Lafutidine, a histamine H2 receptor antagonist with mucosal protective properties, attenuates 5-fluorouracil-induced intestinal mucositis in mice through activation of extrinsic primary afferent neurons

Intestinal mucositis accompanied by severe diarrhea is one of the most common side effects during cancer chemotherapy. Lafutidine, a histamine H2 receptor antagonist with protective properties via sensory afferent neurons, is used for the treatment of upper gastrointestinal diseases. The present study investigated the effects of lafutidine on 5-fluorouracil (5-FU)-induced intestinal mucositis induced in mice. Male C57BL/6 wild-type (WT), sensory deafferented mice, and transient receptor potential vanilloid subfamily 1 knockout (TRPV1-KO) mice were used. Animals were administered 5-FU once daily, while lafutidine and famotidine were administered twice daily for 6 days. Repeated administration of 5-FU caused severe intestinal mucositis, characterized by shortening of villi and destruction of crypts and was accompanied by diarrhea and body weight loss. Daily administration of lafutidine reduced the severity of intestinal mucositis, diarrhea and body weight loss in a dose-dependent manner, while famotidine had no effect on intestinal mucositis. The preventive effects of lafutidine were completely abolished in sensory deafferented and TRPV1-KO mice. Lafutidine significantly suppressed 5-FU-increased MPO activity and inflammatory cytokine expression on day 6, but not apoptosis induction in intestinal crypts on day 1. Lafutidine induced Alcian Blue and PAS-positive mucus production in the small intestine. These findings suggest that lafutidine attenuates 5-FU-induced intestinal mucositis, most likely by increasing mucus production via activation of sensory afferent neurons. Furthermore, intact TRPV1 signaling is essential for the activation of sensory afferent neurons induced by lafutidine. Therefore, lafutidine is more useful than other common antacids for the treatment of intestinal mucositis during cancer chemotherapy.

Key words: intestinal mucositis, diarrhea, body weight loss, cancer chemotherapy, transient receptor potential vanilloid subfamily 1, deafferentiation, myeloperoxidase activity, inflammatory cytokine expression, mucus production
MATERIALS AND METHODS

Animals

This study was carried out in strict accordance with ARRIVE guidelines for reporting experiments involving animals (16). The protocols were approved by the committee on the Ethics of Animal Research of Kyoto Pharmaceutical University (Permit Number: 16-13-010). Eight- to nine-week-old male C57BL/6 mice weighing 20 – 24 g were purchased from SLC Incorporated (Shizuoka, Japan). Mice lacking transient receptor potential vanilloid subfamily 1 (TRPV1) were kindly provided by Dr. Julius D (University of California, San Francisco). All mice were maintained in plastic cages with free access to food and water, and were housed at 22 ± 1°C with a 12-h light/dark cycle.

Assessment of intestinal mucositis

On day 6 following the onset of 5-FU injection, jejunal tissue was washed with cold phosphate-buffered saline (PBS), and stored in RNA later (Ambion, Austin, TX) at 4°C before use. Total RNA was extracted using Sepasor RNA-1 Supper G (Nacalai Tesque, Kyoto, Japan) and reverse transcription (RT) was performed using PrimeScript Reverse Transcriptase (Takara, Shiga, Japan). Quantitative polymerase chain reaction (PCR) was carried out using ABI 7500 (Applied Biosystems, Foster City, CA) with SYBR Premix Ex Taq (Takara). Specific primer sets for β-actin (MA050368), TNF-α (MA097070) and IL-1β (MA025939) were obtained from the Perfect Real-Time Supporting System (Takara). Expression levels of TNF-α and IL-1β mRNA were calculated using the comparative CT (ΔΔCT) method (standardized against β-actin mRNA and expressed as fold-increase from normal group).

