Effect of Lafutidine on Inflammatory Pain

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We investigated the effect of Lafutidine on inflammatory pain. This was done by studying how capsaicin receptors (TRPV 1) affected Lafutidine by pretreating animals with a capsaicin antagonist, capsazepine. We prepared an inflammatory pain model, the complete Freund's adjuvant (CFA) model, and investigated the development of inflammatory pain using behavioral assessments. Lafutidine was administered to the hind paw of experimental rats at 10 and 150 mg/kg, and the effect was investigated using behavioral assessments. We found that although heat-hyperalgesia and mechano-allodynia induced by inflammation were slightly promoted at 10 mg/kg, hypalgesia and hypoesthesia were noted at 150 mg/kg. The latter findings were not seen at 10 mg/kg. Pretreatment with capsazepine inhibited these effects. We concluded that Lafutidine exhibited different actions on inflammatory pain depending on the dose, and that the effects were exhibited through capsaicin-sensitive sensory nerves. Based on these findings, we concluded that TRPV 1 is closely involved in the pain mechanism, and that Lafutidine may be effective for painful disorders. (J Osaka Dent Univ 2006; 40: 31-36)

Key words: Lafutidine; Inflammatory pain; CFA model; Capsaicin receptor

INTRODUCTION

Lafutidine is an antiulcer drug that inhibits gastric acid secretion by H2 receptor antagonism, and promotes the stomach mucosal defense system. Lafutidine differs from famotidine and cimetidine generally used in clinical practice in that it protects the stomach mucosa by increasing blood flow through TRPV 1.1,2 TRPV 1 receives three types of stimulation, capsaicin, heat, and acid. It plays a central role in pain reception, and has also been reported to play an important role in the mechanism of inflammatory pain.3 It is anticipated that drugs that act on TRPV 1 as analgesics will be developed to replace NSAIDs.4 We studied the action of Lafutidine on TRPV 1, and investigated its effect on inflammatory pain.

MATERIALS AND METHODS

1) Animals and preparation of inflammatory pain model

Eighty-four male Sprague-Dawley rats with body weight between 200 g and 250 g were used (Japan SLC, Hamamatsu, Japan). The animals were given food pellets and drinking water ad libitum. The inflammatory pain model was prepared by subcutaneous injection of 100 μL of complete Freund's adjuvant (CFA) (Calbiochem-Novabiochem Corporation, San Diego, CA, USA) into the surface of the left hind paw under diethyl ether anesthesia (Sigma Aldrich Japan, Tokyo). Behavioral assessment was made 24 hours after administration, and rats judged having inflammatory pain based on the criteria described below were used for the experiment. The animals were handled in conformity to 'The Guidelines for Animal Research at Osaka Dental University',5 and the study was approved by the Animal
Experiment Committee of Osaka Dental University (No. 05-04001).

2) Drug administration
Rats confirmed to have inflammatory pain were divided into 7 groups: A group without drug administration (the controls, C group), a group treated with 100 μL of physiological saline alone (S group), groups treated with 10 and 150 mg/kg lafutidine (L 10 and L 150 groups, respectively), groups pretreated with 0.6 mg capsazepine before administration of 10 and 150 mg/kg lafutidine (CL 10 and CL 150 groups, respectively), and a group treated with 0.6 mg capsazepine alone (CPZ group). To confirm that the effect of lafutidine is exerted through capsaicin-sensitive sensory nerves, the animals were pretreated with a TRPV1 antagonist, capsazepine. Lafutidine was dissolved with 1 N hydrochloric acid/1 N sodium hydroxide (4:1) at pH 7.0. It was administered at 10 and 150 mg/kg body weight. Capsazepine (0.6 mg) was dissolved with 100 μL of physiological saline containing 50% dimethylsulfoxide (Table 1).

3) Behavioral assessment
Behavioral assessment was made in a quiet room at 25°C. Withdrawal responses of the rats were measured by mechanical and thermal tests, and mechano-allodynia and heat-hyperalgesia were evaluated. The tests were performed before and after the operation and at 2, 5, 24 and 48 hours after drug administration (Fig. 1).

a) von Frey test (mechano-alldynia)
The rat was placed in a chamber with a metal mesh floor and acclimated to the environment for 30 minutes, followed by measurement using a Dynamic Plantar Aesthesiometer® (UgoBasil, Comerio, Italy). A filament set to increase the weight load to 50 g within 20 seconds was touched to the hind paw 4 times at 5-minute intervals. The weight in grams when the rat elevated the hind paw was recorded. The mean of the weights was regarded as the threshold for that animal. When the threshold calculated by subtracting the threshold before CFA administration from the threshold at 24 hours after CFA administration was negative, the animal was judged as having inflammatory pain.

b) Thermal test (heat-hyperalgesia)
The rat was placed in a chamber and acclimated to the environment for 30 minutes, followed by measurement using a heat stimulation instrument (The Plantar Test®, UgoBasil). The surface of the hind paw was stimulated with heat, and the withdrawal latency before elevation of the hind paw was measured 4 times at 5-minute intervals. The mean of the latency was regarded as the threshold for the animal. When the threshold calculated by subtracting the threshold before CFA administration from the threshold at 2, 5, 24 and 48 hours after lafutidine administration was negative, the animal was judged as having inflammatory pain.

