PHARMACOGENETICS AND GENOMICS

Different dosage regimens of rabeprazole for nocturnal gastric acid inhibition in relation to cytochrome P450 2C19 genotype status

Objective: **For the treatment of gastroesophageal reflux disease, intragastric pH should be lower than 4.0 for no more than 4 hours a day (<16.7%). We aimed to develop optimal dosage regimens for rabeprazole to control nocturnal acidity in relation to cytochrome P450 (CYP) 2C19 genotypes.**

Methods: **Fifteen *Helicobacter pylori*–negative volunteers, comprising 5 homozygous extensive metabolizers (EMs), 6 heterozygous EMs, and 4 poor metabolizers (PMs) of CYP2C19, took placebo and rabeprazole, at a dose of 20 or 40 mg once daily (at 10 PM) for 8 days. Plasma rabeprazole concentrations and 24-hour intragastric pH were determined on days 7 and 8, respectively. Because the nocturnal intragastric pH was lower than 4.0 for more than 16.7% of the time with once-daily regimens in homozygous EMs and heterozygous EMs, they were administered 20 mg rabeprazole twice daily (8 AM and 10 PM) or 10 mg rabeprazole 4 times daily (8 AM, 12:30 PM, 6 PM, and 10 PM).**

Results: **With 40 mg rabeprazole once daily, the median percent time with nocturnal pH lower than 4.0 was less than 16.7% in PMs (9.5% [range, 3.0%-31.1%]) but not in homozygous EMs (45.3% [range, 29.0%-52.2%]) ($P = .043$) and heterozygous EMs (41.3% [range, 33.0%-59.0%]) ($P = .043$). The mean plasma rabeprazole concentrations differed among the different CYP2C19 genotype groups. With 20 mg rabeprazole twice daily and 10 mg rabeprazole 4 times daily, the median percent times with nocturnal pH lower than 4.0 were 5.0% (range, 0.0%-42.0%) and 1.0% (range, 5.0%-7.1%) in heterozygous EMs and 62.0% (range, 10.8%-68.3%) and 14.7% (range, 0.0%-41.7%) in homozygous EMs, respectively, and plasma concentrations were sustained longer than with the once-daily regimens.**

Conclusions: **We propose that rabeprazole dosage regimens for sufficient acid inhibition are 20 mg once daily for PMs, 20 mg twice daily for heterozygous EMs, and 10 mg 4 times daily for homozygous EMs or heterozygous EMs. (Clin Pharmacol Ther 2004;76:290-301.)**

Mitsushige Sugimoto, MD, Takahisa Furuta, MD, PhD, Naohito Shirai, MD, PhD, Masayoshi Kajimura, MD, PhD, Akira Hishida, MD, PhD, Masaharu Sakurai, PhD, Kyoichi Ohashi, MD, PhD, and Takashi Ishizaki, MD, PhD *Hamamatsu and Kumamoto, Japan*

From the First Department of Medicine, Department of Clinical Laboratories, and Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, Hamamatsu, and Department of Clinical Pharmacology and Therapeutics, Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto.

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Reprint requests: Mitsushige Sugimoto, MD, First Department of Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, 431-3192, Japan.

E-mail: *mitsu@hama-med.ac.jp*

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Gastroesophageal reflux disease (GERD) is a common disorder estimated to affect approximately 20% to 50% of the adult population in the Western world.[1-3] Proton pump inhibitors (PPIs), such as rabeprazole, omeprazole, and lansoprazole, are now the first-line drugs for GERD treatment.[4-6] Although in most patients GERD can be controlled within 8 weeks with a PPI at the usual standard dose once daily, approximately 10% to 20% of patients have refractory symptoms.[7] In refractory cases 24-hour intragastric pH monitoring studies have revealed that intragastric pH values during PPI treatment are often lower than 4.0 for certain periods of time, particularly during nighttime, resulting in prolonged and frequent exposure of the esophageal mucosa to refluxed low-pH gastric juice. However, several studies have documented that the duration of intragastric pH lower than 4.0 during a 24-hour period should be approximately 2 to 4 hours (ie, intragastric pH lower than 4.0 should be below 16.7%) for the cure of GERD.[7-10]

Recently, the phenomenon of intragastric pH lower than 4.0 lasting more than 1 hour during the nighttime period despite PPI treatment has been defined as *nocturnal gastric acid breakthrough*, and this is considered to be related to the success or failure of treatment of GERD with a PPI.[9,11-13] To overcome nocturnal gastric acid breakthrough, refractory patients require more intensive treatment for nocturnal acid inhibition, and several trials have been conducted modifying the dosage regimens of PPIs.[5,11-13]

Although the causative mechanism of nocturnal gastric acid breakthrough is unclear, evening dosing with a PPI inhibits nocturnal acid secretion more effectively than does morning dosing.[12] Moreover, it has been shown that, at doses of 10 to 40 mg rabeprazole, once-daily dosing in young healthy volunteers inhibits acid secretion in a dose-dependent manner.[14] These data suggest that nocturnal gastric acid secretion may be associated with plasma PPI concentrations and that modifying the dosage regimens of PPIs might prevent nocturnal gastric acid breakthrough. Although a PPI taken before a meal binds more effectively to activated H^+/K^+–adenosine triphosphatase (ATPase) than when taken in the fasting state, taking a PPI at bedtime can be expected to sustain the plasma PPI levels longer during nighttime and to inhibit nocturnal acid more effectively. To our knowledge, however, there have been no reports regarding plasma PPI concentrations during nocturnal gastric acid breakthrough, and the relationship between nocturnal gastric acid breakthrough and nocturnal plasma PPI concentrations remains unclear.

