Studies on the Mechanism for the Gastric Mucosal Protection by Famotidine in Rats

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ABSTRACT — The effect of famotidine on gastric lesions induced by the decrease in mucosal defensive resistance was investigated in rats and compared with those of cimetidine, pirenzepine and cetraxate. Famotidine (0.03, 0.1 and 0.3 mg/kg, p.o.) inhibited dose-dependently the development of gastric lesions produced by taurocholate-histamine in doses that suppressed histamine-induced acid secretion in pylorus-ligated rats. The H2-antagonist also prevented gastric mucosal lesions induced by taurocholate-serotonin, iodoacetamide, acidified aspirin and acidified ethanol. Cimetidine, pirenzepine and cetraxate showed the inhibitory effects on almost all types of the gastric lesions, but their inhibitory effects were much less potent than those of famotidine. On the other hand, famotidine inhibited the decreases of gastric mucosal blood flow induced by acidified ethanol and the mucosal contents of glycoprotein induced by water immersion restraint stress. In addition, famotidine increased the transgastric potential difference (PD) and promoted the recovery of decreased transgastric PD induced by acidified ethanol in rats. These results suggest that the preventive effect of famotidine on gastric lesions is attributable not only to suppression of acid secretion but to activation of the gastric mucosal defensive mechanisms.

Histamine H2-receptor antagonists, famotidine, cimetidine and ranitidine, have been shown to be effective in preventing the formation of gastric mucosal lesions in rats (1–3) and in promoting the healing in rat (2, 4) and human ulcers (5–7). The mechanisms by which H2-receptor antagonists suppress experimental lesions are thought to be secondary to their potent gastric acid antisecretory activity (1, 3). However, cimetidine and ranitidine blocked acidified aspirin-induced gastric lesions in rats in doses that do not suppress acid secretion (8, 9), suggesting that mechanisms other than inhibition of acid secretion might be involved in their mucosal protective activity.

Famotidine has been reported to be a potent H2-receptor antagonist in vitro (10–12) and in vivo (4, 13) with greater antisecretory activity than cimetidine and ranitidine (3, 14, 15). Famotidine has also been reported to inhibit the gastric mucosal lesions that were dependent on gastric acid (2–4). The aims of this investigation were to examine the effect of famotidine on the formation of gastric mucosal lesions induced by the decrease in mucosal defensive factors in comparison with cimetidine, pirenzepine and cetraxate.
MATERIALS AND METHODS

Materials
Famotidine, cimetidine and pirenzepine hydrochloride were synthesized in our laboratories. Cetraxate hydrochloride was extracted and purified from Neuer® capsule S (Daiichi Pharmaceutical). Each drug was suspended with 0.5% methylcellulose solution and administered to rats at a volume of 5 ml/kg. Drugs used for induction of gastric lesions were sodium taurocholate (Sigma Chemical), histamine dihydrochloride (Wako Pure Chemical), serotonin creatinine sulfate (E. Merck), aspirin (Sanko Pharmaceutical), indomethacin (Yamanouchi Pharmaceutical) and iodoacetamide (Tokyokasei). Taurocholate, histamine and serotonin, and iodoacetamide were dissolved in distilled water, physiological saline and tap water, respectively. Aspirin was suspended with 150 mM HCl. Indomethacin was suspended with physiological saline containing 0.2% Tween 80. Other drugs used were trypsin (Sigma Chemical), glucose (Nacalai Tesque) and glucosamine (Sigma Chemical).

Gastric lesion production
Taurocholate-induced gastric lesion: Male Wistar rats weighing 200 to 230 g were deprived of food but allowed free access to water for 24 hr before the experiments. Acute gastric lesions were induced by taurocholate alone and in the combination with histamine or serotonin. Just after 30 mM taurocholate was given intragastrically in a 1-ml volume, histamine was injected i.p. in a dose of 100 mg/kg or serotonin was injected subcutaneously in a dose of 20 mg/kg. Four hours later, the animals were killed by chloroform, and the stomach was removed. The area (mm²) of each lesion was measured macroscopically, summed per stomach, and used as a lesion index. Drugs were given orally 1 hr before ulcerogens administration.

