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A comparative study on the modes of action of TAK-438, a novel potassium-competitive acid blocker, and lansoprazole in primary cultured rabbit gastric glands

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ABSTRACT

TAK-438 is a novel potassium-competitive acid blocker (P-CAB) type antisecretory agent that reversibly inhibits gastric H^+, K^+-ATPase. Previously, we showed that TAK-438 has superior efficacy compared to lansoprazole, a proton pump inhibitor, in the inhibition of acid secretion *in vivo*. In this study, we investigated the differences in the mode of actions of the two drugs using primary cultured rabbit gastric glands. TAK-438 and lansoprazole inhibited gastric acid formation in acutely isolated gastric glands (IC_{50} values, 0.30 and 0.76 μM, respectively). In cultured gastric glands that were preincubated with TAK-438, the inhibitory effect on forskolin-stimulated acid formation was augmented over the incubation period, whereas the inhibitory effect of lansoprazole was not affected by time of incubation. Next, we evaluated the durations of the actions of TAK-438 and lansoprazole after gastric glands were incubated with either drug for 2 h followed by washout. Even 8 h after the drug washout, TAK-438 at higher concentrations inhibited acid formation, but the inhibitory effect of lansoprazole disappeared immediately after washout. Additionally, only a small amount of [^{14}C] lansoprazole accumulated in resting glands, and this accumulation was enhanced by treatment with 1 μM of forskolin. In contrast, high levels of [^{14}C] TAK-438 accumulated in both resting and forskolin-treated glands. Furthermore, a 2-h preincubation followed by washout demonstrated a slow clearance of [^{14}C] TAK-438 from the glands. These findings suggest that TAK-438 exerts a longer and more potent antisecretory effect than lansoprazole as a result of its high accumulation and slow clearance from the gastric glands.

1. Introduction

Proton pump inhibitors (PPIs), including lansoprazole, omeprazole, rabeprazole, and pantoprazole, inhibit H^+, K^+-ATPase, the acid-secreting enzyme located in the gastric parietal cells, by covalent and irreversible bonds [1,2]. PPIs inhibit gastric acid secretion more strongly and for longer periods of time than histamine H_2 receptor antagonists because they block the final step of gastric acid secretion. PPIs are widely used to treat acid-related diseases, including *gastroesophageal reflux disease* (GERD) and peptic ulcers. Although PPIs are now the mainstay of therapy for acid-related diseases, several areas for improvement still remain [3,4]: (1) as all PPIs are acid-labile and, therefore, are administered in acid-protected formulations, such as enteric-coated tablets, their onset of action and efficacy are greatly affected by gastric emptying; (2) their onset of action is slow, with 3–5 days of treatment needed to achieve full efficacy; (3) there is a large variation in efficacy among patients because of CYP2C19 metabolism [5,6]; and (4) PPIs are unable to continuously control acid secretions for a period of 24 h, even when administered twice daily [7]. Furthermore, their inhibition of nighttime acid secretion is inadequate in some patients [8]. Agents that increase gastric pH more quickly, to a greater extent, and with longer duration compared to PPIs offer clinical advantages.

For improved intragastric pH control, several strategies other than PPIs have been investigated. Potassium-competitive acid blocker (P-CAB) type H^+, K^+-ATPase inhibitors hold promise for better treatment of acid-related diseases. P-CABs, as their name suggests, inhibit H^+, K^+-ATPase activity in gastric parietal cells reversibly and in a potassium-competitive manner. In general, P-CABs are stable in acidic environments, and they do not exhibit acid-dependent activation like PPIs; therefore, an early onset of action is expected with P-CAB treatment [9]. P-CABs bind to the E_2P isoform of the H^+, K^+-ATPase enzyme; this mechanism allows for rapid inhibition of the pump without the need for luminal surface

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acidity because the pump is blocked in mid-cycle. SCH28080 is the first P-CAB prototype that showed a strong inhibitory potential, especially in *in vitro* experiments [10,11]. However, the duration of its antisecretory activity was shorter than that of PPIs or an H_2 blocker in clinical studies [12]. Clinical development of SCH28080 was discontinued because of this shorter duration and its hepatotoxicity [13,14]. AZD0865 is a more recently developed P-CAB type compound that showed strong efficacy, equivalent to esomeprazole, for the healing of reflux esophagitis and for symptom control of non-erosive reflux disease [9,15,16]. However, use of this compound was also discontinued because of its hepatotoxic effect.

Because AZD0865 is *hydrophobic* and a weak base with pK_a of 6.1, Andersson and Carlsson [17] postulated that it theoretically would accumulate 100,000 times higher in the acidic spaces of the parietal cell canaliculi than in the plasma. However, the high concentration of AZD0865 in intracellular canaliculi was only theoretically predicted and was not proven. Moreover, no report has directly reported differences in PPI and P-CAB accumulation within the intracellular canaliculi of parietal cells. Furthermore, the relationship between how much compound accumulates in the parietal cells and the level of inhibition of acid secretion *in vivo* has not been fully investigated in PPIs or P-CABs.

