STEROSELECTIVE METABOLISM OF LANSOPRAZOLE BY HUMAN LIVER CYTOCHROME P450 ENZYMES

KYOUGN-AH KIM, MIN-JUNG KIM, JI-YOUNG PARK, JI-HONG SHON, YOUNG-RAN YOON, SANG-SEOP LEE, KWANG-HYEON LIU, JIN-HO CHUN, MYUNG-HO HYUN, AND JAE-GOOK SHIN

Department of Pharmacology and Pharmacogenomics Research Center (K.-A.K., M.-J.K., J.-Y.P., J.-H.S., Y.-R.Y., S.-S.L., K.-H.L., J.-G.S.), and Department of Preventive Medicine (J.-H.C.), Inje University College of Medicine and Clinical Pharmacology Center, Busan Paik Hospital; and Department of Chemistry, Pusan National University (M.-H.H.), Busan, Korea

(Received February 13, 2003; accepted July 1, 2003)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

The stereoselective metabolism of lansoprazole enantiomers was evaluated by incubation of human liver microsomes and cDNA-expressed cytochrome P450 (P450) enzymes to understand and predict their stereoselective disposition in humans in vivo. The intrinsic clearances (Cl\text{int}) of the formation of both hydroxy and sulfone metabolites from S-lansoprazole were 4.9- and 2.4-fold higher than those from the R-form, respectively. The sums of formation Cl\text{int} of both metabolites were 13.5 and 57.3 μl/min/mg protein for R- and S-lansoprazole, respectively, suggesting that S-lansoprazole would be cleared more rapidly than the R-form. The P450 isomeric selective inhibition study in liver microsomes, and the incubation study of cDNA-expressed enzymes, demonstrated that the stereoselective sulfoxidation is mediated by CYP3A4 and that the hydroxylation is mediated by CYP2C9 and CYP3A4 as well as by CYP2C19. Total Cl\text{int} values of hydroxy and sulfone metabolite formation catalyzed by all these P450 enzymes were consistently higher for S-lansoprazole than for the R-form. The CYP3A4 produced the greatest difference of Cl\text{int} between S- and R-enantiomers, mainly due to a difference of sulfoxidation metabolism (Cl\text{int} 76.5 versus 10.8 μl/min/nmol of P450, respectively), whereas CYP2C19-catalyzed hydroxylation resulted in a minor difference of Cl\text{int} between S- and R-enantiomers (179.6 versus 143.3 μl/min/nmol of P450, respectively). However, the affinity of CYP2C19 on hydroxylation was 5.7-fold higher for S-enantiomer than for the R-form (K_{m} 2.3 versus 13.1 μM), suggesting that the role of CYP2C19 on stereoselective hydroxylation would be more prominent at concentrations around the usual therapeutic level. These findings suggest that both CYP2C19 and CYP3A4 are major enzymes contributing to the stereoselective disposition of lansoprazole, but stereoselective hydroxylation of lansoprazole enantiomers is mainly influenced by CYP2C19, especially at the usual therapeutic doses.

Lansoprazole is a proton pump inhibitor (PPI) that suppresses gastric acid secretion via interaction with H^+/K^+-ATPase in gastric parietal cells (Nagaya et al., 1991). This agent is frequently prescribed for acid-related disorders, such as gastric and duodenal ulcers, Zollinger-Ellison syndrome, and other hypersecretory diseases (Barradell et al., 1992).

All PPIs, including omeprazole, pantoprazole, lansoprazole, and rabeprazole, have a common chiral benzimidazole sulfoxide structure; they have, however, been used as racemic mixtures of the stereoisomers, i.e., S- and R-lansoprazole. All PPIs, except rabeprazole, are extensively metabolized by hepatic P450s, mainly by CYP2C19 and CYP3A4 (Andersson, 1996; Pearce et al., 1996). The disposition of the above PPIs, including lansoprazole, is strongly influenced by CYP2C19 genetic polymorphism (Shon et al., 1997; Ishizaki and Horai, 1999).

