A Study Comparing the Antisecretory Effect of TAK-438, a Novel Potassium-Competitive Acid Blocker, with Lansoprazole in Animals

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Received January 18, 2011; accepted March 15, 2011

ABSTRACT

Proton pump inhibitors (PPIs) are widely used for the treatment of acid-related diseases. However, several medical needs such as suppression of night-time acid secretion and rapid symptom relief remain unmet. In this study, we investigated the effects of 1-[5-[2-fluorophenyl]-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethylene monofumarate (TAK-438), a novel potassium-competitive acid blocker, on acid secretion in rats and dogs under various conditions, in comparison with the PPI lansoprazole [2-[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl][sulfinyl]-1H-benzimidazole], to characterize the antisecretory action of TAK-438. TAK-438 showed a more potent and longer-lasting inhibitory effect than lansoprazole on the histamine-stimulated gastric acid secretion in rats and dogs. A pharmacokinetic study in rats showed that TAK-438 accumulated and was retained in the gastric tissue for more than 24 h, unlike that in the plasma. TAK-438 showed significant antisecretory activity with or without cimetidine pretreatment, in contrast to lansoprazole, which did not show antisecretory activity after cimetidine pretreatment in rats. TAK-438 increased the pH of the gastric perfusate to 5.7 in an unstimulated condition, and this effect was maintained in the presence of subsequent histamine stimulation. On the other hand, lansoprazole also increased the pH in an unstimulated condition, but this effect diminished after histamine stimulation. These results indicated that TAK-438 exerted a more potent and longer-lasting antisecretory effect than lansoprazole through high accumulation and slow clearance from the gastric tissue. In addition, TAK-438 was unaffected by the gastric secretory state, unlike PPIs. Therefore, TAK-438 can provide a novel mechanism of action to improve the present PPI-based treatment of acid-related diseases.

Introduction

Gastric H⁺, K⁺-ATPase is the key enzyme involved in the final step of gastric acid secretion. This enzyme transports H⁺ into the secretory canaliculus of the parietal cell by an electroneutral exchange of H⁺ for K⁺ (Ganser and Forte, 1973; Sachs et al., 1976). H⁺, K⁺-ATPase is localized mainly in the tubulovesicles below the plasma membrane in the resting state of the parietal cell and is recruited to the apical plasma membrane when the parietal cell is functionally activated by gastric acid secretagogues such as histamine, gastrin, or acetylcholine (Parsons and Keeling, 2005).

Proton pump inhibitors (PPIs) such as lansoprazole [2-[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl][methyl][sulfinyl]-1H-benzimidazole], omeprazole, rabeprazole, and pantoprazole inhibit gastric H⁺, K⁺-ATPase by covalently binding to its sulphydryl group, resulting in the inhibition of gastric acid secretion (Sachs et al., 1988; Nagaya et al., 1989; Wolfe and Sachs, 2000). PPIs are widely used in the treatment of acid-related diseases such as gastroesophageal reflux disease and peptic ulcer disease (Graham et al., 2002; Frazzoni et al., 2003; Robinson, 2005) and also in combination with antibiotics to eradicate Helicobacter pylori (Malfertheiner et al., 2003). Although
PPIs show potent inhibitory activity against acid secretion and are clinically used worldwide, there is still hope for refinement or enhancement. Because PPIs bind only to activated H⁺, K⁺-ATPases, it takes 4 to 5 days to achieve maximal acid suppression at therapeutic doses (Dammann and Burkhardt, 1999; Tytgat, 2001). PPIs exert their inhibitory activity after undergoing molecular rearrangement under acidic conditions (Sachs et al., 1995). Thus, their activity decreases under neutral conditions. PPIs have a relatively short plasma half-life (t1/2) and therefore cannot inhibit night-time acid secretion in some patients, even when taken twice daily (Katz et al., 2000; Ang and Fock, 2006).

