Experimental Trial

Changes in Neuroglial Activity in Multiple Spinal Segments after Caudal Epidural Pulsed Radiofrequency in a Rat Model of Lumbar Disc Herniation

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Background: Herniated lumbar discs can induce sciatica by mechanical compression and/or chemical irritation. It was recently reported that neuroglial cellular activity after pulsed radiofrequency (PRF) application to a single dorsal root ganglion (DRG) attenuated neuroglial activity at the corresponding spinal dorsal horn. Recently, caudal epidural PRF has been used to manage neuropathic pain, but evidence of molecular changes after the administration of caudal epidural PRF to attenuate neuropathic pain is lacking, and it has not been determined whether caudal epidural PRF affects neuroglial activity at different spinal levels.

Objectives: Using immunohistochemical methods in a rat model of lumbar disc herniation, the authors investigated the effects of caudal epidural PRF administration on pain-related behavior, on the activations of microglia and astrocytes in spinal cord, and on the expressions of calcitonin gene-related peptide (CGRP) and Transient receptor potential vanilloid 1(TRPV1) in the DRG at the L3, L4, L5, L6, and S1 levels.

Study Design: Controlled animal trial.

Setting: University hospital laboratory.

Methods: Forty-five Sprague-Dawley rats were randomly assigned to a sham-operated group (n = 10) or a nucleus pulposus (NP)-exposed group (n = 35). Rats in the NP-exposed group were further subdivided into a NP-exposed with sham stimulation group (the NP-nonPRF group; n = 13) or a NP exposed with caudal epidural PRF stimulation group (the NP-PRF group; n = 22). Pulsed radiofrequency was administered on postoperative day 10 (POD 10) by placing an electrode in the caudal epidural space through the sacral hiatus and administering 5 Hz of PRF current for 600 seconds (maximum tip temperature 42°C). Rats were tested for mechanical allodynia on POD 10 and on days 7 and 14 after caudal epidural PRF administration (post-PRF). At 14 days post-PRF, sections of the spinal cord from L3, L4, L5, L6, and S1 were immunostained for ionized calcium-binding adapter molecule 1 (Iba1) and glial fibrillary acidic protein (GFAP), and DRGs from the same levels were immunostained for CGRP and TRPV1.

Results: Mechanical withdrawal thresholds increased at 7 days post-PRF ($P = 0.04$), and the immunohistochemical expression of Iba1 in the L5 spinal dorsal horn and of CGRP in the L5 DRG were quantitatively reduced ($P < 0.001$) at 14 days post-PRF. Furthermore, the upregulations of Iba1 at L3, L4, L6, and S1 dorsal horns and CGRP at L6 DRG were also attenuated by caudal epidural PRF ($P < 0.001$).

Limitation: We examined molecular changes only in ipsilateral lumbar regions and at 14 days post-PRF.

Conclusion: Caudal epidural PRF reduced mechanical allodynia and downregulated microglia activity and CGRP expression at the lumbar disc herniated level and in adjacent lumbar spinal levels in a rat model of lumbar disc herniation.

Key words: Caudal, pulsed radiofrequency, multisegmental, lumbar disc herniation, microglia, calcitonin gene-related peptide

Lumbar disc herniation can cause injury to spinal nerve roots and severe radicular pain, characterized by hyperalgesia, allodynia, reduced conduction velocity, and histological changes (1). Radicular pain is one of the most common types of neuropathic pain, and is caused by chemical factors released by nucleus pulposus (NP) and by mechanical compression of a lumbar nerve root (1-6). Furthermore, it has been established that cytokines, such as interleukin-1 (IL-1) (7), interleukin-6 (IL-6) (8), interleukin-8 (IL-8) (8,9), tumor necrosis factor-α (TNF-α) (10-13), and cyclooxygenase-2 (COX-2) (14), are involved in NP-induced nerve root injury and associated radicular pain. Because steroids suppress the expressions of various inflammatory cytokines and chemokines (15), caudal epidural steroid injection (CESI) can be used to treat patients suffering from lumbar radicular pain (16). The CESI technique involves injecting steroids into the epidural space via the sacral hiatus, which is often preferred by non-anesthetists because it carries a lower risk of inadvertent thecal sac puncture or intrathecal injection (17,18). However, in clinical practice, many patients treated in this manner continue to complain of persistent neuropathic pain.

In addition to the inflammatory mediators described above, neuroglial cells, such as astrocytes and microglia in the spinal cord, are also activated after nerve injury and inflammation in dorsal root ganglions (DRGs) (19,20). Glial cells release proinflammatory cytokines that induce the proliferation of other glial cells, and the upregulations of these cytokines are known to be associated with nerve degeneration (21,22). Recent reports have shown a relationship exists between pain and glial activity in the central nervous system, by demonstrating that glial activity and inflammation after nerve injury produce hyperalgesia and allodynia (23,24).

In 1998, Sluijter et al (25) introduced an isothermal radiofrequency treatment—pulsed radiofrequency (PRF)—for the relief of chronic pain. It has been suggested that the electric field generated is responsible for the clinical effects of PRF rather than the temperature generated, and interestingly, PRF does not substantially destroy nerve tissue. It is thought likely that the thermal effects of PFR are of minor importance because only a small region around the electrode tip is affected as temperature rapidly diminishes with distance from the electrode. Furthermore, temperatures around the electrode shaft reportedly remain well below neurodestructive values, and thus, the mild tissue destruction caused by PRF probably results from the high electric fields around the electrode tip and shaft (26). Because of its minimally destructive effects on tissues, PRF has been developed and rapidly adopted in clinical practice. Thus, although the mechanisms underling its effects are poorly understood, the clinically demonstrated effectiveness of PRF makes it an alternative modality for the delivery of radiofrequency current (27-31). More recently, the effectiveness of PRF encouraged some clinicians to attempt the caudal route to manage patients with neuropathic pain. Rohof (32) described 3 cases where caudal epidural PRF was used for the management of post herpetic neuralgia and achieved remarkable long-lasting pain relief.

