of the nerve root with development of severe degeneration in nerve root tissues have been observed<sup>33,34</sup>. In our previous study, gene expression changes in the DRG induced by mechanical and chemical factors differed at 7 days after surgery using the NP model and MC model<sup>35</sup>. The upregulation of insulin-like growth factor 1 (IGF-1) might be a key factor in pain-related behavior induced by mechanical but not chemical factors. This result suggests that each factor induces pain-related behavior via a different mechanism. However, these mechanisms are still unknown, and further studies are needed to define them.

It has been reported that anti-TNF- $\alpha$  antibody reduces pain caused by lumbar disc herniation in both animal<sup>18,36</sup> and clinical studies<sup>15,16</sup>. Symptoms due to lumbar disc herniation were considered to be related to inflammatory cytokines (e.g., TNF- $\alpha$ ) derived from the NP<sup>12,31,37</sup>. We have reported the effect of a monoclonal anti-TNF- $\alpha$  antibody on improving pain-related behavior in the NP model<sup>36</sup>. Therefore, the treatment effect on pain-related behavior associated with mechanical factors was investigated using the MC model in this study. We found that a monoclonal anti-TNF- $\alpha$  antibody improved pain-related behavior in the MC group for 3 days. Homma et al. reported that the focal application of soluble TNF- $\alpha$  receptors on the DRG resulted in reduced mimic-allodynia in the chronic DRG compression model. However, the anti-allodynic effect only continued for the first 3 postoperative days during a 7-day application period<sup>22</sup>. This finding suggests that TNF- $\alpha$  may play an important role only in the early stages of the development of allodynia caused by DRG compression. In the present study, the effect of TNF- $\alpha$  was also temporary. Another study showed that anti-TNF- $\alpha$  antibody reduced allodynia only after onset of allodynia, whereas late administration of the anti-TNF- $\alpha$  antibody did not have an allodynic effect in the NP model<sup>36</sup>. One reason for the temporary effect is that the anti-TNF- $\alpha$  antibody used in the present study may have been rapidly metabolized by the rats. Another reason is that the stage in which TNF- $\alpha$  might play an important role may have occurred before day 10 post-surgery. One limitation of this study was that the effect of repeated

administration of the TNF- $\alpha$  antibody was not investigated. To consider a clinical situation, the effect of an anti-TNF- $\alpha$  antibody on both mechanical and chemical factors should be investigated further in an animal study. In the clinical situation, an anti-TNF- $\alpha$  antibody might also reduce painful symptoms associated with chronic compression of the DRG and nerve root as a result of lumbar canal stenosis. Future clinical studies will be necessary to determine if an anti-TNF- $\alpha$  antibody reduces symptoms caused by lumbar canal stenosis.

In conclusion, MC or NP application to the nerve root decreased the mechanical threshold at different time points. Differences in changes in the withdrawal threshold were not noted between the NP and MC+NP groups. Certain chemical factors in NP may play a critical role in the induction of pain experienced in disc herniation. A monoclonal anti-TNF- $\alpha$  antibody reduced allodynia caused by chronic compression of the DRG. In the clinical situation, an anti-TNF- $\alpha$  antibody might reduce painful symptoms associated with lumbar disc herniation and lumbar canal stenosis.

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