Exacerbation of Nonsteroidal Anti-Inflammatory Drug-Induced Small Intestinal Lesions by Antisecretory Drugs in Rats: The Role of Intestinal Motility

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ABSTRACT

Antisecretory drugs such as histamine H$_2$-receptor antagonists (H$_2$-RAs) and proton pump inhibitors (PPIs) are commonly used for the treatment of gastric and duodenal ulcers induced by nonsteroidal anti-inflammatory drugs (NSAIDs). However, the effects of these drugs on NSAID-induced small intestinal ulcers are not fully understood. The effects of H$_2$-RAs and PPIs on NSAID-induced gastrointestinal lesions and small intestinal motility were examined in rats. Male Wistar rats (180–220 g) were used. Indomethacin (10 mg/kg) was administered orally in fasted or fed rats, and gastrointestinal lesions were examined 24 h after indomethacin administration. Intestinal motility was measured by using a balloon method under urethane anesthesia. Indomethacin produced multiple lesions in the gastric corpus in fasted rats and in the small intestine in fed rats: 1) H$_2$-RAs (cimetidine, ranitidine, and famotidine) and PPIs (omeprazole, lansoprazole, and rabeprazole) markedly inhibited the formation of gastric lesions. 2) The drugs, except for lansoprazole, increased intestinal lesions. 3) H$_2$-RAs augmented the increase in intestinal motility caused by indomethacin, and the effects of H$_2$-RAs on motility and intestinal lesions were markedly inhibited by atropine. 4) Lansoprazole inhibited the formation of intestinal lesions, and the effect was prevented by both pharmacological ablation of capsaicin-sensitive sensory neurons and pretreatment with N-nitro-L-arginine methyl ester, a selective inhibitor of nitric-oxide synthesis. The results suggest that: 1) inhibition of acid secretion by antisecretory drugs may exacerbate NSAID-induced intestinal lesions, 2) H$_2$-RAs further aggravate lesions by increasing intestinal motility via the activation of cholinergic pathways, and 3) lansoprazole protects the intestinal mucosa against NSAID-related ulcerative stimuli.

Introduction

Recent progress in endoscopic techniques, such as capsule endoscopy and double balloon (push) endoscopy, has revealed that nonsteroidal anti-inflammatory drugs (NSAIDs) often cause mucosal lesions not only in the stomach and duodenum but also in the small intestine in humans (Goldstein et al., 2005; Graham et al., 2005; Maiden et al., 2005; Matsumoto et al., 2008). Antisecretory drugs such as histamine H$_2$-receptor antagonists (H$_2$-RAs) and proton pump inhibitors (PPIs) are commonly used for the treatment of upper gastrointestinal mucosal lesions induced by NSAIDs (Leandro et al., 2001; Peura, 2004; Yeomans et al., 2006; Scarpignato and Hunt, 2010; Sugano et al., 2011). However, the effects of the drugs on small intestinal ulcers are not fully understood. We reported previously that cimetidine (CIM) did not prevent but rather aggravated small intestinal lesions induced by indomethacin (IND) in cats despite a marked inhibition of the duodenal lesions (Satoh et al., 2009). Wallace et al. (2011) recently reported that repeated treatment with PPIs for 9 days exacerbated NSAID-induced intestinal lesions in rats.


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ABBREVIATIONS: NSAID, nonsteroidal anti-inflammatory drug; ACh, acetylcholine; ATR, atropine; CAP, capsaicin; CIM, cimetidine; CSSN, capsaicin-sensitive sensory neuron; DIC, diclofenac; FAM, famotidine; H$_2$-RA, histamine H$_2$-receptor antagonist; IND, indomethacin; LI, lesion index; L-NAME, N-nitro-L-arginine methyl ester; LPZ, lansoprazole; NO, nitric oxide; OPZ, omeprazole; PGE$_2$, prostaglandin E$_2$; PPI, proton pump inhibitor; RAN, ranitidine; RPZ, rabeprazole; SNP, sodium nitroprusside; VEH, vehicle.
present study, we examined the effects of typical H₂-RAs [CIM, ranitidine (RAN), and famotidine (FAM)] and PPIs [omeprazole (OPZ), lansoprazole (LPZ), and rabeprazole (RPZ)] on NSAID-induced gastrointestinal lesions in rats and found that the antisecretory drugs, except LPZ, exacerbated intestinal lesions, although all of the drugs markedly inhibited the formation of gastric lesions. The possible mechanism of the exacerbation by antisecretory drugs was examined in relation to the effect of the drugs on intestinal motility.

