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Esomeprazole Inhibits the Pentagastrin-Stimulated Secretion of Gastric Acid in Healthy Japanese Volunteers

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Gastroesophageal reflux disease (GERD) is a common disease, in which the reflux of gastric acid causes mucosal damage of the esophagus and/or troublesome symptoms. Esomeprazole, a proton pump inhibitor, has been used for treatment of GERD in Japan since 2011; namely, only little is known about its effect on gastric acid secretion in Japanese. We, therefore, assessed the relationship between dose and timing of esomeprazole administration and gastric acid inhibition in 11 healthy male Japanese volunteers by directly examining gastric acid secretion capacity. In this randomized, open-label, three-way crossover study, the subjects were dosed with esomeprazole 10 mg or 20 mg once a day (q.d.), or 20 mg twice a day (b.i.d.) for 14 days, and pentagastrin-stimulated gastric acid secretion was measured by endoscopic gastrin test. At steady states, gastric acid inhibition rates were significantly higher in esomeprazole 20 mg b.i.d. (median 100.0%, interquartile range [IQR] 99.4-100%, $P = 0.027$) or 20 mg q.d. (100.0%, IQR 99.7-100%, $P = 0.016$), compared with 10 mg q.d. (98.4%, IQR 84.4-100%). At trough states, esomeprazole 20 mg b.i.d. showed significantly higher gastric acid inhibition (99.6%, IQR 99.0-100%) than did 20 mg q.d. (84.2%, IQR 76.4-88.8%, $P = 0.002$) or 10 mg q.d. (64.9%, IQR 59.1-76.7%, $P = 0.001$). Thus, esomeprazole 20 mg b.i.d. was sufficient to inhibit > 99% gastric acid secretion in healthy subjects. We propose that esomeprazole 20 mg b.i.d. is effective for treating Japanese patients with refractory GERD who require long-lasting gastric acid inhibition.

Keywords: esomeprazole; gastric acid; gastroesophageal reflux disease; healthy volunteer; pentagastrin
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Introduction

Gastroesophageal reflux disease (GERD) is a common disease in which reflux of gastric acid brings mucosal damage and/or troublesome symptoms. The degree of mucosal damage and the frequency of symptoms are dependent on the intragastric pH and the degree of esophageal acid exposure (Joelsson and Johnsson 1989). Therefore, inhibition of gastric acid secretion is a primary strategy for treatment of GERD patients.

Proton pump inhibitors (PPIs), potent acid suppressive agents, are used as the first choice for medical treatment of GERD. PPIs suppress secretion of gastric acid by inactivating H^+/K^+ ATPase in the parietal cells, keep intragastric pH higher, and promote healing of mucosal damage and symptom relief in GERD patients (Shi and Klotz 2008). However, in spite of the high efficacy of PPIs, treatment sometimes results in failure. In some cases, the failure may be due to insufficient achievement of gastric acid inhibition. Therefore, in those cases stricter suppression of gastric acid secretion is required for improving the medication strategies of PPIs.

Esomeprazole is the first single-isomer PPI consisting of the *S*-isomer of omeprazole. Esomeprazole is metabolized to a lesser extent by cytochrome P450 2C19 (CYP2C19), a hepatic enzyme, than the other enantiomer, *R*-omeprazole (Andersson et al. 2001). Its metabolic rate is lower than that of *R*- and racemic omeprazole, resulting in higher area under the curve (AUC) values following administration (Hassan-Alin et al. 2005). In patients with reflux esophagitis, both 40 mg once daily (q.d.) and 20 mg q.d. dosing resulted in a higher rate of mucosal healing than treatment with 20 mg omeprazole q.d. (Kahrilas et al. 2000).