Experimental protocols

Animals were randomly divided into the following five groups to study the effects of lafutidine; normal (n = 8), control (5-FU) (n = 8), 5-FU + lafutidine at 3 mg/kg (n = 8), 10 mg/kg (n = 8) and 30 mg/kg (n = 7). Animals were also divided into the following three groups to study the effects of famotidine; normal (n = 8), control (5-FU) (n = 7) and 5-FU + famotidine (n = 7). In addition, sensory deafferented mice were randomly divided into three groups (normal (n = 7), control (5-FU) (n = 6) and lafutidine (30 mg/kg) (n = 6)), and TRPV1KO mice were randomly divided into three groups (normal (n = 7), control (5-FU) (n = 6) and famotidine (30 mg/kg) (n = 6)).

Lafutidine (3 – 30 mg/kg) and famotidine (30 mg/kg) were administered p.o. twice, 0.5 h before and 7 h after 5-FU injection, for 6 days (day 0 to 5). The normal group received saline (vehicle of 5-FU) and CMC solution (vehicle of lafutidine and famotidine), while the control group was given 5-FU and CMC solution (vehicle of lafutidine).

Immunohistochemical analysis

The intestine was removed from wild-type and TRPV1KO mice, washed with cold PBS and immersed in 4% paraformaldehyde for 2 h at 4°C. Immunohistochemical procedures were performed as described previously (21). Briefly, slide-mounted sections were treated with 10% normal donkey serum for 1 hour at room temperature and then incubated with a sheep polyclonal anti-calcitonin gene related peptide (CGRP) antibody (Enzo Life Scienes, Lausen, Switzerland) for 40 hours at room temperature. To visualise target protein expression, sections were incubated with Alexa Fluor 488-labelled donkey anti-sheep IgG (ThermoFisher Scientific, Waltham, MA) for 4 hour. Immunofluorescence was observed using confocal microscopes (A1R+; Nikon, Tokyo, Japan).

Determination of apoptosis

Twenty hours after the first injection of 5-FU (on day 1), jejunal tissues were fixed with 10% neutralized formalin.
embedded in paraffin, and cut into 4-µm sections. Apoptosis was detected by TUNEL assay using an in situ apoptosis detection kit (Takara) after treatment with proteinase K (Takara). Sections were counter-stained with hematoxylin. The number of cells positive for TUNEL assays were counted under a light microscope at a magnification of 400×, as described previously (9).

Measurement of intestinal mucus production

On day 4 following the onset of 5-FU treatment, jejunum tissues were immersed in Caenoy’s solution for about 2 hours. Tissues were embedded in paraffin, cut into 4-µm sections, and stained with Alcian Blue, pH 2.5 (AB-2.5), and Periodic Acid-Schiff (PAS). Intestinal goblet mucus cells were measured under a light microscope at a magnification of 200×.

Preparation of drugs

Drugs used were 5-FU (Sigma-Aldrich, St. Louis, MO), lafutidine, capsaicin (Wako, Osaka, Japan), carboxymethylcellulose (CMC: Nacalai Tesque), terbutaline (Bricanyl®), AstraZeneca, Osaka, Japan), aminophylline (Neophyllin®, Eisai, Tokyo, Japan) and atropine (Sigma-Aldrich). 5-FU was dissolved in physiological saline at a concentration of 5 mg/mL. Lafutidine was suspended with 0.5% CMC solution at concentrations of 0.3, 1, and 3 mg/mL. Capsaicin was dissolved in a Tween 80-ethanol solution (10% ethanol, 10% Tween and 80% saline, w/v; Wako, Osaka, Japan) for s.c. injection while it was suspended in a 0.5% CMC for topical application. Other agents were dissolved in saline. Each agent was prepared immediately before use and administered i.p., p.o. or s.c. at a volume of 0.1 mL/10 g body weight. Control animals received saline instead of the active agent.

