0022-3565/93/2661-0052$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 266, No. 1
Printed in U.S.A.

Oxidative Metabolism of Omeprazole in Human Liver Microsomes: Cosegregation with *S*-Mephenytoin 4′-Hydroxylation[1]

KAN CHIBA, KAORU KOBAYASHI, KYOKO MANABE, MASAYOSHI TANI, TETSUYA KAMATAKI and TAKASHI ISHIZAKI

Division of Clinical Pharmacology, Clinical Research Institute (K.C., K.K., K.M., T.I.) and Division of General Surgery (M.T.), National Medical Center, Tokyo, Japan; Faculty of Pharmaceutical Sciences (K.K., K.M.), Science University of Tokyo, Tokyo, Japan; and Faculty of Pharmaceutical Sciences (T.K.), Hokkaido University, Sapporo, Japan

Accepted for publication March 29, 1993

ABSTRACT

Oxidative metabolism of omeprazole (OPZ) was studied in 14 human liver microsomes in relation to the 4′-hydroxylation capacity of *S*-mephenytoin. The formation of 5-hydroxyomeprazole and omeprazole sulfone (OPZ-SFN) from OPZ exhibited a biphasic kinetic behavior, indicating that at least two distinct enzymes are involved in either of the metabolic pathways of OPZ. By using a two-enzyme kinetic approach, the activities were described by high (K_{m1} and V_{max1})- and low-affinity components (K_{m2} and V_{max2}). The respective mean (±S.D.) kinetic parameters for 5-hydroxylation and sulfoxidation were: K_{m1} = 6.0 ± 2.4 and 10.2 ± 7.2 μM and V_{max1} = 88.0 ± 70.2 and 66.9 ± 53.9 pmol/mg/min; K_{m2} = 106 ± 127 and 482 ± 472 μM and V_{max2} = 116 ± 88 and 299 ± 131 pmol/mg/min. Among these kinetic parameters, only the V_{max1} of 5-hydroxylation gave a close correlation with the corresponding parameter of *S*-mephenytoin (r_s = 0.911, P < .01). In addition, OPZ and *S*-mephenytoin inhibited competitively each other's metabolism with the respective K_i values of 2.0 and 162 μM. Interestingly, OPZ-SFN also inhibited competitively 4′-hydroxylation of *S*-mephenytoin with a K_i value of 8.2 μM. Moreover, polyclonal antibodies raised against *S*-mephenytoin 4′-hydroxylase (P450 form) partially inhibited the 5-hydroxylation of OPZ, whereas no inhibition was observed for the sulfoxidation. These findings suggest that *S*-mephenytoin 4′-hydroxylase is an enzyme primarily responsible for the 5-hydroxylation of OPZ and further metabolism of OPZ-SFN, but not for the sulfoxidation of OPZ in human liver microsomes. The *in vitro* experiments with human liver microsomes appear to be a useful approach to estimate the *in vivo* disposition of a drug like OPZ of which the metabolism is mediated *via* a polymorphic cytochrome P450 in the human.

OPZ is a selective inhibitor of H^+/K^+-adenosine triphosphatase proton pump in gastric parietal cells (Clissold and Campoli-Richards, 1986; Holt and Howden, 1991; Howden, 1991; Maton, 1991). Its therapeutic potential has been documented as a potent long-acting inhibitor of gastric acid secretion for the treatment of duodenal ulcer, refractory gastroesophageal reflex disease, Zollinger-Ellison syndrome and other hypersecretory conditions (Clissold and Campoli-Richards, 1986; Holt and Howden, 1991; Howden, 1991; Maton, 1991). OPZ is metabolized extensively by the liver primarily *via* oxidation, and its major metabolites in plasma are H-OPZ and OPZ-SFN, and those in urine are H-OPZ and OPZ acid in humans (Howden, 1991; Renberg *et al.*, 1989; Regårdh *et al.*, 1990) (fig. 1). Neither of the metabolites is considered to contribute to the antisecretory effect (Clissold and Campoli-Richards, 1986; Maton, 1991). The pharmacokinetic profile of OPZ has been well characterized in humans (Clissold and Campoli-Richards, 1986; Howden, 1991; Maton, 1991), giving a pronounced interindividual variability: some subjects exhibit the extremely higher plasma levels and longer elimination half-lives than those with average kinetic parameters (Andersson *et al.*, 1990a; Regårdh *et al.*, 1990). Later, those subjects were shown to be the PMs of *S*-mephenytoin (Andersson *et al.*, 1990b).

S-mephenytoin 4′-hydroxylation shows a genetically determined polymorphism (Brøsen, 1990; Küpfer and Preisig, 1984; Wilkinson *et al.*, 1989), and demonstrates a marked interethnic difference in the incidence of the PM phenotype: approximately 3 to 6% of Caucasian (Alván *et al.*, 1990; Brøsen, 1990; Küpfer and Preisig, 1984; Wedlund *et al.*, 1985; Wilkinson *et al.*, 1989) and 13 to 23% of Oriental populations are the PMs of *S*-mephenytoin (Horai *et al.*, 1989; Sohn *et al.*, 1992a,b). Thus, if this pharmacogenetic determinants could have clinical implication, a drug whose metabolism is mediated *via* *CYP2C9* [the P450 form is described in an italicized style throughout the

Received for publication January 11, 1993.
[1] This study was supported by Grant-in-Aid 1-5-1-C from the Japan Health Science Foundation and from the Ministry of Human Health and Welfare, Tokyo, Japan.

ABBREVIATIONS: OPZ, omeprazole; H-OPZ, 5-hydroxyomeprazole; OPZ-SFN, omeprazole sulfone; PM, poor metabolizer; *CYP2C9*, *S*-mephenytoin 4′-hydroxylase; AUC, area under the plasma concentration-time curve; EM, extensive metabolizer; HPLC, high-performance liquid chromatography.

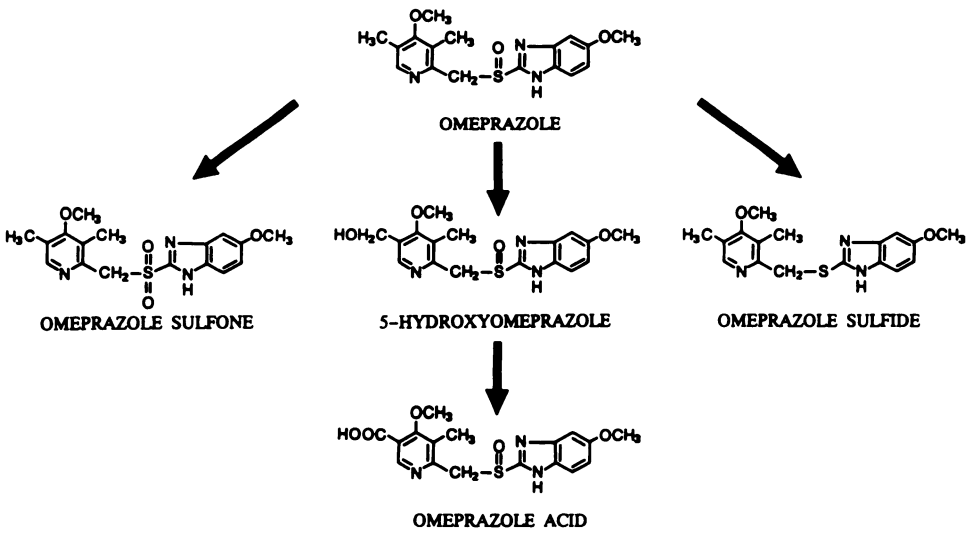

Fig. 1. Metabolic pathways of OPZ in humans.

