Lansoprazole Induces Mucosal Protection through Gastrin Receptor-Dependent Up-Regulation of Cyclooxygenase-2 in Rats

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ABSTRACT

Proton pump inhibitors (PPIs) are antiulcer agents that have both gastric antisecretory and mucosal protective actions. The mechanisms of PPI-induced gastric mucosal protection are not known. The present study was designed to examine the mechanism for lansoprazole-induced gastric mucosal protection in rats. Rats were given 0.5, 5, and 50 mg/kg/day lansoprazole alone or both lansoprazole (50 mg/kg/day) and a specific gastrin receptor antagonist 3R-1-(2,2-diethoxyethyl)-((4-methylphenyl)amino-carbonyl methyl)-3-((4-methylphenyl)ureido-indoline-2-one) (AG-041R) (3, 10, and 30 mg/kg/day) for 14 days. Serum gastrin concentrations were measured. The expression of cyclooxygenases (COX-1 and COX-2) in the gastric mucosa was analyzed using Western blotting and immunohistochemical staining. Another series of rats was used to examine the 1) levels of prostaglandin (PG) E2 in gastric mucosa, 2) influences of the drugs on gastric damage caused by absolute ethanol, and 3) effects of a COX-2-specific inhibitor on PGE2 in the gastric mucosa and the mucosal protection afforded by lansoprazole. Lansoprazole dose dependently increased the serum gastrin concentration and enhanced the mucosal expression of COX-2 but not that of COX-1. Lansoprazole increased gastric mucosal PGE2 and reduced gastric damage caused by ethanol. Concomitant administration of AG-041R abolished the lansoprazole-induced COX-2 expression, and increased mucosal PGE2 and mucosal protection. A specific COX-2 inhibitor blocked the lansoprazole-induced increase in mucosal PGE2 and mucosal protection. Activation of gastrin receptors by endogenous gastrin has a pivotal role in the effects of lansoprazole on COX-2 up-regulation and mucosal protection in the rat stomach.

Proton pump inhibitors (PPIs) are potent antiulcer agents that have both gastric antisecretory and mucosal protective actions (Ruwart et al., 1984; Okabe et al., 1986; Holm, 1988; Bergmann et al., 1992; Kawano et al., 1992; Fukuda et al., 1995; Murakami et al., 1996; Blandizzi et al., 1999). The antisecretory action is due to their inhibitory effects on the H⁺,K⁺-ATPase (proton pump) in parietal cells (Satoh et al., 1989; Nagaya et al., 1990). The mechanisms of PPI-induced gastric mucosal protection are not known. Lansoprazole is a PPI that is widely used to treat peptic ulcers in humans. A previous study reported that the protective effects of lansoprazole were inhibited by pretreatment with indomethacin in gastric lesion models induced by ethanol or ethanol-hydrochloric acid, suggesting that endogenous prostaglandin (PG) synthesis might account for the gastroprotective effects of lansoprazole (Blandizzi et al., 1999). Another study suggested that lansoprazole protects gastric mucosa from ethanol- and acidified taurocholate-induced damage in a dose-dependent manner, with ID₅₀ values of 8.5 mg/kg p.o. (ethanol) and 4.1 mg/kg p.o. (acidified taurocholate). The protective effect of lansoprazole was suppressed by functional ablation of capsaicin-sensitive sensory neurons, or prior administration of indomethacin or a selective inhibitor of nitric oxide synthesis. These findings suggest that endogenous PGs, together with capsaicin-sensitive sensory neurons and nitric oxide, mediate PPI-induced gastric mucosal protection (Murakami et al., 1996).