Aspirin or ethanol-induced gastric lesion: Male Sprague-Dawley rats weighing 210 to 230 g were deprived of food and water for 24 hr before the experiments. Gastric lesions were caused by acidified (150 mM HCl) aspirin in a dose of 150 mg/kg, i.e., or 60% ethanol, i.e., containing 150 mM HCl (acidified ethanol). One hour after i.g.-administration of acidified aspirin or acidified ethanol in a 1-ml volume to rats, the animals were killed by chloroform and the stomach was removed. The length (mm) of each lesion was measured macroscopically, summed per stomach, and used as a lesion index. Drugs were given orally 1 hr before ulcerogens administration.

In another series of experiments to evaluate the involvement of endogenous prostaglandins, indomethacin was subcutaneously injected to rats 1 hr before drug administration.

Iodoacetamide-induced gastric lesion: Male Wistar rats weighing 200 to 230 g were used for production of gastric lesions by iodoacetamide according to the method of Szabo et al. (16). Briefly, rats were placed in individual mesh cages to prevent coprophagy with free access to food and water. Gastric lesion was caused by 0.1% iodoacetamide in drinking water given to rats for 7 days; and on the 8th day, rats were sacrificed by chloroform, and the stomach was removed. The length (mm) of each lesion was measured macroscopically, summed per stomach, and used as a lesion index. Test compounds were given orally once daily to rats during the 7 days.

Gastric acid secretion
Male Wistar rats weighing 200 to 230 g were deprived of food, but allowed free access to tap water for 24 hr before the experiments. With the rats under ether anesthesia, the abdomen was incised, and the pylorus was ligated. They were placed in individual mesh cages to prevent coprophagy. Four hours later, the animals were killed by chloroform, and the gastric contents were analyzed for volume and acidity. Acidity of the gastric juice was determined by titration with 0.05 N NaOH using an automatic titrator (Hiranuma, Comtite-7) to an endpoint pH of 7.0. In another series of experiments to examine the influence of histamine against gastric secretion in pylorus-ligated rats, histamine at a dose of
100 mg/kg was intraperitoneally injected to the rats immediately after the abdomen was sutured. Test compounds were given orally 1 hr before pylorus ligation.

**Measurement of gastric mucosal blood flow**

Male Sprague-Dawley rats weighing 200 to 240 g were deprived of food with free access to water for 24 hr before the experiments. Under urethane (1.25 g/kg, i.p.) anesthesia, the trachea was cannulated and the abdomen was opened. The esophagus was ligated without disturbing the vagus nerves. Gastric mucosal blood flow was measured using a laser Doppler blood flowmeter (TSI, Laserflo; BPM403) according to the method of Saita et al. (17). The optical flow probe was inserted into the stomach through a temporary gastric fistula, which was prepared using a polyethylene tube, in the forestomach and was placed on the gastric mucosa. The probe was mounted in a commercially available balancer (Physio-Tech). Approximately 30 min after anesthesia, 60% ethanol containing 150 mM HCl in a 1-mL volume was intragastrically administered through a gastric fistula. The changes in gastric mucosal blood flow was continuously monitored. Drugs were given orally 30 min before urethane anesthesia.

