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# The neurological safety of epidural parecoxib in rats

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ABSTRACT

Epidural injection of cyclooxygenase-2 inhibitors has been suggested as a useful therapeutic modality in pain management in animal studies and clinical settings. Direct epidural administration of parecoxib, a highly selective cyclooxygenase-2 inhibitor, may have advantages over its parenteral administration regarding required dose, side effects, and efficacy. However, no animal studies have been performed to investigate the possible neurotoxicity of epidurally injected parecoxib. Therefore, the present study was performed to assess the neurotoxicity of epidurally injected parecoxib in rats. Rats ($n = 45$) were randomly divided into three groups: normal saline group (group N, $n = 15$), ethanol group (group E, $n = 15$), and parecoxib group (group P, $n = 15$). 0.3 mL of epidural parecoxib (6 mg) and the same volume of epidural ethanol or normal saline were injected into the epidural space. Neurologic assessment was performed 3, 7 and 21 days after the injection by pinch toe testing. Histologic changes were evaluated for vacuolation of the dorsal funiculus, chromatolytic changes of the motor neurons, neuritis, and meningeal inflammation.

All rats in groups N and P showed normal response to pinch-toe testing and had a normal gait at each observation point. Histological examination showed no evidence suggestive of neuronal body or axonal lesions, gliosis, or myelin sheet damage in group N or P at any time. However, all rats in group E showed sensory-motor dysfunction, behavioral change, or histopathological abnormalities. No neurotoxicity on the spinal cord or abnormalities in sensorimotor function or behavior was noted in rats that received epidural parecoxib.



## 1. Introduction

Cyclooxygenase-2 (COX-2) plays a major role in the inflammatory process by catalyzing the conversion of arachidonic acid into prostaglandins and thromboxane (Yedgar et al., 2006). COX-2 is generally expressed at low levels, but increases in the peripheral and central nervous systems in reaction to tissue damage or inflammation (Dirig et al., 1998). Epidural injection of selective COX-2 inhibitors seemed to affect not only peridural inflammation around the affected nerve roots but also dorsal root ganglion and spinal cord (Kawakami et al., 2002). The presence of constitutive COX-2 expression in neurons of all laminae of the spinal cord provides a rationale for the acute antinociceptive effect of intrathecal COX-2 inhibitors (Yaksh et al., 2001). Intrathecal administration of the COX-2 inhibitor celecoxib was shown to reduce acute inflammation and provide pain relief without hemodynamic or behavioral side effects (Nishiyama, 2006; Deleo et al., 2000).

Epidural injection of COX-2 inhibitors may be therapeutically useful if the drugs and vehicles have no neurotoxic effects. However, a limited number of parenterally administered non-steroidal anti-inflammatory drugs (NSAIDs) may be administered for acute and chronic pain management (Padi et al., 2004). Due to their characteristic hydrophobic properties (Canduz et al., 2007), epidural injection of many NSAIDs may be problematic when used in routine clinical practice. Here, a prodrug approach was applied with parecoxib sodium, a highly water-soluble second-generation selective COX-2 inhibitor, for parenteral administration to address solubility limitations.

By injecting a drug directly into the epidural space, its dosage can be significantly lowered compared to oral intake or parenteral injection. This has the potential to decrease side effects and increase the effectiveness of the drug. Epidural injection of drugs has been widely accepted in the field of pain medicine because it is comparatively less invasive, long-term administration is possible, and the risk of neurotoxicity is minimized. However, preclinical confirmation of the risk of neural toxicity on epidural administration

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of a drug in animal studies is mandatory (Yaksh and Collins, 1989). Although this drug has a wide safety margin in systemicatic use, before epidural or intrathecal administration of the drug in a clinical setting, its safety and effectiveness via this route of administration should be determined in animal experiments.

As no animal studies have been reported to investigate the possible neurotoxicity of spinoaxially injected parecoxib, the present work was performed to determine the neurotoxicity of epidurally injected parecoxib in a rat model of epidural drug injection.

