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The Long-lasting Effect of TU-199, a Novel H^+,K^+-ATPase Inhibitor, on Gastric Acid Secretion in Dogs

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Abstract

We have used Heidenhain-pouch dogs to investigate the effects of (±)-5-methoxy-2-{[(4-methoxy-3,5-dimethylpyrid-2-yl)methyl]sulphinyl}-1*H*-imidazo[4,5-*b*]pyridine (TU-199), an imidazopyridine derivative, on gastric acid secretion stimulated by histamine, carbachol and tetragastrin. We have also investigated the duration of the antisecretory effect of TU-199 using a measurement of intragastric pH for 24 h in gastric fistula dogs whose gastric acid secretion was stimulated by histamine.

Single oral administration of TU-199 (0·1, 0·2 and 0·4 mg kg^{-1}) dose-dependently suppressed gastric acid secretion stimulated by histamine infusion. Oral treatment with TU-199 (0·2, 0·4 and 0·8 mg kg^{-1}) also dose-dependently inhibited acid secretion induced by carbachol and tetragastrin. The inhibitory effect of TU-199 on stimulated gastric acid secretion was more potent than that of omeprazole, a well-known H^+,K^+-ATPase inhibitor in dogs. Repeated oral treatment with TU-199 at a dose of 0·2 mg kg^{-1} once a day for seven days markedly suppressed histamine-stimulated gastric acid secretion in dogs. This inhibitory effect of TU-199 reached a maximum level after three or four doses and was more pronounced than that of omeprazole or lansoprazole. In gastric fistula dogs, the duration of intragastric pH-elevation by administration of TU-199 (0·3 mg kg^{-1}) was much longer than that of omeprazole (0·6 mg kg^{-1}) or lansoprazole (0·9 mg kg^{-1}). The IC50 values (doses resulting in 50% inhibition) of TU-199, omeprazole and lansoprazole with regard to H^+,K^+-ATPase activity in dog gastric mucosal microsomes were 8·6, 8·8 and 9·9 μM, respectively.

These results indicate that TU-199 inhibits gastric acid secretion via suppression of a H^+,K^+-ATPase activity. Our findings also suggest that TU-199 might have potent and long-lasting effects on gastric acid secretion.

TU-199, (±)-5-methoxy-2-{[(4-methoxy-3,5-dimethylpyrid-2-yl)methyl] sulphinyl} - 1*H* - imidazo-[4,5-*b*]pyridine, is a new H^+,K^+-ATPase inhibitor. TU-199, an imidazopyridine derivative, is a slight modification of omeprazole or lansoprazole. Previous studies have shown that omeprazole and lansoprazole inhibit gastric acid secretion more strongly than histamine H_2-receptor antagonists in experimental animals (Larsson et al 1983; Nagaya et al 1991). Further, many studies have reported that H^+,K^+-ATPase inhibitors have a more potent curative activity than histamine H_2-receptor antagonists in various animal models with ulcer (Yamamoto et al 1984).

In this study we compared effects of TU-199 and omeprazole on gastric acid secretion stimulated by histamine, carbachol and tetragastrin in dogs. Because the inhibitory effect of H^+,K^+-ATPase inhibitor on gastric acid secretion is known to be enhanced when the treatment is repeated (Lind et al 1983; Howden et al 1984), experiments were designed to compare the antisecretory effect of TU-199 with that of omeprazole or lansoprazole by repeated administration.

In addition, to determine precisely the duration of the antisecretory effect of TU-199, we studied sequential changes in gastric pH values over 24 h in gastric fistula dogs.

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Materials and Methods

Animals

Experiments were performed on male and female beagle dogs, 6·5–13 kg (Yakken Farm, Hyogo, Japan and Oriental Maruichi Kyoudo, Shizuoka, Japan). Animals were housed under regulated conditions of temperature (20–26°C), relative humidity (30–70%) and illumination (12 h–12 h light–dark cycle). All experiments performed in this study conformed with the guiding principles for the Care and Use of Laboratory Animals approved by the Japanese Association for Laboratory Animal Science.

Drugs and chemicals

TU-199 (Figure 1), omeprazole and lansoprazole were synthesized by our laboratory as test drugs. The drugs were suspended in 0·5% methylcellulose solution containing 0·2% $NaHCO_3$ immediately before administration. Histamine dihydrochloride (histamine; Tokyo Kasei Kogyo, Tokyo, Japan), carbamylcholine chloride (carbachol; Sigma, St Louis, MO) and tetragastrin (Mect, Tokyo, Japan) were used as gastric acid secretion stimulators. Other reagents were the best grade available.

Effects of single administration on gastric acid secretion in Heidenhain-pouch dogs

Heidenhain pouches were prepared in beagle dogs according to the conventional method using a stainless steel gastric fistula tube (Natsume Seisakusho, Tokyo, Japan) under anaesthesia with pentobarbital. The post-operative animals were used for experiments one month after surgery.