TAK-438 is a newly synthesized P-CAB type compound that reversibly inhibits H^+, K^+-ATPase in a potassium-competitive manner [18]. It exerted a stronger and more potent inhibition of acid secretion compared to lansoprazole in *in vivo* experiments, including those in *anesthetized rats*. It was formerly thought that P-CAB type compounds exert a short duration of inhibition because they reversibly inhibit H^+, K^+-ATPase, despite their early onset of action. However, treatment with TAK-438 (1 mg/kg i.v.) has been demonstrated to exert a higher and a longer-lasting elevation in the pH levels of gastric perfusate in histamine-stimulated, anesthetized rats compared to treatment with 3 mg/kg SCH28080 or 10 mg/kg lansoprazole [18]. These data indicate that TAK-438 has stronger potential as an inhibitor of acid secretion than PPIs. In this study, in order to elucidate a mechanism accounting for the longer *duration* of antisecretory activity observed with TAK-438 treatment than with lansoprazole treatment *in vivo*, we investigated the potential differences in the accumulation and clearance properties of the two drugs using cultured rabbit gastric glands. The use of cultured gastric glands, which are different from acutely isolated gastric glands, allowed us to examine accumulation and clearance of these compounds over a few days. Therefore, the cultured gastric glands would enable us to effectively analyse the efficacy and concentration of H^+, K^+-ATPase inhibitors for a long period. This is the first report that clearly compares the accumulation and clearance properties of TAK-438 and lansoprazole using cultured rabbit gastric glands.

2. Materials and methods

2.1. Drugs and chemicals

TAK-438 and lansoprazole were synthesized at the Takeda Pharmaceutical Company Limited. [^{14}C] TAK-438 (specific activity: 5.16 MBq/mg, 2.39 GBq/mmol), [^{14}C] lansoprazole (specific activity: 5.85 MBq/mg, 2.17 GBq/mmol), and [^{14}C-dimethylamine]aminopyrine (1.85 MBq/mL) were obtained from GE Healthcare (Buckinghamshire, England). All chemicals were reagent grade and obtained from Sigma (Tokyo, Japan), Wako (Osaka, Japan), or Invitrogen (Tokyo, Japan) except where otherwise noted.

2.2. Animals

All animal experiments were carried out in accordance with ethical guidelines established by the Animal Care and Use Committee at the Takeda Pharmaceutical Company Limited. Male Japanese white rabbits (Oriental Yeast Co., Osaka, Japan) that were 10–13-weeks-old were used.

2.3. Membrane preparations and the H^+, K^+-ATPase activity assay

Membranes enriched for the tubulovesicles of resting parietal cells were purified from resting rabbit gastric mucosa as described by Omi et al. [19]. Briefly, rabbit stomach mucosa was homogenized in homogenizing buffer (125 mM mannitol, 40 mM sucrose, 1 mM EDTA, and 5 mM PIPES; pH 6.7). The supernatant of the homogenate, obtained after centrifugation at $14{,}500 \times g$ for 10 min, was further centrifuged at $100{,}000 \times g$ for 45 min to obtain the microsomal fraction. The microsomes were suspended in the homogenizing buffer and then layered on top of a sucrose gradient and centrifuged at $100{,}000 \times g$ for 2 h. The material on top of the 21% sucrose layer was harvested and stored at −80 °C until use. Gastric H^+, K^+-ATPase activity was determined by the method of Tsukimi et al. [20] with a slight modification. Briefly, fractionated microsomes (13.5 μg protein) were preincubated for 30 min at 37 °C in the incubation buffer (62.5 mM HEPES–Tris, 6.25 mM KCl, and 2.5 mM $MgSO_4$; pH 6.5), in the presence of test compounds and 20 μM valinomycin. The reaction was initiated by the addition of 2 mM ATP (final concentration), ran at 37 °C for 15 min, and stopped by the addition of 10% chilled trichloroacetic acid. K^+-stimulated activity was estimated in the presence of 6.25 mM KCl and 2.5 mM $MgSO_4$. The amount of released inorganic phosphate was measured by the method of Fiske and Subbarow [21].

2.4. Isolation of gastric glands

Gastric glands were isolated from rabbits using the method of Berglindh and Obrink [22]. Isolated glands suspended in control medium containing 132.4 mM NaCl, 5.4 mM KCl, 1.2 mM $MgSO_4$, 1.0 mM $CaCl_2$, 25 mM HEPES-Na, 2 mg/mL glucose, and 1 mg/mL bovine serum albumin (pH 7.4) were used for the gastric acid formation assay.