## 2. Materials and methods

### 2.1. Subjects

The experimental protocol was reviewed and approved by our institutional animal care and use committee. Male Sprague-Dawley rats (250–300 g) were used in this study. The rats were housed in an animal room with a 12-h light/dark cycle and had free access to food and water. For epidural catheterization, anesthesia was induced by placing a rat in a closed box containing 2% sevoflurane in oxygen (3 L/min) with spontaneous ventilation (Lim et al., 2003; Kim et al., 1998; Choi et al., 2005). Briefly a midline skin incision was made over the spinous processes of the L2–L5 vertebrae. Using fine forceps, the center of the interspinous ligament was pierced, and a PE-10 catheter (15 cm long with an approximate volume of 15 mcL, Natsume, Tokyo, Japan) was inserted and gently advanced about 3 cm caudally. The tip of the catheter was positioned on the dorsal dura mater at L4/L5 level. For epidural injection, the externalized catheter was fixed with resin and maintained in position with a silk suture at the head. To confirm correct catheter positioning, 0.15 mL of 2% lidocaine was injected through the catheter after complete recovery from anesthesia; correct epidural catheter placement was defined as that resulting in paralysis of the hindlimbs, while the forelimbs retained normal motor strength.

### 2.2. Drug preparation

Under general anesthesia, 0.3 mL of preservative-free parecoxib (20 mg of parecoxib dissolved in 1 mL of normal saline; Advanced Tech & Industrial Co., Hong Kong) was injected into the epidural space through the implanted epidural catheter in group P ($n$ = 15). Groups E ($n$ = 15) and N ($n$ = 15) received injections of the same volume of 40% ethanol (pH 5.79) or 0.9% normal saline (pH 5.5, Osmolarity 308 mosm/L), respectively, via the epidural catheter (Choi et al., 2005; Lee et al., 2010). After recovery from anesthesia, the rats were housed individually under a 12-h light/dark cycle.

### 2.3. Functional examination

Neurological function was measured by an investigator who was blinded to the group assignment. Acute toxicity was evaluated at 3 days and chronic toxicity at 7 and 21 days postinjection. Pinch toe testing was used to evaluate motor and sensory deficits (Bajrovic and Sketelj, 1998). The skin of the paw was pinched with fine forceps at 1-mm intervals from the toes to the ankle. A reflexive withdrawal response of the treated paw elicited by pinching the skin was taken as positive evidence of neurological function. Motor function was assessed using a previously devised scoring system (Kawakami et al., 1994) with some modifications according to the following grades: grade 1: normal gait with no evidence of motor paresis, grade 2: normal gait with slight hindpaw deformity, such as plantar flexion of the toes, grade 3: slight gait disturbance with motor weakness or an inverted hindpaw, and grade 4: prominent limping gait with a dropped hindpaw. The rats with motor disturbance of grade 2 or above were considered to have a motor deficit.

### 2.4. Histologic examination

The spinal cord and nerve roots were cropped in five rats from each group 3 days postinjection to evaluate acute toxicity. To assess chronic toxicity, the spinal cords of five rats in each group were cropped on the 7th and 21st day after the injection. The rats were killed under general anesthesia, and the left ventricle was cannulated and perfused with 50 mL of 0.2 M phosphate buffer, followed by 400 mL of freshly prepared 4% paraformaldehyde. The vertebral column was removed from the lower sacral area to the cervical area. Approximately 1-cm length of spinal cord, caudal and rostral to the catheter tip, were obtained. For light microscopy, spinal cords were fixed with 10% neutral formalin solution. The spinal cord was then embedded in paraffin, cut into sections at a thickness of 4–5 μm, mounted, and stained with hematoxylin and eosin (Gradert et al., 2003; Guevara-Lopez et al., 2006). Luxol fast blue stain was added to assess myelin loss. Histological changes were evaluated for vacuolation of the dorsal funiculus, chromatolytic changes of the motor neurons, neuritis, and meningeal inflammation (Yamashita et al., 2003). The degree of vacuolation was graded on a 4-point scale as follows: 0: no vacuolation of the dorsal funiculus, 1: 10% vacuolation of the dorsal funiculus, 2: 10–50% vacuolation of the dorsal funiculus, and 3: >50% vacuolation of the dorsal funiculus (Madsen et al., 1993). A neurofilament stain (monoclonal mouse anti-human neurofilament protein clone. 2F11: code No. M 0762, Dako) was added to higher than grade 1 vacuolated sections to determine axonal damage (Vranken et al., 2005).