Recently, the first enantiomeric drug of the PPI esomeprazole (Nexium) was developed from its racemates. This enantiomer drug has been reported to have a number of advantages over its racemic form (Andersson et al., 2001). First, the sum of intrinsic clearance for the formation of all three major metabolites from S-omeprazole was 3-fold lower than that from R-omeprazole in vitro, suggesting lower clearance and higher plasma concentration of S-omeprazole after administration of the same dose in vivo (Åbelö et al., 2000a). Second, S-omeprazole is less influenced by the CYP2C19 genetic polymorphism because CYP2C19-catalyzed metabolism is minimal, as compared with that of the R-enantiomer (Tybring et al., 1997; Åbelö et al., 2000a), which results in less overall interindividual variability in the disposition, and optimum individual dose, of esomeprazole, as compared with its racemic form.

In our previous study, we found that the plasma concentrations of the R-enantiomer were consistently higher than those of the S-enantiomer in both CYP2C19 extensive metabolizers (EMs) and poor metabolizers (PMs), and that enantiomer-selective lansoprazole dis-
position was influenced less by CYP2C19 genetic polymorphism than by omeprazole, suggesting that CYP2C19-catalyzed metabolism is not the predominant pathway determining the enantioselective disposition of lansoprazole (Kim et al., 2002). The opposite was reported for omeprazole and pantoprazole, since the $S/R$ ratios of AUC, half-life, and maximum plasma concentration ($C_{\text{max}}$) were significantly higher in CYP2C19 PMs than in EMs, suggesting that stereoselective metabolism of these PPIs is highly dependent on CYP2C19 genetic polymorphism (Tybring et al., 1997; Tanaka et al., 2001). These results suggested that CYP2C19-mediated stereoselective metabolism of lansoprazole was different from that of omeprazole and pantoprazole. Ábeló et al. (2000a) have demonstrated the predominant stereoselectivity of CYP2C19-catalyzed omeprazole hydroxylation to the $R$-enantiomer, which causes higher AUC of $S$-omeprazole in CYP2C19 EM subjects, whereas the higher AUC of the $R$-enantiomer was observed in CYP2C19 PM subjects (Tybring et al., 1997). A small number of reports have addressed the stereoselective metabolism of lansoprazole. Based on the results of experiments with rat microsomes, Arimori et al. (1998) proposed that enantioselective disposition of lansoprazole is related to enantioselective metabolism and enantioselective protein binding. Katsuki et al. (2001) reported that CYP3A4 preferentially catalyzed hydroxylation of $S$-lansoprazole, whereas CYP2C19 preferentially catalyzed that of the $R$-enantiomer. However, this was inconsistent with our previous findings in vivo, which indicated that the $R/S$ ratio of oral clearance was higher, and that of AUC was lower, in CYP2C19 PM subjects, as compared with EM subjects (Kim et al., 2002). Katsuki et al. (2001) reported that the $R/S$ ratio of clearance is lower and that of AUC is higher in CYP2C19 PM than in EM subjects.

Although the chemical structures of lansoprazole and omeprazole share a common pyridinyl sulfinyl benzimidazolone backbone with a sulfoxide chiral center, the catalytic sites of both drugs have been reported to be different for CYP2C19-catalyzed hydroxylation: the omeprazole hydroxylation site is on a pyridine group, whereas that of lansoprazole is on a benzimidazole group (Meyer, 1996). These findings raised questions about possible differences in stereoselectivity in the metabolism of both PPIs by human liver microsomes.

The present study, therefore, was performed to elucidate the in vitro metabolism of the lansoprazole enantiomers in detail and compared the results with previous findings concerning omeprazole and its substituted benzimidazole structural analogs (Ábeló et al., 2000a,b) to understand their stereoselective disposition in humans in vivo.

### Materials and Methods

#### Chemicals and Reagents

Lansoprazole, phenacetin, acetaminophen, tolbutamide, dextromethorphan, quinidine, phenytoin, sulfaphenazole, ketocazol, furafylline, chlorzoxazone, $\beta$-NADP, NADPH, EDTA, MgCl$_2$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). $S$-Mephenytoin and $S$-benzylNirvanol were purchased from Ultrafine Chemical Co. (Manchester, UK) and BD Biosciences-Discovery Labware (Bedford, MA), respectively. Acetonitrile and methanol were provided by Fisher Scientific (Pittsburgh, PA). Lansoprazole metabolites were generous gifts from Dr. Ichiro Izeki, Department of Pharmacy, Faculty of Medicine, Tottori University, Yonago, Japan. All other reagents and chemicals were of analytical or HPLC grade. The individual lansoprazole enantiomers, $R$- and $S$-lansoprazole, were separated by chiral semipreparative HPLC on a Chiralpak AS column (10 $\mu$m, 4.6 $\times$ 250 mm; Daicel Chemical Co., Tokyo, Japan), as reported by Katsuki et al. (1996). Enantiomeric purity of each separated enantiomer was >99%.