Statistical analysis

Data are presented as means ± S.E.M. Statistical analyses were analyzed with GraphPad Prism 6.0b (GraphPad Software, La Jolla, CA) using a parametric one-way ANOVA followed by Dunnett’s multiple comparison test and non-parametric Kruskal-

![Graph](image)

**Fig. 1.** Effects of lafutidine (A) and famotidine (B) on body weight loss during 5-FU treatment. Animals were given 5-FU (50 mg/kg, i.p.) once daily while lafutidine (3, 10 and 30 mg/kg) and famotidine (30 mg/kg) were administered twice daily for 6 days. Body weight was determined daily and is shown as a percentage of initial body weight. Data are presented as means ± SE for 7 – 8 mice. Significant difference at P < 0.05; *from control (5-FU alone); #from normal (5-FU-untreated).
Wallis one-way ANOVA, followed by Dunn's multiple comparison test, with $P < 0.05$ regarded as statistically significant.

RESULTS

Effects of lafutidine and famotidine on body weight loss and diarrhea during 5-FU treatment

Repeated administration of 5-FU (50 mg/kg) to animals caused body weight loss (Fig. 1A) and diarrhea (Table 1). Mean body weight was reduced to 82.7 ± 0.8% of initial body weight on day 6. Prominent diarrhea was observed from day 4, and the mean diarrhea score reached 3 (2 – 3) on day 6. In the present study, repeated administration of 5-FU (50 mg/kg) for 6 days did not cause any animal deaths. Twice-daily administration of lafutidine (3 – 30 mg/kg) had no apparent effect on body weight loss, but reduced the severity of diarrhea during 5-FU treatment, in a mostly dose-dependent manner, and a significant effect was observed at a dose of 30 mg/kg. In contrast, daily administration of famotidine (30 mg/kg) had no effect on body weight loss (Fig. 1B) and diarrhea (Table 1) during 5-FU treatment.

Effects of lafutidine and famotidine on 5-FU induced intestinal mucositis

Repeated administration of 5-FU caused severe intestinal mucositis, histologically characterized by shortening of villi and destruction of crypts (Fig. 2A). Villus height shortened to 54.9% when compared to normal animals on day 6, while the destruction of intestinal crypts was evaluated by a decrease in the number of surviving crypts and crypt cells (Fig. 2B). The number of surviving crypts and crypt cells decreased to 40.9% and 51.2% when compared to normal animals, respectively, on day 6. Twice-daily administration of lafutidine dose-dependently mitigated the histological intestinal damage induced by 5-FU. In contrast, twice-daily administration of famotidine (30 mg/kg) had no effect on the histological intestinal damage (Fig. 3A and B).

Effects of lafutidine on changes in myeloperoxidase (MPO) activity and up-regulation TNF-α and IL-1β mRNA induced by 5-FU in the small intestine

Repeated administration of 5-FU caused a marked increase in intestinal MPO activity on day 6 (Fig. 4A). Twice-daily administration of lafutidine (30 mg/kg) significantly attenuated

![Fig. 2. Effects of lafutidine on shortening of villi and destruction of crypts induced by 5-FU in small intestines. Animals were given 5-FU (50 mg/kg, i.p.) once daily while lafutidine (3, 10 and 30 mg/kg) was administered twice daily for 6 days. Intestinal mucositis was evaluated on day 6 following the onset of 5-FU treatment. Histological observations for intestinal villi (100×) and crypts (400×) (A). Height of villi, number of surviving crypts per millimeter, and surviving cells per crypts were measured (B). Data are presented as means ± SE for 7 – 8 mice. Significant difference at $P < 0.05$; *from control (C, 5-FU alone); #from normal (N, 5-FU-untreated).]
Fig. 3. Effects of famotidine on shortening of villi and destruction of crypts induced by 5-FU in small intestines. Animals were given 5-FU (50 mg/kg, i.p.) once daily while famotidine (30 mg/kg) was administered twice daily for 6 days. Intestinal mucositis was evaluated on day 6 following the onset of 5-FU treatment. Histological observations for intestinal villi (100×) and crypts (400×) (A). Height of villi, number of surviving crypts per millimeter, and surviving cells per crypts were measured (B). Data are presented as means ± SE for 7 – 8 mice. Significant difference at P < 0.05; *from control (5-FU); #from normal (5-FU-untreated).

