5-Hydroxylation of Omeprazole by Human Liver Microsomal Fractions from Chinese Populations Related to CYP2C19 Gene Dose and Individual Ethnicity

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ABSTRACT
It has been previously reported that omeprazole (OP) oxidation is mediated by CYP2C19 and CYP3A4 in human livers. In this study, we assessed their relative contributions with human liver microsomal fractions from Chinese populations that were genotyped by CYP2C19 and recruited from two ethnic groups, Han and Zhuang. The kinetics of 5-hydroxyomeprazole (5-OH-OP) formation was best described by the two-enzyme and single-enzyme Michaelis-Menten equations for liver microsomes from CYP2C19 extensive (EMs) and poor metabolizers, respectively. At a low substrate concentration that may be encountered in vivo, the monoclonal antibody to CYP2C8/9/19 strongly inhibited 5-OH-OP formation in EM microsomes, whereas troleandomycin (TAO) eliminated most of the formation at a high substrate concentration. In poor metabolizer microsomes, either TAO or anti-CYP3A4 could alone abolish 5-OH-OP formation. Furthermore, there were differences between homozygous and heterozygous EMs in the percentage of inhibition by TAO and the antibodies. At the low substrate concentration, OP 5-hydroxylation correlated well with S-mephenytoin 4'-hydroxylation and CYP2C19 contents in liver microsomes of 34 Chinese individuals. Moreover, in these individuals, obviously genetic and somewhat ethnic differences in OP 5-hydroxylation were observed between different CYP2C19 genotypes (wt/wt > wt/m1 > m1/m1) and between Han and Zhuang (Han > Zhuang), respectively. The results indicate that CYP2C19 is a high-affinity enzyme for OP 5-hydroxylation by liver microsomes from Chinese individuals and that its contribution is CYP2C19 gene dependent and ethnically related. Similar studies indicate that OP sulfoxidation is mediated mainly by CYP3A4 and independent of CYP2C19 genotype status.

CYP2C19-mediated S-mephenytoin 4'-hydroxylation shows a genetically determined polymorphism, with the PM phenotype representing 2 to 5% of Caucasian populations but 13 to 23% of Oriental populations (Wilkinson et al., 1989; Alván et al., 1990; Xie et al., 1996). A number of drugs, including OP, have been studied to determine whether their in vivo metabolism cosegregates with the polymorphism. OP is metabolized mainly to 5-OH-OP and OPS in human livers (Regårdh et al., 1990). In vitro results indicated that CYP3A4 is active in catalyzing the formation of both 5-OH-OP and OPS in human livers (Regårdh et al., 1990). In vivo studies indicated that OP 5-hydroxylation is under a co-regulatory pharmacogenetic control of S-mephenytoin 4'-hydroxylation (Andersson et al., 1990, 1992; Sohn et al., 1992). In the past a few years, OP has even replaced S-mephenytoin as an in vivo phenotypic probe for CYP2C19 in a few large population studies in Caucasians (Balian et al., 1995; Chang et al., 1995), Blacks (Marinac et al., 1996), and Oriental Korean (Roh et al., 1996) and Indian (Lamba et al., 1998).

In vitro results indicated that CYP3A4 is active in catalyzing the formation of both 5-OH-OP and OPS (Andersson et al., 1993; Curi-Pedrosa et al., 1993; Karam et al., 1996). In particular, Yamazaki et al. (1997) recently reported that the relative contributions of CYP2C19 and CYP3A4 to OP 5-hydroxylation depended on the contents of these two P450 forms in the liver. Because the levels of CYP3A4 have been shown to be more than 20-fold higher than those of CYP2C19 in human liver microsomes (Inoue et al., 1997), it was suggested that CYP3A4 was an important enzyme in the 5-hydroxylation, as well as CYP2C19 (Yamazaki et al., 1997). However, because the expression of CYP3A4 in the liver can vary up to 60-fold between individuals (Forrester et al., 1997).
1992), an inaccurate result might thus be predicted for the population study using OP as the phenotypic probe for CYP2C19. Furthermore, CYP2C19 was suggested to be a high-affinity enzyme (low $K_m$) responsible for OP 5-hydroxylation in human liver microsomes, whereas CYP3A4 was a low-affinity enzyme (high $K_m$) (Andersson et al., 1993; Chiba et al., 1993; Karam et al., 1996). When estimated with those kinetic parameters reported previously, the relative contribution of CYP2C19 to OP 5-hydroxylation was suggested to be predominant in vitro at therapeutically relevant substrate concentrations. Therefore, further assessing the relative contributions of major P450 isoforms to OP metabolism in a large group of livers would help in clarifying the role of OP as an in vivo phenotypic probe for the CYP2C19 genetic polymorphism.