Coded sections from all animals were examined by a neuropathologist blinded to the animal treatment groups.

### 2.5. Statistical analysis

Intergroup comparison of baseline body weight was analyzed using a one-way ANOVA. The changes of body weights from baseline were analyzed using a mixed linear model where the significance of group effect, time effect and the interaction effect between group and time were tested. The adjusted $p$-values were calculated using Bonferroni method for the control of type I error inflation due to multiple comparisons. All statistical analyses were performed using SAS 6.2.

## 3. Results

Both groups P and N exhibited normal weight gain. However, most rats in group E gained weight at less than the normal rate, while a few rats in group E weighed significantly less than they did just prior to the study as noted 7 and 21 days postinjection ($p$ = 0.023, Table 1).

**Table 1**
Body weight changes after epidural drug injection.

| Day after epidural injection | Group N | Group P | Group E |
|---|---|---|---|
| Baseline ($n$ = 15/group) | 290.8 ± 25.9 | 265.2 ± 11.7 | 294.9 ± 26.9 |
| 3rd day ($n$ = 15/group) | 311.1 ± 31.0 | 278.8 ± 14.1 | 282.5 ± 26.2* |
| 7th day ($n$ = 10/group) | 339.8 ± 34.3 | 297.2 ± 9.30 | 300.9 ± 23.9* |
| 21st day ($n$ = 5/group) | 434.8 ± 35.5 | 370.4 ± 18.7 | 346.4 ± 61.2* |

Values are expressed as mean ± SD. Group N: epidural injection of 0.3 mL of normal saline, group P: epidural injection of 0.3 mL (3 mg/mL) of parecoxib, and group E: epidural injection of 0.3 mL of 40% ethanol.
* $p < 0.05$ versus corresponding data of group N and group P.

None of the rats in groups N or P exhibited abnormal behavior during the course of the study. In contrast, all rats in group E displayed reduced activity, lack of appetite, and weight loss. All rats in groups N and P showed normal response to pinch-toe testing and had a normal gait at each observation time point. However, all rats in group E demonstrated an abnormal response to pinch-toe testing, as well as a gait disturbance of grade 2 or more in hindpaw movement.

One rat in group P, two rats in group E and two rats in group N were excluded in the nerve root examination because of poor samples. Histological examination showed no evidence suggestive of neuronal body or axonal lesions, gliosis, or myelin sheet damage in group N or P at any time point (Figs. 1 and 2). We observed mild vacuolation (grade 1) in one rat 3 days post injection, in one rat 7 days post injection, and in two rats 21 days post injection in group P. When we compared the group P with the group N as a negative control, we found no significant differences between them. We also performed Luxol fast blue staining for myelin and immunohistochemical staining for axons in rats showing vacuolation greater than grade 1. Although mild vacuolation was observed in the dorsal funiculus, in groups N and P showing vacuolation greater than grade 1, normal morphology of myelin was observed on sections treated with Luxol fast blue stain. Similar vacuolated sections were also subjected to immunohistochemical staining with an anti-neurofilament antibody (Fig. 3). The axons of all rats in groups N and P (greater than grade 1) were well surrounded with myelin, and no evidence of either demyelinization or degenerative changes was detected and there were no other abnormal pathologic findings. By contrast, in the group E, pale, reduced myelin was seen, and significant axonal degeneration was observed with the Luxol fast blue and immunohistochemical stains, although only mild grade 1 vacuolation was found in the dorsal funiculus.

No chromatolysis or neuritis was observed in groups N or P, but one rat in group E showed severe neuritis and meningeal inflammation ($p = 0.018$, Tables 2 and 3). Ten rats in group E exhibited chromatolytic motor neurons.

## 4. Discussion

The neurotoxicity of parecoxib administered into the epidural space was investigated and no behavioral or histological changes attributable to neurotoxicity in the spinal cord of rats were observed after parecoxib administration.

