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PHARMACOGENETICS

Optimal dose regimens of esomeprazole for gastric acid suppression with minimal influence of the *CYP2C19* polymorphism

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Abstract
Objective In this pilot study, we attempted to determine the optimal dosage regimens of esomeprazole for treatment of GERD with minimal influence of the *CYP2C19* polymorphism through a study of the pharmacokinetics and pharmacodynamics of esomeprazole given at 3 different dosage regimens with the same total daily dose.
Methods Each of the 3genotypes of *CYP2C19*, homozygous extensive metabolizers (homEMs), heterozygous EMS (hetEMs), and poor metabolizers (PMs) were recruited in this clinical trial. Subjects were given a placebo followed by the administration of esomeprazole, at a dose of 40 mg once daily (40QD), 20 mg twice daily (20TD), or 10 mg 4 times daily (10Q4D) for 7 days. Twenty-four-hour and nocturnal intragastric pH and plasma esomeprazole concentrations were all determined on day 7.
Results The pharmacokinetic parameters and dynamic characteristics differed among the 3 *CYP2C19* genotype groups. With esomeprazole 40QD, gastric acid suppression was insufficient to achieve a therapeutic effect, while 20TD and 10Q4D were found to be effective in controlling both daytime and nocturnal gastric acidity for all 3 genotype groups.
Conclusions It was confirmed that intragastric pH values and plasma esomeprazole concentrations potentially depended on the *CYP2C19* genotype status for treatment with esomeprazole. Dosage regimens of divided doses of 20TD or 10Q4D esomeprazole yielded improved antisecretory effects with a minimal influence of *CYP2C19* polymorphisms.

Keywords GERD · Esomeprazole · CYP2C19 · Polymorphism · Intragastric pH

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Introduction

Gastroesophageal reflux disease (GERD) is a common disorder of the gastrointestinal tract estimated to affect approximately 21%~59% of the adult population [1]. Proton pump inhibitors (PPIs) are biotransformed to the active form, sulfonamide, which forms disulfide covalent bonds with cysteine residues of the H^+/K^+-ATPase enzyme in the proton pump, thus inhibiting the final step of acid secretion and are recommended as a first-line therapy for GERD [2]. There is an approximately linear relationship between the healing rate and number of hours for which the gastric pH is controlled to a level above 4.0 [3]. Several authors have also reported that the duration of intragastric pH levels lower than 4.0 during a 24-hour period should be shortened to fewer than 2~4 hours (16.7%) [3–5]. Therefore, esomeprazole at the standard dose of 40 mg once daily for maintaining the intragastric pH above 4.0 for a mean of 14.0 h provides more-effective control of gastric acid at a steady state than a standard dose of lansoprazole (30 mg, 11.5 h) [6, 7], omeprazole (20 mg, 11.8 h) [6, 8–9], pantoprazole (40 mg, 10.1 h) [6, 10], or rabeprazole (20 mg, 12.1 h) [6, 11] in patients with symptoms of GERD. Esomeprazole at 40 mg twice daily provided a mean of

19.2 h of intragastric pH levels > 4.0 versus 14.2 h with 40 mg once daily and 17.5 h with 20 mg twice daily in 25 subjects. Intragastric pH was maintained above 4.0 for similar durations of time during active and sleeping periods for all doses [12].

However, despite the overall efficacy of PPIs, 15%~30% of patients with GERD remain unhealed and/or experience insufficient symptom relief after initial PPI therapy [13]. In failed cases, 24-hour intragastric pH monitoring often reveals levels of < 4.0 lasting for more than 1 hour especially during the nocturnal period, which is defined as nocturnal acid breakthrough (NAB), resulting in frequent exposure of the esophageal mucosa to refluxed low-pH gastric juices [14–16]. Thus it has been recommended that a PPI with an enhanced bioavailability (esomeprazole vs. omeprazole) [8, 9], a higher standard dose (40 mg esomeprazole vs. 30 mg lansoprazole or 20 mg rabeprazole) [10, 11] or twice daily with a standard dose (40 mg esomeprazole twice daily vs. once daily) [7, 12], and a longer half-life (tenatoprazole) [13] would result in more-prolonged inhibition of gastric acid secretion, especially during the night leading to an increase in the healing rate for GERD.

PPIs, including omeprazole, esomeprazole, pantoprazole, and lansoprazole, are hydroxylated by *CYP2C19*, and sulfoxidated to esomeprazole sulfone by *CYP3A4*. Both *CYP2C19* and *CYP3A4* are sequentially but alternatively involved in the metabolism of these PPIs [17]. Recent studies have suggested that inherited genetic polymorphisms of *CYP2C19* may determine the enzyme's activity, and hence may dominate the plasma concentrations of these drugs and the ability to suppress acid production [18–20]. To overcome NAB in patients with inherited genetic polymorphisms of *CYP2C19*, intensive or modified dosage regimens of PPIs are needed. Sugimoto *et al*. [21] proposed that a therapeutic strategy of rabeprazole should be based on the *CYP2C19* genotype status as follows: 20 mg once daily for poor metabolizers (PMs), 20 mg twice daily or 10 mg 4 times daily for heterozygous extensive metabolizers (hetEMs), and 10 mg 4 times daily for homozygous EMs (homEMs) of *CYP2C19*.

Neither genetic nor phenotypic testing for *CYP2C19* activity is routinely used in clinical practice to design appropriate therapeutic regimens. Except for a study by Sheu et al. reporting no significant differences among CYP2C19 genotypes in triple therapy with administration of 40 mg esomeprazole twice daily [22], there have been no reports regarding intragastric pH levels with different dosage regimens of esomeprazole, and the relationship between intragastric pH levels and plasma concentrations of esomeprazole remains unclear in the Taiwanese population. Furthermore, in a study using esomeprazole (40 mg daily) in 205 patients with GERD, it was contrarily found that the healing rate after 4 weeks was not dependent on the *CYP2C19* genotypes [23]. Therefore, pharmacokinetic parameters evaluated by plasma concentration profiles and pharmacodynamic efficacies estimated by intragastric acid suppression, as indicated by the percent time during a 24-hour or a nocturnal period that the intragastric pH is maintained below 4.0, were employed in this study to examine the impacts of *CYP2C19* polymorphisms for different dosage regimens with the same total daily dose and to optimize dosage regimens of esomeprazole for controlling acid secretion with minimal influence by *CYP2C19* polymorphisms.