It has been previously reported that the metabolism of OP in vivo is genetically determined and ethnically dependent (Caraco et al., 1996a). However, the metabolism of OP has not been characterized in vitro with respect to individual CYP2C19 genotype status and investigated in the liver microsomes from Chinese individuals. In this study, we assessed the relative contributions of CYP2C19 and CYP3A4 to OP metabolism with human liver microsomal fractions obtained from Chinese populations that were genotyped for CYP2C19 and recruited from two ethnic groups, Han and Zhuang.

**Experimental Procedures**

**Materials.** OP, 5-OH-OP, OPS, and H259/36 were generous gifts from Astra Hassle AB (Mölndal, Sweden). S-Mephénytoïn and 4'-hydroxymephytoïn were kindly donated by Dr. G.R. Wilkinson (Vanderbilt University School of Medicine, Nashville, TN). NADP, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, and TAO were purchased from Sigma Chemical Co. (St. Louis, MO). Inhibitory monoclonal antibodies to human CYP2C8/9/19 and CYP3A4, and anti-lysozyme monoclonal antibody (HyHel, IgG) as a control for the immunoinhibition experiments were kindly donated by Drs. T. J. Yang and H. V. Gelboin (Laboratory of Molecular Carcinogenesis and Metabolism, National Institutes of Health, Bethesda, MD). Recombinant CYP2C19 expressed in human lymphoblast and goat antibody anti-rat CYP2C11 were from Daichi Pure Chemicals Co. (Tokyo, Japan). All other supplies are of the highest grades available from commercial sources.

**Human Liver Microsomes.** The collection and use of human liver tissue for this study were approved by the Ethics Committee of Hunan Medical University. Adult human liver tissues were obtained from renal transplant donors and patients undergoing partial hepatectomy. Of the 34 liver donors, 17 belonged to the Han majority group residing in the Hunan Province, and the remaining 17 to the Zhuang minority in the southwestern part of the Autonomous Region of Guangxi, China. The selection of candidates for liver sample collection and the collection procedures were described previously (Shu et al., 1998; Xu et al., 1999). All liver samples were shown to have normal histology before use.

Liver donors were genotyped for CYP2C19 by the method of de Morais et al. (1995). Of the 34 liver donors, 18 were genotyped as homozygous EMs (wt/wt), 13 heterozygous EMs (wt/m1), and 3 PMs (m1/m1). No m2 allele was found.

Microsomes were prepared by differential centrifugation (von Bahr et al., 1980). Microsomal protein concentrations were determined by the method of Lowry et al. (1951) and total P450 contents were measured spectrally by the method of Omura and Sato (1964).

**OP Metabolism In Vitro and HPLC Analysis.** OP metabolism in vitro was carried out in 0.1 mM potassium phosphate buffer (pH 7.4) containing 0.5 mg/ml microsomal protein, 0.5 mM NADP, 5 mM glucose 6-phosphate, 1 U/ml glucose-6-phosphate dehydrogenase, 5 mM MgCl$_2$, 0.1 mM EDTA, and a specified concentration of OP, in a final volume of 0.5 ml. Preliminary experiments showed that the formation rates of 5-OH-OP and OPS were linear at 37°C for incubation times up to 30 min and microsomal protein concentrations up to 1.0 mg/ml, respectively. Accordingly, the incubation time of 20 min and the microsomal protein concentration of 0.5 mg/ml were used for the subsequent work. The metabolic reaction was terminated by cooling the samples in ice bath and by adding 3 ml of extraction solution (dichloromethane).