**Measurement of the transgastric potential difference (PD)**

The experimental procedure was essentially the same as that described by Takeuchi and Okabe (18). Male Sprague-Dawley rats weighing 200 to 250 g were deprived of food for 24 hr but water was allowed ad libitum. After anesthesia with urethane (1.25 g/kg, i.p.), the trachea of the rat was cannulated. The abdomen was opened and the stomach was exposed. The esophagus was ligated without disturbing the vagus nerves. A temporary gastric fistula was prepared using a polyethylene tube in the forestomach. The fistula that led to a three-way tap was used for intragastric instillation and removal of gastric contents and for continuous intraluminal perfusion with warmed saline. The pyloroduodenal junction was exposed, and two catheters were passed into the stomach through an incision in the duodenum and were held in place by a ligature around the pylorus. One catheter, which was filled with 4% agar in saturated KCl, served as the intragastric electrode. The circuit for the PD recording was coupled with an indifferent electrode placed in the abdominal cavity filled with saline. Both the intragastric and intraperitoneal electrodes were placed in separate beakers containing saturated KCl solution in which a balanced Ag-AgCl electrode was positioned. The changes in PD were continuously monitored using a recorder (Rikadenki, R-52) connected to the millivoltmeter (Toa Electronics, HM-16S). The second gastric catheter led to a pH glass electrode of the flow type (Toa Electronics, GS-80) to determine the changes in pH of fluids emerging from the pylorus and which was connected to a pH meter (Toa Electronics, HM-16S) for simultaneous measurement of the changes in PD. The whole interior of the stomach was gently rinsed with warmed saline 3 to 4 times, and then 3 mL of saline was instilled into the stomach. The perfusion of warmed saline (2 mL/min) was controlled by a peristaltic pump (Taitec, N-18) located at the middle of entry or exit tube connected to the fistula or to the pH glass electrode.

Approximately 30 min after anesthesia, the perfusion system was interrupted, and the solution in the stomach was withdrawn. The stomach was then exposed for 10 min to 3 mL of acidified ethanol. After acidified ethanol exposure, the stomach was gently rinsed again several times, another 3 mL of saline was instilled, and the perfusion system was resumed. Drugs were given orally 30 min before urethane anesthesia.

**Measurement of the contents of gastric mucosal glycoprotein**

Male Wistar rats weighing 200 to 240 g were deprived of food but allowed free access to tap water for 18 hr before the study. The stress condition was evoked by placing the animals in individual compartments of the spec-
cial stress cages and then immersing them in a water bath at 23°C for 7 hr to the xyphoid level as described by Takagi and Okabe (19). After the animals were killed by chloroform, the stomachs were removed, placed in ice-cold physiological saline solution, and opened along the greater curvature. The gastric corpus wall was then placed between two glass slides and frozen by immersion in hexane cooled in a dry ice-ethanol bath. The gastric mucosa was separated from its underlying muscle layer by pulling the two glass slides apart and then weighed. The frozen mucosa was homogenized in 2.5 ml of water with a Polytron homogenizer. The resultant homogenate was digested with trypsin (0.5 mg) for 20 hr at 37°C and centrifuged to eliminate non-digestable components. A portion of the supernatant was hydrolyzed in conc-HCl in a sealed ampoule at 110°C for 16 hr. Hexoses in the hydrolyzate were assayed colorimetrically using the anthrone-H2SO4 method (20). Hexosamine in the hydrolyzate was also assayed colorimetrically after separation through a Dowex 50 column. Glucose and glucosamine were used as the standard for calibration, respectively.

Statistics
All values represent the mean ± standard error of the mean (S.E.M.), and the statistical significance was determined by analysis of variance (ANOVA) or Student's t-test. The differences between treatment groups were compared by the Neuman-Keuls multiple range test. Probabilities of < 5% (P < 0.05) were considered significant. The doses producing 50% inhibition of acid secretion or gastric lesion (ED50 values) were determined by log-probit analysis from data obtained for 3 doses of each compound.

RESULTS

Effect on taurocholate-histamine and taurocholate-serotonin-induced gastric lesion
Administration of taurocholate at 30 mM/rat, i.g., histamine at 100 mg/kg, i.p., and serotonin at 20 mg/kg, s.c., resulted in only small areas of lesions, with indices of 0.4 ± 0.2, 2.6 ± 0.4 and 3.1 ± 0.5 mm², respectively. When taurocholate was administered to rats in the combination with histamine or serotonin, however, marked gastric lesions were observed with lesion indices of 12.3 ± 1.4 and 7.2 ± 1.0 mm², respectively.