The animals were deprived of food but allowed free access to water for 18 h before the experiment. After placing of the animals in suspended-type dog restrainers, the test drugs or vehicle were orally administered at 0·1–0·8 mg $(3\,mL)^{-1}\,kg^{-1}$. The gastric acid secretion stimulators were administered by intravenous infusion at $7{\cdot}78\,mL\,h^{-1}$, by use of an infusion pump, via a catheter in a hind limb vein for 4 h. The test drugs or vehicle were given orally 30 min before each stimulation. Histamine or carbachol was dissolved in saline, and tetragastrin was diluted with saline. The doses (30–80 $\mu g\,kg^{-1}\,h^{-1}$ for histamine, 4–12 $\mu g\,kg^{-1}\,h^{-1}$ for carbachol and 8–12 $\mu g\,kg^{-1}\,h^{-1}$ for tetragastrin) were chosen individually to induce (approx.) 50% maximum acid secretion in each dog. Gastric juice from the pouch was continuously collected every 15 min for 4 h and analysed for volume and acidity. The acidity of gastric juice was determined by titration to pH 7 with 0·1 M NaOH using phenolphthalein as indicator. Experiments were conducted on groups of four or five animals with a cross-over design.

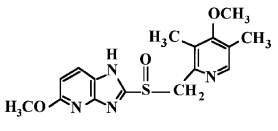

Figure 1. The chemical structure of TU-199.

Effects of repeated administration on gastric acid secretion in Heidenhain-pouch dogs

The test drugs were administered orally once a day for seven days at a dose of 0·2 mg kg^{-1}. Continuous intravenous infusion of histamine for 2 h was started 1 h after administration of the test drugs. Gastric juice from the pouch was continuously collected every 15 min for 2 h and analysed for volume and acidity. Histamine was dissolved in saline for administration at individualized doses (30–45 $\mu g\,kg^{-1}\,h^{-1}$; doses which induced 50% of maximum acid secretion). The acidity of gastric juice was measured by titration to pH 7 with 0·1 M NaOH using phenolphthalein as indicator. Drug efficacy was evaluated on the basis of mean gastric acid output determined between 90 and 120 min after stimulation with histamine. Mean gastric acid output determined for two days before administration of the test drugs was used as a control value. Gastric acid output was determined once a day after withdrawal of the test drugs until it returned to the control level. Five animals were employed using a cross-over design.

Effects on intragastric pH values for 24 h in gastric-fistula dogs

A gastric fistula was prepared at the corpus using a stainless steel fistula tube (Natsume Seisakusho, Tokyo, Japan) under anaesthesia with pentobarbital. Experiments were performed one month after surgery. Feed was supplied once a day between 1300 and 1400 h, but the animals had free access to water. A cross-over study was conducted on groups of six animals with 7-day recovery periods.

An osmotic pump (2ML4; Alza, CA) was filled with histamine and set to release it at a rate of 10 $\mu g\,kg^{-1}\,h^{-1}$. Each pump was implanted in subcutaneous tissue at the poll, under pentobarbital anaesthesia, to keep a stable environment of intragastric acid.

The test materials were administered once a day at 1000 h for seven days from three days after

implantation of the osmotic pump. Gastric pH values were determined every hour for 24 h after the final treatment. TU-199, omeprazole and lansoprazole were administered at doses of 0·3, 0·6 and 0·9 mg kg^{-1}, respectively.

pH values on the gastric mucosal surface were directly determined by inserting pH test-paper (pH 0·0–1·8, 1·8–3·8, 3·8–5·5, and 4·0–9·0; Macherey-Nagel, Düren, Germany) into the gastric fistula tube until it came into contact with the gastric mucosa. pH values measured over 24 h the day before the treatment with the test drug were regarded as the pretreatment baseline.

Effects on the activity of H^+,K^+-ATPase in dog gastric mucosal microsomes

Gastric vesicles were isolated from dog gastric mucosa as described previously (Nagaya et al 1989). Briefly, stomachs resected from dogs were washed in 3 M NaCl solution and the mucosa from the fundic region of the stomach was detached for homogenization in 250 mM sucrose solution. After centrifugation for 30 min at 20 000 *g*, the supernatant was further centrifuged at 78 000 *g* for 30 min to furnish microsome fractions. The microsome fractions were then resuspended in 250 mM sucrose, layered over 7·5% Ficoll (w/w) in 250 mM sucrose, and centrifuged for 3 h at 100 000 *g*. The microsomal band at the interface between two layers was used as gastric vesicle preparation enriched with H^+,K^+-ATPase.

Protein content was determined according to the method of Lowry et al (1951). For determination of gastric H^+,K^+-ATPase activity gastric vesicle (20 μg protein) was suspended in 40 mM Tris–HCl buffer (pH 7·4) in the presence or absence of 15 mM KCl and 10 μg valinomycin. TU-199, omeprazole or lansoprazole (10 μL) dissolved in dimethylsulphoxide (DMSO) was added to the medium (1 mL). After pre-incubation for 20 min at 37°C, Mg-ATP (3 mM) was added to initiate the reaction. After incubation for 20 min the reaction was immediately stopped by adding ice-cold solution containing ammonium molybdate (4·5%) and perchloric acid (12%). Inorganic phosphate produced by ATP hydrolysis was measured by the method of Yoda & Hokin (1970). H^+,K^+-ATPase activity was calculated as the difference between the ATPase activities in the presence and absence of KCl and valinomycin.