text according to the proposed nomenclature and its designation (Nelson *et al.*, 1993)] might be of more clinical concern among Oriental patients than among Caucasian patients. The metabolism of several clinically important drugs has been demonstrated to cosegregate with this hydroxylation polymorphism, including propranolol (Ward *et al.*, 1989), imipramine (Skjelbo *et al.*, 1991), mephobarbital (Küpfer and Branch, 1985), hexobarbital (Knodell *et al.*, 1988; Yasumori *et al.*, 1990), proguanil (Ward *et al.*, 1991), diazepam (Bertilsson *et al.*, 1989; Sohn *et al.*, 1992b) and amitriptyline (Breyer-Pfaff *et al.*, 1992).

Sohn *et al.* (1992a) and Andersson *et al.* (1992) have reported recently that the AUCs of OPZ and OPZ-SFN are significantly greater, whereas the AUC of H-OPZ is significantly less in the PMs than in the EMs of *S*-mephenytoin. Moreover, the former investigators have shown that the urinary excretion of H-OPZ is significantly less in the PMs than in the EMs (Sohn *et al.*, 1992a). These findings suggest that the 5-hydroxylation of OPZ is and further, but yet uncharacterized, metabolism of OPZ-SFN may be mediated by cytochrome P450 responsible for the 4′-hydroxylation of *S*-mephenytoin (*i.e.*, *CYP2C9*), but that the sulfoxidation of OPZ appears to be catalyzed by a different enzyme(s). In the present investigation, we intended to characterize the oxidative metabolism of OPZ in human liver microsomes in order to clarify further the metabolic process of OPZ in relation to the 4′-hydroxylation capacity of *S*-mephenytoin, and wish to discuss the results in the light of clinical implication of an *in vitro* human pharmacogenetic research.

Materials and Methods

Drugs and chemicals. OPZ, OPZ-SFN and H-OPZ were generous gifts from Fujisawa-Astra Ltd. (Osaka, Japan), Fujisawa Pharmaceutical Co. (Osaka, Japan) and Astra Hässle AB (Mölndal, Sweden), respectively. Because OPZ contained 0.2% H-OPZ as a contaminant, it was purified before use as follows: OPZ was dissolved in dichlormethylene and washed 3 times with sodium-phosphate buffer (pH 11.0, 100 mM). By this procedure, more than 95% of H-OPZ was removed. Racemic mephenytoin and 4′-hydroxymephenytoin were kindly donated by Dr. Küpfer (University of Berne, Berne, Switzerland). *S*- and *R*-mephenytoin were separated from the racemic mixture of mephenytoin by a Chiralcel OJ column (10 μm, 4.6 × 250 mm, Daicel Chemical Co. Ltd., Tokyo, Japan) as reported by Yasumori *et al.* (1990). Phenobarbital was donated from Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan). NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Acetonitrile and other reagents of analytical grade were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Human liver microsomes. Fresh human liver samples (n = 14) were obtained from the Japanese patients who underwent a partial hepatectomy at the Division of General Surgery (National Medical Center, Tokyo, Japan) as an excess material removed during surgery on the liver. None of the patients had been phenotyped *in vivo* with the racemic mephenytoin before undergoing the hepatectomy. All the surgeries were performed for the removal of metastatic tumor(s) from the liver. The use of the human liver for the study had been approved by the Institutional Ethics Committee. It took less than 5 min from the removal of liver until collecting and freezing a sample in liquid nitrogen. The liver parenchyma of the nontumor-bearing part used for the study was shown later to be histopathologically normal in all cases. The liver samples obtained from the following liver donors were not included in the study: patients with acute or chronic hepatitis, those with cirrhosis and those who were taking medications known to induce or inhibit the hepatic monooxygenase activity.

Microsomes were prepared by differential centrifugation and the 105,000 × g pellet was washed and resuspended in 50 mM sodium-phosphate buffer (pH 7.4) containing 0.1 mM EDTA. After the determination of protein concentration (Lowry *et al.*, 1951), the suspended microsomes were aliquoted, frozen and kept at −80°C until used.

Incubation and sample preparation. The basic incubation medium contained 0.1 or 0.2 mg/ml of microsomes, 0.5 mM NADP, 2.0 mM glucose-6-phosphate, 1 IU/ml of glucose-6-phosphate dehydrogenase, 4 mM $MgCl_2$, 0.1 mM EDTA, 100 mM potassium-phosphate buffer (pH 7.4) and 2.5 to 400 μM of OPZ or 25 to 400 μM of *S*-mephenytoin in a final volume of 250 μl. The mixture was incubated at 37°C for 20 and 60 min for OPZ and *S*-mephenytoin, respectively, and the reaction was stopped by adding 100 μl of cold acetonitrile. After the termination of incubation, phenacetin (50 μg/ml in methanol) or phenobarbital solution (2 μg/ml in methanol) was added to the sample as an internal standard for OPZ or *S*-mephenytoin, respectively. The mixture was centrifuged at 10,000 × g for 5 min and the supernatant was injected into an HPLC apparatus as described below.

Determination of metabolites. The determination of H-OPZ and OPZ-SFN was performed by using an HPLC method as reported recently from our laboratory (Kobayashi *et al.*, 1992). The HPLC system consisted of a model L-6000 pump (Hitachi Ltd., Tokyo, Japan), a model L-4000 UV detector (Hitachi), a model A*S*-2000 autosampler (Hitachi), a model D-2500 integrator (Hitachi) and a 4.6 mm × 25 cm Capcell Pak C_{18} AG120 column (Shiseido Co. Ltd., Tokyo, Japan). The mobile phase consisted of acetonitrile-sodium-phosphate buffer (0.05 M, pH 8.5) in proportion of 25/75 by v/v, and was delivered at a flow rate of 0.8 ml/min. The column temperature was maintained at 30°C.

The eluate was monitored at the wavelength of 302 nm. The calibration curve was generated from 10 to 100 ng/ml by processing the authentic standard substances through the entire procedure.

The determination of 4′-hydroxymephenytoin was performed by using a simple HPLC method as developed recently in our laboratory (Chiba *et al.*, 1993). Briefly, the HPLC system used was the same as mentioned above, and the mobile phase consisted of acetonitrile-sodium-phosphate buffer (0.05 M, pH 4.0) in proportion of 26/74 by v/v and was delivered at a flow rate of 0.8 ml/min. The eluate was monitored at the wavelength of 204 nm. The calibration curve was generated from 25 to 100 ng/ml by processing the authentic standard substance through the entire procedure.

The quantitation of H-OPZ, OPZ-SFN and 4′-hydroxymephenytoin was made by comparison with the standard curves by using the peak-height ratio method. Intra ($n = 6$)- and interassay ($n = 6$) coefficients of variations were less than 8 and 10% for determining 4′-hydroxymephenytoin and the metabolites of OPZ, respectively.