While esomeprazole has been used worldwide for more than a decade, the usage experience is relatively limited in Japan because esomeprazole in doses of 10 mg q.d. and 20 mg q.d. has been approved in Japan since 2011. Some European and American studies have reported that the dose and timing of esomeprazole administration influenced the length of time with intragastric pH > 4 during the 24 h-monitoring period (Hammer and Schmidt 2004; Katz

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et al. 2004; Wilder-Smith et al. 2010). On the other hand, the effect of the dosing regimen of esomeprazole on gastric acid secretion in Japanese people has been insufficiently examined. Nagashima and Ikushima (2011) investigated the acid-secretion inhibitory effect of esomeprazole (10 mg, 20 mg and 40 mg q.d.) by monitoring the 24-h intragastric pH in healthy Japanese subjects. Sahara et al. (2013) also monitored the 24-h intragastric pH in healthy Japanese subjects treated with esomeprazole 20 mg twice a day (b.i.d.). However, these studies did not directly measure the maximum gastric-acid secretory capacity. Moreover, in these studies, the efficacy for gastric acid inhibition was not compared among different doses and timings of esomeprazole.

In this study, we investigated the effects of various dosages of esomeprazole on inhibition of gastric acid secretion in healthy Japanese volunteers, by measuring gastric acid secretion stimulated with pentagastrin.

Methods

Study design and subjects

This was a randomized, open-label, three-way crossover study in healthy volunteers, conducted in a single center in Japan. The study was conducted in accordance with the Helsinki Declaration, and was approved by the Ethics Committee for Human Research at Tohoku University School of Medicine, Sendai, Japan. All subjects provided written informed consent prior to the initiation of the study. The study was registered at the University Hospital Medical Information Network Center Clinical Trials Registry as number UMIN000009065.

Healthy Japanese males aged 20-65 years old were eligible for inclusion. Subjects were excluded if they had a history of drug allergy or hepatic, renal or cardiac disease, if they had experienced gastrointestinal tract resection or vagotomy, were receiving ongoing therapy for any disease, or were expected to require medication, including acid-suppressive drugs or antacids, during the study period.

Study procedure

Subjects received a randomized sequence of three regimens of esomeprazole: (1) 10 mg q.d. at 8 am before breakfast, (2) 20 mg q.d. at 8 am before breakfast, and (3) 20 mg b.i.d. at 8 am before breakfast and 10 pm before dinner. The dosing period of each regimen was 14 days, separated by a washout period of 14-28 days.

Prior to dosing of esomeprazole, venous blood was collected from the subjects for serological assay of *Helicobacter pylori* (*H. pylori*) infection and genotyping of CYP2C19. Gastric acid secretion capacity was measured by endoscopic gastrin test (EGT), details of which are described later. Once dosing in each regimen was underway, acid secretion capacity at steady state and trough state was measured at 2 pm on day 7 of dosing and at 8 am on day 15 (the day after the end of the dosing period), respectively, using EGT.

Adverse events occurring during the study period were evaluated at each visit. Dosing compliance was assessed by interview at the end of each dosing period.

Measurement of gastric acid secretion capacity

Gastric acid secretion capacity was measured by EGT, as described previously (Iijima et al. 1998). After fasting for at least 10 h, the subjects were intramuscularly injected with 6 μg/kg of pentagastrin (Sigma, St. Louis, MO, USA). About 15 min after the injection, an endoscope was inserted into the stomach. Gastric fluid pooled in the stomach was aspirated and discarded under direct observation with endoscopy. Then, 20 min post-injection, newly secreted gastric juice was collected over a 10-min period until 30 min post-injection. The obtained gastric fluid was measured for volume, and its H^+ concentration was determined by titration. The EGT value was determined as an acid output for the 10-min period, and expressed as H^+ mEq/10 min.

