Pathogenesis of the Earliest Epithelial Cell Damage Induced by Mepirizole and Cysteamine in the Rat Duodenum

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Abstract—Mepirizole (200 mg/kg) and cysteamine (100 mg/kg) induced epithelial cell damage in the proximal duodenum of rats within 30 min after s.c. administration. The injury induced was severe 60 min later. Gastric acid secretion determined in intact animals was stimulated by these agents 30 and 60 min later when the intraluminal pH of the duodenum was significantly decreased. Duodenal blood flow was significantly decreased beginning 5 min after administration up to 60 min. Oral treatment with sodium bicarbonate (300 mg/kg), cimetidine (100 mg/kg), omeprazole or NC-1300 (gastric proton pump inhibitors, 30 mg/kg) and 16,16-dimethyl prostaglandin E_2 (10 μg/kg) protected the epithelium from damage induced by the two duodenal ulcerogens. Epithelial cell damage in the duodenum in response to mepirizole and cysteamine appears to be related to the increased gastric acid secretion followed by lowered intraduodenal pH of the duodenum having decreased blood flow.

We reported that mepirizole, a basic nonsteroidal antiinflammatory agent, induces visible ulcers in the mucosa of the proximal duodenum of rats with a high incidence, usually 12 to 24 hr after administration (1, 2). Microscopically, however, we found epithelial cell damage on the villous tip of the duodenum where the ulcer develops as early as 2 hr after administration of the agent (3). Cysteamine, a well-known duodenal ulcerogen (4), also induced microscopical damage to the epithelial cells of the duodenum 2 hr after administration (5). The location of damage caused by cysteamine was nearly identical to that seen with mepirizole. This study was designed to determine changes in the duodenal epithelium at much earlier times, i.e., 15, 30 and 60 min after subcutaneous (s.c.) administration of mepirizole and cysteamine using scanning electron and light microscopy. To elucidate the pathogenesis of the earliest damage, the effect of these agents on gastric acid secretion, intraluminal pH of the duodenum and stomach, duodenal blood flow, and effects of an antacid, antisecretory agents, and a prostaglandin on the lesion formation were studied.

Materials and Methods

Male Sprague-Dawley rats (220–260 g, Nihon Charles-River) were fasted but allowed free access to tap water for 24 hr before the experiments. Water was withheld for only 2 hr before the experiments. Rats were kept in raised mesh bottom cages to prevent coprophagy. Each study was carried out using 8 animals per group.

Duodenal damage formation: Occurrence of the earliest damage in the epithelial cell on the villous tip of the proximal duodenum was mainly determined by scanning electron microscopy (SEM) and partly by light microscopy. In the SEM study, all experiments were done using our method (3). Briefly, mepirizole (Daiichi, 200 mg/kg) and cysteamine (Sigma, 100 mg/kg), suspended in 1% carboxymethylcellulose (CMC) or the vehicle

alone, was given s.c. in a volume of 1.0 ml/200 g body wt. Under ether anesthesia, the animals were killed 0, 15, 30 and 60 min post-treatment. The stomach and duodenum were immediately removed, opened along the mesenteric side and fixed in phosphate-buffered 5% paraformaldehyde–4% glutaraldehyde for 3 hr at 4 °C. These samples were then postfixed in 1% OsO_4 for 1 hr. After dehydration in graded ethanols, the tissues were subjected to critical point drying with CO_2 (Hitachi, HLP-2), mounted, and then vacuum coated with a palladium-platinum ion sputter (Eicho, IB-3). The samples were then examined for damage using a scanning electron microscope (Hitachi, S-510). The severity of damage (damage index) was divided into four degrees as follows:

0: intact villi
1: focal damage of a few epithelial cells at the villous tips
2: an exfoliation of several epithelial cells at the villous tips without any exposure of the lamina propria.
3: an extensive exfoliation of a number of epithelial cells on multiple villi and exposure of the lamina propria.

The degree of damage was determined by scanning the proximal 1 cm of duodenum, and the most severe damage was recorded for indexing.

In separate experiments, light microscopic study was performed. Animals, treated with mepirizole (200 mg/kg) or cysteamine (100 mg/kg), s.c., were killed 30 min later. The stomach and duodenum were removed and fixed in 10% formalin solution. Tissue sections of the proximal duodenum were stained with hematoxylin and eosin.