2.5. Cell culture

Gastric glands were cultured according to the method described by Chew et al. [23] with slight modifications [24]. Briefly, isolated gastric glands were washed 4 times and incubated for 10 min in medium B (Dulbecco's modified Eagle's medium/F-12 plus 2 mg/mL bovine serum albumin, 100 μg/mL gentamicin; pH 7.4), to which 25 μg/mL amphotericin B was added to prevent yeast infection. Afterward, the glands were washed once in medium A (medium B plus 8 nM EGF, 10 nM hydrocortisone, 800 nM insulin, 3.1 nM sodium selenite, 2.6 μg/mL transferrin, 0.93 μg/mL ethanolamine, 5 μg/mL geneticin, and 8 μg/mL amphotericin B). Glands were incubated for 30 min in a flask precoated with 10% fetal bovine serum/Dulbecco's modified Eagle's medium to exclude fibroblasts. Cells were then plated onto Matrigel (diluted 1:4) with water-coated coverslips and incubated at 37 °C in culture medium A.

2.6. Aminopyrine uptake assay for acutely isolated and cultured rabbit gastric glands

Acid formation in the acutely isolated rabbit gastric glands was monitored by the accumulation of a weak base, [^{14}C] aminopyrine [25]. Briefly, isolated gastric glands were diluted approximately 1:20 with the control medium (10–12 mg wet weight per sample) and then preincubated with vehicle (DMSO) or one of the test compounds at 37 °C for 30 min in a shaking water bath. Forskolin

(an acid secretagogue; 10 μM final concentration) and [^{14}C] aminopyrine were then added, and the glands were further incubated at 37 °C for 30 min. After incubation, each tube was centrifuged at 10,000 rpm for 2 min, and the aliquots of both the supernatant and overnight-dried pellet were counted in a liquid scintillation counter (DPM). The concentrations of the test compounds were 0.01, 0.03, 0.1, and 0.3 μM, and each well had 0.9 mg (wet weight) of gastric glands. For the experiments that examined the pretreatment effects of the drugs, cultured rabbit gastric glands were washed twice with control medium, and the culture medium was completely replaced with the control medium. Then, the cultured gastric glands were pretreated with compounds for 0, 30, 60, and 120 min. Next, 1 μM forskolin and 0.925 kBq/mL [^{14}C] aminopyrine were added, and the glands were further incubated for 30 min at 37 °C. For the measurement of the durations of actions of the compounds, after a 2 h preincubation period with the compounds, the culture dishes were washed twice with culture medium. Then, glands cultured for 0, 4, 8, and 24 h were washed twice with control medium and stimulated with 1 μM forskolin for 30 min in the presence of [^{14}C] aminopyrine at each time point. After incubation, glands were washed with control medium twice and lysed with the sample buffer (0.125 M Tris–HCl, 4% sodium dodecylsulfate, 20% glycerol, and 10% 2-mercaptoethanol; pH 6.8) at 60 °C in an oven. Lysed glands were added to scintillation vials. The dishes were washed with the sample buffer once, and this sample buffer was also added in the vials. The radioactivity levels of the lysed glands were then determined by a liquid scintillation counter (DPM).

2.7. Accumulation and clearance of [^{14}C] TAK-438 and [^{14}C] lansoprazole in cultured gastric glands

In order to directly evaluate the accumulation and clearance of TAK-438 or lansoprazole in the cultured gastric glands, we labeled each compound with ^{14}C. The cultured gastric glands were then treated with each compound. In all experiments, the concentrations of the labeled compounds were 0.01, 0.03, 0.1, and 0.3 μM. For the accumulation assay, cultured glands were incubated for 0, 15, 30, 60, and 120 min in the presence of DMSO (vehicle) or 1 μM forskolin in order to investigate whether stimulation of acid formation affects the accumulation of labeled compounds. To measure the clearance of each compound, cultured glands were pretreated with the labeled compound for 2 h, and then the dishes were washed twice with the culture medium. The glands were further incubated for 0, 4, 8, and 24 h, and the remaining radioactivity of each treatment group was measured by a liquid scintillation counter (DPM).

2.8. Statistics

All data are presented as the mean ± S.E. The results of the acid formation tests were expressed as a percentage of vehicle-treated control values. IC_{50} values were obtained by fitting the curves with a logistic sigmoid model using the SAS system.

3. Results

3.1. The effects of TAK-438 and lansoprazole on rabbit gastric H^+, K^+-ATPase

Rabbit H^+, K^+-ATPase activity was measured in the presence of TAK-438 or lansoprazole using rabbit gastric H^+, K^+-ATPase-rich vesicles. Both TAK-438 and lansoprazole inhibited H^+, K^+-ATPase activity in a concentration-dependent manner (Fig. 1). The IC_{50} values of TAK-438 and lansoprazole at pH 6.5 were 35 nM and 17 μM, respectively.

