E3710, a new proton pump inhibitor, with a long lasting inhibitory effect on gastric acid secretion

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Running title: E3710 a long lasting new PPI

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Number of text page: 35

Number of tables: 5

Number of figures: 7

Number of References: 29

The number of words in abstract: 250

The number of words in Introduction: 438

The number of words in Discussion: 606
List of nonstandard abbreviations: PPI, proton pump inhibitor; ARD, acid related disease; GERD, gastroesophageal reflux disease; NSAID, nonsteroidal anti-inflammatory drugs; DTT, dithiothreitol; MC, methyl cellulose; CI, confidence interval; NAB, nocturnal acid breakthrough

Recommended section assignment: Gastrointestinal, Hepatic, Pulmonary, and Renal
Abstract

We have investigated the pharmacology of E3710, Sodium(R)-2-[4-(2,2-dimethyl-1,3-dioxan-5-yl) methoxy-3, 5- dimethylpyridin-2-yl] methylsulfinyl-1 H-benzimidazol, a new proton pump inhibitor 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 μmol/L. Administering 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 0-2 h and 24-26 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 another representative PPI, esomeprazole (0.2, 0.4, 0.8 and 1.6 mg/kg; n=6) at 0-2 h after administration (ED₅₀= 0.40 mg/kg) and 2.8 times more potent at 24-26 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 above 4 for 82% of a day, compared to 61% 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.
Introduction

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, non-steroidal anti-inflammatory drugs (NSAID)-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, especially about 30% of GERD patients (Fass et al., 2005). GERD is a disorder of the upper gastrointestinal tract characterized by heartburn, regurgitation, epigastric pain and belching, as a result of 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 the quality of life (Wahlqvist et al., 2007). The severity is related to the acid exposure to 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 above 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 keep an appropriate acid control and be useful for the treatment of GERD.

We have newly synthesized E3710, Sodium(R)-2-[4-(2,2-dimethyl-1,3-dioxan-5-yl) methoxy-3, 5- dimethylpyridin-2-yl] methylsulfanyl-1 H-benzimidazol (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 patients (Katz et al., 2006). We compared the effects of E3710 with esomeprazole to predict its clinical efficacy and usefulness. In clinical studies the measurement of 24-h intragastric pH monitoring based on crossover design which includes a placebo is widely used to assess the efficacy of PPIs (Williams et al., 1998; Bruley des Varannes et al., 2004). We also carried out a crossover study of 24-h intragastric pH monitoring in gastric fistula dogs to confirm the long-lasting suppressive effect of E3710 compared to esomeprazole.
Methods

