E3710, a New Proton Pump Inhibitor, with a Long-Lasting Inhibitory Effect on Gastric Acid Secretion

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Received March 3, 2010; accepted May 17, 2010

ABSTRACT

We have investigated the pharmacology of sodium (R)-2-[4-(2,2-dimethyl-1,3-dioxan-5-yl) methoxy-3,5-dimethylpyridin-2-yl]methylsulfinyl-1H-benimidazol (E3710), a new proton pump inhibitor (PPI), and its effect on gastric acid secretion. E3710 irreversibly inhibited H⁺,K⁺-ATPase activity in pig gastric vesicles with an acidic internal environment with an IC₅₀ of 0.28 μM. Administration of E3710 (0.1, 0.2, 0.4, and 0.8 mg/kg; n = 6) intraduodenally in a gastric fistula model in dogs inhibited histamine-stimulated gastric acid secretion at 0 to 2 and 24 to 26 h after administration with ED₅₀ values of 0.18 and 0.22 mg/kg, respectively. The inhibition by E3710 was 2.3 times more potent than that of another representative PPI, esomeprazole (0.2, 0.4, 0.8, and 1.6 mg/kg; n = 6) at 0 to 2 h after administration (ED₅₀ = 0.40 mg/kg) and 2.8 times more potent at 24 to 26 h (ED₅₀ = 0.71 mg/kg). In the gastric fistula dogs, the intragastric pH was ≥4 for 17% (n = 27) of a 24-h period with vehicle alone, but when E3710 was administered, at 0.2 (n = 4), 0.4 (n = 8), and 0.8 mg/kg (n = 5), the pH was ≥4 for 40, 79, and 88% of a day, respectively. The corresponding values for esomeprazole at 0.8 (n = 4) and 1.6 mg/kg (n = 8) were 55 and 59%, respectively. In a crossover study with vehicle, E3710 at 0.4 mg/kg and esomeprazole at 1.6 mg/kg (n = 6), E3710 increased the intragastric pH to >4 for 82% of a day compared with 61% of a day with esomeprazole. These results show that E3710 is a long-acting inhibitor of gastric acid secretion and a promising novel therapy for acid-related diseases, such as gastroesophageal reflux disease.

Proton pump inhibitors (PPIs) are potent inhibitors of H⁺,K⁺-ATPase, the enzyme responsible for the final step in hydrochloric acid secretion by gastric parietal cells (Sachs et al., 2006; Scarpignato et al., 2006), regulation of which is shared by different signaling pathways. PPIs effectively block gastric acid secretion regardless of the type of inducing stimulus and therefore have provided excellent outcomes in the treatment of acid-related diseases (ARDs), such as gastric ulcers, duodenal ulcers, marginal ulcers, gastroesophageal reflux disease (GERD), Helicobacter pylori infection, nonsteroidal anti-inflammatory drug-associated gastrointestinal lesions, upper gastrointestinal bleeding, and Zollinger-Ellison syndrome (Boparai et al., 2008; Shi and Klotz, 2008).

Despite their clinical efficacies and safety, there are still some patients who report that their symptoms are refractory to treatment with PPIs, in particular, approximately 30% of patients with GERD (Fass et al., 2005). GERD is a disorder of the upper gastrointestinal tract characterized by heartburn, regurgitation, epigastric pain, and belching, resulting from the reflux of gastric contents into the esophagus (Castell et al., 2004) and is a chronic, relapsing condition requiring long-term therapy associated with chest pain, asthma, chronic cough, and sleep disturbance, which impair quality of life (Wahlqvist et al., 2007). The severity is related to the acid exposure to the esophagus (Lundell et al., 1999), and the healing rate is known to be well correlated with the percentage of time over a day for which the intragastric pH remains at or greater than 4 (Bell et al., 1992; Armstrong, 2004). PPIs are usually given once a day, but it is clear that better acid control is gained by using them twice daily (Katz et al., 2004). Hence, a long-lasting PPI, even given once a day, may main-
tain appropriate acid control and be useful for the treatment of GERD.