Gastric acid secretion: Animals were killed 30 or 60 min after s.c. administration of mepirizole (200 mg/kg), cysteamine (100 mg/kg) or the vehicle alone. The stomach was exposed, and the esophagus and pylorus were immediately clamped to avoid any leakage of gastric content. The gastric content was collected into the test tube as soon as possible. In the vehicle treated group, the volume was too small to analyze the gastric acidity; thus 1.5 ml of saline, adjusted to pH 7.0 with 0.01 N NaOH, was injected into the stomach. The gastric content was then collected 1 min later and mixed with the previously collected sample. Titratable acidity was determined by automatic titration of the gastric content against 0.1 N NaOH to pH 7.0 (Radiometer). Total acid output was expressed as μEq/stomach.

Intraluminal pH in the duodenum and stomach: Intraluminal pH in the duodenum and stomach was determined after s.c. administration of mepirizole (200 mg/kg), cysteamine (100 mg/kg) or the vehicle alone. Immediately, 30 or 60 min post-treatment, the animals were anesthetized with ether and the abdomen was incised. The duodenum and stomach were exposed and the pylorus was ligated. The duodenum was opened along the antimesenteric side. A tip of a pH test paper (Toyo Roshi) was placed lightly on the luminal surface of the proximal duodenum and the pH of the duodenal juice determined. The stomach was then quickly removed and opened along the greater curvature. After removal of the gastric contents, the pH of the surface of the corpus and antrum was determined similarly as in the case of duodenal pH. Determination of pH in each portion was done in duplicate.

Duodenal blood flow: Duodenal blood flow was continuously determined by the laser Doppler velocimetry method (6), and intermittently by the hydrogen gas clearance method (7). Animals were anesthetized with urethane (1.25 g/kg, i.p.; Nakarai), and a midline laparotomy was performed. The duodenum was then exposed. The probe of the laser Doppler velocimeter was lightly pressed to the serosal side of the duodenum, an area where the damage was induced by the two ulcerogens. Although the blood flow was determined from the serosal side, the flow was empirically known to reflect the mucosal blood flow because of the thinness of the duodenal wall. The laser Doppler velocimeter consisted of a He-Ne laser (2 mW, λ=632.8 nm; Periflux, Sweden). In the case of the hydrogen gas clearance method, a platinum contact electrode was positioned to the proximal duodenal mucosa through a small incision made in the distal part of the duodenum. A reference electrode was placed inside the abdominal cavity. Hydrogen gas (100%) was inhaled every 15 min until the duodenal

mucosa was completely saturated with hydrogen. In separate experiments, the systemic blood pressure in response to cysteamine and mepirizole was determined at the cervical artery using a pressure transducer (Narco Scientific LDI-5) and a polygraph (San-Ei 6W-7I).

Effects of drugs on duodenal damage: Duodenal epithelial cell damage was observed 30 min after the administration of mepirizole and cysteamine; therefore, this period was selected for the following studies. First, the effects of an antacid and antisecretory agents on the damage formation were studied. The antacid sodium bicarbonate ($NaHCO_3$, Tokyo Kasei, 300 mg/kg); a histamine H_2-receptor antagonist, cimetidine (Sigma, 100 mg/kg), gastric proton pump inhibitors, omeprazole (Hässle, 30 mg/kg) and NC-1300 (Nippon Chemiphar, 30 mg/kg), were suspended in 1% CMC and given per os (p.o.) 5, 60, 60 and 60 min before s.c. administration of cysteamine and mepirizole, respectively. The doses of the above drugs are known to neutralize gastric acid and inhibit gastric acid secretion for several hours (8–10). Control animals were given the vehicle alone. Second, the effect of 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$) on the damage formation was studied. $dmPGE_2$ (3 and 10 μg/kg, Ono), first dissolved in a trace of 100% alcohol and then diluted with saline, was given p.o. 30 min before the administration of mepirizole and cysteamine. The dose of $dmPGE_2$ is known to have a cytoprotective effect (i.e., non-antisecretory dose) on the rat gastric mucosa (11). Control animals were given the vehicle alone.

