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Interphenotype differences in disposition and effect on gastrin levels of omeprazole—suitability of omeprazole as a probe for CYP2C19

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1 Fourteen healthy Swedish Caucasian subjects were given 20 mg of omeprazole orally each morning for 8 days. The subjects included five poor metabolisers (PM) of S-mephenytoin, four heterozygous extensive metabolisers (hetEM) and five subjects with a very rapid metabolism (rapidEM).

2 After the first dose, the relative mean areas under the plasma concentration *vs* time curve (AUC) of omeprazole in rapidEM, hetEM and PM were 1:3.7:20 (all different, $P < 0.001$). A similar relation was seen in the AUC(0,10 h) of the sulphone metabolite (1:3:12). Concentrations of hydroxyomeprazole were higher in EM than in PM confirming that the hydroxy, but not the sulphone metabolite, is formed by the S-mephenytoin hydroxylase (CYP2C19). After 8 days of treatment, the differences between groups were similar.

3 After both the first and the eighth doses, the omeprazole/hydroxyomeprazole plasma concentration ratio, determined 3 h after drug intake, correlated with the mephenytoin S/R ratio ($r_s = 0.94$; $P < 0.001$; $n = 14$) suggesting that omeprazole might be used to phenotype for CYP2C19.

4 After the first dose of omeprazole, there was no difference in the AUC(0,10 h) of plasma gastrin between the three groups. From the first to the eighth dose, the AUC(0,10) of gastrin increased significantly in both hetEM and PM, while there was no change in the rapidEM. After the eighth dose, the AUC(0,10) of gastrin correlated significantly with the AUC of omeprazole in plasma ($r_s = 0.79$; $P < 0.01$; $n = 13$).

Keywords omeprazole mephenytoin phenotype pharmacokinetics CYP2C19 gastrin levels

Introduction

Omeprazole is a substituted benzimidazole, which effectively inhibits gastric acid secretion by irreversible binding to the proton pump (H^+,K^+) ATPase in the gastric parietal cell [1]. The drug is rapidly and completely metabolised by the liver. Hydroxyomeprazole and omeprazole sulphone are the two major metabolites in plasma [2].

Polymorphic S-mephenytoin hydroxylation [3] is catalysed by cytochrome P450 2C19(CYP2C19) [4, 5], which metabolises some important drugs, e.g. diazepam [6] and proguanil [7]. The hydroxylation of omeprazole is also related to the hydroxylation of S-mephenytoin [2, 8, 9]. The area under the plasma concentration *vs* time curve (AUC) of oral omeprazole is markedly lower in extensive metabolisers (EM) than in poor metabolisers (PM) of S-mephenytoin both in Caucasian and Oriental subjects [2, 9]. The clearance of omeprazole is higher in Caucasian EM compared with Korean and Chinese EM [2, 9, 10]. As the frequency of subjects with defective S-mephenytoin hydroxylase activity is higher in Orientals (15% PM in China and 13% in Korea) than in Caucasians

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(3% PM in Sweden), there is accordingly a higher frequency of heterozygous EM among Orientals compared with Caucasians [6, 10]. This might be the reason for the slower elimination of omeprazole in Oriental EM compared with Caucasian EM.

Gastrin is secreted from G-cells in the antral part of the stomach. Gastrin exerts a trophic effect on the oxyntic mucosa and stimulates the secretion from parietal cells. High acidity in the antral part of the stomach is known to inhibit gastrin secretion. Inhibition of acid secretion raises the pH in the gastric antrum and secretion of gastrin is augmented. As the degree of acid reduction seems to be related to the AUC of omeprazole [11, 12], it is not surprising that treatment with omeprazole leads to a rise in plasma gastrin level [13]. A significant increase in fasting plasma gastrin levels was observed during long term omeprazole treament, but not after a single dose of the drug [14].

In the present study, we have investigated the disposition of omeprazole and its effect on plasma gastrin levels after single and multiple oral doses in the three groups of S-mephenytoin hydroxylators, i.e. very rapid EM (rapidEM, mephenytoin S/R ratio < 0.05), heterozygous EM (hetEM) and PM. As a genotyping technique for CYP2C19 only became available [15] after this study was completed, we identified heterozygous EM from family studies.

Methods

Subjects

Fourteen healthy white Swedish subjects were recruited from a population study of the S-mephenytoin 4'-hydroxylation polymorphism [16]. None consumed extensive amounts of alcohol and only one was a light smoker. None had taken any drug 1 week before or during the study. They were healthy as assessed by medical history, physical examination, and routine laboratory tests. The study was performed according to the 'Declaration of Helsinki' and all subjects gave their informed consent before participation. The study was approved by the Ethics Committee at Huddinge Hospital.

