Characteristics of the $\text{K}^+$-competitive $\text{H}^+,\text{K}^+$-ATPase inhibitor AZD0865 in isolated rat gastric glands

P. Kirchhoff, K. Andersson, T. Socrates, S. Sidani, O. Kosiek, and J. P. Geibel. Characteristics of the $\text{K}^+$-competitive H$^+,\text{K}^+$-ATPase inhibitor AZD0865 in isolated rat gastric glands. Am J Physiol Gastrointest Liver Physiol 291: G838–G843, 2006. First published June 22, 2006; doi:10.1152/ajpgi.00120.2006.—The gastric H$^+,\text{K}^+$-ATPase of the parietal cell is responsible for acid secretion in the stomach and is the main target in the pharmacological treatment of acid-related diseases. Omeprazole and other benzimidazole drugs, although having delayed efficacy if taken orally, have high success rates in the treatment of peptic ulcer disease. Potassium competitive acid blockers (P-CAB) compete with $\text{K}^+$ for binding to the H$^+,\text{K}^+$-ATPase and thereby inhibit acid secretion. In this study, the in vitro properties of AZD0865, a reversible H$^+,\text{K}^+$-ATPase inhibitor of gastric acid secretion, are described. We used a digital-imaging system and the pH sensitive dye BCECF to observe proton efflux from hand-dissected rat gastric glands. Glands were stimulated with histamine (100 $\mu$M) and exposed to a bicarbonate- and Na$^+$-free perfusate to induce an acid load. H$^+,\text{K}^+$-ATPase inhibition was determined by calculating pH, recovery (dpH/dT) in the presence of omeprazole (10–200 $\mu$M) or AZD0865 (0.01–100 $\mu$M). The efficacies of both drugs were compared. Our data show that acid secretion is inhibited by both the proton pump inhibitor omeprazole and the P-CAB AZD0865. Complete inhibition of acid secretion by AZD0865 had a rapid onset of activation, was reversible, and occurred at a 100-fold lower dose than omeprazole (1 $\mu$M AZD0865 vs. 100 $\mu$M omeprazole). This study demonstrates that AZD0865 is a potent, fast-acting inhibitor of gastric acid secretion, effective at lower concentrations than drugs of the benzimidazole class. Therefore, these data strongly suggest that AZD0865 has great potential as a fast-acting, low-dose inhibitor of acid secretion.

In the resting cell, the pumps are internalized in a system of tubular vesicles, therefore the PPIs can only inhibit the H$^+,\text{K}^+$-ATPases that have already been activated and transferred to the apical surface of the parietal cell (8). Despite their high degree of efficacy and their worldwide clinical use, failure in the treatment of acid-related diseases has been reported, and the degree and speed of onset of symptom relief are important to patients (15). It has been estimated that ~30% of GERD patients remain symptomatic on a standard dose of PPI (5). Furthermore, PPIs have a short plasma half life, which, often leads to a nocturnal acid breakthrough (12). Therapeutic oral doses of PPIs reach steady state and thus achieve their maximal effective levels after 4–5 days with typical dosing regimens (21). This slow and cumulative onset of effect of PPIs relates partly to their ability to inhibit only those pumps that are active when the PPI drug is available. After PPI administration, there is a return of acid secretion that is partly due to de novo synthesis of the enzyme (9). A further limitation of this class is the degree of interpatient variability observed in their effects on acid secretion, which is due to different polymorphisms in the hepatic cytochrome P450 responsible for the metabolism of the drug (2).

Although optimizing pharmacological profiles within the PPI class may provide some clinical benefit, other areas of research may prove to be more fruitful. An alternative strategy for effective inhibition of gastric acid secretion is the use of the potassium-competitive acid blockers (P-CAB). This concept has evolved through recent decades of research into the requirement of K$^+$ for the activation of the H$^+,\text{K}^+$-ATPase. P-CABs are lipophilic, weak bases that have high pK$\alpha$ values and are stable at a low pH. These properties allow them to concentrate to a high degree in parietal cell acid space, thus resulting in a fast onset of action and a direct dose-response relationship. When the P-CAB binds to the H$^+,\text{K}^+$-ATPase, it stabilizes the enzyme in the E$\text{2}$ conformation and, thereby, prevents the movement of H$^+$ ions into the parietal cell canaliculus (4). These agents inhibit H$^+,\text{K}^+$-ATPase by ionically binding at or near the potassium binding site in a K$^+$-competitive manner, thereby blocking gastric acid secretion by a direct and reversible mechanism (11, 22). This results in a very rapid onset of effect, with initial research showing that almost complete acid blockade can be achieved within 30 min of administration (22). In contrast to the PPIs, the reversible inhibitors are active in the absence of stimulated acid secretion and should therefore produce a less variable
onset of the effect, and furthermore, the inhibition will follow the plasma concentration of the drug closely (22, 23).