After the termination of metabolic reaction, H259/36 (24.0 μM in methanol) was added to the incubation mixture as the internal standard for assaying 5-OH-OP and OPS. The mixture was shaken vigorously for 30 s and centrifuged for 10 min (2500g). Then the aliquot of organic layer was transferred to another conical centrifuge tube and evaporated under a gentle stream of nitrogen at 37°C. The residues were reconstituted in the HPLC mobile phase consisting of acetonitrile/methanol/0.01 M pH 8.0 phosphate buffer (43:100:100, v/v/v). The HPLC system included an HP series of 1050 pump, online degasser, variable wavelength detector, and manual injector (Hewlett-Packard Co., Palo Alto, CA). 5-OH-OP and OPS were separated by a 5-μm Kromasil C18 column (4.6 × 250 mm i.d.; Alltech, Dalian, China) and detected at the wavelength of 302 nm. The flow rate of mobile phase was 1.1 ml/min. Retention times of 5-OH-OP, internal standard, OP, and OPS were 5.5, 9.8, 12.2, and 13.5 min, respectively. The limit of detection for both 5-OH-OP and OPS was 0.01 nmol, and the coefficient of variation for intra- and interday reproducibility ranged from 2.9 to 9.5%.

**Kinetic Experiments.** Ten concentrations of OP (1 to 200 μM) were used to characterize the kinetics of OP 5-hydroxylation and sulfoxidation by liver microsomes from Chinese individuals with different CYP2C19 genotypes. Several kinetic models (Schmider et al., 1995) were used to fit the data (Fip perfect, version 5.0; Software Cooperation, Durham, NC). The most appropriate model was determined on the basis of the dispersion of residuals and whether an F test showed a significant reduction (P < .05) in the residual sum of squares. The following two equations best described the kinetics of OP 5-hydroxylation by EM (wt/wt and wt/m1) and PM (m1/m1) microsomes, respectively. Equation 2 was also the best model for the kinetics of OP sulfoxidation:

$$V = V_{max1} \cdot \frac{S}{(K_{m1} + S)} + V_{max2} \cdot \frac{S}{(K_{m2} + S)}$$  \hspace{1cm} (1)

$$V = V_{max} \cdot \frac{S}{(K_m + S)}$$  \hspace{1cm} (2)

**TAO Inhibition Experiments.** TAO was used to inhibit CYP3A4 activity to assess the role of this P450 form in the metabolism of OP. The 50 μM TAO was reported to be selective for CYP3A4 on the basis of $IC_{90}$, $K_m$, and $V_{max}$ values (Pessayre et al., 1983). TAO was prepared in methanol, and the solution was evaporated to dryness before incubation. TAO was preincubated with liver microsomes and the NADPH-generating system for 15 min before the addition of substrate.

**Immunoinhibition Experiments.** The inhibitory monoclonal antibodies specific to CYP2C8/9/19 and CYP3A4 were further used to assess the roles of CYP2C and CYP3A4 in the metabolism of OP, respectively. The antibodies were preincubated with the incubation mixture containing liver microsomes before the addition of substrate. A ratio of antibody/microsomal protein of 1.5 was used to ensure a maximal inhibitory effect on both CYP2C19 and CYP3A4 (Xu et al., 1999). The same amount of anti-lysozyme monoclonal antibody (HyHel, IgG) was added to the control incubations. Due to the limited quantities of the antibodies, single incubations were used.

**Correlation Experiments.** The activities of OP 5-hydroxylation were determined at three substrate concentrations (4, 20, and 100 μM) for the liver microsomes obtained from 34 Chinese individuals. These activities were then correlated with the activities of S-meph-
nystatin 4'-hydroxylation and the protein contents of CYP2C19 in the
liver microsomes. The incubation of S-mephenytoin (250 μM) was
performed as described by Goldstein et al. (1994), and 4'-hydroxyme-
phenytoin formed was measured using HPLC as described by Xie et
al. (1995). The protein contents of CYP2C19 in these livers were
determined by using Western blot analysis as developed by Inoue et
al. (1997), with a minor modification. In our analysis, we used the
anti-rat CYP2C11 antibody prepared from goat instead of rabbit to
probe the human CYP2C19. Immunoblots were scanned with a laser
densitometer (LKB Instruments, Gaithersburg, MD).