Materials and methods

Subjects

Blood samples were obtained from 38 healthy Taiwanese subjects after receiving their written informed consent. DNA was extracted from each subject's leukocytes by a standard phenol-chloroform extraction procedure. Genotyping for *CYP2C19* was performed by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described by de Morais *et al.* [24] with minor modifications. On the basis of genotyping, subjects were classified into one of three genotype groups as follows: homEM (**1/*1*), hetEM (**1/*2* or **1/*3*), or PM (**2/*2*, **3/*3*, or **2/*3*), as previously reported.

In total, 9 subjects without *Helicobacter pylori* infection based on a carbon 13-labeled urea breath test (Taiwan I-SO BIOTEC) were randomly selected and invited to participate in this study. Three homEMs, 3 hetEMs, and 3 PMs exhibited no demographic differences in gender, age, body weight, or height as shown in Table 1. Subjects were blind to their *CYP2C19* genotype status. Their normal health status was judged by a physical examination, electrocardiogram, urinalysis, and screening of the blood chemistry including a complete blood count and liver function test. None of the subjects consumed extensive amounts of alcohol, and all were non-smokers. Written informed consent was obtained from each of the subjects before participation in the study.

Study protocol

All subjects were first administered a placebo (water only) and then 3 different dosage regimens of esomeprazole, each of which provided the same total daily dose of 40 mg. Each subject was administered esomeprazole (Nexium®, AstraZeneca, lot no. 956) as enteric coated pellets in a capsule with 240 mL water for 40 mg once daily (08:00, before breakfast; 40QD), 20 mg twice daily (08:00 and 18:00, before breakfast and dinner; 20TD), and 10 mg four times daily (08:00M, 12:00, 18:00, and 20:00; before breakfast,

Table 1 Demographic characteristics of *Helicobacter pylori*–negative healthy volunteers enrolled in the study with different *CYP2C19* genotypes

	Genotype status	Age (years)	Body weight (kg)	Height (cm)
homEM (n=3)	*1/*1 (n=3) (31.58%)[1]	22.7±1.4 (21~25)	58.9±2.4 (54.2~62.4)	170.3±3.8 (164~177)
hetEM (n=3)	*2/*1 (n=2) (39.47%) *1/*3 (n=1) (13.16%)	23.0±0 (23)	67.3±1.2 (65~69.2)	178.3±2.4 (175~183)
PM (n=3)	*2/*2(n=1) (7.89%) *3/*3(n=1) (2.63) *2/*3(n=1) (5.26%)	21.7±0.8 (20~23)	66.6=3.4 (61.6~73)	176.0±3.1 (172~182)
P value		*NS*	*NS*	*NS*

[1] The allelic frequency of CYP2C19 in the study. NS, statistically insignificant.
Age, body weight, and height are given as the median±S.D. and (range).
1*, wild-type; **2, CYP2C19*2* mutation in exon 5;3, CYP2C19*3* mutation in exon 4; homEM, homozygous extensive metabolizer; hetEM, heterozygous extensive metabolizer; PM, poor metabolizer.

lunch, dinner, and bedtime; 10Q4D) for 7 days. Plasma esomeprazole concentrations and 24-hour intragastric pH levels were determined on day 7 as described below. There was a washout period of 2 weeks between the 3 study periods.

On day 7 of each regimen, all subjects were asked to stay at Taipei Medical University Hospital for 24 hour to collect the blood sample and to measure intragastric pH values. Subjects were provided with 3 meals (breakfast [400 kcal] at 08:30, lunch [650 kcal] at 12:30, and dinner [650 kcal] at 18:30. Total calories were 1700 kcal/day; including 210 g carbohydrates, 85 g protein, and 50 g lipids. Mineral water was allowed at fixed times, but other beverages were not permitted. The nocturnal supine period was defined as 23:00 to 07:00. Patients were instructed to remain in bed during this period of the night regardless of whether they were awake or asleep. Meals, water, and sleep were consumed within the same number of minutes relative to dosing in order to minimize response variability, but other normal daily activities were not restricted. Thus, the collected data referred to similar postural and physiological conditions of the subjects. The study protocol was approved by the Internal Review Board of Taipei Medical University.

Twenty-four-hour intragastric pH monitoring

The pH electrodes were calibrated before recording with standard buffer solutions of pH 4.0 and 7.0 (MMS, The Netherlands). On day 7 of each dosing regimen, an antimony pH electrode (pHersaflex, MMS) was inserted transnasally with local anesthetic lidocaine hydrochloride (Xylocaine Jelly® 2%, AstraZeneca) and was placed 10 cm distal to the lower esophageal sphincter. Each subject had their own electrode, which was placed in the same position during all 4 recordings. The pH values were recorded every 2 seconds with a Orion II (pH recorder, MMS), beginning at 07:30, and was stopped at the following morning for the 24-hour recording. The 24-hour intragastric pH monitoring period was divided into daytime (07:00 to 23:00) and nighttime (23:00 to 07:00). When the recordings were completed, the data were transferred to a computer and stored with dedicated software programs (MMS Investigation and Diagnostic Software, The Netherlands).