Statistical analysis

Data are shown as means ± standard errors of the means (s.e.m.). The statistical significance of differences among groups was determined by use of Dunnett's test or the Tukey–Kramer test. A P value of <0.05 was selected before the study as the level of significance. IC50 and ED50 values (doses resulting in 50% inhibition and 50% protection, respectively) were estimated by fitting to a logistic curve on a non-linear regression model using the least square method. All statistical analysis was conducted with SAS software (SAS Institute, Cary, NC).

Results

Effects of single administration on gastric acid secretion in Heidenhain-pouch dogs

As shown in Figure 2, histamine caused a marked increase in gastric secretion and maximum secretion was observed within 2 h with a steady state of (approx.) 500 μEq per 15 min. Both TU-199 and omeprazole dose-dependently inhibited acid secretion. At a dose of 0·4 mg kg^{-1}, however, the effect of omeprazole showed a tendency to decrease gradually 2 h after the treatment compared with that of TU-199. Average acid outputs during the experiment are shown in Figure 5A. The inhibitory effects of TU-199 and omeprazole on histamine-stimulated acid secretion at doses of 0·2 and

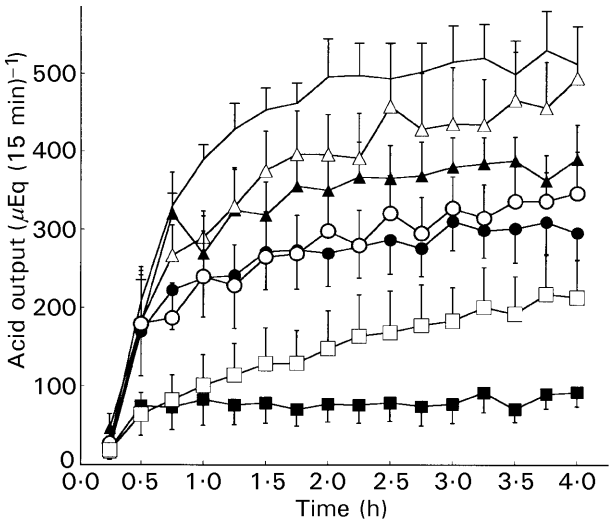

Figure 2. The effects of TU-199 (▲, 0·1 ●, 0·2 ■, 0·4 mg kg^{-1}) and omeprazole (△, 0·1 ○, 0·2 □, 0·4 mg kg^{-1}) on histamine-stimulated acid secretion in Heidenhain-pouch dogs (— = control). Histamine was administered by intravenous infusion at 30–80 μg kg^{-1} h^{-1} for 4 h, starting 30 min after administration of the test drugs or vehicle. Gastric juice was collected every 15 min for 4 h. Each point represents the mean ± s.e.m. of results from five dogs.

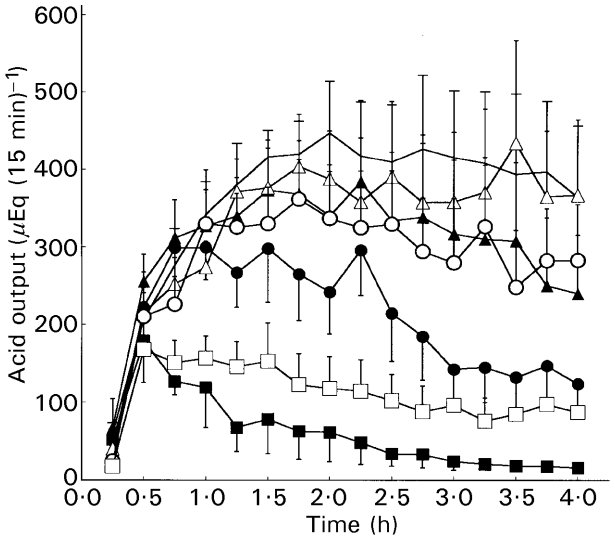

Figure 3. The effects of TU-199 (▲ 0·2; ● 0·4; ■ 0·8 mg kg^{-1}) and omeprazole (△ 0·2; ○ 0·4; □ 0·8 mg kg^{-1}) on carbachol-stimulated acid secretion in Heidenhain-pouch dogs (— = control). Carbachol was administered by intravenous infusion at 4–12 μg kg^{-1} h^{-1} for 4 h, starting 30 min after administration of the test drugs or vehicle. Gastric juice was collected every 15 min for 4 h. Each point represents the mean ± s.e.m. of results from four dogs.

0·4 mg kg^{-1} were statistically significant compared with the control.

As shown in Figure 3, in the control group carbachol-stimulated acid secretion reached a steady state (about 400–450 μEq per 15 min) within 1·5 h after the stimulation. TU-199 and omeprazole had dose-dependent inhibitory effects at doses of 0·2, 0·4 and 0·8 mg kg^{-1}. At a dose of 0·8 mg kg^{-1}, TU-199 almost completely inhibited acid secretion, starting (approx.) 2·5 h after stimulation. The inhibitory effect of TU-199, but not of omeprazole, at a dose of 0·4 mg kg^{-1} was statistically significant, as shown in Figure 5B.