However, few clinical studies have investigated the effects of caudal epidural PRF on neuropathic pain, and little is known of the molecular changes induced by caudal epidural PRF used to treat this pain. Accordingly, we investigated the effects of caudal epidural PRF on pain-related behavior and molecular changes in a rat model of lumbar disc herniation by examining the expressions of ionized calcium binding adapter molecule 1 (Iba1), glial fibrillary acidic protein (GFAP), calcitonin gene-related peptide (CGRP), and transient receptor potential vanilloid 1 (TRPV1) in ipsilateral adjacent segments in a rat model of radicular pain.

Animals

Forty-five male Sprague-Dawley rats (200 – 250 g) were randomly assigned to either a sham-operated group (n = 10) or a NP-exposed group (n = 35). Rats were housed 2 per cage and had free access to water and food. All experiments were conducted in a humane manner in accordance with guidelines issued by the Institutional Animal Care and Use Committee.

Lumbar Disc Herniation and PRF Administration

Rats were anesthetized by injecting Zoletil (Virbac; 50 mg/kg, i.p.). With an animal placed prone, an incision of ~1 cm was made on the dorsal surface of the proximal tail for autologous NP harvesting. The disc between the second and third coccygeal vertebrae of the tail was incised and NP was harvested by curette. A midline dorsal incision was then made over the lumbar spine, multifidus muscles were separated along L4–S1 spinous processes, and left L5 nerve roots and DRGs were exposed through laminectomy. The harvested NP was then implanted next to the left L5 nerve root just proximal to its DRG without mechanical compression. Similar amounts of NP were implanted in all animals.

The sham procedure was performed in an identical manner and included autologous NP harvesting and nerve root exposure but not autologous NP implantation (33-36).

The 35 rats in the NP-exposed group were subdivided into a NP-exposed with sham stimulation group (the NP-nonPRF group; n = 13) or a NP-exposed with caudal epidural PRF stimulation group (the NP-PRF group; n = 22). We assigned more animals in the NP-PRF group to verify the effectiveness of the PRF more clearly. At 10 days after NP implantation, a PRF needle (Cosman RFG 1A generator (Cosman Medical, Inc., Burlington, MA, USA) was inserted at the sacral hiatus and advanced into the caudal epidural space. Correct placement of the PRF needle in the caudal epidural space was confirmed by fluoroscopy using a contrast dye (Fig. 1). After confirming correct needle placement in the caudal epidural space, PRF was administered by applying power at 5 Hz at a pulse width of 5 ms for 600 seconds (32). Currents and voltages were administered at intensities strong enough to elicit minimal tail muscle contraction (mean voltage, 33.0 volts [range, 12 – 52]). For rats in the NP-nonPRF group, electrode placement was conducted in precisely the same manner, but the machine was turned off and radiofrequency stimulation was not applied to the caudal canal.

Pain Behavior Evaluation

Mechanical allodynia of the plantar surfaces of ipsilateral hind paws was tested on postoperative day 10 (POD 10) and 7 and 14 days after caudal epidural PRF administration (post-PRF). Mechanical allodynia was determined by measuring withdrawal response to mechanical stimulation of ipsilateral hind paws with von Frey filaments (North Coast Medical, Inc. North Coast Medical, Inc., Gilroy, CA, USA), which had been calibrated in grams. Rats were placed individually in a clear plastic cage with a metal mesh floor and allowed to adapt to the test environment for 30 minutes. The plantar surface of each hind paw was then stimulated sufficiently to cause slight filament bending for 5 seconds. Filaments were applied in increasing and decreasing thicknesses, until a filament produced a consistent withdrawal response to more than 3 of 5 stimuli. Probability thresholds (50%) of mechanical paw withdrawal were calculated. If no withdrawal response was elicited by the 26 g filament, the mechanical threshold was assigned as 26 g.

Immunohistochemical Examination

To determine the effects of caudal epidural PRF administration on microglial and astrocytic activation in the dorsal horn and CGRP and TRPV1 expressions in DRGs, we euthanized all 35 rats in the NP-nonPRF and NP-PRF groups at 14 days post-PRF. Under anesthesia, a catheter was inserted into

