

Histamine-Induced Villous Damage in the Rat Duodenum

Hironori TANAKA, Koji TAKEUCHI and Susumu OKABE

Department of Applied Pharmacology, Kyoto Pharmaceutical University,
Misasagi, Yamashina, Kyoto 607, Japan

Accepted June 26, 1989

Abstract—A single s.c. administration of histamine dose-dependently (5–20 mg/kg) induced villous damage of the proximal duodenum in 24-hr fasting rats. Time course studies indicate that histamine (20 mg/kg) induced severe exfoliation of the epithelial cells at the villous tips of the duodenal mucosa 0.5 hr after administration. The damage, however, tended to heal with time, and recovery was nearly complete 8 hr later. This villous damage was significantly inhibited by pretreatment with sodium bicarbonate given orally or cimetidine, omeprazole and NC-1300 given subcutaneously. Histamine (20 mg/kg) significantly stimulated gastric acid secretion and lowered the intraduodenal pH for 1 hr. Gastric content was significantly greater than that in the control group for 1 hr after histamine administration, probably due to stimulated gastric secretion and delayed emptying. We conclude that a single administration of histamine induces microscopical duodenal damage by stimulation of gastric acid secretion, but the damage heals with time, probably as a result of the short periods of acid stimulation and delayed emptying.

A single administration of cysteamine or mepirizole induces duodenal ulcers in rats, usually within 12 to 24 hr after administration (1–3). Recently, we found that both cysteamine and mepirizole induce microscopic damage to the villous tips of the proximal duodenum within 2 hr of administration (4, 5). Histamine, a potential gastric secretagogue, is also known to induce penetrating duodenal ulcers 24 hr after s.c. administration of a large dosage (300 mg/rat) or after continuous administration over 48 hr (6, 7). We have reported that histamine, when repeatedly administered to rats at a secretagogue dose 4 times at 1- to 2-hr intervals, induced a high incidence of duodenal damage 7 hr later (8). It was largely villous damage, and the severity was nearly the same as that observed 0.5 hr after administration of cysteamine and mepirizole. In contrast to the damage induced by those agents, however, histamine damage did not advance to the penetrating ulcer stage. The present study was undertaken to determine the pathogenesis of histamine-induced duodenal damage and to discover the reason why this damage does not progress to a serious level.

Materials and Methods

Male Sprague-Dawley rats, weighing 220–250 g (Nihon Charles-River), were subjected to a 24-hr fast but were allowed free access to tap water for up to 2 hr before the experiments. The rats were kept in raised mesh bottom cages to prevent coprophagy. Each study was carried out using 6–8 animals per group.

Induction of duodenal damage: Dose-response and time-course of histamine-induced duodenal damage were determined after s.c. administration of 5, 10, 20 and 40 mg/kg of histamine·2HCl (Nacalai Tesque, dissolved in 10% gelatin) in a volume of 1 ml/200 g body wt. The vehicle alone was administered to the control groups. Duodenal damage was determined according to the method previously described (3). Briefly, animals were killed at 0, 0.5, 2, 4 and 8 hr after histamine administration, and the stomach and duodenum were removed as a single unit. After being opened along the greater curvature and the antimesenteric side, these organs were quickly extended on a glass sheet. The samples were gently washed with ice-cold saline and then put into 5% paraformaldehyde.

hyde-4% glutaraldehyde in phosphate buffer (pH 7.4) for 3 hr at 4°C and then postfixed in 1% OsO₄ for 1 hr. After fixation and dehydration with a graded series of ethanol solutions, the tissues were subjected to critical point drying with CO₂ (HLP-2, Hitachi), mounted and then vacuum-coated with a palladium ion sputter (IB-3; Eichro). The samples were examined for damage under a scanning electron microscope (S-510; Hitachi). The entire duodenum was scanned, and the area showing the most severe damage was recorded for indexing. The damage (damage index) was graded into 4 degrees of severity as follows:

- 0: normal duodenal mucosa
- 1: epithelial cell damage as defined by destruction of cell membrane on the duodenal villous tips
- 2: exfoliation of epithelial cells and exposure of the lamina propria
- 3: low and broad villi due to destruction of the lamina propria