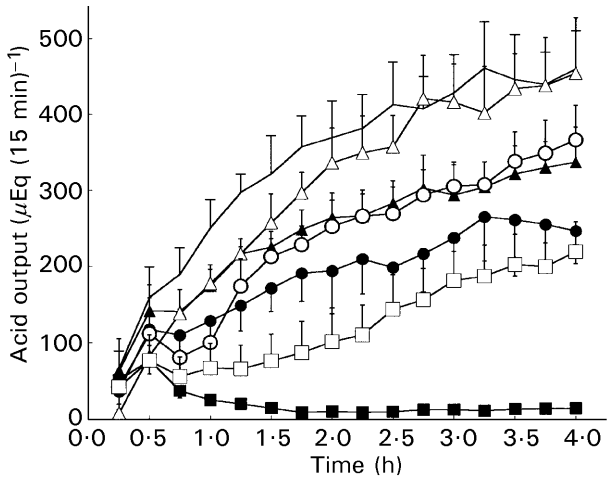

Figure 4. The effects of TU-199 (▲ 0·2; ● 0·4; ■ 0·8 mg kg^{-1}) and omeprazole (△ 0·2; ○ 0·4; □ 0·8 mg kg^{-1}) on tetragastrin-stimulated acid secretion in Heidenhain-pouch dogs (— = control). Tetragastrin was administered by intravenous infusion at 8–12 μg kg^{-1} h^{-1} for 4 h, starting 30 min after administration of the test drugs or vehicle. Gastric juice was collected every 15 min for 4 h. Each point represents the mean ± s.e.m. of results from four dogs.

As shown in Figure 5, a continuous increase in acid secretion with no steady state was observed when the control group was stimulated with tetragastrin. Maximum acid output was 460 μEq per 15 min. TU-199 and omeprazole had dose-dependent inhibitory effects at doses of 0·2, 0·4 and 0·8 mg kg^{-1}. Whereas the inhibitory effect of omeprazole at a dose of 0·2 mg kg^{-1} was weak, the effect of TU-199 at the same dose was statistically significant, as shown in Figure 5C. During the experimental periods, TU-199 at a dose of 0·8 mg kg^{-1} almost completely inhibited tetragastrin-stimulated acid secretion, whereas the same dose of omeprazole did not.

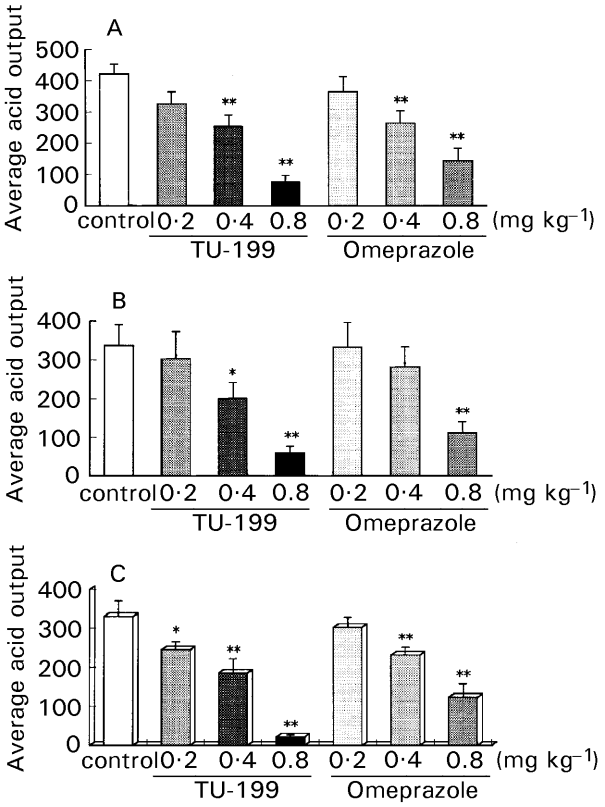

Figure 5. Average acid output (μEq (15 min)$^{-1}$) between 15 min and 4 h after administration of TU-199 or omeprazole to Heidenhain-pouch dogs stimulated by histamine (A, ED50 values (doses resulting in 50% protection) for TU-199 and omeprazole 0·22 and 0·27 mg kg^{-1}, respectively) carbachol (B, ED50 values for TU-199 and omeprazole 0·46 and 0·65 mg kg^{-1}, respectively) or tetragastrin (C, ED50 values for TU-199 and omeprazole 0·38 and 0·62 mg kg^{-1}, respectively). Each column represents the mean ± s.e.m. of results from four dogs. *$P < 0·05$, **$P < 0·01$ compared with control.

Effects of repeated administration on gastric acid secretion in Heidenhain-pouch dogs

The effects of TU-199 ($0{\cdot}2\,mg\,kg^{-1}\,day^{-1}$), omeprazole ($0{\cdot}2\,mg\,kg^{-1}\,day^{-1}$) and lansoprazole ($0{\cdot}2\,mg\,kg^{-1}\,day^{-1}$) on histamine-induced acid output are shown in Figure 6. The inhibitory effects of the three drugs increased gradually and reached a plateau after three or four doses, with (approx.) 90% inhibition by TU-199, 60–70% by omeprazole and 40–50% by lansoprazole. Inhibition of acid output by TU-199 was significantly greater than by omeprazole or lansoprazole. Acid output returned to the control level four or five days after the last treatment with all drugs.