As shown in Figs. 1 and 2, famotidine inhibited taurocholate-histamine and taurocholate-serotonin-induced gastric lesions at p.o. doses of 0.03 to 0.3 mg/kg in a dose-dependent manner with the ED50 values (95% confidence limits) of 0.04 (0.04-0.05) and 0.08 (0.02-0.19) mg/kg, p.o., respectively. Cimetidine (3-30 mg/kg, p.o.) and pirenzepine

Fig. 1. Effects of famotidine, cimetidine, pirenzepine and cetraxate on taurocholate (30 mM)-histamine (100 mg/kg, i.p.)-induced gastric lesion in rats. Each point represents the mean ± S.E.M. for 8-10 rats. The statistical significance was determined by ANOVA (**: P < 0.01).

Fig. 2. Effects of famotidine, cimetidine, pirenzepine and cetraxate on taurocholate (30 mM)-serotonin (20 mg/kg, s.c.)-induced gastric lesion in rats. Each point represents the mean ± S.E.M. for 10 rats. The statistical significance was determined by ANOVA (*: P < 0.05, **: P < 0.01).
(10–100 mg/kg, p.o.) also inhibited dose-dependently these lesions in rats. Cetraxate (100–1000 mg/kg, p.o.) produced dose-dependent inhibition against taurocholate-histamine-induced lesions, whereas cetraxate did not affect taurocholate-serotonin-induced gastric lesions at oral doses of 30 and 100 mg/kg and inhibited them by 68.1% at 300 mg/kg.

**Effect on acidified aspirin-induced gastric lesion**

Intragastric administration of acidified aspirin produced hemorrhagic damage along the long axis of the stomach, and the lesion index was 32.6 ± 5.4 mm. Oral administration of famotidine (0.3 and 1 mg/kg), cimetidine (3 to 30 mg/kg), pirenzepine (10 to 100 mg/kg) and cetraxate (30 to 300 mg/kg) produced a significant inhibition against acidified aspirin-induced lesion in a dose-dependent manner (Fig. 3). The ED₅₀ values for famotidine, cimetidine, pirenzepine and cetraxate were 0.14 (0.12–0.16), 4.1 (3.5–4.7), 30.8 (21.1–45.0) and 45.2 (21.4–95.8) mg/kg, p.o., respectively.

**Effect on acidified ethanol-induced gastric lesion**

When acidified ethanol was given to the control animals, multiple severe lesions were induced in the glandular portion, and the lesion index was 54.0 ± 5.6 mm. Famotidine at 0.3 to 1 mg/kg, p.o., inhibited gastric lesions, and the degrees of inhibition were 52.2 and 44.4%, respectively. Cimetidine at a dose of 100 mg/kg, p.o., inhibited the lesion formation by 53.7%. In contrast, pirenzepine and cetraxate at 10 to 100 mg/kg, p.o., dose-dependently inhibited the lesion formation induced by acidified ethanol with ED₅₀ values of 21.2 (17.6–25.5) and 50.6 (30.0–85.3) mg/kg, p.o. (Fig. 4).

**Effect on iodoacetamide-induced gastric lesion**

In the control animals, mild erosion was induced in the upper glandular portion (mainly in the corpus) by 0.1% iodoacetamide in drinking water for 7 days with a lesion index of 11.9 ± 1.1 mm. Oral administration of famotidine to rats once daily for 7 days significantly showed a preventive effect on iodoacetamide-induced gastric lesion. The degrees of inhibition were 52.9 and 56.3% at the dose of 0.3 and 1 mg/kg, p.o., respectively. Treatment with orally administered pirenzepine and cetraxate also significantly prevented gastric lesions in response to iodoacetamide, the degrees of inhibition being 55.2% by pirenzepine at 100 mg/kg and 58.0% by cetraxate at 300 mg/kg. Cimetidine at a dose of 100 mg/kg once daily for 7 days did not significantly protect the gastric mucosa against...
iodoacetamide-induced lesion, the degree of inhibition being 33.6% (Fig. 5).