Effects of several drugs on duodenal damage: To study the influence of gastric acid secretion on duodenal damage induced with 20 mg/kg of histamine, effects of the following drugs were determined. Sodium bicarbonate (300 mg/kg) was given p.o. 5 min before histamine administration. Cimetidine (100 mg/kg), omeprazole (30 mg/kg) and NC-1300 (30 mg/kg), a proton pump inhibitor (9), were given s.c. 30 min before histamine administration. All the drugs were suspended in 0.5% carboxymethylcellulose and given in a volume of 1 ml/200 g body wt. Animals were killed 0.5 hr after histamine administration, and their duodenum was examined for damage as described above.

Determination of intraduodenal pH and gastric content: Histamine (20 mg/kg) or the vehicle alone was administered s.c., and the animals were killed after 1, 2, 4, 6 and 8 hr. The abdomen was incised along the linea alba, and the stomach and duodenum were exposed. The lower end of the esophagus and the pylorus were immediately clamped to avoid any leakage of gastric contents. The duodenum was opened along the anti-mesenteric side, and the end of a pH test paper (Toyo Roshi) was placed lightly on the luminal surface of the proximal duodenum for

3 sec. Determination of the intraduodenal pH was performed in duplicate. The stomach was then removed, and the gastric contents were collected in graduated test tubes. Gastric samples were centrifuged at 3,000 rpm for 10 min, and the volume and acidity were determined. Titratable acidity was determined by automatic titration with 0.1 N NaOH to pH 7.0, using an autoburette (ABU 80; Radiometer). The amount of acid was calculated by multiplying the acidity and the volume of each sample and expressed as $\mu\text{Eq/stomach}$.

Determination of gastric emptying: Gastric emptying of liquids was quantitated by measuring the total length of the intestine stained with black dye and the volume of the gastric contents 1 hr after p.o. administration of the dye (Noir Negro, Riepe KG). The dye was suspended in 2.5% carboxymethylcellulose (CMC) and given in the volume of 1.5 ml/rat. Histamine (20 mg/kg) or the vehicle alone was administered s.c. 1 hr before dye administration.

Statistics: Data are expressed as means \pm S.E. The mean values were compared using the unpaired Student's *t*-test, and $P < 0.05$ was regarded as being significant.

Results

Damage induction: A single s.c. administration of histamine (5–40 mg/kg) did not produce any macroscopically visible damage for up to 8 hr. However, the agent induced severe exfoliation of the epithelial cells and exposure of the lamina propria to the proximal duodenum in a dose-dependent manner after 0.5 hr (Figs. 1 and 2). The severity of the damage was maximal at 20 mg/kg, and the incidence was 100% at doses over 10 mg/kg. The damage induced by histamine (20 mg/kg) tended to heal within 8 hr after administration (Figs. 3 and 4).

Effects of several drugs on duodenal damage: Oral administration of sodium bicarbonate significantly protected the duodenal mucosa against histamine-induced damage (Figs. 5 and 6). Pretreatment with cimetidine, omeprazole and NC-1300 also markedly prevented the histamine-induced duodenal damage.

Effects of histamine on intraduodenal pH and gastric content: Intraduodenal pH in the

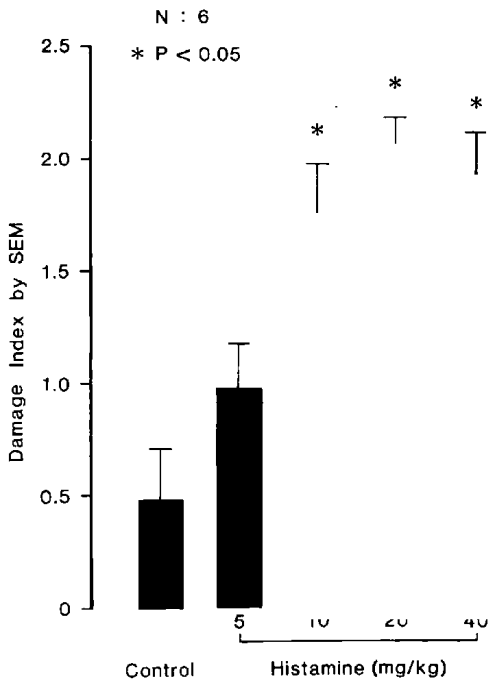


Fig. 1. Dose-response relationship of histamine-induced duodenal damage in rats. Duodenal damage index was determined by scanning electron microscopy (SEM) 0.5 hr after s.c. administration of histamine-2 HCl. Values are means \pm 1 S.E. for 6 rats. * $P < 0.05$ (vs. control).

