INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs), which are used for the treatment of several inflammatory disorders including rheumatoid arthritis (RA), cause gastroduodenal mucosal lesions as an adverse effect (1, 2). Recently, the serious problem of NSAID-induced small intestinal damage has become a topic of great interest to gastroenterologists, since capsule endoscopy and double-balloon enteroscopy are available for the detection of small intestinal lesions. Goldstein et al. (3) reported that small bowel mucosal breaks were induced in 55% of healthy volunteers given naproxen for two weeks, while Maiden et al. (4) reported that ingestion of slow-release diclofenac for two weeks resulted in macroscopic injury to the small intestine in 68% of healthy volunteers. In patients with RA, the use of NSAIDs may be associated with an increased incidence of severe small bowel injury (5, 6). Several factors have been postulated as the pathogenic element of NSAID-induced small intestinal lesions, including intestinal hypermotility, entero bacterial invasion, neutrophil activation, and nitric oxide (NO) overproduction by inducible NO synthase (iNOS), in addition to prostaglandin deficiency (7-14). However, no satisfactory means are currently available for prevention and treatment of these lesions, except for prostaglandin analogs (15).

Proton pump inhibitors (PPI), such as lansoprazole and omeprazole, show a potent anti-secretory effect due to irreversible inhibition of the H+/K+-ATPase in the parietal cell and are indicated for the treatment of gastric acid-related disorders including gastroduodenal ulcers, reflux esophagitis, and NSAID-induced gastric lesions (16, 17). PPIs have a gastroprotective effect, independent of their anti-secretory actions (18-21). In addition, several reports demonstrated that PPIs reduced the severity of intestinal lesions induced by ischemia-reperfusion and NSAIDs in experimental animals and humans (3, 22-24). It is therefore possible that PPIs may be useful for NSAID-related lesions not only in the stomach but also in the small intestine. However, the mechanisms involved in these effects are unclear.

PREVENTION BY LANSOPRAZOLE, A PROTON PUMP INHIBITOR,
OF INDOMETHACIN-INDUCED SMALL INTESTINAL ULCERATION
IN RATS THROUGH INDUCTION OF HEME OXYGENASE-1

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The effect of lansoprazole, a proton pump inhibitor (PPI), on indomethacin-induced small intestinal ulceration was examined in rats, particularly in relation to heme oxygenase (HO)-1. The animals were administered indomethacin (10 mg/kg, p.o.) and killed 24 h later. Lansoprazole (30-100 mg/kg, p.o.) and omeprazole (30-100 mg/kg, p.o.) were given 30 min before the administration of indomethacin, while tin-protoporphyrin IX (SnPP; 30 mg/kg, i.v.), an inhibitor of HO-1, was injected 10 min before indomethacin or lansoprazole. Indomethacin produced hemorrhagic lesions in the small intestine, accompanied with an increase of mucosal invasion of enterobacteria, inducible nitric oxide synthase (iNOS) expression, and myeloperoxidase (MPO) activity in the mucosa. Pretreatment with lansoprazole dose-dependently reduced the severity of the indomethacin-induced intestinal lesions, with suppression of the increased MPO activity, while omeprazole had no effect. Pretreatment with SnPP significantly exacerbated these intestinal lesions and almost totally abolished the protective effect of lansoprazole. The up-regulation of iNOS mRNA expression following indomethacin was suppressed by lansoprazole in a SnPP-inhibitable manner, although the enhanced enterobacterial invasion remained unaffected. The amount of HO-1 protein in the intestinal mucosa was significantly increased by lansoprazole but not by omeprazole. Prior administration of carbon monoxide (CO)-releasing molecule-2 (CORM-2; 10 mg/kg, i.p.) significantly reduced the severity of these lesions and the enhancement of mucosal iNOS mRNA expression induced in the small intestine by indomethacin. These results suggest that lansoprazole prevents indomethacin-induced small intestinal ulceration, and this effect is associated with inhibition of iNOS expression, through up-regulation of HO-1/CO production in the mucosa.