Blood sample collection and esomeprazole analysis

About 8 mL of venous blood was collected by means of an indwelling venous cannula of the cubital vein in heparinized tubes. On day 7 of the once-daily dosage regimen, blood samples were collected before and at 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 5, 7, 10, 12 and 23 hours after the morning dose of esomeprazole. In the divided dosage regimens, blood samples were taken before and at 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 5, 7, 10, 10.33, 10.67, 11, 11.5, 12, 12.5, 13, 15, and 23 hours after the morning dose of esomeprazole on day 7. Any deviation from the stated sampling time was recorded on the form. Plasma samples were immediately separated by centrifugation at 3500 rpm for 10 minutes, and then plasma was transferred to suitably labeled tubes, and stored at -30°C to ensure optimal stability until the HPLC assay. To determine the concentration of esomeprazole, a plasma sample (1 mL) was spiked with 100 μL of an internal solution (Phenacetin, 40 μg/mL) and 100 μL of sodium hydroxide (1 N). The mixture was extracted with 6 mL of ethyl acetate/dichloromethane (5/1, v/v) and centrifuged at 3500 rpm for 10 minutes. The supernatant was transferred to another clean glass tube and evaporated under a stream of nitrogen gas at 50 C until completely dry. The dry residue was reconstituted with 250 μL of the mobile phase, and 100 μL of the supernatant was injected into a Phenyl HYPERSIL column (25 cm×4.6 mm, with a particle size of 5 μm, Thermo). The mobile phase consisted of acetonitrile (A) and 0.5% ammoniumdihydrogen phosphate buffer adjusted to pH 7.5 with an ammonium hydroxide solution (B) graduated from A:B 20:80 to A:B 80:20 within 19 minutes. The flow rate was 1.2 mL/min. The column effluent was monitored by an ultraviolet detector

at a wavelength of 302 nm. Since esomeprazole is the *S*-isomer of omeprazole, the racemic form of omeprazole without resolution was appropriate as the standard to analyze the concentration of esomeprazole by HPLC.

Pharmacokinetic analysis

Concentrations below the limit of quantification were reported as zero. The maximum esomeprazole concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined from the respective observed plasma concentration versus time data. Pharmacokinetic parameters were calculated by noncompartmental methods. The elimination rate constant (k_e) was obtained by a linear regression analysis using at least 3 sampling points of the terminal log-linear declining phase to the last measurable concentration. The elimination half-life ($T_{1/2}$) was calculated as $ln2/k_e$. The area under the curve to the last measurable concentration (AUC_{0-24}) was calculated by the linear trapezoidal rule. The apparent oral clearance (Cl/F) was calculated as the dose/AUC_{0-24}, and the mean residence time (MRT) was calculated as $AUMC_{0-24}/AUC_{0-24}$.

Statistical analysis

Statistically significant differences in pharmacodynamic parameters among the 3 *CYP2C19* genotype groups were determined using the Mann-Whitney *U*-test when significant differences were observed by the Kruskal-Wallis test. Statistically significant differences in mean pharmacokinetic parameters among the 3 genotype groups were determined using one-way analysis of variance (ANOVA) followed by Scheffe's multiple-comparison test. To determine whether pharmacodynamic parameters differed among the 3 dosage regimens, the Wilcoxon signed rank test was used when significant differences were observed by the Friedman test. Statistical differences in the mean pharmacokinetic parameters between the different dosage regimens were determined using a repeated-measure ANOVA followed by Scheffe's multiple-comparison test. All P values were 2-sided, and $P<0.05$ was accepted as indicating statistical significance. All analyses were performed using the statistical software package SAS, Version, 5.1.2600 (SAS Institute, Cary, NC, USA).

Results

In total, 38 subjects were recruited for genotyping after signed informed consent was obtained. According to the genotyping for *CYP2C19*, the allelic frequency was calculated and is listed in Table 1, and 38 subjects were classified into the following genotypes: 12 (12/38) were wild-type homozygous (homEMs, **1/*1*), 15 (15/38) were heterozygous for the *CYP2C19*2* mutation and without the *CYP2C19*3* mutation (hetEMs, **1/*2*), 5 (5/38) were heterozygous for the *CYP2C19*3* mutation and without the *CYP2C19*2* mutation (hetEMs, **1/*3*), 2 (2/38) were heterozygous for both the *CYP2C19*2* and *CYP2C19*3* mutations (PMs, **2/*3*), 3 (3/28) were homozygous for the *CYP2C19*2* mutation and without the *CYP2C19*3* mutation (PMs, **2/*2*), and 1 (1/38) was homozygous for the *CYP2C19*3* mutation and without the *CYP2C19*2* mutation (PMs, *3/*3). Nine subjects (3 for each genotype) with an even distribution among allele subgroups in each genotype were invited to participate in the study according to the protocol and demographic data, and their clinical baseline characteristics were examined and determined not to be significantly different (Table 1). The use of esomeprazole was well tolerated clinically, and no subject dropped out due to serious adverse events. On day 7, a steady state had been reached, so these data are also representative of a longer period of therapy.

Esomeprazole plasma concentrations and pharmacokinetic parameters

The mean plasma concentration versus time curves of esomeprazole in the 3 genotype groups after the last dosing for 40QD and 20TD, and for 10Q4D for 7 days are shown in Fig. 1 a-c, respectively. Pharmacokinetic parameters are provided in Table 2, and the influence of the CYP2C19 genotypes on various pharmacokinetic parameters calculated as the PM/homEM ratio is also listed in Table 2. The mean plasma concentration-time profiles demonstrated by Fig. 1 generally differed among the 3 *CYP2C19* genotype groups as follows: the highest was in PMs, followed by hetEMs and homEMs. The C_{max} value was determined to be statistically insignificantly different among the 3 genotypes at the same dosage regimens, but the mean C_{max} for PMs was the highest among the 3 genotype groups at all 3 dosage regimens, and the mean C_{max} values for homEMs were approximately 1.14-, 1.34-, and 1.42-fold lower than those for PMs at the 40QD, 20TD, and 10Q4D dosages, respectively, as shown in Table 2. Although the C_{max} value for 10Q4D was the lowest of all the treatment regimens in all 3 genotypes, plasma esomeprazole concentrations were sustained throughout the 24-hour period. It was concluded that an impact of *CYP2C19* polymorphisms was shown on the mean C_{max}, with the highest mean C_{max} value being observed for PMs at the 3 different dosage regimens. With the same total daily dose of 40 mg, the higher metabolic activity of *CYP2C19* (homEm > hetEM > PM) would result in the higher extent of reduction in the mean C_{max} value (40QD < 20TD < 10Q4D) following the dosage regimens with more dosage divisions (Fig. 2).