Five of the subjects (four males and one female; age 23 to 32 years; weight 53 to 88 kg) were PM of S-mephenytoin as the urinary S/R enantiomeric ratio of mephenytoin was close to 1 in the 0–8 h urine after the intake of 100 mg racemic mephenytoin (range 0.91–1.10). Another five subjects (four males and one female; age 24 to 33 years; weight 64 to 82 kg) were EM with an S/R ratio less than 0.05 in the 0–8 h urine (rapidEM). The remaining four subjects (three males and one female; age 24 to 33 years; weight 64 to 84 kg) had S/R ratios from 0.27 to 0.75. They were identified as obligate heterozygous EM because each of them has one parent who is a PM (Table 1).

The subjects took 20 mg omeprazole as encapsulated enteric coated granules (Losec, Astra-Hässle) at 08.00 h on 8 consecutive days. After an overnight fast on the first and the eighth day, venous blood samples were drawn before the dose at 08.00 h and then at 1, 2, 3, 4, 6, 8 and 10 h after the intake of omeprazole. Plasma was separated after centrifugation and stored frozen at −20°C until analysis. The subjects fasted until the 3 h sample had been drawn and thereafter a lunch was served.

Table 1 Mephenytoin S/R ratio (0–8 h/24–32 h) in the four heterozygous EM and in their PM parents

Heterozygous EM	*PM parent*
0.75/0.29	1.08/1.06 (father)
0.28/ND	1.08/1.06 (father)
0.32/ND	1.08/1.07 (father)
0.27/ND	0.95/0.98 (mother)

ND = not determined, because the low S/R ratio in the 0–8 h urine showed that they are EM.

Analysis of omeprazole and metabolites

Two assays were used for quantification of omeprazole and metabolites. Analysis was always performed in duplicate.

Omeprazole and the sulphone metabolite were assayed by the h.p.l.c. method of Lagerström & Persson [17], with minor modifications. The chromatographic separation was performed on a silica column, 11.9 cm × 4.0 mm i.d., Superspher Si 60 (E. Merck, Darmstadt, Germany) preceded by a guard column, 1.5 cm × 3.2 mm i.d. (Brownlee Column, Applied Biosystems Foster City, CA, USA) with a mobile phase of dichloromethane containing 5% 2-propanol, 0.8% methanol and 0.175% ammonium hydroxide (25%). The flow rate was 1.0 ml min^{-1} and the absorbance was monitored by u.v. detection at 302 nm. Hydroxyomeprazole was analysed in a similar way (personal communication—Dr Lagerström, Bioanalytical Department, Astra Hässle, Mölndal Sweden). Briefly, 500 µl plasma and the internal standard H259/36 were mixed with 50 µl 1 M NaH_2PO_4 and extracted for 10 min with 1.0 ml dichloromethane containing 1% v/v 1-butanol. After centrifugation 150 µl of the organic phase was injected into the chromatographic system which was the same as that described above. The mobile phase consisted of dichloromethane containing 0.5% 2-propanol and 5.5% methanol, which contained 2.5% ammonium hydroxide (25%). The flow rate was 1.5 ml min^{-1}.

On each day of analysis, standard curves were prepared for both methods by adding different amounts of respective compounds to drug-free plasma in the range of 0–500 nM. At a concentration of 200 nM, the interday coefficients of variation were 1.6–3.2%. The limits of detection for omeprazole, omeprazole sulphone and hydroxyomeprazole were 20 nM, 15 nM and 50 nM respectively. Omeprazole, its two metabolites and the two internal standards were obtained from Astra Hässle.

Measurement of gastrin in plasma

Gastrin was determined by radioimmunoassay according to Nilsson [18], using antibody 4562 (generously supplied by Professor Jens Rehfeld, Denmark) and synthetic human gastrin I (Milab, Malmö, Sweden) was used as tracer. One of the rapid EM had a meal-stimulated gastrin response consistent with G-cell hyperfunction or hyperplasia. A later follow-up with 24 h intragastric acidity measurement, new gastrin determinations, endoscopy and biopsies from the antral mucosa confirmed the diagnosis of diffuse antral G-cell hyperplasia. This subject was excluded from the study of gastrin in plasma.

Pharmacokinetic data and statistics

C_{max} was noted as the maximum concentration measured during the dosage interval. The area under the plasma drug concentration *vs* time curve from 0 to 10 h (AUC(0,10)) was calculated using the linear trapezoidal method. The AUC from 0 h to infinity (AUC) was calculated by extrapolating the AUC to infinity with the rate constant determined from the terminal slope (λ_z) of the log plasma concentration-time curve. The plasma elimination half-life ($t_{1/2}$) and the oral plasma clearance (CL_0) were calculated as 0.693 λ_z and dose/AUC, respectively.

The AUC of gastrin was calculated from 0–3 h (basal fasting), 3–10 h (meal stimulated) and 0–10 h (total). The pharmacokinetic parameters in the three groups, rapidEM, hetEM and PM, are presented as mean ± standard deviation (s.d.). Differences between the rapidEM and hetEM groups, and also between the PM and hetEM groups were tested for statistical significance by Student's unpaired *t*-test. Regression analysis was performed by Spearman's rank correlation. P values < 0.05 were regarded as statistically significant.