#### Human Liver Microsomes and Recombinant P450

Microsomes were prepared from human liver tissues (HL-14, HL-20, and HL-21) obtained, with informed consent, from patients undergoing partial hepatectomy for removal of metastatic tumors at the Department of General Surgery, Busan Paik Hospital (Busan, Korea). The samples were of non-tumor-bearing parenchymal tissue and were confirmed to be histopathologically normal. Tissues obtained from patients who had taken any known P450 inhibitors or inducers in the week before the surgical operation were not used. The Institutional Review Board of our hospital approved the use of human liver tissue. Microsomes were prepared by differential centrifugation of liver homogenate, as described previously (Ko et al., 1997). The resulting microsomal pellets were resuspended at a final protein concentration of 10 mg/ml, in 100 mM phosphate buffer (pH 7.4), containing 1.0 mM EDTA and 5.0 mM MgCl$_2$. Microsomes were stored frozen at $-80^\circ$C until use. Microsomal protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard. Aliquots of microsomes were frozen and stored at $-80^\circ$C until use.

Six different human recombinant P450s, CYP1A2, -2C9, -2C19, -2D6, -2E1, and -3A4 (Supersomes), were purchased from BD Gentest (Woburn, MA). The manufacturer supplied information regarding protein concentration and P450 isoform content.

#### Metabolism of Lansoprazole Enantiomers in Human Liver Microsomes or cDNA-Expressed P450 Isoforms

The optimal conditions for microsomal incubation were determined in the linear range for the formation of 5-hydroxy and sulfone metabolites of lansoprazole. In all experiments, racemic lansoprazole and its enantiomers were dissolved and serially diluted with methanol to the required concentrations; the solvent was subsequently removed by evaporation to dryness, under reduced pressure, with an AES2010 SpeedVac (Savant Instruments, Holbrook, NY).

The incubation mixtures containing either 25 $\mu$g of microsomes (10 mg of protein/ml of stock, prepared from three different human liver microsomal preparations), or 25 $\mu$L of cDNA-expressed P450 (diluted to 200 pmol/ml with phosphate buffer, pH 7.4) and various concentrations of racemic lansoprazole or lansoprazole enantiomer (1–200 $\mu$M), reconstituted in 100 $\mu$L phosphate buffer (pH 7.4), were preincubated for 5 min at 37°C. The reaction was initiated by adding the NADPH-regenerating system (including 1.3 mM NADP, 3.3 mM glucose 6-phosphate, 3.3 mM MgCl$_2$, and 1.0 U/ml glucose-6-phosphate dehydrogenase), and the reaction mixtures (final volume of 250 $\mu$L) were incubated for 60 min at 37°C in a shaking water bath. The reaction was terminated by placing the incubation tubes on ice and adding 100 $\mu$L of acetonitrile. After addition of internal standard (omeprazole, 50 $\mu$L), the mixtures were centrifuged at 1,000g for 5 min at 4°C, and aliquots of the supernatant were injected into an HPLC system.

#### Analytical Procedures

The concentrations of 5-hydroxylansoprazole and lansoprazole sulfone were measured by the reverse-phase HPLC method (Pichard et al., 1995). The system consisted of a Gilson 307 pump, 118 UV detector, and 234 autoinjector (Gilson, Villiers Le Bel, France). The samples were separated on a $\mu$-Bondapak C$_{18}$ column (3.9 mm i.d. $\times$ 30 cm, 10-$\mu$m particle; Waters, Milford, MA) at ambient temperature with a mobile phase consisting of 0.1 M acetate buffer (pH 4.0)/acetonitrile/methanol (64:31:5, v/v) at a flow rate of 0.9 ml/min. The chromatograms were obtained with UV detection set at a wavelength of 285 nm. The Unipoint Analysis System (Gilson) was used to calculate the concentrations of lansoprazole metabolites from the peak area ratios. None of the P450 isoform-selective inhibitors tested in this study interfered with the peaks of lansoprazole metabolites in the inhibition study on lansoprazole metabolism.