PGs have an important role in gastric mucosal defense (Robert et al., 1983; Arakawa et al., 1990). Synthesis of PGs is governed by PG endoperoxide synthase, or cyclooxygenase.
The constitutive isoform (COX-1) is dominantly expressed in platelets, prostate, and stomach. The mitogen-inducible isoform (COX-2) is minimally expressed in normal stomach (Seibert et al., 1994; Kargman et al., 1996). COX-2 expression is enhanced, however, in gastric epithelial cells after growth stimulation in vitro and in gastric epithelium after acid-induced damage in vivo (Tsuji et al., 1996; Sawaoka et al., 1997). We recently demonstrated that COX-2 protein is overexpressed during the healing of gastric lesions and a COX-2-specific inhibitor delays healing in the rat, suggesting an important role for this isoform in gastric ulcer healing (Sun et al., 2000). Therefore, the present study examined the effects of 14-day administration of a PPI, lansoprazole, on gastric mucosal expression of COX-1 and COX-2 and on gastric mucosal protection in rats. In addition, long-term acid suppression might result in elevated gastrin, an important humoral factor in stomach. Gastrin is reported to have fundamental role in stimulating gastric acid secretion. Furthermore, gastrin has protective action on gastric mucosa against ethanol-induced injury (Mercer et al., 1997) and induces growth-promoting effects on diversity of target cells (Yassin, 1999). Various mechanisms, including endocrine, paracrine, and autocrine, have been proposed for gastrin's actions. The mitogenic effects of gastrin are mediated by specific cell surface receptors activated after gastrin binding. The functionally defined receptors for gastrin include cholecystokinin A receptor, which binds gastrin17 sulfated and nonsulfated CCK8 with nearly equal affinities; cholecystokinin C, which is a low-affinity gastrin binding protein; and novel, high-affinity receptors selective for amidated gastrin, processing intermediates of gastrin, or both (Yassin, 1999). We also examined the influences of a gastrin/CCKb receptor antagonist (Ding et al., 1997; Chiba et al., 1998; Fukui et al., 1998; Hakanson et al., 1999) on gastric mucosal expression of cyclooxygenase and gastric mucosal protection.

Materials and Methods

Animals and Agents. Specific pathogen-free male Sprague-Dawley rats (Nippon SLC, Shizuoka, Japan), aged 6 weeks and weighing approximately 150 g, were fed with standard pellet chow and tap water ad libitum. The rats were food-deprived for 24 h but allowed free access to water before sacrifice. All of the experiments were performed according to the Guidelines of the Institutional Committee on Experimental Animals.

Lansoprazole was a gift from Takeda Chemical Industries Co., Ltd. (Osaka, Japan). AG-041R, a potent and specific gastrin receptor antagonist (Ding et al., 1997; Chiba et al., 1998; Fukui et al., 1998; Hakanson et al., 1999), was a gift from Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). NS-386, a specific inhibitor of COX-2 (Putaki et al., 1993a,b; Masferrer et al., 1994; Tsuji et al., 1996), was purchased from Cayman Chemicals (Ann Arbor, MI). The agents were suspended in 0.5% carboxymethylcellulose (CMC; Sigma-Aldrich, St. Louis, MO). The other agents were purchased from Nacalai Tesque Co. (Kyoto, Japan), unless stated otherwise.

Influences of Lansoprazole and AG-041R on Gastric Mucosa: Administration of Test Drugs. The rats were divided into eight groups. Seven animals per group were used for assay for serum gastrin. In these seven animals, four were used for Western analysis and three were used for immunohistochemical study. The other seven animals per group were used for assessment of gastric mucosal injury. Six animals per group were used for prostaglandin assay. The first group received vehicle, 0.5% CMC (1 ml/kg), via a gastric tube once a day for 14 days. Groups 2, 3, and 4 were treated with lansoprazole (0.5, 5, and 50 mg/kg). Groups 5, 6, and 7 were treated with both lansoprazole (50 mg/kg) and the gastrin receptor antagonist AG-041R (3, 10, and 30 mg/kg). The eighth group was administered with AG-041R (30 mg/kg) for 14 days.

Radioimmunoassay of Serum Gastrin Levels. Ten hours after the last administration of the above-mentioned agents, the rats (n = 7) were anesthetized with sevoflurane and blood was drawn by a cardiac puncture. The blood was centrifuged at 2500 g for 10 min, and serum was collected and stored at −20°C until gastrin determination was performed in duplicated manner (Gastrin-RIA kit II; Dainabot, Tokyo, Japan).

Expression of COX-1 and COX-2 in Rat Gastric Mucosa. After the blood samples were collected, the stomach was harvested and opened along the greater curvature. The oxyntic mucosa was scraped with glass slides, and immediately frozen in liquid nitrogen and stored at −80°C for Western blot analysis of COX-1 and COX-2 expression.