Results

The plasma concentrations of omeprazole, hydroxyomeprazole and omeprazole sulphone in the three groups after the first and the eighth omeprazole doses are shown in Figure 1. After the single dose of omeprazole, the AUC of the parent drug differed significantly ($P < 0.001$) between the three groups with a relative ratio of 1:3.7:20 between rapidEM, hetEM and PM (Table 2). Also, omeprazole sulphone kinetics were different in the three groups with hetEM having pharmacokinetic values in between those in rapidEM and PM. For hydroxyomeprazole, the AUC was similar in rapidEM and hetEM, and significantly higher than in PM (Figure 1; Table 2).

The mean values of kinetic parameters derived after the eighth dose showed similar relationships between the three groups as after a single dose (Figure 1; Table 2). In the plasma drawn just before the eighth dose of omeprazole, neither parent drug nor hydroxyomeprazole could be detected. The sulphone could, however, be measured in all PM (mean 192 ± 52 nM) and in three of the four hetEM (37, 31, 82 and < 15 nM), but in none of the rapidEM (< 15 nM). The AUC of the sulphone increased by 42, 160 and 32% from the first to the eighth dose in rapidEM, hetEM and PM, respectively (all $P < 0.05$).

The metabolic ratio of omeprazole, expressed as the ratio between the AUCs of omeprazole and hydroxyomeprazole, as used in two previous studies (2,19), ranged from 0.37–0.58 in the rapidEM group, from 1.12–2.01 in the hetEM group, and from 12.1–55.8 in the PM group. Thus, there was no overlap in the metabolic ratios between the three groups. This metabolic ratio correlated significantly with the mephenytoin S/R ratio ($r_s = 0.96$; $P < 0.001$). To define whether a single blood sample could be used to phenotype subjects with omeprazole, we investigated ratios of omeprazole to hydroxyomeprazole at different time points after omeprazole intake. Both compounds could be measured in all 14 subjects in samples drawn at 3 and 4 h, but not at earlier or later time points after drug intake. As shown in Figure 2 there was a significant correlation between the omeprazole/hydroxyomeprazole ratio at 3 h and the S/R mephenytoin ratio, not only after a single dose, but also after multiple doses (both $r_s = 0.94$; $P < 0.001$), with no overlap between the three groups. The correlation using the 4 h ratio after the single dose was almost identical to that obtained at 3 h ($r_s = 0.89$; $P < 0.01$).

There were no differences in the gastrin AUC(0,10), AUC(0,3) and AUC(3,10) values between the three groups after the single dose except when comparing PM with rapidEM with respect to AUC(3,10) (Figure 3, Table 3). After the eighth dose, however, the three estimates of AUC of gastrin were all about twice as high in both PM and hetEM than in rapidEM (Table 3). In both PM and hetEM, there was a doubling of the AUC of gastrin (all three estimates) after the eighth compared with the first omeprazole dose. However, there was no increase in AUC of gastrin during multiple dosing in rapidEM (Figure 3; Table 3). The AUC(0,10) of gastrin correlated significantly with the AUC of omeprazole after the eighth dose and the first dose ($r_s = 0.79$, $P < 0.01$; Figure 4; $r_s = 0.68$, $P < 0.05$).

Discussion

After the single dose, the AUC of omeprazole in hetEM was almost four-fold higher than that in rapidEM, and about five times lower than in PM. For other kinetic parameters, i.e. the plasma half-life, clearance and C_{max}, there were also significant differences between the three groups as shown in Table 2. Earlier studies have shown that the AUC of omeprazole was different in EM and PM in both Orientals [2,9] and Caucasians [2]. There were also differences between the EM groups in the two populations, i.e. the clearance in Oriental EM was half that in Caucasian EM [10]. As the frequency of