**Statistical Analyses.** Duplicate incubations were used through-
out the present study unless indicated. Determination of the most
appropriate model for the kinetic data described under **Kinetic Ex-
periments.** ANOVA, paired or unpaired Student’s t tests were applied
to analyze data, when appropriate. The correlations between S-
mephenytoin 4’-hydroxylation, CYP2C19 content, and OP 5-hy-
droxylatation in different liver microsomal preparations were deter-
dined by least-squares linear regression. A P value of <.05 was
considered statistically significant.

**Results**

**Kinetic Behaviors of 5-OH-OP and OPS Formation
by Liver Microsomes from Chinese Individuals with
Different CYP2C19 Genotypes.** The kinetics of 5-OH-OP
and OPS formation was studied in liver microsomes from six
subjects (2 wt/wt, 2 wt/m1, and 2 m1/m1). Several enzyme
kinetic models were iteratively fitted to the untransformed
data of each subject until the best fitness was achieved
(Schmider et al., 1996).

We found that the kinetics of microsomal 5-OH-OP forma-
tion in the 4 CYP2C19 EMs (wt/wt and wt/m1) followed the
two-enzyme Michaelis-Menten model (eq. 1), whereas the
kinetics in the 2 PMs (m1/m1) was best described by the
single-enzyme Michaelis-Menten model (eq. 2). The Eadie-
Hofstee plots for 5-OH-OP formation showed a difference in
kinetic behavior between the EM microsomes and the PM
microsomes (Fig. 1). The PM microsomes lacked the high-
affinity component of OP 5-hydroxylase that found in the EM
microsomes. Compared with the EMs, the 5-OH-OP forma-
tion by the PM microsomes was significantly slower, espe-
cially at low substrate concentrations. Furthermore, the con-
cavity of Eadie-Hofstee plots for the EM microsomes, which
suggests involvement of at least two enzymes in the reaction,
was more apparent for the homozygous (wt/wt) than for the
heterozygous (wt/m1). The intrinsic clearances (Vmax/Km) of
the high-affinity component for the homozygous were much
higher than those for the heterozygous. In addition, the high-
affinity component of OP 5-hydroxylase showed an approxi-
ately 6-fold higher intrinsic clearance compared with the
low-affinity component.

With regard to OPS formation, the single-enzyme Michaelis-
Menten model could be used to best describe its kinetics in
all the six livers. No difference in kinetic behavior was found
between the homozygous EMs, the heterozygous EMs, and
the PMs. Representative substrate versus velocity and Eadie-Hofstee plots for OPS formation are shown in Fig. 2.
The individual and mean kinetic parameters for OP metab-
olism are listed in Table 1.

**Effect of TAO on OP Metabolism by Liver Micro-
smes from Chinese Individuals with Different
CYP2C19 Genotypes.** Three substrate concentrations (4,
20, and 100 μM) were used to examine the effect of CYP3A4-
selective inhibitor TAO (50 μM) on 5-OH-OP (Fig. 3A) and
OPS formation (Fig. 3B) in the microsomes of 19 individual
livers (8 wt/wt, 8 wt/m1, and 3 m1/m1). At the 4 μM OP, TAO
had a minor inhibitory effect (<30%) on 5-OH-OP formation
in both the homozygous and the heterozygous EM micro-
somes. However, it could be identified that the mean percent-
age inhibition by TAO was lower in the homozygous EM
microsomes than in the heterozygous EM microsomes (12.6
versus 22.9%, P < .05). With the increase of substrate con-
centration, the inhibition was increased to 37.6 and 45.3% at
20 μM OP, and to 56.5 and 68.4% at 100 μM OP in the
homozygous and the heterozygous, respectively. In the PM
microsomes, however, the addition of TAO almost abolished
5-OH-OP formation at all the three substrate concentrations
examined (>90%).

TAO effectively resulted in the reduction of OPS formation in
the 19 livers. The rate of OPS formation was inhibited by
approximately 94.3, 87.2, and 78.0% at 4, 20, and 100 μM OP,
respectively. The degree of inhibition was almost the same
between different CYP2C19 genotypes (Fig. 3B).