H. pylori status and CYP2C19 polymorphism genotyping

H. pylori infection status was determined by measuring titers of anti-*H. pylori* IgG in the serum samples using an EIA kit (E Plate "Eiken" *H. pylori*-antibody II; Eiken Chemical Co. Ltd., Tokyo, Japan). A genotype of CYP2C19 gene, which encodes a hepatic enzyme involved in metabolism of esomeprazole, was determined using fluorescence correlation spectroscopy (Shinkai et al. 2013). Genomic DNA was extracted from venous white blood cells, and used as a template for polymerase chain reaction (PCR) to specifically amplify fluorescent-labeled DNA fragments with wild-type (wt) or mutant sequence. The PCR products were analyzed by fluorescence correlation spectroscopy to determine CYP2C19 genotype. The two loss-of-function mutations have been reported in the CYP2C19 gene: a G-to-A mutation in exon 5 (m1) and a G-to-A mutation in in exon 4 (m2) (De Morais et al. 1994a, b). The m1 generates an aberrant splice site that results in a premature termination of CYP2C19 protein, while the m2 represents the non-sense mutation that also generates truncated CYP2C19 protein. Accordingly, subjects were classified into homo-EM, hetero-EM and poor metabolizer (PM). Homo-EM subjects have wild-type alleles (wt/wt), whereas PMs carry the m1 mutation allele and/or the m2 mutation allele (m1/m1, m1/m2, or m2/m2). Hetero-EM subjects carry a mutant allele in exon 5 or 4 (wt/m1 or wt/m2) (De Morais et al. 1994a, b).

Statistical analysis

Values of subject profiles were expressed as means ± standard deviation (S.D.). The inhibition rate of gastric acid secretion was calculated in each subject as follows: inhibition rate (%) = (1 – EGT value at steady state or trough state/EGT value at baseline) × 100. Its median and interquartile ranges (IQR) were determined. The differences in inhibition rates between the regimens were evaluated by the Wilcoxon signed-rank test with a kind of closed testing procedure to control overall significant level. That is, the first step was comparison between esomeprazole 10 mg q.d. and 20 mg b.i.d. regimens. When the result was statistically significant, the inhibition rates between 10 mg q.d. and 20 mg q.d. were compared. If the results were significant again, the inhibition rates between 20 mg q.d. and 20 mg b.i.d. were compared. We considered a P value of less than 0.05 to be statistically significant. All statistical analyses were conducted using the SPSS program, version 18 (SPSS, Chicago, IL, USA).

Results

Participants' profiles

Twelve subjects were enrolled and completed the study with no adverse events during the study period. Data for one subject were excluded from the analysis because of *H. pylori* infection, which affects gastric acid secretion. The background characteristics of the remaining subjects

are shown in Table 1. The mean (± S.D.) age was 29.3 ± 8.3 years, and the mean body mass index was 21.6 ± 1.7. Genotyping of CYP2C19 determined that one subject was homo-EM and eight were hetero-EM; the remaining two were classified as having a PM genotype. The mean EGT value at baseline was 4.07 ± 1.42 mEq/10 min. Four of the 11 subjects were current smokers. One subject was non-drinker of alcoholic beverages, while two and eight were regular (> 2 days a week) and occasional/social (≤ 1 day a week) drinkers, respectively.

Inhibition of gastric acid secretion

Inhibition rates of gastric acid secretion at steady and trough states are shown in Fig. 1. At steady state, corresponding to 6 h after the morning dosing, median gastric acid inhibition rates were 98.4% (IQR 84.4-100.0%), 100.0% (IQR 99.7-100.0%) and 100.0% (IQR 99.4-100.0%), for esomeprazole 10 mg q.d., 20 mg q.d. and 20 mg b.i.d. regimens, respectively (Fig. 1A). Comparison of the inhibition rates between the regimens indicated that acid secretion was significantly more inhibited by esomeprazole 20 mg b.i.d. than 10 mg q.d. dosing (P = 0.027). The difference was also significant between the 10 mg q.d. and 20 mg q.d. regimens (P = 0.016), but not between the 20 mg q.d. and 20 mg b.i.d. regimens (P = 0.375).