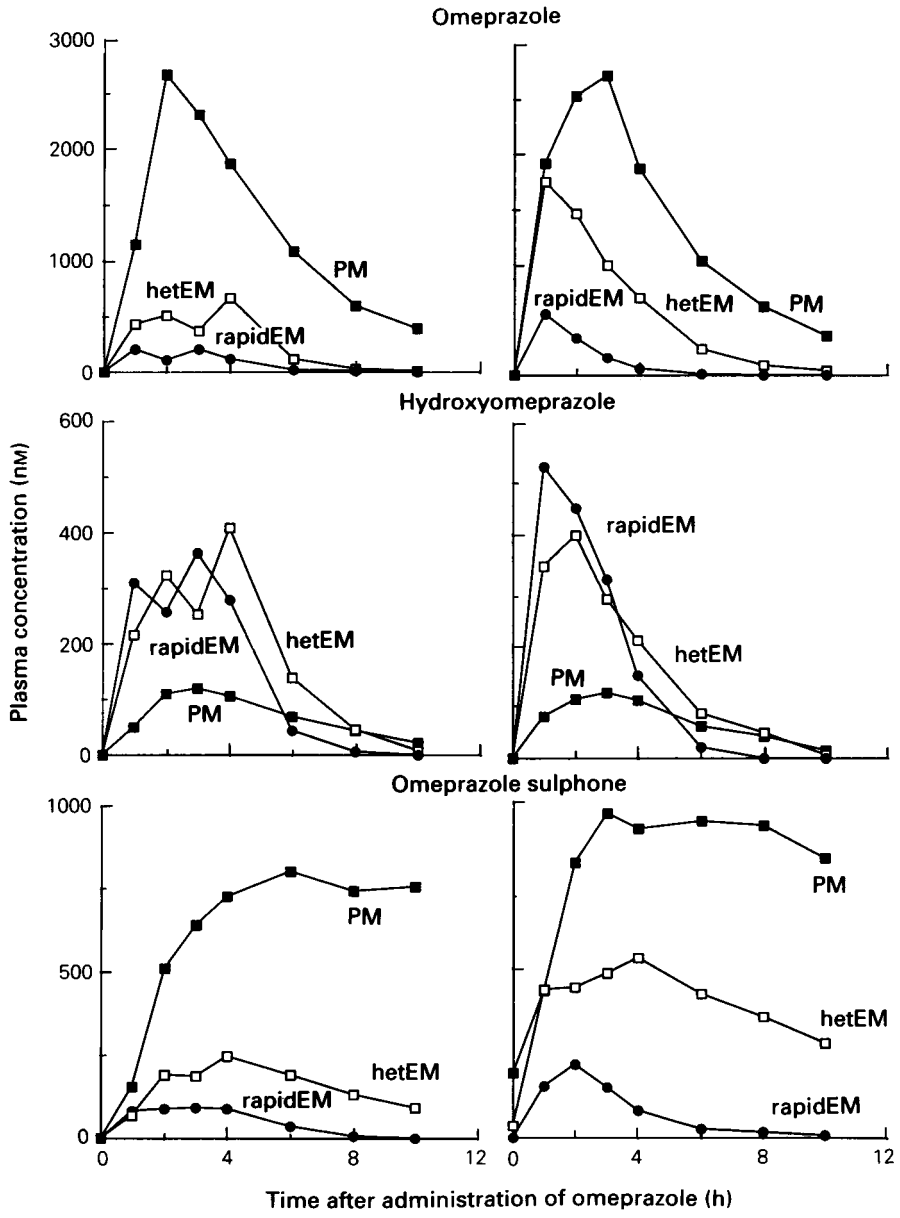

Figure 1 *Left panel*: Mean plasma concentrations of omeprazole, hydroxyomeprazole and omeprazole sulphone in rapid extensive metabolisers (● rapidEM; $n = 5$), heterozygous extensive metabolisers (□ hetEM; $n = 4$) and poor metabolisers (■ PM; $n = 5$) of S-mephenytoin following administration of 20 mg omeprazole.
Right panel: As in the left panel, but the mean plasma concentrations were measured after the eighth dose following administration of 20 mg omeprazole once daily.

mutated *CYP2C19* alleles is higher in Oriental populations, it has been suggested that the slower elimination of omeprazole in the Oriental EM group could be due to the higher proportion of heterozygous EM in this population compared with Caucasians [2, 10]. Our results with hetEM of S-mephenytoin having a slower elimination of omeprazole than rapidEM gives strong support for this hypothesis.

Andersson *et al.* [20] have shown a 60% increase in AUC of omeprazole during multiple dosing in 10 non-phenotyped healthy subjects. In the present study, the AUCs of omeprazole after 8 days were very similar to those on the first day in PM, but in the hetEM group, the AUCs of omeprazole and of the sulphone increased from the first to the eighth dose (Table 2 and Figure 1). The corresponding increase in rapidEM was less pronounced. As shown both *in vivo* [2] and *in vitro* [21, 22], omeprazole and the sulphone metabolite are both metabolised mainly by CYP2C19. The formation of the sulphone from omeprazole is catalysed by CYP3A4 [22]. The reason for a more pronounced accumulation of both omeprazole and the sulphone during multiple dosing in hetEM compared with rapidEM could be that less CYP2C19 is present in heterozygous EM. Therefore, concentration dependent metabolism may start at a lower concentration in this group, than in rapidEM. Another reason for the increase of omeprazole and sulphone in the hetEM is that these compounds are metabolised by the same enzyme and may therefore inhibit each other's

Table 2 Pharmacokinetic parameters of omeprazole and its two major metabolites in rapid extensive metabolisers (rapidEM; $n = 5$), heterozygous EM (hetEM; $n = 4$) and poor metabolisers (PM; $n = 5$) of S-mephenytoin after a single dose and after multiple doses