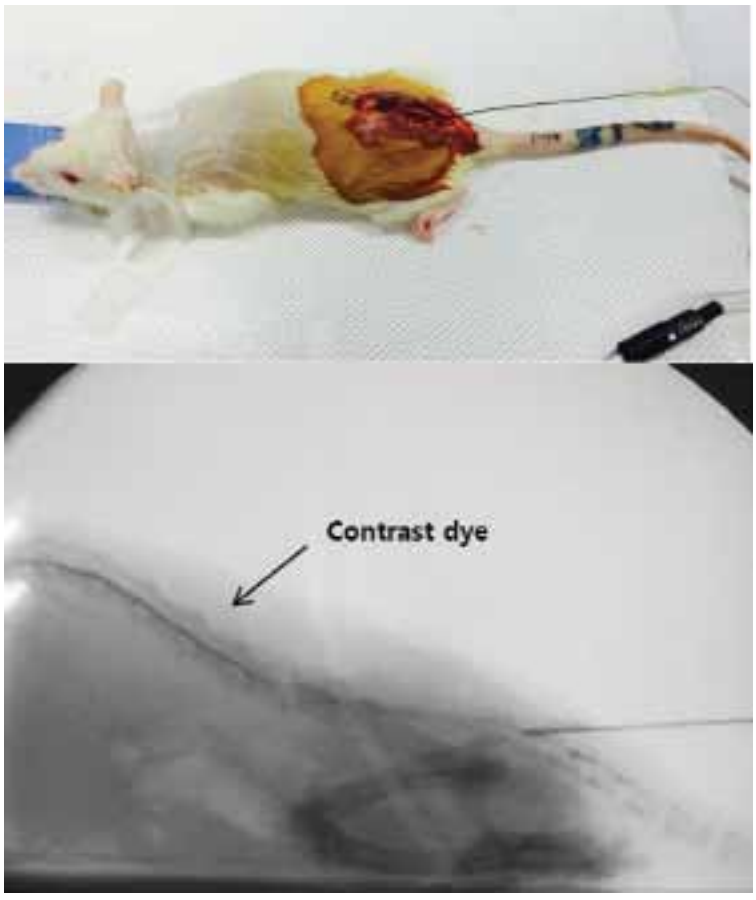

Fig. 1. *Fluoroscopic confirmation of caudal epidural space using contrast.*

the left ventricle, which was then rinsed with 500 mL of saline and fixed with 500 mL of 4% paraformaldehyde (in 0.1 N phosphate buffer [PB]). Spinal cords from L3 to S1 level were removed, post-fixed for 2 days in the same fixative, and stored in 30% sucrose (in PB) for at least 24 hours. Transverse sections (30 μm) of spinal cords and of DRGs (20 μm) were prepared using a cryostat (Leica, Wetzlar, Germany) and stored in PB. All incubation and reaction procedures for multiple immunohistochemical staining were performed at room temperature on a shaker. To enhance antibody penetration into tissues, DRG sections were immersed in 50% ethanol for 30 minutes and rinsed with phosphate buffered saline (PBS; 3x5 minutes). To block nonspecific primary antibody reactions, samples were treated with 10% normal donkey serum (NDS; Jackson Immunoresearch, Westgrove, PA, USA). Tissue sections were incubated overnight in a mixture of the following primary antibodies: mouse anti-ionized calcium-binding adapter molecule 1 (Iba1) (Wako, Japan; 1:1000), mouse anti-glial fibrillary acidic protein (GFAP) (BD Pharmingen, USA; 1:100), anti-transient receptor potential vanilloid type 1 (TRPV1) (Neuromics, Edina, MN, USA; 1:5000), and anti-calcitonin gene related peptide (CGRP) (Enzo, Farmingdale, NY, USA; 1:200). Sections were then rinsed with PBS (3x5 minutes), treated with 2% NDS for 15 minutes, incubated with cy3-conjugated donkey anti-mouse (Jackson Immunoresearch, PA, USA, 1:100), cy3-conjugated donkey anti-goat (Jackson Immunoresearch, PA, USA, 1:100), and Alexa 488-conjugated donkey anti-rabbit (Invitrogen, Eugene, OR, USA, 1:200) antibodies for 3 hours, rinsed with PBS, and mounted using Vectashield (Vector Lab, Burlingame, CA, USA). All antibodies were tested for sensitivity and specificity before the study and were used at manufacturers' recommended dilutions. Immunofluorescent images were acquired using a cooled charge-coupled device (CCD) camera (Olympus DP71, Japan) attached to a light microscope (Olympus BX51, Japan).

Quantitative Image Analysis

To analyze immunoreactions of Iba1 and GFAP in dorsal horns and of CGRP and TRPV1 in DRGs quantitatively, we obtained images from 5 spinal cord sections (for Iba1 and GFAP) from L3, L4, L5, L6, and S1 segments and of 5 DRG sections (for CGRP and TRPV1) from L3, L4, L5, L6, and S1 DRGs per rat. One image (898 X 660 μm) was acquired of each spinal cord section using a CCD camera using the same shutter speed and digital gain. Images were encoded in order to blind the investigator before analysis. Pixels positive for Iba1 and GFAP immunoreactions were segmented by applying an appropriate threshold gray value and area fractions (segmented area/total frame area) were calculated using image analysis software (Leica application suite V4.2, Leica Microsystems, Switzerland). For CGRP and TRPV1, numbers of CGRP- and TRPV1-postive DRG cells were counted. Then, relative area fractions of Iba1 and GFAP immunoreactions and relative cell counts of CGRP and TRPV1-positive DRG cells in ipsilateral L3, L4, L5, L6, and S1 spinal levels of the experimental groups versus L5 level of the sham-operated group were calculated in percentages.

Statistical Analysis

Characteristics and outcomes were summarized using descriptive analysis, quantitative variables are presented as means and standard deviations (SDs) and qualitative variables as frequencies and percentages. Group comparisons of pain behavior evaluations and of Iba1, GFAP, CGRP, and TRPV1 expressions were made using one-way ANOVA when normally distributed or the Kruskal Wallis test when not normally distributed. Multiple comparisons were performed using the Scheffe method. Comparison of pain behavior evaluation results, expressions of Iba1, GFAP, CGRP, and TRPV1 in the NP-nonPRF group and NP-PRF group were analyzed using the 2 sample t-test when normally distributed or the Mann Whitney U test when not normally distributed. *P*-values are provided for statistically significant differences. All tests were 2-sided and P-values of < 0.05 were deemed significant. The analysis was conducted using IBM SPSS ver. 19.0.

Results

Pain Behavior

The mean (SD) mechanical withdrawal thresholds of the NP-exposed group on postoperative day 0 (POD 0) was 22.7 g (5.3). The mechanical withdrawal thresholds of rats with lumbar disc herniation were significantly decreased on ipsilateral sides on POD 10. For rats in the NP-PRF group, mechanical allodynia of ipsilateral hind paws was significantly attenuated at 7 days post-PRF ($P = 0.04$), and tended to be reduced at 14 days after post-PRF ($P = 0.07$). On the other hand, in the NP-nonPRF group, pain was sustained on ipsilateral sides (Fig. 2).