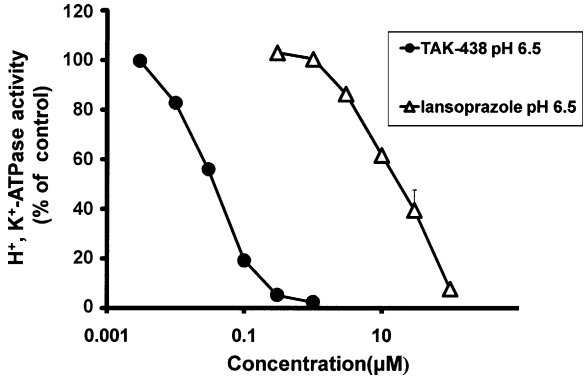

Fig. 1. The effects of TAK-438 and lansoprazole on purified rabbit gastric H^+, K^+-ATPase. Rabbit gastric tubulovesicular membrane was incubated with compounds at 37 °C in the presence of 20 mM valinomycin (pH 6.5). Each point represents mean ± S.E. of 3 different experiments.

3.2. Inhibition of acid formation in acutely isolated gastric glands

At the resting state, aminopyrine accumulation in acutely isolated gastric glands was very low, but in the presence of 10 μM forskolin, aminopyrine uptake dramatically increased. Within the concentration range of 0.01–0.1 μM, TAK-438 did not inhibit forskolin-stimulated acid formation; however, at concentrations of 0.30 μM and above, it strongly inhibited acid formation, and the IC_{50} value was 0.30 μM (Fig. 2). Concentrations of lansoprazole from 0.3 to 3 μM inhibited gastric acid formation in a concentration-dependent manner. The IC_{50} value of lansoprazole was 0.76 μM.

3.3. The effects of preincubation time on the inhibition of forskolin-stimulated acid formation by TAK-438 and lansoprazole in cultured rabbit gastric glands

In the TAK-438-treated group, forskolin-stimulated aminopyrine uptake decreased in a concentration-dependent manner. This was also time-dependent at lower concentrations. Moreover, the IC_{50} value also decreased as the preincubation time was increased (Fig. 3a and Table 1). In contrast, in the lansoprazole-pretreated group (0.01–0.3 μM), forskolin-stimulated acid formation and IC_{50} values were almost the same, irrespective of preincubation time. Especially, the IC_{50} value for TAK-438 was approximately 10-fold lower than that of lansoprazole, irrespective of the preincubation time in cultured glands (Fig. 3 and Table 1).

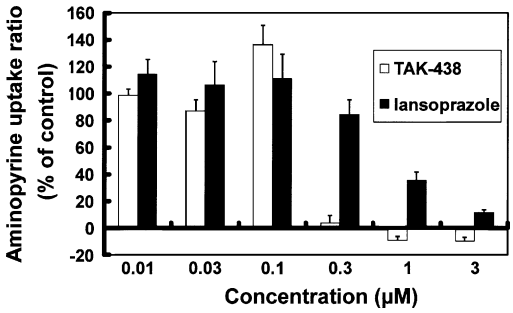

Fig. 2. The effects of TAK-438 and lansoprazole on forskolin-stimulated aminopyrine accumulation in isolated rabbit gastric glands. Aminopyrine uptake was measured in TAK-438 or lansoprazole-treated isolated rabbit gastric glands. Results were expressed as the mean ± S.E. of the percentage of vehicle-treated control. Each point represents mean ± S.E. of 4 different experiments.

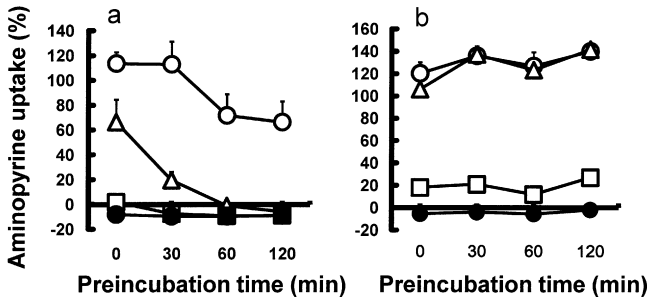

Fig. 3. The effects of preincubation with H^+, K^+-ATPase inhibitors on forskolin-induced aminopyrine accumulation in cultured rabbit gastric glands. Cultured gastric glands were preincubated with 0.01–0.3 μM TAK-438 (a) or lansoprazole (b) for 0–120 min (open circle, 0.01 μM; open triangle, 0.03 μM; open square, 0.1 μM; filled circle, 0.3 μM). After preincubation, [^{14}C] aminopyrine and 1 μM forskolin were added, and acid accumulation was examined by monitoring [^{14}C] aminopyrine uptake in gastric glands. The data represent the mean percentage of vehicle-treated control ± S.E. of 3 independent experiments.

Table 1
IC_{50} values of lansoprazole and TAK-438 in acutely isolated and cultured gastric glands ($N = 4$ acute; $N = 3$ cultured).

Preincubation	IC_{50} of TAK-438 (μM)		IC_{50} of lansoprazole (μM)	
Time (min)	Acute	Cultured	Acute	Cultured
0	N.D.	0.03	N.D.	0.10
30	0.30	0.03	0.76	0.09
60	N.D.	0.01	N.D.	0.10
120	N.D.	0.01	N.D.	0.10

N.D., not determined.