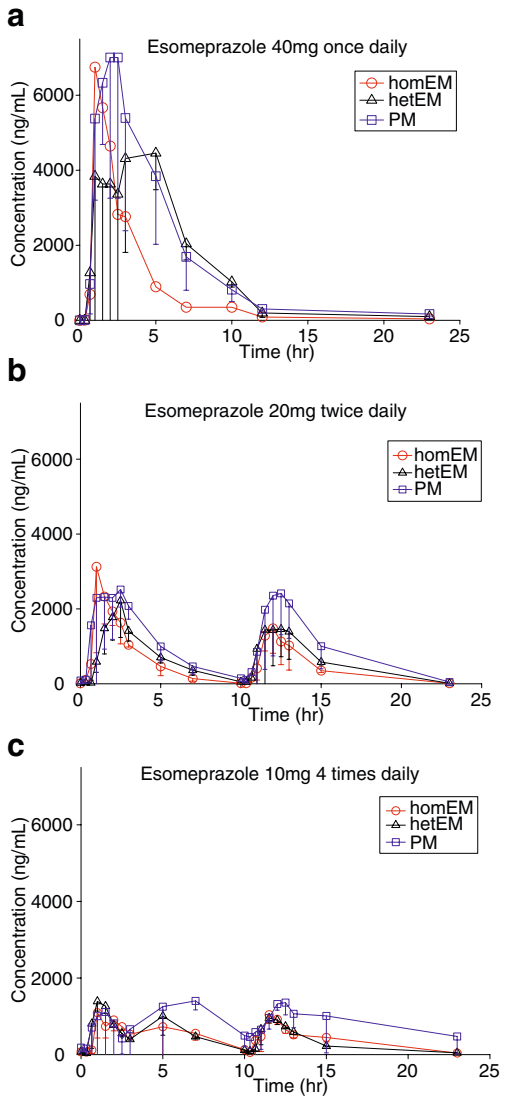

Fig. 1 Mean (±S.D.) 24-hour plasma esomeprazole concentration-time curves with (a) 40 mg once daily (40QD), (b) 20 mg twice daily (20TD), and (c) 10 mg 4 times daily (10Q4D) as a function of the *CYP2C19* genotype status

An insignificant difference in AUC_{0-24} values was obtained among the 3 dosage regimens for the same genotype groups (homEM: P=0.1337; hetEM: P=0.2936; and PM: P=0.9062), and the mean AUC_{0-24} values were the highest for PMs in all 3 regimens examined. The AUC_{0-24} ratios of PMs to homEMs were observed to be 1.48 for 40QD, 1.88 for 20TD, and 2.01 for 10Q4D as shown in Table 2. Similarly, the mean $T_{1/2}$ values within the same *CYP2C19* genotype group did not significantly differ among the 3 dosage regimens. With 40QD, the mean $T_{1/2}$ value for PMs was significantly longer than that for homEMs (P=0.0005) and hetEMs (P=0.0145). The $T_{1/2}$ ratios of PMs/homEMs as shown in Table 2 were 1.60, 1.36, and 1.21 for 40QD, 20TD, and 10Q4D, respectively. Correspondingly, the values of Cl/F for homEMs among the 3 dosage regimens were the highest, and were 1.4-, 1.5-, and 1.8-fold greater than those for PMs at 40QD, 20TD, and 10Q4D, respectively. Although the mean MRT_{0-24} values showed a marginal significance among the 3 different genotypes at 40QD (P=0.0463), 20TD (P=0.0499), and 10Q4D (P=0.0498), the MRT_{0-24} ratios of PMs/homEMs as shown in Table 2 were1.32, 1.14, and 1.24 for 40QD, 20TD, and 10Q4D, respectively. Finally, no significant differences were observed in T_{max} values among different *CYP2C19* genotype groups or among different dosage regimens. It was also concluded that an impact of *CYP2C19* polymorphisms was shown for the mean AUC_{0-24}, $T_{1/2}$, and Cl/F values, with the highest mean value being observed for PMs at the 3 different dosage regimens. Similarly, the higher metabolic activity of *CYP2C19* (homEm > hetEM > PM) would lead to the higher extent of reductions in the mean AUC_{0-24} (40QD < 20TD < 10Q4D) and of elevations in the mean $T_{1/2}$ and Cl/F values (40QD > 20TD > 10Q4D) following the dosage regimens with more dosage divisions in the same total daily dose of 40 mg.

Intragastric pH monitoring

The median intragastric pH versus time curves with different dosage regimens of esomeprazole on day 7 are shown in Fig. 3. The median intragastric pH and the median percent time the pH was < 4 as defined for the 24-hour and nocturnal periods are summarized in Table 3. With administration of a placebo, the median intragastric pH values were observed to be < 4 and the median percent time of pH < 4.0 was > 16.7% in both the 24-hour and nocturnal periods for the 3 genotype groups (P=0.3737). However, gastric acid secretion was significantly suppressed but to different extents after esomeprazole administration to the 3 *CYP2C19* genotype groups with different dosage regimens as shown in Table 3. Median values of the intragastric pH during the 24-hour period increased to > 4 for the 3 genotype groups after administration of esomeprazole at the 3 different dosage regimens, but those during the nocturnal period for the homEM (3.6) and hetEM (3.1) genotypes were not attained when esomeprazole was given as 40QD. It was concluded that treatment with esomeprazole to the 3 genotype groups was effective at maintaining a median intragastric pH level of > 4 during the 24-hour period with any of the 3 different dosage regimens, while the effectiveness of maintaining the median intragastric pH at > 4 during the nocturnal period for all 3 genotype groups could be improved when esomeprazole was given as 20TD and 10Q4D (Fig. 4).