On the other hand, at trough state, which corresponded to just before the morning dosing, median gastric acid inhibition rates were 64.9% (IQR 59.1-76.7%), 84.2% (IQR 76.4-88.8%) and 99.6% (IQR 99.0-100.0%), for esomeprazole 10 mg q.d., 20 mg q.d. and 20 mg b.i.d. regimens, respectively (Fig. 1B). Gastric acid secretion was significantly more inhibited in the esomeprazole 20 mg b.i.d. regimen compared with the 10 mg q.d. regimen (P = 0.001). Significant differences were also seen between the 10 mg q.d. and 20 mg q.d. regimens (P = 0.032), and the 20 mg q.d. and 20 mg b.i.d. regimens (P = 0.002).

In the esomeprazole 20 mg b.i.d. regimen, gastric acid secretion was nearly completely inhibited at both steady and trough states in most subjects (Fig. 2). In contrast, in both the esomeprazole 10 mg q.d. and 20 mg q.d. regimens, the inhibition rate was relatively lower at trough state than at steady state.

Table 1. Baseline characteristics of study participants.

Characteristic	n = 11
Age (years)	29.3 ± 8.3
Height (cm)	174.1 ± 5.2
Body weight (kg)	65.6 ± 7.8
Body mass index (kg/m^2)	21.6 ± 1.7
EGT value (mEq/10 min)	4.07 ± 1.42

Values are indicated as mean ± S.D.
EGT, endoscopic gastrin test.

Discussion

This is the first report to demonstrate the effect of dose and timing of esomeprazole administration on gastric acid

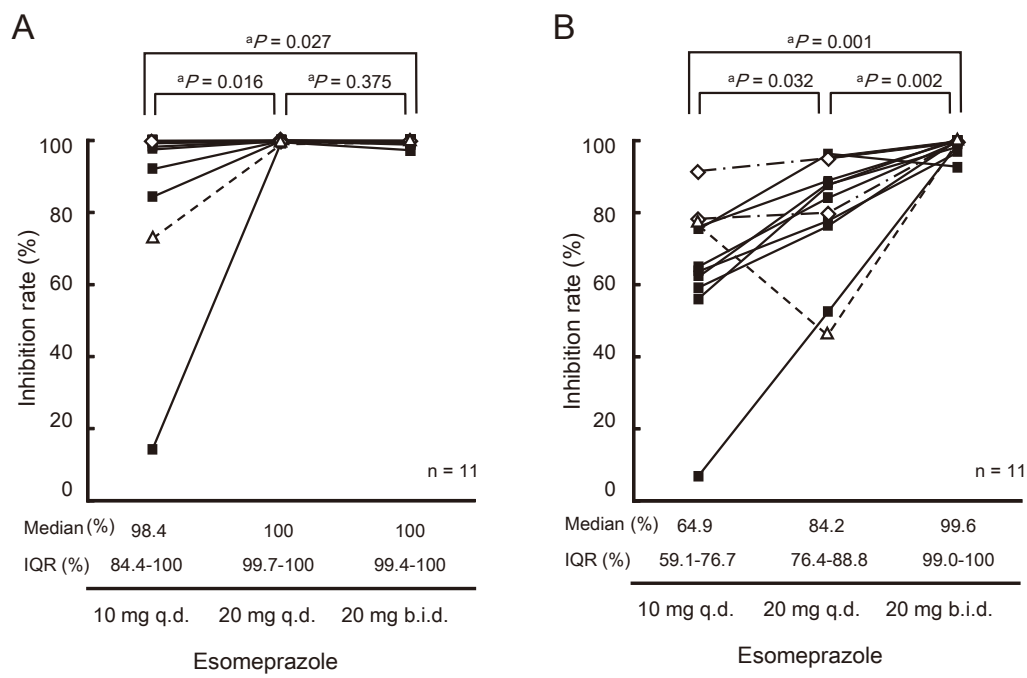

Fig. 1. Comparison of gastric acid inhibition rates among esomeprazole regimens.
Gastric acid inhibition rates in each subject after administration of esomeprazole 10 mg q.d., 20 mg q.d. and 20 mg b.i.d. are indicated. Diamonds, solid squares and triangles represent poor metabolizers, heterozygous extensive metabolizers and homozygous extensive metabolizers, respectively. A: At steady state. B: At trough state. IQR, interquartile range. [a]P values for Wilcoxon signed-rank test between regimens.

