Mucosal Protective Effect of Leminoprazole on Reflux Esophagitis Induced in Rats

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ABSTRACT—We examined the effect of leminoprazole (an acid pump inhibitor) on reflux esophagitis induced in rats. Intragastrically administered leminoprazole significantly and dose-dependently protected the esophageal mucosa against the reflux of gastric contents, without affecting gastric acid secretion. However, it had no effect on the esophagitis when administered intraduodenally, despite its significant inhibition (about 40%) of gastric acid secretion. Omeprazole significantly prevented the development of esophagitis, most probably through potent inhibition of gastric acid secretion. Indomethacin significantly reduced the synthesis of prostaglandin E2 in the esophagus. Since indomethacin pretreatment had no effect on the esophageal protection by leminoprazole, omeprazole or sucralfate, the involvement of endogenous prostaglandins can be ruled out as a possible underlying mechanism. Intragastrically, but not intraduodenally, administered sucralfate significantly prevented the esophagitis even at a dose not affecting gastric acid secretion. These results strongly suggest that both leminoprazole and sucralfate protect the esophageal mucosa directly.

Keywords: Leminoprazole, Omeprazole, Sucralfate, Reflux esophagitis

There is increasing evidence that gastric acid pump inhibitors exert a much more favorable effect on reflux esophagitis than H₂-blockers through their profound and long-lasting antisecretory activities (1-3). Even in animal experiments, both omeprazole and lansoprazole provided marked protection of the esophageal mucosa against the reflux of gastric contents (4, 5). We developed a new gastric acid pump inhibitor, leminoprazole (NC-1300-O-3), that has antisecretory, mucosal protective and ulcer healing effects in rats (6-9). The present study was performed to determine if leminoprazole protects the esophageal mucosa against the reflux of gastric juice. Omeprazole and sucralfate were used as reference drugs.

MATERIALS AND METHODS

Animals

Eight-week-old male Sprague-Dawley rats (Charles-River Japan, Kanagawa), weighing 250-270 g, were fasted for 18 hr. Drinking water was freely available to the animals up to 2 hr before the experiments. All animals were kept in raised mesh-bottom cages to prevent coprophagy. Four to ten rats were included in each group.

Esophagitis induction

Under ether anesthesia, the abdomen was incised along the midline and then both the pylorus and limiting ridge (transitional region between the forestomach and corpus) were simultaneously ligated according to the method described by Nakamura et al. (10). Consequently, the total capacity of the stomach to preserve the gastric juice was greatly diminished, resulting in the reflux of gastric juice into the esophagus. Two, 3, 4 or 6 hr later, the animals were killed with an overdose of ether, and then the esophagus and stomach were removed as a single unit. The total area (mm²) of lesions that had developed in the esophagus was determined under a dissecting microscope (×10) and graded as follows: 0, no visible lesions; 1, a few erosions; 2, total area of lesions ≤30 mm²; 3, total area of lesions ≥31 mm²; 4, perforation. The test drugs were administered either intragastrically (i.g.) by gastric intubation or intraduodenally (i.d.) immediately after ligation of the pylorus and limiting ridge. To determine whether or not endogenous prostaglandins are involved in the protective mechanism of drugs, indomethacin (5 mg/kg) was pretreated subcutaneously (s.c.) 1 hr before ligation of the pylorus and limiting ridge.
Gastric secretory study

It was rational to examine the effects of drugs using a preparation involving the ligation of the pylorus and limiting ridge. However, there is often leakage of gastric contents from the mouth nearly 2 hr after ligation of these portions. Therefore, we used a preparation with only the pylorus ligated. The abdomen was incised under ether anesthesia and only the pylorus was ligated. Four hours later, the animals were killed with an overdose of ether. The gastric contents were collected and its volume (ml/rat) and acidity (mEq/l) were determined. Total acidity was determined by automatic titration of the gastric juice against 0.1 N NaOH to pH 7.0 (Comitie 5; Hiranuma, Tokyo). Acid output was expressed as µEq/hr. In the case of sucralfate administered i.g., only the volume and pH of the contents were determined, acidity not being determined because sucralfate itself consumes NaOH to some extent. The test drugs were administered at 3-300 mg/kg, i.g. or i.d. immediately after pylorus ligation. The volume of the drug or vehicle was 0.5 ml/200 g or 1.0 ml/200 g of body weight for i.g. or i.d. treatment, respectively.