Key words: intestinal lesions, indomethacin, heme oxygenase-1, lansoprazole, carbon oxide, nonsteroidal anti-inflammatory drugs, nitric oxide
Heat shock proteins (HSPs) play an important role in mucosal defense of gastrointestinal tract (25). Heme oxygenase (HO)-1, known as HSP 32, is ubiquitously distributed in mammalian cells and tissues and is potently induced by various stimuli, such as oxidative stress and pathological conditions (26). Several agents reportedly exhibit cytoprotective action in the gastrointestinal mucosa through induction of HO-1 (27, 28). Recently, Becker et al. (29) showed that PPIs induced HO-1 in gastric and endothelial cells in vitro, resulting in protection of these cells against oxidative stress. These findings suggest a possibility that the protective and antioxidative effects of PPIs may be accounted for by the induction of HO-1.

The aim of the present study was to examine the effect of PPIs such as lansoprazole and omeprazole on indomethacin-induced small intestinal ulceration in rats, particularly in relation to HO-1.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (220-240 g, Nippon Charles River, Shizuoka, Japan) were used. The experiments were performed using 5-8 rats per group without fasting. All experimental procedures were approved by the Experimental Animal Research Committee of Osaka Medical College and Kyoto Pharmaceutical University.

Induction of small intestinal ulceration by indomethacin

Small intestinal ulceration was induced by oral administration of indomethacin according to our previous study (30). Animals were assigned randomly to the following 10 groups (n=5-6 for each) indomethacin alone, indomethacin+lansoprazole (30, 60 and 100 mg/kg), indomethacin+omeprazole (30 and 100 mg/kg), indomethacin+lansoprazole (100 mg/kg)+tin-protoporphyrin IX (SnPP: an inhibitor of HO-1; 30 mg/kg), indomethacin+SnPP, indomethacin+carbon monoxide releasing molecule (CORM)-2 (1 and 10 mg/kg), Lansoprazole and omeprazole were given p.o. 30 min before the administration of indomethacin, while SnPP was injected i.v. under light ether anesthesia, 10 min before the administration of lansoprazole or indomethacin. CORM-2 was injected i.v. under light ether anesthesia, 10 min before the administration of indomethacin+carbon monoxide releasing molecule (CORM)-2 (SnPP: an inhibitor of HO-1; 30 mg/kg), indomethacin+SnPP, indomethacin+carbon monoxide releasing molecule (CORM)-2 (1 and 10 mg/kg). Lansoprazole and omeprazole were given p.o. 30 min before the administration of indomethacin, while SnPP was injected i.v. under light ether anesthesia, 10 min before the administration of lansoprazole or indomethacin. CORM-2 was given i.p. twice, 30 min before and 6 h after the administration of indomethacin. Briefly, animals were given indomethacin p.o. at a dose of 10 mg/kg and killed 24 h later under deep ether anesthesia. The small intestines (stomach to ileum) were excised and treated with 2% formalin for fixation of the tissue walls, opened along the anti-mesenteric attachment, and the area of macroscopically visible lesions was measured under a dissecting microscope with square grids (10x), summed per small intestine, and used as a lesion score. To delineate the damage, 1% Evans blue (Sigma-Aldrich, St. Louis, MO) solution was injected i.v. in a volume of 0.5 ml/animal 0.5 h before sacrifice. The person measuring the lesions did not know the treatments given to the animals.

Measurement of myeloperoxidase activity

Myeloperoxidase (MPO) activity in the intestinal mucosa was measured according to a modified method of Krawisz et al. (31). In brief, the small intestine was excised 24 h after the administration of indomethacin, and the intestinal mucosa was weighed, homogenized in a 50 mM phosphate buffer containing 0.5% hexadecyl-trimethyl-ammonium bromide (pH 6.0) and centrifuged at 2000 rpm for 10 min at 4°C. MPO activity in the supernatant was determined using o-dianisidine dihydrochloride (Sigma-Aldrich). The changes in absorbance at 450 nm were recorded on a microplate reader (VERSAmax; Molecular Device, Sunnyvale, CA). The MPO activity was obtained from the slope of the reaction curve, based on the following equation: specific activity (µmol H2O2/min/mg protein) = (OD/min)/(OD/µmol H2O2 × mg protein).