Statistics: Data are expressed as the mean± S.E. The mean values of SEM index were compared with the control value by the χ^2 test, and $P<0.05$ was regarded as significant. The

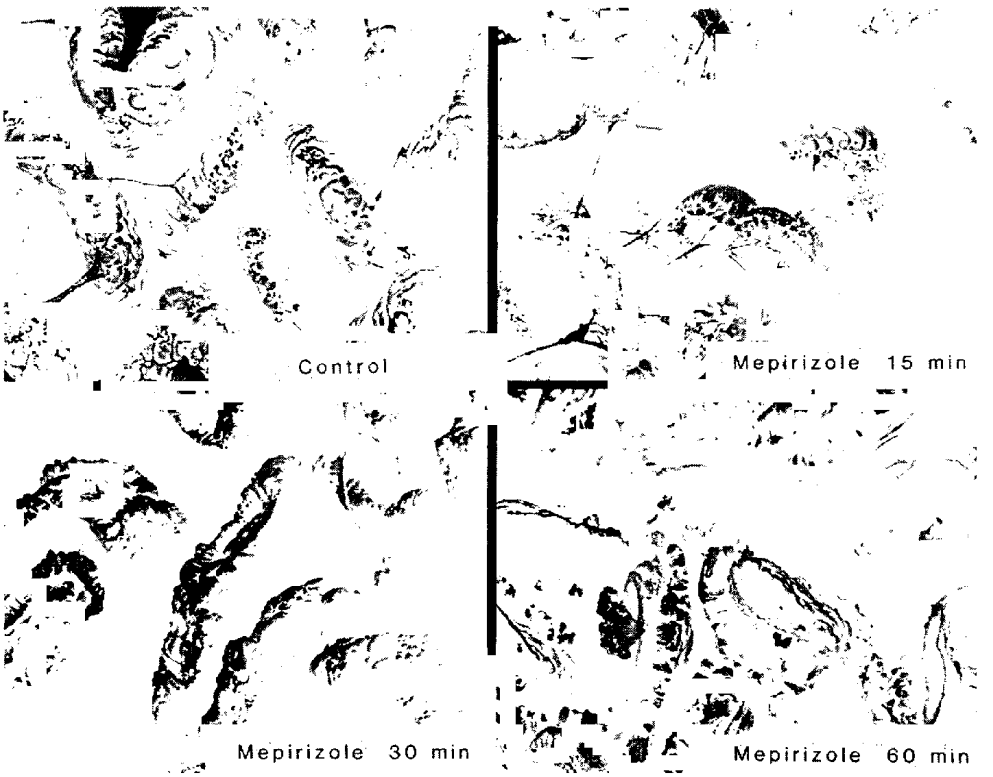

Fig. 1. Scanning electron micrographs showing normal duodenal villi and epithelial cell damage induced at 15, 30 and 60 min after s.c. administration of 200 mg/kg of mepirizole. Note that the earliest cellular damage was clearly observed in many villi of the proximal duodenum 30 min after mepirizole administration and became severe with time.

mean values of gastric secretion, intraluminal pH and duodenal blood flow were compared with the control values by Student's *t*-test, and $P<0.05$ was regarded as significant.

Results

Duodenal epithelial cell damage: Both mepirizole (200 mg/kg) and cysteamine (100 mg/kg) induced little or no change to the epithelial cells of the rat duodenal villi 15 min after administration. At 30 min, however, epithelial cell damage, exfoliation of cells and exposure of the lamina propria were detected (Figs. 1–3). The lesions were located in the antimesenteric part of the proximal duodenum. The degree of damage progressed with time, i.e., marked exfoliation of the epithelial cells from the villous tip was observed at 1 hr (Fig. 4).

Gastric acid secretion: In the control group, only a small amount of gastric contents was detected. However, both mepirizole (200 mg/kg) and cysteamine (100 mg/kg) significantly increased the volume and total acid output as determined 30 and 60 min later (Table 1). Titratable acidity was also significantly increased by these agents, except for the acidity observed 30 min after cysteamine administration. Increase in the volume and acid output in response to cysteamine were significantly higher than those observed with mepirizole.

Intraluminal pH in the stomach and duodenum: The mean intraluminal pH in the duodenum, antrum and corpus was 6.72, 1.73 and 1.56, respectively. Thirty or 60 min after the administration of mepirizole (200 mg/kg) or cysteamine (100 mg/kg), the luminal pH was significantly decreased in the duodenum as well as in the antrum and corpus (Table 2).