Effects on intragastric pH values for 24 h in gastric fistula dogs

Figure 7 shows gastric pH values determined in dogs for 24 h in the absence or presence of stimulation by histamine by means of an osmotic pump. Non-histamine-stimulated gastric pH values were neutral except for several hours after feeding. When stimulated with $10\,\mu g\,kg^{-1}\,h^{-1}$ histamine, however, gastric pH values were maintained at (approx.) 3 or below during the 24-h monitoring period. Continuous stimulation by histamine caused no change in general signs or symptoms of dogs during the experimental periods. Gastric pH values were monitored again for 24 h after treatment with the test drug for seven days (Figure 8). The pH values after treatment with all three drugs increased in gastric fistula dogs, compared with the pretreatment baseline. The pH $\geq$ 3 holding times for 24 h after treatment with each drug are shown in Figure 9A. The pH $\geq$ 3 holding time produced by TU-199 was significantly longer than that produced by

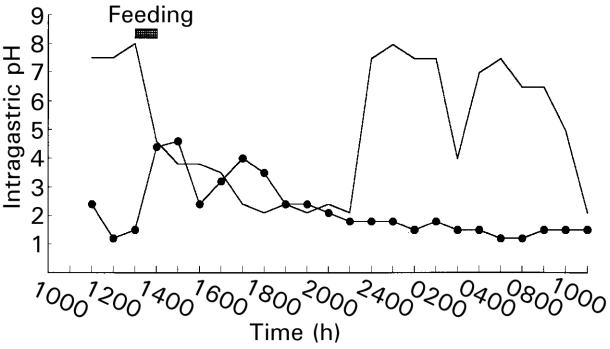

Figure 7. Profiles of intragastric pH for 24 h in gastric fistula dogs. The intragastric pH values were determined every hour in the presence (●) or absence (—) of stimulation by histamine at $10\,\mu g\,kg^{-1}\,h^{-1}$. Feed was given as indicated by the bold horizontal bar.

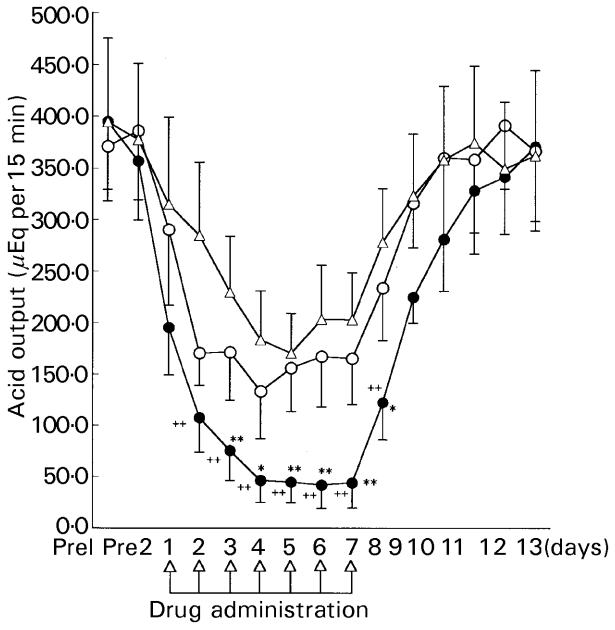

Figure 6. Effects of repeated administration of $0{\cdot}2\,mg\,kg^{-1}$ TU-199 (●), omeprazole (○) or lansoprazole (△) on histamine-induced acid output in Heidenhain-pouch dogs. The test drugs were orally administered for seven days at a dose of $0{\cdot}2\,mg\,kg^{-1}$. Continuous intravenous infusion of histamine ($30–45\,\mu g\,kg^{-1}\,h^{-1}$) for 2 h was started 1 h after administration of the test drugs. Acid output was calculated on the basis of the mean gastric acid output determined between 90 and 120 min after stimulation with histamine. The mean gastric acid output determined for two days before administration of the test drugs was used as the control. Each point represents the mean ± s.e.m. of results from five dogs.

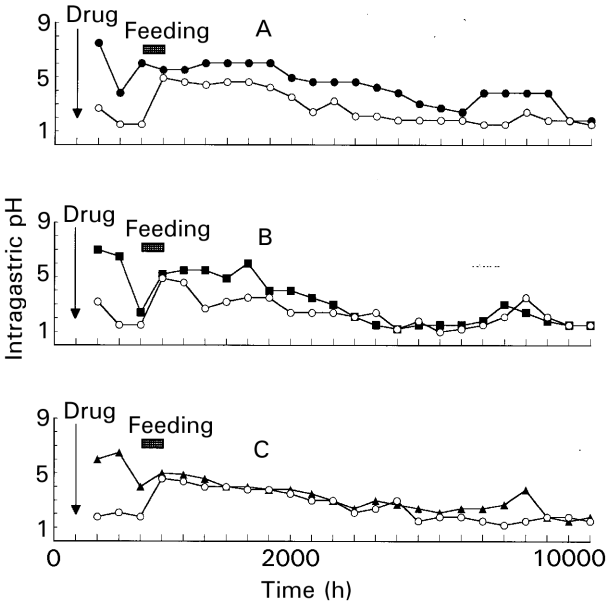

Figure 8. Representative profiles of the effects of oral administration of (A) TU-199, $0{\cdot}3\,mg\,kg^{-1}$ (●; ○ = pretreatment), (B) omeprazole, $0{\cdot}6\,mg\,kg^{-1}$ (■; ○ = pretreatment) and (C) lansoprazole, $0{\cdot}9\,mg\,kg^{-1}$ (▲; ○ = pretreatment), where indicated by the arrows, on intragastric pH for 24 h in histamine-stimulated gastric fistula dogs after repeated administration of the test drugs for seven days. Normal baseline was determined the day before starting treatment with test drug. Feed was given as indicated by the bold horizontal bar.