PPIs such as omeprazole and lansoprazole are mainly metabolized by hepatic cytochrome P450 (CYP) 2C19, and there are genetic differences in the activity of this enzyme.[15] Previous studies have shown that the CYP2C19 genotypes can be classified into homozygous extensive metabolizers (EMs), heterozygous EMs, and poor metabolizers (PMs).[16-19] In PMs the plasma PPI concentrations are markedly increased and the pharmacodynamic effects of PPIs (eg, omeprazole and lansoprazole) are enhanced in comparison with those in heterozygous EMs or homozygous EMs.[20-23] In contrast, the metabolic disposition of rabeprazole was reported to differ from that of other PPIs, because rabeprazole is reduced mainly via a nonenzymatic pathway, with minor CYP2C19 and CYP3A4 involvement.[19,24] Acid inhibition by rabeprazole was, therefore, considered to be less influenced by CYP2C19 genotypes in comparison with other PPIs.[22,25] However, several recent reports have shown that plasma rabeprazole concentrations differ significantly among the different CYP2C19 genotypes (ie, highest in PMs, intermediate in heterozygous EMs, and lowest in homozygous EMs)[22,26,27] and that acid inhibition by rabeprazole dose depends on CYP2C19 genotypic status.[26,28] Whether acid inhibition after repeated doses of rabeprazole depends on CYP2C19 genotype remains controversial.[22,25,29] It also remains unclear whether differences in CYP2C19 genotype status influence the incidence of nocturnal gastric acid breakthrough during rabeprazole treatment.[30]

With this background in mind, we aimed to determine the relationship between intragastric pH and plasma rabeprazole concentrations during daytime and nighttime periods, with the intention of developing optimal dosage regimens for the control of nocturnal acid secretion in relation to CYP2C19 genotypes.

METHODS

Subjects and CYP2C19 genotyping

After written informed consent was obtained, 44 healthy Japanese subjects underwent a CYP2C19 genotyping test by use of a polymerase chain reaction–restriction fragment length polymorphism method with allele-specific primers for identifying the CYP2C19 wild-type (**1*) gene and the 2 mutated alleles, *CYP2C19*2* (**2*) in exon 5 and *CYP2C19*3* (**3*) in exon 4. On the basis of the results, subjects were classified into 1 of 3 genotype groups as follows: homozygous EM (**1/*1*), heterozygous EM (**1/*2* or **1/*3*), or PM (**2/*2*, **3/*3*, or **2/*3*), as previously reported.[18,31,32] Of the 40 subjects (14 homozygous EMs, 22 heterozygous EMs, and 4 PMs) who were free

Table I. Demographic characteristics of *H pylori*–negative healthy male volunteers with different CYP2C19 genotypes

Genotype group	*No.*	*CYP2C19 genotype*	*Age (y)*	*Body weight (kg)*	*Height (cm)*
Homozygous EMs	5	**1/*1* (n = 5)	19 (19-22)	60 (58-65)	171 (169-175)
Heterozygous EMs	6	**1/*2* (n = 4) **1/*3* (n = 2)	19 (19-26)	68 (60-70)	179 (170-182)
PMs	4	**2/*2* (n = 1) **3/*3* (n = 1) **2/*3* (n = 2)	20 (19-22)	66 (57-78)	175 (167-182)
P value			NS	NS	NS

Age, body weight, and height are given as median and range.
**1*, Wild-type; **2*, *CYP2C19*2* mutation in exon 5; **3*, *CYP2C19*3* mutation in exon 4; EM, extensive metabolizer; PM, poor metabolizer, NS, not significant.

of *Helicobacter pylori* infection on the basis of serologic testing (HM-CAP kit; Enteric Products Inc, Westbury, NY) and the carbon 13–urea breath test, 5 homozygous EMs and 6 heterozygous EMs, who were randomly selected, and all 4 PMs were invited to participate in the study. There were no significant differences in age, body weight, or height among the 3 genotype groups (Table I). None of the subjects consumed extensive amounts of alcohol or smoked, and none took any medications for at least 2 weeks before or during the study.

Study protocol

The end point of this study was to control the percent time of nocturnal intragastric pH lower than 4.0 to be less than 16.7%, because this value is considered sufficient for the control of GERD.[7,8] All subjects were first administered a placebo and then the 2 different doses of rabeprazole (Pariet; Eisai Co Ltd, Tokyo, Japan). Each subject was administered rabeprazole once daily at a dose of 20 or 40 mg at 10 PM for 8 days in a randomized, double-blind, crossover fashion. Plasma rabeprazole concentrations and 24-hour intragastric pH monitoring were determined on day 7 and day 8, respectively, as described later. Because nocturnal blood sampling may affect the status of sleep and may also affect nocturnal intragastric pH levels, blood sample collection and intragastric pH monitoring were performed on different days. All subjects were provided with 3 meals a day (breakfast [2100 kJ] at 8 AM, lunch [4284 kJ] at 12:30 PM, and dinner [3510 kJ] at 6 PM). Mineral water was allowed as desired, but no other beverages were permitted. There was a washout period of at least 2 weeks between the 2 study periods.

Because nocturnal acid inhibition was inadequate in homozygous EMs and heterozygous EMs, as described subsequently (median percent time of nocturnal pH lower than 4.0 greater than 16.7%), alternative divided dosage regimens for rabeprazole were tried as follows: 20 mg twice daily (8 AM and 10 PM) or 10 mg 4 times daily (8 AM, 12:30 PM, 6 PM, and 10 PM) for 8 days. Written informed consent was again obtained from each subject before participation in the study. Approval for the study protocol was given in advance by the Human Institutional Review Board of the Hamamatsu University School of Medicine, Hamamatsu, Japan.