In contrast to PPIs, a new class of acid suppressants known as potassium-competitive acid blockers (P-CABs) or acid pump antagonists inhibit gastric H⁺, K⁺-ATPase in a K⁺-competitive and reversible manner (Vakil, 2004; Andersson and Carlsson, 2005; Geibel, 2005). 3-(Cyanoethyl)-2-methyl-8-(phenylmethoxy)imidazo[1,2-a]pyridine (SCH28080), a prototype P-CAB, binds to the phosphoenzyme with an extracytosolic conformation of the monovalent cation site (E₂P) of the H⁺, K⁺-ATPase and is strictly K⁺-competitive (Mandel and Sachs, 1990). This mechanism allows rapid inhibition of the pump without the need for acidity at its luminal surface because the pump is blocked in midcycle. Several structural derivatives, e.g., imidazo[1,2-a]pyridines such as SCH28080 (Wallmark et al., 1987) and 8-[(2,6-dimethylbenzyl)amino]-N-[2-hydroxyethyl]-2,3-dimethylimidazo[1,2-α]pyridine-6-carboxamide (AZD0865) (Gedda et al., 2007), pyrimidines (Yu et al., 2004), imidazopyridazines (Simon et al., 2007), and pyrrolopyridazines such as 7-(4-fluorobenzyloxy)-2,3-dimethyl-1-[(1S,2S)-2-methylcyclopropyl]methyl]-1H-pyrrolo[2,3-d]pyridazine (CS-526) (Ito et al., 2007), have been evaluated as P-CABs. These compounds have higher pKa values than PPIs and are stable at a low pH. Therefore, P-CABs are highly concentrated in the strongly acidic compartment of the gastric parietal cell at the luminal surface of H⁺, K⁺-ATPase and exert a less variable onset of their effect, because unlike PPIs, they do not require a gastroprotective formulation (Wurst and Hartmann, 1996). P-CABs exhibit rapid onset of inhibition of acid secretion based on rapid achievement of their peak plasma concentrations. A complete effect was achieved on the first day of administration (Andersson and Carlsson, 2005). However, these P-CABs are not used clinically worldwide because of their short duration of action and hepatic toxicity (Parsons et al., 1995). Thus, their activity decreases under neutral conditions. PPIs have a relatively short plasma half-life (t1/2) and therefore cannot inhibit night-time acid secretion in some patients, even when taken twice daily (Katz et al., 2000; Ang and Fock, 2006).

Materials and Methods

Chemicals

TAK-438 and lansoprazole were manufactured by Takeda Pharmaceutical Company Limited, Japan (Osaka, Japan). 1-[5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrolo-3-yl]-N-methyl-[14C]methanamine monofumarate ([14C]TAK-438) with a specific radioactivity of 4.59 MBq/mg was synthesized by GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). TAK-438 and [14C]TAK-438 were suspended in 0.5% methylcellulose solution for oral or intraperitoneal administration. Lansoprazole was suspended in 0.5% methylcellulose solution containing 1% NaHCO₃ or 0.5% methylcellulose solution for oral or intraperitoneal administration. TAK-438 and lansoprazole were intravenously administered as a solution in a mixture of N,N-dimethylacetamide and polyethylene glycol 400 in a ratio of 1:1 (v/v). [14C]TAK-438 was dissolved in physiological saline for intravenous injection. Histamine 2HCl was obtained from Wako Pure Chemicals (Osaka, Japan). All other reagents and solvents were of the best grade available.

Animals

All experiments were performed in accordance with ethical guidelines established by the Animal Care and Use Committee of Takeda Pharmaceutical Company Limited. Seven- to 8-week-old male Sprague-Dawley rats (CLEA Japan, Inc., Tokyo, Japan; Charles River Laboratories Japan, Inc., Ibaraki, Japan) and male Beagle dogs (Orbital Yeast Co., Ltd., Tokyo, Japan) were used. Male Beagle dogs were fasted for more than 14 h before the experiment but were given free access to water. In the Heidenhain pouch dog experiment, we constructed a gastric pouch according to the Heidenhain method in male Beagle dogs (Heidenhain, 1879). In brief, the dogs were anesthetized with sodium pentobarbital, and the abdominal cavity was opened. After exposing the stomach, a portion of the greater curvature opposite the splenic hilum was converted into a pouch with adequate blood supply from the intact gastroepiploic artery. The main body of the stomach was reconstituted, while the pouch was drained into an implanted metal cannula. After closing the pouch, the cannula was brought out of the abdominal cavity through the left lateral abdominal wall. Animals were allowed to recover from surgery for a minimum of 4 weeks. Heidenhain pouch dogs were fasted for more than 14 h before the experiment but were given free access to water. Each dog was used for the lansoprazole and TAK-438 experiments with intervals of 1 and 2 weeks, respectively.

Pharmacological Study

Histamine-Stimulated Acid Secretion in Anesthetized Rats.

Drugs and the vehicle were administered orally (2 ml/kg) to rats. The pylorus was ligated after anesthetization with urethane (1.2 g/kg i.p.), and the abdomen was closed. Next, histamine 2HCl (30 mg/kg/10 ml) was subcutaneously injected 1 h after drug and vehicle administration. In a time course study, pylorus ligation, abdomen closure, and histamine injection under urethane anesthesia were performed 1, 5, 9, and 24 h after drug and vehicle administration. Three hours after histamine administration, the rats were sacrificed by CO₂ asphyxiation, and their stomachs were removed. Gastric contents were collected and centrifuged at 3000 rpm for 10 min. The volume of each sample was measured, and the acid concentration...
was determined by an automatic titrator (COM-555SC; Hiranuma Sangyo Co., Ltd., Ibaragi, Japan) set to a pH of 7.0 with 0.1 M NaOH, and the total acid output during the 3-h period was calculated.