**Table 1. Effects of lafutidine and famotidine on diarrhea score during 5-FU treatment.**

<table>
<thead>
<tr>
<th></th>
<th>Number of animals</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8</td>
<td>0 (0 – 0)</td>
<td>0 (0 – 0)</td>
<td>0 (0 – 0)</td>
</tr>
<tr>
<td>Control (5-FU)</td>
<td>8</td>
<td>2 (1 – 3)</td>
<td>3 (1 – 3)</td>
<td>3 (2 – 3)</td>
</tr>
<tr>
<td>5-FU</td>
<td>8</td>
<td>(0 – 3)</td>
<td>(0 – 3)</td>
<td>(0 – 3)</td>
</tr>
<tr>
<td>+ Lafutidine (3 mg/kg)</td>
<td>8</td>
<td>2 (0 – 3)</td>
<td>2 (1 – 3)</td>
<td>2 (0 – 3)</td>
</tr>
<tr>
<td>+ Lafutidine (10 mg/kg)</td>
<td>8</td>
<td>2 (0 – 3)</td>
<td>1* (0 – 3)</td>
<td>2* (0 – 2)</td>
</tr>
<tr>
<td>+ Lafutidine (30 mg/kg)</td>
<td>7</td>
<td>(0 – 3)</td>
<td>(0 – 3)</td>
<td>(0 – 2)</td>
</tr>
<tr>
<td>Normal</td>
<td>8</td>
<td>0 (0 – 0)</td>
<td>0 (0 – 0)</td>
<td>0 (0 – 0)</td>
</tr>
<tr>
<td>Control (5-FU)</td>
<td>8</td>
<td>2 (0 – 2)</td>
<td>3 (2 – 3)</td>
<td>3 (3 – 3)</td>
</tr>
<tr>
<td>5-FU + Famotidine (30 mg/kg)</td>
<td>7</td>
<td>1 (0 – 2)</td>
<td>3 (1 – 3)</td>
<td>3 (2 – 3)</td>
</tr>
</tbody>
</table>

Animals were given 5-FU (50 mg/kg, i.p.) once daily while lafutidine (3, 10 and 30 mg/kg) and famotidine (30 mg/kg) were administered twice daily for 6 days. Diarrhea was scored on day 4 – 6 using five-grade scale (0 to 4). Data are presented as median (min – max) for 7 – 8 mice. Significant difference at P < 0.05; *from control (5-FU); #from normal (5-FU-untreated).
Fig. 4. Effects of lafutidine on increase in MPO activity and up-regulation of TNF-α and IL-1β mRNA expressions induced by 5-FU in the small intestine. Animals were given 5-FU (50 mg/kg, i.p.) once daily while lafutidine (30 mg/kg) was administered twice daily for 6 days. Activity of intestinal MPO (A), expression of TNF-α and IL-1β mRNAs (B) were determined on day 6 following the onset of 5-FU treatment. Expression levels of each mRNA were standardized against β-actin mRNA and are expressed as fold-increase from normal group. Data are presented as means ± S.E for 5 – 8 mice. Significant difference at P < 0.05; * from control (5-FU alone); # from normal (5-FU-untreated).

Table 2. Effects of lafutidine on diarrhea score during 5-FU treatment in sensory deafferented and TRPV1KO mice.

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory deafferented mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>0 (0 – 0)</td>
<td>0 (0 – 0)</td>
</tr>
<tr>
<td>Control (5-FU)</td>
<td>6</td>
<td>1# (0 – 2)</td>
<td>2# (2 – 3)</td>
</tr>
<tr>
<td>5-FU + Lafutidine</td>
<td>6</td>
<td>1 (1 – 3)</td>
<td>2 (2 – 2)</td>
</tr>
<tr>
<td>TRPV1KO mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>0 (0 – 0)</td>
<td>0 (0 – 0)</td>
</tr>
<tr>
<td>Control (5-FU)</td>
<td>7</td>
<td>3# (2 – 3)</td>
<td>3# (3 – 3)</td>
</tr>
<tr>
<td>5-FU + Lafutidine</td>
<td>6</td>
<td>1 (2 – 3)</td>
<td>3 (2 – 3)</td>
</tr>
</tbody>
</table>

Sensory deafferented and TRPV1KO mice were given 5-FU (50 mg/kg, i.p.) once daily while lafutidine (30 mg/kg) was administered twice daily for 6 days. Diarrhea was scored on day 4 – 6 using five-grade scale (0 to 4). Data are presented as median (min – max) for 6 – 7 mice. Significant difference at P < 0.05; # from normal (5-FU-untreated).
5-FU-induced increase in MPO activity with an inhibition of 59.7%.