Sample collection and rabeprazole assays. On day 7 of the once-daily dosage study, blood samples were collected before and at 1, 2, 3, 5, 7, 10, and 24 hours after the bedtime dose of rabeprazole. In the divided dosage regimens, blood samples were taken before and at 1, 2, 3, 5, 7, 10, and 24 hours after both morning and bedtime doses. Blood samples were centrifuged at 3000 rpm for 10 minutes immediately after collection. The plasma samples (1 mL each) were placed in covered storage tubes containing 100 μL of a 1% diethylamine solution. Plasma rabeprazole concentrations were determined by use of an HPLC method.[24] In brief, a 100-μL aliquot of internal standard solution (0.1% diethylamine in methanol solution) and 1 mL of Britton-Robinson buffer (pH 10.38) were added to each 110-μL sample. After the addition of 4 mL ethyl acetate, the mixture was centrifuged at 3500 rpm for 5 minutes. The combined organic layer was transferred to a glass tube and evaporated until dry under nitrogen gas. The residue of the extract was dissolved in 100 μL of 0.1% diethylamine in methanol. A 30-μL aliquot was injected onto the HPLC column. The mobile phase consisted of 280 mL of acetonitrile and 720 mL of 0.1-mol/L phosphate buffer adjusted to pH 7.00 with phosphoric acid. The flow rate was 1.4 mL/min. The column effluent was monitored by the ultraviolet detector at a wavelength of 288 nm.

Intragastric pH measurement. On day 8, after overnight fasting, either a glass pH electrode (Chemical Instruments Co Ltd, Tokyo, Japan) or an antimony pH electrode (Medtronic Functional Diagnostics, Inc, Shoreview, Minn) was inserted transnasally and placed 5 cm distal to the gastric cardia. Intragastric pH data were recorded by a MEMORY pH METER (Chemical Instruments Co Ltd) or a Digitrapper MK III (Synectics Medical AB, Stockholm, Sweden). After recording, the data were transferred to a computer and stored until analysis.

Pharmacokinetic data analysis. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) of rabeprazole were determined directly from the observed data. C_{max} in divided dosage regimens was defined as the nocturnal C_{max} after the bedtime dose of rabeprazole. The terminal elimination rate constant (k_e) was obtained by linear regression analysis by use of at least 3 sampling points of the terminal log-linear declining phase to the last measurable concentration. The terminal elimination half-life ($t_{1/2}$) was calculated with the following equation: $t_{1/2} = \ln 2/k_e$. The area under the plasma concentration–time curve from 0 to 24 hours ($AUC_{0\text{-}24}$) after a dose of rabeprazole was calculated from each individual concentration-time profile by use of the linear trapezoidal method.

Data analysis

The pharmacodynamic parameters are given as the median and range, and the pharmacokinetic parameters are given as the mean ± SEM. The median intragastric pH values for the daytime period (7 AM to 11 PM), the nighttime period (11 PM to 7 AM), and every hour were obtained from the raw pH values. Statistically significant differences in pharmacodynamic parameters among the 3 CYP2C19 genotype groups were determined by use of 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 by use of 1-way ANOVA followed by the Scheffé 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 use of the Friedman test. Statistical differences in the mean pharmacokinetic parameters between the different dosage regimens were determined by use of a repeated-measures ANOVA followed by the Scheffé multiple comparison test. All *P* values were 2-sided, and $P < .05$ was taken to indicate statistical significance.

RESULTS

Intragastric pH profiles

When placebo was administered, there were no significant differences in intragastric pH profiles among the 3 genotype groups (Fig 1, *A*). With once-daily rabeprazole at a dose of 20 or 40 mg, median intragastric pH levels in the PMs were the highest of the 3 groups throughout the 24-hour period, followed by the heterozygous EMs, with the homozygous EMs demonstrating the lowest levels. A median pH higher than 4.0 was attained throughout the 24-hour period in PMs with once-daily rabeprazole, at both 20- and 40-mg doses, but not during the nighttime in homozygous EMs and heterozygous EMs (Fig 1, *B* and *C*). The latter 2 groups were then administered 20 mg rabeprazole twice daily and 10 mg rabeprazole 4 times daily for 8 days. With 20 mg twice daily, a median pH higher than 4.0 was attained in heterozygous EMs throughout the 24-hour period, whereas the median pH attained in homozygous EMs was insufficiently elevated, particularly during the nighttime (Fig 1, *D*). When rabeprazole was administered at 10 mg 4 times daily, however, a median pH higher than 4.0 was attained during almost the entire 24 hours in homozygous EMs, as well as in heterozygous EMs (Fig 1, *E*).

Fig 2 summarizes the median nocturnal intragastric pH values with the different dosage regimens. In homozygous EMs the median nocturnal pH attained with 20 mg once daily was 2.4, which was significantly lower than that attained with 40 mg once daily (4.4) ($P = .043$). The median pH attained with 10 mg 4 times daily (5.8) was significantly higher than that attained with any of the other dosage regimens (all $P = .043$) in homozygous EMs. In heterozygous EMs the median nocturnal pH attained with 20 mg once daily (4.6) was comparable to that attained with 40 mg once daily (4.7) ($P = .178$), whereas a significantly higher median nocturnal pH was attained with 20 mg twice daily (5.8) ($P = .043$), as well as 10 mg 4 times daily (5.9) ($P = .043$). In contrast, a high median nocturnal pH value (6.1) was achieved with 20 mg once daily in PMs.

