ROLES OF THROMBOXANE A2 AND LEUKOTRIENE B4 IN PAIN-RELATED BEHAVIOR INDUCED BY NUCLEUS PULPOSUS IN THE RAT

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Introduction
It has been reported that biologically active substances such as prostaglandins, thromboxanes and leukotrienes, which are metabolites involved in the arachidonic acid cascade, are present in herniated disc samples obtained from patients with lumbar disc herniation. However, little is known concerning the relationships between these substances and clinical symptoms including radicular pain. We demonstrated that autologous nucleus pulposus, relocated to the lumbar nerve root in the rat, produces time-dependent reversible mechanical hyperalgesia, which is thought to be a pain-related behavior in peripheral neuropathic pain models, and which is 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. It has been reported that nucleus pulposus applied on the nerve root induces loss of blood flow in the nerve root and delayed nerve conduction velocity, and that leukocytes play a role in pathophysiological mechanism of pain-related behavior induced by nucleus pulposus. Thromboxane A2 (TXA2) induces not only platelet aggregation, but also blood vessel contraction. Leukotriene B4 (LTB4) is a potent chemotactic agent, which recruits neutrophils and lymphocytes to the site of inflammation. Therefore, it is possible that TXA2 and LTB4 play a significant role in nerve root dysfunction including radicular pain in lumbar disc herniation. The purpose of this study was to examine the roles of TXA2 and LTB4 in hyperalgesia induced by 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-seven 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 3 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 laminectomy. In the NP group (n=24), after amputation of the tail, the nucleus pulposus obtained from the amputated tail was relocatet on the exposed nerve roots. A permanent catheter was placed to permit epidural injections at L4-5 in all rats except those of the control group. Motor function and reflex responses to noxious mechanical and thermal stimuli to both hind paws were measured in all rats preoperatively and by 14 days postoperatively (PO). The percentage difference between both hind paw responses to noxious stimuli was computed so that negative percentages reflected hyperalgesia, and positive percentages represented hypoalgesia. At 7 days PO, the NP group was divided into 3 subgroups (each n=8). In the NP+S, NP+T and NP+L groups, 0.25 ml of physiological saline, TXA2 synthase inhibitor (OKY-046Na, 0.1 mg/kg) and LTB4 receptor antagonist (ONO-4057, 3 mg/kg) were injected into the epidural space through the implanted catheter, respectively. Thirty minutes, 3 and 7 days after epidural injections, motor function and sensitivities to noxious mechanical and thermal stimuli to both hind paws were measured in 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 and classified by 2 independent examiners in a blind manner. Data obtained from these measurements were analyzed by ANOVA and Student's t-test for statistical analysis. A p value < 0.05 was considered significant.

Results
None of the rats in any group had motor paresis of their hindpaws. In the control and sham groups, rats exhibited normal response to noxious mechanical stimuli. Rats in the NP group exhibited evidence of mechanical hyperalgesia in the ipsilateral hindpaws at 5 and 7 days PO (p<0.05). After epidural injection of physiological saline, rats in the NP+S group had evidence of mechanical hyperalgesia, which lasted until sacrifice at 2 weeks PO (p<0.05). In the NP+L group, there was a tendency toward decrease in mechanical hyperalgesia at 30 minutes after epidural injection of LTB4 receptor antagonist, compared with that in the NP+T group. At 3 and 7 days after epidural injection, rats in the NP+T and NP+L groups exhibited decrease in mechanical hyperalgesia, compared with the NP+S group (p<0.05). There were no significant differences in sensitivities to noxious mechanical stimuli between the control and sham groups at 10 and 14 days PO or the NP+L and NP+T groups at 3 and 7 days after epidural injection (Figure). There were no significant differences in sensitivities to thermal noxious stimuli among the groups. Histological examination revealed the formation of granulation tissues accompanied by numerous monocyteic cells in the NP+S group. Inflammatory reactions and granulation tissue were less pronounced in the NP+L and NP+T groups than in the NP+S group.

Discussion
Epidural injection of LTB4 receptor antagonist in this rat model resulted in decrease in mechanical hyperalgesia 30 minutes after injection, which was not completely abolished. We previously reported that the hyperalgesia was completely abolished immediately after epidural injection of phospholipase A2 inhibitor. It is thus possible that not only LTB4 but also prostaglandins involved in the arachidonic acid cascade are related to the hyperalgesia induced by nucleus pulposus. Although TXA2 synthase inhibitor did not affect mechanical hyperalgesia immediately after the epidural injection, decreased mechanical hyperalgesia at 3 and 7 days after epidural injections was observed in the NP+L and NP+T groups, which exhibited less pronounced inflammatory reactions around the nerve root. Not only recruitment, aggregation and activation of neutrophils induced by LTB4 but also the platelet aggregation and blood vessel contraction induced by TXA2 thus appear to be related to the inflammatory process produced by application of nucleus pulposus to the nerve root. Epidural injection of LTB4 receptor antagonist and/or TXA2 synthase inhibitor may be therapeutically useful, and may attenuate painful radiculopathy due to lumbar disc herniation. In conclusion, it is possible that TXA2 and LTB4 play significant roles in the mechanical hyperalgesia induced by autologous nucleus pulposus.

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

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