	First dose			*Eighth dose*			*Difference between first and eighth dose P value*		
	rapidEM	*hetEM*	*PM*	*rapidEM*	*hetEM*	*PM*	*rapidEM*	*hetEM*	*PM*
Omeprazole									
AUC (nMh)	715 ± 253***	2628 ± 147	14273 ± 3983***	1130 ± 513**	5984 ± 2305	14842 ± 3513**	NS	0.055	NS
$t_{1/2}$ (h)	0.71 ± 0.23*	1.91 ± 0.24	2.68 ± 0.69**	0.70 ± 0.15***	1.31 ± 0.21	2.44 ± 0.35***	NS	NS	NS
CL (l h^{-1} kg^{-1})	1.24 ± 0.44**	0.31 ± 0.04	0.06 ± 0.01***	0.81 ± 0.29**	0.15 ± 0.04	0.06 ± 0.01**	< 0.05	< 0.05	NS
C_{max} (nM)	393 ± 179***	1006 ± 136	3000 ± 1067**	678 ± 434*	1958 ± 727	3544 ± 1355	NS	NS	NS
Hydroxyomeprazole									
AUC (nMh)	1468 ± 330	1794 ± 420	772 ± 316**	1570 ± 329	1622 ± 99	710 ± 179***	NS	NS	NS
C_{max} (nM)	618 ± 205	581 ± 144	143 ± 53***	708 ± 302	445 ± 75	133 ± 33***	NS	NS	NS
Omeprazole sulphone									
AUC(0,10 h) (nMh)	515 ± 142***	1555 ± 513	6254 ± 3131**	731 ± 176***	4048 ± 1390	8265 ± 2682*	< 0.05	< 0.05	< 0.05
C_{max} (nM)	158 ± 55**	287 ± 99	919 ± 399**	208 ± 56**	646 ± 199	1159 ± 503	NS	NS	< 0.05

The data are given as mean ± s.d. After the first dose and the eighth dose, both rapidEM and PM, respectively, were compared with hetEM using an unpaired *t*-test, *< 0.05, **< 0.01, ***< 0.001. In the column to the right the differences between the kinetic parameters obtained from the first and the eighth dose are presented for the three groups (paired *t*-test). NS = not significant.

elimination. Such an interaction has been demonstrated *in vitro* [22]. In the PM group, there was no significant increase in AUC of omeprazole during multiple dosing, but the AUC of the sulphone increased by 32% ($P < 0.05$). This increase of the sulphone AUC might be explained by the presence of the sulphone in the samples drawn before the eighth dose (Figure 1), which may be due to the long half-life of this metabolite in PM. We can thus conclude that there is an accumulation in hetEM and to a lesser extent in rapidEM, possibly due to partial saturation of CYP2C19. In PM, who lack this enzyme, there is no accumulation of the parent drug.

In contrast to omeprazole and the sulphone, the AUC of hydroxyomeprazole was similar in hetEM and rapidEM both after the first and the eighth dose, and there was no accumulation during multiple dosing (Figure 1, Table 2). The hydroxy metabolite formed by CYP2C19 is further metabolised by CYP3A4 to hydroxyomeprazole sulphone [24]. The rate of formation of the hydroxyomeprazole is different, but the rate of elimination is probably the same in these three groups.

Methods for determination of the CYP2D6 genotype have been available for many years [23–25]. de Morais *et al.* [15] have shown recently that the principal defect in PM of S-mephenytoin is a single G→A mutation in exon 5 of the *CYP2C19* gene creating an aberrant splice site. This accounted for about 75% of the mutated alleles in both Caucasian and Japanese PM [15]. This technique was not available when the present investigation was performed. Therefore, obligate heterozygous EM had to be identified from family studies (Table 1). Homozygous EM can only be identified with certainty by DNA analysis and not by family studies. In this study we have chosen subjects with an S/R ratio < 0.05, and they probably represent a subgroup of very rapid homozygous EM. It cannot be excluded, however, that this group also includes subjects with multiple copies of an active gene as previously demonstrated for the *CYP2D6* gene locus in ultrarapid hydroxylators of debrisoquine [26, 27].

Although mephenytoin is a widely used probe drug for CYP2C19 activity in humans [28, 29], some concern has been raised [30–32]. It has been shown [2, 19] that the ratio of omeprazole and hydroxyomeprazole AUC values separates EM from PM. In the present study we have confirmed this. Using the omeprazole/hydroxyomeprazole ratio in the 3 h plasma sample there was no overlap between the three groups studied. This indicates that this ratio can be used for phenotyping and that the rapidEM and hetEM might possibly be separated in this way. However, to prove this, a population study needs to be performed. There seemed to be an even better separation between hetEM and rapidEM in the 3 h sample drawn after the eighth dose (Figure 2; right). To give eight doses is inconvenient in healthy subjects, but might be used in patients treated with the drug. We suggest that one single blood sample (taken 3 h after 20 mg omeprazole) could be used for phenotyping for CYP2C19.