With once-daily rabeprazole at doses of 20 and 40 mg, the median daytime and nocturnal pH values in PMs were both significantly higher than those in heterozygous EMs ($P = .043$ for each) and homozygous EMs ($P = .043$ for each), respectively (Fig 2 and Table II). The median nocturnal pH value in heterozygous EMs with 20 mg twice daily was significantly higher than that in homozygous EMs ($P = .043$). The median daytime and nocturnal pH values with 10 mg 4 times daily in homozygous EMs were 5.6 and 5.8, respectively, and were not significantly different from those in heterozygous EMs (6.4 and 5.9,

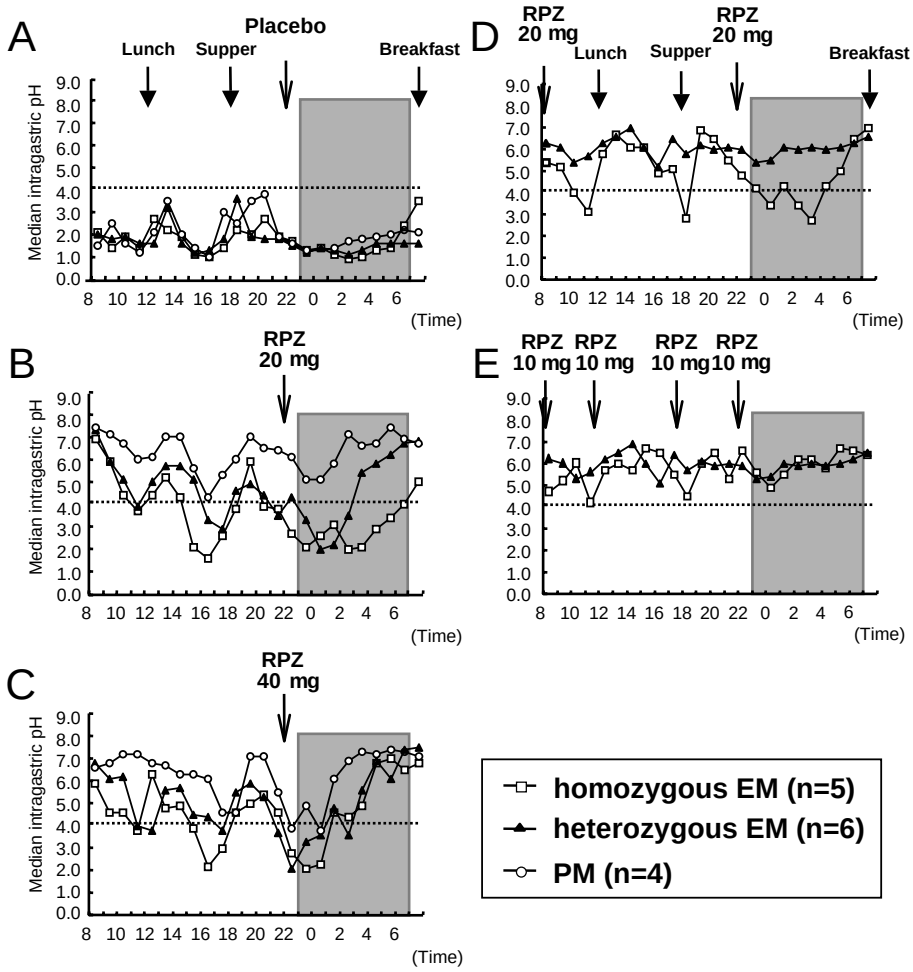

Fig 1. Median 24-hour intragastric pH profiles with placebo once daily (**A**), 20 mg rabeprazole (RPZ) once daily (**B**), 40 mg RPZ once daily (**C**), 20 mg RPZ twice daily (**D**), and 10 mg RPZ 4 times daily (**E**) as a function of CYP2C19 genotype. With once-daily regimens, the pH levels in extensive metabolizers (EMs) were often lower than 4.0, particularly during nighttime (**B** and **C**). With 20 mg twice daily in heterozygous EMs (**D**) and 10 mg 4 times daily in all EMs (**E**), sufficiently elevated pH was attained throughout the 24-hour period. PMs, Poor metabolizers.

respectively). Fig 3 summarizes the median percent times of nocturnal pH lower than 4.0 with the different dosage regimens as a function of CYP2C19 genotype status. Although the median percent times of intragastric pH lower than 4.0 during nighttime attained with once-daily rabeprazole at a dose of 20 or 40 mg and with 20 mg twice daily were 78.8% or 45.3% and 62.0%, respectively, in homozygous EMs, with 10 mg 4 times daily, the corresponding value was 14.3%, which was significantly shorter than that with any of the former 3 regimens (all $P = .043$). In heterozygous EMs the median percent times of intragastric pH lower than 4.0 during nighttime attained with once-daily rabeprazole at doses of 20 and 40 mg were 51.0% and 41.3%, whereas those attained with 20 mg twice daily and 10 mg 4 times daily were 5.0% and 1.3%, respectively, both of which were shorter than those attained with the 2 once-daily dosage regimens. In PMs the median percent times of nocturnal pH lower than 4.0 attained with once-daily rabeprazole at doses of 20 or 40 mg were 4.5% and 9.5%, respectively, both sufficient and comparable with each other.

With 20 mg rabeprazole once daily, the median percent times of pH lower than 4.0 during the daytime and nighttime periods in PMs were both significantly less than those in homozygous EMs or heterozygous EMs ($P = .043$ for each) (Fig 3 and Table II). Similarly, with 40 mg