3.4. A comparison of the recovery of acid formation after treatment with TAK-438 or lansoprazole in cultured gastric glands

The recovery of forskolin-stimulated acid formation was examined after the cultured gastric glands were incubated with TAK-438 or lansoprazole for 2 h followed by replacement of the incubation buffer with control buffer. In the TAK-438-treated groups, acid formation was inhibited in a concentration-dependent manner immediately after the buffer exchange (Fig. 4a), and the forskolin-stimulated acid formation recovered slowly. It took almost 24 h for full recovery in the 0.3 μM TAK-438-treated group. However, in the lansoprazole-treated groups, there was no inhibition of acid formation immediately after washout, even in the groups treated with high concentrations of drug (Fig. 4b).

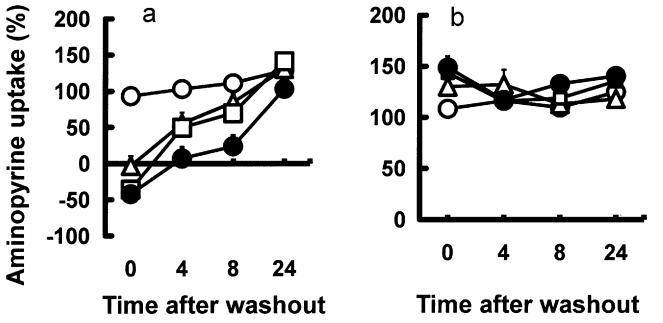

Fig. 4. The durations of inhibition of aminopyrine accumulation by lansoprazole or TAK-438 in cultured gastric glands. Cultured gastric glands were pretreated with 0.01–0.3 μM TAK-438 (a) or lansoprazole (b) for 120 min (open circle, 0.01 μM; open triangle, 0.03 μM; open square, 0.1 μM; filled circle, 0.3 μM). After preincubation, culture medium was exchanged with fresh medium, and the glands were further incubated for 0–24 h. Acid accumulation was measured by monitoring [^{14}C] aminopyrine uptake in gastric glands. Results are expressed as mean ± S.E. for 3 different experiments.

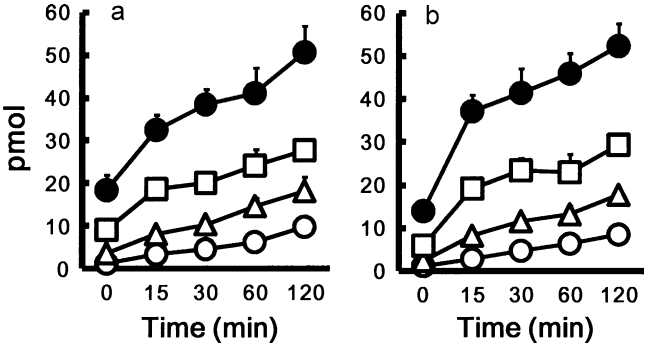

Fig. 5. [^{14}C]-labeled TAK-438 accumulation in cultured rabbit glands. Cultured gastric glands were incubated with [^{14}C] TAK-438 (0.01–0.3 μM; open circle, 0.01 μM; open triangle, 0.03 μM; open square, 0.1 μM; filled circle, 0.3 μM) for 0–120 min. [^{14}C] TAK-438 accumulation was observed in both the vehicle-treated (a) and forskolin-treated (b) groups. There was no apparent difference in [^{14}C] TAK-438 accumulation between the 2 groups. Results are expressed as mean ± S.E. for 5 different experiments.

3.5. The accumulation of TAK-438 and lansoprazole in cultured rabbit gastric glands

In experiments on cultured gastric glands incubated with [^{14}C] TAK-438 for 0–2 h, the amount of radioactivity accumulated in the gastric glands increased in a time- and concentration-dependent manner, irrespective of the presence of forskolin (1 μM) (Fig. 5a and b). Moreover, there was no difference in accumulation between the resting group and forskolin-treated group. The addition of [^{14}C] lansoprazole to resting cultured gastric glands resulted in the increase of radioactivity in a time- and concentration-dependent manner (Fig. 6a). When the cultured rabbit gastric glands were incubated with [^{14}C] lansoprazole in the presence of forskolin, the accumulated radioactivity increased at all concentrations in comparison to the vehicle-treated groups (Fig. 6b).