Effect on basal and histamine-stimulated acid secretion in pylorus-ligated rats

Famotidine given orally at doses of 0.1, 0.3 and 1 mg/kg inhibited basal gastric secretion in a dose-dependent manner with ED$_{50}$ values (95% confidence limits) of 0.45 (0.21–0.95) mg/kg in pylorus-ligated rats. Cimetidine (10–100 mg/kg, p.o.), pirenzepine (10–100 mg/kg, p.o.) and cetraxate (30–300 mg/kg, p.o.) also inhibited dose-dependently acid secretion with ED$_{50}$ values of 23.3 (10.0–54.2), 21.9 (19.0–25.3) and 143.9 (132.3–156.6) mg/kg, p.o., respectively.

Total acid output was increased by approximately 20% compared with basal acid output by i.p. injection of histamine at a dose of 100 mg/kg in pylorus-ligated rats. Famotidine and cimetidine inhibited gastric acid secretion induced by histamine in a dose-dependent manner with ED$_{50}$ values of 0.06 (0.04–0.09) and 6.8 (5.5–8.3) mg/kg, p.o., respectively. In contrast, pretreatment with pirenzepine and cetraxate showed a weak antisecretory effect on histamine-stimulated acid secretion in pylorus-ligated rats and the degrees of inhibition were 22.6 ± 4.8% at 30 mg/kg, p.o., of pirenzepine and 43.0 ± 9.7% at 100 mg/kg, p.o., of cetraxate (Table 1).

Effect on gastric mucosal blood flow

The basal blood flow of the gastric mucosa was 112.2 ± 4.4 ml/100 g tissue/min in anesthetized rats. Oral administrations of famotidine (0.1 to 1 mg/kg, p.o.) and cimetidine (30 and 100 mg/kg, p.o.) had no significant effect on the basal blood flow (data not shown). As shown in Fig. 6, intragastric application of acidified ethanol produced a sustained decrease in gastric mucosal blood flow. Famotidine, given orally at doses of 0.1 and 0.3 mg/kg, p.o., about 1 hr before acidified ethanol, pre-

![Fig. 5. Effects of famotidine, cimetidine, pirenzepine and cetraxate on 0.1% iodoacetamide-induced gastric lesion in rats. Iodoacetamide in drinking water (0.1%) was given to rats for 7 days and on the 8th day, rats were sacrificed. Test compounds were given once daily to rats during the 7 days. Each point represents the mean ± S.E.M. for 10 rats. The statistical significance was determined by ANOVA (*: P < 0.05, **: P < 0.01).](image)

**Table 1.** Effects of famotidine, cimetidine, pirenzepine and cetraxate on basal and histamine-stimulated acid secretion in pylorus-ligated rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>Basal secretion</th>
<th>Histamine-stimulated secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED$_{50}$ value (mg/kg, p.o.)</td>
<td></td>
</tr>
<tr>
<td>Famotidine</td>
<td>0.45$^{a}$</td>
<td>0.06$^{a}$ $^{(0.04–0.09)b}$</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>23.3 $^{(10.0–54.2)}$</td>
<td>6.8 $^{(5.5–8.3)}$</td>
</tr>
<tr>
<td>Pirenzepine</td>
<td>21.9 $^{(19.0–25.3)}$</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Cetraxate</td>
<td>143.9 $^{(132.3–156.6)}$</td>
<td>&gt; 1000</td>
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</table>