Similarly, repeated administration of 5-FU caused the up-regulation of TNF-α and IL-1β mRNA expressions (Fig. 4B). Twice-daily administration of lafutidine (30 mg/kg) significantly attenuated 5-FU-induced up-regulation of TNF-α and IL-1β mRNAs with the inhibition of 65.3 and 50.1%, respectively.

**Effects of lafutidine on 5-FU induced mucositis in sensory deafferented and TRPV1KO mice**

In sensory deafferented mice, repeated administration of 5-FU caused intestinal mucositis, characterized by shortening of villi and destruction of crypts accompanied by severe diarrhea and body weight loss (Fig. 5 and Table 2). Severity of 5-FU-induced intestinal mucositis was almost the same with normal (capsaicin-untreated) mice. Twice-daily administration of (30 mg/kg) failed to reduce the severity of intestinal mucositis in sensory deafferented mice.

Calcitonin gene-related peptide (CGRP)-positive sensory neurons were abundantly detected in the intestinal mucosa of both wild-type and TRPV1KO mice (Fig. 6A). The distribution of sensory neurons was not affected by deficiency of TRPV1. In TRPV1KO mice, repeated administration of 5-FU also caused intestinal mucositis, accompanied by severe diarrhea and body weight loss (Fig. 6B, 6C and Table 2). Severity of 5-FU-induced intestinal mucositis was almost the same with wild-type mice. Twice-daily administration of lafutidine (30 mg/kg) failed to reduce the severity of intestinal mucositis in TRPV1KO mice.

**Effects of lafutidine on 5-FU-induced apoptosis in the intestinal crypt**

TUNEL-positive apoptotic cells were mostly confined to intestinal crypts at 24 h after the first injection of 5-FU (day 1) (Fig. 7), as reported previously (20). Twice-daily administration of lafutidine (30 mg/kg) did not have any effect on 5-FU-induced apoptosis in the intestinal crypts.
Effects of lafutidine on mucus production in small intestine

In normal mice, Alcian Blue and PAS-positive mucus was detected in goblet cells and on the surface of the jejunum (mucus gel layer) (Fig. 8A). Twice-daily administration of lafutidine (30 mg/kg) significantly increased the number of goblet cells and showed a tendency to increase the mucus gel layer on day 4. Repeated administration of 5-FU markedly decreased the number of goblet cells (to 46.1% of normal mice) and mucus gel layer on day 4 (Fig. 8B). Twice-daily administration of lafutidine (30 mg/kg) significantly recovered 5-FU-induced decreases in goblet cells and showed a tendency to recover the decrease in the mucus gel layer on day 4.

DISCUSSION

Although 5-FU is one of the most commonly used clinical chemotherapeutic agents, it is well known that this agent frequently induces intestinal mucositis accompanied by severe diarrhea (1, 2). The etiology of this mucositis is not fully understood and there are no effective prevention or treatment methods. It is well-established that H₂ receptor antagonists and proton pump inhibitors are clinically useful for the prevention and treatment of gastrointestinal toxicities during cancer chemotherapy (22, 23). As these drugs potently reduce gastric acid secretion, their utility is considered to be limited in upper gastrointestinal toxicity. Thus, the potential effects of these drugs on intestinal mucositis accompanied by severe diarrhea remain uncertain.