We have synthesized sodium (R)-2-[4-(2,2-dimethyl-1,3-dioxan-5-yl) methoxy-3,5-dimethylpyridin-2-yl] methyl-sulfinyl-1H-benzimidazol (E3710) (Fig. 1), a new PPI with a long-lasting inhibitory effect. Of the PPIs currently available, esomeprazole has been reported to provide the best control of intragastric pH over a 24-h period (Calvet and Gomollón, 2005) and has been demonstrated to provide good results in resolving the underlying GERD in patients (Katz et al., 2006). We compared the effects of E3710 with those of esomeprazole to predict its clinical efficacy and usefulness. In clinical studies, 24-h intragastric pH monitoring based on a crossover design that includes a placebo is widely used to assess the efficacy of PPIs (Williams et al., 1998; Bruley des Varannes et al., 2004). We also performed a cross-over study of 24-h intragastric pH monitoring in gastric fistula dogs to confirm the long-lasting suppressive effect of E3710 compared with that of esomeprazole.

Materials and Methods

Materials. E3710 was synthesized at Eisai (Ibaraki, Japan). Esomeprazole magnesium trihydrate was purchased from Kemprotec Ltd. (Middlesbrough, UK). Valinomycin, NaDAD, gramicidin, histamine, famotidine, and porcine cerebral cortex Na+-K+-ATPase were purchased from Sigma-Aldrich (St. Louis, MO). ATP was purchased from the Oriental Yeast Co. Ltd. (Tokyo, Japan), 3-(cyanomethyl)-2-pyrindine (SCH28080) and dithiothreitol (DTT) were purchased from Nacalai Tesque (Kyoto, Japan), and [14C]aminopyrine was purchased from GE Healthcare (Ibaraki, Japan). For in vitro studies, E3710, esomeprazole, SCH28080, and famotidine were dissolved in methanol and ouabain was dissolved in distilled water. The concentrations of reagents used in in vitro experiments are expressed as final concentrations. In in vivo studies, E3710 and esomeprazole were suspended in 0.5% methyl cellulose (MC).

Animals. Male 11-week-old New Zealand White rabbits (Kitayama Labs, Nagano, Japan) and male 1- to 3-year-old mongrel dogs (Kitayama Labs, Gifu, Japan) were maintained at a temperature of 23°C (20–26°C) and a humidity of 55% (40–70%) and on a 12-h light/dark cycle. All experiments were approved by the Animal Care and Use Committee at the Eisai Tsukuba Research Laboratories (Ibaraki, Japan).

Measurement of H⁺,K⁺-ATPase Activity Isolated from Pig Gastric Mucosa. H⁺,K⁺-ATPase was prepared from fresh samples of pig gastric mucosa (Fujisaki et al., 1993) and stored at less than −70°C until use. The H⁺,K⁺-ATPase preparation (10 μg of protein/ml) was incubated with the methanol vehicle alone or with either E3710 or esomeprazole at 0.3, 1, 3, 10, and 30 μM in 1 mM Pipes-Tris buffer (pH 6.1) and at 1, 3, 10, and 30 μM in 40 mM Tris-HCl buffer (pH 7.4) for 30 min at 37°C. Either 15 mM KCl or distilled water and 1 μg/ml gramicidin were added to the samples for 10 min and then 3 mM Mg-ATP (pH 7.4) was added for a further 10 min. After the enzyme reaction was stopped by addition of a cold solution of 4.5% ammonium molybdate and 60% perchloric acid, the amount of inorganic phosphorus released from ATP was determined using the method described previously (Yoda and Hok, 1970) and a double-beam spectrophotometer (U-2001; Hitachi, Ltd., Tokyo, Japan). To measure the inhibitory effects of the drugs under acidic conditions, gastric microsomal membranes enriched in H⁺,K⁺-ATPase were isolated in vesicular form, and the accumulation of H⁺ in the presence of ATP, Mg⁺², K⁺, and valinomycin was measured (Lee and Forte, 1978). This H⁺,K⁺-ATPase preparation (10 μg of protein/ml) was mixed with 0.01, 0.03, 0.1, 0.3, 1, or 3 μM E3710 or esomeprazole, or with the ethanol vehicle alone in 36 mM Tris-HCl buffer (pH 7.4) containing 150 mM KCl or 150 mM NaCl, 1 mM glutathione, and 6 μg/ml valinomycin. The reaction was started by the addition of 2 mM Mg-ATP, and H⁺,K⁺-ATPase activity was measured for 30 min at 37°C. The inhibitory effects of E3710 and esomeprazole on the H⁺,K⁺-ATPase activity were determined during the 10-min period from 20 to 30 min. H⁺,K⁺-ATPase activity was measured using the coupled enzyme method, in which the hydrolysis of ATP is coupled to the oxidation of NaDAD (Mori et al., 1990), using a microplate spectrophotometer (SpectraMax 250; Molecular Devices, Sunnyvale, CA).

