Introduction

The pathophysiologic mechanisms of painful radiculopathy secondary to herniated nucleus pulposus are still not clearly known. We demonstrated that autologous nucleus pulposus, relocated to the lumbar nerve root in the rat, produces time-dependent and reversible mechanical hyperalgesia, which is thought to be a pain-related behavior in peripheral neuropathic pain models, and which is completely abolished by epidural injection of phospholipase A2 inhibitor. It is thus possible that the mechanical hyperalgesia induced by nucleus pulposus is related to the production of arachidonic acid and its metabolites, for which phospholipase A2 is a rate-limiting enzyme.

Cyclooxygenase-2 (COX-2) is thought to play a major role in inflammatory processes by catalyzing the conversion of arachidonic acid to prostaglandins and thromboxane, and has been localized primarily to inflammatory cells and tissues. It has been reported that COX-2 is present in herniated disc samples obtained from patients with lumbar disc herniation. Although it is possible that COX-2 plays significant roles in nerve root dysfunction including radicular pain in lumbar disc herniation, little is known concerning the relationships between COX-2 and clinical symptoms including radicular pain. The purpose of this study was to evaluate whether epidural injection of COX-2 inhibitor is safe and abolishes hyperalgesia in our rat model, and to examine the role of COX-2 in mechanisms of radicular pain following herniated nucleus pulposus in the rat.

Materials and Methods

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at our institution. In total, thirty-five male Sprague Dawley rats, each weighing 250 grams, were used for this study. All surgical procedures were performed with deep sodium pentobarbital anesthesia (50 mg/kg, i.p.). Rats were divided into four experimental groups. Rats in which only the tail was amputated served as the control group (n = 5). In the sham group (n = 8), the tail was amputated and then the left L4 and 5 nerve roots were exposed after partial laminectomies. In the NP group (n = 22), after amputation of the tail, the nucleus pulposus obtained from the amputated tail was relocated on the exposed nerve roots. Six rats in the NP group, in which a dural tear was intentionally made, served as the DT group. A permanent catheter, made from a single-lumen polyethylene tube (SP-10, inner diameter 0.28 mm, outer diameter 0.61 mm), was placed to permit epidural injections at the L2-3 interlaminar space in all rats except those of the control group. The tip of the catheter was placed on the dorsal dura mater at L4-5. The operative fields were closed in layers with 4-0 nylon sutures. Motor function and reflex responses to noxious mechanical and thermal stimuli to both hindpaws were measured in all rats preoperatively and by 14 days postoperatively (PO). The percentage difference between both hindpaw responses to noxious stimuli was computed so that negative percentages reflected hyperalgesia and positive percentages represented hypoalgesia. After these measurements at 7 days PO, the sham, NP and DT groups were divided into two similar subgroups. In the sham+vehicle, NP+vehicle and DT+vehicle subgroups (n = 4, 8, and 3, respectively), 0.25 ml of vehicle alone (50% dimethyl sulfoxide/25% cyclolestrin) were injected into the epidural space through the implanted catheter, respectively. Equally, in the sham+anti-COX, NP+anti-COX and DT+anti-COX subgroups (n = 4, 8, and 3, respectively), 0.25 ml of COX-2 inhibitor (0.1 mg/kg SC-236 dissolved in the vehicle) were injected. One hour and 3 and 7 days after epidural injections, motor function and sensitivities to noxious mechanical thermal stimuli were measured in all rats. All rats for which the nucleus pulposus obtained from the tail was relocated on the nerve roots were killed at 2 weeks PO. The specimens on the treated nerve roots were fixed routinely in a buffered formalin solution and paraffin-embedded, 5-µm-thick sections were subsequently stained with haematoxylin-eosin. The specimens were examined microscopically for number of inflammatory cells and the formation of granulation tissue and classified by 2 independent examiners in a blind manner. Data obtained from these measurements were analyzed by ANOVA and Student’s t-test. P values < 0.05 were considered significant.

Results

None of the rats in the control, sham or NP groups exhibited motor paresis of their hindpaws until 14 days PO. However, rats in the DT group developed spasm in both hindpaws immediately after epidural injection at 7 days PO and severe motor paresis remained. In the control and sham groups, rats exhibited normal response to noxious mechanical stimuli. Rats in the NP and DT groups exhibited evidence of mechanical hyperalgesia in the ipsilateral hindpaws at 3 and 7 days PO (p<0.05). After epidural injection of vehicle, rats in the NP+vehicle group exhibited evidence of mechanical hyperalgesia, which lasted until sacrifice at 2 weeks PO (p<0.05). In the NP+anti-COX group, there was a tendency toward decrease in mechanical hyperalgesia one hour after epidural injection of COX-2 inhibitor, compared with the NP+vehicle group. At 3 and 7 days after epidural injection, rats in the NP+anti-COX group exhibited decrease in mechanical hyperalgesia, compared with the NP+vehicle group (p<0.05) (Figure). There were no significant differences in sensitivities to thermal noxious stimuli among the groups. Inflammatory reaction and granulation tissue were less pronounced in the NP+anti-COX group than in the NP+vehicle group.

Discussion

Since the COX-2 inhibitor utilized in this study has extremely low solubility in aqueous solvents, we used 50% dimethyl sulfoxide with 25% 2-hydroxy-propyl-β-cyclodextrin as a vehicle. In rats with intentional dural tear, severe motor paresis remained after epidural injection. The vehicle thus might have exerted toxic neurobehavorial effects on the cauda equina and/or the spinal cord through the injured dura matter. COX-2 inhibitor with higher water solubility may be needed for safe clinical use, although epidural injection induced neither motor paresis nor sensory deficits in rats without intentional dural tear. Epidural injection of COX-2 inhibitor in our rat model resulted in decrease in mechanical hyperalgesia in the NP+anti-COX group, which exhibited less pronounced inflammatory reaction around the nerve root at 7 days after injection. Prostaglandins and thromboxane, which are produced by COX-2 in inflammatory cells, thus appear to be related to the inflammatory process produced by application of nucleus pulposus to the nerve root. Epidural injection of COX-2 inhibitor may be therapeutically useful if the vehicle used has no neural toxicity, and may attenuate painful radiculopathy due to lumbar disc herniation.

Figure Changes in sensitivity to mechanical noxious stimuli over time. -%: Hyperalgesia, * p < 0.05.