Kinetics of the formation of metabolites. The formation rates of H-OPZ and OPZ-SFN were linear at 37°C for the incubation time of up to 30 min when 10 or 200 μM of OPZ and 0.1 mg/ml of microsomal protein coexisted. Neither H-OPZ nor OPZ-SFN was formed in the absence of the NADPH-generating system, microsomes or OPZ. A linear relationship was also observed between the production rates of the metabolites of OPZ in 20 min and protein concentration of up to 0.2 mg/ml. Accordingly, the kinetic studies were performed at 37°C and with a 20-min incubation time at a protein concentration of 0.1 or 0.2 mg/ml.

The formation rate of 4′-hydroxymephenytoin was linear at 37°C for up to 90 min when 200 μM of *S*-mephenytoin and 0.1 mg/ml of microsomal protein were present. A linear relationship was also observed between the rate of metabolite production in 60 min and protein concentration for up to 1.0 mg/ml. Accordingly, the kinetic studies were performed at 37°C with a 60-min incubation time and at a protein concentration of 0.1 to 1.0 mg/ml.

Michaelis-Menten kinetic parameters for the formation of H-OPZ and OPZ-SFN were estimated by fitting the data to the following equation:

$$V = V_{max1} \cdot S/(K_{m1} + S) + V_{max2} \cdot S/(K_{m2} + S)$$

where V is the velocity of the formation of H-OPZ or OPZ-SFN, S is the concentration of OPZ in the incubation mixture, K_{m1} and K_{m2} are the affinity constants for the high- and low-affinity components and V_{max1} and V_{max2} are the maximum enzyme velocities for the high- and low-affinity components, respectively. The kinetic parameters were estimated initially by the graphic analysis of Eadie-Hofstee plots, and the values obtained were used as the first estimate for the nonlinear least-squares regression analysis, MULTI (Yamaoka *et al.*, 1981), in which unweighted raw data were fitted to the model equation.

The enzyme kinetic parameters (K_m, V_{max} and V_{max}/K_m without the numerical subindices) for the formation of 4′-hydroxymephenytoin were estimated by the linear regression analysis by using unweighted raw data, because it followed a simple Michaelis-Menten kinetic behavior (*i.e.*, a one-enzyme kinetic approach) as reported previously (Hall *et al.*, 1987; Jurima *et al.*, 1985; Meier *et al.*, 1985).

Inhibition studies. Inhibition of OPZ 5-hydroxylation by *S*-mephenytoin was studied with the substrate concentrations of 5, 10 and 15 μM in the presence of six concentrations of *S*-mephenytoin over a range of 0 to 1 mM. Inhibition of *S*-mephenytoin 4′-hydroxylation by OPZ or OPZ-SFN was studied with the substrate concentrations of 0.1, 0.2 and 0.4 mM in the presence of six concentrations of OPZ or OPZ-SFN over a range of 0 to 100 μM. Apparent K_i (inhibition constant) values were determined by Dixon plots (Dixon and Webb, 1964).

Immunoinhibition. Rabbit antibodies raised against *CYP2C9* were prepared and assessed for the specificity as described previously (Komori *et al.*, 1988). The immunoinhibition of 5-hydroxylation and sulfoxidation of OPZ was examined by preincubating a human liver microsomal sample (0.1 mg/ml) with various concentrations (0 to 40 μl/mg of microsomal protein) of preimmune serum or antiserum in 0.1 M potassium-phosphate buffer (pH 7.4) for 30 min at room temperature. OPZ (25 μM) and other components of the incubation mixture were added, and the reactions were carried out as described above.

Statistical analysis. Spearman's rank correlation (r_s) was calculated where appropriate. Results are expressed as the mean ± S.D. throughout the text.

Results

Typical Eadie-Hofstee plots for the formation of H-OPZ and OPZ-SFN are shown in figure 2, A and B, respectively. Both the 5-hydroxylation and sulfoxidation of OPZ gave a biphasic relationship in the 14 different human liver microsomes. Because the data suggest that at least two enzymes are involved in the 5-hydroxylation and sulfoxidation of OPZ in human liver microsomes, the kinetic parameters for the formation of H-OPZ and OPZ-SFN were estimated by assuming that both of the metabolic reactions are catalyzed by the two enzymes. Although the data are not shown, Eadie-Hofstee plots for the formation of 4′-hydroxymephenytoin showed that a simple, one-enzyme kinetic analysis is applicable for all of the microsomal samples studied.

The mean (±S.D.) kinetic parameters with the observed ranges derived from the Michaelis-Menten's theoretical analysis by using the two-enzyme kinetic approach (K_{m1}, K_{m2}, V_{max1} and V_{max2}) and the respective ratios or intrinsic clearances (V_{max1}/K_{m1} and V_{max2}/K_{m2}) for H-OPZ and OPZ-SFN and those derived from the one-enzyme kinetic approach of 4′-hydroxylation of *S*-mephenytoin (K_m, V_{max} and V_{max}/K_m) obtained from the 14 different human liver microsomes are summarized in table 1 (the individual kinetic data of the high-affinity component are listed in table 2 in the Appendix).

To assess which of the components of OPZ metabolism would be related to the activity of *S*-mephenytoin 4′-hydroxylase, the correlation analyses were performed between the individual Michaelis-Menten parameters of OPZ metabolism and the corresponding individual values of *S*-mephenytoin 4′-hydroxylation. Among the parameters examined, only the V_{max1} of 5-hydroxylation gave a close correlation ($r_s = 0.911$, $P < .01$) with the corresponding parameter (V_{max}) of *S*-mephenytoin 4′-hydroxylation (fig. 3A). The linear regression analysis indicated

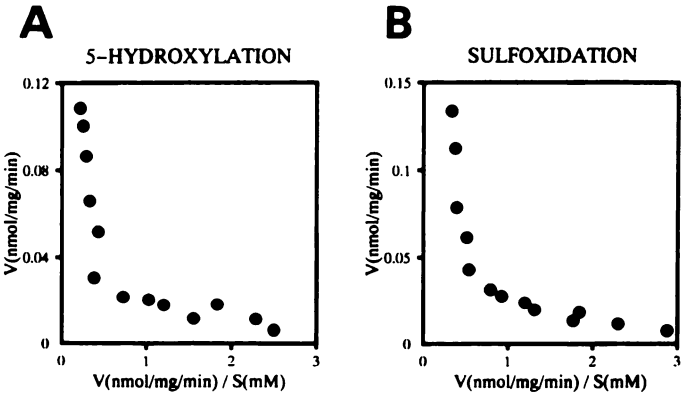

Fig. 2. Representative Eadie-Hofstee plots for 5-hydroxylation (A) and sulfoxidation (B) of OPZ in human liver microsomes. The following parameters were calculated from these data: for 5-hydroxylation, K_{m1} = 2.9 μM; K_{m2} = 528 μM; V_{max1} = 14.3 pmol/mg/min; and V_{max2} = 197 pmol/mg/min; for sulfoxidation, K_{m1} = 5.0 μM; K_{m2} = 1645 μM; V_{max1} = 19.9 pmol/mg/min; and V_{max2} = 582 pmol/mg/min. V, velocity of the metabolite's formation; S, the substrate concentration.