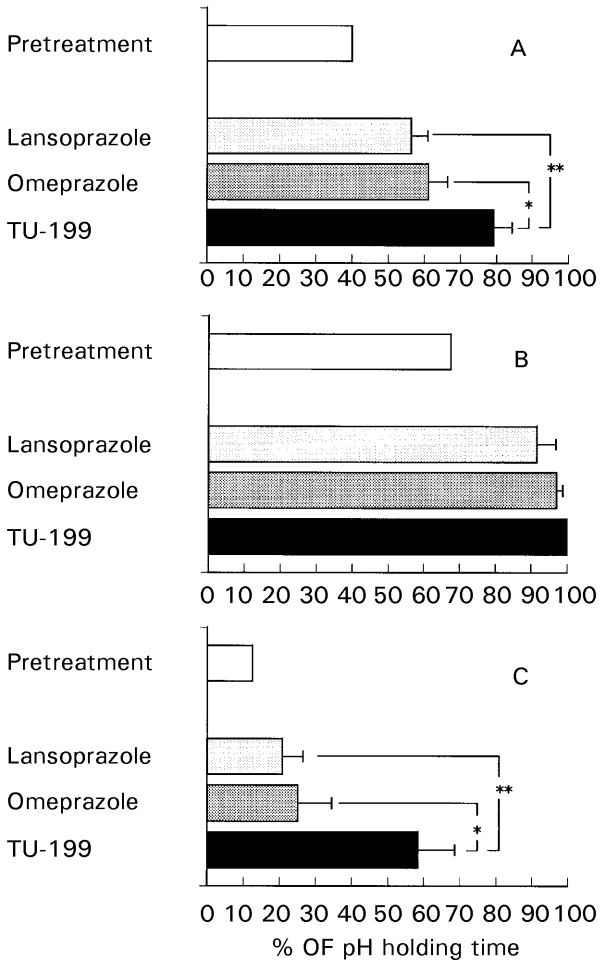

Figure 9. The pH $\geq$ 3 holding time (A) for 24 h, (B) for 12 h (first half) and (C) for 12 h (second half) after repeated administration of test drugs once daily at 1000 h for seven days under histamine-stimulation. TU-199, omeprazole and lansoprazole were administered at 0·3, 0·6 and 0·9 mg kg^{-1}, respectively. pH values determined for 24 h the day before starting treatment with the test drugs were regarded as normal. Each bar represents the mean ± s.e.m. of results from six dogs. *$P < 0.05$, **$P < 0.01$, statistically significant differences.

omeprazole or lansoprazole. Results obtained during the 24-h monitoring periods are shown in Figure 9B and C. During the first 12-h period (Figure 9B), the pH $\geq$ 3 holding time was apparently longer for all three test groups than for the control group, although there were no significant differences among the three test material groups. In the second 12-h period (Figure 9C), however, the pH $\geq$ 3 holding time for the TU-199 group was significantly longer than that for the omeprazole and lansoprazole groups.

Effects on the activity of H^+,K^+-ATPase in dog gastric mucosal microsomes

Inhibition of H^+,K^+-ATPase activity in dog gastric microsomes by TU-199, omeprazole and lansoprazole are shown in Figure 10. TU-199, omeprazole and lansoprazole at concentrations of 10^{-6} to 10^{-4} M dose-dependently inhibited H^+,K^+-ATPase activity. The IC50 values for TU-199, omeprazole and lansoprazole were 8·6, 8·8 and 9·9 μM, respectively. The inhibitory potency of TU-199 was almost the same as that of omeprazole and lansoprazole.

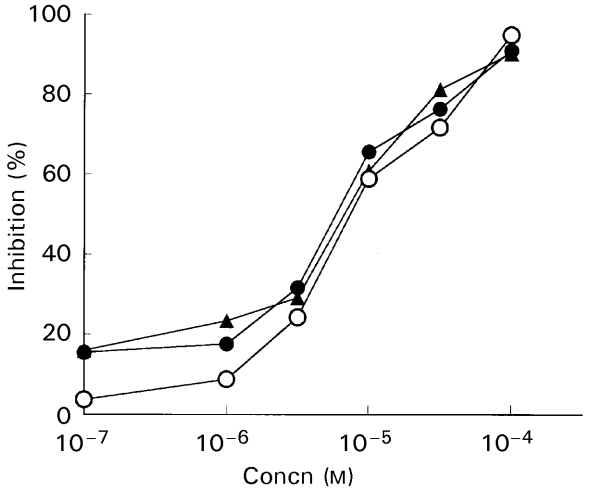

Figure 10. Effects of TU-199 (●), lansoprazole (▲) and omeprazole (○) on H^+,K^+-ATPase activity in dog gastric microsomes. Gastric vesicle (20 μg protein) was suspended in 40 mM Tris–HCl buffer (pH 7·4) in the presence or absence of 15 mM KCl and 10 μg valinomycin. After pre-incubation for 20 min at 37°C, 3 mM Mg-ATP was added to initiate reaction. Reaction was stopped 20 min later. H^+,K^+-ATPase activity was calculated as the difference between ATPase activity in the presence and absence of KCl and valinomycin. Results are expressed as percentage inhibition of control. The IC50 values (doses resulting in 50% inhibition) were 8·6, 8·8 and 9·9 μM for TU-199, lansoprazole and omeprazole, respectively.