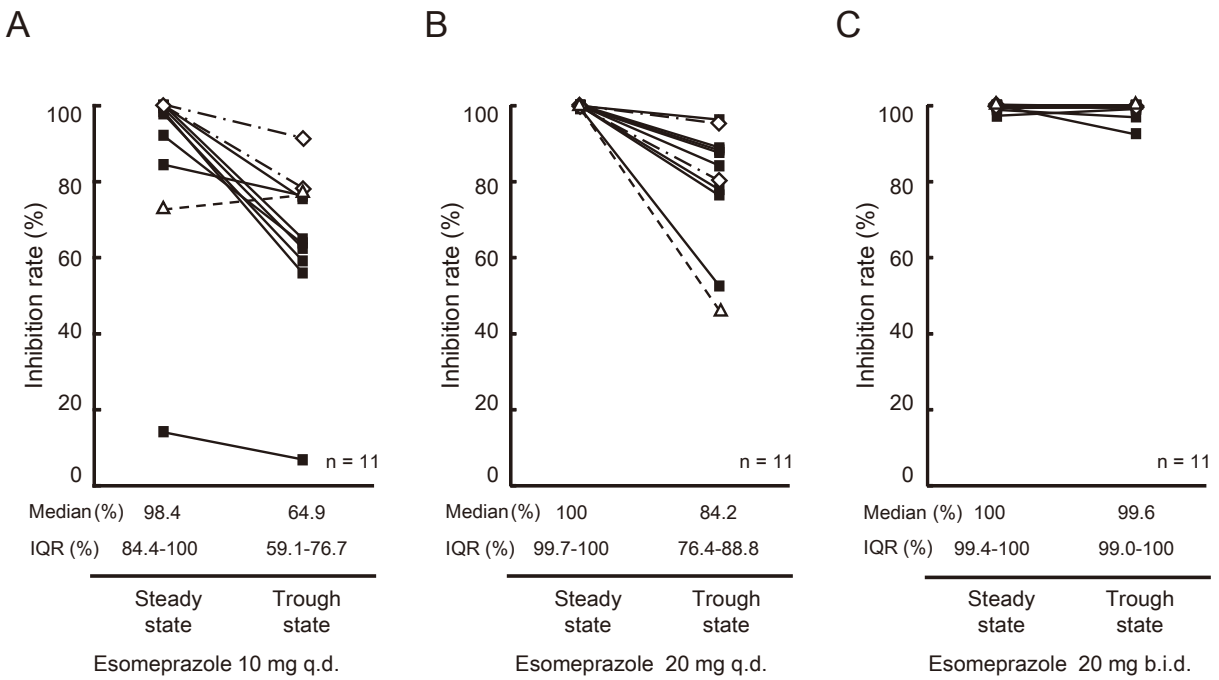

Fig. 2. Gastric acid inhibition rates at steady and trough states.
Gastric acid inhibition rates in each subject at steady and trough states are indicated. Diamonds, solid squares and triangles represent poor metabolizers, heterozygous extensive metabolizers and homozygous extensive metabolizers, respectively. A: Esomeprazole 10 mg q.d. B: Esomeprazole 20 mg q.d. C: Esomeprazole 20 mg b.i.d. IQR, interquartile range.

inhibition in Japanese subjects by directly measuring pentagastrin-stimulated gastric acid secretion. In this cross over study, we quantified gastric acid secretion capacity by EGT. EGT is a rapid and reproducible method enabling measurement of gastric acid secretion capacity, with good correlation to both the conventional peak acid output and maximum acid output methods (Iijima et al. 1998).