Duodenal blood flow: Both mepirizole (200 mg/kg) and cysteamine (100 mg/kg) de-

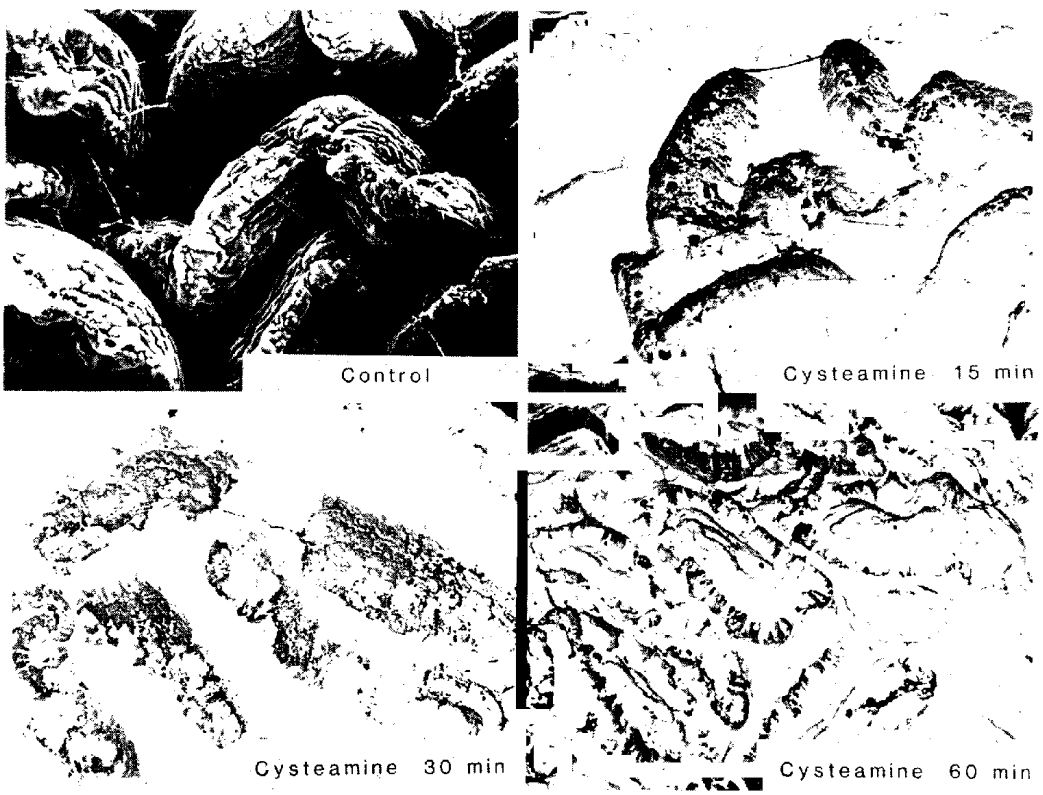

Fig. 2. Scanning electron micrographs showing normal duodenal villi and epithelial cell damage induced at 15, 30 and 60 min after s.c. administration of 100 mg/kg of cysteamine. Similar to mepirizole, the earliest damage was observed 30 min after administration of cysteamine.

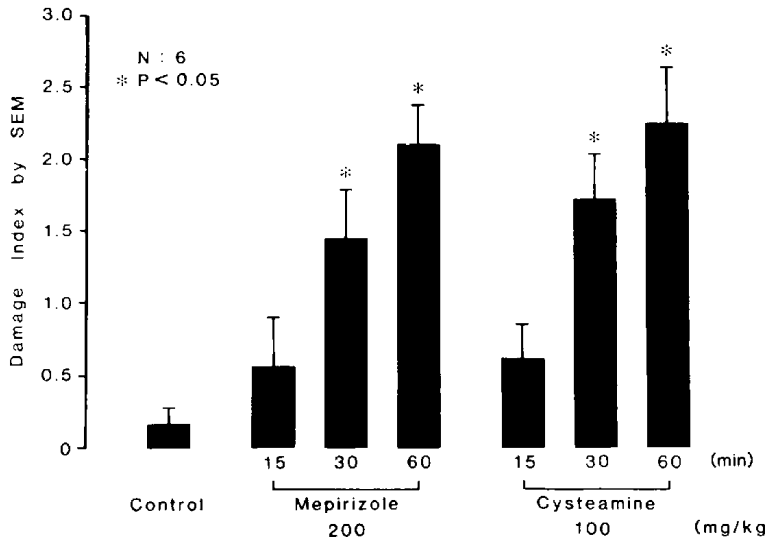

Fig. 3. Time-course of epithelial cell damage of the rat duodenum after s.c. administration of mepirizole and cysteamine. The degree of damage was determined by scanning electron microscopy (SEM). Data represent the mean±one S.E.M. *Significantly different from the control values at $P<0.05$.

