Cyclooxygenase-2 Inhibitor SC-236 Attenuates Mechanical Allodynia Following Nerve Root Injury in Rats


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Summary: Low back pain is a common problem, affecting approximately two-thirds of the adult population. Of these individuals, a significant percentage will exhibit symptoms of radicular pain or sciatica. The purpose of this study was to determine the effect of one systemic (2 mg/kg) or intrathecal (0.2 mg/kg) dose of a selective cyclooxygenase-2 inhibitor (SC-236) in decreasing existing mechanical allodynia in a rat model of radiculopathy. Gait disturbance and mechanical allodynia (increased response to non-noxious von Frey monofilament stimuli) were assessed daily until the rats were killed 7 days after surgery. Robust mechanical allodynia developed in the rats in all groups except for those in the sham group by day 1 after surgery. Mechanical allodynia was significantly lower in the rats that received the systemic or the intrathecal dose of SC-236 than in those in the vehicle control group (analysis of variance followed by Bonferroni multiple comparison test, \( p = 0.002 \)). The intrathecal drug route of administration produced greater attenuation in allodynia than the systemic dose, supporting a central mechanism of action of the cyclooxygenase-2 inhibitor (\( p = 0.002 \)). The hypothesis that cyclooxygenase-2 is involved in spinal nociceptive processing after a nerve root injury was supported by this study. In addition, these data support continued basic science research to further elucidate central inflammatory processes that follow nerve root injury.

Currently, radicular pain in humans is managed by surgical, physical, and pharmacological interventions with no accepted standard treatment. To understand the pathophysiological mechanisms underlying radiculopathy, animal models of nerve root injury have been developed (13,24,26,28,32,33). In this study, we sought to determine whether systemic or spinal administration of a selective cyclooxygenase-2 inhibitor, SC-236, decreases mechanical allodynia (a behavioral measure of persistent pain in which an increased sensitivity to non-noxious stimuli is observed) in an established rodent model of radiculopathy (13).

Nonsteroidal anti-inflammatory drugs are a mainstay for the treatment of acute and chronic inflammatory pain, fever reduction, dysmenorrhea, transient ischemic attacks, and unstable angina. It has been estimated that more than 100 million people worldwide take these drugs on a regular basis (3). They are, however, associated with significant side effects, including gastrointestinal ulceration and life-threatening renal failure, especially in the elderly. To decrease these adverse effects, a new type of nonsteroidal anti-inflammatory drug, the selective cyclooxygenase-2 inhibitors, has been developed (30,31). Most currently available nonsteroidal anti-inflammatory drugs inhibit both the constitutive cyclooxygenase-1 and the inducible cyclooxygenase-2 enzyme activities that are expressed in settings of inflammation. The inhibition of cyclooxygenase, the enzyme responsible for the biosynthesis of prostaglandins and other related autacoids, is the principal mechanism of action of nonsteroidal anti-inflammatory drugs. Cyclooxygenase-2 is normally present in low levels but rises in the peripheral and central nervous system in response to injury or inflammation (34). Two cyclooxygenase-2 inhibitors, meloxicam and celecoxib, have shown efficacy in the treatment of pain in osteoarthritis and rheumatoid arthritis (19,27). Cyclooxygenase-2 inhibitors appear to be as effective as current nonsteroidal anti-inflammatory drugs, with less toxicity in patients with limited previous nonsteroidal anti-inflammatory use (3).

Of particular interest to our investigation of persistent radicular pain is the finding that peripheral and central cyclooxygenase-2 expression is increased by a number of proinflammatory cytokines including interleukin (IL)-1 and tumor necrosis factor (TNF). We have considerable evidence that spinal proinflammatory cytokines such as IL-1β and TNF are involved...
in pain behaviors following peripheral nerve or lumbar nerve root injuries in the rat (6-8). Our working hypothesis states that injury to nerve roots leads to an increase in inflammatory mediators in the central nervous system that manifests as the clinical syndrome of radiculopathy. These immune mediators may include elements of the classic arachidonic acid cascade (phospholipase A2, lipoxin, and cyclooxygenases) or proinflammatory cytokines such as IL-1, IL-6, and TNF, or all three.

An experimental animal approach was used to determine whether clinically available cyclooxygenase-2 inhibitors such as celecoxib (Celebrex), rofecoxib (Vioxx), and meloxicam would demonstrate clinical efficacy in low back pain associated with radiculopathy. Due to the long half-life of the cyclooxygenase-2 inhibitor used in the present study (estimated to be 1-2 weeks), it was possible to perform a single, systemic or intrathecal drug administration in the experimental design.