$^{a}$The doses producing 50% inhibition of acid secretion (ED$_{50}$ values) were determined by log-probit analysis from data obtained for 3 doses of each compound. $^{b}$Figures in parentheses indicate the 95% confidence limits from 5 to 10 animals.
Fig. 6. Effects of famotidine and cimetidine on acidified (150 mM HCl) ethanol (60%)-induced decrease in gastric mucosal blood flow in anesthetized rats. Test drugs were orally administered 1 hr before acidified ethanol treatment. The gastric mucosal blood flow (GMBF) immediately before acidified ethanol was designated as 100%. Each point represents the mean ± S.E.M. for 4–5 rats. The statistical significance was determined by Student’s t-test (*: P < 0.05, **: P < 0.01). (-O-) HCI-ethanol control; (-0-) famotidine, 0.1 mg/kg, p.o.; (-D-) famotidine, 0.3 mg/kg, p.o.; (-•-) famotidine, 1 mg/kg, p.o.; (-A-) cimetidine, 30 mg/kg, p.o.; (-□-) cimetidine, 100 mg/kg, p.o.

vented the decrease in blood flow induced by acidified ethanol; and rather, at the higher dose of 1 mg/kg, the blood flow increased after acidified ethanol. Oral cimetidine also prevented the acidified ethanol-induced decrease in blood flow at 30 and 100 mg/kg, p.o., and increased blood flow at 100 mg/kg, p.o.

Effect on the transgastric PD

The transgastric PD (mucosa negative) was significantly increased by oral administration of famotidine at a dose of 1 mg/kg. The PD was significantly decreased in response to intragastric application of 3 ml acidified ethanol for 10 min, and it decreased after removal of the solution from the stomach and remained lowered for about 1 hr. Famotidine at a dose of 1 mg/kg promoted the recovery of decreased transgastric PD. On the other hand, cimetidine (100 mg/kg, p.o.) did not affect the basal PD or the responses caused by acidified ethanol (Fig. 7). Although the basal pH was significantly higher in the drug-treated groups, the pH changes were not influenced by famotidine (0.3 and 1 mg/kg, p.o.) and cimetidine (100 mg/kg, p.o.) after acidified ethanol exposure (data not shown).

Fig. 7. Effects of famotidine and cimetidine on luminal potential difference before and after exposure to acidified (150 mM HCl) ethanol (60%) for 10 min in anesthetized rats. Test drugs were orally administered 1 hr before acidified ethanol treatment. Each point represents the mean ± S.E.M. for 5 rats. The statistical significance was determined by Student’s t-test (*: P < 0.05, **: P < 0.01). (-O-) HCI-ethanol control; (-0-) famotidine, 0.3 mg/kg, p.o.; (-□-) famotidine, 1 mg/kg, p.o.; (-•-) cimetidine, 100 mg/kg, p.o.
Effect on the contents of gastric mucosal glycoprotein

The contents of gastric mucosal hexosamine and hexose were 372.7 ± 19.3 and 168.7 ± 6.8 μg/100 mg tissue in normal rats, respectively, and were reduced to 76.5 and 79.5% by water immersion restraint stress. Famotidine at 1 mg/kg, p.o., inhibited the decreases of hexosamine and hexose induced by stress by 73.7 and 76.9%, respectively. Cimetidine at 30 mg/kg, p.o., also inhibited the decreases of these glycoproteins by 53.9 and 64.7%, respectively.

Involvement of endogenous prostaglandins

The formation of acidified ethanol-induced gastric mucosal lesions was aggravated from 55.6 ± 4.5 to 90.2 ± 3.2 mm by the pretreatment with indomethacin (5 mg/kg, s.c.) in rats. Oral famotidine at a dose of 1 mg/kg and cimetidine at a dose of 100 mg/kg showed almost the same inhibitory proportion on indomethacin-acidified ethanol-induced lesions as that on acidified ethanol induced lesions. On the contrary, the inhibitory effect of 20% ethanol was significantly reduced by the pretreatment with indomethacin (Fig. 8).