Table 1. Effects of mepirizole and cysteamine on gastric acid secretion of intact rats

Treatments	Time after administration (min)	No. of rats	Volume (ml/rat)	Titratable acidity (mEq/l)	Total Acid output (μEq/stomach)
Control	30	8	0.1±0.02	48.5±10.6	5.1±1.8
Mepirizole (200 mg/kg)	30	8	0.7±0.1*	75.7±6.6*	52.2±8.4*
Cysteamine (100 mg/kg)	30	8	1.3±0.2*†	71.7±10.1	101.7±15.7*†
Control	60	8	0.1±0.01	56.0±7.3	5.9±0.6
Mepirizole (200 mg/kg)	60	8	1.4±0.1*	86.7±6.2*	122.5±12.5*
Cysteamine (100 mg/kg)	60	8	2.8±0.2*†	89.2±5.3*	257.5±30.2*†

Data represent the mean±S.E. *,†Statistically significant difference from the controls or mepirizole-treated groups, respectively, at $P<0.05$.

creased the duodenal blood flow and duodenal mucosal blood flow in the anesthetized rats (Fig. 5A, B). The reduction was observed from 5 or 15 min post-treatment and lasted for 60 min. The degree of reduction was about 10% as determined by the laser Doppler velocimetry method and about 25–30% when determined by the hydrogen gas clearance method. Systemic blood pressure was also reduced by about 10–20% with the two agents.

Effects of drugs on damage formation: Pretreatment with sodium bicarbonate (300 mg/kg), cimetidine (100 mg/kg), omeprazole (30 mg/kg), and NC-1300 (30 mg/kg) potently inhibited the damage formation induced by both mepirizole and cysteamine (Fig. 6). The rate of inhibition by the above agents was 97.9%, 98.6%, 96.6% and 81.4% for mepirizole-induced damage and 95.9%, 90.1%, 87.2% and 70.9% for cysteamine-induced damage. Prior administration of

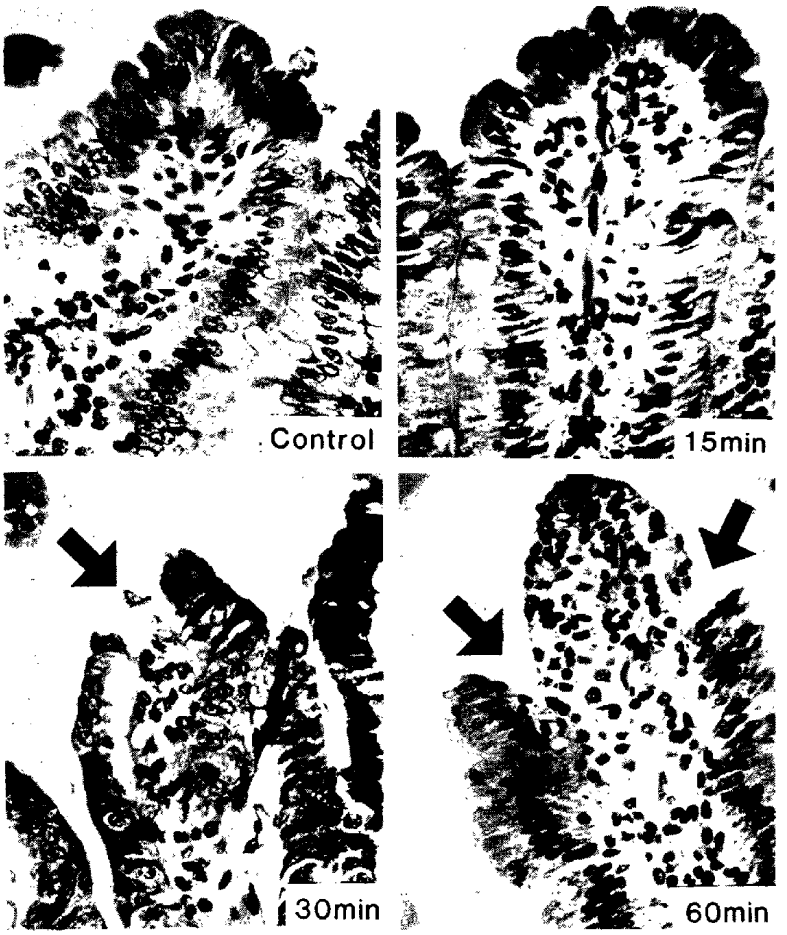

Fig. 4. Light micrographs of rat duodenal villi after s.c. administration of 200 mg/kg of mepirizole. Epithelial cell damage in the villous tip and cell exfoliation (arrows) were seen 30 and 60 min after the administration of mepirizole, respectively.