The gastric mucosal samples were homogenized in phosphate-buffered saline containing 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS. Protein concentration of the homogenate was measured using protein assay reagent (BCA kit; Pierce Chemical, Rockford, IL). The tissue homogenates, 100 μg protein/lane, were electrophoresed in a 10% SDS-polyacrylamide gel, and transferred onto polyvinylidene difluoride membranes (Immobilon; Millipore Corporation, Bedford, MA) using a semidry transfer cell (Bio-Rad, Hercules, CA). The blots were pretreated with Tris-buffered saline containing 5% nonfat dry milk, 1% albumin, and 0.1% Tween 20, and then incubated overnight at 4°C with antibodies for COX-1 and COX-2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Filters were washed three times and incubated with a horseradish peroxidase-conjugated secondary antibody against goat IgG (Dako, Glostrup, Denmark), developed using a commercial enhanced chemiluminescence system (Amersham Biosciences UK Ltd., Little Chalfont, Buckinghamshire, UK), and exposed to films (Hyperfilm; Amersham Biosciences UK Ltd., Little Chalfont, Buckinghamshire, UK). Expression of COX-1 and COX-2 was semiquantified using a densitometric scanner. The density of the proteins is expressed as percentage of the control treated with CMC only.

For immunohistochemical analysis, gastric specimens were taken from two groups of rats (n = 3/group) treated with CMC or 50 mg/kg lansoprazole and fixed in 10% buffered formalin. Sections (4 μm in thickness) cut from paraffin-embedded tissues were deparaffinized. Sections were then microwave in citrate buffer, pH 6.1, at 95°C, for 10 min for antigen retrieval. Endogenous peroxidase activity was quenched by incubation in 3.0% H2O2 in methanol for 30 min. Non-specific binding was blocked with 3% normal rabbit serum in phosphate-buffered saline, and the tissues were incubated with primary antibodies preabsorbed with pure blocking COX-1 and COX-2 (Dako, Glostrup, Denmark), developed using a commercial enhanced chemiluminescence system (Amersham Biosciences UK Ltd., Little Chalfont, Buckinghamshire, UK), and exposed to films (Hyperfilm; Amersham Biosciences UK Ltd., Little Chalfont, Buckinghamshire, UK.). Expression of COX-1 and COX-2 was semiquantified using a densitometric scanner. The density of the proteins is expressed as percentage of the control treated with CMC only.

For immunohistochemical analysis, gastric specimens were taken from two groups of rats (n = 3/group) treated with CMC or 50 mg/kg lansoprazole and fixed in 10% buffered formalin. Sections (4 μm in thickness) cut from paraffin-embedded tissues were deparaffinized. Sections were then microwave in citrate buffer, pH 6.1, at 95°C, for 10 min for antigen retrieval. Endogenous peroxidase activity was quenched by incubation in 3.0% H2O2 in methanol for 30 min. Non-specific binding was blocked with 3% normal rabbit serum in phosphate-buffered saline, and the tissues were incubated with primary goat polyclonal antibodies against COX-1 and COX-2 (1:200 dilution) overnight at 4°C in 1.5% normal rabbit serum. The sections were stained according to the avidin-biotin complex method using a commercial kit (Vectorstain kit; Vector Laboratories, Burlingame, CA) and visualized using 3,3' diaminobenzidine (DAB) (Vectorstain DAB kit; Vector Laboratories). The specimens were subsequently counterstained with hematoxilin. Other sections were incubated with the antibodies preabsorbed with pure blocking COX-1 and COX-2 peptides (Santa Cruz Biotechnology, Inc.) and then treated with the secondary antibody and stained using the avidin-biotin complex-DAB method.

Measurement of Prostaglandin E2 Levels in Gastric Mucosa. Further experiments were conducted to determine whether lansoprazole and the gastrin receptor antagonist influence PG E2 synthesis in rat gastric mucosa. Ten hours after the final administration of each reagent, all rats, six per group, were anesthetized with sevoflurane, intragastrically administered saline containing 100 μM indomethacin and 10 mM EDTA to block excess PG produc-
tion in gastric mucosa, and immediately laparotomized. The stomach was harvested and the exoytic mucosa was scraped with glass slides and immediately frozen in liquid nitrogen. The tissue was weighed and homogenized at 4°C in cold ethanol, and the homogenate was acidified to pH 4 using diluted HCl and centrifuged. PGE$_2$ in the supernatant was purified using C$_{18}$ solid phase extraction cartridges (Sep-Pak; Waters, Millford, MA) and eluted with ethyl acetate containing 1% methanol. PGE$_2$ was solidified using a rotary evaporator, reconstituted in a buffer, and measured by enzyme immunoassay (Cayman Chemicals). PGE$_2$ levels in the gastric mucosa were expressed as picograms of PGE$_2$ per gram of wet tissue.