3.6. The clearance of TAK-438 and lansoprazole from cultured rabbit gastric glands

In the resting state, the residual amount of radioactivity derived from [^{14}C] TAK-438 after washout of incubation medium increased in a concentration-dependent manner. In the [^{14}C] TAK-438-treated groups (0.1 and 0.3 μM), the amount of radioactivity remaining in the cultured gastric glands decreased gradually depending on the incubation time: in the 0.3 μM-treated group, 28% of the radioactivity measured at 0 h still remained 24 h after

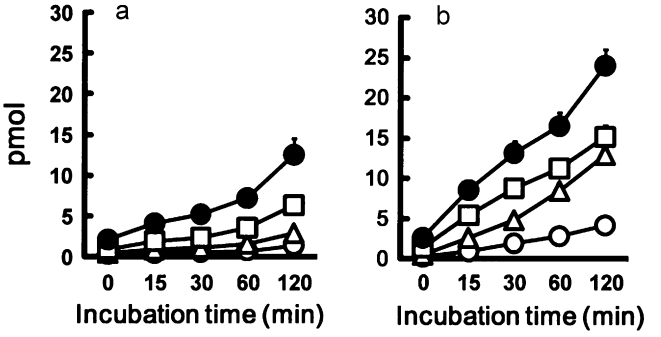

Fig. 6. [^{14}C]-labeled lansoprazole accumulation in cultured rabbit glands. Cultured gastric glands were incubated with [^{14}C] lansoprazole (0.01–0.3 μM; open circle, 0.01 μM; open triangle, 0.03 μM; open square, 0.1 μM; filled circle, 0.3 μM) for 0–120 min. In the forskolin-treated group (b), [^{14}C] lansoprazole accumulation was increased compared to the vehicle-treated (DMSO) group (a). Results are expressed as mean ± S.E. for 5 different experiments.

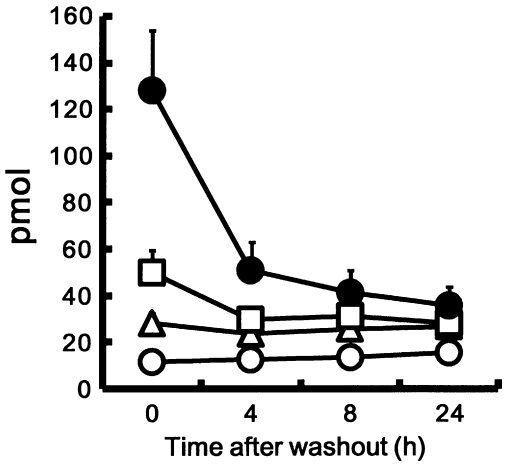

Fig. 7. The duration of [^{14}C] TAK-438 accumulation in cultured gastric glands. Cultured gastric glands were incubated with [^{14}C] TAK-438 (0.01–0.3 μM; open circle, 0.01 μM; open triangle, 0.03 μM; open square, 0.1 μM; filled circle, 0.3 μM) for 120 min. [^{14}C] TAK-438 accumulation lasted for more than 8 h at the highest concentration (0.3 μM). Results are expressed as mean ± S.E. for 3 different experiments.

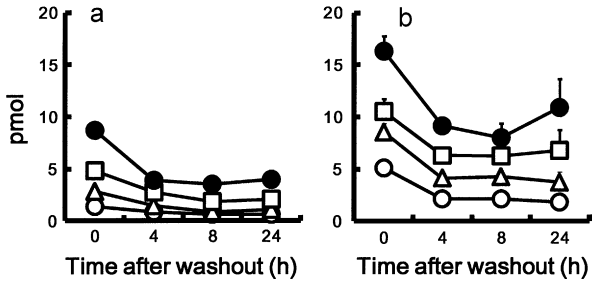

Fig. 8. The duration of [^{14}C] lansoprazole accumulation in cultured gastric glands. Cultured gastric glands were incubated with [^{14}C] lansoprazole (0.01–0.3 μM; open circle, 0.01 μM; open triangle, 0.03 μM; open square, 0.1 μM; filled circle, 0.3 μM) for 120 min in the presence of a vehicle (a) or 1 μM forskolin (b). Results are expressed as mean ± S.E. for 4 different experiments.

washout (Fig. 7). In the [^{14}C] lansoprazole-treated group, the remaining radioactivity derived from [^{14}C] lansoprazole after forskolin treatment was higher than that of the vehicle-treated group (Fig. 8a and b). Moreover, in the 0.3 μM [^{14}C] lansoprazole-treated group, 66.8% of the radioactivity present at 0 h remained at 24 h. Although the absolute level of radioactivity remaining in the [^{14}C] lansoprazole-treated group was much smaller than that of the [^{14}C] TAK-438-treated group, the relative proportion of the remaining radioactivity in the [^{14}C] lansoprazole-treated group at 24 h compared to the baseline level (0 h) was larger than that in [^{14}C] TAK-438-treated group.