Measurement of mucosal HO-1 content

The animals were sacrificed under deep ether anesthesia 3, 6, 12, and 24 h after the administration of lansoprazole (100 mg/kg) or 6 h after the administration of omeprazole (100 mg/kg) or CORM-2 (10 mg/kg). The small intestine was removed, weighed and stored at -80°C until use. Tissue samples were homogenized in extraction buffer containing a protease inhibitor cocktail (Complete Mini, Roche Applied Science, Mannheim, Germany) with a teflon-glass homogenizer and centrifuged at 20000g for 30 min at 4°C. After the supernatant of each sample had been evaporated with nitrogen gas, the residue was reconstituted in assay buffer and used for determination of HO-1. The concentration of HO-1 was measured with an enzyme-linked immunosorbent assay (ELISA) (Stressgen, Ann Arbor, MI).

Immunohistochemical staining for HO-1

Immunostaining of HO-1 in the small intestine was performed 6 h after the administration of lansoprazole. The small intestine was removed, embedded in O.C.T. compound (Miles, Elkhart, IN) and rapidly frozen in carbon dioxide gas. Cryostat sections (CM1510, Leica, Wetzlar, Germany) cut serially at a thickness of 10 µm were mounted on MAS-coated slides (Matsunami, Osaka, Japan), treated with 4% paraformaldehyde, and stained with rabbit anti-human HO-1 polyclonal antibody (Stressgen) in PBS containing 0.3% Triton X-100. Immunohistochemical staining was performed with a streptavidin-biotin peroxidase method according to the manufacturer's instructions with a VECTASTAIN ABC kit (Vector, Burlingame, CA). The sections were treated with 0.03% 3,3’-diaminobenzidine (Sigma-Aldrich) containing 0.005% hydrogen peroxide. Counterstaining was performed with hematoxylin (Merck, Darmstadt, Germany). Stained slides were observed with a light microscope (BX50, Olympus, Tokyo, Japan) at 200- and 400-fold magnifications.

RT-PCR for iNOS mRNA

Expression of iNOS mRNA was analyzed by RT-PCR. In brief, the animals were sacrificed under deep ether anesthesia 6 h after the administration of indomethacin. The small intestine was removed, frozen in acetone/dry ice, and stored at -80°C until use. Total RNA was extracted from the mucosa of the small intestine with Sepasol RNA-I (Nacalai Tesque, Kyoto, Japan). First-strand cDNA primed by random hexamers was reverse-transcribed with ReverTra Ace (TOYOBO, Osaka, Japan). For RT-PCR, an aliquot of the first-strand cDNA sample served as a template in 35 cycles of PCR reaction with 1 min of denaturation at 95°C and 1 min of extension at 68°C using the Advantage 2 polymerase mixture (BD Biosciences Clontech, Palo Alto, CA). The sequences of the sense and antisense PCR primers for iNOS were 5’-GGTTCACAGTCTTGGGAAAG-3’ and 5’-CA GGTTCTCCCCAGGTAGTGA-3’, respectively, giving rise to a 780-bp PCR product, and those for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a housekeeping gene, were 5’-GAACGGAACGTACCAGGATC-3’ and 5’-TGAAGTCCACACCCCGTGGT-3’, respectively, giving rise to a 311-bp PCR product. The PCR products from 3 rats were mixed and then the mixture was electrophoresed on a 1.8% agarose gel in Tris-EDTA-acetic acid buffer (40 mM Tris, 2 mM EDTA and 20 mM acetic acid, pH 8.1), and the gel was stained with
ethidium bromide and photographed (BioDoc-It Imaging System, UVP, Upland, CA).

**Determination of intestinal mucus content**

The amount of mucus secreted in the small intestine was determined by periodic acid-Schiff (PAS) staining. The animals were sacrificed under deep ether anesthesia 6 h after the administration of indomethacin, and the small intestines were removed. The tissue was fixed in Carnoy’s fluid (ethanol: acetic acid: chloroform = 6:1:3) for 24 h, embedded in paraffin, and sectioned at a thickness of 8 µm. PAS staining was subsequently performed according to the conventional method. The area of PAS staining in the small intestine was measured using ImageJ 1.40 (National Institutes of Health, Bethesda, MD).

**Measurement of enterobacterial count in the intestinal mucosa**

The animals were sacrificed under deep ether anesthesia 6 h after the administration of indomethacin, and their small intestines were removed. After the intestine was rinsed with sterile saline, the mucosa was scraped, weighed, and homogenized in 1 ml sterile PBS per 100 mg wet tissue. Aliquots of the homogenate were placed on GAM agar (Nissui, Osaka, Japan) and incubated at 37°C for 24 h under anaerobic conditions (BBL GasPack Pouch Anaerobic System, Becton Dickinson, MD). Plates containing between 10 and 200 colony-forming units (CFU) were analyzed to determine enterobacterial numbers, and the number of enterobacteria that invaded in the small intestine was expressed as log CFU/g tissue.