Median values of the percent of time the pH was < 4 during the 24-hour period for the 3 genotype groups were < 16.7% (8.0%~12.3%) when esomeprazole was given as 20TD, and medium values were > 16.7% (26.7%~32.5%)

when administered as 40QD, while it was slightly > 16.7% (20.8%) only when given as 10Q4D to the PM genotype. During the nocturnal period, median values of the percent of time the pH was < 4 were much more greater than 16.7% (35.6%~65.3%) when administered as 40QD to the 3 genotype groups and was slightly > 16.7% (23.0% and 24.1%) when given as either 20TD or 10Q4D to the hetEM genotype. It was concluded that treatment of the 3 genotype groups with esomeprazole was effectively improved with the 20TD and 10Q4D dosage regimens by attaining a median percent of time the pH was > 4 which of > 83.3% during the 24-hour and nocturnal periods, whereas it might be less effective when esomeprazole was given as 40QD to all 3 genotype groups.

Discussion

In this study, the pharmacokinetic parameters, including C_{max}, $T_{1/2}$, AUC_{0-24}, CL/F, and MRT_{0-24}, were found to be dependent on the *CYP2C19* genotype status with different extents of influence at different dosage regimens. The higher metabolic activity of *CYP2C19* (homEm > hetEM > PM), the higher extent of reduction (C_{max}, AUC_{0-24}, and MRT_{0-24},) or of elevation ($T_{1/2}$ and CL/F) in their mean values resulted following dosage regimens with more dosage divisions for the same total daily dose of 40 mg. The median intragastric pH levels decreased in the order of PM > hetEM > homEM. Treatments of the 3 genotype groups were effective at maintaining the median intragastric pH level at > 4 during the 24-hour period with any one of the 3 different dosage regimens, while the effectiveness at maintaining the median intragastric pH at > 4 during the 24-h and nocturnal periods for all 3 genotype groups could be improved to a greater extent when esomeprazole was given as a divided-dosage regimen (20TD and 10Q4D). Mean values of pharmacokinetic parameters, intragastric pH, and the percent of time and the time the pH was > 4 were observed to follow the trend described above although some of them were determined to be statistically insignificant, which might render such a conclusion indeterminate. Since the study was conducted using short-term (i.e., 7 days) repeated doses of esomeprazole, and only 9 healthy volunteers without *H. pylori* infection were used instead of a patient group, the study design might not have a sufficient sample size to detect large variabilities with statistical significance. Thus, the therapeutic effects of esomeprazole as a function of the

Table 2 Mean (±SD) pharmacokinetic values for esomeprazole with different dosage regimens

Dosage regimen	homEM	hetEM	PM
Esomeprazole, 40 mg once daily (40QD)			
C_{max} (ng/mL)	6746.6±3119.1	6465.0±4068.8	7714.6±3258.0 (*1.14*)[1]
$T_{1/2}$ (h)	1.5±0.02[†]	1.5±0.2[†]	2.4±0.3 (*1.60*)
T_{max} (h)	1.0±0	2.3±1.2	1.7±0.3
AUC_{0-24}(h*ng/mL)	16208.7±4504.9	21037.5±11050.4	24059.2±10234.0 (*1.48*)
MRT_{0-24} (h)	2.8±0.4[*]	4.1±0.7[*]	3.7±0.04 (*1.32*)
Cl/F (L/h)	2.6±0.6	2.2±1.1	1.9±0.8 (*0.73*)
Esomeprazole, 20 mg twice daily (20TD)			
C_{max} (ng/mL)	2498.9±1868.6	2359.3±881.7	3354.6±1764.0 (*1.34*)
$T_{1/2}$ (h)	1.4±0.2	1.3±0.3	1.9±0.3 (*1.36*)
T_{max} (h)	1.7±0.6	2.1±0.7	1.9±0.6
AUC_{0-24}(h*ng/mL)	11016.0±4505.6	12642.5±4945.3	20723.2±11948.7 (*1.88*)
MRT_{0-24} (h)	2.9±0.5[**]	3.3±0.5[**]	3.3±0.5 (*1.14*)
Cl/F (L/h)	3.6±1.1	3.0±1.0	2.4±1.5 (*0.67*)
Esomeprazole, 10 mg 4 times daily (10Q4D)			
C_{max} (ng/mL)	1356.5±382.3	1386.5±27.7	1921.1±877.8 (*1.42*)
$T_{1/2}$ (h)	1.4±0.4	1.7±0.3	1.7±0.5 (*1.21*)
T_{max} (h)	2.7±2.1	2.2±2.4	8.0±3.6
AUC_{0-24}(h*ng/mL)	10123.1±2661.9	9887.3±1002.7	20304.6±11529.0 (*2.01*)
MRT_{0-24} (h)	8.8±0.3[***]	7.7±0.3[***]	10.9±2.1 (*1.24*)
Cl/F (L/h)	2.0±0.2	2.0±0.5	1.1±0.6 (*0.55*)

[1] Ratio of PM/homEM; *AUC*, area under the plasma concentration-time curve; C_{max}, peak plasma drug concentration; T_{max}, time to reach the peak concentration following drug administration; $T_{1/2}$, time taken for the blood concentration to drop to 50% of C_{max}; *MRT*, mean residence time.

[†] $P<0.02$ (vs. PM) by 1-way ANOVA followed by Scheffe's multiple comparison test.