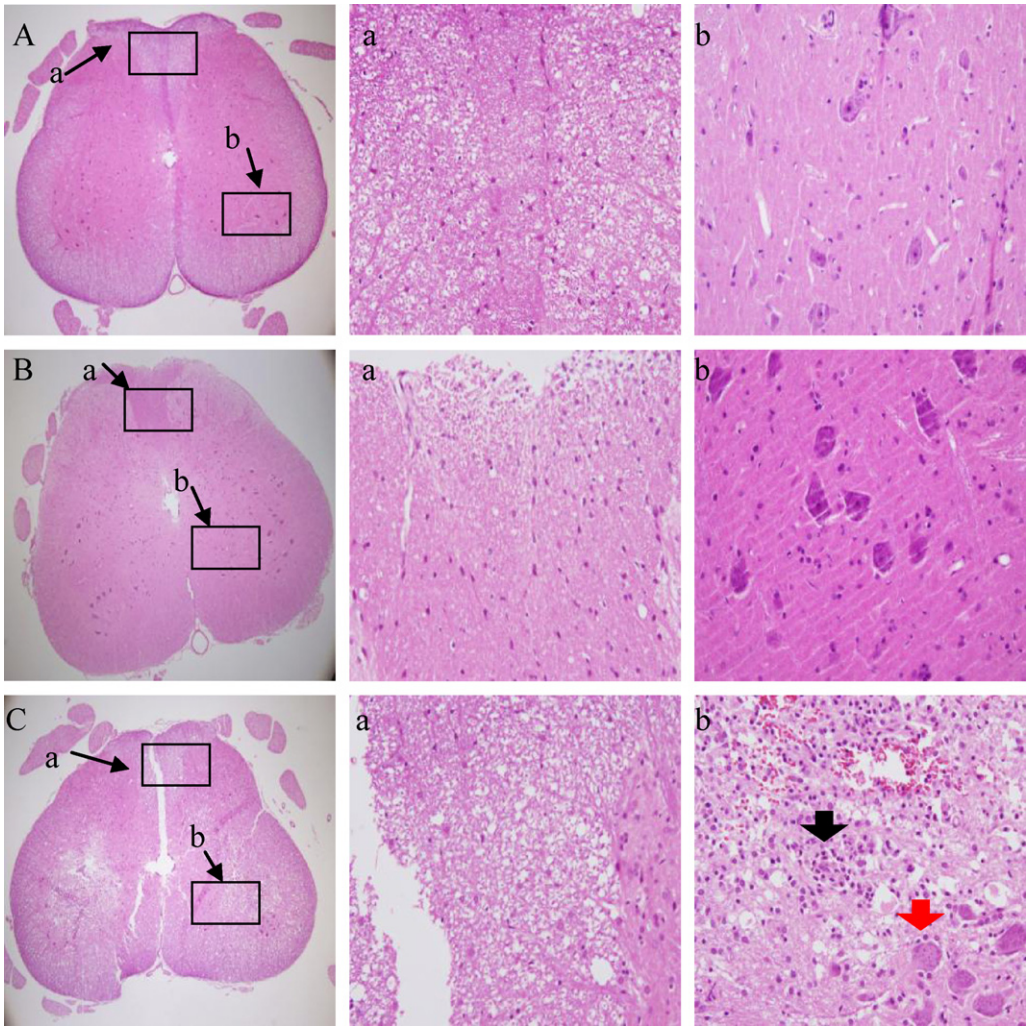


**Fig. 1.** Degree of vacuolization in dorsal funiculus. Infiltration in the spinal cord light microphotographs of normal saline (A), parecoxib (B), and ethanol groups (C), respectively (L5 level, hematoxylin and eosin stain, original magnification, 40X). Neither vacuolation of the dorsal funiculus (a) nor neurons with chromatolytic appearance in the ventral horn (b) are observed in group N (A; b, arrow) (original magnification, 400×). The dorsal funiculus has a normal appearance or only mild vacuolation (B; a). Many large motor neurons are observed with clear Nissl staining in the ventral horn (B; b, arrow) (original magnification, 400×). In group E, the dorsal funiculus shows moderate to severe vacuolation and motor neurons with a chromatolytic appearance in the ventral horn (C; b, arrow) (original magnification, 400×).