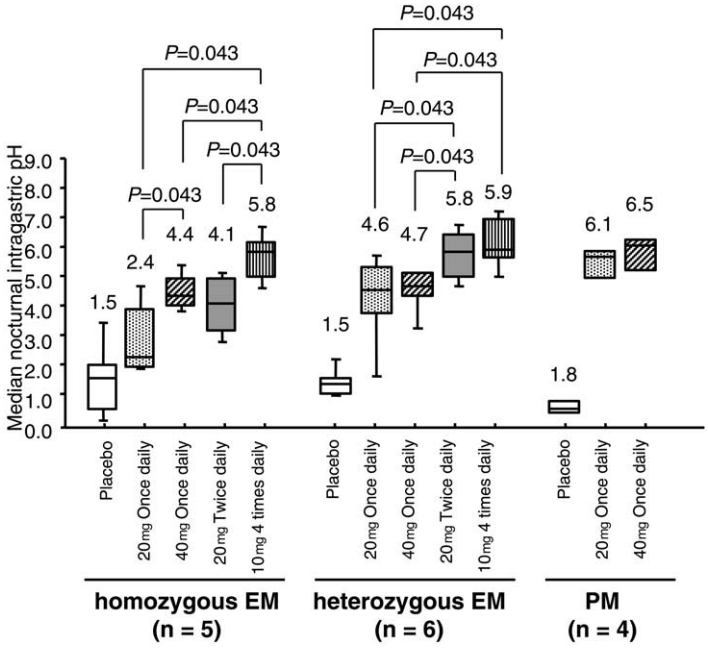

Fig 2. Median nocturnal intragastric pH values with different dosing regimens of rabeprazole in 3 CYP2C19 genotype groups. In homozygous EMs the median nocturnal pH attained by 10 mg 4 times daily was highest. The median nocturnal pH values attained by divided-dose regimens in heterozygous EMs and with 20 mg once daily in PMs were sufficiently elevated. Differences compared with placebo were all significant but have been omitted.

Table II. Median intragastric pH values and median percent times of intragastric pH <4.0 during daytime period on day 8 with different rabeprazole dosage regimens as function of CYP2C19 genotype status

	Median intragastric pH			*Median percent time of pH <4.0*		
Dosage regimen	*Homozygous EMs*	*Heterozygous EMs*	*PMs*	*Homozygous EMs*	*Heterozygous EMs*	*PMs*
Placebo	2.1 (1.6-3.7)	2.3 (1.5-2.4)	1.9 (1.5-3.2)	81.9% (48.3%-94.1%)	83.3% (78.3%-97.1%)	92.7% (73.5%-93.7%)
Rabeprazole, 20 mg once daily	3.8* (1.9-5.4)	4.6* (4.1-5.1)	6.0 (5.0-6.7)	56.3%* (19.5%-80.8%)	34.3%* (29.0%-40.8%)	14.5% (2.0%-31.9%)
Rabeprazole, 40 mg once daily	4.3* (3.8-5.1)	4.7* (3.2-6.0)	5.9 (5.1-6.5)	44.0% (23.0%-49.7%)	31.0% (24.8%-57.5%)	9.5% (2.0%-21.0%)
Rabeprazole, 20 mg twice daily	5.0 (4.0-6.6)	6.1 (4.9-6.4)	—	30.5%† (4.1%-56.0%)	2.5% (1.0%-25.1%)	—
Rabeprazole, 10 mg 4 times daily	5.4 (4.9-7.4)	6.2 (5.0-6.5)	—	10.3% (1.7%-33.1%)	2.7% (0.1%-22.5%)	—

Median intragastric pH values and median percent time of intragastric pH <4.0 are given as median and range.
*$P < .05$ (versus PMs) by use of the Mann-Whitney U test when a significant difference was observed with the Kruskal-Wallis test.
†$P < .05$ (versus heterozygous EMs) by use of the Mann-Whitney U test.

once daily, the median percent times of pH lower than 4.0 during the daytime and nighttime periods in PMs were significantly less than those in homozygous EMs or heterozygous EMs ($P = .043$ for each).

Pharmacokinetic parameters

In all of the treatment regimens, the mean plasma concentration–time curves differed among the 3 CYP2C19 genotype groups as follows: They were

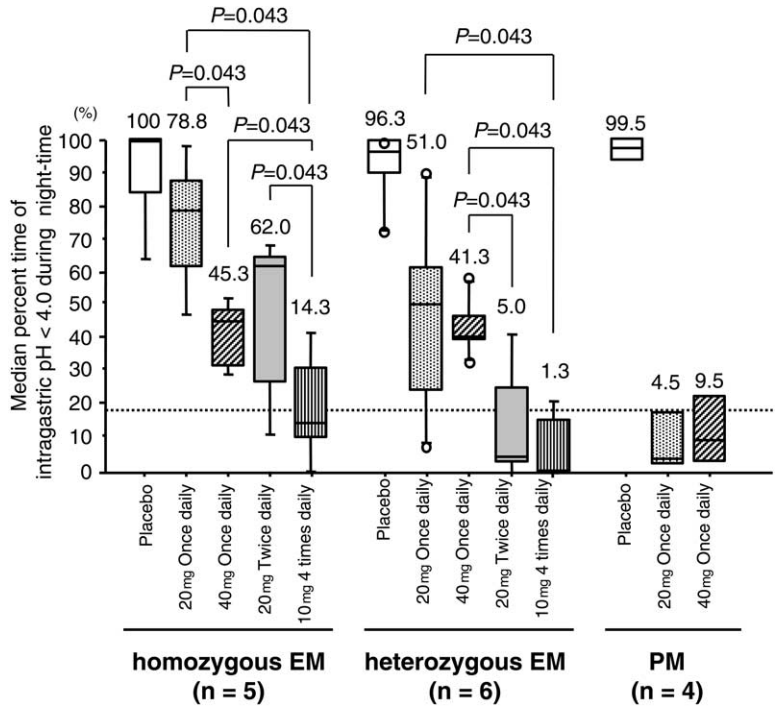

Fig 3. Median percent time of nocturnal intragastric pH lower than 4.0 with different rabeprazole dosage regimens in the 3 CYP2C19 genotype groups. A sufficient median percent time of nocturnal pH lower than 4.0 (<16.7%) was achieved with 20 mg once daily (4.5%) in PMs, 20 mg twice daily (5.0%) in heterozygous EMs, and 10 mg 4 times daily (14.3%) in homozygous EMs. Differences compared with placebo were all statistically significant but have been omitted.