Effects of Lansoprazole and AG-041R on Ethanol-Induced Gastric Mucosal Injury. A separate experiment was designed to determine whether 14-day administration of lansoprazole and/or the gastrin receptor antagonist could prevent or attenuate absolute ethanol-induced gastric mucosal injury. Ten hours after the final administration of lansoprazole and/or AG-041R, all rats ($n = 7$/group) were administered with 1 ml of absolute ethanol through an orogastric tube. One hour later, the rats were sacrificed and laparotomized. The stomach was harvested, opened along to the greater curvature, extended on a plastic board, and photographed. The areas of macroscopic hemorrhages and erosions were assessed by planimetry. The ulcer index was expressed as a percentage of the lesion area to the total gastric glandular area.

For histological assessment, the gastric corpus wall was fixed in phosphate-buffered formalin, sectioned, and paraffin-embedded. Semithin sections were deparaffinized, stained with hematoxylin and eosin, and examined under a light microscope by a pathologist without knowledge of to which group the specimen belonged. The specimens were coded and assessed according to the criteria of Whittle et al. (1990). In brief, a 1-cm length of each histological section was assessed for epithelial cell damage (a score of 1); glandular disruption, vasoscongestion, or edema in the upper mucosa (a score of 2); hemorrhagic damage in the mid- to lower mucosa (a score of 3); and deep necrosis and ulceration (a score of 4). Each section was evaluated on a cumulative basis to give the histological score, the maximum score thus being 10.

Influences of NS-398 on PGE$_2$ Synthesis in Gastric Mucosa and Mucosal Protection Afforded by Lansoprazole. To examine whether a specific COX-2 inhibitor influences prostaglandin synthesis in gastric mucosa, rats were treated orally with 10 mg/kg NS-398 10 h after the final administration of 0.5% CMC or 50 mg/kg lansoprazole. Controls received the corresponding vehicle. One hour later, the rats were sacrificed and laparotomized. The stomach was harvested, opened along to the greater curvature, extended on a plastic board, and photographed. The areas of macroscopic hemorrhages and erosions were assessed by planimetry. The ulcer index was expressed as a percentage of the lesion area to the total gastric glandular area.

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Statistical Analyses. Data were shown as mean ± S.E.M. from more than six rats per group and were then analyzed by analysis of variance with Dunnett’s multiple comparison test. A probability value of less than 0.05 was considered statistically significant.

Results

Serum Gastrin Levels. The fasting serum gastrin level was higher in the lansoprazole groups than in the CMC-treated control group. Lansoprazole increased the serum gastrin level in a dose-dependent manner. Serum gastrin was higher in rats given 30 mg/kg AG-041R than in the control group, possibly due to the inhibition of gastric acid secretion by the agent. Concomitant administration of lansoprazole and AG-041R significantly increased the serum gastrin levels compared with control. There is no statistical significance on serum gastrin between the lansoprazole 50 mg/kg group in the absence of AG-401R and in the presence of lansoprazole 50 and 3 mg/kg AG-401R group (Fig. 1).

Expression of COX-1 and COX-2 in Rat Gastric Mucosa. In the control rats treated with 0.5% CMC, the gastric mucosa expressed a small amount of COX-2 and strongly expressed COX-1. In the groups treated with lansoprazole (0.5, 5, and 50 mg/kg) for 14 days, the expression of COX-1 did not change significantly compared with that of the controls. The expression of COX-2 was enhanced in the animals after daily administration of lansoprazole for 14 days. The level of COX-2 immunoreactivity was increased by lansoprazole. This increase in COX-2 expression was reduced almost back to the control level by concomitant administration of AG-041R. There was a dose-dependent reduction in COX-2 expression with increasing dose of AG-401R. COX-1 expression was not affected by the gastrin receptor antagonist (Fig. 2).

Localization of COX-1 and COX-2 in gastric mucosa was determined by immunohistochemical staining (Figs. 3 and 4). Strong COX-1 immunoreactivity was found mainly in parietal cells and other gastric epithelial cells in both the control (Fig. 3A) and lansoprazole-treated rats (Figs. 3B and 4A), as described previously (Jackson et al., 2000). COX-1 was also expressed in less extent in other types of cells in submucosal areas (Fig. 3A). The microscopic study also confirmed that the treatment with lansoprazole did not affect expression of COX-1 in rats. This immunostaining disappeared with the pretreatment of antibody with the blocking peptide, indicating that the immunoreactivity was specific to COX-1.