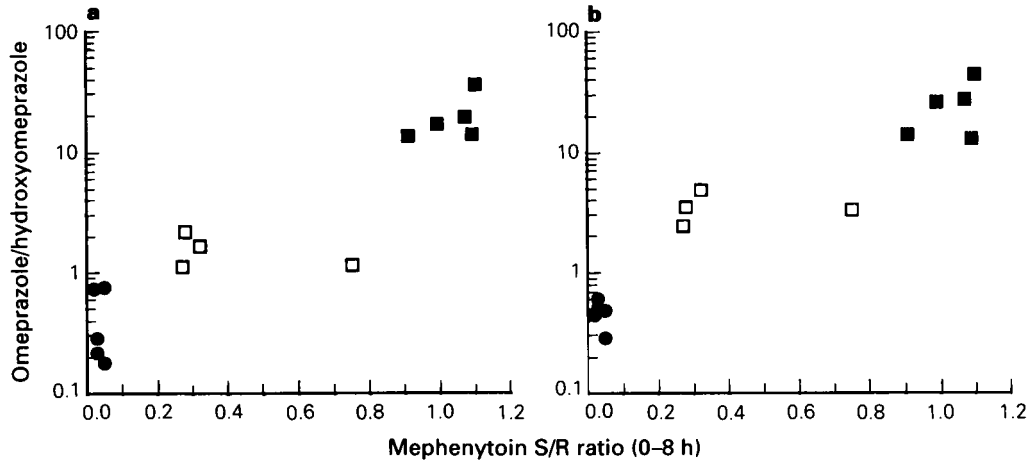

Figure 2 a) Relationship between plasma concentration ratio of omeprazole to hydroxyomeprazole at 3 h after a single dose of omeprazole *vs* the mephenytoin S/R ratio in 0–8 h urine in the three groups. (r_s = 0.94; $P < 0.001$; n = 14). The mean logarithmic ratio of omeprazole to hydroxyomeprazole was markedly different between hetEM and PM($P < 0.001$), and between hetEM and rapidEM ($P < 0.01$) (unpaired t-test).
b) As in the left panel, but the data refer to the eighth omeprazole dose (r_s = 0.94, $P < 0.001$; n = 14).
Symbols are the same as in Figure 1.

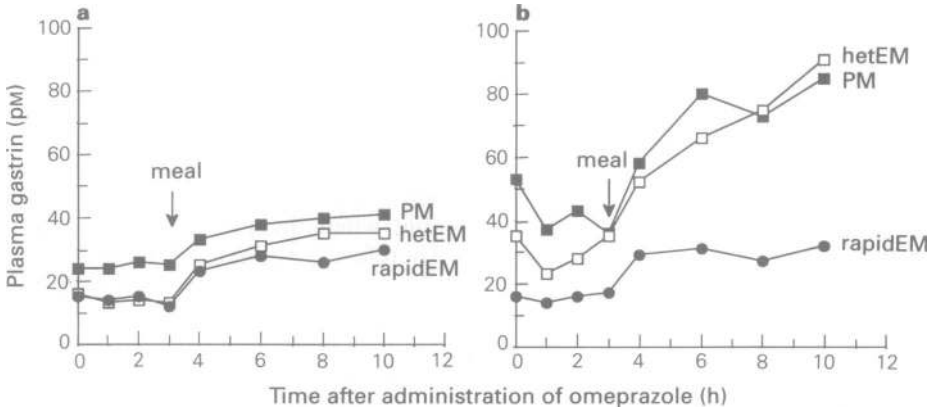

Figure 3 a) Mean gastrin levels in the three groups following a single 20 mg omeprazole dose. There was no significant difference between the three groups either before or after drug administration.
b) Mean gastrin levels after the eighth dose of omeprazole (five PM, four hetEM and four rapidEM). For statistics see Table 3.

Table 3 Mean (± s.d.) of the area under the gastrin concentration *vs* time curve (AUC) in the three groups after single and multiple doses. PM and hetEM were compared with rapidEM after first and eighth dose, respectively (left column; unpaired t-test). In the columns to the right the AUCs after first and eighth doses are compared within each group (paired t-test)

	AUC (pMh)			*Comparison of first dose to eighth dose (P value)*		
	rapidEM	*hetEM*	*PM*	*rapidEM*	*hetEM*	*PM*
AUC(0,10 h)						
1st dose	221 ± 56	254 ± 79	333 ± 85	NS	< 0.05	< 0.01
8th dose	246 ± 62	555 ± 179*	621 ± 116***			
AUC(0,3 h)						
1st dose	43 ± 8	42 ± 11	75 ± 38	NS	< 0.05	< 0.05
8th dose	47 ± 16	87 ± 29	125 ± 21***			
AUC(3,10 h)						
1st dose	167 ± 34	212 ± 70	259 ± 49*	NS	< 0.05	< 0.01
8th dose	197 ± 48	468 ± 152*	496 ± 99***			

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS = not significant.