highest in PMs, intermediate in heterozygous EMs, and lowest in homozygous EMs (Fig 4, *A-D*). With the once-daily regimens of rabeprazole, the mean C_{max} in the homozygous EMs was the lowest of the 3 genotype groups, followed by that in heterozygous EMs, with that in PMs being the highest (Table III). In all genotype groups, the values for C_{max} with 20 mg and 40 mg once daily increased in a dose-dependent manner. Although the C_{max} for 10 mg 4 times daily was the lowest of all of the treatment regimens, in both homozygous EMs and heterozygous EMs, the plasma rabeprazole concentrations were sustained throughout the 24-hour period with dosing 4 times daily. The mean C_{max} during daytime with 20 mg twice daily tended to be higher than that during the nighttime in both homozygous EMs and heterozygous EMs (compare the 2 peaks in Fig 4, *C*). No significant differences were seen in t_{max} values among the different CYP2C19 genotype groups or among the different dosage regimens ($P = .555$). With 40 mg once daily, the mean $t_{1/2}$ value in PMs was significantly longer than that in homozygous EMs ($P = .0014$) and heterozygous EMs ($P = .0014$). However, mean $t_{1/2}$ values within the same CYP2C19 genotype group did not differ significantly between the different dosage regimens (Table III).

In all of the CYP2C19 genotype groups, mean $AUC_{0\text{-}24}$ values increased in a dose-dependent manner from 20 mg once daily to 40 mg once daily (Table III). In all of the regimens tested, mean $AUC_{0\text{-}24}$ values were highest in PMs, intermediate in heterozygous EMs, and lowest in homozygous EMs and showed significant differences between the 3 genotype groups, with relative ratios of 1.0, 1.6, and 2.6 for 20 mg once daily in homozygous EMs, heterozygous EMs, and PMs, respectively ($P = .0062$ and $P = .014$ versus homozygous EMs), and 1.0, 1.6, and 4.2, respectively, for 40 mg once daily ($P = .044$ and $P = .014$ versus homozygous EMs). No significant differences in mean $AUC_{0\text{-}24}$ values were seen among the dosage regimens (40 mg once daily, 20 mg twice daily, and 10 mg 4 times daily) in either the homozygous EM or heterozygous EM groups.

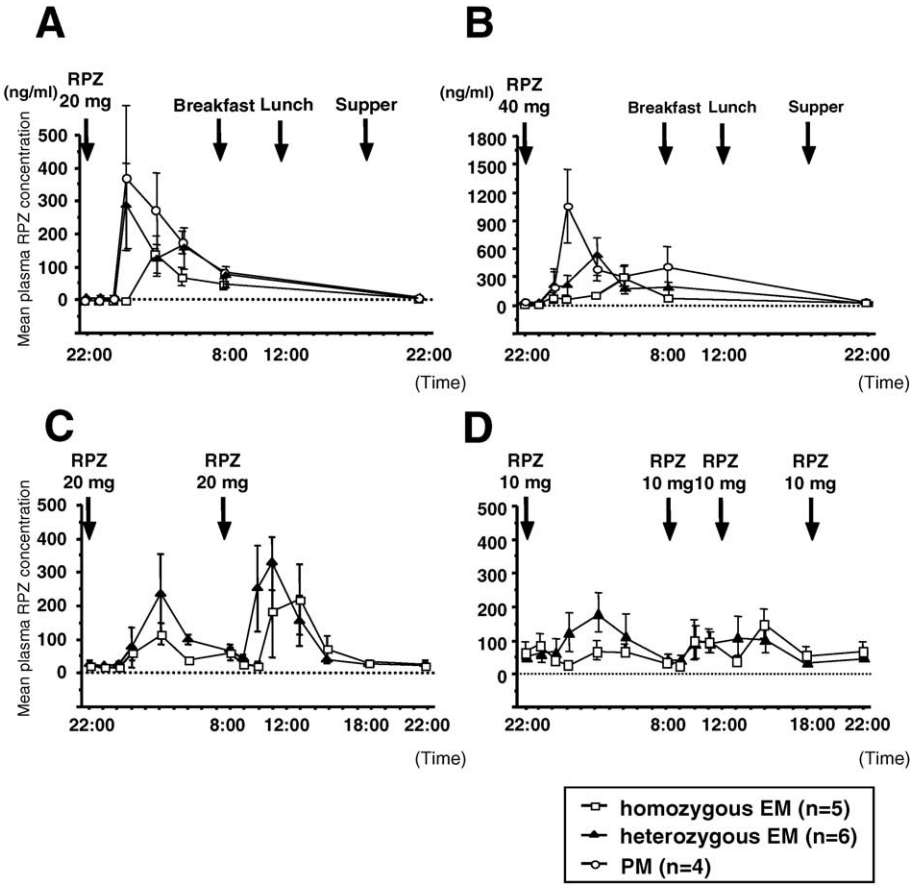

Fig 4. Mean (±SE) 24-hour plasma rabeprazole (RPZ) concentration-time courses with 20 mg once daily (**A**), 40 mg once daily (**B**), 20 mg twice daily (**C**), and 10 mg 4 times daily (**D**) as a function of CYP2C19 genotype status. Plasma concentrations increased in a dose-dependent manner. Although plasma concentrations with 10 mg 4 times daily were the lowest, they were sustained throughout the 24-hour period in all EMs.