Table 2. Effects of mepirizole and cysteamine on intraluminal pH of the duodenum, antrum and corpus of intact rats

Treatments	Time after administration (min)	No. of rats	pH Values Duodenum	Antrum	Corpus
Control	30	8	6.7±0.1	1.7±0.1	1.6±0.1
Mepirizole (200 mg/kg)	30	8	4.3±0.8*	0.9±0.1*	0.9±0.1*
Cysteamine (100 mg/kg)	30	8	4.8±0.5*	0.8±0.03*	0.8±0.04*
Control	60	8	6.8±0.1	1.7±0.1	1.6±0.1
Mepirizole (200 mg/kg)	60	8	4.7±0.4*	1.0±0.1*	0.9±0.1*
Cysteamine (100 mg/kg)	60	8	5.1±0.4*	0.9±0.1*	0.9±0.1*

Data represent the mean±S.E. *Statistically significant difference from the controls at $P<0.05$.

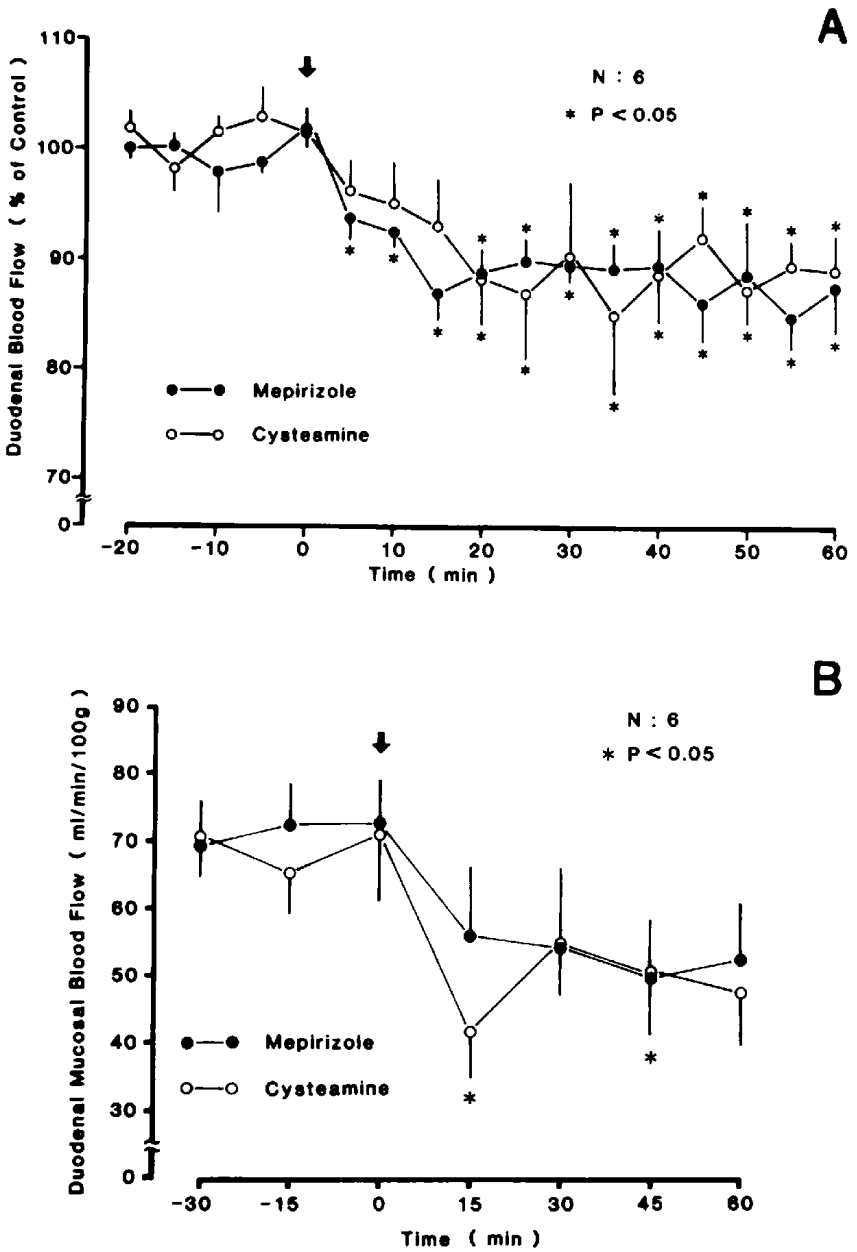

Fig. 5. Changes in duodenal blood flow measured by the laser Doppler velocimetry method (A) and duodenal mucosal blood flow measured by hydrogen gas clearance (B). Data represent the mean±one S.E.M. *Significantly different from the pretreatment values at $P<0.05$.