TABLE 1

Michaelis-Menten kinetic parameters of 5-hydroxylation and sulfoxidation of OPZ and of 4′-hydroxylation of S-mephenytoin in 14 different human liver microsomes

Data are expressed as mean ± S.D. K_{m1} and V_{max} refer to Michaelis-Menten parameters for the high-affinity component, whereas K_{m2} and V_{max2} refer to the low-affinity component of the formation of 5-hydroxyomeprazole or OPZ-SFN. In the case of S-mephenytoin 4′-hydroxylation, only K_m and V_{max} (without the numerical subindices) are listed because it follows a simple Michaelis-Menten kinetic behavior. The values in parenthesis indicate the ranges observed.

Metabolite	K_{m1} or K_m	V_{max1} or V_{max}	V_{max1}/K_{m1} or V_{max}/K_m	K_{m2}	V_{max2}	V_{max2}/K_{m2}
	μM	pmol/mg/min	μl/mg/min	μM	pmol/mg/min	μl/mg/min
5-Hydroxyomeprazole	6.0 ± 2.4 (1.0–9.2)	88.0 ± 70.2 (7.9–212)	13.1 ± 8.7 (3.0–30)	106 ± 127 (17.3–528)	116 ± 88 (17.3–323)	2.6 ± 2.2 (0.4–7.5)
OPZ-SFN	10.2 ± 7.2 (2.3–25)	66.9 ± 53.9 (9.9–210)	6.8 ± 3.0 (1.8–13)	482 ± 472 (65.3–1645)	299 ± 131 (119–582)	1.7 ± 1.3 (0.2–4.7)
4′-Hydroxymephenytoin	102 ± 78 (33–348)	68.5 ± 46.1 (15–173)	1.2 ± 1.1 (0.06–4.29)			

OPZ-SFN = omeprazole sulfone.

that the regression line passes through the near-origin ($Y = 1.37 \cdot X - 5.9$, $r = 0.901$, $P < .01$). Because the K_{m1} of 5-hydroxylation did not show a significant correlation (fig. 3B), the V_{max1}/K_{m1} exhibited a rather weaker, but significant ($P < .01$), correlation with the V_{max}/K_m of S-mephenytoin 4′-hydroxylation ($r_s = 0.771$, $P < .01$). Other parameters (*i.e.*, K_{m2} and V_{max2}) of 5-hydroxylation and all of those of sulfoxidation did not show any significant correlation with the kinetic parameters of S-mephenytoin 4′-hydroxylation (data not shown).

Although we did not phenotype any of the patients before their hepatectomy, the three liver microsomes (HL-8, -9 and -11) might have been obtained from the patients with a PM phenotype of S-mephenytoin. This is because they showed a lower V_{max} and higher K_m, and consequently a lower V_{max}/K_m as compared with those of the remaining 11 samples (see "Appendix"). These three samples showed a 9-fold smaller mean V_{max1} of OPZ 5-hydroxylation than that of the remaining 11 samples (12.1 ± 3.8 *vs.* 108.7 ± 65.3 pmol/mg/min), whereas the mean K_m value of the three samples was rather smaller than that of the remainders.

Because the observations described above suggested that the same enzyme (*i.e.*, *CYP2C9*) is involved in the high-affinity component of OPZ 5-hydroxylation *vs.* S-mephenytoin 4′-hydroxylation, we studied the mutual inhibition of OPZ and S-mephenytoin between each other's microsomal metabolism. The results were observed in a *vice versa* fashion (fig. 4, A and B): the addition of OPZ to microsomal incubations resulted in a competitive inhibition of S-mephenytoin 4′-hydroxylation (fig. 4A) with an inhibitory constant (K_i) of 2.0 μM, which was smaller but comparable to the K_m value of the high-affinity component of OPZ 5-hydroxylation activity in this microsomal sample studied (7.2 μM). In addition, S-mephenytoin inhibited competitively the 5-hydroxylation of OPZ with a K_i value of 162 μM (fig. 4B), which was 4 times greater than the K_m value of the 4′-hydroxylation activity of S-mephenytoin in this microsomal sample studied (40 μM). Interestingly, OPZ-SFN also inhibited competitively the 4′-hydroxylation of S-mephenytoin with a K_i value of 8.2 μM (fig. 5).

Shown in figure 6 are the data on the inhibition of the 5-hydroxylation (fig. 6A) and sulfoxidation (fig. 6B) of OPZ in human liver microsomes by an antiserum raised against *CYP2C9* (Komori *et al.*, 1988). No inhibition was observed when the microsomes were incubated with preimmuno serum or when the formation of OPZ-SFN was measured in the presence of this antiserum (fig. 6B). However, the antiserum produced a partial inhibition of the 5-hydroxylation of OPZ (−58% compared with the preimmuno serum) with a concentration at which more than 90% of the microsomal activity for the 4′-hydroxylation of S-mephenytoin was blocked (fig. 6A).

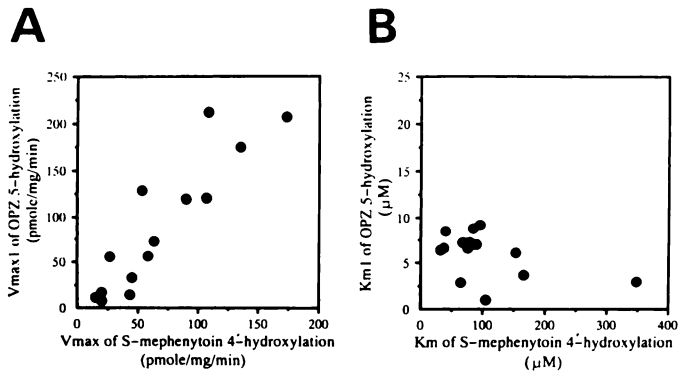

Fig. 3. Relationships between the Michaelis-Menten kinetic parameters for the high-affinity component of 5-hydroxylation of OPZ, V_{max1} (A) and K_{m1} (B), and those (V_{max} and K_m) of 4′-hydroxylation of S-mephenytoin in 14 different human liver microsomes. The correlation coefficient (r_s) between V_{max1} and V_{max} was statistically significant ($r_s = .911$, $P < .01$) and the linear regression line for the relationship between V_{max1} and V_{max} was $Y = 1.37 \cdot X - 5.9$ ($r = 0.901$, $P < .01$), whereas the correlation between K_{m1} and K_m was not ($r_s = 0.280$, $P > .05$).