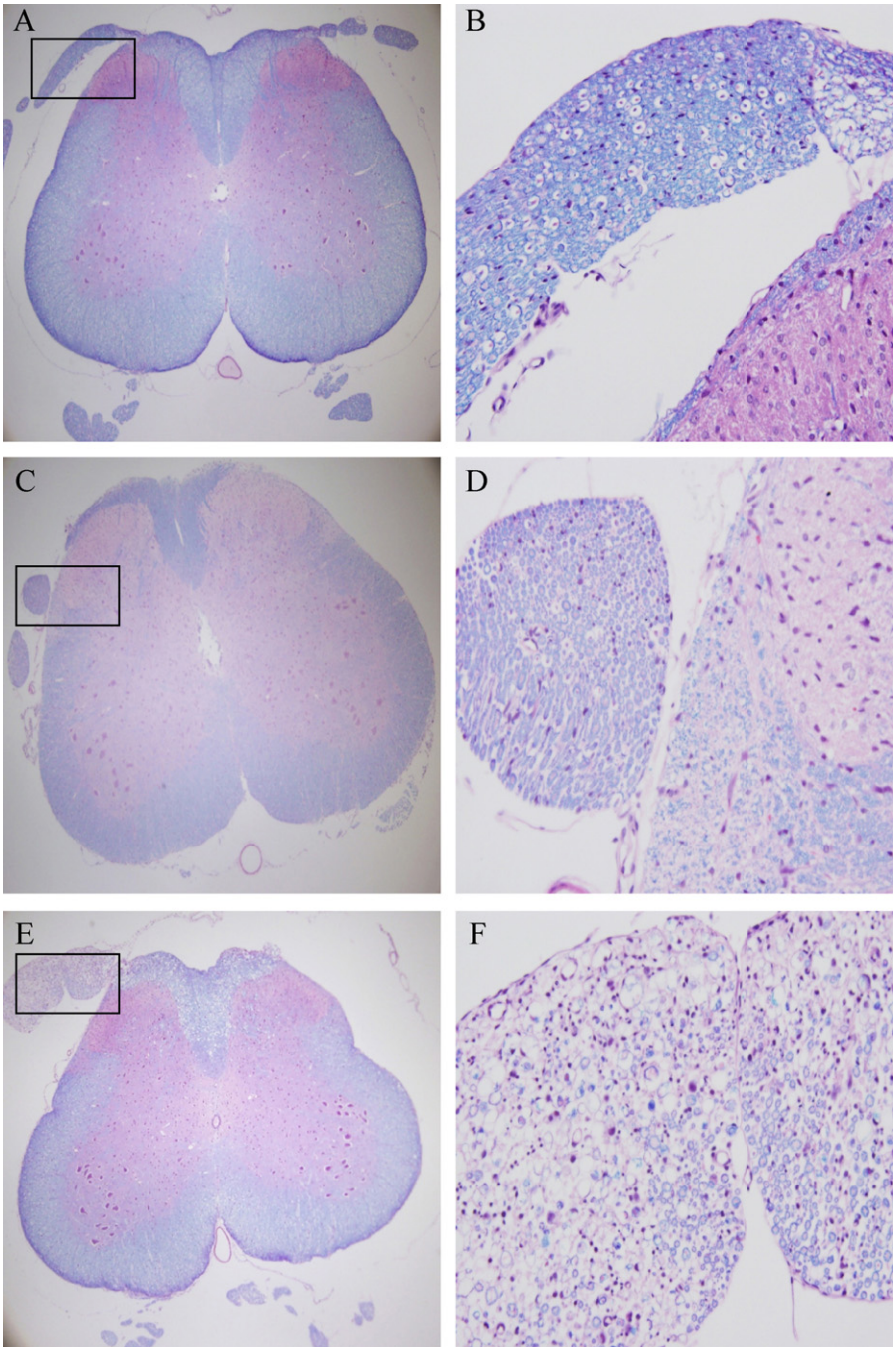


**Fig. 2.** Degree of myelin loss in dorsal funiculus. Spinal cord light microphotographs of group N, group P and group E after the epidural injection of 0.3 mL of normal saline (A and B), parecoxib (C and D) and ethanol (E and F), respectively (L5 level, Luxol fast blue stain). The figures on the right (400×) are higher magnification views the same areas indicated by squares in the adjacent figures on the left (40×). Normal morphology of myelin is observed (original magnification, 400×) (A–D). However, in epidural ethanol group (E and F), pale and diminished myelin are seen.