Measurement of Gastric Acid Secretion in Isolated Rabbit Gastric Glands. Gastric glands were prepared from the rabbit gastric mucosa as described previously (Berglindh and Obrink, 1976). These preparations of gastric glands were incubated with 0.03, 0.1, 0.3, 1, or 10 μM E3710 or esomeprazole or famotidine (for histamine stimulation) or with 0.3, 1, 3, 10, 30, and 100 μM famotidine (for db-cAMP stimulation) in a solution containing 0.1 μCi/ml [14C]aminopyrine in a shaking water bath at 37°C for 30 min. The secretagogue 1 mM db-cAMP or 0.1 mM histamine was then added and incubation was continued for a further 30 min. The supernatant and the pellet were separated by centrifugation at (15,000 rpm, 4°C, 1 min). Then, the levels of radioactive [14C]aminopyrine present in the supernatant and the pellet were measured using a liquid scintillation counter (Tri-Carb 2007TR; PerkinElmer Life and Analytical Sciences, Waltham, MA). The ratio of the weak base [14C]aminopyrine in the supernatant and pellet was used as a measure of the acid-secretory activity in the gastric glands (Sack and Spannery, 1982).

Measurement of Na⁺,K⁺-ATPase Activity from Porcine Cerebral Cortex. Na⁺,K⁺-ATPase (10 μg of protein/ml) prepared from porcine cerebral cortex was mixed with 0.3, 1, 3, 10, 30, and 100 μM E3710 or esomeprazole or 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μM ouabain or vehicle alone in 125 mM Tris-HCl buffer (pH 7.4) containing 50 mM KCl and 100 mM NaCl. The Na⁺,K⁺-ATPase activity was calculated as the difference between the total ATPase activity and the Mg²⁺-ATPase activity, which was defined as the ATPase activity in the presence of 1 mM ouabain. The reaction was started by the addition of 3 mM Mg-ATP, and the Na⁺,K⁺-ATPase activity was measured for 30 min. The effects of E3710, esomeprazole, and ouabain on Na⁺,K⁺-ATPase activity were determined during the 10-min period from 20 to 30 min. The Na⁺,K⁺-ATPase activity was also evaluated as described for the coupled enzyme method (Mori et al., 1990).

Investigation of the Inhibitory Mechanism on H⁺,K⁺-ATPase. To confirm the inhibitory mechanism of E3710 on H⁺,K⁺-ATPase activity as a PPI, we compared it with that of SCH28080, an acid pump antagonist, which inhibits H⁺,K⁺-ATPase based on a mechanism different from that of PPIs. We incubated 10 μg of protein/ml H⁺,K⁺-ATPase with 30 μM E3710, 10 μM esomeprazole, 10 μM SCH28080, or methanol, together with 0.1, 0.3, 1, or 3 μM DTT or distilled water as a control in 1 mM Pipes-Tris buffer (pH 6.1) for 30 min at 37°C. Either 15 mM KCl or distilled water and 1 μg/ml gramicidin were added for 10