Discussion

We have suggested recently on the basis of our results from an *in vivo* human panel study that the 5-hydroxylation of OPZ is mediated by the cytochrome P450 isozyme responsible for the polymorphic 4′-hydroxylation of S-mephenytoin (Sohn *et al.*, 1992a). The current data derived from the *in vitro* experiments with human liver microsomes are consistent with this suggestion and provide further evidence to support this contention. The most prominent finding we observed is a close correlation between the V_{max} of high-affinity component (*i.e.*, V_{max1}) of the 5-hydroxylation of OPZ and that (*i.e.*, V_{max}) of S-mephenytoin 4′-hydroxylation (fig. 3A), with the linear regression line of the relationship passing through the near-origin. In addition, the liver microsomes which might have been obtained from the patients with a PM phenotype of S-mephenytoin showed a 9-fold smaller mean V_{max1} of OPZ 5-hydroxylation than that of the others. These observations strongly suggest that *CYP2C9* is a principal enzyme responsible for the high-affinity component of OPZ 5-hydroxylation in human liver microsomes. However, the K_{m1} values of OPZ 5-hydroxylation gave no significant correlation with the K_m values of S-mephenytoin 4′-hydroxylation (fig. 3B). Although the reason(s)

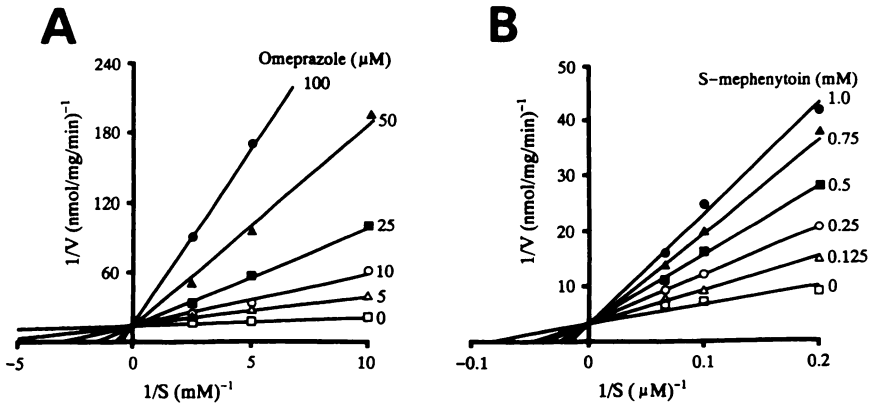

Fig. 4. A, Inhibition of *S*-mephenytoin 4′-hydroxylation by OPZ in human liver microsomes. The following parameters were calculated from this and other data by using this microsomal sample: for OPZ 5-hydroxylation, K_{m1} = 7.2 μM; K_{m2} = 238 μM; V_{max1} = 129 pmol/mg/min; and V_{max2} = 324 pmol/mg/min; for *S*-mephenytoin 4′-hydroxylation, K_m = 68 μM and V_{max} = 53 pmol/mg/min; K_i = 2.0 μM. B, Lineweaver-Burk plots for the inhibition of OPZ 5-hydroxylation by *S*-mephenytoin in human liver microsomes. The following parameters were calculated from this and other data by using this microsomal sample: for OPZ 5-hydroxylation, K_{m1} = 8.5 μM; K_{m2} = 17.3 μM; V_{max1} = 207 pmol/mg/min; and V_{max2} = 128 pmol/mg/min; for *S*-mephenytoin 4′-hydroxylation, K_m = 40 μM and V_{max} = 173 pmol/mg/min; *Ki* = 162 μM. The plotted data are expressed as the averaged values obtained from n = 2. V, velocity of the metabolite's formation; S, the substrate concentration.

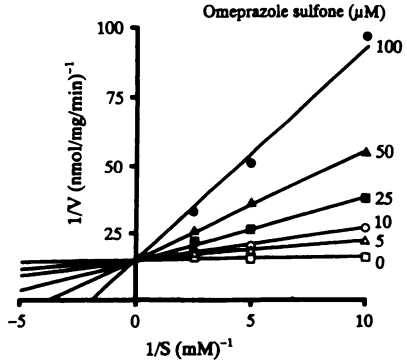

Fig. 5. Lineweaver-Burk plots for the inhibition of *S*-mephenytoin 4′-hydroxylation by OPZ-SFN in human liver microsomes. The kinetic parameters obtained from this and other data by using this microsomal sample are the same as described in the legend of figure 4A except for the K_i value of 8.2 μM. The plotted data are expressed as the averaged values obtained from n = 2. V, velocity of the metabolite's formation; S, the substrate concentration.

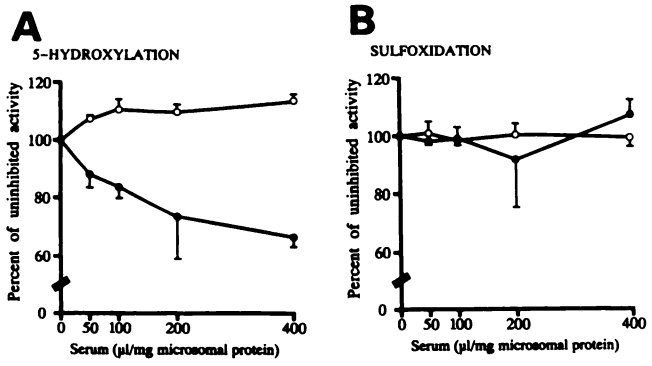

Fig. 6. Effect of an antiserum raised against *CYP2C9* (●) or preimmuno serum (○) on OPZ 5-hydroxylation (A) and sulfoxidation (B) by human liver microsomes. The values are plotted as the mean ± S.D. obtained from n = 4.

for this observation remains unclear, the possibility is not totally negated that a minor, but high-affinity, enzyme other than *CYP2C9* would exist in the human liver microsomes. Nonetheless, it is difficult to separate such a minor enzymatic component from others with the kinetic analysis as used in the present study. This appears to be a limitation of the experiments conducted by using human liver microsomes in which the multiple forms of P450 are present.

Another supportive evidence for the suggestion as mentioned above is a mutual competitive inhibition of OPZ and *S*-mephenytoin between each other's microsomal hydroxylation (fig. 4, A and B). A partial immunoinhibition of OPZ 5-hydroxylation by polyclonal antibodies raised against *CYP2C9* (fig. 6) is considered to be an additional supportive evidence that *CYP2C9* is responsible for the biotransformation of OPZ to H-OPZ. However, it remains unknown why *S*-mephenytoin inhibited the 5-hydroxylation of OPZ with a K_i value greater than the K_m of *S*-mephenytoin 4′-hydroxylation as well as why the extent of inhibition by polyclonal anti-*CYP2C9* for OPZ 5-hydroxylation was less than that for the 4′-hydroxylation of *S*-mephenytoin. Nonetheless, it is assumed to be, at least partly, due to an involvement of multiple (*i.e.*, at least two) enzymes in the 5-hydroxylation pathway of OPZ in human liver microsomes. Otherwise, in the case of the immunoinhibition, the possibility exists that the different affinities to *CYP2C9* (K_{m1} of OPZ = 6.0 ± 2.4 μM and K_m of *S*-mephenytoin = 102 ± 78 μM, table 1) may lead to the differences in the magnitude of the inhibition by the antibodies.

Another interesting observation in the present study was that OPZ-SFN also inhibited competitively *S*-mephenytoin 4′-hydroxylation with its K_i value comparable to the K_i of OPZ (fig. 5). This observation indicates that OPZ-SFN is a substrate for *CYP2C9*. In a previous study (Sohn *et al.*, 1992a), we found that the mean AUC of OPZ-SFN is about 6 times greater in the PMs than that in the EMs of *S*-mephenytoin and the mean half-life value of OPZ-SFN correlates significantly with that of OPZ. Coupled with these findings, the current data strongly suggest that further metabolism of OPZ-SFN to a yet uncharacterized metabolite would be catalyzed by *CYP2C9*. We assume that the same structural position as OPZ (*i.e.*, 5-methyl group of pyridine ring, fig. 1) should be the most likely site of the oxidation of OPZ-SFN in the view of the chemical structure of OPZ-SFN similar to OPZ.