P<0.05 (vs. PM) at *40 mg once daily; **20 mg twice daily; ***10 mg 4 times daily, by repeated-measures ANOVA followed by Scheffe's multiple comparison test

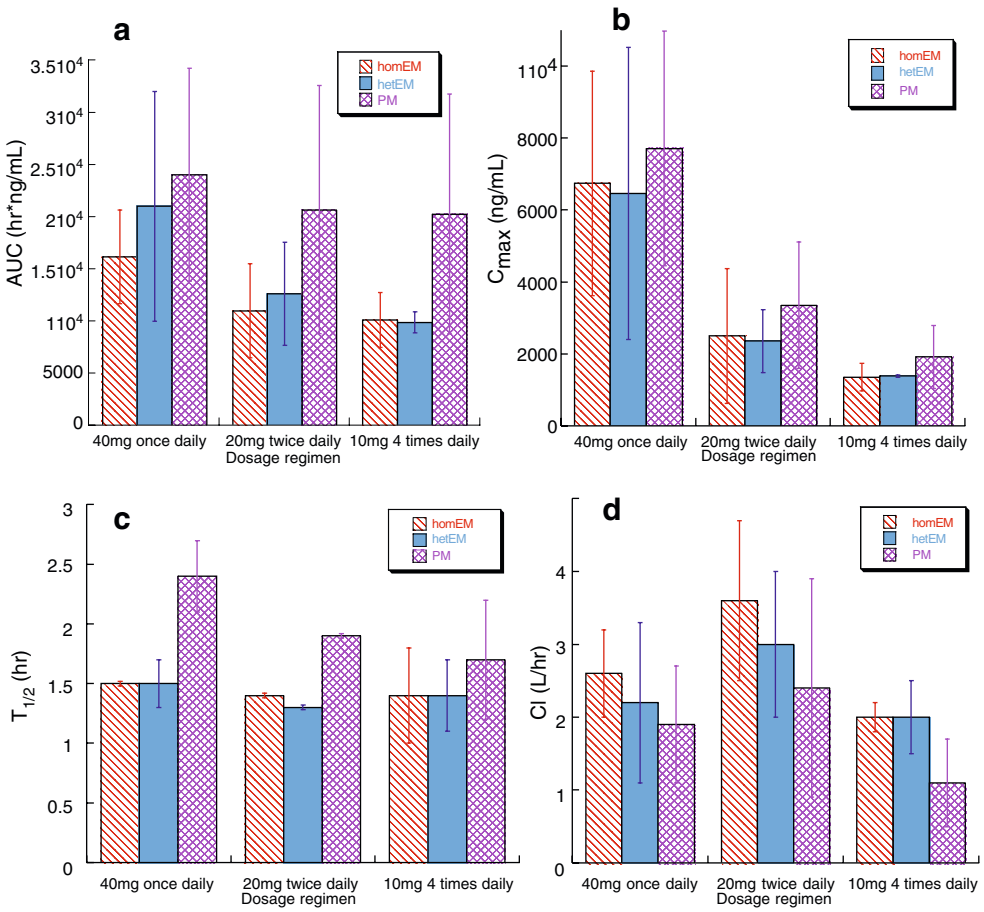

Fig. 2 Area under the curve (AUC_{0-24}) (a), peak plasma esomeprazole concentration (C_{max}) (b), half-life ($T_{1/2}$) (c), and clearance (Cl/F) (d) of the 3 *CYP2C19* genotypes with different dosage regimes

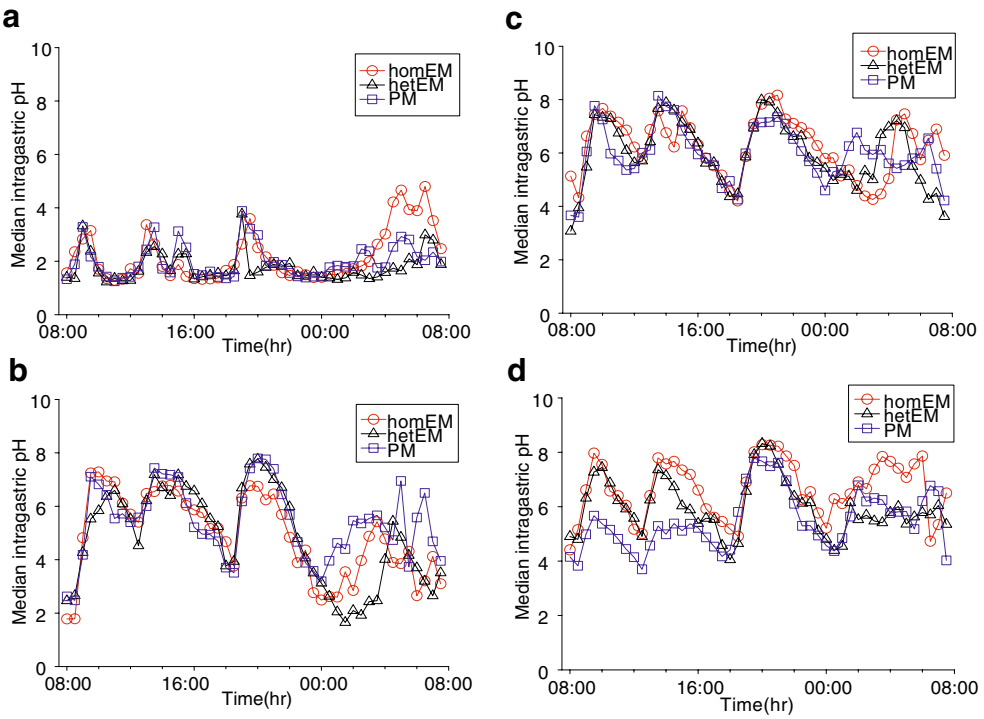

Fig. 3 Median 24-hour intragastric pH profiles with (a) a placebo, (b), 40 mg esomeprazole once daily (40QD); (c), 20 mg esomeprazole twice daily (20TD); and (d), 10 mg esomeprazole 4 times daily (10Q4D) as a function of the *CYP2C19* genotype

Table 3 Median intragastric pH values and median percent of time the intragastric pH was < 4.0 during nighttime and a 24-hour period with different dosage regimens as a function of the *CYP2C19* genotype status