Immunostaining for COX-2 was minimal or negligible in control rats treated with 14-day administration of CMC (Fig. 3D). After 14-d administration of lansoprazole, however, COX-2 immunoreactivity was strongly detected from the neck to the bottom of the gastric glands (Figs. 3E and 4B).
When the antibody was preincubated with the blocking COX-2 peptide and applied to the sections, no immunoreactive signals appeared (Fig. 3F).

**PGE$_2$ Levels in Gastric Mucosa.** Gastric mucosal PGE$_2$ level was higher in the lansoprazole groups than in the CMC-treated control group. Lansoprazole increased gastric mucosal PGE$_2$ levels in a dose-dependent manner. Concomitant administration of AG-041R dose dependently suppressed lansoprazole-induced increase in gastric mucosal PGE$_2$ (Fig. 5).

**Effects of Lansoprazole and AG-041R on Ethanol-Induced Gastric Mucosal Injury.** In the rats given 0.5% CMC, significant hemorrhagic streaks and erosions developed mainly in the gastric corpus mucosa 1 h after the intragastric administration of absolute ethanol. In the groups administered lansoprazole (0.5, 5, and 50 mg/kg), however, the ulcer index was significantly lower than in the control group treated with CMC. On the other hand, there were no significant differences in the ulcer index between the group administered CMC and the group administered AG-041R. The ulcer index was significantly larger in the group administered both lansoprazole and AG-041R than in that of lansoprazole alone (Fig. 6).

Histological assessment demonstrated that hemorrhagic damage to mid- to lower mucosa was occasionally associated with deep necrosis in rats treated with CMC and then absolute ethanol. In contrast, in the groups treated with lansoprazole and then ethanol, the gastric mucosal injury was restricted to glandular disruption and damage within the upper part of the mucosa. These results indicated that lansoprazole protects gastric mucosa from ethanol-induced injury. On the other hand, there were no significant differences in the histological scores between the group administered CMC and the group administered AG-041R. The histological score was significantly larger, however, in the group given both lansoprazole and AG-041R than in that of lansoprazole alone (Fig. 7). Consequently, treatment with a gastrin receptor antagonist inhibited gastric mucosal protection by lansoprazole.
Effects of NS-398 on PGE$_2$ Synthesis in Gastric Mucosa and Mucosal Protection Afforded by Lansoprazole.

Treatment with NS-398 significantly reduced lansoprazole-induced mucosal PGE$_2$ formation (Fig. 8). There were no significant differences in the ulcer index between the group administered CMC and the group given CMC followed by administration of NS-398. Therefore, NS-398 did not aggravate the ethanol-induced macroscopic injury of the stomach. The ulcer index was significantly larger in the group administered lansoprazole followed by NS-398 than in that of lansoprazole alone.

Discussion

The present study demonstrated that lansoprazole protected gastric mucosa from ethanol-induced injury in rats. PGE$_2$, one of the dominant PGs in gastric mucosa and an endogenous mediator of gastric mucosal protection, increased after 14-day administration of lansoprazole. Lansoprazole also increased serum gastrin levels in rats, consistent with clinical findings of increased levels of serum gastrin in humans during PPI therapy, possibly because of the marked decrease in gastric acid secretion.

**Fig. 4.** Enlarged pictures of COX-1 (A) and COX-2 (B) immunoreactivities in gastric mucosa in a rat treated with lansoprazole. COX-1 immunoreactivity is found mainly in parietal cells and other gastric epithelial cells in lansoprazole-treated rats (Figs. 4A). After 14-day administration of lansoprazole, however, COX-2 immunoreactivity is strongly detected from the neck to the bottom of the gastric glands (Figs. 4B).

**Fig. 5.** Influences of lansoprazole and AG-041R on mucosal PGE$_2$ in rats. *p < 0.05; **p < 0.005 versus the control group. +p < 0.02; +++, p < 0.005 versus the group treated with 50 mg/kg lansoprazole. Data are shown as mean ± S.E.M. (n = 6/group). Gastric mucosal PGE$_2$ level is higher in the lansoprazole groups than in the CMC-treated control group. Lansoprazole increases gastric mucosal PGE$_2$ levels in a dose-dependent manner. Concomitant administration of AG-041R dose dependently suppresses lansoprazole-induced increase in gastric mucosal PGE$_2$.