**Histamine-Stimulated Acid Secretion in Heidenhain Pouch Dogs.** Drugs and the vehicle were administered orally (0.2 ml/kg) to the dogs in a blind manner. Histamine 2HCl (30 μg/kg) was subcutaneously injected a day before and 1, 3, 6, 24, and 48 h after drug and vehicle administration. Gastric juice from the pouch was collected for a 90-min period after each dose of histamine. The volume of gastric juice was measured, and the acid concentration was determined as described above. The total acid output during the 90-min period from each time point was calculated and expressed as a percentage of the predosing value measured 1 day before the administration.

**Effect of Cimetidine Pretreatment on the Inhibitory Activity against Histamine-Stimulated Acid Secretion in Rats.** To transiently inhibit acid secretion and increase the gastric pH (change in gastric acid secretory state), 30 mg/kg cimetidine was intravenously injected into the rats. Fifteen minutes after cimetidine or vehicle administration, 0.7 mg/kg TAK-438, 1 mg/kg lansoprazole, or the vehicle were intraperitoneally administered to the rats. Four hours after drug or vehicle administration, the rats were anesthetized with urethane (1.2 g/kg i.p.) and the pylorus was ligated. The abdomen was then closed, and histamine 2HCl (30 mg/kg/10 ml) was subcutaneously injected. The rats were sacrificed by CO2 asphyxiation, and their stomachs were removed 3 h after histamine administration. The gastric contents were collected, and the total acid output during the 3-h period was calculated as described above. In a preliminary experiment, the pH of the gastric surface was measured using pH paper (Merck, Darmstadt, Germany) after intravenous administration of 30 mg/kg cimetidine.

**Measurement of the pH of the Gastric Perfusate in Anesthetized Rats.** The animals were anesthetized with urethane (1.2 g/kg i.p.), the abdomen was opened, and the stomach was exposed. Cannulas were introduced into the stomach from the duodenum and forestomach, and the esophagus was ligated. The stomach was perfused with saline at a rate of 0.5 ml/min, and the pH of the gastric perfusate was continuously measured with a glass electrode (9691-15C and 2461A-15T; Horiba, Kyoto, Japan) interfaced to a signal transduction unit (PowerLab; AD Instruments, Colorado Springs, CO). When the pH stabilized, 1 mg/kg TAK-438, 10 mg/kg lansoprazole, or the vehicle were intravenously administered. Histamine 2HCl (8 mg/kg/h) was intravenously infused via the cervical vein 30 min after drug or vehicle administration. The pH of the perfusate was measured for 5 h after administration of the drug or vehicle. Data were analyzed using the Chart program supplied with the PowerLab system.

**Pharmacokinetic Study**

**Dosing and Sample Collection.** [14C]TAK-438 and unlabeled TAK-438 were orally or intravenously administered to fasted animals. Five, 10 (only for intravenous dosing), 15, and 30 min, and 1, 2, 3, 4, 6, 8, and 24 h after dosing, blood was withdrawn from the tail vein of the rats, the abdominal aorta of the rats under light anesthesia, and the femoral vein of the dogs. Blood was then centrifuged. The supernatant was evaporated under a nitrogen gas stream. The residues were reconstituted with a reconstitution solution, and the sample solution was injected into the HPLC system.

**Measurement of Radioactivity.** Radioactivity in plasma and organic solvent extracts was measured using liquid scintillation counters (LSC-5100 and LSC-6100; Aloka Co., Ltd., Tokyo, Japan, and Tri-Carb 2100TR, PerkinElmer Life and Analytical Sciences, Waltham, MA). In the on-line detection during high-performance liquid chromatography (HPLC; Shimadzu, Kyoto, Japan), radioactivity was measured with an RI detector (model 625TR; PerkinElmer Life and Analytical Sciences) to which the liquid scintillator (Ultima-Flo AP; Life and Analytical Sciences) was attached.