Fig. 1 Protocol. After confirming there was no difference among the rats in the behavioral assessments, the CFA model was surgically prepared. The development of inflammatory pain was confirmed after 24 hours by the behavioral assessments. Capsazepine was administered to the CL 10, CL 150 and CPZ groups. Lafutidine was administered 30 minutes later. Assessments were performed at 2, 5, 24 and 48 hours after lafutidine administration.

Table 1 Experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Lafutidine(mg/kg)</th>
<th>Capsazepine(mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>L 10</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>L 150</td>
<td>10</td>
<td>0.6</td>
</tr>
<tr>
<td>CL 10</td>
<td>150</td>
<td>0.6</td>
</tr>
<tr>
<td>CL 150</td>
<td>150</td>
<td>0.6</td>
</tr>
<tr>
<td>CPZ</td>
<td>-</td>
<td>0.6</td>
</tr>
</tbody>
</table>

No drugs were administered to the control(C) group. The saline(S) group was treated with the solvent of lafutidine, physiological saline, alone. The L 10 group was treated with 10 mg/kg lafutidine, and the CL 10 group was treated with 0.6 mg capsazepine followed by 10 mg/kg lafutidine. The L 150 group was treated with 150 mg/kg lafutidine, and the CL 150 group was treated with 0.6 mg capsazepine followed by 150 mg/kg lafutidine. The CPZ group was treated with capsazepine alone.
threshold at 24 hours after CFA administration was negative, the animal was judged as having inflammatory pain.

4) Statistical analysis
For statistical analysis, intragroup and intergroup repeated measures of ANOVA and the post hoc test (Fisher’s PLSD) were performed; *p < 0.05 was regarded as significant.

RESULTS

a) von Frey test
Figures 2-1 and 2-2 show the von Frey test results. The reaction threshold was significantly decreased in the L 10 group at 5, 24 and 48 hours after administration compared with the controls. No significant differences were noted in the S, CL 150 or CPZ group compared with the controls. In the L 150
group, the reaction threshold was significantly increased at 2, 24 and 48 hours after administration compared with the controls. No significant differences were noted in the S, CL 150 or CPZ group compared with the controls.

**b) Thermal test**

Figures 3–1 and 3–2 show the thermal test results. No significant differences were noted among the groups or time points. In the L 150 group, the reaction threshold was significantly increased at 2, 5, 24 and 48 hours after administration compared with the controls. No significant differences were noted in the S, CL 10 or CPZ group compared with the controls.

**DISCUSSION**

Several ion channel-type receptor genes related to pain reception have recently been cloned. Ion channel-type receptors receive nociceptive stimulations at sensory nerve endings, open the channel, and induce depolarization and excitation of neurons. In this way, nociceptive stimulation is transmitted. Capsaicin receptors (TRPV 1), which are ion channel-type receptors, receive three types of stimulation, capsaicin, heat, and acid. They have been attracting attention as molecules that play a central role in pain reception. Expression of TRPV 1 has been confirmed not only in small and intermediate neurons of the spinal dorsal root ganglia and trigeminal ganglion (unmyelinated C fibers and Aδ fibers), but also in sensory nerve endings, the skin, tongue, and brain. These findings suggest its involvement in pain reception in various regions. In addition, close involvement of TRPV 1 in the inflammatory pain mechanism has been reported, confirming that inflammation-related substances regulate TRPV 1 activity.

It has been suggested that pain is produced in the early stage of inflammation as a result of TRPV 1 activity that is increased by inflammation-related substances released from inflammatory tissue. It has also been suggested that changes in TRPV 1 expression is involved in delayed inflammatory pain and the persistence of and sensitization to pain. For these reasons, drugs that act on TRPV 1 may be effective in reducing inflammatory pain. It is expected that such drugs may be developed as analgesics to replace NSAIDs.

Lafutidine is an H₂ receptor antagonist used as a therapeutic drug for stomach ulcers and as a pre-anesthetic medication. It increases mucosal protective factors through TRPV 1 and has an inhibitory effect on gastric acid secretion through H₂ receptors. The mediation through TRPV 1 is considered a novel mechanism different from that of previous H₂ receptor antagonists. The drug induces stomach mucosal vasodilatation through TRPV 1, which increases blood flow and protects the stomach mucosa.