**Effects of Anti-CYP2C8/9/19 and Anti-CYP3A4 on OP
Metabolism by Liver Microsomes from Chinese Indi-
viduals with Different CYP2C19 Genotypes.** Effects of
anti-CYP2C8/9/19 and anti-CYP3A4 on microsomal 5-hy-

Fig. 1. Eadie-Hofstee plots for OP
5-hydroxylation in liver microsomes
from homozygous EMs (●), heterozy-
gous EMs (▲), and PMs (■) with re-
spect to CYP2C19. Human liver mi-
croscope samples HL-5 (A), HL-35
(B), HL-39 (C), HL-44 (D), HL-24 (E),
and HL-34 (F) were used. The values
are the mean of duplicate incuba-
tions.
hydroxylation and sulfoxidation of OP were observed with the microsomes from the same 19 livers examined in TAO inhibition experiments, but only at a low substrate concentration of 4 μM OP (Fig. 4). The addition of monoclonal anti-CYP2C8/9/19 strongly inhibited OP 5-hydroxylation in either the homozygous or the heterozygous EM microsomes. Moreover, the inhibition in the homozygous EM microsomes was more apparent than in the heterozygous EM microsomes (86.6 versus 72.9%, P < .05). Similar to the results of TAO inhibition experiments at 4 μM OP, monoclonal anti-CYP3A4 had a minor inhibitory effect on the 5-hydroxylation in the EM microsomes, and the inhibition in the homozygous was less apparent than in the heterozygous (11.7 versus 22.4%, P < .05). Moreover, this antibody eliminated 5-OH-OP formation in both the EM microsomes and the PM microsomes (>90%). However, little inhibition by anti-CYP2C8/9/19 toward OP sulfoxidation was observed (<10%).

Correlations between Microsomal S-Mephenytoin 4′-Hydroxylation, CYP2C19 Contents, and OP 5-Hydroxylation in the Livers of Chinese Individuals. The microsomal activities of OP 5-hydroxylation were measured for 34 livers, at a low (4 μM), a medial (20 μM), and a high substrate concentration (100 μM), respectively. The CYP2C19 activities, which were reflected with the activities of S-mephenytoin 4′-hydroxylation (Goldstein et al., 1994), and CYP2C19 protein contents in these livers were also determined. A good correlation (r = 0.82, P < .001) was found between S-mephenytoin 4′-hydroxylation and OP 5-hydroxylation at the low substrate concentration of 4 μM (Fig. 5A). However, the correlation coefficient between these two metabolic reactions was decreased to r = 0.57 (P < .01) at 20 μM OP (Fig. 5B), and even to a statistically insignificant r = 0.27 at 100 μM OP (Fig. 5C). Similar correlations were found between OP 5-hydroxylation and CYP2C19 contents at different substrate concentrations (r = 0.83, 0.55, and 0.21; the low, medial, and high substrate concentrations, respectively; data not shown).

### Genetic and Ethnic Differences in the Activity of OP 5-Hydroxylation by Liver Microsomes from Chinese Individuals.

The microsomal activities of OP 5-hydroxylation at the low substrate concentration of 4 μM OP were further compared in the 34 livers with respect to their CYP2C19 genotypes and ethnicity (Table 2). The livers had a varying microsomal activity of OP 5-hydroxylation that was from none in a PM liver to 243 pmol/min/nmol of P450 in a homozygous EM liver. The average activity in the homozygous was 1.5-fold as much as in the heterozygous (155 ± 64 versus 102 ± 52 pmol/min/nmol of P450, P < .05). We observed that the three PM livers had a very low turnover number of 5-ΟΗ-ΟΡ compared with most of the EM livers. However, the formation of 5-ΟΗ-ΟΡ was also seriously deficient in one heterozygous EM liver. In addition, of the 34 livers, the 17 Han livers showed a tendency to have a higher OP 5-hydroxylation activity compared with the 17 Zhuang livers. In particular, the activity for the homozygotes of Han was significantly higher than for those of Zhuang (188 ± 63 versus 125 ± 56 pmol/min/nmol of P450, P < .05). The 5-hydroxylation activities at medial (20 μM) and high (100 μM) substrate concentrations were also compared, but no statistically significant genetic and/or ethnic difference was found (data not shown).