Materials and Methods

Ethics Approval. Experimental protocols were approved by the Animal Research Committees at Kyoto Pharmaceutical University (Kyoto, Japan).

Animals. Seven-week-old male Wistar rats (Shimizu Laboratories, Shizuoka, Japan) weighing 180 to 220 g were used. The animals were given rat chow pellets (MF; Oriental-Yeast, Tokyo, Japan) and had free access to water during the experimental periods unless otherwise specified.

Drugs. The following drugs and chemicals were used: acetylcholine (ACh), aminophylline, atropine (ATR), capsaicin (CAP), carboxymethylcellulose, cimetidine, diclofenac (DIC), dimethylsulfoxide, famotidine, Freund’s complete adjuvant (killed Mycobacterium tuberculosis, H37Ra), indomethacin, lansoprazole (Wako Pure Chemicals, Osaka, Japan), N-nitro-l-arginine methyl ester (l-NAME; Sigma, St. Louis, MO), omeprazole (Wako Pure Chemicals), prostaglandin E₂ (PGE₂; Ono, Osaka, Japan), rabeprazole sodium (Boche Scientific, New Brunswick, NJ), ranitidine, NaHCO₃ (Wako Pure Chemicals), sodium nitroprusside (SNP; Sigma), terbutaline (Sigma), Tween 50, and urethane (Wako Pure Chemicals). Drugs for oral or intravenous injection were dissolved in saline containing 10% dextrose containing 1% NaHCO₃ for oral administration. H₂-RAs for intravenous injection were dissolved in saline containing 10% dimethyl sulfoxide. The drugs were prepared just before the experiment.

Induction of Gastric Lesions. IND (10 mg/kg) was administered orally after a 16-h fast in normal rats. Antisecretory drugs were given orally; PPIs were administered once (30 min before IND), and H₂-RAs were administered twice (30 min before and 6 h after IND). The animals were sacrificed by ether overdose 24 h after IND administration. The stomachs were removed, filled with 10 ml of 1% formalin solution, and then immersed in the same formalin solution for 15 min. The stomachs were opened along the greater curvature. The length (mm) of the individual lesions in the corpus was measured under a dissecting microscope (SZ-PT; Olympus, Tokyo, Japan) with a 1-mm-square grid eyepiece (10×), and the sum of the lengths of all the lesions in each stomach was used as the lesion index (LI). The person measuring the lesions did not know the treatments given to the animals.

Induction of Small Intestinal Lesions. Rats were given regular chow pellets (CE-2; Japan Clea, Osaka, Japan). IND (10 mg/kg) or DIC (10 mg/kg) was administrated orally in unfasted rats. Antisecretory drugs were administered, and the animals were sacrificed according to the same schedule as for the induction of gastric lesions. The small intestines were spread out on paper and opened along the antimesenteric side, and the contents were removed. The length (mm) of the individual lesions was measured under a dissecting microscope with a 1-mm-square grid eyepiece (10×), and the sum of the lengths of all the lesions in each intestine was used as the LI.

Induction of Arthritis by Adjuvant. Arthritis in rats was induced by the method described previously (Kato et al., 2007). In brief, 50 μl of Freund’s complete adjuvant (10 mg/ml of killed M. tuberculosis suspended in paraffin oil) was injected into the plantar region of the right hindpaw under ether anesthesia, and 2 weeks later the animals were studied.

Pharmacological Ablation of Capsaicin-Sensitive Sensory Neurons. Pharmacological ablation of capsaicin-sensitive sensory neurons (CSSNs) was performed according to the method described previously (Takeuchi et al., 1991). CAP was dissolved in saline containing 10% ethanol and 10% Tween 80. Rats received subcutaneous injections of CAP (20, 30, and 50 mg/kg) under ether anesthesia for 3 consecutive days 2 weeks before the experiment. The animals were treated twice with terbutaline (0.1 mg/kg i.m.) and aminophyllin (20 mg/kg i.m.) 30 min before and just after CAP injection to prevent the respiratory impairment associated with CAP injection. The functional ablation of CSSN was confirmed 2 weeks after CAP treatment by the lack of protective wiping movements after the instillation of a drop of CAP (0.1 mg/ml) into one eye.