**Preparation of drugs**

Lansoprazole, indomethacin, CORM-2 (Sigma-Aldrich), omeprazole (Wako, Osaka, Japan) and SnPP (Frontier Scientific, Logan, UT) were used. Indomethacin, lansoprazole and omeprazole were suspended in a carboxymethylcellulose (CMC, Nacalai Tesque) solution. CORM-2 was first dissolved in dimethylsulfoxide (DMSO) and then diluted in saline (to a final concentration of 1% DMSO), while SnPP was first dissolved in 8.4% NaHCO₃ solution and then diluted in saline. All drugs were prepared immediately before use and administered p.o. and i.p. in a volume of 0.5 ml/100 g body weight or i.v. in a volume of 0.1 ml/100 g body weight. Control animals received CMC as the vehicle, in place of indomethacin.

**Statistics**

Data are presented as the mean±SE from 5 to 8 rats per group. Statistical analyses were performed with a two-tailed Dunnett’s multiple comparison test, and values of P<0.05 were regarded as significant.

**RESULTS**

**Effect of lansoprazole on indomethacin-induced small intestinal lesion**

Oral administration of indomethacin (10 mg/kg) caused severe hemorrhagic lesions in the small intestine within 24 h, mostly in both the jejunum and the ileum, the lesion score being 335.7±19.9 mm² (Fig. 1). Lansoprazole (30, 60, and 100 mg/kg, p.o.) given 0.5 h prior to indomethacin decreased the severity of these intestinal lesions in a dose-dependent manner, and a significant effect was observed at 60 mg/kg and 100 mg/kg, the inhibition being 57.9% and 84.1%, respectively. Pretreatment of the animals with omeprazole (30 and 100 mg/kg, p.o.), however, had no effect on the severity of these lesions, the inhibition being 6.8% and 3.1%, respectively. As shown in Fig. 2, the protective effect of lansoprazole (100 mg/kg, p.o.) on indomethacin-induced intestinal lesions was almost totally attenuated by prior administration of SnPP (30 mg/kg, i.v.), the inhibitor of HO; the lesion score was 221.3±31.6 mm², which was significantly greater than that (48.0±15.2 mm²) in the animals pretreated with lansoprazole alone. Pretreatment with SnPP by itself significantly exacerbated these intestinal lesions in response to indomethacin.

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**Fig. 1.** Effects of lansoprazole and omeprazole on indomethacin-induced intestinal lesions in rats. Animals were given indomethacin (10 mg/kg) p.o. and sacrificed 24 h later. Lansoprazole (30, 60, and 100 mg/kg) and omeprazole (30 and 100 mg/kg) were given p.o. 30 min before the administration of indomethacin. Data are presented as the mean±SE from 6 rats. *Significant difference from vehicle, at P<0.05.

**Fig. 2.** Effect of SnPP on the protective effect of lansoprazole against indomethacin-induced intestinal lesions in rats. Animals were given indomethacin (10 mg/kg) p.o. and sacrificed 24 h later. Lansoprazole (100 mg/kg) was given p.o. 30 min before the administration of indomethacin, while SnPP (30 mg/kg) was given i.v. under light ether anesthesia, 10 min before the administration of indomethacin or lansoprazole. Data are presented as the mean±SE from 6 rats. Significant difference at P<0.05; *from vehicle; # from indomethacin plus lansoprazole.
The mucosal MPO activity in the intestinal mucosa of control rats given CMC was 0.064±0.008 µmol H₂O₂/min/mg protein (Table 1). Oral administration of indomethacin caused a marked increase in mucosal MPO activity (0.324±0.063 µmol H₂O₂/min/mg protein), about 5-fold greater than that in control rats. The increase in MPO activity following indomethacin treatment was significantly suppressed by pretreatment of the animals with lansoprazole (100 mg/kg, p.o.), the inhibition being 60.7%.