Microglia, Astrocytes, CGRP, and TRPV1

Immunohistochemical examination of L5 dorsal horns for Iba1 at 14 days post-PRF revealed immunostaining for microglia was elevated through lamina I-V, prominent at lamina II and III in the NP-nonPRF group, but significantly attenuated at whole dorsal horn in the NP-PRF group ($P < 0.001$) (Fig. 3). Immunoreactivity for CGRP at L5 DRG at 14 days post-PRF also revealed that CGRP-positive cells were elevated in the NP-nonPRF group, but significantly attenuated in DRGs in the NP-PRF group ($P < 0.001$) (Fig. 4). Moreover, at 14 days post-PRF, increased Iba1 and CGRP expressions were also observed at L3, L4, L6, and S1 in the NP-nonPRF group, but increased Iba1 expressions were significantly lower in the ipsilateral L3, L4, L6, and S1 dorsal horns in the NP-PRF group ($P < 0.001$) (Fig. 3). Furthermore, CGRP expression in ipsilateral L6 DRG was also significantly lower in the NP-PRF group ($P < 0.001$) (Fig. 4). Immunostaining for GFAP in the dorsal horn at 14 days post-PRF tended to be lower at whole laminae in the NP-PRF group than in the NP-nonPRF group ($P > 0.05$) (Fig. 5). TRPV1 expressions in DRGs at day 14 post-PRF were not significantly different in the NP-nonPRF and NP-PRF groups ($P > 0.05$) (Fig. 6).

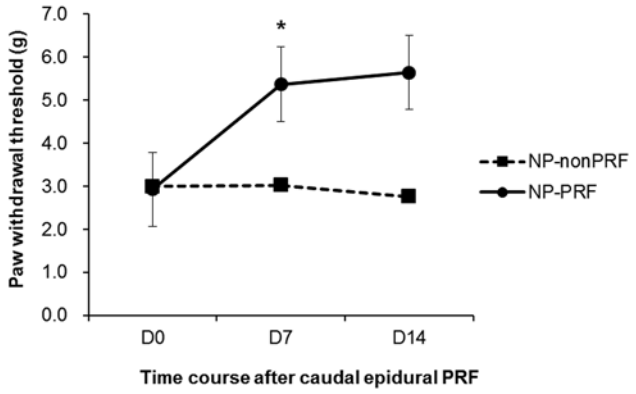

Fig. 2. *Effects of caudal epidural pulsed radiofrequency (PRF) on mechanical hyperalgesia in a rat model of lumbar disc herniation, induced by implantation of NP at the left L5 nerve root. Caudal epidural PRF was administered 10 days after surgery (D0). Withdrawal thresholds of ipsilateral paws were significantly reduced 7 days after caudal PRF administration and showed reduced tendencies until 14 days after PRF administration. D = days, NP-nonPRF = NP-exposed with sham stimulation; NP-PRF = NP-exposed with caudal epidural PRF. * $P < 0.05$.*

Discussion

In a rat model of lumbar disc herniation, mechanical withdrawal thresholds were increased at 7 days post-PRF. In addition, the multisegmental upregulation of Iba1 positive microglia in dorsal horns was attenuated in ipsilateral L3, L4, L5, L6, and S1 dorsal horns post-PRF. Furthermore, multisegmental increases in CGRP expression were also attenuated in ipsilateral L5 and L6 DRGs post-PRF.

Radicular pain caused by disc herniation is mediated by chemical and mechanical factors, which are referred to as primarily inflammatory mediators (1,3,4,37,38). Furthermore, it has been proposed that cytokines and chemokines play major roles in the chemical pathomechanisms of radicular pain (9,11,39). In general, corticosteroids are believed to suppress various inflammatory cytokines and chemokines, and clinically, transforaminal epidural injection of corticosteroids are commonly administered under fluoroscopy and CESI to patients with lumbar radicular pain (16,40-45). However, some patients continue to experience persistent neuropathic pain. Recently, PRF was advocated for the treatment of acute and chronic neuropathic pain of spinal nerve root origin (28,31,46). In a previous study, we showed PRF administration to the DRG reduced mechanical allodynia and downregulated microglia activity and pERK expression in the spinal dorsal horn in a rat model of lumbar disc herniation (46). Recently, caudal route administration of PRF has been used to manage severe neuropathic pain. Initially, caudal epidural PRF was used to control coccygeal pain. Atim et al (47) reported that in patients with coccygodynia unresponsive to classic treatment protocols, the caudal epidural PRF method achieved long-term reductions in pain scores. Interestingly, Rohof (32) suggested caudal epidural PRF produced remarkable longer-lasting pain relief in dermatomes far removed from sacral segments. More specifically, caudal epidural PRF resulted in pain relief in patients with chronic neuropathic pain (one patient with failed back surgery syndrome and 2 patients with Complex Regional Pain Syndrome (CRPS), and he also reported caudal epidural PRF provided immediate pain relief in 2 of 3 patients with post herpetic neuralgia. However, the numbers of patients included in these previous reports were limited and the mechanisms underlying the efficacy of caudal epidural PRF treatment for neuropathic pain control remained unclear.