Determination of the Role of Endogenous NO. To examine the possible involvement of endogenous NO in the intestinal mucosal protective action of LPZ, 20 mg/kg of l-NAME (a selective inhibitor of NO synthesis) was administered subcutaneously 30 min before the oral administration of LPZ, CAP, or PGE₂.

Measurement of Intestinal Motility. Intestinal motility was determined by using a miniature balloon according to the method described previously (Takeuchi et al., 2002). In brief, rats were anesthetized with urethane (1.25 g/kg i.p.), and a midline incision was performed to expose the small intestine. A thin, water-filled balloon, made from silicone rubber and attached to a polyethylene catheter, was then introduced into the ileum via a small incision made approximately 10 cm oral to the ileocecal junction. The volume in the balloon was adjusted to give an initial resting pressure of approximately 10 to 15 cm of H₂O, which was not sufficient to cause active distension of the intestinal wall. After allowing the preparation to rest for 30 min, intestinal motility was monitored on a personal computer (Dynabook, Satellite A20; Toshiba, Tokyo, Japan) as intraluminal pressure changes by using a pressure transducer and a DC Strain Amplifier (AS2101; NEC, Tokyo, Japan). After basal motor activity had stabilized (≥ 1 h after the operation), the effects of the drugs were examined. The area of contraction (area under the curve) of the small intestine was measured every 30 min by using a Power Lab System 4/25 (ADInstruments, Nagoya, Japan) and a computer software program (Chart and Scope; ADInstruments) and was expressed as centimeters of H₂O/30 min.

Statistics. All data are expressed as mean ± S.E.M. Differences between groups were analyzed by Student’s t test for paired group comparisons, or analysis of variance (Dunnett’s multiple range test) if more than two variables were considered, with the significance level set at p < 0.05.

Results

Effect of Drugs on IND-Induced Gastric Lesions

In fasted rats, IND (10 mg/kg p.o.) produced multiple linear lesions in the corpus mucosa at 24 h post-treatment.

Effect of H₂-RAs

Effect of CIM. The LI in the vehicle (VEH)-treated group was 25.6 ± 5.3 mm (n = 7). The LIs were decreased by pretreatment with CIM (30–300 mg/kg p.o., times two) dose-dependently (Table 1, experiment 1). The effects at doses of 30 mg/kg or more were significant (p < 0.05 or 0.01).

Effects of RAN and FAM. The LI in the VEH-treated group was 20.0 ± 3.0 mm (n = 7). Both RAN (3–30 mg/kg p.o., times two) and FAM (1–10 mg/kg p.o., times two) inhibited the formation of gastric lesions dose-dependently (Table 1, experiment 2). The effects at doses more than 10 mg/kg (RAN) and 3 mg/kg (FAM) were significant (p < 0.05 or 0.01).
**Effect of PPIs**

**Effects of LPZ.** The LI in the VEH-treated group was 25.9 ± 5.9 mm (n = 7). LPZ (3–30 mg/kg p.o.) decreased the LI in a dose-dependent manner (Table 2, experiment 1). The effects were significant at doses of 10 mg/kg or higher (0.01).

**Effects of OPZ and RPZ.** The LI in the VEH-treated group was 17.3 ± 2.3 mm (n = 7). Both OPZ (10–100 mg/kg p.o.) and RPZ (10–100 mg/kg p.o.) inhibited the formation of gastric lesions, and the maximal effects were observed at 30 mg/kg with each drug. The effects at doses of 30 and 100 mg/kg were significant (p < 0.05 or 0.01) (Table 2, experiment 2).

**Effect of Drugs on IND-Induced Intestinal Lesions**

In fed rats, IND (10 mg/kg p.o.) produced multiple lesions in the middle and lower parts of the small intestine at 24 h post-treatment (Fig. 1A).

**Effect of H₂-RAs**

**Effect of CIM.** The LI in the VEH-treated group was 91.7 ± 14.3 mm (n = 7). The LIs were increased by pretreatment with CIM (30–300 mg/kg p.o., times two) dose-dependently (Fig. 2, experiment 1). The effects at doses of 100 and 300 mg/kg were significant (p < 0.05 and 0.01).

**Effects of RAN and FAM.** The LI in the VEH-treated group was 80.2 ± 12.1 mm (n = 7). Both RAN (3–30 mg/kg p.o., times two) and FAM (1–10 mg/kg p.o., times two) increased the formation of intestinal lesions dose-dependently (Fig. 2, experiment 2). The effects at doses of 10 mg/kg or more were significant (p < 0.05 or 0.01).