Effect of lansoprazole on expression of HO-1 protein in small intestine

The amount of HO-1 protein in the intestine of control rats was 0.02±0.01 ng/mg protein, as determined by ELISA (Fig. 3). Levels of HO-1 in the intestinal mucosa were apparently increased by administration of lansoprazole (100 mg/kg, p.o.), and the maximal response was observed 6 h later, the values being 0.15±0.07 ng/mg protein. However, omeprazole (100 mg/kg, p.o.) did not affect the mucosal amount of HO-1, and the value observed 6 h later was 0.03±0.02 ng/mg protein, which was not significantly different from that of control rats. Likewise, CORM-2 (10 mg/kg, i.p.) alone did not affect the mucosal amount of HO-1, the values being 0.02±0.01 ng/mg protein. Immunohistochemically, the expression of HO-1 was observed only weakly at the surface of the intestinal epithelium of control rats. When the animals were given lansoprazole (100 mg/kg, p.o.) and the small intestine was examined 6 h later, the expression of HO-1 was enhanced markedly in the intestinal epithelium and slightly in the lamina propria (Fig. 4).

Table 1. Effect of lansoprazole on the increase in small intestinal MPO activity induced by indomethacin in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>MPO Activity µmol H₂O₂/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC</td>
<td>6</td>
<td>0.064 ± 0.008</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>6</td>
<td>0.324 ± 0.063*</td>
</tr>
<tr>
<td>IM + Lansoprazole</td>
<td>6</td>
<td>0.166 ± 0.022#</td>
</tr>
</tbody>
</table>

Animals were given indomethacin (10 mg/kg, p.o.) and sacrificed 24 h later. Lansoprazole (100 mg/kg) was given p.o. 30 min before the administration of indomethacin. Data are presented as the mean±SE from 6 rats. Significant difference at P<0.05; *from CMC; #from indomethacin (IM)

Fig. 3. Effects of lansoprazole and omeprazole on mucosal HO-1 content in the rat small intestine. Animals were given lansoprazole (100 mg/kg) p.o. and sacrificed at various time points up to 24 h later, while those given omeprazole (100 mg/kg) p.o. or CORM-2 (10 mg/kg) i.p. were sacrificed 6 h later. The mucosal HO-1 content in the small intestine was determined by ELISA. Data are presented as the mean±SE from 5-8 rats. *Significant difference from control, at P<0.05.

Fig. 4. Immunohistochemistry for HO-1 expression in the rat small intestine. Animals were given lansoprazole (100 mg/kg) p.o. and sacrificed 6 h later. A and D: vehicle alone, B and E, lansoprazole, C and F: negative control (without anti-HO-1 antibody), A, B, and C: magnification x200, D, E, and F: magnification x400. Note that the expression of HO-1 was observed only weakly at the surface of the epithelium in the small intestine (A) of a control rat but enhanced markedly at the surface of the epithelium and slightly in the lamina propria following lansoprazole (B, E).
Indomethacin (10 mg/kg, p.o.) up-regulated iNOS mRNA expression in the small intestine when examined 6 h after administration. Animals were given indomethacin 10 mg/kg p.o., sacrificed 6 h later, and the expression of iNOS mRNA was examined by RT-PCR. Lansoprazole (100 mg/kg) was given p.o. 30 min before the administration of indomethacin, while SnPP (30 mg/kg) was given i.v. under light ether anesthesia, 10 min before the administration of indomethacin or lansoprazole.

Effect of lansoprazole on changes in mucus content and enterobacterial count in small intestinal mucosa

Orally administered indomethacin (10 mg/kg) significantly decreased the amount of mucus in the small intestine by 33.1% (Table 2). Lansoprazole (100 mg/kg, p.o.) had no effect on either basal or indomethacin-decreased mucus content in the small intestine. Indomethacin (10 mg/kg, p.o.) caused a marked increase in the mucosal invasion of enterobacteria, and the bacterial count in the mucosa 6 h after administration was 7.44±0.25 log CFU/g tissue, which was about 10-fold greater than that (6.64±0.12 log CFU/g tissue) in control rats (Table 2). Prior administration of lansoprazole (100 mg/kg, p.o.) did not significantly affect the enhanced mucosal invasion of enterobacteria in response to indomethacin, the bacterial count in the mucosa 6 h after administration being 7.08±0.13 log CFU/g tissue, which was significantly higher than that in control rats.