4. Discussion

In our previous study, TAK-438 showed superior efficacy compared to lansoprazole in *in vitro* experiments using purified porcine gastric H^+, K^+-ATPase and in *in vivo* experiments using conscious and *anesthetized rats* [18]. In particular, the duration of acid inhibition of TAK-438 was found to be longer than that of lansoprazole or SCH28080. P-CAB type H^+, K^+-ATPase inhibitors, such as SCH28080, have been generally thought to have shorter *in vivo* durations of inhibition because they inhibit H^+, K^+-ATPase reversibly by formation of ionic bonds [26]. Although TAK-438 inhibited H^+, K^+-ATPase reversibly, it exerted a stronger and longer inhibition of acid secretion *in vitro* and *in vivo* compared to lansoprazole, suggesting that TAK-438 had different pharmacological properties from other P-CABs developed to date. We aimed to clarify the mode of action of TAK-438 and were able to clearly show that TAK-438 accumulates and persists in larger quantities than lansoprazole in cultured rabbit gastric glands, leading to a stronger and longer inhibition of acid formation. The inhibitory nature of TAK-438 on acid secretion appears to be associated with the physiology of gastric parietal cells as discussed below.

Acid formation in both acutely isolated and cultured gastric glands were examined; TAK-438 was superior to lansoprazole in its efficacy in both experiments (Figs. 2 and 3). The IC_{50} values of the inhibitors were larger in acutely isolated glands partly because forskolin concentration in the acute experiment was 10 times higher than that in the cultured experiment. Chew [27] reported that the optimal concentration of forskolin in the acute experiment was 10 μM. In the case of cultured glands, we found that 1 μM forskolin exhibited a maximal acid accumulation (data not shown).

There were large differences between TAK-438 and lansoprazole with regard to the effect of preincubation time on forskolin-induced aminopyrine accumulation, as well as in the amount of accumulation of each compound in cultured gastric glands. In cultured gastric glands, the inhibitory efficacy of lansoprazole on gastric acid formation was independent of preincubation time, whereas the inhibition by TAK-438 increased proportional to preincubation time (Fig. 3). In experiments that measured the accumulation of labeled TAK-438 or lansoprazole (Figs. 5 and 6), the stimulation of acid formation did not affect TAK-438 accumulation. In contrast, lansoprazole accumulation was dependent on acid stimulation. The radioactivity levels of accumulated [^{14}C] lansoprazole (treated with forskolin) and [^{14}C] TAK-438 (treated with DMSO) at maximum concentrations (120 min, 0.3 μM) were 24 and 51 pmol, respectively. These findings indicate that the amount of TAK-438 that was accumulated in the gastric glands was about twice that of the forskolin-stimulated lansoprazole group, and this accumulation was not affected by glandular acid formation.

There was also a difference between TAK-438 and lansoprazole in the recovery of acid formation and the clearance of the compounds from the gastric glands. In resting cultured gastric glands, preincubation with lansoprazole did not inhibit forskolin-stimulated acid formation, even immediately after washout (Fig. 4). In TAK-438-treated glands, TAK-438 accumulated in the resting cultured gastric glands, and forskolin-stimulated acid formation was inhibited in a concentration-dependent manner for more than 8 h after washout. In the TAK-438-treated group (0.3 μM), acid formation recovered to preincubation levels 24 h after washout. Beales and Calam [28] and Chew et al. [23] reported that the number of gastric parietal cells does not increase in these culture conditions. These data suggest that TAK-438 inhibited gastric acid formation by directly inhibiting gastric H^+, K^+-ATPase, and not by a nonspecific effect. Regarding clearance of [^{14}C] lansoprazole and [^{14}C] TAK-438 from cultured gastric glands (Figs. 7 and 8), the amount of accumulated [^{14}C] lansoprazole in the forskolin-treated group was higher than that in the resting group, and the ratio of remaining [^{14}C] lansoprazole at 24 h (compared to the 0 h baseline) was larger than that of [^{14}C] TAK-438 at 0.3 μM. These phenomena may be explained by the findings that lansoprazole is converted to its active form, AG-2000, in the acidic environment, and AG-2000 is covalently bound to cysteine residue of H^+, K^+-ATPase [29,30]. Therefore, radioactivity derived from lansoprazole can persist for a long time, especially in stimulated glands. Although the remaining ratio of lansoprazole in the stimulated glands at 24 h after washout was greater than that of TAK-438, the remaining amount of TAK-438 at 24 h was much larger than that of lansoprazole. These results

could account for the long duration of inhibition of acid formation by TAK-438 in cultured gastric glands.