**Fig. 6.** Influences of lansoprazole and AG-041R on macroscopic gastric damage induced by ethanol. *p < 0.05; **p < 0.01; and ***p < 0.005 versus the control group. +p < 0.02 versus the group treated with 50 mg/kg lansoprazole. Data are shown as mean ± S.E.M. (n = 7/group). In the groups predosed lansoprazole (0.5, 5, and 50 mg/kg), ethanol-induced gastric damage is significantly smaller than in the control group pretreated with CMC. On the other hand, there are no significant differences in the ulcer index between the group predosed CMC and the group predosed AG-041R. The ulcer index is significantly larger in the group predosed both lansoprazole and AG-041R than in that of lansoprazole alone.

**Fig. 7.** Influences of lansoprazole and AG-041R on histological gastric damage induced by ethanol. *p < 0.05; **p < 0.01; and ***p < 0.005 versus the control group. +p < 0.02 versus the group treated with 50 mg/kg lansoprazole. Data are shown as mean ± S.E.M. (n = 7/group). The 14-day administration with lansoprazole (0.5, 5, and 50 mg/kg) significantly reduced microscopic damage after ethanol administration compared with the control group pretreated with CMC. On the other hand, there are no significant differences in the ulcer index between the group predosed CMC and the group predosed AG-041R. The ulcer index is significantly larger in the group predosed both lansoprazole and AG-041R than in that of lansoprazole alone.
The present study demonstrated that repeated administration with lansoprazole for 14 days significantly enhanced gastric mucosal PGE$_2$.

In the present study, 14-day administration of lansoprazole also associated with a significant increase in fasting serum gastrin compared with vehicle. Indeed, several studies have suggested that long-term administration of lansoprazole elevates serum gastrin (Muller et al., 1989; Brunner et al., 1995). Consequently, AG-041R, the gastrin-specific receptor antagonist (Ding et al., 1997; Chiba et al., 1998; Fukui et al., 1998; Hakanson et al., 1999), was used to clarify the involvement of gastrin and its receptor on mucosal protection induced by 14-day administration of lansoprazole. No direct interactions have been reported between lansoprazole and AG-041R (Ding et al., 1997; Chiba et al., 1998; Fukui et al., 1998; Hakanson et al., 1999). The study clearly indicated that AG-041R suppresses the increase in gastric mucosal PGE$_2$ after long-term administration with lansoprazole, suggesting an important role for gastrin and its receptors in gastric mucosal PG production induced by lansoprazole. Furthermore, AG-041R also abolished lansoprazole-induced gastric mucosal protection against ethanol. Thus, lansoprazole might induce serum gastrin, stimulate gastric mucosal production of PGE$_2$, and participate in gastric mucosal protection in rats. The effects of PPIs on COX-2 induction in organs other than stomach are not yet available in the literature. However, the present data suggest that the effects of PPIs on COX-2 in stomach are mediated by gastrin. Thus, PPIs may induce COX-2 only in upper gastrointestinal tract that has cells expressing receptors for gastrin.

The present study also examined the source of PGs in rat gastric mucosa after 14-day administration of lansoprazole. Because COX is the rate-limiting enzyme for PG production, the influence of lansoprazole on the expression of the two isoforms of COX was examined. An initial study by Seibert et al. (1997) suggested that COX-1 mRNA was readily detectable in all normal tissue examined, especially in stomach and others, but that levels of COX-2 mRNA were substantially lower, with the exception of the brain. Several studies, including the present study, have shown that unstimulated gastric mucosa express a lower level of COX-2, whereas gastric mucosal epithelium expresses this isoform after various stimuli (Sawaoka et al., 1997; Sun et al., 2000). In the present study, lansoprazole enhanced the expression of mitogen-inducible COX-2, but not that of constitutive COX-1 in rat gastric mucosa. AG-041R abolished lansoprazole-induced COX-2 expression, but not that of COX-1 in rat gastric mucosa. These findings indicate that lansoprazole induces gastric mucosal production of PGs by enhancing expression of COX-2. Furthermore, the lansoprazole-induced increase in gastric mucosal PGE$_2$, was blocked by NS-398, a specific COX-2 inhibitor. NS-398 also attenuated mucosal protection induced by lansoprazole. Taken together, lansoprazole induces COX-2-dependent mucosal PG production and mucosal protection in the rat stomach mediated by endogenous gastrin and gastrin receptors. Gastrin receptors are localized at parietal cells, mast cells, and enterochromaffin-like cells in rat corpus mucosa. On the other hand, immunohistochemical evaluation of COX-2 indicated that COX-2 is up-regulated...
not only in parietal cells but also in mucosal neck cells. Although the precise mechanism for gastrin-induced COX-2 in the stomach is not known, one source of PG production could be parietal cells that express gastrin receptors. Gastrin up-regulates heparin-binding epidermal growth factor (EGF)-like growth factor in RGM1 gastric epithelial cells (Kobayashi et al., 1996) transfected with the gene for gastrin receptor (Miyazaki et al., 1999). Several ligands for EGF receptors such as EGF and transforming growth factor-α up-regulate COX-2 in several gastrointestinal cell lines, including RGM1 in vitro (Sawaoka et al., 1997, 1999). Therefore, future studies should investigate whether gastrin up-regulates COX-2 via stimulating expression of one of the EGF-receptor ligands in rat stomach in vivo.