**Effect of PPIs**

**Effect of LPZ.** The LI in the VEH-treated group was 110.0 ± 18.0 mm (n = 7). LPZ (3–30 mg/kg p.o.) decreased the LI in a dose-dependent manner, and the effect at 30 mg/kg was significant (p < 0.01) (Fig. 3, experiment 1). The effect of subcutaneous LPZ on intestinal lesions was examined. The LI in the VEH-treated group was 146.1 ± 16.3 mm (n = 7). LPZ at doses of 3, 10, and 30 mg/kg significantly (p < 0.01) decreased the LI by 40, 65, and 57% (n = 7), respectively.

**Effects of OPZ and RPZ.** The LI in the VEH-treated group was 123.6 ± 8.0 mm (n = 7). Neither OPZ (10 and 30 mg/kg p.o.) nor RPZ (10 and 30 mg/kg p.o.) affected the formation of intestinal lesions, but the drugs at a dose of 100 mg/kg increased lesion formation significantly (p < 0.01 and 0.05, respectively) (Fig. 3, experiment 2).

**Effect of Drugs on DIC-Induced Intestinal Lesions**

In fed rats, DIC (10 mg/kg p.o.) produced multiple lesions in the middle and lower parts of the small intestine at 24 h post-treatment (Fig. 1B).

**Effect of H₂-RAs.** The LI in the VEH-treated group was 195.7 ± 8.5 mm (n = 7). The LIs were increased by pretreat-
Exacerbation of Intestinal Ulcer by Antisecretory Drugs

Effect of Drugs on IND-Induced Intestinal Lesions in Arthritic Rats

Arthritis markedly increased IND-induced intestinal lesions.

Effect of H2-RAs. The LI in the VEH-treated group was 178.6 ± 17.9 mm (n = 7). CIM (100 mg/kg p.o., times two), RAN (30 mg/kg p.o., times two), and FAM (10 mg/kg p.o., times two) increased the LI by 63 (p < 0.01), 54, and 41%, respectively (Table 4, experiment 1).

Effect of PPIs. The LI in the VEH-treated group was 248.9 ± 18.5 mm (n = 7). LPZ (30 mg/kg p.o.) decreased the LI significantly (p < 0.01) by 43%, but neither OPZ (100 mg/kg p.o.) nor RPZ (100 mg/kg p.o.) affected the formation of lesions (Table 4, experiment 2).

Effect of ATR on the RAN-Induced Increase in Small Intestinal Lesion Formation Caused by IND

The effect of ATR on IND-induced intestinal lesions in RAN-treated and untreated rats was examined to elucidate the mechanism of lesion enhancement by RAN. ATR was administered orally 30 min before RAN (30 mg/kg p.o., times two) or VEH. The LI in the VEH-treated group was 115.5 ± 9.2 mm (n = 7). ATR (1–10 mg/kg p.o., times two) decreased the LI in a dose-dependent manner, and the effects at 3 and 10 mg/kg were significant (p < 0.01) (Fig. 3). RAN (30 mg/kg p.o., times two) significantly (p < 0.01) increased the formation of intestinal lesions; the LI was 256.6 ± 15.1 mm (n = 7). The increase was markedly prevented by pretreatment with ATR (1–10 mg/kg p.o., times two) (Fig. 4).

Effect of H2-RAs on Motility of the Small Intestine

In urethane-anesthetized rats, the ileum usually did not show spontaneous strong contractions. IND (10 mg/kg s.c.) gradually increased motility starting at approximately 1 h after treatment (Fig. 5). A similar motility-enhancing effect was also observed after the administration of DIC (10 mg/kg s.c.) (data not shown). Injection of CIM (10 and 30 mg/kg i.v.), RAN (3 and 10 mg/kg i.v.), and FAM (3 and 10 mg/kg i.v.) mildly increased ileal motility. When the H2-RAs were administered intravenously 2 h after IND, they produced marked contractions of the small intestine (Fig. 5). Among the three H2-RAs tested, RAN showed the most prominent effect. Furthermore, when RAN (10 mg/kg i.v.) was administered 30 min before IND (10 mg/kg s.c.), it markedly increased the motor-stimulating effect of IND (Fig. 6A). The motor indexes at 30 and 60 min after IND in the group treated with RAN were significantly (p < 0.05) larger than those of the VEH-treated group (Fig. 6B). The increases in motility caused by treatment with IND with or without RAN were abolished by the administration of ATR (10 mg/kg i.v.) (Fig. 6). Both CIM (30 mg/kg i.v.) and FAM (10 mg/kg i.v.) also increased IND-induced motility, and the effect of CIM was significant (p < 0.05) (Fig. 7).

