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

Yasunobu Hori, Jun Matsukawa, Toshiyuki Takeuchi, Haruyuki Nishida, Masahiro Kajino, and Nobuhiro Inatomi

Pharmaceutical Research Division (Y.H., J.M., T.T., H.N., N.I.), and Chemistry, Manufacturing, and Controls Center (M.K.), Takeda Pharmaceutical Company Limited, Osaka, Japan

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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-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine 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][sulfanyl]-1H-benzimidazol-1-yl]pyridine-6-carboxyamide; AUCacid, area under the acid secretion-time curve from 0 to 25.5 h; AUCconc, area under the plasma concentration-time curve from 0 to 24 h; BA, bioavailability; HPLC, high-performance liquid chromatography; CI, confidence intervals.

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][sulfanyl]-1H-benzimidazole], omeprazole, rabeprazole, and pantoprazole inhibited gastric H⁺, K⁺-ATPase by covalently binding to its sulfhydryl 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

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.
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 (t½) 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-(Cyanomethyl)-2-methyl-8-(phenylimethoxy)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 (Mendlein 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., imidazopyridines such as SCH28080 (Wallmark et al., 1987) and 8-[(2,6-dimethylbenzyl)amino]-N-[2-hydroxyethyl]-2,3-dimethylimidazo[1,2-a]pyridine-6-carboxamide (AZD0865) (Gedda et al., 2007), pyrimidines (Yu et al., 2004), imidazaphenyridines (Simon et al., 2007), and pyrrolopyridazines such as 7-(4-fluorobenzoxo)-2,3-dimethylimidazo[1,2-a]-pyridine-6-carboxamide (CS-526) (Ito et al., 2007), have been evaluated as P-CABs. These compounds have higher pKᵢ 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 and Keeling, 2005; Kahrlas et al., 2007).

We have discovered a novel P-CAB, 1-[5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine monofumarate (TAK-438), which is a pyrrole derivative with a chemical structure that completely differs from that of the P-CABs developed to date. TAK-438 demonstrated a potent inhibitory effect on the gastric H⁺, K⁺-ATPase in a reversible and K⁺-competitive manner, and the effect was unaltered by pH in vitro. TAK-438 exerted a potent and long-lasting inhibitory activity on the gastric acid secretion in rats (Hori et al., 2010). High levels of TAK-438 accumulated in the resting and activated gastric glands in vitro. In addition, slow clearance of TAK-438 was observed, and the inhibitory effect of TAK-438 was maintained even after the glands were washed out (Matsukawa et al., 2011).

In this study, we evaluated the effect of TAK-438 on the gastric acid secretion in rats and dogs under various conditions and on the pH of rat gastric perfusate in comparison with lansoprazole to characterize the antisecretory effects of TAK-438. In addition, we analyzed the pharmacokinetics of TAK-438 in rats and dogs to clarify whether the long-lasting activity of TAK-438 could be explained by its pharmacokinetic properties.

### 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-pyrrol-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 (Oriental Yeast Co., Ltd., Tokyo, Japan), each weighing 9.2 to 10.4 kg, were used in the experiments. They were fed laboratory chow (CR-LPF for rats, Oriental Yeast Co., Ltd.; CD-5M for dogs, CLEA Japan, Inc.), had free access to water, and were housed in temperature- and humidity-controlled rooms (18–28°C, 40–70%) with 12-h light-dark cycles. The rats were fasted for 24 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 gastropiploic 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 pre-dosing 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 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 to obtain the plasma fraction. In the experiment measuring plasma and stomach concentrations of TAK-438 in rats, blood and stomach samples were obtained 0.25, 1, 2, and 24 h after dosing. The gastric contents were washed out, and the stomach was homogenized in distilled water to prepare a homogenate at a concentration of 20% (w/v). The plasma and stomach tissues were frozen at –80°C until analysis.