**Results**

**Effect of TAK-438 and Lansoprazole on Histamine-Stimulated Gastric Acid Secretion in Anesthetized Rats.** In a preliminary experiment, gastric acid secretion in urethane-anesthetized rats was negligible in the absence of a secretagogue. When gastric acid secretion was stimulated with histamine, the acid output was approximately 155 to 525 μEq/3 h. TAK-438 at doses of 1, 2, and 4 mg/kg (all doses are shown as the free base), orally inhibited histamine-stimulated acid secretion in a dose-dependent manner (Fig. 1A), and complete inhibition was observed at the 4 mg/kg dose. Lansoprazole at 1, 2, and 4 mg/kg p.o. also inhibited histamine-stimulated gastric acid secretion in a dose-dependent manner (Fig. 1B), and the inhibition at the 4 mg/kg dose was potent but incomplete. The ID50 values and their 95% confidence intervals (CI) were calculated by logistic regression analysis. In the dose-response study in rats, differences between each drug treatment group and the vehicle group were tested for statistical significance by the one-tailed Shirley-Williams test, and p values less than 0.025 were considered significant. In the time course and acid secretory state studies in rats, differences between the drug treatment and vehicle groups were tested for statistical significance by the two-tailed Student’s t test, and p values less than 0.05 were considered significant. In the study of Heidenhain pouch dogs, we used the 4 × 4 Latin square to allocate treatments for a cross-over design. The area under the acid secretion-time curve from 0 to 25.5 h after dosing (AUCacid) was calculated by the trapezoidal rule. Analysis of variance was performed for the cross-over study design and followed using intergroup comparison by contrast analysis. A closed testing procedure was used sequentially for evaluating intergroup comparison between the vehicle control group and high-, middle-, or low-dose groups in descending order. The analysis was performed at the two-tailed significance level of 0.05. Values for maximum plasma concentration (Cmax) and the time taken to reach Cmax (Tmax) were directly noted from the data. Plasma t1/2 and the area under the plasma concentration-time curve from 0 to 24 h after dosing (AUCarea) were calculated by linear regression analysis and the trapezoidal rule, respectively. Bioavailability (BA) was determined after dose normalization.
Pouch Dogs. Stimulated Gastric Acid Secretion in Heidenhain administration. significant inhibition was observed 9 to 12 or 24 to 27 h after inhibitory action declined over a period of time (Fig. 2B). No acid secretion 1 to 4 and 5 to 8 h after administration, but its zole at 4 mg/kg p.o. strongly inhibited histamine-stimulated was 40% 24 to 27 h after administration (Fig. 2A). Lansopra- inhibition of TAK-438 was sustained, and the inhibition rate 5 to 8, and 9 to 12 h after administration. The significant strongly inhibited histamine-stimulated acid secretion 1 to 4, zole exhibited equivalent potency. TAK-438 at 3 mg/kg p.o. TAK-438 and 4 mg/kg lansopra-, or vehicle. Gastric contents were collected 3 h after histamine administration, and the total acid output was calculated. Each column represents the mean ± S.E. from seven or eight rats. Statistical significance of the difference was determined by the one-tailed Shirley-Williams test. *, p < 0.025 versus vehicle.

**Time Course of the Inhibitory Effect of TAK-438 and Lansoprazole on Histamine-Stimulated Acid Secretion in Rats.** According to the ratio of the ID_{50} values of TAK-438 and lansoprazole, 3 mg/kg TAK-438 and 4 mg/kg lansoprazole, or vehicle. Lansoprazole at 4 mg/kg p.o. strongly inhibited histamine-stimulated acid secretion 1 to 4, 5 to 8, and 9 to 12 h after administration. The significant inhibition of TAK-438 was sustained, and the inhibition rate was 40% 24 to 27 h after administration (Fig. 2A). Lansoprazole at 4 mg/kg p.o. strongly inhibited histamine-stimulated acid secretion 1 to 4 and 5 to 8 h after administration, but its inhibitory action declined over a period of time (Fig. 2B). No significant inhibition was observed 9 to 12 or 24 to 27 h after administration.

**Effect of TAK-438 and Lansoprazole on Histamine-Stimulated Gastric Acid Secretion in Pouch Dogs.** TAK-438 at doses of 0.1 to 1 mg/kg p.o. inhibited histamine-stimulated acid secretion in a dose-dependent manner, and the inhibitory effect lasted for more than 48 h (Fig. 3A). One milligram/kilogram TAK-438 showed complete inhibition 1, 3, and 6 h after administration. A statistically significant inhibitory effect on AUC_{acid} was observed at 0.1 to 1 mg/kg (Fig. 4A), and the ID_{50} value of TAK-438 for AUC_{acid} was 0.21 mg/kg (95% CI, 0.17–0.24 mg/kg). Lansoprazole at doses of 0.3 to 3 mg/kg p.o. inhibited histamine-stimulated acid secretion in a dose-dependent manner, but the inhibitory effect disappeared 48 h after administration (Fig. 3B). Three milligram/kilogram lansoprazole demonstrated almost complete inhibition 1 h after administration; however, the inhibitory effect diminished at 3 h. A statistically significant inhibitory effect on AUC_{acid} was observed at 0.3 to 3 mg/kg (Fig. 4B), but the ID_{50} value of lansoprazole for AUC_{acid} was more than 3 mg/kg.