Several P-CABs are currently undergoing clinical development, including CS-526 (R-105266), soraprazan (BY-359), and AZD0865. In our protocol, we used AZD0865 for detailed characterization and evaluation as a competitive inhibitor of gastric H⁺,K⁺-ATPase in isolated gastric glands. AZD0865 is a substitute imidazopyridine and inhibits H⁺,K⁺-ATPase activity in a K⁺-competitive manner. It concentrates in the acidic compartment of the parietal cell and has a luminal site of action. Available data suggest that the full effect is achieved on the first day of dosing, and similar effects are achieved with repeated dosing (1). These properties would offer improvement for patients with GERD and other acid-related diseases; however, the efficacy and tolerability of these drugs has to be investigated in large clinical studies.

The aim of the present study is to characterize the K⁺ competitive H⁺,K⁺-ATPase inhibitor AZD0865 in isolated rat gastric glands. Therefore, we investigated the dose-dependent inhibition of acid secretion for AZD0865 compared with omeprazole and, furthermore, the onset of inhibition of acid secretion by AZD0865.

MATERIALS AND METHODS

Animals. Sprague-Dawley rats (150–250 g, Charles River Laboratory) were housed in climate- and humidity-controlled, light-cycled rooms, fed standard chow with free access to water, and handled according to the humane practices of animal care established by the Yale Animal Care. Prior to experiments, animals were fasted for 12 h with free access to water.

Isolation of gastric glands. Following removal of the stomach, the stomach was opened longitudinally, and the corpus and antrum were isolated and sliced into 0.5-cm square sections and washed with cold Ringer’s solution to remove residual food particles. The tissues were isolated and sliced into 0.5-cm square sections and washed with cold 7.4 at 37°C using either NaOH or KOH.

Histamine (100 μmol) exposure induced an alkalization rate of 0.008 pH unit/min (Fig. 1B). Intracellular alkalization stimulated by histamine (100 μmol) in the absence of extracellular Na⁺ is a function of H⁺,K⁺-ATPase, because it can be blocked by the specific inhibitor AZD0865 (10 μmol) (Fig. 1C). To show that this effect is due to the K⁺ competitive inhibition by AZD0865, we used also the well-known drug Schering 28080, which belongs to the same class of P-CABs. Figure 1D shows that Schering 28080 (100 μM) abolished the histamine-induced acid secretion to a rate of 0.008 ± 0.0009 pH unit/min. Figure 2 shows that proton efflux can also be induced by other secretagogues, either by 100 μM pentagastrin

Table 1. Compositions of solutions used for intracellular pH measurements in single rat gastric glands

<table>
<thead>
<tr>
<th></th>
<th>Standard HEPES</th>
<th>Na⁺-Free HEPES</th>
<th>Na⁺-Free HEPES + NH₄Cl</th>
<th>High K⁺ Calibration</th>
</tr>
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<tbody>
<tr>
<td>NaCl</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>NMDG</td>
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<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>20</td>
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<td>20</td>
<td>20</td>
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<tr>
<td>KCl</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>MgSO₄</td>
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<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>CaCl₂</td>
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<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glucose</td>
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<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
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<td>pH</td>
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<td>7.4</td>
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<td>7.4</td>
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All concentrations are given in millimolars. All solutions were titrated to pH 7.4 at 37°C using either NaOH or KOH. N-methyl-o-glucamine (NMDG) was titrated with HCl.
The next experiments showed that this alkalinization was inhibited by specific inhibitors of the H\(^+/\)K\(^-\)-ATPase, either omeprazole or AZD0865, demonstrating that the observed proton efflux was due to the H\(^+\),K\(^-\)-ATPase activity.