DISCUSSION

Peptic ulcer is well accepted to be caused by an imbalance between the mucosal defensive factors and the aggressive factors of acid and pepsin (21). The mechanisms by which an H2-receptor antagonist, cimetidine, suppresses experimental lesions are thought to be secondary to its potent gastric acid antisecretory activity (1). Famotidine has also been reported to inhibit the gastric mucosal lesions induced by water immersion-restraint stress, indomethacin and pylorus-ligation that were all dependent on gastric acid (2–4). A muscarinic M1-receptor antagonist, pirenzepine, has been shown to inhibit gastric secretion, improve gastric mucosal microcirculation, and protect gastric mucus contents, resulting in the prevention of gastric lesion formation (22–24). It is reported that the cytoprotection by pirenzepine might not be due to endogenous prostaglandins but be due to a direct action on the gastric mucosa (25). It is also reported that the prevention by cetrazate of experimental gastric lesions may be attributable to its ability to reinforce the gastric mucosal defensive factors such as microcirculation (26) and mucus glycoproteins (27) through a release of endogenous prostaglandins (28), although cetrazate has been shown to exert an inhibitory effect on the gastric secretion in pylorus-ligated rats (29).

In the present study, acute gastric lesions were introduced by taurocholate-histamine, taurocholate-serotonin, iodoacetamide, acidified aspirin and acidified ethanol in rats. Taurocholate is one component of bile acids that may cause gastric lesion by the retrogressive influx from the duodenum to the stomach (30), and it has been reported to induce gastric erosion by the consecutive administration to rats (31). Histamine develops gastric lesion as a result of a marked vascular disturbance in the gastric wall as well as an increase in acid secretion (32). Although single administration
of taurocholate or histamine to rats showed only a little influence on the gastric mucosa, marked lesions were observed after the administration of taurocholate in combination with histamine. Famotidine and cimetidine inhibited taurocholate-histamine-induced gastric lesions in rats at doses that suppressed histamine-stimulated acid secretion in pylorus-ligated rats. Pirenzepine and cetraxate also inhibited these lesions at doses that suppressed basal acid secretion, although these compounds did not affect histamine-stimulated secretion. It is, therefore, suggested that the antisecretory effects of famotidine, cimetidine, pirenzepine and cetraxate are mainly responsible for their antiulcer activity in the taurocholate-histamine induced lesion model.

Serotonin causes gastric lesions by platelet aggregation, followed by fibrous thrombi formation and vasoconstriction, followed by a disturbance in the peripheral circulation (33), without affecting gastric acid secretion (33, 34). Iodoacetamide is a sulfhydryl (SH) blocking agent and is thought to cause gastric lesion by a counteraction on the endogenous prostaglandins, resulting in the depression of the mucosal defensive factors (35). Therefore, the formation of taurocholate-serotonin- and iodoacetamide-induced lesions seemed to be dependent on defensive factors rather than gastric acid. Famotidine and other drugs inhibited these gastric lesions in doses suppressing basal or histamine-stimulated acid secretion. It is known that acid is likely to penetrate the injured gastric mucosa and to make the lesion worse after the mucosal epithelium is damaged by the ulcerogenic agents (36). The control of acid secretion, therefore, should help to reduce mucosal lesions induced by taurocholate-serotonin and iodoacetamide. It is reported that the antisecretory effect of cimetidine is shorter than that of famotidine in dogs (3) and humans (37, 38). This could account for the observation that cimetidine did not show any significant influence on iodoacetamide-induced gastric lesions.