### Discussion

This is, to our knowledge, the first study characterizing the in vitro enzyme kinetic behaviors for the formation of 5-ΟΗ-ΟΡ and OPS from OP in human liver microsomes of Chinese individuals with different CYP2C19 genotypes. We observed biphasic enzyme kinetics for 5-ΟΗ-ΟΡ formation in EM microsomes. This result indicates clearly that at least two enzymes are responsible for this reaction that possesses high- and low-affinity components. However, the 5-hydroxylation in PM microsomes lacked the high-affinity component and displayed monophasic enzyme kinetics. Our kinetic data from the genotyped liver microsomes thus are in good agreement with a previous understanding that CYP2C19 accounts for the high-affinity component for OP 5-hydroxylation (Andersson et al., 1993; Chiba et al., 1993; Karam et al., 1996; Lasker et al., 1998). Furthermore, the intrinsic clearance of the high-affinity component for OP 5-hydroxylation is about 7.1 times that of the low-affinity component, suggesting that CYP2C19 is the predominant enzyme in catalyzing
the reaction in vivo. Of the four EM livers used to characterize enzyme kinetics, it was interesting to find that the homozygous showed a more typical Eadie-Hofstee plot for the two-enzyme model compared with the heterozygous. This was mainly due to the higher activities of OP 5-hydroxylation at low substrate concentrations in the homozygous (Fig. 1).

In fact, the kinetics of the heterozygous could also be well described by the single-enzyme Michaelis-Menten model, although the fitness was not as good as that by the two-enzyme model. As discussed below, it has been reported that gene dose has an effect on CYP2C19 activity (homozygous EMs, heterozygous EMs, PMs; de Morais et al., 1995; Xiao et al., 1996). Thus, the genotype-related differences in CYP2C19 activity may generally lead to different apparent enzyme kinetics for a metabolic reaction mediated by CYP2C19 and other enzyme(s) between the livers of different genotypes.

It has been proposed that the affinity of CYP2C19 for OP, and therefore its ability to be inhibited by OP, is lower in Chinese subjects than in Caucasian subjects (Caraco et al., 1999).

**Table 1**

<table>
<thead>
<tr>
<th>Liver Samples</th>
<th>5-OH-OP Formation</th>
<th>OPs Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(K_m)</td>
<td>(V_{max})</td>
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<tr>
<td>Homozygous EMs (genotype)</td>
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<td></td>
</tr>
<tr>
<td>HL-5 (wt/wt)</td>
<td>3.5</td>
<td>198</td>
</tr>
<tr>
<td>HL-35 (wt/wt)</td>
<td>4.2</td>
<td>240</td>
</tr>
<tr>
<td>Heterozygous EMs (genotype)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HL-39 (wt/m1)</td>
<td>3.8</td>
<td>78</td>
</tr>
<tr>
<td>HL-44 (wt/m1)</td>
<td>9.8</td>
<td>276</td>
</tr>
<tr>
<td>PMs (genotype)</td>
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<td></td>
</tr>
<tr>
<td>HL-24 (m1/m1)</td>
<td>59</td>
<td>525</td>
</tr>
<tr>
<td>HL-34 (m1/m1)</td>
<td>129</td>
<td>382</td>
</tr>
<tr>
<td>Mean</td>
<td>5.3</td>
<td>198</td>
</tr>
<tr>
<td>S.D.</td>
<td>3.0</td>
<td>86</td>
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</table>

**Fig. 3.** Effect of CYP3A4-selective inhibitor TAO (50 \(\mu M\)) on OP 5-hydroxylation (A) and sulfoxidation (B) at different substrate concentrations (4, 20, and 100 \(\mu M\)) in different liver microsomes genotyped for CYP2C19. The liver microsomes of 19 Chinese individuals were used, including 8 wt/wt, 8 wt/m1, and 3 m1/m1. The values are the mean inhibition percentage (±S.D.).