As specific tracts that convey nociceptive impulses in the spinal cord were noted, this suggested that blocking these pathways directly by spinal or epidural administration of analgesic drugs may be possible (Hodgson et al., 1999).

In the present study, preservative-free parecoxib (6 mg) was administered into the epidural space of rats, which would be equivalent to an epidural dose of 1200 mg in humans. Moreover, the 6-mg dose of epidural parecoxib administered may be equivalent to parenteral administration of about 12,000 mg in an adult human (the recommended NSAID dose by the epidural route is one-tenth of its parenteral dose and that for the oral route is three to four times the parenteral dose). Based on the results of a previous study on intravenous parecoxib (10 mg/kg), which led to 70% and 90% inhibition of thermal and mechanical hyperalgesia, respectively (Padi et al., 2004), the 6 mg epidural dose may be equivalent to a parenteral dose of 200 mg/kg. Thus, an epidural dose of 6 mg was considered sufficient for the present neurological study.

Using fluoroscopy, the total spread for 0.3 mL of contrast medium in the spinal segment in rats was 10–11 segments, which was sufficient to affect the entire spinal cord segment (Choi et al., 2005). Therefore, doses were administered in a constant volume of 0.3 mL.

Neurotoxicity was assessed by examining sensory, motor, and behavioral changes. A nociceptive pinch test was used to examine