control group was about 6.8, and the volume and the amount of acid were about 0.2 ml/stomach and 8 μ Eq/stomach, respectively. One hour after histamine administration, intraduodenal pH dropped significantly to about 3.8, and both the volume and amount of acid in gastric contents significantly increased (Table 1). Intraduodenal pH, however, returned to control levels thereafter. Increases in the volume and amount of acid were observed even 2 hr after histamine administration, but these changes disappeared 4 and 8 hr later.

Effect of histamine on gastric emptying:

The black dye given p.o. to the control group reached the end of the small intestine within 1 hr. At that time, the remaining gastric content was about 0.4 ml/stomach. When histamine administration followed drug pre-treatment, there was no significant difference between the control and histamine-treated groups as to the length of small intestine stained with dye (Table 2). However, the gastric contents in the histamine-treated group were significantly greater than that in the control group.

Table 1. Effects of histamine on intraduodenal pH and gastric secretion in rats

	Time after histamine administration (hr)				
	0	1	2	4	8
Intraduodenal pH	6.8 \pm 0.1	3.8 \pm 0.4*	6.3 \pm 0.3	6.6 \pm 0.1	6.8 \pm 0.1
Gastric content					
Volume (ml/stomach)	0.2 \pm 0.1	1.4 \pm 0.1*	1.1 \pm 0.3*	0.2 \pm 0.1	0.2 \pm 0.1
Amount of acid (μ Eq/stomach)	8.1 \pm 1.8	141.3 \pm 12.0*	97.4 \pm 27.9*	5.4 \pm 1.0	10.3 \pm 3.3

Histamine was given s.c. at 20 mg/kg. Values are means \pm S.E. for 8 rats. * $P < 0.05$ (vs. 0 hr).

Table 2. Effects of histamine on gastric emptying in rats

Treatment	No. of rats	Total length of intestine with dye (mm)	% change	Gastric content (ml/stomach)	% change
Control	8	61.9 \pm 3.8		0.4 \pm 0.1	
Histamine	8	59.5 \pm 3.2	-3.9	1.5 \pm 0.2*	+275.0

Histamine was given s.c. at 20 mg/kg, and the dye was given p.o. 1 hr after histamine administration. Values are means \pm S.E. * $P < 0.05$ (vs. control).