Materials. E3710 was synthesized at Eisai (Ibaraki, Japan). Esomeprazole magnesium trihydrate was purchased from Kemprotec Ltd. (Middlesbrough, UK). Valinomycin, NADH, gramicidin, histamine, famotidine and porcine cerebral cortex Na⁺,K⁺-ATPase were purchased from Sigma-Aldrich (St. Louis, MO, USA). ATP was purchased from the Oriental Yeast Co. Ltd. (Tokyo, Japan), SCH28080 and dithiothreitol (DTT) were purchased from Nacalai Tesque Inc (Kyoto, Japan) and [¹⁴C]-aminopyrine was purchased from Amersham-Biosciences (Piscataway, NJ, USA). Ouabain and dibutyryl-cAMP (db-cAMP) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, 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 experiment are expressed as final concentration. 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 1-3 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 below −70°C until use. The H⁺,K⁺-ATPase preparation (10 μg protein/mL) was
incubated with the methanol vehicle alone or with either E3710 or esomeprazole at 0.3, 1, 3, 10 and 30 μmol/L in 1 mmol/L Pipes–Tris buffer pH 6.1, and at 1, 3, 10 and 30 μmol/L in 40 mmol/L Tris-HCl buffer pH 7.4 for 30 min at 37°C. Either 15 mmol/L KCl or distilled water and 1 μg/mL gramicidin were then added to the samples for 10 min and then 3 mmol/L Mg–ATP (pH 7.4) was added for a further 10 min. After stopping the enzyme reaction by adding 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 Hokin, 1970) and using 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, Mg²⁺, K⁺ and valinomycin was measured (Lee and Forte, 1978). This H⁺,K⁺-ATPase preparation (10 μg protein/mL) was mixed with 0.01, 0.03, 0.1, 0.3, 1 or 3 μmol/L E3710 or esomeprazole, or with the methanol vehicle alone, in 36 mmol/L Tris–HCl buffer pH 7.4 containing 150 mmol/L KCl or 150 mmol/L NaCl, 1 mmol/L glutathione and 6 μg/mL valinomycin. The reaction was started by the addition of 2 mmol/L 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 NADH (Morii et al., 1990), using a microplate spectrophotometer (SpectraMax 250; Molecular Devices Corporation,
Measurement of gastric acid secretion in isolated rabbit gastric glands. Gastric glands were prepared from the rabbit gastric mucosa as previously described (Berglindh and Öbrink, 1976). These preparations of gastric glands were incubated with 0.03, 0.1, 0.3, 1, 3 or 10 μmol/L E3710 or esomeprazole or famotidine (for histamine stimulation) or with 0.3, 1, 3, 10, 30 and 100 μmol/L 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 secretagogues 1 mmol/L db-cAMP or 0.1 mmol/L histamine were then added, and incubated 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; Packard Instrument Co., Meriden, CT, USA). 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 Spenney, 1982).

Measurement of Na⁺,K⁺-ATPase activity from porcine cerebral cortex. Na⁺,K⁺-ATPase (10 μg protein/mL) prepared from porcine cerebral cortex was mixed with 0.3, 1, 3, 10, 30 and 100 μmol/L E3710 or esomeprazole, or 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 μmol/L ouabain or vehicle alone in 125 mmol/L Tris-HCl buffer pH 7.4 containing 50 mmol/L KCl, 100 mmol/L 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 mmol/L ouabain. The reaction was started by the addition of 3
mmol/L 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 (Morii 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 with that of SCH28080, an acid pump antagonist, which inhibits H⁺,K⁺-ATPase based on a different mechanism from PPIs. We incubated 10 μg protein/mL H⁺,K⁺-ATPase with 30 μmol/L E3710, 10 μmol/L esomeprazole, 10 μmol/L SCH28080 or methanol, together with 0.1, 0.3, 1 or 3 μmol/L DTT, or distilled water as a control, in 1 mmol/L Pipes–Tris buffer pH 6.1 for 30 min at 37°C. Either 15 mmol/L KCl or distilled water and 1 μg/mL gramicidin were added for 10 min and then, 3 mmol/L Mg–ATP (pH 7.4) was added for a further 10 min, with the samples at 37°C throughout. After stopping the enzyme reaction, 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 previously reported (Lorentozn et al., 1985; Nagaya et al., 1989). The H⁺,K⁺-ATPase (100 μg protein/mL) was preincubated with 100 μmol/L E3710, 100 μmol/L esomeprazole, 10 μmol/L SCH28080 or vehicle for 30 min at 37°C in 2 mmol/L Pipes–Tris buffer (pH 6.1). Then, H⁺,K⁺-ATPase activity was measured under two conditions: with or without dilution of the reaction mixture. Either 15 mmol/L KCl or distilled water, 1 μg/mL gramicidin and 3 mmol/L Mg–ATP (pH 7.4) were added, followed by incubation for 10 min under the undiluted
condition (60 μg protein/mL H⁺,K⁺-ATPase, 60 μmol/L E3710, 60 μmol/L esomeprazole, 6 μmol/L SCH28080) or 20 min under the diluted condition (3 μg protein/mL H⁺,K⁺-ATPase, 3 μmol/L E3710, 3 μmol/L esomeprazole, 0.3 μmol/L SCH28080). After stopping the enzyme reaction, the amount of phosphorous 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 the 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 x 5 cross-over study design for both drugs including the vehicle and was carried out over two 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 were 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 were collected every 20 min. The volume of gastric juice was determined, and then the concentration of acid was measured by titrating 0.5 mL gastric juice against 0.04 mol/L 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 secretion (mEq/20 min) = volume of gastric juice (ml/20
min) × acid concentration (mEq/ml). The inhibitory effects of the drugs were measured for the 0-2 h time period after administration on day 1 and the 24-26 h time period after administration on day 2.