Effects of AZD0865 and omeprazole on gastric acid secretion. Omeprazole and AZD0865 inhibited H\(^+\),K\(^-\)-ATPase activity in a dose-dependent manner (Fig. 3, A and B). In this protocol, acid secretion was stimulated by histamine and expressed as ΔpH/min (0.056 ± 0.008). Therefore, the gastric glands were incubated with histamine (15 min), and 100 μM histamine was added throughout the whole superfusion. To investigate the inhibitory potency of both omeprazole and AZD0865, we used different concentrations of omeprazole (10–200 μM) and AZD0865 (0.01–100 μM). Each drug was present during the entire experiment, including the histamine incubation period of 15 min. The inhibitory efficacy of AZD0865 was much stronger than the inhibition by omeprazole. Figure 3, A and B, shows that inhibition of acid secretion by omeprazole and AZD0865 is a dose-dependent effect. AZD0865 abolished the acid secretion as strongly as omeprazole at a 100-fold lower concentration (1 μM AZD0865 vs. 100 μM omeprazole). The IC\(_{50}\) values of AZD0865 and omeprazole were 0.3 and 80 μM, respectively (Fig. 4).

Fig. 1. Original tracing of acid secretion and inhibition by AZD0865, a reversible H\(^+\),K\(^-\)-ATPase inhibitor of gastric acid secretion. Single rat gastric glands were isolated and loaded with the pH-sensitive dye BCECF to measure intracellular pH over single parietal cells, and the pH recovery rate was calculated from the slope after an acid load using the NH\(_4\)Cl prepulse technique as described previously. A: original tracing of an intracellular pH measurement demonstrating the resting parietal cell without alkalinization (proton efflux) after removing Na\(^+\) out of the perfusion bath. B: intracellular alkalinization stimulated by histamine (100 μM) in the absence of extracellular Na\(^+\). C: histamine-stimulated acid secretion is a function of H\(^+\),K\(^-\)-ATPase, because it can be blocked by the specific inhibitor AZD0865 (10 μM). Histamine was present during the entire experiment and for a 15-min incubation time prior to the perfusion. D: histamine-stimulated acid secretion could also be inhibited by Schering 28080 (100 μM) that was applied during the entire experiment.

Fig. 2. Acid secretion stimulated by different secretagogues. The control shows a low basal proton efflux without any stimulation (0.011 ± 0.002 pH unit/min) (n = 32 cells/5 glands/4 animals). Histamine (100 μM) caused a strong intracellular alkalinization (0.056 ± 0.008 pH unit/min) (n = 210 cells/37 glands/32 animals). Carbachol (100 μM) induced a rapid proton efflux (0.058 ± 0.006 pH unit/min) (n = 78 cells/8 glands/7 animals). Pentagastrin had nearly the same rate of acid secretion (0.059 ± 0.011 pH unit/min) (n = 48 cells/4 glands/4 animals).
Fast and reversible inhibition of acid secretion by AZD0865. AZD0865 is a reversible proton pump inhibitor that binds to the H⁺,K⁺-ATPase in a K⁺-competitive manner. We investigate these characteristics of the drug in our in vitro setting. Therefore, we stimulated acid secretion with histamine (100 μM) during the entire experiment. By adding 10 μM AZD0865 to the perfusion bath, we showed that acid secretion was abolished after less than 30 s. During the same experiment, we removed the drug from the superfusion bath, and the acid secretion was visible again after 2–3 min (Fig. 5A). With the proton pump inhibitor omeprazole (200 μM), the inhibition of acid secretion was irreversible and had no rapid onset of effect (Fig. 5B). To confirm these results, we used another member of the potassium competitive inhibitors, Schering 28080. Adding 100 μM Schering 28080 during the histamine-induced recovery phase resulted in an immediate inhibition of acid secretion (Fig. 5C).

AZD0865 inhibits acid secretion independently of the stimulatory drug. In this series of experiments, we used pentagastrin, carbachol, or histamine, each in a concentration of 100 μM during the entire experiment. After stimulation with each of these single drugs, we observed similar rates of proton efflux (Fig. 2).