$dmPGE_2$ also dose-dependently inhibited the damage induced by the two duodenal ulcerogens (Fig. 7). The inhibition was statistically significant at 10 μg/kg and was 78.5% for the mepirizole-induced damage and 79.8% for the cysteamine-induced injury.

Discussion

We obtained evidence that mepirizole induced epithelial cell damage of the rat

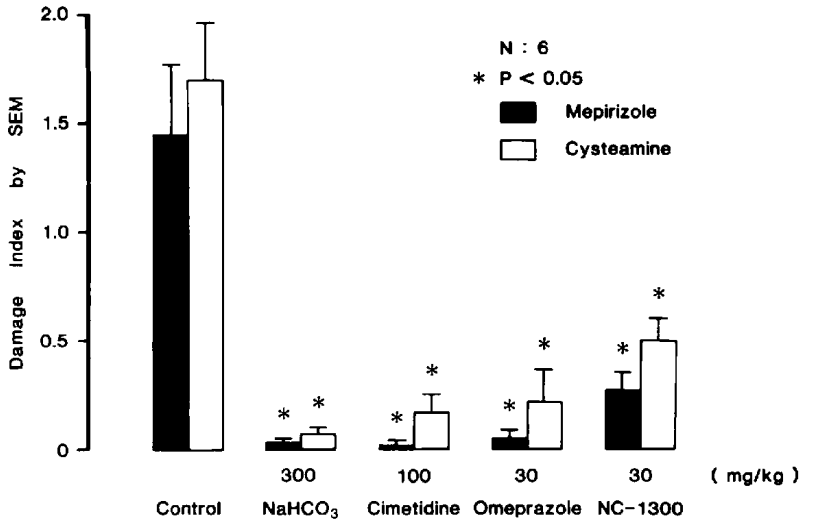

Fig. 6. Effects of sodium bicarbonate ($NaHCO_3$), cimetidine, omeprazole and NC-1300 on mepirizole and cysteamine-induced epithelial cell injury in the rat duodenum. Damage was induced 30 min after s.c. administration of duodenal ulcerogens. Each drug was given p.o. 5, 60, 60 or 60 min before administration of mepirizole and cysteamine. Data represent the mean±one S.E.M. *Significantly different from the control values at $P<0.05$.

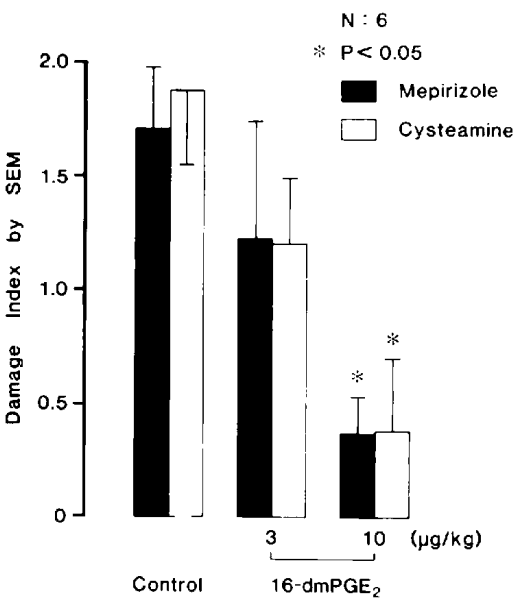

Fig. 7. Effects of $dmPGE_2$ on mepirizole- and cysteamine-induced epithelial cell damage in the rat duodenum. The lesion was measured 30 min after s.c. administration of 200 mg/kg of mepirizole and 100 mg/kg of cysteamine. $dmPGE_2$ was given p.o. 30 min before each ulcerogen. Data represent the mean ±one S.E.M. *Significantly different from the control values at $P<0.05$.