Fig. 1. Chemical structure of E3710 (sodium (R)-2-[4-(2,2-dimethyl-1,3-dioxan-5-yl) methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl-1H-benzimidazol).
min and then 3 mM Mg-ATP (pH 7.4) was added for a further 10 min, with the samples at 37°C throughout. After the enzyme reaction was stopped, the amount of phosphorus released from ATP was determined. The reversibility of the inhibition of H⁺,K⁺-ATPase by E3710 was investigated using the dilution method as reported previously (Lorentzon et al., 1985; Nagaya et al., 1989). The H⁺,K⁺-ATPase (100 μg of protein/ml) was preincubated with 100 μM E3710, 100 μM esomeprazole, 10 μM SCH28080, or vehicle for 30 min at 37°C in 2 mM Pipes-Tris buffer (pH 6.1). Then, H⁺,K⁺-ATPase activity was measured in two conditions with or without dilution of the reaction mixture. Either 15 mM KCl or distilled water, 1 μg/ml gramicidin, and 3 mM Mg-ATP (pH 7.4) were added, followed by incubation for 10 min under the undiluted condition (60 μg of protein/ml H⁺,K⁺-ATPase, 60 μM E3710, 60 μM esomeprazole, or 6 μM SCH28080) or 20 min under the diluted condition (3 μg of protein/ml H⁺,K⁺-ATPase, 3 μM E3710, 3 μM esomeprazole, or 0.3 μM SCH28080). After the enzyme reaction was stopped, the amount of phosphorus released from ATP was determined. In both experiments, H⁺,K⁺-ATPase activity was also evaluated in a same way as described previously (Yoda and Hokin, 1970).

Measurement of Histamine-Stimulated Gastric Acid Secretion in Gastric Fistula Dogs. Twelve dogs underwent surgery to create gastric fistulae and were divided into two groups: one received 0.1, 0.2, 0.4, or 0.8 mg/kg E3710 or the 0.5% MC vehicle alone (n = 6) and the other received 0.2, 0.4, 0.8, or 1.6 mg/kg esomeprazole or the 0.5% MC vehicle alone (n = 6). Each experiment used a 6 × 5 crossover design study for both drugs including the vehicle and was performed over 2 consecutive days. On day 1, gastric acid secretion was stimulated by intravenously infusing 50 or 75 μg/kg/min histamine over 180 min, and gastric juice was collected every 20 min. Sixty minutes after the start of histamine infusion, 0.5% MC, E3710, or esomeprazole was administered intraduodenally. On day 2, 24 h after 0.5% MC, E3710, or esomeprazole administration, histamine was infused intravenously over 120 min, and gastric juice was collected every 20 min. The volume of gastric juice was determined, and then the concentration of acid was measured by titrating 0.5 ml of gastric juice against 0.04 M NaOH solution to pH 7.0 using a Titration Workstation (Radiometer Analytical SAS, Lyon, France). The gastric acid output was calculated using the following formula: gastric acid concentration (milliequivalents per min) = volume of gastric juice (milliliters per 20 min) × acid concentration (milliequivalents per milliliter). The inhibitory effects of the drugs were measured for the 0- to 2-h time period after administration on day 1 and the 24- to 26-h time period after administration on day 2.

Measurement of Intragastric pH over 24 h in the Gastric Fistula Dogs. We carried out three separate intragastric stimulations according to the standard clinical trial methodology for PPIs (Williams et al., 1998; Bruley des Varannes et al., 2004), using histamine stimulation at a time appropriate for “breakfast” followed by two feedings at times appropriate for “lunch” and “dinner,” to ascertain whether E3710 was likely to be clinically useful and have long-term efficacy. After infusion of histamine intravenously for 40 min (as breakfast), 0.5% MC, 0.2, 0.4, or 0.8 mg/kg E3710, or 0.8 or 1.6 mg/kg esomeprazole was administered intraduodenally. The measurement of intragastric pH over 24 h commenced at approximately 10:00 AM, and values was recorded every 10 s using an ambulatory pH monitoring system (PH-1012G; Chemical Instruments Co., Ltd., Tokyo, Japan) carried in a canine jacket. During this time, the dogs could move freely and had free access to drinking water. Two meals, each of ~225 g of DS-A pellet diet (Oriental Yeast Co. Ltd.), were offered to each animal separately at approximately 1:00 PM (as lunch) and 6:00 PM (as dinner). Before and after each experiment, the pH electrode was calibrated with standard buffer solutions at pH 4.01 and 6.86. The intragastric pH data were downloaded to a computer and analyzed using the W-IPC pH analysis program (Chemical Instruments Co. Ltd.). After a dose estimation study, 6 × 3 crossover design studies using 0.5% MC, 0.4 mg/kg E3710, and 1.6 mg/kg esomeprazole (n = 6) were performed to confirm the long-lasting inhibitory effects of E3710 compared with those of esomeprazole.