**MATERIALS AND METHODS**

The experiments were performed with use of male Holtzman-strain rats, each weighing 200-250 g at surgery, housed individually under United States Department of Agriculture and Association for Assessment and Accreditation of Laboratory Animal Care-approved conditions with 12-12 hours light-dark cycle and free access to food and water. All experimental procedures were approved by the Dartmouth College Institutional Animal Care and Use Committee.

**Surgical Procedure**

All surgical procedures were performed with the animal under inhalation anesthesia (induced at 4% and maintained at 2% halothane in 100% O2). The radiculopathy model in the rat has been previously described (13). Briefly, the spinal root, dorsal root ganglia, and the adjacent dura mater on the left side at L5 were carefully exposed by hemilaminectomy in each rat with the aid of a surgical microscope. Five 0.3-cm pieces of 4-0 chromic gut ligature were laid adjacent to the root and secured by two loose ligatures of 5-0 chromic gut (Fig. 1). The muscle layers and incision were closed with 3-0 silk suture and staples, respectively.

**Drug Preparation**

The cyclooxygenase-2 inhibitor SC-236 (MW 401.8) was obtained from G.D. Searle (St. Louis, MO, U.S.A.). Due to the extremely low solubility in aqueous solvents, SC-236 was dissolved in 100% dimethyl sulfoxide for intraperitoneal administration (25 mM solution was administered in a volume of 50 µl [2 mg/kg]). There were no obvious adverse side effects after intraperitoneal administration of the drug in solution. Due to the neural side effects of 100% dimethyl sulfoxide, for all intrathecal injections SC-236 was dissolved in dimethyl sulfoxide and then diluted 1:1 with 50% 2-hydroxy-propyl-β-cyclodextrin (Sigma-Aldrich, St. Louis, MO, U.S.A.) in sterile water, following the method previously reported by Dirig et al. (9). The systemic and intrathecal doses were based on previously published in vivo studies (9).

**Experimental Protocol**

Four distinct combinations of treatments were investigated. For rats in the intraperitoneal group (n = 8), at day 4 after surgery a one-time dose of 2 mg/kg of SC-236 (dissolved in 100% dimethyl sulfoxide, 50 µl volume) was intraperitoneally injected. For rats in the intrathecal group (n = 12), at 4 days after surgery, the surgical wound was opened and 0.2 mg/kg of SC-236 (dissolved in 100% dimethyl sulfoxide and then diluted 1:1 with 50% 2-hydroxy-propyl-β-cyclodextrin in sterile water, 10 µl volume) was intrathecally injected by passing a PE-10 catheter through an incision in the exposed dura mater to a position 3 mm rostral to the incision. The PE-10 catheter was pulled out, and standard incision closing procedures were followed. For rats in the vehicle control group (n = 12), the same combination of dimethyl sulfoxide and cyclodextrin was intrathecally administered by the same protocol as for rats in the intrathecal group. For rats in the sham group (n = 7), the L5 nerve roots were exposed but not injured. SC-236 was administered with the same dose, protocol, and time point as used with rats in the intrathecal group. Mechanical allodynia was assessed in all rats daily until they were killed at day 7 after surgery. All behavioral testing was performed by an experimenter blinded to the treatment.

**Mechanical Sensitivity**

Tactile sensitivity (mechanical allodynia) was measured as the frequency of foot-withdrawals elicited by a defined mechanical stimulus, i.e., 2 and 12 g von Frey filaments (Stoelting, Wood Dale, IL, U.S.A.) (4,5). The animals were tested for 3 days preoperatively to acclimate them to the behavioral testing apparatus and the experimenter and to obtain baseline values. They were subjected to three sequential series of 10 tactile stimulations to the plantar surface of the ipsilateral (nerve root injured) hindpaw with consecutive use of 2 and 12 g von Frey filaments. Baseline (before lesion) responsiveness was minimal or absent as confirmed from testing sessions prior to the surgery. Mechanical allodynia was assessed by recording the total number of responses elicited during three successive trials (10 stimulations per each filament) separated by at least 10 minutes for a total possible score of 50 per filament.

**Statistical Analysis**

All data obtained from the observations of mechanical sensitivity were presented as the mean of animals per treatment.