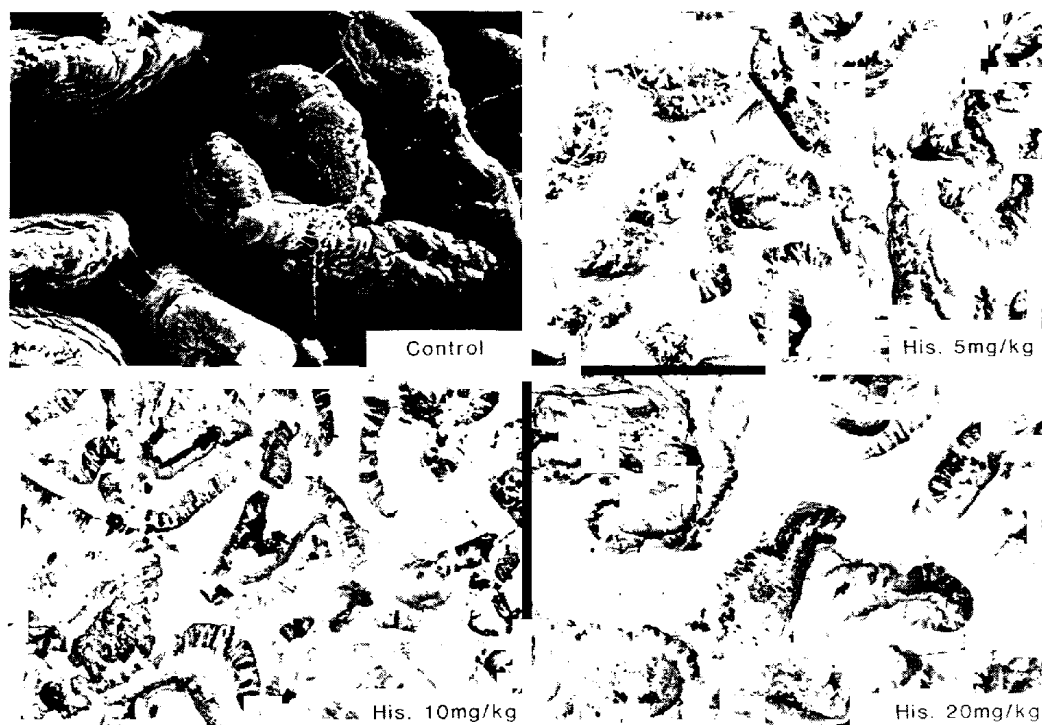


Fig. 2. Scanning electron micrographs of duodenal damage in rats induced by s.c. administration of histamine (His.) ($\times 200$). Animals were killed 0.5 hr after histamine administration. Note that severe exfoliation of epithelial cells in the upper part of the villi and exposure of the lamina propria in the duodenum were evident at doses of 10 or 20 mg/kg of histamine.

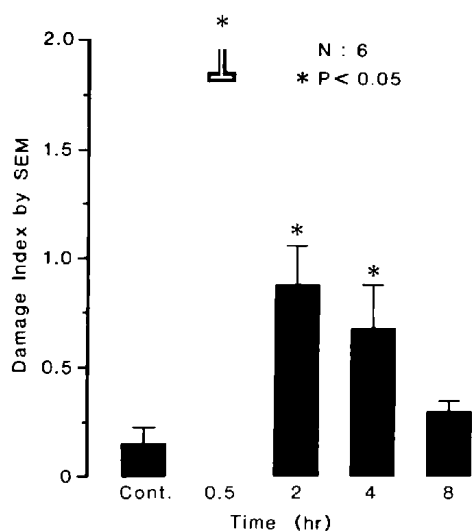


Fig. 3. Time course of histamine-induced duodenal damage in rats. Duodenal damage index was determined by scanning electron microscopy (SEM). Note that the damage was maximal 0.5 hr after histamine administration and healed within 8 hr.

Discussion

These results indicated that even a single administration of histamine can induce, in a dose-dependent manner, damage to the villous tips of the proximal duodenum of rats within 0.5 hr of administration. As expected, histamine significantly stimulated gastric acid secretion when 20 mg/kg of the agent was given, and the effect persisted for 2 hr. In addition, the intraduodenal pH was significantly lowered by histamine, although transiently, for only 1 hr, suggesting that the duodenum could not sufficiently neutralize the inflowing gastric acid. Pretreatment with an acid neutralizing agent and antisecretory agents clearly inhibited the histamine-induced duodenal damage. Therefore, the villous damage in the duodenum may be caused by the stimulatory activity of histamine on gastric acid secretion, resulting in acidification of the duodenum.