To elucidate the motility-enhancing effect of the H2-RAs, the effects of H2-RAs on motility caused by ACh were examined. ACh (1, 3, and 10 mg/kg s.c.) increased intestinal motility in a dose-dependent manner. CIM (30 mg/kg i.v.), RAN (10 mg/kg i.v.), and FAM (10 mg/kg i.v.) all enhanced the increase in intestinal motility caused by ACh (3 mg/kg s.c.) (Fig. 8A), and the effects of CIM and RAN were significant (p < 0.05 and 0.01, respectively) (Fig. 8B).

Table 3

Effect of antisecretory drugs on small intestinal lesions induced by DIC in rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Lesion Index</th>
<th>Lesion Index % of VEH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEH</td>
<td>195.7 ± 8.5</td>
<td>100.0 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>CIM</td>
<td>236.7 ± 17.8</td>
<td>120.7 ± 9.1</td>
<td></td>
</tr>
<tr>
<td>RAN</td>
<td>311.7 ± 20.3**</td>
<td>159.2 ± 10.4</td>
<td></td>
</tr>
<tr>
<td>FAM</td>
<td>272.9 ± 15.6*</td>
<td>139.4 ± 8.0</td>
<td></td>
</tr>
<tr>
<td>Expt 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEH</td>
<td>232.3 ± 20.2</td>
<td>100.0 ± 8.7</td>
<td></td>
</tr>
<tr>
<td>LPZ</td>
<td>121.6 ± 12.8**</td>
<td>52.4 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>OPZ</td>
<td>279.7 ± 13.9</td>
<td>120.6 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>RPZ</td>
<td>310.0 ± 27.9*</td>
<td>134.0 ± 12.0</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01 vs. VEH (Dunnett’s test).
Effect of Pharmacological Ablation of CSSN and L-NAME on the Mucosal Protective Action of LPZ

In VEH-treated rats, IND (10 mg/kg p.o.) caused multiple lesions in the small intestine; the LI was 95.9 ± 11.7 mm (n = 7). LPZ (10 mg/kg p.o.), CAP (10 mg/kg p.o.), and PGE$_2$ (0.1 mg/kg p.o.) significantly (p < 0.01) inhibited the formation of intestinal lesions by 50, 63, and 63% (n = 7), respectively (Fig. 9, experiment 1). Denervation of CSSN markedly increased the formation of intestinal lesions induced by IND (10 mg/kg p.o.); the LI in the VEH-treated group was 197.7 ± 32.5 mm (p < 0.05 versus VEH in experiment 1). The denervation almost completely abolished the inhibitory effects of LPZ and CAP, but the inhibitory effect of PGE$_2$ was still observed (Fig. 9, experiment 2).

To examine the possible involvement of endogenous NO in the protective action of LPZ in the small intestine, 20 mg/kg of L-NAME (a selective inhibitor of NO synthesis) was administered subcutaneously 30 min before oral administration of LPZ, CAP, or PGE$_2$. Administration of L-NAME (20 mg/kg s.c.) produced a marked increase in the formation of intestinal lesions induced by IND (10 mg/kg p.o.); the LI in VEH-treated rats was 194.4 ± 14.8 mm (p < 0.01 versus VEH in experiment 1). The administration of L-NAME almost completely abolished the inhibitory effects of LPZ and capsaicin, but a significant effect of PGE$_2$ was still observed (p < 0.05) (Fig. 9, experiment 2). The effect of L-NAME on SNP (NO donor) induced inhibition of intestinal lesions caused by IND was examined. SNP (10 mg/kg s.c.) significantly (p < 0.01) inhibited the formation of intestinal lesions by 50% (n = 7) and also decreased the lesions significantly (p < 0.05) by 40.2% (n = 7) after L-NAME treatment.