Determination of prostaglandin E₂

Synthesis of prostaglandins E₂ in the esophagus was determined by the method of Lee and Feldman (11, 12). Briefly, under ether anesthesia, the esophagus was isolated, weighed and placed in a vial that contained 1 ml of Tris buffer (50 mmol/l, pH 8.4) and was kept cool on ice. The specimen was minced for about 15 sec with scissors, washed and centrifuged at 9,000 x g for 15 sec. The supernatant was discarded and an additional 1 ml of Tris buffer was added to the vial. Each sample was mixed with a vortex mixer for 60 sec at 25°C and then centrifuged again at 9,000 x g for 15 sec. The supernatants were stored at -80°C, and then prostaglandin E₂ generated was determined by an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA). Prostaglandin E₂ was expressed as pg/mg tissue/min. Esophageal samples were obtained immediately before and 0.5 hr or 4 hr after indomethacin treatment (5 mg/kg, s.c.).

Drugs

Leminoprazole and omeprazole (provided by Nippon Chemiphar, Tokyo) were suspended in 0.5% carboxymethylcellulose. Sucralfate (Chugai, Tokyo) was suspended in distilled water. Indomethacin (Sigma, St. Louis, MO, USA) was suspended in a small amount of Tween 80 (Wako, Osaka) and 0.5% carboxymethylcellulose.

Statistical analysis

Statistical analysis was performed by the Kruskal-Wallis rank test and two-tailed Dunnett's multiple comparison test, values of P<0.05 being regarded as significant. ED₅₀ values and confidence limits were calculated from the dose-inhibition relationships by the least squares method.

RESULTS

Effects of test drugs on reflux esophagitis

With the surgical procedure used in this study, reflux esophagitis developed in the thoracic esophagus in 7 of 8 animals 3 hr after ligation (Fig. 1). However, both the severity and incidence of esophagitis were increased when each esophagus was examined 4 hr later. The incidence of perforation was about 50%. Nearly the same result was obtained at 6 hr after ligation, so we selected 4 hr as the experimental time. Both leminoprazole (30 or 60 mg/kg) and omeprazole (60 mg/kg) administered i.g. significantly prevented the development of esophagitis (Fig. 2). The ED₅₀ values of these drugs were 12.7 mg/kg (8.0-19.2) and 25.9 mg/kg (20.4-32.8), respectively. Sucralfate (10, 30, 100 or 300 mg/kg) administered i.g. also significantly prevented esophagitis. Neither leminoprazole (10, 30 or 60 mg/kg) nor sucralfate (100 or 300 mg/kg) administered i.d. significantly prevented esophagitis. Neither leminoprazole (10, 30 or 60 mg/kg) nor sucralfate (100 or 300 mg/kg) administered i.d. prevented the development of esophagitis (Fig. 3). In contrast, omeprazole (30 or 60 mg/kg) administered i.d. significantly prevented it. The ED₅₀ value was 5.9 mg/kg (2.6-9.4). Pretreatment with indomethacin had no influence on the protective effect of leminoprazole (60 mg/kg, p.o.), omeprazole (60 mg/kg, p.o.) or sucralfate (300 mg/kg) (Fig. 4).
Effects of leminoprazole, omeprazole and sucralfate on reflux esophagitis induced in rats. The drugs were administered intragastrically (i.g.) 5 min after simultaneous ligation of the pylorus and limiting ridge. *Significantly different from the control values, at P < 0.05.