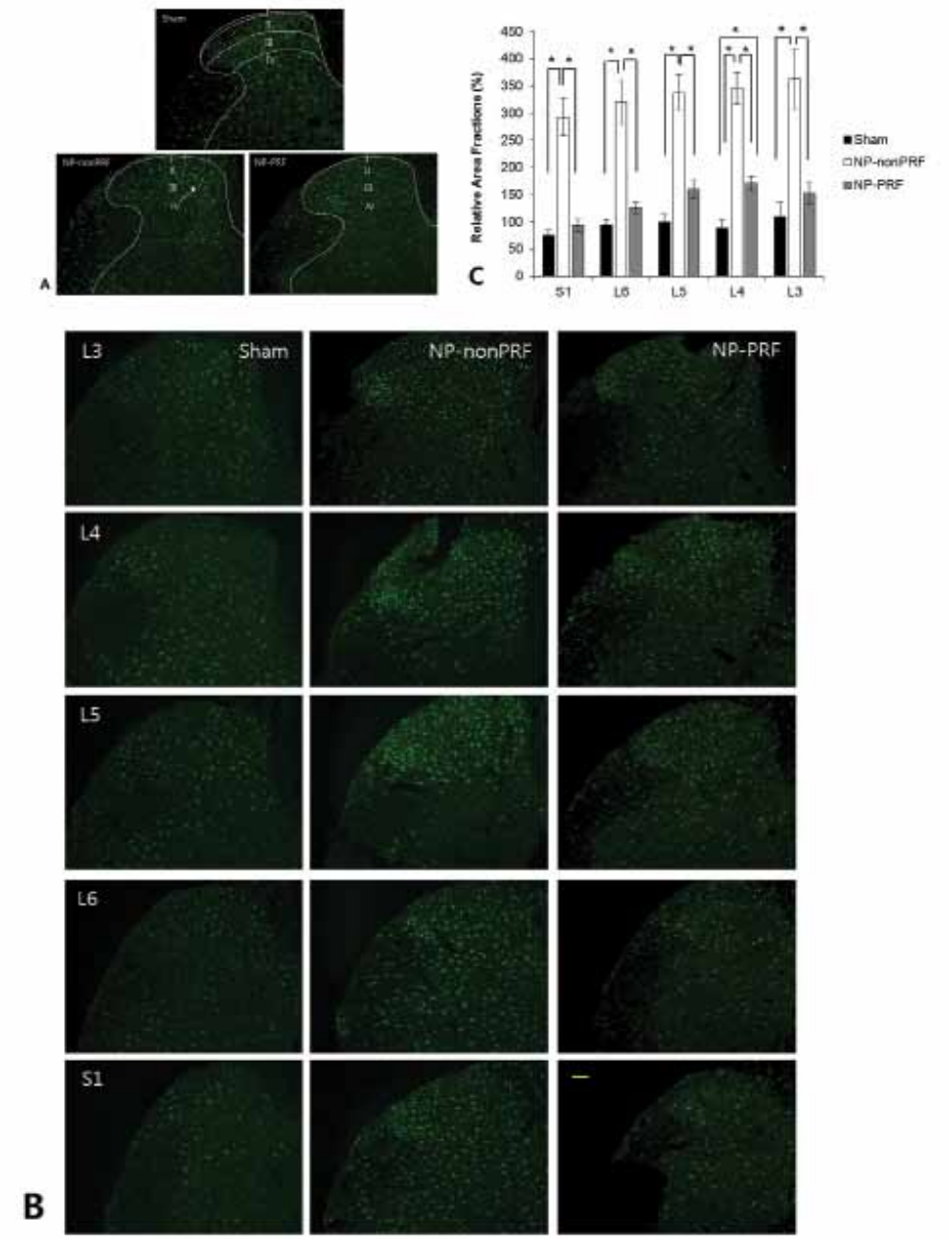

Fig. 3. *Immunohistochemical staining for Iba1 in ipsilateral dorsal horns at 14 days after caudal epidural pulsed radiofrequency (PRF) application. Changes of Iba1 immunoreactivity at L5 level of spinal cord of sham, NP-nonPRF, and NP-PRF animals (A), multi-segmental changes of Iba1 immunoreaction in the spinal dorsal horn at L3, L4, L5, L6, and S1 spinal levels (B), and the area ratio for Iba1 immunoreaction in dorsal horn (C). A: Immunoreactivity for Iba1 was increased in dorsal horns through lamina I-V in NP-nonPRF group and it was prominent at lamina II-III (arrow). Increase of Iba1 immunoreactivity was attenuated at whole dorsal horn after PRF application. B: Increase of Iba1 immunoreactivity in NP-nonPRF and attenuation in NP-PRF group was observed in the dorsal horn through the cord level of L3 to S1. C: In the NP-PRF group, relative area fractions of Iba1 immunoreactions were significantly lower in L5 dorsal horns (the NP implantation level) and also in ipsilateral L3, L4, L6, and S1 dorsal horns than in the NP-nonPRF group. Results are presented as means ± SEMs. *P < 0.05; Bar = 100 μm*