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**FIG. 1.** Schematic of the spinal root injury model: the spinal root, dorsal root ganglia (DRG), and the adjacent dura mater on the left side at L5 were exposed by hemilaminectomy. Five 0.3-cm pieces of 4-0 chromic gut ligature were laid adjacent to the root and secured by two loose ligatures of 5-0 chromic gut (see arrow).
Statistical evaluation was then performed on these group averages. To compare the time-dependent curves among the groups, a repeated analysis of variance (ANOVA) with a Bonferroni multiple comparison was used. Additionally, the data from the tactile stimulation were analyzed by a one-way ANOVA at each time point. Differences between individual means were determined with a Bonferroni multiple comparison test. \( P < 0.05 \) for intergroup differences and \( p < 0.02 \) for the post hoc test were defined as significant.

**RESULTS**

**Mechanical Sensitivity**

The rats rarely demonstrated baseline stimulation responses with 2 or 12 g von Frey filaments before the surgery. Therefore, we defined the elevated behavioral responses after surgery compared with baseline measurement as allodynia (increased sensitivity to a normally non-noxious stimulus). Robust allodynia developed in rats in all groups except those in the sham group by day 1 after surgery (2 g von Frey: intraperitoneal group = 10.0 ± 3.6, intrathecal group = 10.2 ± 5.0, and vehicle control group = 12.5 ± 5.7; 12 g von Frey: intraperitoneal group = 21.9 ± 5.4, intrathecal group = 22.7 ± 4.8, and vehicle control group = 23.8 ± 3.1) and remained elevated until day 4 after surgery. There was no statistical difference between the rats in the three groups at day 4 after surgery (one-way ANOVA). Mechanical allodynia with use of 2 g von

**FIG. 2.** Time course of tactile sensitivity with use of von Frey filaments following L5 nerve root injuries (A: Use of 2 g von Frey filament and B: use of 12 g von Frey filament). For each time point, all animals were exposed to a total of 30 stimulations/filament. The average number of evoked foot-withdrawal responses is recorded for each group. Intraperitoneal (IP) group (n = 8): At day 4 after surgery, 2 mg/kg of SC-236 was intraperitoneally injected. Intrathecal (IT) group (n = 12): At 4 days after surgery, the surgical wound was opened and 0.2 mg/kg of SC-236 was intrathecally injected. Vehicle control group (n = 12): The same combination of dimethyl sulfoxide and cyclo-dextrin was intrathecally administered with use of the same protocol as in the intrathecal group. Sham group (n = 7): The L5 nerve roots were exposed but not injured. SC-236 was administered with use of the same dose, protocol, and time point as in the intrathecal group. *Significant difference compared with the vehicle control group (one-way analysis of variance [ANOVA] with Bonferroni multiple comparison test, \( p < 0.002 \)). #Significant difference as compared with the intraperitoneal group (one-way ANOVA with Bonferroni multiple comparison test, \( p < 0.002 \)).
Frey filaments was significantly lower in rats in the intrathecal group at days 5 and 6 compared with those in the vehicle control group (repeated ANOVA with Bonferroni multiple comparison test, p = 0.002) (Fig. 2).

Mechanical allodynia with use of the 12 g filament was significantly attenuated at days 5, 6, and 7 compared with the control group (repeated ANOVA with Bonferroni multiple comparison test, p = 0.002). Although rats in the intraperitoneal treatment group demonstrated a statistically significant decrease in allodynia with use of the 2 or 12 g filaments at day 6 after surgery, at day 7 there was no significant difference in the time course as compared with rats in the vehicle control group (repeated-measures ANOVA). At day 7 after surgery, there was a statistically significant difference in resultant allodynia between rats in the intraperitoneal and intrathecal groups with use of the 12 g filament (one-way ANOVA with Bonferroni multiple comparison test, p = 0.002). The rats that underwent the sham procedure and received intrathecal SC-236 demonstrated only a minimal response to von Frey stimulation after the surgery. No significant changes in tactile sensitivity and motor function were observed after SC-236/vehicle administration in rats in the sham group.