**Effects of test drugs on gastric secretion**

Leminoprazole (3 and 10 mg/kg) administered i.g. had little or no effect on gastric acid output (Fig. 5). At 30 and 60 mg/kg, however, it significantly increased the volume of the gastric contents (11.2±0.6 ml/rat and 11.9±0.7 ml/rat vs 7.2±0.9 ml/rat in the controls), without any effect on the acid output. Omeprazole (10 mg/kg) administered i.g. significantly inhibited the acid output by 40.9%. At 30 or 60 mg/kg, it caused a significant reduction in the acid output; the inhibition of acid output amounted to 59.8% and 63.1%, respectively. The volume of the gastric contents significantly or insignificantly
Fig. 4. Effect of pretreatment with indomethacin on the protective effects of leminoprazole, omeprazole and sucralfate on reflux esophagitis induced in rats. Indomethacin (5 mg/kg) was administered subcutaneously (s.c.) 1 hr before ligation of the pylorus and limiting ridge. The drugs were administered 5 min after ligation. *Significantly different from the control values, at P < 0.05.

Fig. 5. Effects of leminoprazole, omeprazole and sucralfate on gastric acid secretion in pylorus-ligated rats (4 hr). The drugs were administered intragastrically (i.g.) 5 min after pylorus ligation. Values are means±1 S.E.M. for 8 rats. *Significantly different from the control values, at P < 0.05.
decreased with these doses, i.e., by about 20%. Sucralfate (10, 30 or 100 mg/kg) administered i.g. had little or no effect on the pH of the gastric contents. At 300 mg/kg, it caused a significant increase in the volume of the gastric contents (12.0 ml vs 8.0 ml in the controls). The pH of the contents was also increased to 2.2 vs 1.3 in the controls. When administered i.d., however, both leminoprazole (60 mg/kg) and omeprazole (60 mg/kg) significantly inhibited the acid output by 42.4% and 93.6%, respectively (Fig. 6). The volume of the gastric contents was significantly reduced by omeprazole, but tended to be decreased by leminoprazole. Sucralfate (300 mg/kg, i.d.) slightly increased the volume of the gastric contents and acid output compared with the control values. The pH was 1.4 in both the sucralfate-treated and control groups.

Effect of indomethacin on prostaglandin E2 synthesis
The prostaglandin E2 synthesis in the normal esophagus was 9.42 ± 2.85 pg/mg tissue/min. The synthesis was markedly reduced at 0.5 hr and 4 hr after indomethacin treatment by 69.3% and 85.2%, respectively (Table 1). The inhibition observed at 4 hr was statistically significant.

DISCUSSION
The results indicate that leminoprazole, omeprazole and sucralfate markedly protect the esophageal mucosa from the reflux of gastric juice, depending on the route of administration. Therefore, the underlying mechanisms seem to be different from each other. First, the mechanism of action of i.g. administered leminoprazole appears to be unrelated to the gastric secretory conditions because gastric acid secretion was not inhibited with that route. Rather, the volume of the gastric juice increased with the drug. This increased volume with leminoprazole was observed in our previous studies, although at that time it was administered orally for 4 weeks (7). The mechanism underlying this increase in the volume remains unknown. In our preliminary study, increased permeability of the esophageal mucosa, as judged from the Evans blue, was ob-

Table 1. Effect of indomethacin pretreatment on prostaglandin E2 synthesis in the rat esophagus

<table>
<thead>
<tr>
<th>Time</th>
<th>Prostaglandin E2 synthesis (pg/mg tissue/min)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.42 ± 2.85</td>
<td>0</td>
</tr>
<tr>
<td>0.5 hr</td>
<td>2.89 ± 1.49</td>
<td>69.3</td>
</tr>
<tr>
<td>4 hr</td>
<td>1.39 ± 0.69*</td>
<td>85.2</td>
</tr>
</tbody>
</table>