The location and degree of villous damage

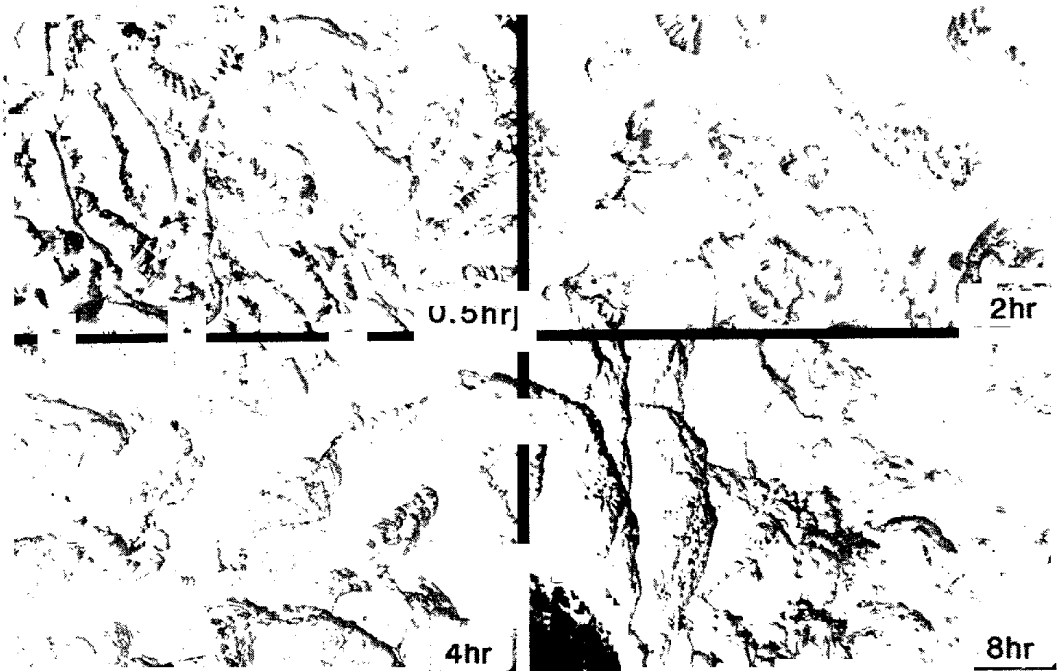


Fig. 4. Scanning electron micrographs of duodenal damage in rats observed 0.5, 2, 4 and 8 hr after histamine administration ($\times 200$).

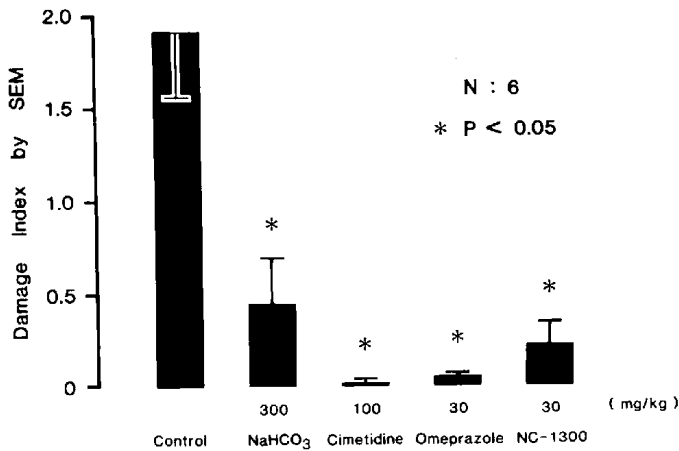


Fig. 5. Effects of several agents on histamine induced duodenal damage in rats. Sodium bicarbonate was given p.o. 5 min before administration of histamine (20 mg/kg). Other agents were given s.c. 0.5 hr before histamine administration. Note that all agents significantly protected the duodenal mucosa against histamine-induced damage.

in the proximal duodenum 0.5 hr after administration appeared to be identical with that observed from use of the potential duodenal ulcerogens, cysteamine and mepirazole. As shown in this study, however, the damage

induced by histamine was no longer evident 8 hr after administration. Feil et al. (10) recently showed that rabbit duodenum with epithelial damage induced by acid (200 mM) was largely healed within 9 hr postadminis-