Discussion

In the present study we examined the effects of H$_2$-RAs (CIM, RAN, and FAM) and PPIs (OPZ, LPZ, and RPZ) on NSAID-induced gastrointestinal lesions in rats. Although all
of the drugs inhibited the formation of gastric lesions induced by IND in fasted rats, they showed differing effects on IND-induced small intestinal lesions in fed rats. That is, all of the H$_2$-RAs increased lesion formation in a dose-dependent manner at doses that inhibited gastric lesions, but LPZ decreased intestinal lesions dose-dependently. OPZ and RPZ both increased lesion formation significantly at the high dose of 100 mg/kg. Similar effects of the drugs on the small intestine were observed when another NSAID, DIC, was used instead of IND. Because DIC is commonly used in patients with arthritis and other diseases, the present results suggest a possibility that antisecretory drugs may aggravate NSAID-induced intestinal lesions in humans. The effects of the drugs on intestinal lesions were further examined in rats with adjuvant-induced arthritis, because NSAIDs are often used in arthritic patients. Arthritis aggravated IND-induced small intestinal lesions as reported previously (Kato et al., 2007). In rats with adjuvant-induced arthritis, lesion formation was still increased by H$_2$-RAs. Together, the results suggest that both H$_2$-RAs and high doses of OPZ and RPZ can exacerbate NSAID-induced intestinal lesions.

The question remains as to how antisecretory drugs exacerbate NSAID-induced small intestinal lesions, in light of the fact that gastric acid does not seem to play any role in lesion formation. Although several different factors related to the pathogenesis of NSAID-induced intestinal ulcers have been suggested, we have proposed the importance of hypermotility of the small intestine and indigestive solid components of food such as cellulose in the formation of intestinal lesions induced by NSAIDs (Satoh, 2010; Takeuchi et al., 2010). We reported that IND increased intestinal motility in rats (Takeuchi et al., 2002) and cats (Satoh et al., 2009) and decreased the mucus content of the small intestine in rats (Kunikata et al., 2002). Under conditions where the amount of surface mucus is decreased by NSAIDs, it is possible that indigestive solid components of food may cause physical damage to the mucosal surface when the intestine is strongly contracted by NSAIDs and accelerate the invasion of intestinal bacteria and irritants (bile acids, etc.) into the mucosa. Therefore, it is conceivable that strong inhibition of gastric acid secretion by drugs may exacerbate NSAID-induced intestinal lesions via increasing the amount of undigested solid components of food in the small intestine by dyspepsia in the stomach. Although there are no data that directly support this possibility in the present study, such a mechanism may explain why antisecretory drugs exacerbate intestinal lesions induced by NSAIDs, especially at high doses; this in turn suggests that strong and sustained inhibition of gastric acid secretion may have a deleterious effect on NSAID-induced intestinal lesion. Although LPZ has strong antisecretory activity (Satoh et al., 1989), it did not increase, but rather decreased, intestinal lesion formation. This finding may be explained by the protective action of LPZ on the intestinal mucosa, as we discuss in detail below.

The second possibility is that the drugs may affect intestinal motility and thereby exacerbate intestinal lesion formation. In the present study, we found that both IND and DIC increased intestinal motility in anesthetized rats. We examined the effects of H$_2$-RAs on intestinal motility and found that H$_2$-RAs markedly enhanced the motor-stimulating effect of IND. It has been reported that some H$_2$-RAs such as RAN and CIM have an inhibitory effect on acetylcholinesterase (Cheah et al., 1985; Aono et al., 1986) and stimulate intestinal motility (Ueki et al., 1993; Bortolotti et al., 1995). In the present study, H$_2$-RAs increased intestinal motility caused by exogenous ACh as well as by IND, and RAN showed the most prominent effect among the three H$_2$-RAs in accordance with their acetylcholinesterase-inhibitory activi-
ties (Cheah et al., 1985; Aono et al., 1986). As mentioned above, RAN increased IND-induced intestinal lesions, and the effects of RAN on motility and the formation of intestinal lesions were markedly prevented by treatment with ATR. These results indicate that H$_2$-RAs increase NSAID-induced intestinal lesions, at least in part, by increasing intestinal motility via the activation of cholinergic pathways caused by the inhibition of acetylcholinesterase.