duodenal mucosa within 30 min after s.c. administration. We also found that cysteamine caused similar cell damage within 30 min. Pfeiffer et al. (12, 13) recently reported that cysteamine did not induce lesion in the proximal duodenum at 30 min after administration, in tissues examined using scanning electron and light microscopy. The discrepancy between these findings may be due to differences in the experimental conditions, i.e., we injected cysteamine s.c. in a dose of 100 mg/kg to male rats, whereas in the other study, it was given p.o. in a dose of 700 mg/kg to female rats. It is likely that the absorption of cysteamine is much more rapid through the s.c. route in male rats than the p.o. route in female rats so that the damage of epithelial cells may occur much earlier. Indeed, previous dose-response studies demonstrated that cysteamine injected s.c. produced duodenal ulcers and mortality faster than after p.o. administration (14). Pfeiffer et al. (13) also found that cysteamine induced in 30 min intracellular changes at the villous tips, e.g., apical endoplasmic reticulum swelling and loss of cytoplasmic ground substance. They suggested that these cytotoxic changes may precede the mucosal attack by intraluminal

damaging factors following cysteamine. Whether or not such intracellular cytotoxicity also precedes the mepirizole-induced ulcers needs further investigation.

The damage induced by mepirizole and cysteamine was similar with regard to the time of development, severity and location in the duodenum, hence the damage may share a common mechanism of pathogenesis, at least in the beginning of duodenal ulceration. We (1) demonstrated that mepirizole ulcers induced 24 hr after administration were prevented to a great extent by pretreatment with antacid and antisecretory agents, thereby suggesting the participation of gastric acid sometime during the ulceration. Other investigators (15–18) suggested that gastric acid is a prerequisite for cysteamine ulcerogenesis. The following evidence appears to support the proposal that the gastric acid factor is involved in both the mepirizole and cysteamine-induced ulcerogenesis. First, gastric acid secretion when determined in intact animals was significantly increased 30 and 60 min following the administration of mepirizole and cysteamine. Concerning the results of cysteamine, our data are consistent with the results of Szabo et al. (17, 18) who found the increased gastric acid secretion in female rats 1 hr after p.o. or s.c. administration of 150 to 280 mg/kg of cysteamine. Cysteamine delays gastric emptying in rats (19, 20). We also found that mepirizole at the dose of 200 mg/kg markedly delayed gastric emptying and suppressed gastric motility in rats (21). Therefore, it is most likely that the increased gastric contents after administration of the two duodenal ulcerogens resulted in the combination of stimulated gastric secretion and delayed gastric emptying. These phenomena suggest that delayed gastric emptying greatly contributes to their ulcerogenecity. Second, the intraluminal pH of the proximal duodenum was significantly decreased 30 and 60 min later. The volume of gastric contents in cysteamine-treated animals was about 1 to 3 ml/animal so that the intraabdominal pressure might have intermittently forced out a certain amount of acidic contents into the duodenum through the contracted pylorus. In the case of mepirizole-treated animals, it appears that the physical pressure of body weight on the distended stomach by marked prostration pushed out the gastric contents into the duodenum. Third, antacid and antisecretory agents prevented the development of early lesion and duodenal ulcers. Therefore, gastric acid is probably a crucial component for both mepirizole and cysteamine duodenal ulcerogenesis. Certainly, the decrease in acid neutralizing capacity of the duodenal mucosa by cysteamine (22–24) and mepirizole (3) might increase the susceptibility of the mucosa to the inflowed acid.

Ikeda et al. (25) reported that cysteamine given s.c. in a dose of 350 mg/kg significantly decreased the duodenal blood flow in anesthetized rats when determined by the hydrogen clearance method. We also confirmed that the duodenal blood flow was decreased during 60 min starting from 5 min after administration of mepirizole and cysteamine. Since the systemic blood pressure was reduced by the two agents, it is possible that the reduced blood flow may be caused by the reduction of the systemic blood pressure. Leung et al. (26) reported that the slight reduction of duodenal blood flow in anesthetized rats increased the susceptibility of the duodenal mucosa to gastric acid. In addition, they (27) demonstrated that while $dmPGE_2$ in a dose of 10 μg/kg did not increase the gastric blood flow in anesthetized rats, it did maintain the flow against ethanol-induced cessation. Thus, the reason why $dmPGE_2$ at the non-antisecretory dose significantly prevented the damage formation induced by ulcerogens may be related to the maintenance of duodenal blood flow against reduction.

We conclude that epithelial cell damage in the proximal duodenum induced within 30 to 60 min by the two duodenal ulcerogens may be caused by an increased gastric acid secretion followed by a reduction in the intraluminal pH in the duodenum having a decreased mucosal blood flow.

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