**Pharmacokinetics of TAK-438F in Rats and Dogs.** After oral administration of TAK-438 at 0.3 mg/kg in dogs, TAK-438 concentrations in the plasma reached 17 ng/ml (C_{max}) at 0.3 h (T_{max}). The concentrations decreased with a t_{1/2} of 2.6 h; the AUC_{conc} of TAK-438 was 27 ng·h/ml. After intravenous administration of TAK-438 at 0.75 mg/kg, the plasma concentration of TAK-438F was 60 ng·ml at 5 min. The concentrations decreased with a t_{1/2} of 1.2 h; the AUC_{conc} was 99 ng·h/ml. BA of TAK-438F after oral administration in rats was 10% (Table 1). After oral administration of TAK-438 at 0.3 mg/kg in dogs, T_{max}, C_{max}, t_{1/2}, and AUC_{conc} were 1.0 h, 30 ng/ml, 1.1 h, and 68 ng·h/ml, respectively. After intravenous administration of TAK-438 at 0.1 mg/kg, C_{5 min}, t_{1/2}, and AUC_{conc} were 26 mg/ml, 1.2 h, and 44 ng·h/ml, respectively. BA of TAK-438F after oral administration in dogs was 52% (Table 1).

**Concentrations of TAK-438F in the Plasma and Stomach of Rats.** After oral administration of TAK-
inhibition of gastric acid secretion by cimetidine (H₂ receptor antagonist) on the antisecretory activity of TAK-438 and lansoprazole was investigated. In a preliminary experiment, intravenous administration of TAK-438 at 1 mg/kg increased the pH of the gastric perfusate to 5.7 at 30 min after administration, and the increase in pH was sustained for more than 4 h after the initiation of histamine infusion. Intravenous administration of lansoprazole at 10 mg/kg increased the pH of the gastric perfusate to 5.2 at 30 min after administration; however, the pH decreased after the initiation of histamine infusion. The pH values 60 and 180 min after the start of histamine infusion and was maintained throughout the duration of the infusion. Intravenous administration of TAK-438 at 1 mg/kg increased the pH of the gastric perfusate to 6.5 at 30 min after administration; then the pH returned to 1.9, normal condition, 4 h after administration. Intraperitoneal administration of TAK-438 at 0.7 mg/kg significantly inhibited histamine-stimulated acid secretion after pretreatment with or without cimetidine (Fig. 6A). The inhibition rates of TAK-438 with or without cimetidine pretreatment were 76 or 64%, respectively. On the other hand, intraperitoneal administration of lansoprazole at 1 mg/kg significantly inhibited histamine-stimulated acid secretion, but the inhibitory activity of lansoprazole was not observed after pretreatment with cimetidine (Fig. 6B).

**Effect of Cimetidine Pretreatment on the Inhibitory Activity of TAK-438 and Lansoprazole against Histamine-Stimulated Acid Secretion in Rats.** It is well known that the inhibitory effect of PPIs on acid secretion is influenced by the gastric acid secretory state (De Graaf and Woussen-colle, 1986). In this study, the effect of the transient inhibition of gastric acid secretion by cimetidine (H₂ receptor antagonist) on the antisecretory activity of TAK-438 and lansoprazole was investigated. In a preliminary experiment, intravenous administration of 30 mg/kg cimetidine increased the gastric pH to 6.5 at 30 min after administration; then the pH returned to 1.9, normal condition, 4 h after administration. Intraperitoneal administration of TAK-438 at 0.7 mg/kg significantly inhibited histamine-stimulated acid secretion after pretreatment with or without cimetidine (Fig. 6A). The inhibition rates of TAK-438 with or without cimetidine pretreatment were 76 or 64%, respectively. On the other hand, intraperitoneal administration of lansoprazole at 1 mg/kg

**Discussion**

We reported previously that TAK-438 is a novel P-CAB that inhibits H⁺, K⁺-ATPase in a reversible and K⁺-competitive manner and exerts potent inhibition of gastric acid secretion in rats (Hori et al., 2010). The results of this study showed that in rats and dogs TAK-438 exerts a more potent and longer-lasting inhibitory effect on gastric acid secretion
TABLE 1
Pharmacokinetic parameters of TAK-438F in rats and dogs given a single oral and intravenous dose of [14C]TAK-438 or TAK-438
Data show mean value ± S.E. (rats, n = 3; dogs, n = 4) except BA (mean). The \( C_{\text{max}} \) after intravenous administration denotes the \( C_{\text{min}} \).