Statistical Analysis. In in vitro experiments, the mean IC₅₀ and 95% confidence interval (CI) were calculated based on the IC₅₀ values generated from separated sigmoid curves. In vivo experiments, ED₅₀ values were calculated by linear regression, and the potency ratio with 95% CIs was calculated using a two-by-three assay. Differences in the intragastric pH between E3710 and 0.5% MC or esomeprazole and 0.5% MC were analyzed using Dunnett’s multiple range test in a dose escalation study. Intragastric pH was evaluated using one-way analysis of variance followed by Tukey’s multiple comparison test in a crossover study. Two-sided p < 0.05 was considered statistically significant. Statistical analyses were performed using the SAS software package (version 8.1; SAS Institute Japan Ltd., Tokyo, Japan).

Results

Inhibitory Effect of E3710 on H⁺,K⁺-ATPase Activity Isolated from Pig Gastric Mucosa. IC₅₀ values of E3710 and esomeprazole under different pH conditions are summarized in Table 1. The inhibitory effects of E3710 on H⁺,K⁺-ATPase activity were dependent on pH condition. In pig gastric vesicles with an acidic internal environment, E3710 and esomeprazole had an inhibitory effect with IC₅₀ values of 0.28 and 0.53 μM, respectively (Fig. 2A). At pH 6.1, E3710 and esomeprazole had an inhibitory effect with IC₅₀ values of 4.2 and 2.3 μM, respectively (Fig. 2B). At pH 7.4, E3710 and esomeprazole both showed weak inhibitory effects with IC₅₀ values >30 μM (Fig. 2C).

Inhibitory Effect of E3710 on Acid Secretion in Isolated Rabbit Gastric Glands. IC₅₀ values of E3710, esomeprazole, and famotidine are summarized in Table 2. Both E3710 and esomeprazole inhibited acid secretion stimulated by both db-cAMP (Fig. 3A) and histamine (Fig. 3B). In contrast, famotidine selectively inhibited acid secretion stimulated by histamine.

Effect of E3710 on Porcine Cerebral Cortex Na⁺,K⁺-ATPase. When we used Na⁺,K⁺-ATPase purified from porcine cerebral cortex, we showed that ouabain inhibited its activity with an IC₅₀ value of 0.43 μM (95% CI, 0.41–0.45). In contrast, both E3710 and esomeprazole were very poor inhibitors of this enzyme with IC₅₀ values greater than 100 μM.

Effects of DTT and Dilution on the Inhibition of H⁺,K⁺-ATPase Activity by E3710. E3710 at 30 μM and esomeprazole at 10 μM inhibited H⁺,K⁺-ATPase activity by 96.5 and 95.8%, respectively. When DTT was added, inhibition of H⁺,K⁺-ATPase by E3710 and esomeprazole was prevented in a concentration-dependent manner. DTT at 3 μM

### Table 1

<table>
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<tr>
<th>Acidity</th>
<th>IC₅₀</th>
<th>pH 6.1</th>
<th>pH 7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3710</td>
<td>0.28 (0.17–0.44)</td>
<td>4.2 (3.8–4.7)</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>0.53 (0.47–0.59)</td>
<td>2.3 (2.1–2.5)</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>
SCH28080 inhibited H⁺,K⁺-ATPase activity by 93.0% after dilution (Fig. 4D). In contrast, 10⁻⁷.0% after dilution (Fig. 4D). DTT had no effect on its ability to inhibit H⁺,K⁺-ATPase (Fig. 4A) and esomeprazole (Fig. 4B). In contrast, SCH28080 completely suppressed the inhibitory effects of both E3710 and esomeprazole (Fig. 4C). In dilution studies, 100 μM E3710 inhibited H⁺,K⁺-ATPase activity by 55.4% and by 58.5% when the reaction mixture was diluted (Fig. 4D). Likewise, esomeprazole inhibited H⁺,K⁺-ATPase activity by 91.1% when undiluted and by 93.0% after dilution (Fig. 4D). In contrast, 10 μM SCH28080 inhibited H⁺,K⁺-ATPase activity by 80.5% but by 7.0% after dilution (Fig. 4D).