DISCUSSION

A number of animal models have been developed to study the pathophysiological sequelae of lumbar radiculopathy (13,24,26,28,32,33). In 1934, mixer and Barr provided the first comprehensive report of the association between herniated nucleus pulposus and radiculopathy (29). The concept that compression of a nerve root by a herniated intervertebral disc produced radicular pain was later challenged by electrophysiologic experiments demonstrating that acute compression of the nerve roots did not cause sustained repetitive firing (20,35). Of interest to the genesis of animal models of neurophy etiology of radiculopathy, Howe et al. may be credited with first using chromic gut ligation to induce local inflammation, demyelination, and axonal degeneration (20). This chromic gut ligation procedure was utilized to develop the well-established chronic constriction injury model of neuropathic pain (2) and the radiculopathy model used in the present study. Further evidence of an inflammatory response in the dural sac, spinal cord, and nerve roots was observed following injection of autologous nucleus pulposus into the epidural space of the dog (28). Olmarker et al. (32) and Kayama et al. (26) supported these findings by demonstrating neurophysiologic changes in nerve roots exposed to nucleus pulposus.

We and others hypothesize that rodent models of radiculopathy/sciatica produce similar nerve root and central pathological sequelae of a herniated intervertebral disc concomitant with mechanical root compression (13,17,23,28,32). This is supported by the recent finding that when autologous disc material is extruded by a posterolateral approach in a new radiculopathy model and is placed directly on the L5 nerve root, the resultant mechanical allodynia and thermal hyperalgesia are identical to that observed in the chronic gut model used in the present study (22). Early in the development of the chronic gut model, it was determined that the addition of root ligation with inflammatory chronic gut was more effective than silk ligation in modeling lumbar radiculopathy in humans (24). This finding directly implicates inflammation in the etiology of radiculopathy. In addition, the steroid betamethasone and a phospholipase A2 (PLA2) inhibitor attenuated hyperalgesia in this model (17,25). Previously, in our investigation of spinal neuroimmune changes after root injury, we observed spinal glial (microglial and astrocytic) activation and increased cytokine (IL-1β) expression in this radiculopathy model (13). More recently, expression of major histocompatibility complex class II and CD4+ were also observed in the spinal cord after the chronic gut nerve root injury. These observations further support a direct, central neuroimmunologic response to radiculopathy (21). Moreover, we recently reported that centrally administered methotrexate, an immunomodulator and anti-inflammatory agent, reduced mechanical allodynia in the same animal model (14). When our results are considered collectively, they provide compelling evidence for a central, neuroinflammatory process in the generation and maintenance of radicular pain.

In the present study, we extend this hypothesis and report that a selective cyclooxygenase-2 inhibitor attenuates mechanical allodynia after lumbar nerve root injury. Intraperitoneal administration of the cyclooxygenase-2 inhibitor demonstrated efficacy in reducing allodynia after the lumbar nerve root lesion. Spinal, intrathecal administration of the drug was more effective in attenuating allodynia than an intraperitoneal, systemic injection, however, and this supports a central mechanism of action.

Certainly, the role of cyclooxygenases in the function and pathology of the central nervous system spans many arenas. In particular, the concept of neuroinflammation has gained increasing attention in neurodegenerative diseases such as Alzheimer's and multiple sclerosis. In the nervous system, prostanoids are well recognized as mediators in a variety of processes that include stress modulation and hyperalgesia. It has been demonstrated that cyclooxygenase-2 is expressed in different cell types in the central nervous system after a variety of stimuli (30). For example, intraplantar injection of carrageenan and complete
Freund's adjuvant increase cyclooxygenase-2 expression in the spinal cord, and the hyperalgesia associated with these peripheral inflammatory models is attenuated following administration of a selective cyclooxygenase-2 inhibitor (9,15,16,36). The demonstration of cyclooxygenase expression in astrocytes (18) and microglia (1,10-12) is germane to our findings of extensive glial activation in the lumbar spinal cord after lumbar nerve root injury (13).

In conclusion, the present results demonstrate that cyclooxygenase-2 expression is involved in spinal nociceptive processing after a nerve root injury. Our enthusiasm for the direct clinical relevance of these results should be dampened, since it has not been proved that any animal model of radiculopathy is a predictor of clinical success or failure of potential novel analgesics. Additionally, anecdotal observations of the use of clinically available oral cyclooxygenase-2 inhibitors have not shown its efficacy in reducing radicular pain. A provocative explanation may be that systemic concentrations are not high enough to alter central inflammatory processes that occur in radiculopathy. Even though cyclooxygenase-2 inhibitors are unlikely to be the analgesic panacea as originally described, these data support further basic science research in elucidating central inflammatory processes that follow nerve root injury.

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