To our knowledge, this is the first report describing the metabolism of OPZ in human liver microsomes. The results suggest that at least two distinct enzymes are involved in each of the 5-hydroxylation and sulfoxidation of OPZ in human liver microsomes (fig. 2). When compared between the high- and low-affinity components, the former is considered to dominate the 5-hydroxylation, because the mean V_{max}/K_m of the high-affinity component was about 5 times greater than that of the low-affinity component (*i.e.*, $V_{max1}/K_{m1} >> V_{max2}/K_{m2}$) (table

1). However, it remains possible that a low-affinity enzyme(s) may also be involved in its *in vivo* metabolism when the activity of *CYP2C9* is decreased or deficient. In fact, we observed in the previous study that the mean urinary excretion of H-OPZ in the PMs reaches approximately 40% of the EMs at 24 hr after a p.o. administration of OPZ (Sohn *et al.*, 1992a), suggesting the possibility that an enzyme(s) other than *CYP2C9* may catalyze the 5-hydroxylation of OPZ in the individuals whose activity of *CYP2C9* is deficient. The low-affinity OPZ 5-hydroxylase which exists in human liver microsomes as indicated in the present study may function as an alternative OPZ-metabolizing enzyme when the activity of *CYP2C9* is decreased or deficient. It should be worthy to note that the difference in the p.o. clearance of OPZ between the EMs and PMs of *S*-mephenytoin decreases with advancing the multiple dosing as a result of a saturable metabolism of OPZ in the EMs (Andersson *et al.*, 1992). In this case, *CYP2C9* would be saturated and the low-affinity enzyme could be responsible, in part, for the *in vivo* metabolism of OPZ in the EMs of *S*-mephenytoin. The findings to support the abovementioned assumptive consideration have been reported for *CYP2D6*, another genetically determined drug-metabolizing isozyme (Brøsen, 1990; Eichelbaum and Gross, 1990), in the metabolism of a tricyclic antidepressant, imipramine (Brøsen, 1990), and a serotonin reuptake inhibitor, paroxetine (Sindrup *et al.*, 1992).

As concerned with the sulfoxidation of OPZ, the high-affinity component appears to dominate the metabolic process in human liver microsomes, because the mean V_{max1}/K_{m1} for the formation of OPZ-SFN was about 5 times greater than that of the low-affinity component (*i.e.*, V_{max2}/K_{m2}) (table 1). In addition, the mean K_m of the low-affinity component (K_{m2}) is approximately 50 times greater than that of the high-affinity component (K_{m1}) (table 1). Therefore, the high-affinity component should be the enzyme(s) primarily responsible for the *in vivo* sulfoxidation of OPZ in human liver microsomes. When the mean V_{max}/K_m values for the high-affinity component was compared between the sulfoxidation and 5-hydroxylation of OPZ, the mean value for the sulfoxidation was less than half of the 5-hydroxylation (table 1), suggesting that the sulfoxidation is a less efficient metabolic process of OPZ compared with the 5-hydroxylation. Although to what extent OPZ is metabolized *in vivo* to OPZ-SFN remains unknown, the present observation is consistent with an *in vivo* finding that 5-hydroxylation is the dominant metabolic pathway of OPZ in humans, accounting for 51% of the metabolites of OPZ excreted in the 0- to 2-hr urine (Renberg *et al.*, 1989).

What enzyme(s) would be involved in the sulfoxidation of OPZ in humans cannot be assessed by the present study. However, *CYP2C9* does not appear to take part in this metabolic process of OPZ, because the kinetic parameters for the formation of OPZ-SFN did not show any significant correlation with those of 4′-hydroxymephenytoin. In addition, polyclonal anti-*CYP2C9* did not inhibit the sulfoxidation of OPZ (fig.6). These findings are consistent with the observation that the mean AUC ratio of OPZ-SFN to OPZ is significantly greater in the PMs than in the EMs of *S*-mephenytoin (Sohn *et al.*, 1992a), implying that the formation of OPZ-SFN from OPZ is apparently not impaired in the PMs. Therefore, other cytochrome P450(s) and/or predictably flavin-containing monooxygenase would be involved in the sulfoxidation of OPZ in humans.

Ultimately, we observed that OPZ and OPZ-SFN are the potent inhibitors of *S*-mephenytoin 4′-hydroxylase, as expected from their respective K_i values of 2.0 and 8.2 μM, which are the lowest ones among the K_i values for *S*-mephenytoin 4′-hydroxylation reported previously (Hall *et al.*, 1987; Inaba *et al.*, 1985; Wilkinson *et al.*, 1989). Therefore, it follows that OPZ should reduce the clearance of drugs of which the metabolism is mediated *via CYP2C9*. In fact, Gugler and Jensen (1985) reported that the mean elimination half-life of diazepam was increased by 130% and the mean total body clearance was reduced by 55% after a 9-day administration of OPZ (40 mg daily). The metabolism of diazepam has been reported to cosegregate with *S*-mephenytoin 4′-hydroxylation polymorphism (Bertilsson *et al.*, 1989; Sohn *et al.*, 1992b). Thus, a caution should be exercised when OPZ is administered with such drugs (*e.g.*, imipramine, amitriptyline or propranolol) that their metabolic disposition has been demonstrated to cosegregate with the 4′-hydroxylation of *S*-mephenytoin (Breyer-Pfaff *et al.*, 1992; Skjelbo *et al.*, 1991; Wilkinson *et al.*, 1989).

In conclusion, the present study on an *in vitro* experiment by using human liver microsomes has provided further evidence that *CYP2C9* is primarily responsible for the formation of H-OPZ and possibly for further metabolism of OPZ-SFN, but not for the sulfoxidation of OPZ in humans. Thus, both OPZ and OPZ-SFN may interact with several therapeutic agents of which the metabolism is mediated *via CYP2C9*. An emphasis should be made that the kinetic analysis of the metabolism of a drug in an *in vitro* experiment by using human liver microsomes as described herein may be a useful approach to understand further and scrutinize an *in vivo* drug disposition in humans.

Appendix

Because pharmacogenetic data are characterized by the interindividual variability in the kinetic parameters of a probe drug which might be overlooked in the mean values, the individual data on Michaelis-Menten kinetic parameters for the high-affinity component (K_{m1} and V_{max1}) of the 5-hydroxylation and sulfoxidation of OPZ and those (K_m *and* V_{max}) for 4′-hydroxylation of *S*-mephenytoin are listed in table 2. In addition, one may assume that some of the 14 liver microsomes obtained from the Japanese patients should be of a PM phenotype of *S*-mephenytoin, because the frequency of PMs of *S*-mephenytoin 4′-hydroxylation is exceedingly greater in a Japanese population (*i.e.*, 22.5%) (Horai *et al.*, 1989) compared with the frequency (*i.e.*, approximately 3 to 6%) of PMs reported from European and North American Caucasian populations (Alván *et al.*, 1990; Brøsen, 1990; Küpfer and Preisig, 1984; Wedlund *et al.*, 1985; Wilkinson *et al.*, 1989). For this reason we wish to discuss on this point as below.