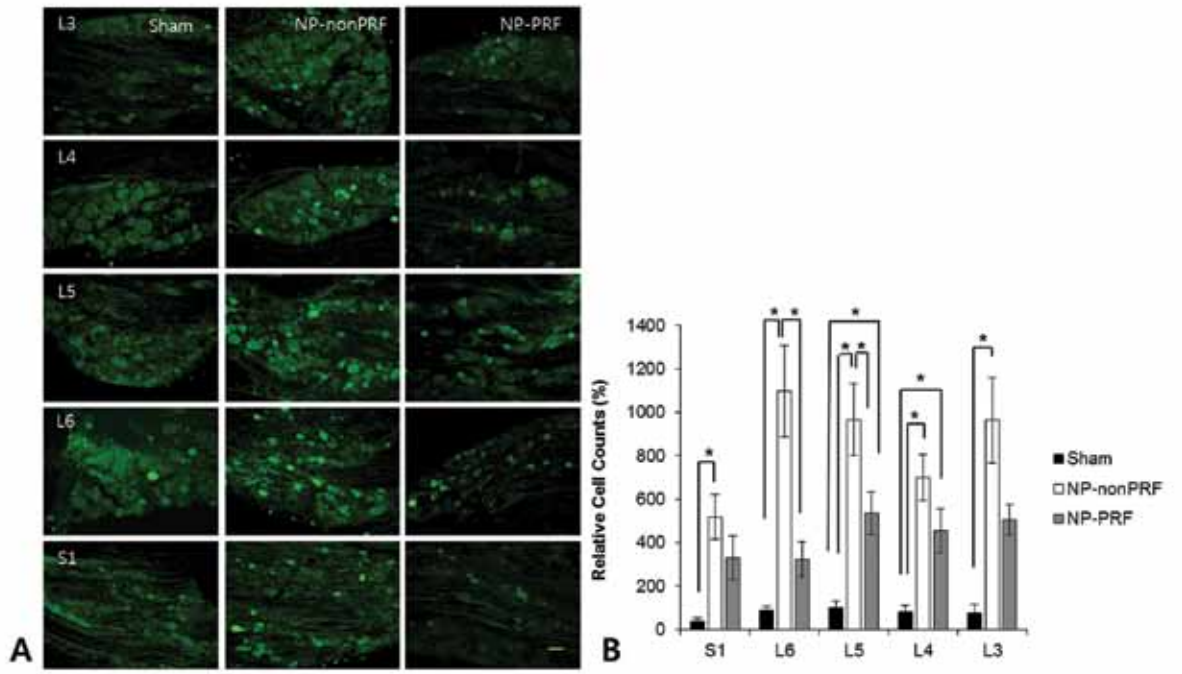

Fig. 4. *Immunohistochemical staining for CGRP in ipsilateral dorsal root ganglia (DRG) at 14 days after caudal epidural pulsed radiofrequency (PRF) application. Multi-segmental changes of CGRP immunoreaction in DRG at L3, L4, L5, L6, and S1 spinal levels (A) and relative cell counts of CGRP-positive DRGs (B). A: CGRP expression showed decreasing tendencies in DRGs at multi-segmental in the NP-PRF group than in the NP-nonPRF group. B: Relative cell counts of CGRP-positive DRGs were significantly lower in L5 DRG (the NP implantation level) and in ipsilateral L6 DRG in the NP-PRF group than in the NP-nonPRF group. Results are presented as means ± SEMs. *P < 0.05; Bar = 100 μm*

In the present study, we sought to identify neuroglial changes in dorsal horns and DRGs at multiple segments in the lumbar spine and to provide an explanation for the pain relief documented after caudal epidural PRF administration by using a rat model of radicular pain.

Microglia are the resident macrophages of the central nervous system (CNS) and contribute to the development of chronic neuropathic pain by releasing a variety of mediators, including proinflammatory cytokines and chemokines that influence pain signaling (48-50). It has been suggested in previous studies that NP application induces glial activity in the spinal cord and that these activated glia might play a crucial role in pain transmission in the spinal dorsal horn (34,51,52). In the present study, immunoreactivity for Iba1 was increased in ipsilateral L5 dorsal horns through lamina I-V, predominant at lamina II and III in the NP-nonPRF group. Increase of Iba1 immunoreactivity was attenuated at whole dorsal horns after caudal epidural PRF application. Our study showed the same results that nociceptive neurons in the superficial laminae of the dorsal horn play an important role in the processing of peripheral noxious stimuli. Higuchi et al (53) reported that exposure of the DRG in rats to PRF currents showed a significant increase in c-Fos-immunoreactive neurons in the superficial laminae I and II, and a few c-Fos-immunoreactive cells also were found in lamina V. Interestingly, our study showed that the expression of Iba1-positive microglia were also obviously lower in L3, L4, L6, and S1 dorsal horns post-PRF. Furthermore, similar results were obtained for CGRP. These multisegmental CGRP increases were reduced post-PRF in ipsilateral L5 and ipsilateral L6 DRGs. Recently, it was reported that the effects of NP on nerve roots are closely associated with cytokines such as TNF-α and COX-2 (5,14). TNF-α induces the production of inflammatory neuropeptides, such as substance P (SP) and CGRP, and induces the release of SP and CGRP from peripheral terminals of the