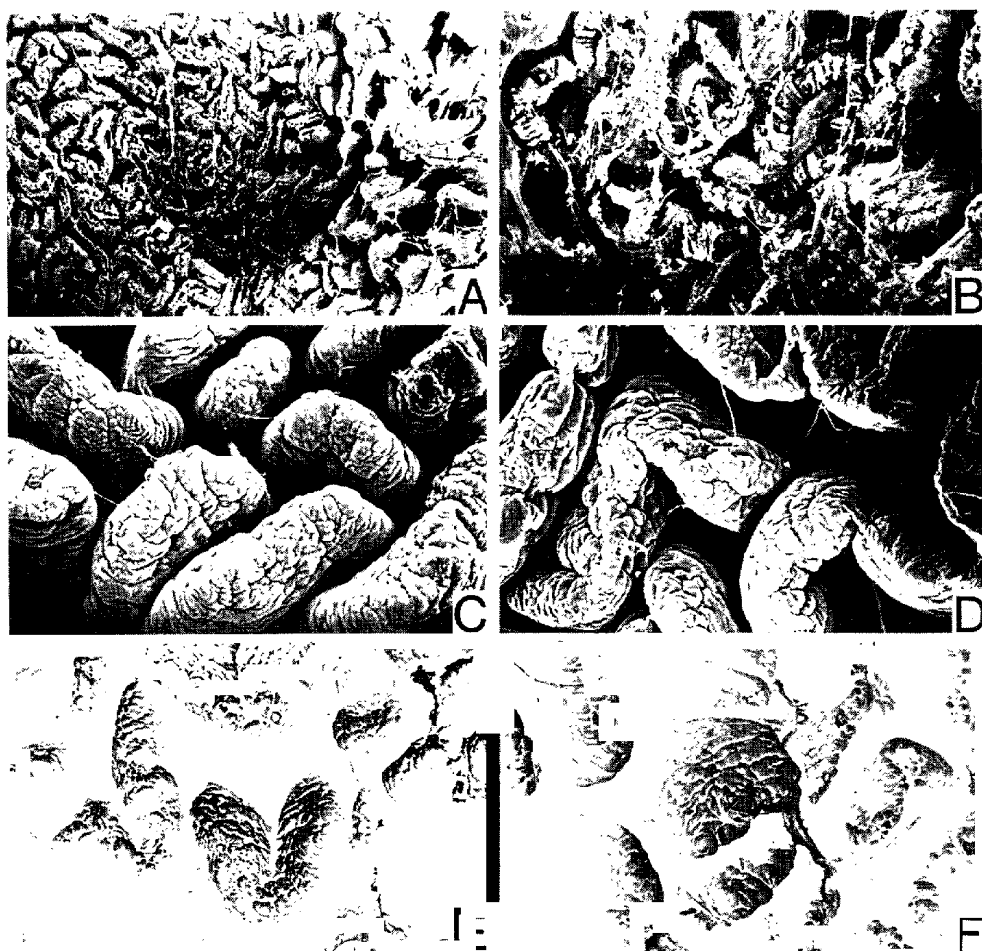


Fig. 6. Scanning electron micrographs of duodenal mucosa in rats treated with the vehicle alone and several agents together with histamine. Sodium bicarbonate (300 mg/kg, p.o.) (C, $\times 200$). Cimetidine (100 mg/kg, s.c.) (D, $\times 200$), omeprazole (30 mg/kg, s.c.) (E, $\times 200$), and NC-1300 (30 mg/kg, s.c.) (F, $\times 200$) apparently protected the duodenal mucosa against histamine-induced damage. (A, $\times 40$) and (B, $\times 200$) are controls.

tration, and suggested that rapid mucosal repair seen after acid injury was responsible. The degree of reduction of intraduodenal pH observed 1 hr after cysteamine and mepirizole was much the same as that observed with histamine (11). There is a question as to why the damage induced by histamine did not develop into ulcers, as was seen with the duodenal ulcerogens. In the case of cysteamine and mepirizole, the accumulation of gastric juice in the stomach persisted for 6 to 8 hr after administration. In addition, the lowering of the intraduodenal pH did persist

for 6 hr after administration. It is most likely that the accumulation of gastric juice for longer periods might be caused by delayed gastric emptying. This accumulated gastric juice gradually empties into the duodenum which has an attenuated neutralizing capacity due to reduced HCO_3^- secretion (4, 12). The corrosive action of gastric acid on the duodenal mucosa results in villous damage, and finally, in penetrating ulcers. It is reported that histamine given i.p. significantly delayed gastric emptying in rats; i.e., the delay at 20 mg/kg being about 70% in comparison with

controls taken as 100% (13). This delayed emptying might be contraction of the gastroduodenal junction (14) and by inhibition of gastric motility by histamine, as we described previously (15). We found that the remaining gastric content was significantly greater than that in the control group for 1 hr postadministration. This may be caused by stimulation of gastric secretion and delayed emptying. In any event, the presence of gastric content was shorter than that observed with cysteamine and mepirizole. Histamine given s.c. at 20 mg/kg had no effect on duodenal HCO_3^- secretion in rats (16). Taken together, we conclude that a single administration of histamine induces duodenal mucosal damage simply by stimulation of gastric acid secretion, but that the damage does not advance to the ulcerated state because of the brief periods of hypersecretion of gastric acid and inhibition of gastric emptying.