DISCUSSION

We found that nocturnal acid suppression with 20 mg and 40 mg rabeprazole administered at bedtime was insufficient in healthy *H pylori*–negative homozygous EM and heterozygous EM volunteers and that intragastric pH values and the plasma rabeprazole concentrations significantly depended on the CYP2C19 genotype status. We also demonstrated that the median percent times of nocturnal pH lower than 4.0 were greater in homozygous EMs and heterozygous EMs than in PMs. Divided doses of 40 mg rabeprazole daily (ie, 20 mg twice daily or 10 mg 4 times daily) yielded plasma rabeprazole concentrations that were sustained throughout the 24-hour period, resulting in clinically sufficient nocturnal acid inhibition (median percent time of nocturnal pH lower than 4.0 less than 16.7%) and the prevention of nocturnal gastric acid breakthrough without an increase in $AUC_{0\text{-}24}$ values. On the basis of our findings, we propose a therapeutic strategy for the control of nocturnal acid secretion by rabeprazole based on the CYP2C19 genotype status as follows: 20 mg once daily for PMs of CYP2C19, 20 mg twice daily or 10 mg 4 times daily for heterozygous EMs, and 10 mg 4 times daily for homozygous EMs.

Although the metabolism of rabeprazole was previously reported to be less affected by CYP2C19 in comparison with that of omeprazole and lansoprazole,[19,24] this study and recent studies have clearly demonstrated significant CYP2C19 genotype–dependent differences in the pharmacokinetics and pharmacodynamics of rabeprazole, as well as lansoprazole and omeprazole.[17,20,22,23,33,34] In this study the mean $t_{1/2}$ value in PMs was significantly longer than that in homozygous EMs or heterozygous EMs, indicating that

Table III. Mean (±SE) pharmacokinetic values for rabeprazole with different 8-day dosage regimens

Dosage regimen	*Homozygous EMs*	*Heterozygous EMs*	*PMs*
Rabeprazole, 20 mg once daily			
C_{max} (ng/mL)	194.2 ± 27.2	448.6 ± 63.7**	573.7 ± 124.7*
$t_{1/2}$ (h)	0.93 ± 0.05	1.00 ± 0.04	1.71 ± 0.69
t_{max} (h)	6.4 ± 1.0	4.7 ± 0.8	4.5 ± 1.0
$AUC_{0\text{-}24}$ (ng · h/mL)	875.5 ± 98.6	1685.3 ± 253.8**	2276.5 ± 355.2*
Rabeprazole, 40 mg once daily			
C_{max} (ng/mL)	316.1 ± 87.0	710.0 ± 189.3	1307.4 ± 197.5†
$t_{1/2}$ (h)	0.90 ± 0.03	0.97 ± 0.02	2.86 ± 0.60**††
t_{max} (h)	5.8 ± 0.8	5.3 ± 1.1	4.8 ± 1.8
$AUC_{0\text{-}24}$ (ng · h/mL)	1552.2 ± 281.4	3273.2 ± 605.7*	6646.3 ± 858.5*†
Rabeprazole, 20 mg twice daily			
C_{max} (ng/mL)	23.1 ± 26.4	287.3 ± 101.5	—
$t_{1/2}$ (h)	0.99 ± 0.03	1.02 ± 0.04	—
t_{max} (h)	5.0 ± 0.6	5.4 ± 0.8	—
$AUC_{0\text{-}24}$ (ng · h/mL)	1531.4 ± 304.8	2207.1 ± 275.5	—
Rabeprazole, 10 mg 4 times daily			
C_{max} (ng/mL)	98.8 ± 23.29	196.6 ± 58.0	—
$t_{1/2}$ (h)	0.91 ± 0.02	0.97 ± 0.03	—
t_{max} (h)	5.8 ± 0.5	4.0 ± 0.9	—
$AUC_{0\text{-}24}$ (ng · h/mL)	1581.6 ± 273.1	1916.3 ± 255.4	—

Data are given as mean ± SE. C_{max}, Maximum plasma concentration; $t_{1/2}$, terminal elimination half-life, t_{max}, time to maximum plasma concentration; AUC_{0-24}, area under plasma concentration–time curve from 0 to 24 hours.

*$P < .05$ (versus homozygous EMs).

**$P < .01$ (versus homozygous EMs).

†$P < .05$ (versus heterozygous EMs).

††$P < .001$ (versus heterozygous EMs) by use of 1-way ANOVA followed by Scheffé's multiple comparison test.

rabeprazole is more rapidly metabolized in homozygous EMs and heterozygous EMs than in PMs. Therefore plasma rabeprazole concentrations could not be maintained during the interval between doses in EMs when rabeprazole was administered once daily. In EMs, as compared with PMs, H^+,K^+-ATPase newly generated or activated in gastric parietal cells after the rapid elimination of rabeprazole is able to secrete gastric acid, resulting in insufficient acid inhibition. With multiple doses, however, plasma rabeprazole concentrations were sustained throughout the 24-hour period, suggesting that newly generated or activated H^+,K^+-ATPase can be inactivated consistently throughout the 24-hour period. In this study, when rabeprazole was administered as 40 mg once daily to EMs and 20 mg twice daily to homozygous EMs, plasma rabeprazole concentrations before and at 1 and 2 hours after dosing were often below detectable levels. With 20 mg twice daily in heterozygous EMs and 10 mg 4 times daily in heterozygous EMs and homozygous EMs, plasma rabeprazole concentrations were sustained above 10 ng/mL throughout the 24-hour period and sufficient acid suppression was achieved. C_{max} values over the 24-hour period with 10 mg 4 times daily were 60.81 ± 9.49 ng/mL in homozygous EMs and 75.58 ± 5.39 ng/mL in heterozygous EMs. Although the minimum effective concentration of rabeprazole could not be determined in our study, we assume that the plasma rabeprazole concentration for sufficient nocturnal acid inhibition should be sustained at or above 60 ng/mL throughout the 24-hour period, as observed in our study. Although the mean $AUC_{0\text{-}24}$ values for 40 mg as a once-daily dose and in divided doses did not differ significantly, acid inhibition attained with 10 mg 4 times daily was more potent than that with 40 mg once daily or 20 mg twice daily in homozygous EMs. We are accordingly tempted to assume that, to maintain the plasma PPI concentration higher than a certain threshold level throughout the 24-hour period, a multiple-dosage regimen would be more effective for nocturnal acid inhibition than simply increasing the C_{max} or the $AUC_{0\text{-}24}$ value by increasing the dose of a PPI as a single dose.