We studied the action of lafutidine through TRPV 1, and investigated how it affected inflammatory pain in an inflammatory pain model, the CFA model. Based on behavioral assessments, we found that lafutidine significantly promoted mechano-allodynia in the von Frey test beginning 5 hours after administration at the low dose of 10 mg/kg. This effect persisted until 48 hours after administration. Although no significant changes were noted at 10 mg/kg with the thermal test, heat-hyperalgesia was slightly promoted 5 hours after administration.

Administration of a TRPV 1 antagonist, capsaicin, inhibited lafutidine-induced promotion of mechano-allodynia and heat-hyperalgesia, suggesting that these effects are mediated by TRPV 1. Increased sensitivity of TRPV 1 in tissues with CFA-induced inflammation may have promoted mechano-allodynia and heat-hyperalgesia. There have been reports that increases in inflammation-related substances during tissue injury, inflammation and nerve injury (ATP, bradykinin and neurotrophic factor (NGF)) tend to increase sensitivity of nociceptive receptors including TRPV 1, and that this increased sensitivity is involved in induction of pain and abnormal pain sensation. These have also been reports that TRPV 1-expressing unmyelinated C fibers and myelinated Aδ fibers increase during inflammation, and that inflammation-related substances promote axon transport of TRPV 1 from spinal dorsal root ganglion cells to
sensory nerve endings, increasing the TRPV1 expression level in the nerve endings.\textsuperscript{15}

Studies on changes in TRPV1 expression level in the CFA model have reported a significant increase after administration of CFA.\textsuperscript{12, 16} This suggests that CFA increases TRPV1 in the inflammatory region, which increases the action of lafutidine and promotes mechano-allodynia and heat-hyperalgesia. Results of the thermal test indicated that administration of a TRPV1 antagonist, capsazepine, after CFA administration reduced heat-hyperalgesia. This may have been due to inhibition of heat-hyperalgesia by capsazepine. Reports have shown that capsazepine does not improve carrageenan-induced inflammation, but rather inhibits inflammation-induced hyperalgesia,\textsuperscript{17} and that capsazepine inhibits formalin- and capsaicin-induced pain reactions.\textsuperscript{18} At a high dose of 150 mg/kg, significant increases in the reaction thresholds were noted in both the mechanical and thermal tests two hours after administration of lafutidine. The tests indicate the presence of mecanoa-hypoesthesia and heat-hypoalgesia. The conditions persisted for 48 hours. These effects were inhibited by capsazepine, suggesting that the effects were exerted through capsaicin-sensitive sensory nerves similar to the situation at 10 mg/kg.

Regarding the increases noted in the reaction thresholds, it has been reported that capsaicin acts as neurotoxin, and that massive administration of capsaicin to neonatal rats induces neuronal loss.\textsuperscript{19} It has also been reported that TRPV1-expressing cells exposed to capsaicin for several hours swelled markedly and died,\textsuperscript{6} suggesting that lafutidine in high concentrations is a neurotoxin that can induce neuronal loss or death, as well as mecanoa-hypoesthesia and heat-hypoalgesia.

It has been reported that exposure of sensory nerve cells to capsaicin induces 'desensitization', in which repeated administration and massive administration decrease electric flow responses.\textsuperscript{1} It is thought that administration of a high concentration of lafutidine might desensitize sensory nerve cells. Clinically, this action is utilized in capsaicin cream which is used for treatment of neuralgia that occurs followings herpes zoster\textsuperscript{20} and for treatment of pain associated with diabetic neuropathy and rheumatic neuropathy.\textsuperscript{21}

Based on our findings, we concluded that lafutidine exhibits different effects depending on dose through capsaicin-sensitive sensory nerves, and that it affects inflammatory pain. Lafutidine has also been reported effective for glossalgia, a chronic pain in the oral field.\textsuperscript{22} It also increases blood flow thorough capsaicin-sensitive sensory nerves, exhaustion of neurotransmitters, and neurotoxin-induced anesthesia.\textsuperscript{22, 23} We have reported that lafutidine is effective in the CCI model, a neuropathic pain model.\textsuperscript{24} Another H\textsubscript{2} receptor antagonist, famotidine, was not effective, suggesting that capsaicin-sensitive sensory nerves mediate this action. Although the details of the mechanism of the lafutidine action on TRPV1 are unclear, these experiments suggest that lafutidine exhibits various actions through TRPV1. Further investigation of this issue should be productive.

CONCLUSION

We investigated the effect of an H\textsubscript{2} receptor antagonist, lafutidine, on inflammatory pain. At 10 mg/kg, it promoted mechano-allodynia and heat-hyperalgesia, and at 150 mg/kg, induced mecanoa-hypoesthesia and heat-hypoalgesia. These effects were inhibited by a TRPV1 antagonist, capsazepine, suggesting that the effects were exerted through capsaicin-sensitive sensory nerves. Based on these findings, we concluded that TRPV1 is closely involved in the pain mechanism, and that lafutidine may be effective for painful disorders.

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