**Fig. 4.** Effects of monoclonal antibodies anti-CYP2C8/9/19 and anti-CYP3A4 on OP 5-hydroxylation (A) and sulfoxidation (B) at a low substrate concentration (4 \(\mu M\)) in different liver microsomes genotyped for CYP2C19. Also see the legend to Fig. 3.
individuals (8.6 1996b). However, the $K_m$ values of the high-affinity component for OP 5-hydroxylation in this study (5.3 ± 3.0 μM) are in a range similar to those in the liver microsomes from Japanese (6.0 ± 2.4 μM; Chiba et al., 1993) and Caucasian individuals (8.6 ± 5.6 μM; Andersson et al., 1993). This may indicate a similar affinity of CYP2C19 for OP in these three ethnic groups. The degree of variability and absolute values of other kinetic parameters for OP 5-hydroxylation were also similar to those previously reported (Andersson et al., 1993; Chiba et al., 1993). However, we observed monophasic enzyme kinetics for OPS formation, whereas the other investigators reported biphasic kinetics (Andersson et al., 1993; Chiba et al., 1993). CYP3A4 has been previously identified as the principal enzyme responsible for OP sulfoxidation (Andersson et al., 1993; Karam et al., 1996). In this study, the observations of almost inhibition of sulfoxidation by TAO and anti-CYP3A4 but was not affected by anti-CYP2C8/9/19. Interestingly, the percentage inhibition by anti-CYP2C8/9/19 was found to be larger in the homozygous EM microsomes than in the heterozygous EM microsomes, and vice versa by TAO and anti-CYP3A4, suggesting that the percentage contribution of CYP2C19 to OP 5-hydroxylation, and hence that of CYP3A4, is related to CYP2C19 gene dose. It has been shown that CYP2C19 contents in the homozygous EM microsomes are somewhat higher than those in the heterozygous EM microsomes (Inoue et al., 1997). Moreover, we recently demonstrated an obvious gene dosage effect of CYP2C19 on enzyme protein expression in the livers of 42 Chinese individuals (wt/wt: 9.2 ± 3.5 pmol/mg of protein; wt/ m1: 5.8 ± 3.9 pmol/mg of protein; m1/m1: not determined; Shu et al., 2000). Thus, the present results, to some extent, are consistent with those of Yamazaki et al. (1997) who reported that the contributions of CYP2C19 and CYP3A4 to OP 5-hydroxylation depended on their protein contents in the liver. However, both our kinetic and inhibition results indicate that at therapeutically relevant substrate concentrations, for example at 4 μM, OP 5-hydroxylation is mediated predominantly via CYP2C19 in EM livers, with only a minor contribution of CYP3A4. Furthermore, the good correlations between OP 5-hydroxylation (at 4 μM OP), S-mephenytoin 4′-hydroxylation, and CYP2C19 contents confirm that OP can be used as an in vivo phenotypic probe for CYP2C19 to replace mephenytoin, which

![Fig. 5. Correlation between S-mephenytoin 4′-hydroxylation and OP 5-hydroxylation at different OP concentrations (A, 4 μM; B, 20 μM; C, 100 μM) in the liver microsomes of 34 Chinese individuals.](https://www.aspetjournals.org/jpet/955/1/fig5.png)

**TABLE 2**

Comparison of microsomal OP 5-hydroxylation at a low substrate concentration (4 μM) in liver samples of genotyped Chinese from two ethnic groups, Han and Zhuang

<table>
<thead>
<tr>
<th>CYP2C19 Genotype</th>
<th>Han (n)</th>
<th>Zhuang (n)</th>
<th>Total Samples (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt/wt</td>
<td>188 ± 63* (9)</td>
<td>125 ± 56* (10)</td>
<td>155 ± 64 (19)</td>
</tr>
<tr>
<td>wt/m1</td>
<td>107 ± 54* (6)</td>
<td>97 ± 59 (6)</td>
<td>102 ± 52 (12)</td>
</tr>
<tr>
<td>m1/m1</td>
<td>29 ± 28* (2)</td>
<td>26 (1)</td>
<td>22 ± 20* (3)</td>
</tr>
<tr>
<td>wt/wt + wt/m1 + m1/m1</td>
<td>140 ± 79 (17)</td>
<td>109 ± 56 (17)</td>
<td>124 ± 69 (34)</td>
</tr>
</tbody>
</table>

* Different from Han ethnic group (P < .05).
† Different from wt/wt (P < .05).
‡ Different from wt/wt and wt/m1 (P < .05).

$n$, number of samples examined.
is no longer approved for marketing in most countries due to the occurrence of idiosyncratic reactions.