**Analytical Methods**

**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.

**Measurement of Unchanged Compound.** The free base of TAK-438 (TAK-438F) in the plasma was quantified by HPLC/liquid chromatography-tandem mass spectrometry. Ammonium acetate and diethyl ether were added to the plasma, and the samples were mixed and centrifuged. The organic layers were evaporated to dryness under a nitrogen gas stream. The residues were reconstituted with a reconstitution solution, and the sample solution was injected into the liquid chromatography-tandem mass spectrometry system (API 4000 and 4000 Q TRAP; Applied Biosystems by Life Technologies, Tokyo, Japan). [14C]TAK-438F in the plasma and stomach was extracted using acetonitrile, and the samples were mixed and centrifuged. The supernatant was evaporated until dry, under a nitrogen gas stream. The residues were reconstituted with acetonitrile, and the sample solution was injected into the HPLC system.

**Statistics.** Data were expressed as mean ± S.E. and calculated using the Excel software program (Microsoft, Redmond, WA). 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 Shiry-ley-Williams 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 (AUCₜₐcdc) 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 sequentively 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 (AUCₚₐcdc) were calculated by linear regression analysis and the trapezoidal rule, respectively. Bioavailability (BA) was determined after dose normalization.

**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 of TAK-438 and lansoprazole were 0.86 mg/kg (95% CI, 0.69–1.05 mg/kg) and 1.14 mg/kg (95% CI, 0.79–1.78 mg/kg), respectively. The ratio of the ID₅₀ values of TAK-438 and lansoprazole was 1:1.33.
**Fig. 1.** Effect of TAK-438 (A) and lansoprazole (B) on histamine-stimulated acid secretion in rats. Anesthetized rats were administered TAK-438, lansoprazole, and the vehicle orally 1 h before pylorus ligation and histamine 2HCl (30 mg/kg s.c.) 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 seven or eight rats. Statistical significance of the difference was determined by Student’s t test. *, p < 0.05 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 exhibited equivalent potency. TAK-438 at 3 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 effect 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 Heidenhain 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 h after administration; however, the inhibitory effect diminished at 3 h. 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 [^{14}C]TAK-438 at 2 mg/kg to rats, TAK-438F 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 1.3 h; the AUC_{acid} of TAK-438F was 27 ng · h/ml. After intravenous administration of [^{14}C]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_{acid} 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_{acid} were 1.4 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_{min}, t_{1/2}, and AUC_{acid} were 26 ng/ml, 1.2 h, and 44 ng · h/ml, respectively. BA of TAK-438F after oral administration in dogs was 52% (Table 1).

<table>
<thead>
<tr>
<th>Time after drug administration (h)</th>
<th>Inhibition (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–4</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>5–8</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>9–12</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>24–27</td>
<td>100</td>
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</tr>
</tbody>
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**Fig. 2.** Time course of the inhibitory effect of TAK-438 (A) and lansoprazole (B) on histamine-stimulated acid secretion in rats. Pylorus ligation and histamine 2HCl (30 mg/kg s.c.) administration was performed 1, 5, 9, and 24 h after the administration of 3 mg/kg TAK-438, 4 mg/kg lansoprazole, or vehicle. Gastric contents were collected 3 h after histamine administration, and the total acid output was calculated. The acid output in the vehicle group was 142 to 576 µEq/3 h. Each column represents the mean ± S.E. from four or five rats. Statistical significance of the difference was determined by Student’s t test. *, p < 0.05 versus vehicle at each time period.
438 at 2 mg/kg to rats, TAK-438F concentrations in the plasma at 0.25, 1, 2, and 24 h were 23, 11, 5, and 0 ng/ml, respectively (Fig. 5). The concentrations of TAK-438F in the stomach at 0.25, 1, 2, and 24 h were 2412, 957, 941, and 20 ng/g, respectively (Fig. 5). The concentrations of TAK-438F in the plasma at 0.25, 1, 2, and 24 h were 23, 11, 5, and 0 ng/ml, respectively (Fig. 5).

**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 significantly inhibited histamine-stimulated acid secretion, but the inhibitory activity of lansoprazole was not observed after pretreatment with cimetidine (Fig. 6B).