	Median intragastric pH[1]			Median time (%) the pH was < 4 (h, %)		
	homEM	hetEM	PM	homEM	hetEM	PM
Nocturnal						
Placebo	2.7 (2.2–3.2)	1.7 (1.1–2.3)	2.1 (1.7–2.6)	6.44 (80.5)[2] (5.7–8.0; 69.6–100)[3]	7.66 (95.8) (7.0–8.0; 87.5–100)	7.35 (91.9) (6.1–8.0; 76.5–100)
40 mg once daily (40QD)	3.6 (3.1–4.0)	3.1 (1.8–4.6)	4.9 (2.5–6.4)	5.22 (65.3*) (4.3–6.6; 53.5–82.6)	5.64 (70.5**) (2.9–7.8; 36.8–97.4)	2.85 (35.6***) (0.9–6.4; 10.9–80.4)
20 mg twice daily (20TD)	5.6 (5.6–5.7)	5.5 (4.5–6.3)	5.8 (5.1–6.2)	0.86 (10.8*) (0.1–1.1; 7.6–13.7)	1.84 (23.0**) (0.6–4.1; 7.0–50.7)	1.01 (12.6***) (0.5–2.4; 0.6–30.4)
10 mg 4 times daily (10Q4D)	6.6 (6.3–7.2)	5.5 (3.7–6.6)	5.8 (5.4–6.2)	0.73 (9.1*†) (0.3–1.2; 4.1–15.2)	1.93 (24.1**) (0.4–4.8; 4.8–59.4)	0.91 (11.4***) (0.6–1.5; 7.0–19.2)
24-hour						
Placebo	2.2 (2.0–2.5)	1.8 (1.3–2.2)	2.0 (1.8–2.3)	21.24 (88.5) (19.8–23.1; 82.6–96.4)	22.78 (94.9) (21.9–23.4; 91.3–97.4)	21.89 (91.2) (19.9–22.9; 82.8–95.5)
40 mg once daily (40QD)	4.9 (4.7–5.2)	4.9 (3.7–6.0)	5.4 (4.8–6.3)	7.80 (32.5*) (6.6–9.4; 27.4–39.1)	7.73 (32.2**) (4.6–9.4; 19.1–39.0)	6.41 (26.7***) (2.4–9.2; 10.2–38.4)
20 mg twice daily (20TD)	6.3 (6.2–6.4)	6.0 (5.5–6.5)	6.0 (5.5–6.3)	1.92 (8.0*) (1.0–2.6; 4.1–10.9)	3.65 (15.2**) (1.9–6.0; 8.0–24.8)	2.95 (12.3***) (0.5–4.2; 2.0–17.5)
10 mg 4 times daily (20Q4D)	6.6 (6.4–7.1)	6.0 (5.4–6.3)	5.5 (4.0–6.7)	1.85 (7.7*†) (0.6–2.6; 2.6–11.0)	3.29 (13.7**) (1.5–6.0; 6.2–24.8)	4.99 (20.8***) (1.5–12.0; 6.2–49.8)

[1] Median intragastric pH values are given as the median and range.
[2] The time the intragastric pH was < 4 is given as the median value, with its range in parentheses.
[3] The percent of time the intragastric pH was < 4 is given as the median value with its range in parentheses.
$P<0.05$ (vs. the placebo) in *homEM; **hetEM; and ***PM, by the Wilcoxon signed-rank test, and significant differences were determined by the Friedman test
† $P<0.01$ (vs. 40 mg once daily) by the Wilcoxon signed-rank test, and significant differences were determined by the Friedman test.

CYP2C19 genotype need to be reevaluated in further studies designed using a large patient group with gastrointestinal disorders who are undergoing longer-term dosing regimens. This study should be viewed as a preliminary basis for further studies.

Enzymatic metabolism of esomeprazole at a daily dose of 40 mg by either *CYP2C19* (first-pass metabolism to 5-OH esomeprazole) or *CYP3A4* (intestinal metabolism to esomeprazole sulfone and first-pass metabolism to esomeprazole sulfone and 5-OH esomeprazole sulphone) could simply be assumed to follow Michaelis-Menten kinetics, which expects the half-life ($T_{1/2}$) to depend on the dose and the percentage metabolized (or the area under the plasma concentration-time curve to increase more than proportionately) to decrease with an increase in the dose [26]. For those *CYP2C19* genotype groups with different metabolic activities, pharmacokinetic parameters were rationally expected to be influenced to different extents by different dosage regimens at the same daily dose, of which a lower dose was available to metabolic processes with various metabolic rates with an increasing dose division (40QD > 20TD > 10Q4D). The observation of this study can be explained by that the higher metabolic activity of *CYP2C19* (homEm > hetEM > PM) should lead to the higher extent of reduction (C_{max}, $AUC_{0–24}$, and $MRT_{0–24}$,) or of elevation ($T_{1/2}$ and CL/F) in their mean values from dosage regimens with more dosage divisions.

In this study, values of the $AUC_{0–24}$, median intragastric pH, and median percent of time the pH was > 4.0 increased by dividing the dosing with a lower extent for those *CYP2C19* genotypes with higher metabolic activity, even though the total daily dose was equivalent. Although the intragastric pH at 10Q4D did not follow this trend, the antisecretory effect of esomeprazole 10Q4D was still better than that at 40QD in this study. In addition, acid suppression with 40QD esomeprazole was insufficient with the median intragastric pH being < 4 and a higher percent of time the pH was < 4 for those *CYP2C19* genotypes with higher metabolic activity. Divided doses of 40QD esomeprazole (i.e., 20TD or 10Q4D) resulted in clinical improvement in acid inhibition (median intragastric pH > 4 and median percent of time the nocturnal pH was < 4 or close to 16.7%). Our data showed that esomeprazole administered at 20TD or 10Q4D was potentially effective at inhibiting gastric acid secretion for all 3 *CYP2C19* genotypes.