The fact that gene dose has an effect on drug metabolism has been described previously. Broly et al. (1991) reported that genetic ratio of heterozygous EMs of CYP2D6 is higher than that of homozygous EMs. We also found that gene dose affects the metabolism of S-mephenytoin and diazepam in vivo (de Morais et al., 1995; Xiao et al., 1996; Qin et al., 1999). In the present study, we observed that at the therapeutically relevant substrate concentration of 4 μM, the mean 5-OH-OP formation of 19 homozygous EM livers was significantly higher than that of 12 heterozygous EM livers, showing a gene dose effect on the metabolism of OP. Gene dose effect may result in differential inhibition of the affected drug-metabolizing enzyme(s) by substrates or inhibitors between subjects with different genotypes, as demonstrated in our inhibition studies. In addition, we recently found that the in vivo induction of CYP2C19 by rifampicin is gene dose dependent (Feng et al., 1998). However, the clinical implication of the gene dose effect remains to be further explored. Furuta et al. (1998) recently reported that CYP2C19 genotype status is associated with cure rates for Helicobacter pylori infection and peptic ulcer with OP and amoxicillin, which is in line with the present results and gives an example of the application of genotyping test to clinic. Furthermore, the higher proportion of heterozygous CYP2C19 EMs in Oriental subjects is suggested to be a cause of the differences between Caucasian, Chinese, and Korean subjects in the metabolism of OP and diazepam (Andersson et al., 1992; Bertilsson and Kalow, 1993; Ishizaki et al., 1994; Qin et al., 1999).

However, between a Chinese group and a Caucasian group with a similar proportion of CYP2C19 heterozygotes, OP metabolism was still decreased in the former compared with the latter, suggesting possible environmental effects such as diet and/or other genetic effects on OP metabolism (Caraco et al., 1996a,b). In this study, we unexpectedly found that in the homozygous EM microsomes, there was a significant difference in 5-OH-OP formation at 4 μM OP between Han and Zhuang, which are two distinct ethnic groups in China but that has a similar genetic origin. Accordingly, the CYP2C19 dose effect on OP 5-hydroxylation within Zhuang was less obvious than within Han. These results indicate that the CYP2C19 dose-dependent 5-hydroxylation of OP can be affected by individual ethnicity to a significant extent. In agreement with this, it has been previously reported that ethnic differences in CYP2C19 activity exist between different Chinese populations with similar CYP2C19 allele frequencies (Shu and Zhou, 2000). Further studies are needed to determine the exact reason for such ethnic differences.

In summary, we conclude that the polymorphic CYP2C19 is the high-affinity enzyme responsible for OP 5-hydroxylation by human liver microsomes from Chinese individuals, and that CYP3A4 is a low-affinity enzyme contributing little to this metabolic reaction at therapeutically relevant substrate concentrations in EM microsomes but the principal 5-hydroxylase in PM microsomes. Furthermore, the contribution of CYP2C19 to OP 5-hydroxylation is gene dose dependent and ethnically related, and OP may provide a convenient in vivo phenotypic probe for CYP2C19. The work also confirms that OP sulfoxidation is exclusively mediated via CYP3A4.
genotype and phenotype determined by omeprazole in a Korean population. Phar-
macogenetics 6:547–551.
Schmider J, Greenblatt DJ, Harmatz JS and Shader RI (1996) Enzyme kinetic
modelling as a tool to analyse the behavior of cytochrome P450 catalyzed reactions:
Interindividual variations in the levels and activities of cytochrome P-450s in liver
analysis and inhibition of amitriptyline N-demethylation in human liver micro-
Shu Y and Zhou HH (2000) Individual and ethnic differences in CYP2C19 activity in
kinetics and metabolism of omeprazole in extensive and poor metabolizers of
S-mephenytoin 4'-hydroxylation recruited from an oriental population. J Pharma-
Wilkinson GR, Guengerich FP and Branch RA (1989) Genetic polymorphism of
determination of urinary 4'-hydroxymephenytoin, a metabolic marker for the hepatic
polymorphisms of debrisoquine and S-mephenytoin oxidation metabolism in Chi-
(1999) Evidence for involvement of polymorphic CYP2C19 and 2C9 in the N-
demethylation of sertraline in human liver microsomes. Br J Clin Pharmacol
Yamazaki H, Inoue K, Shaw PM, Checovich WJ, Guengerich FP and Shimada T
(1997) Different contributions of cytochrome P450 2C19 and 3A4 in the oxidation
of omeprazole by human liver microsomes: Effects of contents of these two forms in
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