Table 2. Effect of lansoprazole on the mucus content and the number of enterobacteria in the rat small intestine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mucus Content</th>
<th>Number of Enterobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAS-positive</td>
<td>log CFU/g tissue</td>
</tr>
<tr>
<td></td>
<td>staining</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mucosa (%)</td>
<td></td>
</tr>
<tr>
<td>CMC</td>
<td>15.1±1.0</td>
<td>6.64±0.12</td>
</tr>
<tr>
<td>Indomethacin (IM)</td>
<td>10.1±1.3*</td>
<td>7.44±0.25*</td>
</tr>
<tr>
<td>IM + Lansoprazole</td>
<td>10.4±0.4*</td>
<td>7.08±0.13*</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>13.1±0.9</td>
<td>—</td>
</tr>
</tbody>
</table>

Animals were given indomethacin (10 mg/kg, p.o.) and sacrificed 6 h later. Lansoprazole (100 mg/kg) was given p.o. 30 min before the administration of indomethacin. Data are presented as the mean±SE from 5-6 rats. *Significant difference from CMC, at P<0.05.

DISCUSSION

The results of the present study showed that lansoprazole reduced the severity of indomethacin-induced intestinal lesions, together with the suppression of the increase in MPO activity and iNOS mRNA expression, in agreement with the recent studies by others (23, 24). Given that gastric acid does not participate in the pathogenesis of NSAID-induced small intestinal lesions, the present findings are very interesting, because lansoprazole, an inhibitor of acid secretion, prevents these lesions, independently of its antisecretory action. Clinically, PPIs and prostaglandin analogs are the drugs of first choice for the prevention of NSAID-induced peptic ulcers and bleeding. However, patients cannot continue to take misoprostol (a prostaglandin analog), since this agent...
frequently causes adverse events such as diarrhea, abdominal pain, and bloating. Thus, it is assumed that lansoprazole may be a safe, economical, and novel treatment for preventing the gastric and small intestinal lesions induced by NSAIDs.

We found in the present study that lansoprazole significantly reduced the severity of small intestinal lesions caused by indomethacin. These results are partly consistent with the findings by Pozzoli et al. (24) who showed that both lansoprazole and omeprazole were effective against indomethacin-induced intestinal lesions. However, we could not confirm a protective effect of omeprazole against these intestinal lesions. Kato et al. (32) also reported the failure of omeprazole to prevent these intestinal lesions in rats. The reason for the different results on the omeprazole effect remains unknown, but it may be due to the different experimental conditions such as the dosing schedule and so on. In the present study, omeprazole was administered once, 30 min before indomethacin, while in their study this agent was administered 3 times, 12 h and 0.5 h before and after the administration of indomethacin. Since PPIs are used clinically in a once-daily dosing regimen for the treatment of several upper gastrointestinal disorders, the dosing schedule used in our study is likely to reflect more accurately the clinical use of PPIs. The clinical study using capsule endoscopy in healthy volunteers revealed that omeprazole did not prevent small intestinal lesions caused by naproxen. Further studies are certainly needed for PPIs on protective effect in the small intestine.

The role of HO-1 in the protective action of several drugs has been studied without consistent results (33, 34). In the present study, we demonstrated that the protective effect of lansoprazole against indomethacin-induced small intestinal lesions was brought about by induction of HO-1, suggesting an involvement of HO-1 in the intestinal mucosal defense system. This idea was also supported by the findings that the protective effect of lansoprazole against these lesions was almost totally abrogated by pretreatment with SnPP, an inhibitor of HO-1. It was also found that lansoprazole, but not omeprazole, enhanced the expression of HO-1 in the small intestinal mucosa, predominantly in the epithelium and somewhat in the lamina propria. The effects of PPIs on HO-1 induction remain controversial; Becker et al. (29) showed that both omeprazole and lansoprazole strongly induced HO-1 expression in gastric epithelial cells in vitro, while Takagi et al. (35) recently reported that omeprazole weakly induced the expression of HO-1 in gastric epithelial cell lines, though a potent expression was induced by lansoprazole. The present results in the rat small intestine are consistent with those of Takagi et al. (35) in gastric epithelial cells, but further studies are needed to clarify these discrepancies in the effects of PPIs on HO-1 induction.