**Measurement of intragastric pH over 24h 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 feeds at times appropriate for “lunch” and “dinner”, to ascertain whether E3710 was likely to be clinically useful and have long term efficacy. After infusing 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 around 10:00, and was recorded every 10 s using an ambulatory pH monitoring system (PH-101ZG; 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 DS-A pellet diet (Oriental Yeast Co., Ltd., Tokyo), were offered to each animal separately at about 13:00 (as lunch) and 18:00 (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 x 3 cross-over design studies, using 0.5% MC, 0.4 mg/kg E3710 and 1.6 mg/kg esomeprazole (n=6), were carried out to confirm the long-lasting inhibitory effects of E3710, compared to
esomeprazole.

**Statistical Analysis.** In *in vitro* experiments, the mean IC$_{50}$ and 95% confidence intervals (CI) were calculated based on the IC$_{50}$ values generated from separated sigmoid curves. In *in vivo* experiments, ED$_{50}$ values were calculated by linear regression and the potency ratio with 95% CIs were calculated using 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 cross over study. Two-sided probability (p) values <0.05 were 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.
IC_{50} values of E3710 and esomeprazole under different pH condition were 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 inhibited with IC_{50} values of 0.28 μmol/L and 0.53 μmol/L, respectively (Fig. 2A). At pH 6.1, E3710 and esomeprazole inhibited with IC_{50} values of 4.2 μmol/L and 2.3 μmol/L, respectively (Fig. 2B). At pH 7.4, E3710 and esomeprazole both showed weak inhibitory effects with IC_{50} values > 30 μmol/L (Fig. 2C).

Inhibitory effect of E3710 on acid secretion in isolated rabbit gastric glands. IC_{50} values of E3710, esomeprazole and famotidine were summarized in Table 2. Both E3710 and esomeprazole inhibited acid secretion stimulated by both db-cAMP (Fig. 3A) and histamine (Fig. 3B). By 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 IC_{50} value of 0.43 μmol/L (95% CI 0.41–0.45). In contrast, both E3710 and esomeprazole were very poor inhibitors of this enzyme with IC_{50} values greater than 100 μmol/L.