### Inhibitory Effect of E3710 on Histamine-Stimulated Gastric Acid Secretion in Gastric Fistula Dogs

The effects of E3710 and esomeprazole on histamine-stimulated gastric acid secretion in gastric fistula dogs are summarized in Figs. 5 and 6, respectively. E3710 and esomeprazole both inhibited gastric acid secretion in a dose-dependent manner. E3710 at 0.4 and 0.8 mg/kg fully inhibited gastric acid secretion within 1 h of administration (Fig. 5A). Even 24 h after administration, these sustained inhibitory effects were still observed after histamine stimulation (Fig. 5B). Esomeprazole also inhibited histamine-stimulated gastric acid secretion 1 h after administration (Fig. 6A). However, 24 to 26 h after administration, these inhibitory effects were not sustained in the way seen with E3710 (Fig. 6B). The ED₅₀ values for E3710 (liner regression range 0.1, 0.2, and 0.4 mg/kg) and esomeprazole (liner regression range 0.2, 0.4, and 0.8 mg/kg) during 0 to 2 and 24 to 26 h after administration are shown in Table 3. The potency ratios for E3710 to esomeprazole during 0 to 2 and 24 to 26 h after administration were 2.3 (95% CI, 1.9–2.6) and 2.8 (95% CI, 2.2–3.6), respectively.

### Effects of E3710 on Intragastric pH over 24 h in Gastric Fistula Dogs

The experimental protocol is summarized in Fig. 7A. In gastric fistula dogs monitored over 24 h, E3710 and esomeprazole both elevated the mean intragastric pH in a dose-dependent manner and increased the percentage of time for which the intragastric pH was substantially greater than pH 4 during the same time period. The mean intragastric pH in the esomeprazole-treated group, although the difference was not statistically significant. In both the E3710- and esomeprazole-treated groups the intragastric pH gradually dropped, after the maximal pH-elevating effects had been reached. The intragastric pH in the esomeprazole-treated group dropped to less than 4 just after midnight (between 1:00 and 3:00 AM), whereas in the E3710-treated group it remained substantially greater than pH 4 during the same time period. The percentage of time for which the intragastric pH was ≥4 over the whole 24-h period was significantly longer in the E3710-treated group than in the esomeprazole-treated group (Table 5).

### Discussion

E3710, a new PPI, potently suppressed histamine-stimulated gastric acid secretion even 24 h after administration.
Fig. 3. Inhibitory effects of E3710, esomeprazole, and famotidine on acid secretion in isolated rabbit gastric glands. A, db-cAMP stimulation. B, histamine stimulation. The ratio of the weak base [14C]aminopyrine in the supernatant and pellet was used as a measure of the acid-secretory activity in the gastric glands. ○, E3710; ●, esomeprazole; △, famotidine. Each data point represents the mean from four independent experiments performed in duplicate.

Fig. 4. Effects of DTT and dilution on the inhibition of H⁺,K⁺-ATPase activity with E3710, esomeprazole, and SCH28080. A–C, the effects of DTT on the inhibition of H⁺,K⁺-ATPase activity by E3710 at 30 μM (A), esomeprazole at 10 μM (B), and SCH28080 at 10 μM (C) were investigated. D, reversibility of the inhibition of H⁺,K⁺-ATPase activity by E3710 at 100 μM, esomeprazole at 100 μM, and SCH28080 at 10 μM was investigated with dilution (−) or without dilution (+). Each data point represents the mean ± S.E.M. from three independent experiments performed in duplicate.

Fig. 5. Effects of E3710 on histamine-stimulated gastric acid secretion in gastric fistula dogs. A, gastric acid output changes on day 1. Gastric acid secretion was stimulated by intravenous histamine infusion. Then, 1 h after the start of histamine infusion, 0.5% MC or E3710 was intraduodenally administered (indicated by arrow). B, gastric acid output changes on day 2. Twenty-four hours after the administration of 0.5% MC or E3710, gastric acid secretion was stimulated by intravenous histamine infusion. ○, 0.5% MC; ●, 0.1 mg/kg E3710; △, 0.2 mg/kg E3710; ▲, 0.4 mg/kg E3710; □, 0.8 mg/kg E3710. Results are expressed as the mean ± S.E.M. of six dogs (6 × 5 crossover study).
Histamine infusion

Effects of E3710 and esomeprazole on histamine-stimulated gastric acid secretion in gastric fistula dogs