C_{max} decreased from the 40QD dose to the divided dosing, but mean $AUC_{0–24}$ values did not significantly differ, and acid inhibition with 10Q4D or 20TD was more potent than that with 40QD in all of 3 genotypes. Many studies [19–22] have found that increasing the dose of PPIs

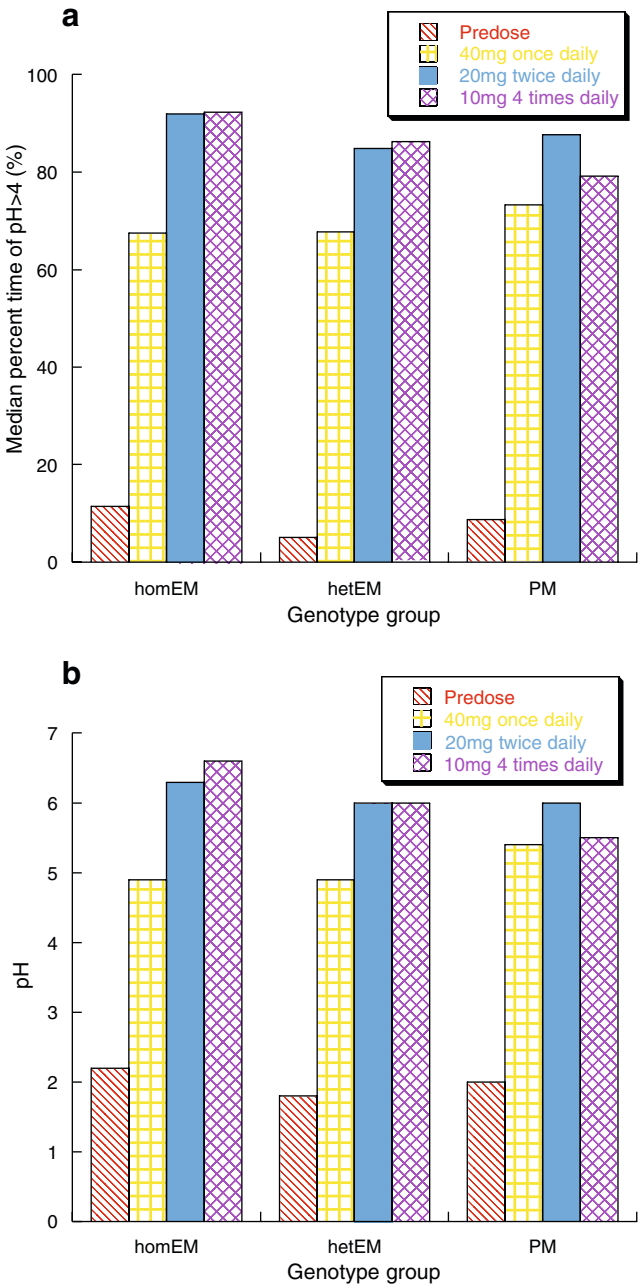

Fig. 4 Median percent of time the pH was > 4.0 (a), and median pH during the 24-hour period (b) with different dosage regimes with the 3 *CYP2C19* genotypes

does not completely suppress acid breakthrough, and its mechanism is thought to be unrelated to plasma PPI concentrations. It was tempting to assume that to maintain the plasma concentration higher than a certain threshold level throughout the 24-hour period, a multiple dosage regimen would be more effective for acid inhibition than raising the C_{max} or AUC_{0-24} value by increasing the dose of PPIs as a single dose.

Proton pumps, however, are continuously being renewed, and approximately 25% of the pumps are replaced by newly synthesized ones each day [25]. These observations suggest that at least 12.5% of the proton pumps could be activated during the nocturnal period, even if a high morning dose of PPI had completely inhibited all proton pumps in the morning, because 12.5% of the proton pumps are newly synthesized and available for activation in a 12-hour period. Subsequent doses will inactivate additional active pumps, including those that were not inactivated the previous day, as well as newly synthesized pumps. Our present study demonstrated that a PPI, esomeprazole, controlled nocturnal gastric acidity more effectively when it was divided into 2 (20TD) or 4 doses (10Q4D). This observation is consistent with previous reports, which demonstrated that the acid-suppressive effect of PPIs increased when the doses were divided into morning and evening ones. Therefore, in long-term maintenance therapy for acid-related diseases, split doses of PPIs may be considered effective regimens to control nocturnal acidity and intensive treatment with esomeprazole is recommended for patients who are extensive metabolizers (particularly homEM) and who are refractory to the usual dose of PPIs. If the *CYP2C19* genotype status is not determined, a dosage regimen with minimal influence of *CYP2C19* polymorphism of esomeprazole should be expected to be that with 20TD or 10Q4D.

This observation obviously agreed with a report describing the significant dependence of intragastric pH values and plasma concentrations of rabeprazole (which is less metabolized by *CYP2C19* to esomeprazole) on the *CYP2C19* genotype status [21], but it seemed to conflict with 2 previous reports. One study concluded that 40 mg esomeprazole twice daily for triple therapy may improve *H. pylori* eradication compared to omeprazole-based therapy, but only for the *CYP2C19* genotype of homEMs [22], and another one described how esomeprazole-induced healing of GERD was unrelated to the *CYP2C19* genotypes when esomeprazole was administered at 40 mg daily for 4 weeks, which was explained by the metabolic shift toward the *CYP3A4*-mediated pathway [23]. Treatment of the former using 40 mg esomeprazole twice daily was ascribed to the divided dosage regimens as defined in this study which were expected to significantly improve the inhibition of acid secretion, especially for the *CYP2C19* genotype of homEMs. Treatment of the latter using 40 mg esomeprazole daily for 4 weeks was ascribed as a dosage regimen of 40QD as that in this study, but for a much longer period of time. As observed in this study at 40QD, median intragastric pH values were all > 4, and the median percent of times the pH was < 4 during 24-h period were all about 30% for the 3 *CYP2C19* genotype groups. If this pharmacodynamic effect could be related to the efficacy of clinical healing of GERD, then this study could reach the same conclusion as that of esomeprazole-induced healing of GERD being unrelated to the *CYP2C19* genotype status.

Therefore, results of both studies basically do not conflict with those of this study.

Conclusions

It was found that the *CYP2C19* polymorphism significantly affected the intragastric pH and the percent of time the pH was > 4 (pharmacodynamics) and the plasma esomeprazole concentrations (pharmacokinetics) of esomeprazole at different dosage regimens to different extents. Fortunately, treatment with an optimal dosage regimen of esomeprazole with dose division had a minimal influence on acid inhibition by the *CYP2C19* polymorphism. While this study has its limitations, it is anticipated that it can serve as a basis for further studies on treatment strategies of GERD using esomeprazole.

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