It has been reported that Gram-negative enterobacteria such as Escherichia coli play an important role in the formation of intestinal lesions induced by NSAIDs (Uejima et al., 1996; Reuter et al., 1997). Wallace et al. (2011) recently reported that PPIs (OPZ and LPZ) given to rats subcutaneously twice a day for 9 days increased the formation of small intestinal lesions induced by NSAID (naproxen) occurred after marked suppression of acid secretion had been achieved by repeated administration of the PPIs in the latter study. Kuroda et al. (2006) first reported that LPZ had an inhibitory effect on IND-induced small intestinal lesions in rats and suggested that LPZ prevented intestinal lesions by anti-inflammatory and antioxidant mechanisms, because LPZ inhibited IND-induced increase of myeloperoxidase activity and thiobarbituric acid-reactive substance in the intestinal mucosa. Higuchi et al. (2009) and Yoda et al. (2010) reported that LPZ, but not OPZ, prevented IND-induced intestinal lesions in rats and suggested that LPZ protected the intestinal mucosa by up-regulation of hemooxygenase-1 production in the mucosa. We reported previously that LPZ protects the gastric mucosa by a mechanism independent from its antisecretory action, and both CSSN and NO are involved in the protective mechanism of LPZ on the gastric mucosa (Murakami et al., 1996). We have also reported that both CSSN and NO play an important role in protecting the small intestinal mucosa against ulcerative stimuli triggered by NSAIDs (Kato et al., 2000; Mizoguchi et al., 2001; Tanaka et al., 2001). In the present study, the inhibitory effects of LPZ and CAP on IND-induced intestinal lesions were markedly abolished by pharmacological ablation of CSSN and pretreatment with l-NAME. On the other hand, the inhibitory effect of PGE$_2$ was still observed under these conditions, and SNP, an NO microflora in the present study, it is questionable whether administration of PPIs and H$_2$-RAs once or twice would cause significant changes in the intestinal microflora within 24 h. Therefore, at present, it is difficult to draw conclusions about the role of enterobacteria in the effects of H$_2$-RAs and PPIs on NSAID-induced intestinal lesions observed in this study.

In contrast to the findings in subacute studies by Wallace et al. (2011), in the present studies LPZ did not increase, but rather decreased, the formation of intestinal lesions induced by IND in rats. The difference in the results of the present study and those of Wallace et al. may be explained by the differences in experimental methods in both studies as described in the previous section; especially, the first administration of NSAID (naproxen) occurred after marked suppression of acid secretion had been achieved by repeated administration of the PPIs in the latter study. Kuroda et al. (2006) first reported that LPZ had an inhibitory effect on IND-induced small intestinal lesions in rats and suggested that LPZ prevented intestinal lesions by anti-inflammatory and antioxidant mechanisms, because LPZ inhibited IND-induced increase of myeloperoxidase activity and thiobarbituric acid-reactive substance in the intestinal mucosa. Higuchi et al. (2009) and Yoda et al. (2010) reported that LPZ, but not OPZ, prevented IND-induced intestinal lesions in rats and suggested that LPZ protected the intestinal mucosa by up-regulation of hemooxygenase-1 production in the mucosa. We reported previously that LPZ protects the gastric mucosa by a mechanism independent from its antisecretory action, and both CSSN and NO are involved in the protective mechanism of LPZ on the gastric mucosa (Murakami et al., 1996). We have also reported that both CSSN and NO play an important role in protecting the small intestinal mucosa against ulcerative stimuli triggered by NSAIDs (Kato et al., 2000; Mizoguchi et al., 2001; Tanaka et al., 2001). In the present study, the inhibitory effects of LPZ and CAP on IND-induced intestinal lesions were markedly abolished by pharmacological ablation of CSSN and pretreatment with l-NAME. On the other hand, the inhibitory effect of PGE$_2$ was still observed under these conditions, and SNP, an NO...
donor, decreased intestinal lesion formation significantly even after treatment with L-NNAME. These results suggest that LPZ protects the intestinal mucosa against NSAID-induced ulcerative stimuli via mechanisms involving CSSN and NO, as seen in the stomach.

The present results suggest that 1) strong and sustained inhibition of gastric acid secretion by antisecretory drugs may exacerbate the formation of NSAID-induced small intestinal lesions, 2) in addition to possessing antisecretory activity, H2-RAs may aggravate intestinal lesions by increasing intestinal motility via activation of cholinergic pathways, and 3) LPZ can protect the intestinal mucosa, and both CSSN and NO may be involved in the protective mechanism. Finally, it is concluded that antisecretory drugs, with the exception of LPZ, are essentially inappropriate for the treatment of intestinal damage caused by NSAIDs.

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Authorship Contributions
Participated in research design: SATOH and TAKEUCHI. Conducted experiments: SATOH and AMAGASE. Performed data analysis: SATOH and AMAGASE. Wrote or contributed to the writing of the manuscript: SATOH and TAKEUCHI.

References


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