<table>
<thead>
<tr>
<th>Dosing Route</th>
<th>Dose</th>
<th>( T_{\text{max}} )</th>
<th>( C_{\text{max}} )</th>
<th>( t_{\frac{1}{2}} )</th>
<th>( \text{AUC}_{\text{conc}} )</th>
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<td>Rat</td>
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<td>1.3 ± 0.1</td>
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<td>60 ± 6</td>
<td>1.2 ± 0.0</td>
<td>99 ± 4</td>
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</tr>
<tr>
<td>Dog</td>
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<td>30 ± 6</td>
<td>1.1 ± 0.1</td>
<td>68 ± 16</td>
<td>52</td>
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<tr>
<td></td>
<td>Intravenous</td>
<td>0.1 ± 0.0</td>
<td>26 ± 2</td>
<td>1.2 ± 0.1</td>
<td>44 ± 2</td>
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Fig. 5. Concentrations of TAK-438F in plasma and the stomach after oral administration of 2 mg/kg [14C]TAK-438 in rats. Plasma and stomach samples were obtained 0.25, 1, 2, and 24 h after dosing. Plasma and gastric concentrations of TAK-438F were shown as ng/ml and ng/g, respectively. Each point represents the mean ± S.E. from three rats.

Fig. 6. Effect of cimetidine pretreatment on the inhibitory activity of TAK-438 (A) and lansoprazole (B) against histamine-stimulated acid secretion in rats. Cimetidine (30 mg/kg) or vehicle was injected intravenously. Fifteen minutes later, TAK-438 at 0.7 mg/kg, lansoprazole at 1 mg/kg, or vehicle were intraperitoneally administered. Pylorus ligation and histamine 2HCl (30 mg/kg s.c.) was administered 4 h after drug or vehicle administration. Gastric contents were collected 3 h after histamine administration, and the total acid output was calculated. Each column represents the mean ± S.E. from 9 or 10 rats. The statistical significance of the difference was determined by Student’s t test. *: \( p < 0.05 \) versus vehicle.

Fig. 7. Effect of TAK-438 and lansoprazole on the pH of the gastric perfusate in anesthetized rats. The stomach was perfused with saline at a rate of 0.5 ml/min, and the pH was measured continuously. TAK-438 at 1 mg/kg, lansoprazole at 10 mg/kg, or vehicle was administered intravenously after the pH stabilized. Histamine 2HCl (8 mg/kg/h) was intravenously infused 30 min after drug or vehicle administration. Each point represents the mean ± S.E. from four rats.

than lansoprazole. It was reported that the duration of the inhibitory effect of CS-526 and SCH28080, the aforementioned P-CABs, on gastric acid secretion was shorter than that of PPIs in rats and dogs (Long et al., 1983; Ito et al., 2007), and the effect of TAK-438 was more potent and had a longer duration than that of SCH28080 in rats (Hori et al., 2010). These findings clearly indicate that TAK-438 is a novel P-CAB that exerts a stronger and longer-lasting inhibitory effect on gastric acid secretion than PPIs and the aforementioned P-CABs. Pharmacokinetic studies of TAK-438 in rats showed that the concentration of TAK-438F in the stomach was much higher than that in the plasma after oral administration of [14C]TAK-438. TAK-438F was present in the gastric tissue even 24 h after administration; however, it rapidly disappeared from the plasma. The long-lasting antisecretory effect of TAK-438 seems to be associated with its high concentration in the stomach, but this effect cannot be explained by plasma concentration. The plasma \( t_{\frac{1}{2}} \) of TAK-438 in dogs was 1.1 h, which is also short. TAK-438 may exert a potent and long-lasting antisecretory effect through high accumulation and long retention in the gastric glands because it strongly accumulates in cultured rabbit gastric glands and shows an inhibitory effect on forskolin-stimulated acid formation for more than 8 h after washout (Matsukawa et al., 2011). P-CABs are instantly protonated in an acidic environment; they bind to and inhibit H+, K+-ATPase in this protonated form (Andersson and Carlsson, 2005). The pH values of P-CABs such as SCH28080 and A2D0865 are 5.6 and 6.1, respectively, whereas those of PPIs range from 3.8 to 5.0 (Bell et al., 1992; Gedda et al., 2007). This property allows P-CABs to concentrate more than PPIs in acidic environments such as the secretory canaliculi of the gastric parietal
unaffected by ambient pH; it inhibited gastric H+ secretion with or without cimetidine pretreatment. TAK-438 was also unaffected by the gastric secretory state in vivo. The pKa values of TAK-438 range from 3.8 to 5.0. It has been reported that the uptake of PPIs into gastric glands was higher in acidic conditions when stimulated with an acid secretagogue than that in neutral conditions (Felle-nius et al., 1982; Matsukawa et al., 2011). Thus, the effect of PPIs is altered by the gastric acid secretory state (De Graef and Woussen-colle, 1986). In this study, lansoprazole showed a significant antisecretory effect without cimetidine pretreatment, but this effect disappeared when cimetidine was administered before lansoprazole. PPIs cannot be transformed to their active compounds under neutral conditions because of pretreatment with cimetidine. Furthermore, PPIs cannot accumulate in the secretory canaliculi of the parietal cell under neutral conditions because the pKa values of PPIs range from 3.8 to 5.0. It has been reported that the uptake of PPIs into gastric glands was higher in acidic conditions when stimulated with an acid secretagogue than that in neutral conditions (Felle-nius et al., 1982; Matsukawa et al., 2011). Thus, the effect of PPIs is altered by the gastric acid secretory state. On the other hand, TAK-438 showed a significant antisecretory effect with or without cimetidine pretreatment. TAK-438 was unaffected by ambient pH; it inhibited gastric H+, K+-ATPase activity and accumulated in cultured gastric glands in acidic and neutral conditions in vitro (Hori et al., 2010; Matsukawa et al., 2011). The results in this study indicate that inhibitory effect of TAK-438 on gastric acid secretion is also unaffected by the acid secretory state in vivo. The pKa value of TAK-438 is 9.4, which is higher than that of PPIs and the aforementioned P-CABs, thus only TAK-438 exists mostly in the protonated active form in gastric glands even in neutral conditions and exerts its inhibitory activities for gastric acid secretion.