There was a pronounced interindividual variability in the kinetic parameters of the 4′-hydroxylation capacity of *S*-mephenytoin in our liver samples obtained from the 14 Japanese patients who underwent a partial hepatectomy (see table 2). Because about 23% of a Japanese population are the PMs of *S*-mephenytoin (Horai *et al.*, 1989), a similar frequency of the PM phenotype might have been expected to be seen in the liver samples used in the present *in vitro* study. The three (HL-8, -9 and -11) liver microsomes (3/14, 21.4%) showed a relatively lower V_{max} (15, 20 and 20 pmol/mg/min) and higher K_m (166, 105 and 348 μM), and consequently a lower V_{max}/K_m (0.10, 0.20 and 0.06 μl/mg/min, respectively) as compared with those of the remaining 11 samples (table 2). Nevertheless, it remains totally unknown as to whether the former three liver samples would have been obtained from the patients with a PM phenotype of *S*-mephenytoin, because none of the patients had been phenotyped *in vivo* with the racemic mephenytoin before undergoing their hepatectomy.

Finally, the somewhat discrepant results on the formation of 5-

TABLE 2

Individual data on Michaelis-Menten kinetic parameters of 5-hydroxylation and sulfoxidation of OPZ and 4′-hydroxylation of S-mephenytoin in 14 different human liver microsomes

K_{m1} and V_{max1} refer to Michaelis-Menten parameters for the high-affinity component of 5-hydroxyomeprazole or OPZ-SFN. In the case of S-mephenytoin 4′-hydroxylation, only K_m and V_{max} (without the numerical subindices) are listed because it follows a simple one-enzyme Michaelis-Menten kinetic behavior.

Human Liver	Formation of 5-Hydroxyomeprazole			Formation of OPZ-SFN			Formation of 4′-Hydroxymephenytoin		
	K_{m1}	V_{max1}	V_{max1}/K_{m1}	K_{m1}	V_{max1}	V_{max1}/K_{m1}	K_m	V_{max}	V_{max}/K_m
	μM	pmol/mg/min	μl/mg/min	μM	pmol/mg/min	μl/mg/min	μM	pmol/mg/min	μl/mg/min
HL-1	7.2	129	17.9	4.1	53	12.9	68	53	0.79
HL-2	7.0	213	30.3	11.2	125	11.1	90	108	1.20
HL-3	6.6	175	26.4	18.9	126	6.7	76	135	1.77
HL-4	2.9	14	5.0	5.0	20	4.0	65	43	0.65
HL-5	7.2	56	7.8	4.0	17	4.3	69	58	0.85
HL-6	6.6	73	11.0	16.7	102	6.1	37	63	1.72
HL-8	3.7	11	3.0	5.3	34	6.4	166	15	0.10
HL-9	1.0	8	7.9	24.6	43	1.8	105	20	0.20
HL-11	3.0	17	5.6	6.5	34	5.2	348	20	0.06
HL-15	6.4	120	18.8	22.1	210	9.5	33	90	2.70
HL-17	8.5	207	24.5	7.4	75	10.1	40	173	4.29
HL-18	9.2	56	6.0	5.4	34	6.3	96	27	0.28
HL-19	8.8	121	13.7	2.3	10	4.3	85	107	1.25
HL-20	6.1	33	5.5	8.9	55	6.2	153	45	0.29

OPZ-SFN = omeprazole sulfone.

hydroxyomeprazole *vs.* 4′-hydroxymephenytoin (*e.g.*, V_{max1} *vs.* V_{max}, respectively) were observed in the two (HL-4 and -18) liver samples (table 2). The reason(s) for this observation remains totally obscure. When one accepts that the HL-18 might have been of a PM phenotype S-mephenytoin, the PM frequency becomes 28.6% in the Japanese liver microsomes used in the present study.

Acknowledgments

The authors are thankful to Mrs. Tomie Chiba and Mitsuko Echizen for their excellent secretarial assistance.

References

ALVÁN, G., BECHTEL, P., ISELIUS, L. AND GUNDERT-REMY, U.: Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. Eur. J. Clin. Pharmacol. **39:** 533–537, 1990.

ANDERSSON, T., CEDERBERG, C., EDVARDSSON, G., HEGGELUND, A. AND LUNDBORG, P.: Effect of omeprazole treatment on diazepam plasma level in slow *versus* normal rapid metabolizers of omeprazole. Clin. Pharmacol. Ther. **47:** 79–85, 1990a.

ANDERSSON, T., REGÅRDH, C.-G., DAHL-PUUSTINEN, M.-L. AND BERTILSSON, L.: Slow omeprazole metabolizers are also poor S-mephenytoin hydroxylators. Ther. Drug Monit. **12:** 415–416, 1990b.

ANDERSSON, T., REGÅRDH, C.-G., LOU, Y.-C., ZHANG, Y., DAHL, M.-L. AND BERTILSSON, L.: Polymorphic hydroxylation of S-mephenytoin and omeprazole metabolism in Caucasian and Chinese subjects. Pharmacogenetics **2:** 25–31, 1992.

BERTILSSON, L., HENTHORN, T. K., SANZ, E., TYBRING, G., SÄWE, J. AND VILLÉN, T.: Importance of genetic factors in the regulation of diazepam metabolism: Relationship to S-mephenytoin, but not debrisoquin, hydroxylation phenotype. Clin. Pharmacol. Ther. **45:** 348–355, 1989.

BREYER-PFAFF, U., PHANDL, B., NILL, K., NUSSER, E., MONNEY, C., TONZIER-PEREY, M., BAETTIG, D. AND BAUMANN, P.: Enantioselective amitriptyline metabolism in patients phenotyped for two cytochrome P-450 isozymes. Clin. Pharmacol. Ther. **52:** 350–358, 1992.

BRØSEN, K.: Recent developments in hepatic drug oxidation. Implications for clinical pharmacokinetics. Clin. Pharmacokinet. **18:** 220–239, 1990.

CHIBA, K., MANABE, K., KOBAYASHI, K., TAKAYAMA, Y., TANI, N. AND ISHIZAKI, T.: A simple assay of S-mephenytoin 4-hydroxylase activity in human liver microsomes and its preliminary application. Eur. J. Clin. Pharmacol., in press, 1993.

CLISSOLD, S. P. AND CAMPOLI-RICHARDS, D. M.: Omeprazole: A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in peptic ulcer disease and Zollinger-Ellison syndrome. Drugs **32:** 15–47, 1986.

DIXON, M. AND WEBB, E. C.: Enzyme kinetics. *In* Enzymes, ed. by M. Dixon and E. C. Webb, pp. 5–166, Academic Press, Orland, 1964.

EICHELBAUM, M. AND GROSS, A. S.: The genetic polymorphism of debrisoquine/sparteine metabolism—Clinical aspects. Pharmacol. Ther. **46:** 377–394, 1990.

GUGLER, R. AND JENSEN, J. C.: Omeprazole inhibits oxidative drug metabolism—Studies with diazepam and phenytoin *in vivo* and 7-ethoxycoumarin *in vitro*. Gastroenterology **89:** 1235–1241, 1985.

HALL, S. D., GUENGERICH, F. P., BRANCH, R. A. AND WILKINSON, G. R.: Characterization and inhibition of mephenytoin 4′-hydroxylase activity in human liver microsomes. J. Pharmacol. Exp. Ther. **240:** 216–222, 1987.

