The interaction of 5-hydroxytryptamine and tumor necrosis factor-alpha to nucleus pulposus induced pain-related behavior in rats.

+4Kobayashi, H; 1Sekiguchi, M; 1Kato, K; 1Kikuchi, S; 1Konno, S
+4Department of Orthopaedic Surgery, Fukushima medical University, Fukushima City, Japan
hirokish@fmu.ac.jp

Introduction: Lumbar disc herniation (LDH) is a major cause of sciatica. The herniated disc induces sciatica by both mechanical and chemical factors. Several studies have demonstrated that various proinflammatory cytokines, monoamine derived substances, and other factors play crucial roles in the chemical pathogenesis of sciatica in LDH. Serotonin (5-hydroxytryptamine [5-HT]), one of the monoamines, is an inflammatory mediator released from platelets in injured and inflamed tissues. Exogenous 5-HT on the nerve root induces pain related behavior in rats. A selective 5-HT2A receptor antagonist was shown to improve sciatica in both experimental and clinical studies. Tumor necrosis factor-alpha (TNF), one of the inflammatory cytokines, is present in the nucleus pulposus (NP). Exogenous TNF applied on the DRG also induces pain-related behavior in rats. A selective inhibition of TNF prevents NP-induced pain related behavior, pathological changes in nerve root, and reduction of nerve conduction velocity. However, little is known about how much and when these chemical factors may affect pain-related behavior induced by nucleus pulposus applied to the nerve root in rats. The purpose of this study was to examine the effects of exogenous 5-HT and TNF applied to the nerve root on pain-related behavior and immunohistochemical changes in DRG. In order to examine the interaction of 5-HT with TNF, the expression of TNF, TNF receptor 1, and 5-HT2A receptors in DRGs were evaluated, and the release of endogenous 5-HT in plasma was measured using rat models.

Material & Methods: The experiment was carried under the control of the Animal Care and Use Committee in accordance with the Guidelines for Animal Experiments of Fukushima Medical University and Japanese Government Law Concerning the Protection and Control of Animals.

Animals and Anesthesia A total of 230 adult female Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) weighing 180 to 250 g were used. Surgical Procedure The L5 DRG and spinal nerve were exposed by L5/6 partial laminectomy. Rats were divided into 6 groups as described following the table 1. A 27-gauge needle connected to a micro syringe was inserted into the perineural space of the nerve root just distal to the DRG, and the substances described under “Experimental Groups” were slowly injected. In the NP group, rats received autologous NP, which was harvested from a coccygeal vertebral disc, to the DRG and were injected 100µL of normal saline to the nerve root.

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<th>Table 1: Experimental groups</th>
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<td>group</td>
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<td>5-HT group</td>
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Behavioral Test Von Frey tests were used for pain-related behavior testing. The hind paw withdrawal response to von Frey hair (Stoelting, Wood Dale, IL) stimulation of the plantar surface of the footpads was determined at 2, 7, 14, 21, and 28 days after surgery.

Immunohistochemical analyses of ATF3 and CGRP
Immunohistologic examinations were performed in each group (n = 5) at 2, 7, 14, 21, and 28 days after surgery. The expressions of activating transcription factor 3 (ATF3), a marker of nerve injury, and calcitonin gene related peptide (CGRP), an inflammatory neuropedete, were evaluated using immunohistochemistry.

Immunoblotting for TNF, TNFR1, and 5-HT2A Receptors
The expressions of TNF, TNF receptor 1 (TNFR1), and 5-HT2A receptors in the left L5 DRG were examined using Western blotting in each group (n = 5) at 2, 7, and 14 days after surgery.

High Performance Liquid Chromatography (HPLC) Analysis of Plasma 5-HIAA
5-hydroxyindole acetic acid (5-HIAA), a metabolite of 5-HT, in plasma was measured in TNF, NP, and control groups at 2, 7, and 14 days after surgery.

Results: Behavioral Test
In the NP group, the mechanical withdrawal thresholds were decreased during the 28 days after surgery (Figure 1). In the control group, the thresholds were not decreased for 28 days. The thresholds in the NP group significantly decreased for 28 days compared with the control group (p < 0.05). In the 5-HT and TNF groups, the thresholds were significantly decreased during the 7 days after surgery compared with the control group (p < 0.01). The thresholds in the two groups increased from 14 days to 28 days after surgery and there was no significant difference compared with the control group. In the combination group, the thresholds were significantly decreased during the 21 days after surgery compared with the control group (p < 0.05).

Immunohistochemical analyses of ATF3 and CGRP
ATF3-IR neurons increased for 2 days in the 5-HT, TNF, and combination groups, whereas increased for 7days in the NP group compared with the control group (p < 0.05). CGRP-IR neurons significantly increased in the 5-HT, TNF, combination, and the NP groups at day7 compared with the control group (p < 0.05).

Immunoblotting for TNF, TNFR1, and 5-HT2A Receptors
TNF bands in the DRGs were detected at 26kDa. In the 5-HT and NP groups, the expression level of TNF was significantly increased during 2 days and 7 days after surgery in comparison with the control group (p < 0.05). TNFR1 bands were detected at 55kDa. In the 5-HT and NP groups, the expression level of TNF receptor 1 was significantly increased during 2 days and 7 days after surgery in comparison with the control group, (p < 0.05). 5-HT2A receptor bands were detected at 53kDa. In comparison with the control group, the expression level of 5-HT2A receptors was significantly increased during the 7 days after surgery in the TNF and NP groups (p < 0.05).

HPLC Analysis of Plasma 5-HIAA
At 2 days after surgery, concentrations of plasma 5-HIAA in the NP group were significantly increased compared with the control group (p < 0.05). On the other hand, concentrations of plasma 5-HIAA in the TNF group were not significantly increased compared with the control group at any time points.

Discussion: We demonstrated that exogenous 5-HT and TNF applied to the nerve root induce pain-related behavior, nerve damage, and inflammation. These effects only last for a short period when compared with combination of them and NP. 5-HT induces TNF and TNFR1, TNF induces 5-HT2AR in this LDH rat model. These results suggest that 5-HT and TNF interact each other and prolong the reduction of mechanical threshold in a rat LDH model. To modulate the interaction between 5-HT and TNF may exert more specific therapeutic effects for treating sciatica in LDH patients.

Figure 1: Changes in the mechanical withdrawal threshold of the foot pad in rats. Data are means ± SE (n=12 for each group). *p < 0.05, †p < 0.01, compared with the control group.