Prostaglandin synthesis was determined 0.5 hr and 4 hr after indomethacin (5 mg/kg, subcutaneously) treatment. Data are means ± S.E.M. for 4 rats. *Significantly different from the control values, at P < 0.05.
served after i.g. administration of the drug. As shown in this study, the i.d. administration of leminoprazole at 60 mg/kg tended to decrease the volume of the gastric juice. Therefore, the increase in the volume of the gastric juice after i.g. administration appears to be due to direct irritation of the gastric mucosa by leminoprazole. There is a possibility that the protective action of leminoprazole is due to the dilution of gastric contents since the gastric juice volume was significantly increased at 30 and 60 mg/kg. The degree of the increase in the volume was quite similar at these doses. However, the development of esophagitis was not significantly inhibited by 30 mg/kg of the drug. Therefore, the mechanism of action of leminoprazole would not be simply due to the dilution of gastric juice, but due to a specific protective effect. It was found that the gastric acid output was reduced by about 40% when leminoprazole was administered i.d. at 60 mg/kg. Of note was that even at 60 mg/kg, leminoprazole had little or no effect on esophagitis. This result strongly suggests that about 40% of acid inhibition is not enough to prevent the development of esophagitis. Once again, this finding rules out the possible participation of gastric acid in the mechanism underlying the protective activity of the i.g. administered drug. Leminoprazole has a cytoprotective effect against necrotizing agent-induced gastric lesions (6). Therefore, it is likely that this protection of the esophagus and the stomach might involve the same mechanism.

Similar to leminoprazole, omeprazole extensively prevented the development of esophagitis on i.g. administration, as reported by Goto and Kishi (5). It significantly inhibited the gastric acid output by about 60% when administered p.o. at 30 or 60 mg/kg. Since the pylorus was ligated, the antisecretory effect of omeprazole might mainly comprise its direct effect on parietal cells and partly, if any, that of the absorbed drug. Thus, it is possible that the protective effect of omeprazole is partly due to this antisecretory effect. Although the degree of inhibition of acid is nearly the same with 30 and 60 mg/kg, the protection observed with 30 mg/kg was insignificant. Therefore, the protection by 60 mg/kg may be partly related to the mucosal protection, which is identical to the gastric protection by this drug (13, 14). In contrast to leminoprazole, omeprazole administered i.d. markedly protected the esophagus from the reflux of gastric contents. The dose that inhibited esophagitis is one that inhibits gastric secretion by more than 90%. Therefore, this protection might be purely caused by the inhibition of acid secretion.

Sucralfate is known to be quite effective for the treatment of human reflux esophagitis (15, 16). As expected, sucralfate administered i.g. markedly prevented the development of experimentally-induced esophagitis. As reported previously (17) and confirmed in this study, this drug apparently increased the volume and pH of the gastric contents when administered i.g. at 300 mg/kg. These results indicate that the mucosal protection is partly related to the buffering action of the drug. However, 10 or 30 mg/kg of sucralfate had no effect on the volume or pH, but it significantly protected the esophagus. In addition, sucralfate administered i.d. at 300 mg/kg showed no effect on esophagitis and tended to increase the volume of the gastric contents. These results suggest that i.g. administered sucralfate acts through a direct protective mechanism, which was confirmed in the case of the protection against necrotizing agent-induced gastric lesions (18, 19). It is possible that both leminoprazole and sucralfate prevent esophagitis through similar mechanisms.

As expected, indomethacin markedly reduced the synthesis of prostaglandin E2 in the esophagus for 4 hr. Since indomethacin pretreatment had no effect on the protective effect of leminoprazole, omeprazole or sucralfate (i.g.) against esophagitis, the participation of endogenous prostaglandins in the underlying mechanism could be ruled out.

We conclude that leminoprazole is a promising drug for the treatment of reflux esophagitis when administered via the oral route.

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