Discussion

In this study we compared the pharmacological activity of TU-199 in dogs with the activity of other H^+,K^+-ATPase inhibitors. Single oral treatment with TU-199 inhibited stimulated gastric acid secretion in Heidenhain-pouch dogs more strongly than did treatment with omeprazole. TU-199 treatment at 0·4 mg kg^{-1} inhibited histamine-stimulated acid secretion most strongly. The inhibitory potency of TU-199 on histamine-stimulated acid secretion was stronger than that on carbachol- and tetragastrin-stimulated acid secretion in dogs. Although the reason for this is unclear, Satoh et al (1989) have suggested that the proton-pump inhibitor AG-1749 can suppress acid secretion by other mechanisms in addition to the inhibition of H^+,K^+-ATPase. Therefore, the precise mechanism of suppression remains to be elucidated.

After repeated oral administration the potency of TU-199 in Heidenhain-pouch dogs was greater than that of omeprazole or lansoprazole. From our findings, therefore, the clinical efficacy of TU-199 on ulcers can be expected to be comparable with or greater than that of omeprazole or lansoprazole. In this study, there was no observation of a rebound phenomenon on gastric acid secretion when treatment of TU-199 was discontinued, but further studies are needed to clarify the rebound of TU-199 in detail.

In an in-vitro study using H^+,K^+-ATPase obtained from dog gastric mucosal microsome fractions, the inhibitory potency of TU-199 was almost the same as that of omeprazole or lansoprazole. These results demonstrate that TU-199 can inhibit acid secretion by inhibiting H^+,K^+-ATPase. However, there was no difference among the IC50 values of the three drugs for inhibition of H^+,K^+-ATPase. On the basis of these data, therefore, it is difficult to explain the strong antisecretory effect of TU-199 in dogs, compared with that of omeprazole or lansoprazole. In our preliminary study pharmacokinetic investigation of TU-199 in dogs demonstrated that its oral bioavailability was relatively high and that the half-life of this drug was longer than those of omeprazole and lansoprazole (data not shown). Therefore, that TU-199 suppressed gastric acid secretion in dogs more strongly than omeprazole or lansoprazole in this study might be because of their different pharmacokinetic profiles.

In recent years a convenient 24-h pH monitoring method, which determines changes in gastric acid secretion under physiological conditions (Fimmel et al 1985; Reynolds et al 1986), has become clinically popular and has made it possible to obtain detailed information on the relationship between reduction in gastric acidity and ulcer healing rate. Inhibition of gastric acid secretion during the night has been regarded as the most important factor in accelerating ulcer healing, but recent study demonstrates that it is most important to reduce gastric acidity constantly during the entire day to achieve quick healing. Burget et al (1990), who analysed data from more than 14 000 duodenal ulcer patients, reported that the optimum acid suppression therapy should aim to increase the intragastric pH to >3 for a period of 18–20 h/day for healing to take place within 3–4 weeks.

To confirm clearly the clinical usefulness of TU-199, we studied the duration time of inhibition of gastric acid secretion produced by this drug in fistula dogs using the 24-h intragastric pH monitoring method. A previous study demonstrated that gastric pH in dogs was significantly higher than in man (Lui et al 1986). Postius et al (1991) reported that pantoprazole, a novel H^+,K^+-ATPase inhibitor, can cause a sequential intragastric pH-elevation under conditions of drug-stimulated gastric acid secretion in dogs. In our study pH values in dog stomachs were maintained in the neutral range except for several hours after feeding. In contrast, during histamine-stimulated acid secretion pH values were < 3 (approx.) in dog stomachs. From these observations, in this study we used the histamine as a gastric acid stimulator. TU-199 was administered at dose of $0{\cdot}3\,mg\,kg^{-1}$ on the basis of findings obtained in our previous studies, even though clinical studies of TU-199 in patients with peptic ulcer disease have shown that the optimum dose of TU-199 is 10 mg (data not shown). Therefore, omeprazole and lansoprazole were administered at a dose of 0·6 and $0{\cdot}9\,mg\,kg^{-1}$ because the clinical doses of omeprazole and lansoprazole are 20 and 30 mg, respectively. Because the effects of omeprazole and other H^+,K^+-ATPase inhibitors have been shown to be reinforced as treatment is repeated (Lind et al 1983; Howden et al 1984), the efficacy of repeated treatment with the test drugs was evaluated on the 7th day of treatment, by which time a steady state is believed to be achieved.

