5-HT2A receptor antagonist attenuates pain-related behavior in a rat lumbar disc herniation model

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Introduction: The herniated disc induces painful radiculopathy by both the mechanical deformation and biochemical irritations to the nerve root and dorsal root ganglions (DRG). Serotonin (5-hydroxytryptamine [5-HT]) is a monoamine that is released from platelets and mast cells in injured and inflamed tissues. Exogenous 5-HT applied to the rat nerve root induces pain-related behavior. Moreover, the application of nucleus pulposus from the herniated disc can induces 5-HT production and expression of 5-HT2A receptors in the adjacent DRG. Thus, 5-HT has recently been shown to be a potential therapeutic target against radiculopathy in lumbar disc herniation (LDH). However, little is known about the efficacy of 5-HT inhibition for treating radiculopathy. The present study was designed to evaluate the effect of 5-HT2A receptor antagonist on pain-related behavior, the endogenous 5-HT production in plasma, and the expression of 5-HT2A receptors in DRG in a rat model of LDH.

Materials and Methods: Surgical Procedure. A total of 90 adult female Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) were used. The left L5 nerve root, DRG, and spinal nerve were exposed by L5/6 partial laminectomy with great care taken to avoid trauma to tissue. Nucleus pulposus harvested from the tail was applied onto the left L5 nerve root just distal to the DRG. 5-HT2A receptor antagonist (sarpogrelate hydrochloride [SPG]; Mitsubishi Pharma Corp., Osaka, Japan) was administered orally once a day from 1 to 21 days postoperatively as described following the table.

<table>
<thead>
<tr>
<th>Group</th>
<th>dose (mg/kg)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP</td>
<td>vehicle</td>
<td>30</td>
</tr>
<tr>
<td>NP + Placebo group</td>
<td>vehicle</td>
<td>30</td>
</tr>
<tr>
<td>NP + Low dose SPG group</td>
<td>1 mg/kg</td>
<td>30</td>
</tr>
<tr>
<td>NP + High dose SPG group</td>
<td>10 mg/kg</td>
<td>30</td>
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Behavioral test. The hind paw withdrawal response to von Frey hair stimulation of the plantar surface of the left footpads was determined in all groups (n=10, each group) at 2, 5, 7, 9, 12, 14, 16, 19 and 21 days after surgery. All behavioral tests were performed by a technician who was unaware of the experimental groupings. Data were analyzed for statistical significance using ANOVA followed by Tukey’s post-hoc test (p value <0.05).

High-performance Liquid Chromatography Analysis of Plasma 5-hydroxyindole Acetic Acid. High-performance liquid chromatography (HPLC) examination of plasma were performed in all groups (n=5 in each group at 2, 7, 14, and 21 days after surgery). Rats were anesthetized and blood samples (4.0 mL) were collected by heart puncture through a polyethylene tube and mixed with 1/10 volume of 1.5% disodium dihydrogen ethylenediamine tetraacetate dehydrate (EDTA-2Na). To obtain platelet-poor-plasma (PPP), the samples were centrifuged at 4000g for 10 minutes. 5-hydroxyindole acetic acid (5-HIAA), the metabolite of 5-HT, in PPP was measured by HPLC coupled with electrochemical detection. The lower detection limit of the method was 0.1 ng/mL. Data were analyzed for statistical significance using ANOVA followed by Tukey’s post-hoc test (p value <0.05).

Immunoblotting for 5-HT2A receptors. Immunoblotting examinations were performed in all groups (n=5 at 2, 7, 14, and 21 days after surgery; same animals were used for HPLC experiments). L5 DRG tissues were harvested and homogenized in lysis buffer. Twenty micrograms of total protein was loaded and electrophoresed in 10% Tris-glycine SDS-polyacrylamide gel, and then transferred to polyvinylidene difluoride filter membranes (Millipore, Billerica, MA). The membranes were incubated with anti-5-HT2A receptor (1:150; Abcam Inc., Cambridge, MA) and mouse anti-β-actin antibodies (1:5000; Sigma, Saint Louis, MO). Positive bands were visualized using an enhanced chemiluminescence system and analyzed by ratio against internal control β-actin using Image J software. As a positive control for these bands, we also analyzed 5-HT2A receptor immunoreactivity in naïve rat (n=1) brain lysate. Data were analyzed for statistical significance using ANOVA followed by Tukey’s post-hoc test (p value <0.05).

Results: Behavioral test. Rats in all groups showed stable conditions before surgery in response to mechanical stimulation. In the high-dose (10 mg/kg) SPG group, the mechanical withdrawal thresholds were significantly recovered from 5 to 21 days after surgery compared with the control group (p<0.05 at day 19, p<0.01 at day 5, 7, 9, 12, 16, and 21), and there were no significant differences at day 14. There were no significant differences between the low-dose (1 mg/kg) SPG group and control group during the 21 days after surgery (Figure 1).

HPLC analysis for plasma 5-HIAA. At all time-points, there were no significant differences in all groups.

Immunoblotting for 5-HT2A receptors. In the low-dose SPG group, the expressions of 5-HT2A receptors were inhibited after 14 days. In the high-dose SPG group, the expressions of 5-HT2A receptors were inhibited during the 21 days after surgery compared with the control group (Figure 2).

Discussion: In the present study, we demonstrate that 5-HT2A receptor antagonist (sarpogrelate hydrochloride) attenuated pain-related behavior and paradoxically suppressed the expression of 5-HT2A receptors in the DRG, but did not affect the 5-HT production. Unlike other G protein-coupled receptors, 5-HT2 class receptors have shown to be down-regulated by many antagonists (2). Sarpogrelate hydrochloride might have potential to attenuate painful radiculopathy due to down-regulation of 5-HT2A receptors expressed on the DRG. Our results offer a promising new pharmaceutical options for treat painful radiculopathy in lumbar disc herniation.


Figure 1: Changes in the mechanical withdrawal threshold of the foot pad in rats. Data are means ± SD (n=10 for each group). *p<0.05, **p<0.01, compared with the control group.

Figure 2: Expression of 5-HT2A receptor proteins in left L5 DRG tissues determined by Immunoblotting. Positive bands were analyzed by ratio against internal control β-actin. As a positive control for these bands, naïve rat brain lysate (RB) was used. Data are means ± SD (n=5 for each group). *p<0.05, **p<0.01 compared with the control group.