Figure 6A, shows that pentagastrin-induced acid secretion (0.059 ± 0.011 pH unit/min) was reduced 76% by 200 μM omeprazole (0.014 ± 0.002 pH unit/min) and 81% by 10 μmol AZD0865 (0.011 ± 0.001 pH unit/min). Also, the carbachol-stimulated proton efflux (0.058 ± 0.006 pH unit/min) was reduced 76% by 200 μM omeprazole (0.015 ± 0.002 pH unit/min) and 79% by 10 μM AZD0865 (0.012 ± 0.001 pH unit/min); Fig. 6B. Acid secretion caused by histamine and the dose-dependent inhibition by AZD0865 and omeprazole is shown in Fig. 3, A and B.

**DISCUSSION**

In this study, we examined the dose-dependent inhibition of the gastric H⁺,K⁺-ATPase by the P-CAB AZD0865 and compared it with the classic proton pump inhibitor omeprazole. Furthermore, we tried to evaluate the onset of effect of AZD0865.

We employed the single gastric gland superfusion in vitro technique to investigate mechanisms of acid secretion on the cellular level. Acid secretion was induced by the classically known secretagogues histamine, carbachol, and pentagastrin, all of which led to a robust proton extrusion via the H⁺,K⁺-ATPase compared with the basal acid secretion in the resting, unstimulated gland (Fig. 2).

In subsequent studies, we examined the inhibitory effects of omeprazole and AZD0865 on the secretagogue-sensitive gastric acid secretion. Here, we confirmed the inhibitory potency of AZD0865 and omeprazole on histamine-induced acid secretion. We determined that either a concentration of 1 μM AZD0865 or 100 μM omeprazole was necessary to cause an 70–80% inhibition of secretagogue-induced proton extrusion via the H⁺/K⁺-ATPase. Furthermore, our experiments revealed that AZD0865 is able to inhibit acid secretion at a 100-fold lower concentration than omeprazole.

As mentioned in the introduction, proton pump inhibitors have a delayed onset of acute effect, and the full inhibitory effect is slow and needs several dose cycles. For example, omeprazole reaches only 30% of inhibition of acid secretion on the first day of treatment (6). Our further investigations try to...
examine the fast onset of AZD0865 and its reversibility, because treatments that provide faster onset of effect and increased duration of action would offer improvements for patients with GERD and other acid-related disorders. In fact, as shown in Fig. 5A, we were able to inhibit secretagogue-dependent acid secretion by addition of 10 μM AZD0865. On the other hand, histamine-induced acid secretion continued after removal of AZD0865 from the superfusion bath, demonstrating the reversible nature of the binding to the H^+\text{,K}^+\text{-ATPase (Fig. 5A)}.

These results confirm the existing data of the fast onset of inhibition of acid secretion by P-CABs (13). In humans, AZD0865 was able to inhibit acid secretion, 1 h after oral dosing, to 95% (1). P-CABs are absorbed rapidly and exhibit a classically dose-effect relationship with a dose-dependent duration increasing the intragastric pH (22). In humans, more than 95% inhibition was sustained for up to 15 h for 0.8 and 1 mg/kg doses (1). Our experiments show that there is no
difference in the inhibitory characteristics of AZD0865 between the classical acid-stimulating agents (histamine, carbachol, and pentagastrin). Therefore, we assume that P-CABs are not interfering with the intracellular pathway of the acid secretory process and act only on the H\(^+\)K\(^-\)ATPase as the final enzyme responsible for secreting protons, and forming HCl in the lumen of the stomach.

In summary, our findings indicate that AZD0865 offers a more rapid and elongated inhibition of gastric acid secretion at much lower concentrations than conventional proton pump inhibitors. AZD0865 is a reversible and fast-acting inhibitor of acid secretion in single gastric glands. Compared with omeprazole, AZD0865 inhibits acid secretion with a faster onset of effect and in a 100-fold lower dose. Such treatments may provide significant benefits to patients with GERD. Future studies investigating the efficacy of P-CABs in the clinical setting are awaited and will help to define their place in the treatment of acid-related diseases.

**REFERENCES**

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