Pepsin, which plays an important role in digesting foods in the stomach, is activated at $pH \geq 3$ (approx.). The activity of pepsin falls rapidly between pH 4 and pH 5 (Samloff 1989). Krier (1990) reported that at $pH < 3$ there was a significantly higher incidence of acute stress ulcerations. Therefore, pH 3 is believed to be a physiologically critical point, and the maintenance of pH at or above this level is generally regarded as one of the most important objectives in ulcer therapy. The current study therefore compared the $pH \geq 3$ holding times of the three test material groups. The $pH \geq 3$ holding time was significantly longer for the TU-199 group than for the omeprazole and lansoprazole groups, and differences were particularly marked during the second 12-h period, as is shown in Figure 9. Thus our results indicate that inhibition of gastric acid secretion by TU-199 is stronger and more long-lasting than that by omeprazole or lansoprazole. In addition, pantoprazole (Fitton & Wiseman 1996) and rabeprazole (Prakash & Faulds 1998) are clinically used to treat peptic ulcer disease. Our findings are therefore of interest in relation to the pharmacological profile of pantoprazole or rabeprazole.

In conclusion, this study indicates that TU-199 exerts an inhibitory effect on gastric acid secretion via suppression of H^+,K^+-ATPase activity. Our findings also suggest that TU-199 might have

potent and long-lasting effects on gastric acid secretion and promote the healing of ulcers in man.

References

Burget, D. W., Chiverton, S. G., Hunt, R. H. (1990) Is there an optimal degree of acid suppression for healing of duodenal ulcers? A model of the relationship between ulcer healing and acid suppression. Gastroenterology 99: 345–351

Fimmel, C. J., Etienne, A., Cilluffo, T., von Ritter, C., Gasser, T., Rey, J. P., Caradonna-Moscatelli, P., Sabbatini, F., Pace, F., Buhler, H. W. (1985) Long-term ambulatory gastric pH monitoring: validation of a new method and effect of H_2-antagonists. Gastroenterology 88: 1842–1851

Fitton, A., Wiseman, L. (1996) Pantoprazole. A review of its pharmacological properties and therapeutic use in acid-related disorders. Drugs 51: 460–482

Howden, C. W., Forrest, J. A., Reid, J. L. (1984) Effects of single and repeated doses of omeprazole on gastric acid and pepsin secretion in man. Gut 25: 707–710

Krier, C., Bohrer, H., Jurs, G., Warth, S., Just, O. H. (1990) Continuous intragastric pH measurement in intensive care patients treated with ranitidine and tube feeding. Anasth. Intensivther. Notfallmed. 25: 10–13

Larsson, H., Carlsson, E., Junggren, U., Olbe, L., Sjostrand, S. E., Skanberg, I., Sundell, G. (1983) Inhibition of gastric acid secretion by omeprazole in the dog and rat. Gastroenterology 85: 900–907

Lind, T., Cederberg, C., Ekenved, G., Haglund, U., Olbe, L. (1983) Effect of omeprazole—a gastric proton pump inhibitor—on pentagastrin-stimulated acid secretion in man. Gut 24: 270–276

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Lui, C. Y., Amidon, G. L., Berardi, R. R., Fleisher, D., Youngberg, C., Dressman, J. B. (1986) Comparison of gastrointestinal pH in dogs and humans: implications on the use of the beagle dog as a model for oral absorption in humans. J. Pharm. Sci. 75: 271–274

Nagaya, H., Satoh, H., Kubo, K., Maki, Y. (1989) Possible mechanism for the inhibition of gastric $(H^+ + K^+)$-adenosine triphosphatase by the proton pump inhibitor AG-1749. J. Pharmacol. Exp. Ther. 248: 799–805

Nagaya, H., Inatomi, N., Satoh, H. (1991) Differences in the antisecretory actions of the proton-pump inhibitor AG-1749 (lansoprazole) and the histamine H_2-receptor antagonist famotidine in rats and dogs. Jpn J. Pharmacol. 55: 425–436

Postius, S., Brauer, U., Kromer, W. (1991) The novel proton pump inhibitor pantoprazole elevates intragastric pH for a prolonged period when administered under conditions of stimulated gastric acid secretion in the gastric fistula dog. Life Sci. 49: 1047–1052

Prakash, A., Faulds, D. (1998) Rabeprazole. Drugs 55: 261–267

Reynolds, J. R., Walt, R. P., Hardcastle, J. D., Clark, A. G., Smart, H. L., Langman, M. J. (1986) 24-hour intragastric pH: continuous monitoring or nasogastric aspiration? Digestion 33: 219–224

Samloff, I. M. (1989) Peptic ulcer: the many proteinases of aggression. Gastroenterology 96: 586–595

Satoh, H., Inatomi, N., Nagaya, H., Inada, I., Nohara, A., Nakamura, N., Maki, Y. (1989) Antisecretory and antiulcer activities of a novel proton pump inhibitor AG-1749 in dogs and rats. J. Pharmacol. Exp. Ther. 248: 806–815

Yamamoto, O., Okada, Y., Okabe, S. (1984) Effects of a proton pump inhibitor, omeprazole, on gastric secretion and gastric and duodenal ulcers or erosions in rats. Dig. Dis. Sci. 29: 394–401

Yoda, A., Hokin, L. E. (1970) On the reversibility of binding of cardiotonic steroids to a partially purified (Na^+,K^+)-activated adenosinetriphosphatase from beef brain. Biochem. Biophys. Res. Commun. 40: 880–886