We reported previously the effect of TAK-438 on the pH of the gastric perfusate under histamine stimulation; the pH was approximately 2 when TAK-438 was administered to anesthetized rats (Hori et al., 2010). In this study, we administered TAK-438 and lansoprazole with an approximate pH of 4 without any secretagogue stimulation and measured the pH of the gastric perfusate. Furthermore, we evaluated the effect of both agents on the change in pH caused by intravenous infusion of histamine initiated 30 min after drug administration. Intravenous administration of TAK-438 at 1 mg/kg increased the pH from 4 to 5.7 30 min after TAK-438 administration. It was noteworthy that the increase in pH by TAK-438 was unaffected by subsequent histamine infusion, and the pH was maintained at approximately 6 for more than 4 h. The potent and long-lasting antisecretory activity of TAK-438 was maintained even when gastric acid secretion was stimulated after TAK-438 administration. On the other hand, intravenous administration of lansoprazole at 10 mg/kg increased the pH, but this effect diminished after histamine infusion was initiated. The pH decreased to 2.9 90 min after lansoprazole administration. In our previous study, the same dose of lansoprazole given under the pH approximately 2 condition by histamine infusion increased the pH; however, it decreased to 4.4 90 min after lansoprazole administration (Hori et al., 2010). The effect of lansoprazole was attenuated when it was administered under the basal acid condition compared with its effect in the actively acid-secreting state. PPIs are activated in acidic condition, and the active compound can inhibit only the H+, K+-ATPase that has already been transferred to the apical membrane and activated (Forte and Yao, 1996). The resting proton pumps are internalized in a tubulovesicle that is not transferred to the apical membrane, and PPIs cannot inhibit these resting proton pumps because the active form of PPIs cannot penetrate the membrane (Nagaya et al., 1990). The results of this study suggest that lansoprazole inhibited the H+, K+-ATPase that had been activated, but it failed to inhibit the H+, K+-ATPase newly transferred to the apical membrane and activated at certain periods of time after lansoprazole administration. The latter finding can be explained by the short plasma t1/2 of PPIs and their rapid degradation in the secretory canaliculi. PPIs exhibit maximal activity after treatment with daily doses for 4 to 5 consecutive days, and they cannot completely control night-time acid secretion (Tytgat, 2001; Ang and Fock, 2006). These shortcomings of PPIs correlate with their property of inhibiting only activated H+, K+-ATPase (Parsons and Keeling, 2005). In contrast, TAK-438 maintained its potent antisecretory activity even when gastric acid secretion was stimulated after TAK-438 administration. The results of the pharmacokinetic studies show that TAK-438 can remain in the gastric tissue in high concentrations after its plasma concentration becomes zero. It is thought that TAK-438 can be present for a long time in the protonated form regardless of the pH in the secretory canaliculi. Therefore, TAK-438 binds to the H+, K+-ATPase newly transferred to the apical membrane and maintains its potent antisecretory activity. As a result of these properties, TAK-438 has a potential to exert its full inhibitory activity from the first dose and provide complete control of night-time acid secretion in patients with gastroesophageal reflux disease. Furthermore, the potent and long-lasting antisecretory activity of TAK-438 may be effective for patients having Barrett’s esophagus or scleroderma or those who are refractory to or insufficiently controlled by treatment with PPIs (Katz et al., 2006).

In conclusion, TAK-438 exerted a more potent and longer-lasting antisecretory effect than lansoprazole through high accumulation and slow clearance from the gastric tissue. The effect of TAK-438 was unaltered by the gastric secretory state, unlike that of PPIs. Therefore, TAK-438 can provide a novel mechanism of action for improving the present PPI-based treatment of acid-related diseases.