Fig. 6. Effects of esomeprazole on histamine-stimulated gastric acid secretion in gastric fistula dogs. A, gastric acid output changes on day 1. Gastric acid secretion was stimulated by 3-h intravenous histamine infusion. Then, 1 h after the start of histamine infusion, 0.5% MC or esomeprazole was intraduodenally administered (indicated by arrow). B, gastric acid output changes on day 2. Twenty-four hours after the administration of 0.5% MC or esomeprazole, gastric acid secretion was stimulated by 2 h of intravenous histamine infusion. ○, 0.5% MC; △, 0.2 mg/kg esomeprazole; ▲, 0.4 mg/kg esomeprazole; ▣, 0.8 mg/kg esomeprazole; ■, 1.6 mg/kg esomeprazole. Data are expressed as the mean ± S.E.M. of six dogs (6 × 5 crossover study).

TABLE 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean pH/24 h</th>
<th>%Time with pH ≥4/24 h</th>
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<tr>
<td>0.5% MC</td>
<td>27</td>
<td>3.2 ± 0.1</td>
<td>17 ± 3</td>
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<tr>
<td>E3710 0.2 mg/kg</td>
<td>4</td>
<td>3.7 ± 0.2</td>
<td>40 ± 6**</td>
</tr>
<tr>
<td>E3710 0.4 mg/kg</td>
<td>8</td>
<td>5.1 ± 0.1**</td>
<td>79 ± 3**</td>
</tr>
<tr>
<td>E3710 0.8 mg/kg</td>
<td>5</td>
<td>5.5 ± 0.1**</td>
<td>88 ± 2**</td>
</tr>
<tr>
<td>Esomeprazole 0.5 mg/kg</td>
<td>4</td>
<td>4.0 ± 0.2*</td>
<td>55 ± 3**</td>
</tr>
<tr>
<td>Esomeprazole 1.6 mg/kg</td>
<td>8</td>
<td>4.3 ± 0.1**</td>
<td>59 ± 4**</td>
</tr>
</tbody>
</table>

* p < 0.01 vs. the 0.5% MC (Dunnett’s multiple range test).
** p < 0.001.
* p < 0.05 vs. 0.5% MC.
* p < 0.01 vs. 0.5% MC.

TABLE 4

Effects of E3710 and esomeprazole on 24-h intragastric pH in gastric fistula dogs

After 40 min of intravenous histamine infusion at approximately 10:00 AM, 0.5% MC, E3710, or esomeprazole was administered intraduodenally, and the measurement of intragastric pH was commenced. Intragastric pH was measured for 24 h under free moving and free access to drinking water. The total food allowance per day was divided into two equal portions, which were separately offered to each dog at approximately 1:00 PM and 6:00 PM. Data are expressed as the mean ± S.E.M.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean pH/24 h</th>
<th>%Time with pH ≥4/24 h</th>
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</thead>
<tbody>
<tr>
<td>0.5% MC</td>
<td>27</td>
<td>3.3 ± 0.2</td>
<td>20 ± 5</td>
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<tr>
<td>E3710 0.4 mg/kg</td>
<td>8</td>
<td>5.3 ± 0.3**</td>
<td>82 ± 5**</td>
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<tr>
<td>Esomeprazole 1.6 mg/kg</td>
<td>6</td>
<td>4.6 ± 0.3*</td>
<td>61 ± 7**</td>
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</table>

* p < 0.01 vs. 0.5% MC.
** p < 0.001.
* p < 0.05 vs. 1.6 mg/kg esomeprazole (Tukey’s multiple comparison test).

TABLE 5

Effects of E3710 and esomeprazole on 24-h intragastric pH in gastric fistula dogs in a crossover study

After 40 min of intravenous histamine infusion, 0.5% MC, E3710 at 0.4 mg/kg, or esomeprazole at 1.6 mg/kg was administered intraduodenally, and the measurement of intragastric pH was commenced at approximately 10:00 AM. Intragastric pH was measured for 24 h under free moving and free access to drinking water. The total food allowance per day was divided into two equal portions, which were separately offered to each dog at approximately 1:00 PM and 6:00 PM. Data are expressed as the mean ± S.E.M. (n = 6; 6 × 3 crossover study).