The mechanism by which aspirin induces gastric damage is thought to be the inhibition of prostaglandin biosynthesis (39) and the disruption of the gastric mucosal barrier followed by back-diffusion of hydrogen ions, stasis in the proximal gastric mucosal microcirculation, and increased vascular permeability (40). Ethanol has been shown to produce gastric lesions with concomitant impairments in such factors as mucus (41) and mucosal circulation (42). It was reported that famotidine and cimetidine did not inhibit the gastric lesions induced by such severe conditions as 600 mM HCl and absolute ethanol (43, 44). In the present study, aspirin and ethanol were administered to rats together with sufficient amounts of exogenous acid (150 mM HCl) in order to exclude the possibility that the antisecretory activity may be responsible for the protective effect of test drugs. Famotidine and cimetidine inhibited acidified aspirin- and acidified ethanol-induced gastric lesions like pirenzepine (25) and cetraxate (28), which have been shown to possess cytoprotective activity. The degree of inhibitory effects of famotidine and cimetidine on acidified ethanol-induced gastric lesion were not so marked (approximately 50%) but significant. In these gastric lesions induced by acidified aspirin and acidified ethanol, we used Sprague-Dawley rats instead of Wistar rats because the lesion formation in Sprague-Dawley rats was more marked than that in Wistar rats (data not shown). This may be due to the difference of acid secretion between these rats, that is, Sprague-Dawley rats used in this study secreted gastric acid much more than Wistar rats, indicating that endogenous gastric acid plays a role in the pathogenesis of even gastric lesions induced by aspirin and ethanol in combination with exogenous acid. It is, therefore, possible that the prevention by famotidine and cimetidine of acidified aspirin- and acidified ethanol-induced gastric lesions may be partly attributable to their ability to suppress acid secretion. From these results, in any case, mechanisms other than suppression of acid secretion might be involved, at least in part, in the mucosal protection produced by famotidine and cimetidine.

Therefore, in the next series of experiments,
the effect of famotidine on the mucosal defensive factors was examined in rats. Famotidine and cimetidine have been shown to inhibit the decrease in gastric mucosal blood flow induced by hemorrhagic shock in rats (45, 46) and to promote the transfer of bicarbonate into the gastric lumen in dogs with gastric pouch (47, 48). In the present study, famotidine and cimetidine inhibited the decreases in gastric mucosal blood flow induced by acidified ethanol and in mucosal contents of glycoproteins (hexose and hexosamine) by water immersion restraint stress. Furthermore, famotidine, not cimetidine, increased the transgastric PD and promoted the recovery of decreased PD induced by acidified ethanol in rats. Therefore, it is suggested that there are some differences between the mucosal defensive mechanisms strengthened by famotidine and cimetidine. Our data also show that the inhibitory effects of famotidine and cimetidine on the acidified ethanol-induced gastric lesion were not influenced by prior administration of indomethacin, indicating that endogenous prostaglandins are not involved in the mechanisms by which these H2-antagonists protected the gastric mucosa against the injurious action of acidified ethanol.

Famotidine, cimetidine and pirenzepine inhibited almost all types of the gastric lesions used in the present study in rats at doses that suppressed basal or histamine-stimulated acid secretion in pylorus-ligated rats. On the contrary, cetraxate inhibited acidified aspirin- and acidified ethanol-induced gastric lesions at non-antisecretory doses, indicating that cetraxate has a gastric cytoprotective activity similar to that of prostaglandins. Thus, the inhibitory effect of famotidine, like cimetidine and pirenzepine, on gastric lesions seemed to be attributable to both its antisecretory and cytoprotective activities, and the inhibitory effect on acid secretion may be predominantly involved in the antulcer effect of famotidine.

In summary, famotidine has been shown to inhibit the gastric lesion formations induced by taurocholate-histamine, taurocholate-serotonin, acidified aspirin, acidified ethanol and iodoacetamide; and it is superior to cimetidine, pirenzepine and cetraxate in rats. The preventive effect of famotidine is thought to be attributable not only to the suppression of acid secretion, but to the activation of the mucosal defensive factors such as mucosal blood flow, mucus glycoproteins and bicarbonate secretion. Moreover, the results of the present study indicate that the mucosal protection by famotidine is not mediated through endogenous prostaglandins, but may be due to a direct action on the gastric mucosa like the actions of cimetidine and pirenzepine.

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