Acknowledgments

We thank Dr. Yasuhiro Tsukimi for helpful suggestions and Toshima Kyutoku and Kozo Matsushita for technical assistance.
De Graef J and Woussen-Colle MC (1986) Influence of the stimulation state of the
References
chi, and Inatomi.

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References
therapeutic strategy for the treatment of acid-related diseases. Pharmacol Ther
Ang TL and Fock KM (2006) Nocturnal acid breakthrough: clinical significance and
suppression for management of gastro-esophageal reflux disease. Digestion 51(Suppl 1):
59–67.
Dammann HG and Burkhardt F (1999) Pantoprazole versus omeprazole: influence on
meal-stimulated gastric acid secretion. Eur J Gastroenterol Hepatol 11:1277–
1282.
De Graef J and Woussen-Colle MC (1986) Influence of the stimulation state of the
parietal cells on the inhibitory effect of omeprazole on gastric acid secretion in
Fellenius E, Elander B, Wallmark B, Helander HF, and Berglind T (1982) Inhibition of
acid secretion on isolated gastric glands by substituted benzimidazoles. Am J Physiol
243:G505–G510.
Forte JG and Yao X (1996) The membrane-recruitment-and-recycling hypothesis of
Frazzoni M, De Micheli E, Grisendi A, and Savarino V (2003) Effective intra-
gastric administration of omeprazole to prevent in long-term users of nonsteroidal anti-
inflammatory drugs: results of a double-blind, randomized, multicenter, active- and
Ganser AL and Forte JG (1973) K+-stimulated ATPase in purified microsomes of
action of AZD9085, a K+-competitive inhibitor of gastric H+-K+-ATPase. Biochem
Pharmacol 73:198–205.
5265.
Graham DY, Agrawal NM, Campbell DR, Haber MM, Collis C, Lukasik NL, Huang
B, and NSAI-Associated Gastric Ulcer Prevention Study Group (2002) Ulcer
prevention in long-term users of nonsteroidal anti-inflammatory drugs: results of a
Heidenhain R (1879) Uber die absonderung der fundusdruehen des magens. Arch
pyrrolo[3,2-3]-N-methylmethanamine monofumarate (TAK-438), a novel and potent
Ito K, Kinoshita K, Tomizawa A, Inaba F, Morikawa-Inamata Y, Makino M, Tabata
7-(4-fluorobenzoyloxy)-2,3-dimethyl-1-[(5S,2S)-2-methylcyclopropyl]methyl]-1H-
Kabirul CS, Deict D, Lauritsen K, Malfurtheiner P, Denison H, Franzén S, and
Katz PO, Hatlebakk JG, and Castell DO (2000) Gastric acidity and acid break-
through with twice-daily omeprazole or lansoprazole. Aliment Pharmacol Ther
14:709–714.
Katz PO, Scheiman JM, and Barkun AN (2006) Review article: acid-related disease—
what are the unmet clinical needs? Aliment Pharmacol Ther 23:9–22.
Long JF, Chiu PJ, Derelanko MJ, and Steinberg M (1983) Gastric antisecretory and
cytoprotective activities of SCH28080. J Pharmacol Exp Ther 226:114–120.
Malfurtheiner P, Mossier J, Fischbach W, Layer P, Leodolter A, Stolte M, Demleit-
Mendlein J and Sachs G (1996) Interaction of a K+-competitive inhibitor, a substitu-
ted imidazol[1,5a]pyridine, with the phospho- and dephosphoenzyme forms of
Nagaya H, Satoh H, Kudo K, and Maki Y (1989) Possible mechanism for the
inhibition of gastric (H + K+)-adenosine triphosphatase by the proton pump in-
Nagaya H, Satoh H, and Maki Y (1996) Possible mechanism for the inhibition of acid
Parsons M and Keeling DJ (2005) Novel approaches to the pharmacological block-
Robinson M (2005) Proton pump inhibitors: update on their role in acid-related
non-electrogenic H+-2-methylcyclopropyl]-1-[5-(2-Fluorophenyl]-pyrrolo[2,3-
of the gastric acid pump: the H+-K+-ATPase. Annu Rev Pharmacol Toxicol 35:
277–295.
Simon WA, Herrmann M, Klein T, Shin JM, Huber R, Senn-Bilfinger J, and Postius
Vakil N (2004) Review article: new pharmacological agents for the treatment of
Wallmark B, Brivin C, Frykland J, Munson K, Jackson R, Mendlein J, Rabon E,
and Sachs G (1987) Inhibition of gastric H+ -K+-ATPase and acid secretion by
SCH28080, a substituted pyridyl(1,2-
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