HOLT, S. AND HOWDEN, C. W.: Omeprazole: Overview and opinion. Dig. Dis. Sci. **36:** 385–393, 1991.

HORAI, Y., NAKANO, M., ISHIZAKI, T., ISHIKAWA, K., ZHOU, H.-H., ZHOU, B.-J., LIAO, C.-L. AND ZHANG, L.-M.: Metoprolol and mephenytoin oxidation polymorphisms in Far Eastern Oriental subjects: Japanese *versus* mainland Chinese. Clin. Pharmacol. Ther. **46:** 198–207, 1989.

HOWDEN, C. W.: Clinical pharmacology of omeprazole. Clin. Pharmacokinet. **20:** 38–49, 1991.

INABA, Y., JURIMA, M., MAHON, W. A. AND KALOW, W.: *In vitro* inhibition studies of two isozymes of human liver cytochrome P-450. Mephenytoin p-hydroxylase and sparteine monooxygenase. Drug Metab. Dispos. **13:** 443–448, 1985.

JURIMA, M., INABA, T. AND KALOW, W.: Mephenytoin metabolism *in vitro* by human liver. Drug Metab. Dispos. **13:** 151–155, 1985.

KNODELL, R. G., DUBEY, R. K., WILKINSON, F. R. AND GUENGERICH, F. P.: Oxidative metabolism of hexobarbital in human liver: Relationship to polymorphic S-mephenytoin 4′-hydroxylation. J. Pharmacol. Exp. Ther. **245:** 845–849, 1988.

KOBAYASHI, K., CHIBA, K., SOHN, D.-R., KATO, Y. AND ISHIZAKI, T.: Simultaneous determination of omeprazole and its metabolites in plasma and urine by reversed-phase high-performance liquid chromatography with an alkaline-resistant polymer-coated C_{18} column. J. Chromatogr. **579:** 299–305, 1992.

KOMORI, M., HASHIZUME, T., OHI, H., MIURA, T., KITADA, M., NAGASHIMA, K. AND KAMATAKI T.: Cytochrome P-450 in human liver microsomes: High-performance liquid chromatographic isolation of three forms and their characterization. J. Biochem. **104:** 912–916, 1988.

KÜPFER, A. AND BRANCH, R. A.: Stereoselective mephobarbital hydroxylation cosegregates with mephenytoin hydroxylation. Clin. Pharmacol. Ther. **38:** 414–418, 1985.

KÜPFER, A. AND PREISIG, R.: Pharmacogenetics of mephenytoin: A new drug hydroxylation polymorphism in man. Eur. J. Clin. Pharmacol. **26:** 753–759, 1984.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. AND RANDALL, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275, 1951.

MATON, P. N.: Omeprazole. N. Engl. J. Med. **324:** 965–975, 1991.

MEIER, U. T., DAYER, P., MALE, P.-J., KRONBACH, T. AND MEYER, U. A.: Mephenytoin hydroxylation polymorphism: Characterization of the enzymatic deficiency in liver microsomes of poor metabolizers phenotyped *in vivo*. Clin. Pharmacol. Ther. **38:** 488–494, 1985.

NELSON, D. R., KAMATAKI, T., WAXMAN, D. J., GUENGERICH, F. P., ESTABROOK, R. W., FEYEREISEN, R., GONZALEZ, F. J., COON, M. J., GUNSALUS, I. C., GOTOH, O., OKUDA, K. AND NEBERT, D. W.: The P450 superfamily: Update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. DNA Cell Biol. **12:** 1–51, 1993.

REGÅRDH, C. G., ANDERSSON, T., LAGERSTROM, P. O., LUNDBORG, P. AND SKANBERG, I.: The pharmacokinetics of omeprazole in humans—A study of single intravenous and oral doses. Ther. Drug Monit. **12:** 163–172, 1990.

RENBERG, L., SIMMONSSON, R. AND HOFFMANN, K.-J.: Identification of two main urinary metabolites of [^{14}C] omeprazole in humans. Drug Metab. Dispos. **17:** 69–76, 1989.

SINDRUP, S. H., BRØSEN, K. AND GRAM, L. F.: Pharmacokinetics of the selective

serotonin reuptake inhibitor paroxetine: Nonlinearity and relation to the sparteine oxidation polymorphism. Clin. Pharmacol. Ther. **51:** 288–295, 1992.

SKJELBO, E., BRØSEN, K., HALLAS, J. AND GRAM, L. F.: The mephenytoin oxidation polymorphism is partially responsible for the N-demethylation of imipramine. Clin. Pharmacol. Ther. **49:** 18–23, 1991.

SOHN, D.-R., KOBAYASHI, K., CHIBA, K., LEE, K.-H., SHIN, S.-G. AND ISHIZAKI, T.: Disposition kinetics and metabolism of omeprazole in extensive and poor metabolizers of *S*-mephenytoin 4′-hydroxylation recruited from an Oriental population. J. Pharmacol. Exp. Ther. **262:** 1195–1202, 1992a.

SOHN, D.-R., KUSAKA, M., ISHIZAKI, T., SHIN, S.-G., JANG, I.-J., SHIN, J.-G. AND CHIBA, K.: Incidence of S-mephenytoin hydroxylation deficiency in a Korean population and the interphenotypic differences in diazepam pharmacokinetics. Clin. Pharmacol. Ther. **52:** 160–169, 1992b.

WARD, S. A., HELSBY, N. A., SKJELBO, E., BRØSEN, K., GRAM, L. F. AND BRECKENRIDGE, A. M.: The activation of the biguanide antimalarial proguanil co-segregates with the mephenytoin oxidation polymorphism—A panel study. Br. J. Clin. Pharmacol. **31:** 689–692, 1991.

WARD, S. A., WALLE, T., WALLE, U. K., WILKINSON, F. R. AND BRANCH, R. A.: Propranolol's metabolism is determined by both mephenytoin and debrisoquin hydroxylase activities. Clin. Pharmacol. Ther. **45:** 72–79, 1989.

WEDLUND, P. J., ASLANIAN, W. S., JACQZ, E., MCALLISTER, C. B., BRANCH, R. A. AND WILKINSON, G. R.: Phenotypic differences in mephenytoin pharmacokinetics in normal subjects. J. Pharmacol. Exp. Ther. **234:** 662–669, 1985.

WILKINSON, G. R., GUENGERICH, F. P. AND BRANCH, R. A.: Genetic polymorphism of S-mephenytoin hydroxylation. Pharmacol. Ther. **43:** 53–76, 1989.

YAMAOKA, K., TANIGAWARA, Y., NAKAGAWA, T. AND UNO, T.: A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobio. Dyn. **4:** 879–885, 1981.

YASUMORI, T., MURAYAMA, N., YAMAZOE, Y. AND KATO, R.: Polymorphism in hydroxylation of mephenytoin and hexobarbital stereoisomers in relation to hepatic P-450 human-2. Clin. Pharmacol. Ther. **47:** 313–322, 1990.

Send reprint requests to: Takashi Ishizaki, M.D., Ph.D., Clinical Research Institute, National Medical Center, Toyama 1-21-2, Shinjuku-ku, Tokyo 162, Japan.