HO is the rate-limiting enzyme in heme degradation, which generates CO, free iron, and biliverdin (28, 36). Three isoforms of HO have been identified, one of which, HO-1, is known to be a stress-responsive protein induced by various stimuli and play an important role in antioxidative, anti-inflammatory, and protective actions. Several studies have demonstrated that CO, not biliverdin/bilirubin, is a key molecule mediating the protective action of HO-1 (37-39). CORM-2, a transition metal carbonyl compound that has the ability to release CO in biological systems, has been demonstrated to exhibit anti-inflammatory and protective actions mediated by CO (40, 41). We observed in this study that CORM-2, given i.p. twice 30 min before and 6 h after indomethacin, significantly reduced the intestinal ulcerogenic response to this NSAID. Furthermore, lansoprazole up-regulated HO-1 expression in the intestinal mucosa and protected this tissue against indomethacin-induced lesions, in a SnPP-inhibitable manner. Thus, it is likely that CO produced by HO-1 may account largely for the intestinal protective effect of lansoprazole, although the involvement of biliverdin/bilirubin cannot be totally excluded.

In the present study, lansoprazole suppressed the enhanced expression of iNOS mRNA in the small intestine following indomethacin treatment, one of the important pathogenic events in indomethacin-induced small intestinal lesions (8, 11, 12, 42). We showed a correlation between the increase of iNOS activity and NO production with time following the administration of indomethacin, although the activity of cNOS remained unchanged (12, 31). In addition, Tanaka et al. (12) reported that the severity of the indomethacin-induced intestinal lesions was aggravated by prior administration of L-NAME, a nonselective cNOS and iNOS inhibitor, but prevented by later administration of this agent, both in an L-arginine-reversible manner, suggesting a dual role of endogenous NO in the pathogenesis of these lesions a protective effect of CO and NO and a proulcerogenic effect of iNOS/NO. Since the present study clearly showed that lansoprazole suppressed the up-regulation of iNOS expression induced by indomethacin, it is easily speculated that this agent also prevents both the increase of iNOS activity and NO production following indomethacin, without the actual measurement of iNOS protein or its enzymatic activity.

At present, the mechanism by which lansoprazole suppressed the enhanced iNOS expression following indomethacin remains speculative. It is known that this process is causally associated with enterobacterial invasion in the mucosa (8, 11, 12, 42). Since lansoprazole had no effect on bacterial invasion following indomethacin treatment, it is assumed that the inhibitory effect on iNOS expression is due to mechanisms other than suppression of bacterial invasion. It is known that CO released by CORM-2 inhibited NO and tumor necrosis factor (TNF)-α production in mouse macrophages and microglial cell lines (43, 44) and that the anti-inflammatory action of CO may be related to iNOS down-regulation in the human colon cancer cell line DLD-1 and intestinal inflammation (45). Furthermore, it has been shown that the suppressive effect of CO on iNOS induction and cytokine production was brought about by inhibition of NF-κB activation (46, 47). We recently reported that indomethacin caused the activation of NF-κB in the small intestine and that tacrolimus, an immunosuppressive agent, prevented both intestinal lesions and iNOS expression following indomethacin by inhibiting NF-κB activation (48). In the present study, we found that the increase in iNOS mRNA expression was suppressed by CORM-2. Thus, it is assumed that the intestinal protective effect of lansoprazole through upregulation of HO-1/CO may be attributable to suppression of iNOS induction caused by inhibition of NF-κB activation.

Intestinal mucus has an important role in the prevention of enterobacterial invasion into the intestinal mucosa. We previously reported that irsogladine, a mucosal protective drug, reduced the severity of intestinal lesions as well as the up-regulation of iNOS mRNA expression induced by indomethacin through an increase in mucus production (49). In the present study, lansoprazole did not affect the mucus content and had no effect on mucosal invasion of enterobacteria following indomethacin treatment. These findings confirm a relationship between these two events and suggest that the protective effect of lansoprazole in the small intestine is accounted for by factors or events other than changes in mucus secretion or bacterial invasion.

Taking all of the present and previous findings together, it is concluded that lansoprazole protects the small intestine against indomethacin-induced lesions, and this prophylactic effect may be associated with the inhibition of iNOS expression, probably through upregulation of HO-1/CO production in the mucosa. It is therefore assumed that lansoprazole may be useful for preventing the adverse effects of NSAIDs not only in the stomach but also in the small intestine.

Conflict of interests: None declared.
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