Effects of DTT and dilution on the inhibition of H^+,K^+-ATPase activity by E3710.
E3710 at 30 μmol/L and esomeprazole at 10 μmol/L 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 \(\mu\)mol/L completely suppressed the inhibitory effects of both E3710 (Fig. 4A) and esomeprazole (Fig. 4B). In contrast, SCH28080 at 10 \(\mu\)mol/L inhibited H\(^+\),K\(^+\)-ATPase activity by 68.1%, but DTT had no effect on its ability to inhibit H\(^+\),K\(^+\)-ATPase (Fig. 4C). In dilution studies, 100 \(\mu\)mol/L E3710 inhibited H\(^+\),K\(^+\)-ATPase activity by 55.4% and by 58.5% when the reaction mixture was diluted (Fig. 4D). Similarly, esomeprazole inhibited H\(^+\),K\(^+\)-ATPase activity by 91.1% when undiluted and by 93.0% after dilution (Fig. 4D). In contrast, 10 \(\mu\)mol/L 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 Fig. 5 and Fig. 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 24-26 h after administration these inhibitory effects were not sustained in the way seen with E3710 (Fig. 6B). The ED\(_{50}\) 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 0-2 h and 24-26 h after
administration are shown in Table 3. The potency ratios for E3710 to esomeprazole during 0-2 h and 24-26 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 24h in gastric fistula dogs.** Experimental protocol was 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 equal to or above pH4, compared to the 0.5% MC control (Table 4). In a crossover study E3710 and esomeprazole maintained a higher intragastric pH than the 0.5% MC control (Fig. 7B). E3710 rapidly elevated the intragastric pH, in a similar way to esomeprazole, but E3710 maintained a high intragastric pH for 24 h, in contrast to esomeprazole. The mean intragastric pH seen in the E3710 group was higher than in the esomeprazole group, although the difference was not statistically significant. In both the E3710- and esomeprazole-treated groups the intragastric pH gradually dropped, after the maximum pH-elevating effects had been reached. The intragastric pH in the esomeprazole-treated group dropped below 4 just after midnight (between 1:00 to 3:00), while in the E3710-treated group it remained substantially above pH4 during the same time period. The percentage of time for which the intragastric pH was equal to or above 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 and provided prolonged control of intragastric pH>4 over a 24 h period in gastric fistula dogs. These results proactively indicate that E3710 would substantiate an ideal therapy for ARDs, as a potent and long-acting PPI in the future.

Classical PPIs are activated by protonation and irreversibly inhibit H⁺,K⁺-ATPase mediated through the interaction with cysteine groups (Lorentzon et al., 1985; Nagaya et al., 1989; Scarpignato et al., 2006). We compared inhibitory effects of E3710 with those of esomeprazole (PPI), famotidine (histamine H₂ receptor antagonist) which inhibits acid secretion based on selective histamine H₂ receptor block, and SCH28080 (acid pump antagonist) which inhibits H⁺,K⁺-ATPase with reversible and in 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⁺,K⁺-ATPase under acidic condition and its inhibitory effect relatively decreases under neutral pH condition 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 that, unlike 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 PPIs, and is unlikely to those of histamine H₂ receptor antagonist and acid pump antagonist.
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 \(^{14}\text{C}]\text{E3710}\) 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 \textit{in vitro}, we consequently hypothesized that other \textit{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 time have a greater negative impact on QOL as a result of interrupting sleep patterns and increasing the risk of esophageal and respiratory complications (Shaker et al., 2004). Furthermore, nocturnal acid breakthrough (NAB), defined as a period of intragastric pH above 4 for more than 1 h at night during PPI therapy (Peghini et al., 1998), has been suggested as a possible refractory causes for GERD. E3710 clearly provided better control of intragastric pH in gastric fistula dogs, thereby showing a potential to prevent nocturnal GERD symptoms and NAB better than existing PPIs. Potent acid neutralization also may leads to faster resolution of symptoms, faster healing of lesions, better responses in severe lesions and less frequent relapses in GERD patients (Sonnenberg, 2004).

Apart from GERD, NSAID-related ulcers, gastrointestinal bleeding, and \textit{Helicobacter pylori} eradication also still remain unmet medical needs (Katz et al., 2006; Scarpignato and
Pelsini, 2006). As an 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 a potent and a 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 owing to a rapid and an effective treatment for ARDs in the future.
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Legends for figures

**Fig. 1.** The chemical structure of E3710. Sodium(R)-2-[4-(2,2-dimethyl-1,3-dioxan-5-yl) methoxy-3, 5- dimethylpyridin-2-yl] methylsulfinyl-1 H-benzimidazol

**Fig. 2.** Inhibitory effects of E3710 and esomeprazole on pig gastric H⁺,K⁺-ATPase activity. Inhibitory effects of E3710 on H⁺,K⁺-ATPase activity were estimated under different pH condition. A: Acidic condition. Inhibitory effects under more acidic condition compared with pH 6.1 by addition of ATP and valinomycin were evaluated. B: pH 6.1 and C: pH 7.4. Inhibitory effects under pH 6.1 and 7.4 were evaluated in each pH conditioned preincubation assay medium. ○: E3710, ●: esomeprazole. Each data represents mean from 3 independent experiments in performed in duplicate.