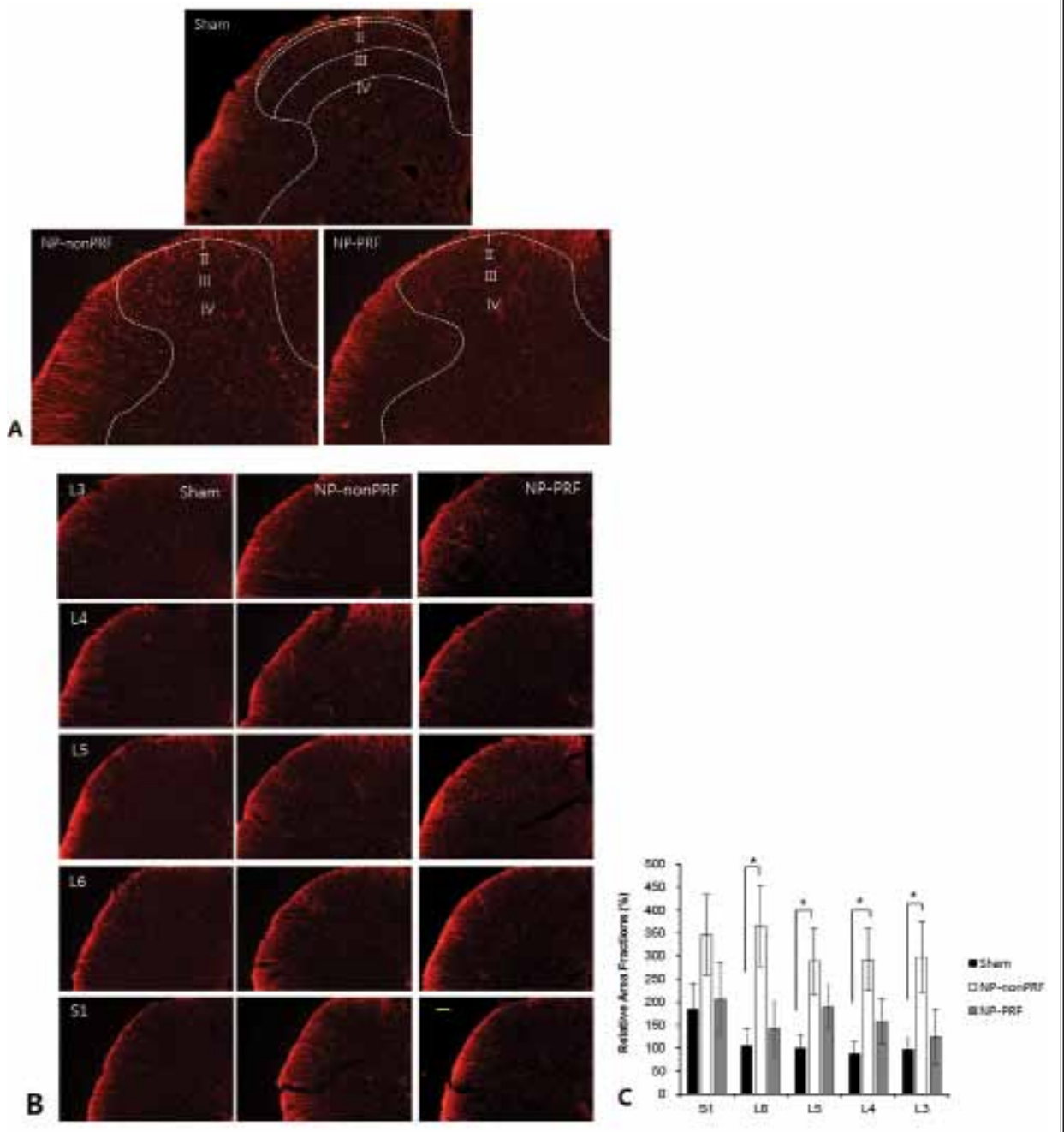

Fig. 5. *Immunohistochemical staining for GFAP in ipsilateral dorsal horns at 14 days after caudal epidural pulsed radiofrequency (PRF) application. Changes of GFAP immunoreactivity at L5 level of spinal cord of sham, NP-nonPRF, and NP-PRF animals (A), multi-segmental changes of GFAP immunoreaction in the spinal dorsal horn at L3, L4, L5, L6, and S1 spinal levels (B), and the area ratio for GFAP immunoreaction in dorsal horn (C). A-B: Immunoreactivity for GFAP was increased in whole laminae of dorsal horns at L3-S1 segments in NP-nonPRF group and decreased after PRF application. C: GFAP immunoreactivity tended to decrease, but no significant difference was found between the NP-nonPRF group and the NP-PRF group. Results are presented as means ± SEMs; Bar = 100 μm*

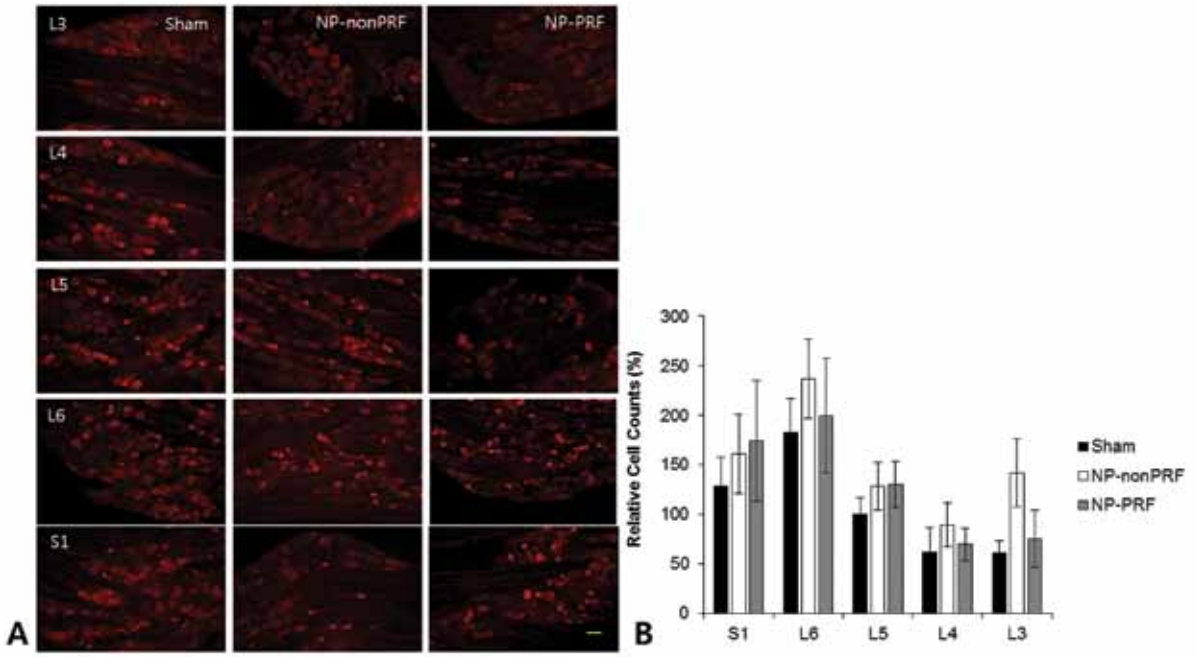

Fig. 6. *Immunohistochemical staining for TRPV1 in ipsilateral dorsal root ganglia (DRG) at 14 days after caudal epidural pulsed radiofrequency (PRF) application. Multi-segmental changes of TRPV1 immunoreaction in DRG at L3, L4, L5, L6, and S1 spinal levels (A) and relative cell counts of TRPV1-positive DRGs (B). TRPV expressions in the NP-PRF and NP-nonPRF groups were no significant different from that in the sham-operated group. Results are presented as means ± SEMs;* Bαρ = *100 μm*