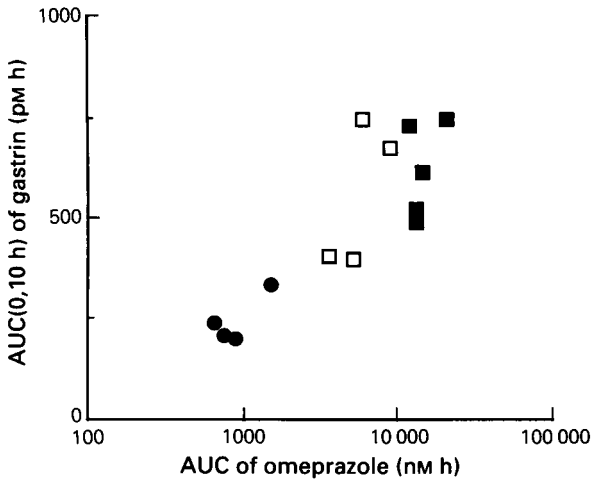

Figure 4 AUC(0,10 h) of gastrin *vs* AUC of omeprazole after the eighth dose of omeprazole in the three groups ($r_s = 0.79$, $P < 0.01$; $n = 13$).
Symbols are the same as in Figure 1.

Festen *et al.* [14] gave 30 mg omeprazole daily and showed that basal and meal-stimulated serum gastrin was increased after 7 and 14 days of treatment. However, no increase was seen after a shorter period of treatment, which is probably due to the fact that the gastrin increase is secondary to decreased gastric acidity. Our results are similar to those of Festen *et al.* but also demonstrate that gastrin release is concentration dependent (Figures 3 and 4). To confirm the shape of the concentration-effect curve, lower (e.g. 10 mg) and higher (40 mg) doses of omeprazole need to be given to the same subjects. A close relationship between the inhibition of pentagastrin induced acid secretion and the AUC of omeprazole after giving the drug at doses of 0, 20, 40, 60 and 80 mg has been documented [11]. Thus, when 20 mg omeprazole is given daily to Swedish subjects, there should be little effect on gastrin in the majority of subjects, i.e. homozygous EM. In heterozygous EM and PM, an increase of gastrin would, however, be expected to be more pronounced. Theoretically, the same dose of omeprazole given to Orientals should have a more pronounced effect on gastrin as the incidence of both PM and heterozygous EM is higher in this population [16].

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References

1 Fellenius E, Berglindh T, Sachs G, *et al.* Substituted benzimidazoles inhibit gastric acid secretion by blocking (H^+, K^+) ATPase. *Nature* 1981; **290**: 159–161.

2 Andersson T, Regårdh CG, Lou YC, Zhang Y, Dahl ML, Bertilsson L. Polymorphic hydroxylation of S-mephenytoin and omeprazole metabolism in Caucasian and Chinese subjects. *Pharmacogenetics* 1992; **2**: 32–37.

3 Wilkinson GR, Guengerich FP, Branch RA. Genetic polymorphism of S-mephenytoin hydroxylation. In *Pharmacogenetics of drug metabolism*, ed Kalow W, New York: Pergamon Press, 1992: 657–685.

4 Wrighton SA, Stevens JC, Becker GW, VandenBranden M. Isolation and characterization of human liver cytochrome P450 2C19: correlation between 2C19 and S-mephenytoin 4'-hydroylation. *Arch Biochem Biophys* 1993; **306**: 240–245.

5 Goldstein JA, Faletto MB, Romkes-Sparks M, *et al.* Evidence that CYP2C19 is the major S-mephenytoin 4'-hydroxylase in humans. *Biochemistry* 1994; **33**: 1743–1752.

6 Bertilsson L, Kalow W. Why are diazepam metabolism and polymorphic S-mephenytoin hydroxylation associated with each other in white and Korean populations but not in Chinese populations? *Clin Pharmac Ther* 1993; **53**: 608–610.

7 Ward SA, Helsby NA, Skjelbo E, Brosen K, Gram LF, Breckenridge AM. The activation of the biguanide antimalarial proguanil co-segregates with the mephenytoin oxidation polymorphism—a panel study. *Br J clin Pharmac* 1991; **31**: 689–692.

8 Andersson T, Regårdh CG, Dahl-Puustinen ML, Bertilsson L. Slow omeprazole metabolisers are also poor S-mephenytoin hydroxylators. *Ther Drug Monit* 1990; **12**: 415–416.

9 Sohn DR, Kobayashi K, Chiba K, Lee KH, Shin SG, Ishizaki T. Disposition kinetics and metabolism of omeprazole in extensive and poor metabolizers of S-mephenytoin 4'-hydroxylation recruited from an Oriental population. *J Pharmac exp Ther* 1992; **262**: 1195–1202.

10 Ishizaki T, Sohn DR, Kobayashi K, *et al.* Interethnic differences in omeprazole metabolism in the two S-mephenytoin hydroxylation phenotypes studied in Caucasians and Orientals. *Ther Drug Monit* 1994; **16**: 214–215.

