WFC Award Winning Paper

Attenuation Effect of Spinal Manipulation on Neuropathic and Postoperative Pain Through Activating Endogenous Anti-Inflammatory Cytokine Interleukin 10 in Rat Spinal Cord

Xue-Jun Song, MD, PhD,[a] Zhi-Jiang Huang, PhD,[b] William B. Song,[c] Xue-Song Song, MD, PhD,[b] Arlan F. Fuhr, DC,[a] Anthony L. Rosner, PhD,[d] Harrison Ndtan, PhD,[e] and Ronald L. Rupert, DC[d]

Abstract

Objectives: The purpose of this study was to investigate roles of the anti-inflammatory cytokine interleukin (IL) 10 and the proinflammatory cytokines IL-1β and tumor necrosis factor α (TNF-α) in spinal manipulation–induced analgesic effects of neuropathic and postoperative pain.

Methods: Neuropathic and postoperative pain were mimicked by chronic compression of dorsal root ganglion (DRG) (CCD) and decompression (de-CCD) in adult, male, Sprague-Dawley rats. Behavioral pain after CCD and de-CCD was determined by the increased thermal and mechanical hypersensitivity of the affected hindpaw. Hematoxylin and eosin staining, whole-cell patch clamp electrophysiological recordings, immunohistochemistry, and enzyme-linked immunosorbent assay were used to examine the neural inflammation, neural excitability, and expression of c-Fos and PKC as well as levels of IL-1β, TNF-α, and IL-10 in blood plasma, DRG, or the spinal cord. We used the activator adjusting instrument, a chiropractic spinal manipulative therapy tool, to deliver force to the spinous processes of L_5 and L_6.

Results: After CCD and de-CCD treatments, the animals exhibited behavioral and neurochemical signs of neuropathic pain manifested as mechanical allodynia and thermal hyperalgesia, DRG inflammation, DRG neuron hyperexcitability, induction of c-Fos, and the increased expression of PKCγ in the spinal cord as well as increased level of IL-1β and TNF-α in DRG and the spinal cord. Repetitive Activator-assisted spinal manipulative therapy significantly reduced simulated neuropathic and postoperative pain, inhibited or reversed the neurochemical alterations, and increased the anti-inflammatory IL-10 in the spinal cord.

Conclusion: These findings show that spinal manipulation may activate the endogenous anti-inflammatory cytokine IL-10 in the spinal cord and thus has the potential to alleviate neuropathic and postoperative pain. (J Manipulative Physiol Ther 2016;39:42-53)

Key Indexing Terms: *Trauma; Nervous System; Ganglia; Spinal; Pain; Spinal manipulation; Interleukin-10; Interleukin-1beta*

Injury and inflammation to the nerve and tissues within or adjacent to the lumbar intervertebral foramen (IVF) can cause a series of pathologic changes, which may contribute to the pathogenesis of chronic low back pain.[1-6] After injury or inflammation, chemical factors (eg, cytokines, nerve growth factors, inflammatory mediators) release, activate, or change the properties of the dorsal root ganglion (DRG) neurons within the IVF and spinal dorsal horn neurons. These changes may contribute to chronic pain.[4,5,7-11] To better understand the mechanisms of low back pain due to nerve injury and IVF inflammation, we previously developed an animal model of chronic compression of DRG (CCD)[4,12] and an IVF inflammation model produced by in vivo delivery of inflammatory mediators into the IVF at L_5.[13-15] Rats with

[a] Professor, Parker University, Parker Research Institute, Dallas, TX.
[b] Research Scientist, Parker University, Parker Research Institute, Dallas, TX.
[c] (Volunteer) Research Assistant, Parker University, Parker Research Institute, Dallas, TX.
[d] Research Consultant, Parker University, Parker Research Institute, Dallas, TX.
[e] Associate Professor, Parker University, Parker Research Institute, Dallas, TX.
Submit requests for reprints to: Xue-Jun Song, MD, PhD, Professor, Parker Research Institute, 2540 Walnut Hill Lane, Dallas, TX 75229. (e-mail: *song@parker.edu*).
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CCD or IVF inflammation at L_4 and/or L_5 exhibited measurable pain and hyperalgesia, and the affected DRG neurons became more excitable. Mechanisms underlying chronic pain remain elusive, and the effective clinical approaches for reliving chronic pain are very limited.