<table>
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<tr>
<th>Treatment</th>
<th>Mean pH/24 h</th>
<th>%Time with pH ≥4/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% MC</td>
<td>3.3 ± 0.2</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>E3710 0.4 mg/kg</td>
<td>5.3 ± 0.3**</td>
<td>82 ± 5**</td>
</tr>
<tr>
<td>Esomeprazole 1.6 mg/kg</td>
<td>4.6 ± 0.3*</td>
<td>61 ± 7**</td>
</tr>
</tbody>
</table>

* p < 0.01 vs. 0.5% MC.
** p < 0.001.
* p < 0.05 vs. 1.6 mg/kg esomeprazole (Tukey’s multiple comparison test).

Classic PPIs are activated by protonation and irreversibly inhibit H^+\textsuperscript{+}, K^-\textsuperscript{-}ATPase mediated through the interaction with cysteine groups (Lorentzon et al., 1985; Nagaya et al., 1989; Scarpignato et al., 2006). We compared the inhibitory effects of E3710 with those of esomeprazole (PPI), famotidine (histamine H_{2} receptor antagonist), which inhibits acid secretion based on select histamine H_{2} receptor blocker, and SCH28080 (acid pump antagonist), which inhibits H^+\textsuperscript{+}, K^-\textsuperscript{-}ATPase in a reversible and a potassium-competitive manner (Scott et al., 1987; Andersson and Carlsson, 2005), to clarify the inhibitory mode of E3710 on acid secretion. First, E3710 potently inhibited H^+\textsuperscript{+}, K^-\textsuperscript{-}ATPase under acidic conditions, and its inhibitory effect relatively decreases under neutral pH conditions similar to esomeprazole. Second, E3710 also acted at a...
stage during the process of gastric acid secretion after cAMP production, which is similar to the mode of action of esomeprazole but unlike that of famotidine in isolated rabbit gastric glands. Third, the inhibitory effect of E3710 on H⁺,K⁺-ATPase activity was antagonized by DTT and was not reversed by diluting the concentration of the drug in the medium, similar to esomeprazole. These results indicated that the inhibitory mode of E3710 on acid secretion might be based on proton pump inhibition and is unlikely to be those of histamine H₂ receptor antagonists and acid pump antagonists.

To clarify the long-lasting inhibitory mechanism, we compared the reversibility of E3710-inhibited H⁺,K⁺-ATPase activity by DTT with that of esomeprazole and investigated the binding site of E3710 for H⁺,K⁺-ATPase. No obvious difference between these two PPIs was observed regarding reversibility, and [³⁵S]E3710 binds to cysteine 815 in H⁺,K⁺-ATPase, which is in common with other PPIs such as esomeprazole (data not shown). We thus identified no apparent differences between E3710 and esomeprazole in vitro and consequently hypothesized that other in vivo factors, such as pharmacokinetic parameters or distribution, may account for the prolonged inhibitory effects of E3710 on gastric acid secretion in gastric fistula dogs.

Although GERD affects patients during the day as well as the night, the symptoms during the night have a greater negative impact on quality of life as a result of interrupting sleep patterns and increasing the risk of esophageal and respiratory complications (Shaker et al., 2004). Furthermore, nocturnal acid breakthrough, defined as a period of intragastric pH >4 for more than 1 h at night during PPI therapy (Peghini et al., 1998), has been suggested as a possible refractory cause for GERD. E3710 clearly provided better control of intragastric pH in gastric fistula dogs, thereby showing a potential to prevent nocturnal GERD symptoms and nocturnal acid breakthrough better than existing PPIs. Potent acid neutralization also may lead to faster resolution of symptoms, faster healing of lesions, better responses in severe lesions, and less frequent relapses in patients with GERD (Sonnenberg, 2004).

Apart from GERD, nonsteroidal anti-inflammatory drug-related ulcers, gastrointestinal bleeding, and H. pylori eradication also still remain unmet medical needs (Katz et al., 2006; Scarpignato and Pelosi, 2006). Because optimizing control of intragastric pH would also be feasible for these disorders, E3710 could be expected to offer improved clinical outcomes for patients with these intractable ARDs.

In conclusion, E3710, a newly synthesized PPI, may achieve potent and long-lasting suppression of gastric acid production, an adequate intragastric pH control. As a consequence, E3710 would provide a cost-effective and a valid therapy for ARDs in the future.

References