**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, Δ: famotidine. Each data represents mean from 4 independent experiments in performed in duplicate.

**Fig. 4.** Effects of DTT and dilution on the inhibition of H⁺,K⁺-ATPase activity with E3710, esomeprazole and SCH28080. The effects of DTT on the inhibition of H⁺,K⁺-ATPase activity by E3710 at 30 μmol/L (A), esomeprazole at 10 μmol/L (B) and SCH28080 at 10
μmol/L (C) were investigated. D: Reversibility of the inhibition of H⁺,K⁺-ATPase activity by E3710 at 100 μmol/L, esomeprazole at 100 μmol/L and SCH28080 at 10 μmol/L were investigated with dilution (-) or without dilution (+). Each data point represents the mean ± SEM 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. The gastric acid secretion was stimulated by 3 h of 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 h after the administration of 0.5% MC or E3710, gastric acid secretion was stimulated by 2 h of intravenous histamine infusion. ○: 0.5% MC, ●: E3710 0.1 mg/kg, △: E3710 0.2 mg/kg, ▲: E3710 0.4 mg/kg, □: E3710 0.8 mg/kg. Results are expressed as the mean ± SEM of 6 dogs (6 x 5 cross-over study).

**Fig. 6.** Effects of esomeprazole on histamine-stimulated gastric acid secretion in gastric fistula dogs. A: Gastric acid output changes on day 1. The gastric acid secretion was stimulated by 3 h of 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 h after the administration of 0.5% MC or esomeprazole, gastric acid secretion was stimulated by 2 h of intravenous histamine infusion. ○: 0.5% MC, △: esomeprazole 0.2 mg/kg, ▲: esomeprazole 0.4 mg/kg, □:
esomeprazole 0.8 mg/kg, ■: esomeprazole 1.6 mg/kg. Data are expressed as the mean ± SEM of 6 dogs (6 x 5 cross-over study).

**Fig. 7.** Effects of E3710 and esomeprazole on 24-h intragastric pH in gastric fistula dogs. A: Experimental protocol. B: Median 24-h intragastric pH profiles of E3710 and esomeprazole in gastric fistula dogs. After 40 min of intravenous histamine infusion, 0.5% MC, E3710 at 0.4 mg/kg or esomeprazole at 1.6 mg/kg was intraduodenally administered and the measurement of intragastric pH was commenced at about 10:00. 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 2 equal portions, which were separately offered to each dog at about 13:00 and 18:00 (indicated by triangle). Each data were expressed form 6 dogs (6 x 3 cross-over study).
Table 1. Inhibitory effects of E3710 and esomeprazole on pig gastric H⁺,K⁺-ATPase activity

<table>
<thead>
<tr>
<th></th>
<th>Acidic condition</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>

Inhibitory effects of E3710 and esomeprazole on H⁺,K⁺-ATPase activity were summarized under different pH condition. Acidic condition: Inhibitory effects under more acidic condition compared with pH 6.1 by the addition of ATP and valinomycin were evaluated. Each data represents mean from 3 independent experiments in performed in duplicate. The 95% CIs are expressed in the parenthesis.
Table 2. Inhibitory effects of E3710 and esomeprazole on acid secretion stimulated by db-cAMP and histamine in isolated rabbit gastric glands

<table>
<thead>
<tr>
<th></th>
<th>db-cAMP (μmol/L)</th>
<th>Histamine (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3710</td>
<td>0.40 (0.28-0.59)</td>
<td>0.27 (0.12-0.58)</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>0.53 (0.29-0.99)</td>
<td>0.41 (0.22-0.74)</td>
</tr>
<tr>
<td>Famotidine</td>
<td>&gt;100</td>
<td>0.35 (0.25-0.48)</td>
</tr>
</tbody>
</table>

Each data represents mean from 4 independent experiments in performed in duplicate.