Spinal manipulative therapy (SMT) has been recognized as an effective approach for reliving certain chronic pain and used for treating patients with chronic pain syndromes such as low back pain.[16-18] Mechanisms underlying the clinical effects of SMT are poorly understood but are thought to be related to mechanical, neurophysiologic, and reflexogenic processes.[16-20] In addition to traditional manual SMT, instruments such as the activator adjusting instrument (AAI) have been used to produce spinal mobilization.[21] The AAI was developed to precisely control the speed, force, and direction of the adjustive thrust to produce a safe, reliable, and controlled force for manipulation of osseous spinal structures.[22,23] Activator evolved in response to currently knowledge in biomechanical and neurophysiologic categories of investigation.[21,24,25] We have previously demonstrated the treatment effects of SMT as performed using the AAI (Activator-assisted spinal manipulative therapy [ASMT]) on pain and hyperalgesia produced by DRG inflammation using the IVF inflammation model in adult rats with outcomes being assessed through behavioral, electrophysiological, pathologic, molecular biological approaches.[15] However, the mechanisms underlying the ASMT-induced analgesic effects remain unknown.

The purpose of this study was to examine the possible mechanisms that may underlie ASMT-induced analgesic effect using a small animal model of CCD and relief of CCD (decompression of CCD [de-CCD]). This study investigated if repetitive ASMT could suppress neuropathic pain after CCD and the postoperative pain after de-CCD, reduce the increased excitability of CCD and de-CCD DRG neurons, attenuate the DRG inflammation, and inhibit induction of c-Fos and expression of PKC in the spinal dorsal horn.

Methods

Animals

All experimental procedures were conducted in concordance with the recommendations of the International Association for the Study of Pain and the National Institute of Health Guide for the Care and Use of Laboratory Animals. The procedures were reviewed and approved by the Institutional Animal Care Committee, Parker University Research Institute. Adult, male, Sprague-Dawley rats (200-250 g weight at start of the experiment, n = 96) were used in this study. They were housed in groups of 4 to 5 in 40 × 60 × 30 cm plastic cages with soft bedding and free access to food and water under a 12-/12-hour day/night cycle. The rats were kept 3 to 5 days under these conditions before and up to 28 to 35 days for some animals after surgery. All surgeries were done under anesthesia induced by sodium pentobarbital (40 mg/kg, intraperitoneal injection, supplemented as necessary).

Models of CCD and de-CCD

The CCD was mimicked by surgically implanting a stainless steel rod unilaterally into the intervertebral foramen at L_4 and L_5. The procedure was modified from what we have previously described.[12] In brief, 48 rats were anesthetized, and a midline incision was made from L_4 to L_6. On the left side, the paraspinal muscles were separated from the mammillary process and the transverse process, and the L_4 and L_5 IVF was exposed. A fine, sharp, stainless steel needle, 0.4 mm in diameter with a right angle to limit penetration, was inserted approximately 4 mm into the IVF at L_4 and L_5, at a rostral direction at an angle of approximately 30° to 40° to the dorsal mid line and −10° to −15° to the vertebral horizontal line. Once the needle was withdrawn, a stainless steel rod, L shaped, 4 mm in length and 0.6 mm in diameter, was implanted into the IVFs. The insertion was guided by the mammillary process and the transverse process and oriented as described for the needle. As the rod was moved over the ganglion, the ipsilateral hind leg muscles typically exhibited 1 or 2 slight twitches. After the rods were in place, the muscle and skin layers were sutured.

We observed pain behavioral changes as well as the accompanied pathologic, cellular, molecular biological changes after relief of DRG compression, that, decompression of CCD (de-CCD) (n = 24 of 48 CCD rats), which was mimicked by withdrawing the previously inserted rods (de-CCD). The protocol of de-CCD was similar to that we have described.[4] The rats that previously received CCD were again anesthetized, the paraspinal muscles separated, and L_4 and L_5 IVF exposed. We carefully found and examined the location of the rod previously implanted. As the rod was gently touched, the ipsilateral hind leg muscles exhibited slight twitches as well. The rod was then carefully withdrawn, and the wound was sutured. The rod was withdrawn from 24 rats on the 10th day after surgery. We presumed that IVF volume reduction and DRG compression induced by a rod insertion were restored and relieved, respectively, after the rod withdrawal. Surgical sham control (Sham) was performed in a separate 24 rats. The surgical procedure was identical to that described in CCD model but without needle stick or rod insertion. An oral antibiotic, augmentin, was administered in the drinking water for each rat (7.52 g in 500 mL) after surgery for 7 days.

Activator-Assisted Spinal Manipulative Therapy

The AAI, delivers short-duration (<0.1 ms) mechanical force, manually assisted spinal manipulative thrusts. The