dorsal horn (54,55). CGRP is a marker of sensory neurons that are mainly involved in pain perception. Moreover, these observed downregulations of microglia and CGRP with time after caudal epidural PRF administration followed a course similar to that of pain behavior attenuation, which suggests they may be responsible for the analgesic effect of caudal epidural PRF, and that the mechanism responsible for reductions of radicular pain caused by lumbar disc herniation involves reduced neuroglial expression in spinal segments.

We suggest molecular changes in adjacent lumbar spinal segments perturb synaptic homeostasis in our neuropathic pain model and that caudal epidural PRF attenuates these perturbations. It has been previously reported glial changes following peripheral nerve injury are associated with increased sprouting of primary afferent nociceptive fibers (C and A-δ fibers) entering the spinal cord (56), morphological changes in nerve myelination and DRG architecture (57), and with the down-regulations of glial amino acid transporters (58,59), and that these morphologic and molecular structural changes underlie the relation between neuro-glial plasticity changes and peripheral sensitization and induce adaptive plasticity facilitating neuropathic pain transmission (60,61). Furthermore, superficial laminae of dorsal horns of the spinal cord represent nodal points for the modulation and integration of peripheral sensory stimuli through complex networks involving glutamate receptors and local inhibitory GABAergic interneurons (62). During our studies, we found that lumbar disc herniation upregulated microglial activity and CGRP expression in many adjacent and ipsilateral lumbar spinal segments (63). Cirillo et al (64) observed the onset of reactive gliosis following spared nerve injury (as evidenced by increases in Iba1 and GFAP) was paralleled by remarkable changes in the expressions of glial and neuronal neurotransmitter transporters, as indicated by down-regulations of glial amino acid transporters and up-regulations of neuronal glutamate transporter, neuronal vesicular GABA transporter, and the GABAergic neuron marker. In addition, the authors found relations between reactive astrogliosis and mechanisms underlying the perturbation of synaptic circuitry in a peripheral nerve injury model (64), and notably,

these molecular changes were substantially reduced by caudal epidural PRF administration. Moreover, the decrease of microglial activation may contribute to the synaptic modulation at the synapse and induce expression of c-Fos protein in neurons of substantia gelatinosa which are the main neurons for pain modulation (65). Caudal epidural PRF also reduces mechanical allodynia, which is a hallmark of neuropathic behavior following nerve injury. Hagiwara el al (66) suggested that the analgesic action of PRF involved the enhancement of noradrenergic and serotonergic descending pain inhibitory pathways. They found the analgesic effects of PRF were significantly inhibited by intrathecal administration of the alpha 2-adrenoceptor antagonist and the serotonergic receptor antagonists (66). Thus, we suggest that the beneficial effect of caudal epidural PRF administration is due to reductions in reactive neuroglia levels and the restoration of synaptic homeostasis after lumbar disc herniation. We also conjecture that caudal epidural PRF may partially enhance the descending inhibitory pathways and reduce the mechanical allodynia.

In the present study, we found no significant difference between GFAP immunoreactivities in the NP-PRF and NP-nonPRF groups. Several studies have reported astrocyte activation and GFAP expression in various animal models of neuropathic pain (67-69). Li et al (70) explored GFAP expression in bilateral L5 DRGs and spinal cords using an immunohistochemical approach after applying NP to left L5 DRGs, and observed GFAP-immunoreactive astrocytes in bilateral spinal cord dorsal horns but no significant difference between GFAP expressions in their NP and sham groups or in ipsilateral and contralateral DRGs. In a previous study using in a rat model of lumbar disc herniation, we found reactive astrocytes with thickened processes in the dorsal horn after NP implantation were unaffected by PRF administration, although mechanical allodynia was significantly attenuated (46). TRPV1 receptors have been shown to be molecular integrators of nociceptive stimuli at peripheral nerve endings, but their roles in the modulation of synaptic transmission at the spinal cord level remain unresolved (71). TRPV1 has been localized to small-diameter, unmyelinated C-fibers and medium-diameter, thinly myelinated A-δ fibers in DRGs (72,73), and spinal TRPV1 receptors have been shown to play important roles in the modulation of nociceptive transmission, especially under pathologic conditions (74-77). In the present study, TRPV1 expression was not significantly attenuated by caudal epidural PRF administration, and thus, we suggest astrocytes and TRPV1 did not play a major role in the molecular changes underlying pain-related behavior in our rat model of disc herniation.

Conclusion

Summarizing, our results regarding pain relief of neuropathic pain by caudal epidural PRF administration corroborate previously published results. At 7 and 14 days post-PRF, mechanical allodynia was diminished as determined by withdrawal thresholds. We also found microglial activation and CGRP expression were attenuated by caudal epidural PRF administration at adjacent lumbar spinal levels as well as at the lumbar disc herniation level. To the best of our knowledge, this is the first report to describe molecular changes in the dorsal horn and DRG after caudal epidural PRF administration in a model of radicular pain. However, we observed molecular changes only in ipsilateral lumbar regions and at 14 days post-PRF. Our results suggest caudal epidural PRF administration downregulates microglial activity and CGRP expression at multiple lumbar segments and that these downregulations are possibly correlated with pain attenuation. More detailed study of the mechanism responsible for this attenuation of radicular pain by caudal epidural PRF administration is needed.

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