In the present study, we demonstrated that lafutidine, a histamine H₂ receptor antagonist with mucosal protective properties, significantly attenuates 5-FU-induced intestinal mucositis in mice. Lafutidine is clinically used for treatment of upper gastrointestinal diseases such as peptic ulcers and reflux esophagitis in Japan and India (10-12). In addition, anti-secretory agents including histamine H₂ receptor antagonists and proton pump inhibitors are clinically used for prevention and
treatment of anti-cancer agent-induced upper gastrointestinal toxicities including gastric bleeding, nausea and vomiting. Thus, lafutidine is expected to be useful for both upper and lower gastrointestinal side effects during cancer chemotherapy.

Consistent with the findings from many previous studies (1-3, 24, 25), repeated administration of 5-FU caused severe intestinal mucositis, histologically characterized by shortening of villi and destruction of crypts, accompanied by systemic symptoms such as diarrhea and body weight loss. Twice-daily administration of lafutidine dose-dependently reduced the severity of intestinal mucositis such as shortening of villi and destruction of crypts induced by 5-FU. Lafutidine also significantly reduced the severity of diarrhea, but failed to suppress body weight loss during 5-FU treatment. We previously demonstrated that the occurrence of diarrhea is closely related to the severity of intestinal mucositis (9, 24). However, the present results support the notion that body weight loss may be caused by various systemic toxicities of 5-FU rather than intestinal mucositis. In contrast, twice-daily administration of famotidine, another H₂ receptor antagonist without any influence on sensory neurons, had no effect on 5-FU-induced intestinal mucositis, body weight loss and diarrhea. We previously showed that the dose of famotidine used in this study potently inhibited gastric acid secretion (26). These findings suggest that the protective effects of lafutidine on 5-FU-induced intestinal mucositis are independent of H₂ receptor blockade and its anti-secretory action.

Several studies have demonstrated the increased expression and production of inflammatory cytokines such as TNF-α and IL-1β, as well as increased MPO activity in chemotherapy-induced intestinal mucositis (7, 27-29). In the present study, we also observed that repeated administration of 5-FU caused up-regulation of TNF-α and IL-1β expression and increase in MPO activity in the small intestine on day 6. Twice-daily administration of lafutidine significantly suppressed 5-FU-induced these changes, suggesting that the protective effects of lafutidine on 5-FU-induced intestinal mucositis may be attributable to inhibition of inflammatory responses.

Lafutidine, a newer histamine H₂-receptor antagonist, has a unique component structurally that differs from conventional H₂-receptor antagonists and promotes gastric mucosal defense mechanisms, including mucus secretion (13). Several studies have shown that lafutidine protected the gastrointestinal mucosa including the esophagus, stomach, small intestine and large intestine other than anti-secretory actions (10-12, 30, 31). Furthermore, the mucosal protective effects of lafutidine were found to be mediated via capsaicin-sensitive sensory afferent neurons, similar to capsaicin, because the lafutidine-induced protective action was mostly abrogated by chemical ablation of sensory afferent neurons (11, 12, 30-32). In the present study, we observed that the ameliorative effects of lafutidine on 5-FU-induced intestinal mucositis were completely abrogated by sensory deafferentation caused by a large dose of capsaicin pretreatment. These findings suggest that lafutidine suppresses 5-FU-induced intestinal mucositis via activation of sensory afferent neurons.

Interestingly, twice-daily administration of lafutidine failed to suppress 5-FU-induced intestinal mucositis in TRPV1KO mice. TRPV1 is known to be a receptor for capsaicin and is mostly expressed in extrinsic sensory afferent neurons in gastrointestinal tracts (33, 34). Therefore, it is considered that capsaicin-induced mucosal protective effect could be mediated by TRPV1 expressed in sensory afferent neurons. In contrast, Nishihara et al. (35) reported that lafutidine alone had no influence.
on the release of CGRP from the stomach, but enhanced the release induced by a submaximal dose of capsaicin. They further showed that lafutidine had no effect on the specific binding of \[^3\text{H}\]-resiniferatoxin to TRPV1. Similarly, we previously reported that lafutidine had no effect by itself on the concentration of intracellular Ca\(^{2+}\) in rat TRPV1-transfected HEK239 cells, although capsaicin evoked significant increases in intracellular Ca\(^{2+}\) in these cells, and that capsazepine, a TRPV1 antagonist, did not significantly influence the protective effects of lafutidine (36). These results indicate that lafutidine activates capsaicin-sensitive afferent neurons independently via TRPV1. However, the present results clearly showed that administration of lafutidine failed to prevent 5-FU-induced intestinal mucositis in sensory TRPV1KO mice as well as in deafferented mice. The present findings reveal that intact TRPV1 signaling is essential for the activation of sensory afferent neurons induced by lafutidine. On the other hand, TRPV1 sensitization is reportedly mediated by adrenergic and prostacyclin/IP receptor activations (37, 38). Thus, it is possible that the lafutidine-induced activation of sensory afferent neurons may also be partly mediated via adrenergic and prostacyclin/IP receptor pathways.