**Effect of TAK-438 and Lansoprazole on the pH of the Gastric Perfusate in Anesthetized Rats.** The effect of TAK-438 and lansoprazole on the pH of the gastric perfusate is shown in Fig. 7. The pH of saline in this experimental condition was 6.0 to 6.3 and that of the gastric perfusate without a secretagogue was approximately 4.2. The pH of the gastric perfusate was unaffected by vehicle administration for 30 min; the pH decreased after the initiation of intravenous infusion of histamine. The pH was 2.2 at 60 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 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 initiation of histamine infusion were 3.6 and 2.9, respectively.

**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.
Cimetidine (+)

The data show mean value ± S.E. (rats, n = 3; dogs, n = 4) except BA (mean). The C\textsubscript{max} after intravenous administration denotes the C\textsubscript{max} after intravenous administration.

**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

<table>
<thead>
<tr>
<th>Dosing Route</th>
<th>Dose</th>
<th>T\textsubscript{max}</th>
<th>C\textsubscript{max}</th>
<th>t\textsubscript{1/2}</th>
<th>AUC\textsubscript{0-24}</th>
<th>BA</th>
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</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Oral</td>
<td>2</td>
<td>0.3 ± 0.0</td>
<td>17 ± 3</td>
<td>1.3 ± 0.1</td>
<td>27 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intra.</td>
<td>0.75</td>
<td>60 ± 6</td>
<td>1.2 ± 0.0</td>
<td>90 ± 4</td>
</tr>
<tr>
<td>Dog</td>
<td>Oral</td>
<td>0.3</td>
<td>1.0 ± 0.0</td>
<td>30 ± 6</td>
<td>1.1 ± 0.1</td>
<td>68 ± 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intra.</td>
<td>0.1</td>
<td>26 ± 2</td>
<td>1.2 ± 0.1</td>
<td>44 ± 2</td>
</tr>
</tbody>
</table>

**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\textsubscript{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\textsubscript{+}, K\textsuperscript{+}-ATPase in this protonated form (Andersson and Carlsson, 2005). The pH\textsubscript{50}-values of P-CABs such as SCH28080 and AZD0865 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...
cell. The $pK_a$ of TAK-438 is 9.4, higher than that of the aforementioned P-CABs. Thus TAK-438 is highly concentrated and remains in the protonated form in the secretory canaliculi of the gastric parietal cell. Furthermore, the binding site of TAK-438 to the gastric H⁺, K⁺-ATPase has been demonstrated using the predictive three-dimensional model (Shin et al., 2010). The model demonstrated hydrogen binding of the sulfonil moiety of TAK-438 to Tyr799 and deep penetration caused by methylamine binding to the carboxylic acid residues containing the proton that binds at the site enclosed by Glu795, Glu820, and Asp824, which has been extruded by entry of Lys791 and replaced by K⁺ during the transport cycle. The simulation from this model suggests that TAK-438 binds tightly to H⁺, K⁺-ATPase and shows a long duration of action despite reversible binding.

PPIs are prodrugs that exert their inhibitory activity after undergoing molecular rearrangement under acidic conditions in parietal cells. This rearrangement allows them to covalently bind to gastric H⁺, K⁺-ATPase (Sachs et al., 1995). Therefore, PPIs need acidic conditions to inhibit acid secretion, and this inhibitory activity is strongly affected 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 $pK_a$ values of PPIs range from 3.8 to 5.0. It has been reported that the uptake of PPIs into gastric glands is higher in acidic conditions when stimulated with an acid secretagogue than that in neutral conditions (Fellenius 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⁺ secretion with or without cimetidine pretreatment. TAK-438 was highly concentrated under acidic conditions in parietal cells. Furthermore, the binding site of TAK-438 to the gastric H⁺, K⁺-ATPase has been demonstrated using the predictive three-dimensional model (Shin et al., 2010). The model demonstrated hydrogen binding of the sulfonil moiety of TAK-438 to Tyr799 and deep penetration caused by methylamine binding to the carboxylic acid residues containing the proton that binds at the site enclosed by Glu795, Glu820, and Asp824, which has been extruded by entry of Lys791 and replaced by K⁺ during the transport cycle. The simulation from this model suggests that TAK-438 binds tightly to H⁺, K⁺-ATPase and shows a long duration of action despite reversible binding.

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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 (Fortet and Yeo, 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 already been 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 $t_{1/2}$ 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 Keling, 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.

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