11 Lind T, Cederberg C, Ekenved G, Haglund U, Olbe L. Effect of omeprazole – a gastric proton pump inhibitor on pentagastrin stimulated acid secretion in man. *Gut* 1983; **24**: 270–276.

12 Cederberg C, Thomson ABR, Mahachai V, *et al.* Effect of intravenous and oral omeprazole on 24-hour intragastric acidity in duodenal ulcer patients. *Gastroenterology* 1992; **103**: 913–918.

13 Olbe L, Cederberg C, Lind T, Olausson M. Effect of omeprazole on gastric acid secretion and plasma gastrin in man. *Scand J Gastroenterology* 1989; **24** (suppl 166) 27–32.

14 Festen HPM, Thijs JC, Lamers CBHW, *et al.* Effect of oral omeprazole on serum gastrin and serum pepsinogen I levels. *Gastroenterology* 1984; **87**: 1030–1034.

15 de Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA. The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J biol Chem* 1994; **269**: 15419–15422.

16 Bertilsson L, Lou YQ, Du YL, *et al.* Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquine and S-mephenytoin. *Clin Pharmac Ther* 1992; **51**: 388–397.

17 Lagerström PO, Persson BA. Determination of omeprazole and metabolites in plasma and urine by liquid chromatography. *J Chromatography* 1984; **309**: 347–356.
18 Nilsson G. Increased plasma gastrin levels in connection with inhibition of gastric acid responses to sham feeding following bulbar perfusion with acid in dogs. *Scand J Gastroenterol* 1975; **10**: 273–277.
19 Andersson T. *Pharmacokinetics of omeprazole in man.* Ph. D. thesis at the University of Göteborg, Medical faculty, 1991.
20 Andersson T, Andren K, Cederberg C, Lagerström PO, Lundborg P, Skånberg I. Pharmacokinetics and bioavailability of omeprazole after single and repeated oral administration in healthy subjects. *Br J clin Pharmac* 1990; **29**: 557–563.
21 Chiba K, Kobayashi K, Manabe K, Tani M, Kamataki T, Ishizaki T. Oxidative metabolism of omeprazole in human liver microsomes: Cosegregation with S-mephenytoin 4'-hydroxylation. *J Pharmac exp Ther* 1993; **266**: 52–59.
22 Andersson T, Miners JO, Tassaneeyakul W, *et al.* Identification of human liver cytochrome P450 isoforms mediating omeprazole metabolism. *Br J clin Pharmac* 1993; **36**: 521–531.
23 Heim M, Meyer UA. Genotyping of poor metabolisers of debrisoquine by allele-specific PCR amplification. *Lancet* 1990; **336**: 529–532.
24 Broly F, Gaedigk M, Heim M, Eichelbaum M, Morike K, Meyer UA. Debrisoquine/sparteine hydroxylation genotype and phenotype: analysis of common mutations and alleles of CYP2D6 in a European population. *DNA cell Biol* 1991; **10**: 545–558.
25 Dahl ML, Johansson I, Porsmyr Palmertz M, Ingelman-Sundberg M, Sjöqvist F. Analysis of the CYP2D6 gene in relation to debrisoquin and desipramine hydroxylation in a Swedish population. *Clin Pharmac Ther* 1992; **51**: 12–17.
26 Bertilsson L, Dahl ML, Sjöqvist F, *et al.* Molecular basis for rational megaprescribing in ultrarapid hydroxylators of debrisoquine. *Lancet* 1993; **341**: 63.
27 Johansson I, Lundqvist E, Bertilsson L, Dahl ML, Sjöqvist F, Ingelman-Sundberg M. Inherited amplification of an active gene in the cytochrome P450 *CYP2D* locus as a cause of ultrarapid metabolism of debrisoquine. *Proc Natl Acad Sci USA* 1993; **90**: 11825–11829.
28 Küpfer A, Roberts RK, Schenker S, Branch RA. Stereoselective metabolism of mephenytoin in man. *J Pharmac exp Ther* 1981; **218**: 193–198.
29 Wedlund PJ, Aslanian WS, McAllister CB, Wilkinson GR, Branch RA. Mephenytoin hydroxylation deficiency in Caucasians: Frequency of a new oxidative drug metabolism polymorphism. *Clin Pharmac Ther* 1984; **36**: 773–780.
30 Relling MV, Ayers D, Heideman RL. Mephenytoin phenotyping: lack of haematologic effect and timing of urine collections. *Pharmacogenetics* 1991; **1**: 42–49.
31 Zhang Y, Blouin RA, McNamara P, Steinmetz J. Wedlund PJ. Limitation to the use of the urinary S/R-mephenytoin ratio in pharmacogenetic studies. *Br J clin Pharmac* 1991; **31**: 350–352.
32 Tybring G, Bertilsson L. A methodological investigation on the estimation of the S-mephenytoin hydroxylation phenotype using the urinary S/R ratio. *Phamacogenetics* 1992; **2**: 241–243.

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