PPIs, including lansoprazole, are used in patients with various gastroduodenal disorders. PPIs are acid-labile, and clinical formulae for PPIs are encapsulated granules or coated tablets. The formulation of lansoprazole used in the present study was purified powder, neither encapsulated nor coated against intragastric degradation. In addition, the lowest dose of lansoprazole used in the study of lansoprazole was 0.5 mg/kg, which is equivalent to 30 mg in subjects weighing 60 kg. Nevertheless, the results clearly indicated that, at doses ranging from 0.5 to 50 mg/kg, lansoprazole efficiently increases serum gastrin, enhances COX-2 expression and PG production in gastric mucosa, and protects gastric mucosa. These findings might be relevant to the long-term effects of the drug in human subjects. It has been suggested that omeprazole, another PPI, accelerates the healing of gastric ulcers via an increase in gastrin secretion possibly mediated by the trophic actions of the peptide (Ito et al., 1994). However, there are differences between PPIs in general and synthetic PGs with respect to their mucosal protective actions. For example, PGs protect gastric mucosa from necrotizing stimuli at doses that do not inhibit gastric acid secretion. On the other hand, PPIs inhibit gastric acid secretion more potently than synthetic prostaglandins. Therefore, gastric protective actions of PPIs have been attributed to their potent inhibitory effect on acid secretion (Bergmann et al., 1992; Blandizzi et al., 1999). However, in the present study, the long-term administration of lansoprazole protected gastric mucosa from absolute ethanol, one of the necrotizing stimuli with the increase in endogenous PGE₂. The clinical implications of lansoprazole-induced gastric mucosal protection remain to be investigated.

Localization of COX-1 and COX-2 in rat gastric mucosa should be discussed. Jackson et al. (2000) reported that COX-1 immunoreactivity is expressed in parietal cells and other components in gastric mucosa in human subjects. These findings were confirmed in the present study. In contrast, the long-term treatment with lansoprazole enhanced the expression of COX-2 in gastric epithelial cells that did not necessarily express gastrin/CCKb receptors. These findings strongly suggest that gastrin indirectly enhances the expression of COX-2 in gastric mucosa. Recently, it was reported that gastrin up-regulates growth factors such as heparin-binding EGF-like growth factor in epithelial cells expressing gastrin/CCKb receptors (Miyazaki et al., 1999; Kinoshita and Ishihara, 2000). Expression of COX-2 in gastric epithelium in response to growth factors and mitogens has been well documented (Sawaoka et al., 1997, 1999). Consequently, long-term treatment with lansoprazole may enhance COX-2 expression and exert cytoprotective activity via gastrin-dependent transactivation of a growth factor and its receptor. Precise mechanisms for gastrin-dependent COX-2 up-regulation remain to be investigated in future studies.

In conclusion, the present results demonstrate that intragastric administration with lansoprazole increases serum gastrin levels, induces COX-2 expression in gastric mucosa, elevates gastric mucosal PGE₂, and protects gastric mucosa from necrotizing agents in rats. Treatment with a specific gastrin receptor antagonist or a specific COX-2 inhibitor abolishes the lansoprazole-induced increase in gastric mucosal PGE₂ and protection of gastric mucosa from acute injury. In this experimental rat ulcer model, the mucoprotective effects of lansoprazole depend on COX-2, which is induced by lansoprazole. Thus, activation of gastric receptors by endogenous gastrin has a pivotal role in the effects of lansoprazole on COX-2 up-regulation and PGE₂-mediated gastric mucosal protection.

References


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