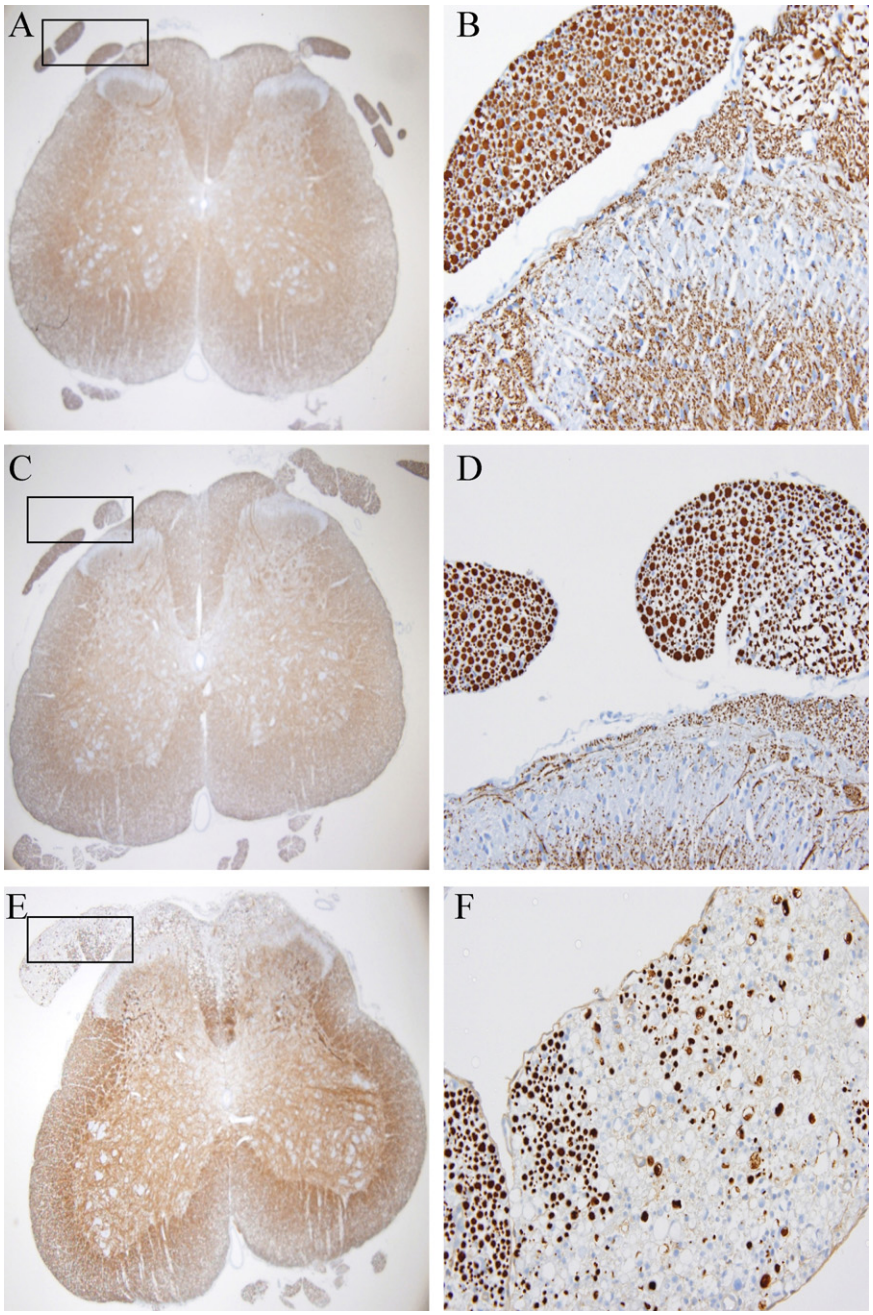


**Fig. 3.** Degree of axonal degeneration in dorsal funiculus. Spinal cord light microphotographs of group N, group P and group E after the epidural injection of 0.3 mL of normal saline (A and B), parecoxib (C and D) and ethanol (E and F), respectively (L5 level, Immunohistochemical staining). The figures on the right (400×) are higher magnification views of the same areas indicated by squares in the adjacent figures on the left (40×). No significant axonal degeneration is noted although they showed mild vacuolation in hematoxylin and eosin stain (A–D). However, E and F show significant axonal degeneration. Immunohistochemical staining was performed with anti-neurofilament antibody (original magnification, 400×).

segmental and peripheral nerves function to the hind paw skin. Rats in the present study that were administered parecoxib epidurally showed no findings of neuronal damage, such as persistent motor function changes or sensory loss. None of the rats in group P (or group N) showed motor or sensory deficits or any abnormal histological findings at any time point. In the present study, all rats in group E exhibited reduced activity and appetite, reduced weight gain, and acute and chronic neurotoxicity findings on histopathological examination. Histopathological examination of the group E animals indicated vacuolation of the dorsal funiculus and central chromatolysis of the motor neurons in the lumbar spinal cord, suggesting possible dorsal and ventral root damage (Yamashita et al., 2003).

Whether the vacuolation occurred due to degeneration of the axon or myelin sheath is not yet clear (Takenami et al., 2005). Sections with vacuolation higher than grade 1 were treated with Luxol fast blue stain and with anti-neurofilament antibody, and both the axon and myelin sheath were normal.

Spinal COX-2, but not COX-1, inhibition mediates a strong antihyperalgesic action after peripheral inflammation and spinal hyperalgesic action via spinal NK-1/NMDA receptor activation (Takenami et al., 2005). In clinical use, patients with post-laminectomy syndrome showed satisfactory pain relief with epidural indomethacin without neurological deficits (Aldrete, 2003). Epidural ibuprofen shows a spinal site of action without systemic absorption, which may explain its antinociceptive action

**Table 2**
Neuropathological findings of the spinal cord under light microscopic examination following epidural drug injection.

| Day after epidural injection | Grade of vacuolation | Group N | Group P | Group E |
|---|---|---|---|---|
| 3rd day ($n=5$) | 0 | 5 | 4 | 0 |
| | 1 | 0 | 1 | 1 |
| | 2 | 0 | 0 | 2 |
| | 3 | 0 | 0 | 2 |
| 7th day ($n=5$) | 0 | 4 | 4 | 0 |
| | 1 | 1 | 1 | 0 |
| | 2 | 0 | 0 | 2 |
| | 3 | 0 | 0 | 3 |
| 21st day ($n=5$) | 0 | 4 | 3 | 0 |
| | 1 | 1 | 2 | 0 |
| | 2 | 0 | 0 | 1 |
| | 3 | 0 | 0 | 4 |

Values are expressed as number of rats. Group N: epidural injection of 0.3 mL of normal saline, group P: epidural injection of 0.3 mL (3 mg/mL) of parecoxib, and group E: epidural injection of 0.3 mL of 40% ethanol. The degree of vacuolation was graded on a 4-point scale as follows: 0: no vacuolation of the dorsal funiculus, 1: 10% vacuolation, 2: 10–50% vacuolation, and 3: >50% vacuolation (17).