The 95% CIs are expressed in the parenthesis.
Table 3. ED50 values of E3710 and esomeprazole on histamine-stimulated gastric acid secretion in gastric fistula dogs

<table>
<thead>
<tr>
<th></th>
<th>ED50 (mg/kg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2 h</td>
<td>24-26 h</td>
</tr>
<tr>
<td>E3710</td>
<td>0.18 (0.15-0.20)</td>
<td>0.22 (0.19-0.27)</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>0.40 (0.37-0.43)</td>
<td>0.71 (0.58-0.99)</td>
</tr>
</tbody>
</table>

The potency ratios for E3710 to esomeprazole during 0-2 h and 24-26 h after administration were 2.3 (95% CI: 1.9 - 2.6) and 2.8 (95% CI: 2.2 - 3.6), respectively. Data are expressed as the mean of 6 dogs (6 x 5 cross-over study). The 95% CIs are expressed in the parenthesis.
Table 4. Effects of E3710 and esomeprazole on 24 h intragastric pH in gastric fistula dogs

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

After 40 min of intravenous histamine infusion at about 10:00, 0.5% MC, E3710 or esomeprazole was intraduodenally administered 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 2 equal portions, which were separately offered to each dog at about 13:00 and 18:00. Data are expressed as the mean ± SEM. **p < 0.01, ***p < 0.001 versus the 0.5% MC (Dunnett’s multiple range test).
Table 5. Effects of E3710 and esomeprazole on 24 h intragastric pH in gastric fistula dogs in cross over study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean pH/24 h</th>
<th>% of 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>

After 40 min of intravenous histamine infusion, 0.5% MC, E3710 at 0.4 mg/kg or esomeprazole at 1.6 mg/kg was intraduodenally administered and the measurement of intragastric pH was commenced at about 10:00. 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 2 equal portions, which were separately offered to each dog at about 13:00 and 18:00. Data are expressed as the mean ± SEM (N=6, 6 x 3 cross-over study). **p<0.01, ***p<0.001 versus the 0.5% MC; #p<0.05 versus the esomeprazole 1.6 mg/kg (Tukey’s multiple comparison test).
Fig. 1.
Fig. 3.

A

\[
\text{[\textsuperscript{14}C]aminopyrine ratio (\% of control)}
\]

\[
\text{Concentration (\textmu mol/L)}
\]

B

\[
\text{[\textsuperscript{14}C]aminopyrine ratio (\% of control)}
\]

\[
\text{Concentration (\textmu mol/L)}
\]
Fig. 4.

A

Remaining enzyme activity (%)

Concentration of DTT (μmol/L)

B

Remaining enzyme activity (%)

Concentration of DTT (μmol/L)

C

Remaining enzyme activity (%)

Concentration of DTT (μmol/L)

D

Remaining enzyme activity (%)

Dilution

E3710 (100 μmol/L) Esomeprazole (100 μmol/L) SCH28080 (10 μmol/L)
Fig. 5.

A

Histamine infusion

Acid output (mEq/20 min)

Time after administration (hr)

B

Histamine infusion

Acid output (mEq/20 min)

Time after administration (hr)
Fig. 6.
Fig. 7.

A

Histamine i.v. infusion for 40 min

0.5% MC
E3710
Esomeprazole (i.d. administration)

0 (at about 10:00) 24hr

24-h intragastric pH measurement

Feed at 13:00
Feed at 18:00
Feed: 225g/head/time

Lighting 12-h light (7:00-19:00)/12-h dark (19:00-7:00)

B

Intragastric pH

--- 0.5% MC
--- E3710 0.4 mg/kg
--- Esomeprazole 1.6 mg/kg

Time of day

10:00 12:00 14:00 16:00 18:00 20:00 22:00 00:00 02:00 04:00 06:00 08:00 10:00