This study revealed a superior effect of esomeprazole 20 mg b.i.d. in terms of inhibition of gastric acid secretion compared with esomeprazole 20 mg q.d. and 10 mg q.d. At steady state, esomeprazole 20 mg q.d. and 20 mg b.i.d. almost completely and more strongly inhibited gastric acid secretion compared with 10 mg q.d. At trough state, esomeprazole 20 mg b.i.d. was significantly more effective at inhibiting acid secretion than 10 mg q.d. or 20 mg q.d. These results suggest that esomeprazole 20 mg b.i.d. is more effective for achieving prolonged and strong gastric acid inhibition in healthy Japanese subjects.

The effect of increasing the dose and frequency of esomeprazole on gastric acid inhibition in this study corresponds with the results of previous western studies using 24-h pH monitoring. Wilder-Smith et al. (2010) reported that esomeprazole 40 mg q.d. significantly increased the time with intragastric pH > 4 during the 24-h monitoring period, daytime period, and night-time period compared with esomeprazole 20 mg q.d., except during the daytime period with before-dinner dosing. They also showed that esomeprazole 20 mg b.i.d. significantly improved the duration of acid inhibition compared with 20 mg q.d. before breakfast and 20 mg q.d. before dinner (Wilder-Smith et al. 2010). Further, in comparison with 40 mg q.d. before breakfast, the esomeprazole 20 mg b.i.d. regimen significantly increased the time with intragastric pH > 4 during the 24-h monitoring period and during the night-time period, and reduced events of nocturnal acid breakthrough (Hammer and Schmidt 2004; Katz et al. 2004; Wilder-Smith et al. 2010). These previous results suggest that both increased dose and increased dosing frequency may improve the acid-inhibitory effects of esomeprazole.

It is important to maintain higher intragastric pH for a longer time during a 24-h period for effective treatment of GERD (Bell et al. 1992). In our study, both esomeprazole 20 mg q.d. and 20 mg b.i.d. achieved median gastric acid inhibition rate of 100% at steady state. However, at trough state, just prior to the breakfast dosing, the inhibition rate was significantly higher with esomeprazole 20 mg b.i.d. (median 99.6%) than with 20 mg q.d. (median 84.2%). Some patients may have a potential risk factor for refractory GERD, such as delayed gastric emptying, which may jeopardize the effect of PPI (Fass and Gasiorowska 2008). In such patients, esomeprazole 20 mg q.d. might be insufficient to inhibit gastric acid secretion completely. Residual gastric acid may prevent the healing of reflux esophagitis. Although the approved dose of esomeprazole in Japan is 20 mg q.d. for reflux esophagitis at this time, an esomeprazole 20 mg b.i.d. regimen may be an effective option for treatment of Japanese patients whose disease has not improved using standard-dose PPI treatment.

This study had several limitations. Because the study was conducted in healthy subjects, the results may not be directly extrapolated to patients with acid-related diseases including GERD. The sample size was too small to allow the effects of esomeprazole dosages and timings to be analyzed in relation to CYP2C19 genotypes; however, a previous study reported that the efficacy of esomeprazole for GERD was less affected by CYP2C19 genotype than that of omeprazole (Schwab et al. 2005). In addition, this study included relatively young male subjects. Such characteristics may differ from the Japanese patient population with GERD, because in Japan, GERD is predominant in females and prevalent in the elderly (Fujiwara and Arakawa 2009). However, the use of healthy younger subjects may strengthen the reliability of the results by reducing confounding biases due to various health problems. Nevertheless, the effect of different esomeprazole regimens should be confirmed by a clinical trial conducted in Japanese GERD patients.

In conclusion, steady state gastric acid secretion was almost completely inhibited in Japanese subjects administered esomeprazole 20 mg q.d. or 20 mg b.i.d. At trough state, the inhibition rate of acid secretion was significantly higher for esomeprazole 20 mg b.i.d. compared with both esomeprazole 10 mg q.d. and 20 mg q.d., suggesting that the esomeprazole 20 mg b.i.d. regimen will be effective in treating refractory cases of GERD requiring long-lasting inhibition of gastric acid secretion.

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Conflict of Interest

The authors declare no conflict of interest.

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