References

- 1 Selye, H. and Szabo, S.: Experimental model for production of perforating duodenal ulcers by cysteamine in the rat. *Nature* **244**, 458–459 (1973)
- 2 Robert, A., Nezamis, J.E., Lancaster, C. and Badalamenti, J.N.: Cysteamine-induced duodenal ulcers: a new model to test antiulcer agents. *Digestion* **11**, 199–214 (1974)
- 3 Okabe, S., Ishihara, Y., Inoo, H. and Tanaka, H.: Mepirizole-induced duodenal ulcers in rats and their pathogenesis. *Dig. Dis. Sci.* **27**, 242–249 (1982)
- 4 Tanaka, H., Ueki, S., Ohno, T., Takeuchi, K. and Okabe, S.: Pathogenic mechanisms involved in mepirizole-induced duodenal damage in the rat. *Japan. J. Pharmacol.* **42**, 383–396 (1986)
- 5 Tanaka, H., Ueki, S., Takeuchi, K. and Okabe, S.: Effects of indomethacin on the duodenal mucosa of rats: comparative study with cysteamine. *Japan. J. Pharmacol.* **42**, 539–548 (1986)
- 6 Robert, A. and Standish, W.L.: Production of duodenal ulcers, in rats, with one injection of histamine. *Fed. Proc.* **32**, 322 (1973)
- 7 Robert, A., Stout, T.J. and Dale, J.E.: Production by secretagogues of duodenal ulcers in the rat. *Gastroenterology* **59**, 95–102 (1970)
- 8 Tanaka, H., Kuwahara, Y. and Okabe, S.: Augmentation of cysteamine and mepirizole-induced lesion in the rat duodenum and stomach by histamine or indomethacin. *Japan. J. Pharmacol.* **41**, 545–549 (1986)
- 9 Okabe, S., Higaki, E., Higuchi, T., Sato, M. and Hara, K.: Biochemical and pharmacological analysis of 2-[(2-dimethylaminobenzyl)sulfinyl] benzimidazole (NC-1300), a new proton pump inhibitor. *Japan. J. Pharmacol.* **40**, 239–249 (1986)
- 10 Feil, W., Wenzl, E., Vattay, P., Starlinger, M., Sogukoglu, T. and Schiessel, R.: Repair of rabbit duodenal mucosa after acid injury in vivo and in vitro. *Gastroenterology* **92**, 1973–1986 (1987)
- 11 Tanaka, H., Takeuchi, K. and Okabe, S.: Role of accumulated gastric contents on duodenal ulceration caused by mepirizole and cysteamine in rats. *Gastroenterology* **96**, A504 (1989)
- 12 Ohe, K., Okada, Y., Fujiwara, T., Inoue, M. and Miyoshi, A.: Cysteamine-induced inhibition of acid neutralization and the increase in hydrogen ion back-diffusion in the duodenal mucosa. *Dig. Dis. Sci.* **27**, 250–256 (1983)
- 13 Scarpignato, C., Coruzzi, G. and Bertaccini, G.: Effect of histamine and related compounds on gastric emptying of the conscious rat. *Pharmacology* **23**, 185–191 (1981)
- 14 Bertaccini, G., Coruzzi, G., Molina, E. and Chiavarini, M.: Action of histamine and related compounds on the pyloric sphincter of the rat. *Rendic. Gastroenterol.* **9**, 163–168 (1977)
- 15 Takeuchi, K., Nishiwaki, H., Okada, M. and Okabe, S.: Mucosal protective action of histamine against gastric lesions induced by HCl in rats: importance of antigastric motor activity mediated by H_2 -receptors. *J. Pharmacol. Exp. Ther.* **245**, 632–638 (1988)
- 16 Takeuchi, K., Furukawa, O., Tanaka, H. and Okabe, S.: A new model of duodenal ulcers induced in rats by indomethacin plus histamine. *Gastroenterology* **90**, 636–645 (1986)