(Wang et al., 1995). Epidural analgesic administration is an effective and efficient method for relief of spinal cord-mediated pain.

Parecoxib showed noticeable and similar anti-inflammatory effects, and was a more potent antihyperalgesic when administered intravenously in comparison to ketorolac in rats (Lauretti et al., 1998). If parecoxib shows no neurotoxicity, its epidural administration may be useful in pain management as it shares central and peripheral antinociceptive effects with other NSAIDs. As corticosteroids have powerful anti-inflammatory effects, epidural corticosteroids are routinely used in clinical practice to alleviate pain caused by herniated discs, degenerative disc disease, or spinal stenosis. Administration of corticosteroids into the epidural space for the treatment of lower back pain with radiculopathy is often used (Bogduk, 1999). However, questions remain concerning other indications, such as dosage (Lauretti et al., 1998), frequency (Bogduk, 1999), and duration of administration (Winnie and Hartman, 1972). These concerns have impeded the obvious therapeutic potential of corticosteroids, and thus the development of an alternative medication with anti-inflammatory action would be advantageous. Synergism between intrathecal morphine and parecoxib was reported, and the effects of morphine potentiated antinociception during visceral nociception by parecoxib in mice (Pinnardi et al., 2005). Combination therapy with intrathecal morphine and intrathecal parecoxib is in clinical use for acute pain management. Thus, NSAIDs have been shown to have opioid-sparing effects. Therefore, the side effects of opioids, such as respiratory depression, urinary retention, and pruritus, may be averted or diminished and the risk of pulmonary and thrombotic complications may be reduced. As NSAIDs act via enzyme systems rather than receptors, the risks of tolerance, resistance, and addiction are low (Wang et al., 1995).

**Table 3**
Incidences of chromatolysis, meningeal inflammation at the spinal cord and nerve roots.

| Day after epidural injection | | Chromatolysis | Meningeal inflammation | Neuritis |
|---|---|---|---|---|
| 3rd day ($n=5$) | Group N | 0 | 0 | 0 |
| | Group P | 0 | 0 | 0 |
| | Group E | 3* | 0 | 0 |
| 7th day ($n=5$) | Group N | 0 | 0 | 0[a] |
| | Group P | 0 | 0 | 0[a] |
| | Group E | 3* | 0 | 0[a] |
| 21th day ($n=5$) | Group N | 0 | 0 | 0[a] |
| | Group P | 0 | 0 | 0 |
| | Group E | 4* | 1 | 1[a] |

Values are expressed as number of rats showing an abnormal response over the total. Group N: epidural injection of 0.3 mL of normal saline, group P: epidural injection of 0.3 mL (3 mg/mL) of parecoxib, and group E: epidural injection of 0.3 mL of 40% ethanol. In one rat of group E, severe meningeal inflammation was detected. In one rat of group E, severe neuritis was detected.

[a] Originally five rats included in each group ($n=5$). However, we had to exclude some rats in the nerve root examination because of poor samples ($n=4$).

* $p<0.05$ versus corresponding data of groups N and P.

This study was subject to several limitations. First, no analysis of the histopathology of nerve roots, which have been shown to be vulnerable to injury, was performed. Some studies have indicated that neurotoxic lesions were induced by intrathecal drugs, which were generally confined to the nerve roots (Sakura et al., 1995), some only observing them in the spinal cord (Ravindran et al., 1982), while others noted them in both (Ready et al., 1985). The spinal cord was evaluated in the present study because it was directly adjacent to the site of epidural injection where the concentration of the injected agent would be greatest. Second, As prolonged exposure to selective COX-2 inhibitors has been reported to be associated with an increased risk of adverse cardiovascular outcomes in clinical practice (Noor, 2003), all subjects received single doses of paracoxib. Repeated administration should be considered in future studies to determine drug toxicity. Third, we did not study the distribution of these agents in CSF. More study needs to be done in this area before being applied to humans.

## 5. Conclusion

No pathological evidence of neurotoxicity associated with epidural injection of parecoxib into rats was observed in the present study. The results presented here indicate no injury to the spinal cord, although further studies in larger species are necessary to assess the full therapeutic scope of parecoxib, and to confirm whether similar neurotoxic-sparing effects could be realized in multiple doses of parecoxib and intrathecal administration.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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