Esophageal mucosal injury in patients with GERD varies according to the intragastric pH values during a 24-hour period; that is, the duration of intragastric pH

higher than 4.0 during a 24-hour period required for the cure of GERD is estimated to be approximately 20 to 22 hours (>83.3%).[7,8] A 24-hour intraesophageal pH monitoring study in patients with high-grade GERD (eg, grade C or D) revealed frequent episodes of reflux of gastric acid throughout the 24-hour period, particularly during the nighttime,[9] and, therefore, the control of nocturnal acid secretion is important for the cure of high-grade GERD. In this study we observed that the duration of intragastric pH lower than 4.0 during the 24-hour period attained by 40 mg once daily was minimal in PMs (13.0%) but not in heterozygous EMs (38.1%) and homozygous EMs (42.0%). Sufficient acid inhibition was achieved, however, with 20 mg twice daily in heterozygous EMs (16.0%), and a similar effect was observed with 10 mg 4 times daily in both homozygous EMs (11.9%) and heterozygous EMs (2.0%). With these results in mind, we recommend that patients with GERD refractory to standard PPI treatment should undergo CYP2C19 genotype testing and be treated with 10 mg rabeprazole 4 times daily for homozygous EMs or heterozygous EMs or with 20 mg twice daily for heterozygous EMs.

One of the reasons GERD may be refractory to PPI treatment is nocturnal gastric acid breakthrough.[11-13] However, the causative mechanism of nocturnal gastric acid breakthrough remains obscure. Because increasing the dose of a PPI at bedtime does not completely suppress nocturnal gastric acid breakthrough,[10] its mechanism has been considered to be unrelated to plasma PPI concentrations. Nocturnal gastric acid breakthrough typically occurs at approximately 6 to 7.5 hours after the evening PPI dose (ie, between 1 and 2 AM).[11,12] In homozygous EMs and heterozygous EMs, PPIs are usually eliminated completely within 6 to 10 hours after dosing, as was observed in this study and previous studies.[16,17,22,23,26,27,34-36] In this study the incidence of nocturnal gastric acid breakthrough was greatest in homozygous EMs (100% [5/5 subjects]), followed by heterozygous EMs (83% [5/6]), and was lowest in PMs (25% [1/4]) with 40 mg once daily. This order of decreasing incidence parallels that of the pharmacokinetic parameters of rabeprazole in the different CYP2C19 genotype groups. Moreover, frequent or divided doses sustained plasma rabeprazole levels longer, thereby yielding sufficient acid inhibition during nighttime, even in homozygous EMs and heterozygous EMs. We, therefore, assume that, for the control of nocturnal gastric acid breakthrough, plasma PPI concentrations must be sustained above a certain threshold throughout the 24-hour period. We believe that multiple-dosage regimens for PPIs may be a useful therapeutic strategy for white patients refractory to standard PPI therapy, because the prevalence of homozygous EMs is much greater in white patients (70%-75%) than in Asian patients (30%-40%).[18,37,38]

Sufficient eradication rates for *H pylori* infection were achieved by treatment with either lansoprazole (30 mg) or rabeprazole (10 mg) plus amoxicillin (INN, amoxicilline) (500 mg) 4 times daily for 2 weeks as second-line therapy in homozygous EMs.[39-41] Similar results were recently reported from Europe.[42] Interestingly, a regimen of 20 mg rabeprazole plus 1000 mg amoxicillin twice daily has not yielded satisfactory cure rates (59.6%).[43] The reason for this observation may be explained by the findings of our current study, as follows: 20 mg rabeprazole twice daily does not yield sufficient acid inhibition throughout the 24-hour period in homozygous EMs as shown, so antibiotics do not remain stable and bioavailable. Indeed, the incidence of homozygous EMs is reported to be higher in patients with failed *H pylori* eradication.[40,41,43,44] On the contrary, in this study, 10 mg rabeprazole 4 times daily attained a sufficient acid inhibition in homozygous EMs. This may be the reason why several reports have attained high re-eradication rates of *H pylori* (96.8%-100%) with dual therapy comprising a PPI plus amoxicillin 4 times daily.[39-42] We, therefore, strongly recommend dual therapy with a PPI plus an antibiotic to which *H pylori* is sensitive, in 4 divided doses, as a rescue treatment strategy for patients in whom the initial treatment regimen has failed to eradicate *H pylori* infection.[33,45]

Finally, our results must be interpreted within the following limitations and cautions. These results were obtained from short-term repeated-dosage regimens in a limited number of CYP2C19-genotyped healthy *H pylori*–negative volunteers, not in patients with acid-related diseases. The therapeutic effects of rabeprazole as a function of CYP2C19 genotype will, therefore, need to be re-evaluated in an appropriate study of patients with acid-related diseases undergoing long-term treatment. This study should accordingly be viewed as the preliminary basis for further studies. Nevertheless, an individualized or optimized dosage regimen with rabeprazole, as a model PPI, based on the individual's CYP2C19 genotype status is a valid therapeutic proposal for overcoming nocturnal gastric acid breakthrough. We strongly recommend the following dosage regimens for patients with GERD refractory to treatment with the usual standard dose of rabeprazole: 20 mg once daily in PMs, 20 mg twice daily or 10 mg 4 times daily in heterozygous EMs, and 10 mg 4 times daily in homozygous EMs, as noted earlier. A PPI

dosage regimen of 4 times daily is unlikely to be popular and will be unacceptable for some patients. Development of a slow-release form of an existing PPI or a new PPI with a longer plasma elimination half-life would be highly desirable. We hope that this study spurs on the development of such a new PPI.

None of the authors has a conflict of interest related to this study.

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