The mechanism of gastric acid secretion, especially the translocation mechanism of gastric H^+, K^+-ATPase, is dependent on the acid secretory state. The mode of action of PPIs has been investigated in detail [1]. In the resting state of parietal cells, most of the H^+, K^+-ATPase enzymes are located in intracellular vesicles called tubulovesicles [31], and the pH of the secretory canaliculi, where secreted acid accumulates, is maintained at 3–4 [32]. Upon stimulation of acid secretion, H^+, K^+-ATPase enzymes translocate from the tubulovesicles to the apical membrane and act as acid pumps, which reduces the pH of the canaliculi to approximately 1. Lansoprazole is considered to act as follows. In resting parietal cells, the pH of secretory canaliculi is not low enough to fully convert lansoprazole to its active form, AG-2000 [29]. Moreover, the amount of H^+, K^+-ATPase localized on the apical membrane is small [33]; therefore, the amount of AG-2000 in resting parietal cells is small ([^{14}C] lansoprazole, even at the highest concentration at 2 h, showed only 12.5 pmol), and preincubation with lansoprazole did not produce any inhibitory effects on acid formation (Fig. 4). In contrast, in stimulated parietal cells, the pH of the secretory canaliculi is low enough to fully convert lansoprazole to AG-2000, and the amount of H^+, K^+-ATPase that translocates to the apical membrane is large [33]. Thus, large amounts of covalent bonds form between AG-2000 and translocated H^+, K^+-ATPase enzymes, and a larger amount of AG-2000 can remain in the stimulated parietal cells. Although the activation mechanism of PPIs (protonation of PPIs and the formation of covalent bonds with H^+, K^+-ATPase) has been studied using gastric mucosal microsomes [29], the activation mechanism of PPIs with regard to physiological stimulation has not been previously studied in gastric glands. This is the first study to report the proton requirement for activation of PPIs using cultured gastric glands.

TAK-438 is considered to act as follows. The amount of accumulated TAK-438 in gastric glands was larger than that of lansoprazole, regardless of the stimulation of acid secretion. In TAK-438-preincubated resting gastric glands, the inhibitory effect was increased in proportion to the preincubation time. This can be explained by the strong TAK-438 accumulation, which depended on the preincubation time. The duration of the inhibitory effect of TAK-438 was associated with the remaining amount of TAK-438 in the gastric glands. As TAK-438 does not need acid activation or to form covalent bonds with H^+, K^+-ATPase in order to exert its inhibitory effect, TAK-438 would be expected to exert a rapid, acid-inhibitory effect, irrespective of the amount of H^+, K^+-ATPase on the apical membrane and the pH of secretory canaliculi. Compared to lansoprazole, TAK-438 accumulated more and remained longer in the glands, likely due to its molecular properties, especially its pK_a. The pK_a of TAK-438 is 9.37, which is much higher than that of lansoprazole (pK_a = 3.83) [1] and other P-CABs, such as AZD0865 (pK_a = 6.1) [17]. Thus, TAK-438 is considered to be able to accumulate in high concentration in resting cultured gastric glands because the intragastric pH of resting gastric glands is acidic with a pH of 3–4 [32]. Shin et al. [34] reported that the dissociation of TAK-438 from H^+, K^+-ATPase was much slower than that of other P-CABs having an imidazopyridine structure; half of TAK-438 binding was dissociated by 0.3 M KCl at 3 h and by 10 mM KCl at 12.5 h. These findings seem to explain the stronger and longer *in vivo* acid-inhibitory effect of TAK-438 compared to lansoprazole.

Several clinical advantages of TAK-438 over PPI are considered on the basis of the differences in mechanism of action between PPIs and TAK-438. PPIs can inhibit only activated H^+, K^+-ATPase, and their inhibitory activities are significantly affected by the acid secretory state [3] as well as their recycling and *de novo* synthesis of H^+, K^+-ATPase [31,35]. On the other hand, TAK-438 accumulates in the secretory canaliculi of parietal cells independent of the acid secretory state and does not require acid activation for its activity. Therefore, TAK-438 can be prescribed whenever required and a rapid onset of action can be expected, whereas PPIs need to be prescribed before meals because acid secretion must be stimulated for maximum PPI efficacy [36]. Furthermore PPIs require 3–5 days to achieve a steady suppression of acid secretion [4]. Nighttime acid secretion cannot be completely inhibited by PPIs because uninhibited H^+, K^+-ATPase secretes acids at night [37]. On the other hand, TAK-438 accumulates in the secretory canaliculi of parietal cells, has a slow clearance rate and has the potential to inhibit nighttime acid secretion in GERD patients.

In summary, this study using rabbit gastric glands clearly showed the following: (1) both TAK-438 and lansoprazole inhibited acid formation in gastric glands in a concentration-dependent manner; (2) the inhibitory effect of lansoprazole did not change with preincubation time, whereas the effect of TAK-438 was augmented depending on preincubation time, and moreover, only the TAK-438-treated groups showed sustained inhibitory effect after washout; and (3) in the experiment using [^{14}C]-labeled compounds, lansoprazole accumulation in cultured gastric glands was increased in the presence of the acid secretagogue forskolin, whereas, TAK-438 accumulated in gastric glands in larger quantities than lansoprazole independent of forskolin. Clearance of the accumulated TAK-438 from the gastric glands was slow. Thus, the stronger potency and the longer duration of action of TAK-438 observed *in vivo* [18] could be explained by the larger accumulation and slow clearance of this compound from the gastric glands. Potent and consistent inhibition of acid secretion by TAK-438 renders this compound clinically promising compared to PPIs for acid-related diseases, such as gastroesophageal reflux disease, peptic ulcers, and for *Helicobacter pylori* eradication. Further studies of TAK-438 are warranted.

Disclosures

None declared.

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