Apoptosis in addition to hypoproliferation is considered to be involved in the pathogenesis of 5-FU-induced intestinal mucositis. Indeed, we previously reported that 5-FU increased the number of apoptotic cells and decreased the number of proliferative cells in the intestinal crypts, and that these responses were most evident 24 hours after the first injection (9). Thus, apoptosis in addition to hypoproliferation is important in the initial event for the course of 5-FU-induced intestinal mucositis. However, in the present study, lafutidine failed to prevent the induction of apoptosis by 5-FU. It is unlikely that the protective effects of lafutidine on 5-FU-induced intestinal mucositis may be accounted for by prevention of early apoptosis induction. These findings suggest that lafutidine suppresses the progression of intestinal mucositis after early damage, including apoptosis in the intestinal crypt.

Mucus production plays an important role in the physiological defense of the gastrointestinal mucosa. Several
studies have demonstrated that decreased mucin, a major component of mucus, is a cause of intestinal mucositis induced by chemotherapeutic agent (14, 39). To confirm the involvement of mucus production, we examined the effects of lafutidine on mucus production, particularly in the progression of 5-FU-induced intestinal mucositis. The administration of lafutidine increased the number of Alcian Blue and PAS-positive goblet cells, protected against 5-FU-induced mucosal injury and reduced mucus production, even on day 4. Thus, it is likely that lafutidine suppresses the progression of 5-FU-induced intestinal mucositis via mucus production after the initial event.

It is known that capsaicin-sensitive afferent neurons store and release calcitonin gene-related peptide (CGRP), the predominant neurotransmitter of spinal afferents in the rat stomach. The release of CGRP exerts gastroprotective actions such as hyperemia and mucus secretion in part through endogenous nitric oxide (40-42). Recently, Kwiecien et al. (43) showed that sensory afferent neurons releasing CGRP contributes to carbon monoxide-induced gastric protection via gastric hyperemia and prevention of oxidative stress. Further, we previously reported that the protective action of lafutidine on indomethacin-induced small intestinal injury was abrogated by chemical ablation of capsaicin-sensitive sensory neurons. Avidin B, a NO synthase inhibitor (12, 44). In the present study, we also observed that administration of lafutidine failed to prevent 5-FU-induced intestinal mucositis in sensory deafferented mice, and confirmed that lafutidine protected the intestinal mucosa from 5-FU-induced injury via the activation of capsaicin-sensitive afferent neurons.

We previously reported that serotonin 5-HT3 receptor antagonists and Japanese herbal medicine saireito significantly attenuated 5-FU-induced intestinal mucositis without any influence on the anti-tumor effects of 5-FU (9, 25). Thus, it is unlikely that the anti-tumor effects of 5-FU are dependent on the pathogenesis of intestinal mucositis. However, the influence of lafutidine on the anti-tumor effects of 5-FU cannot be completely ruled out at present. Further studies are necessary to confirm this point before clinical use.

Lafutidine can attenuate 5-FU-induced intestinal mucositis, most likely by increasing mucus production via activation of sensory afferent neurons. Therefore, lafutidine is more useful than other common antacids for the treatment of intestinal mucositis during cancer chemotherapy.

Conflicts of interest: None declared.

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