#### Inhibition of Lansoprazole Metabolite Formation

The inhibitory effects of known P450 isoform-selective inhibitors on the formation of 5-hydroxy and sulfone metabolites of lansoprazole were evaluated to determine the P450 isoform(s) involved in each metabolic pathway. The formation rates of 5-hydroxylansoprazole and lansoprazole sulfone from lansoprazole racemate or lansoprazole enantiomers were determined from the reaction mixtures incubated in the presence or absence of known P450 isoform-selective inhibitors (Clarke, 1998; Suzuki et al., 2002). The P450 isoform-selective inhibitors used were furafylline (10 $\mu$L) for CYP1A2, sulfaphenazole for CYP2C9 (10 $\mu$L), $S$-benzylNirvanol (1 $\mu$L) and $S$-mephenytoin (100 $\mu$L) for CYP2C19, quinidine for CYP2D6 (10 $\mu$L), and ketoconazole for CYP3A4 (1 $\mu$L). Except for the addition of P450 isoform-selective inhibitors, all other incubation conditions were as used routinely in our laboratory and were similar to those described previously (Shin et al., 1999, 2002).

#### Data Analysis

Results are expressed as means ± S.D. of estimates obtained from three different liver microsome preparations in duplicate experiments. The apparent kinetic parameters for the formation of lansoprazole metabolites ($K_{m}$ and $V_{\text{max}}$) were determined by fitting the Michaelis-Menten...
equation to unweighted data on the formation rate of metabolites and substrate concentrations of racemic lansoprazole or its enantiomers by nonlinear least-squares regression, using the program WinNonLin (Pharsight Corporation, Mountain View, CA). In microsomal incubation studies, the formation rates of S-hydroxylansoprazole from racemic lansoprazole and its enantiomers were best fitted to the Michaelis-Menten kinetics with a one-enzyme kinetic model: $V = V_{\text{max}} \cdot [S]/K_m + [S]$. A sigmoid $V_{\text{max}}$ model, i.e., $V = V_{\text{max}} \cdot [S]/[S]+K_m + \gamma$, showed the best fit to the data on the formation rate of lansoprazole sulfone, where $V$ is the velocity of the reaction at substrate concentration $S$, $V_{\text{max}}$ is the maximum velocity of each enzyme, and $K_m$ is the substrate concentration at which the reaction velocity is 50% of $V_{\text{max}}$. In the sigmoid $V_{\text{max}}$ model, the slope factor $\gamma$ indicates the parameter determined by the sigmoidicity of the curve. Intrinsic clearance of the in vitro incubation was calculated as $Cl_{\text{int}} = V_{\text{max}}/K_m$. In the incubation study of cDNA-expressed P450 isoforms, a simple Michaelis-Menten model was fitted to the unweighted data on the formation rate of both metabolites to estimate the enzyme kinetic parameters.

**Prediction of Cl int in Human Liver Microsomes Using Data from cDNA-Expressed P450 Isoforms.** The predicted $Cl_{\text{int}}$ values in human liver microsomes were calculated using enzyme kinetic parameters estimated from the results of incubation studies of cDNA-expressed enzymes. As described in the previous report of Abelo et al. (2000a), we adapted the information on the average content of each P450 isoform in a microsomal preparation from the reports of Shimada et al. (1994) and Yamazaki et al. (1997). Briefly, the percentages of each P450 in human liver microsomes were estimated as 2% for CYP2C19, 18% for CYP2C9, and 28% for CYP3A4. Since the total content in a typical preparation is 0.46 nmol/mg of protein, the protein contents of each P450 isoform were estimated to be 0.0092, 0.083, and 0.129 nmol/mg of protein for CYP2C19, CYP2C9, and CYP3A4, respectively. These values were multiplied by $Cl_{\text{int}}$ estimated from each cDNA-expressed P450 isoform.

**Results**

The formation of both hydroxy and sulfone metabolites from lansoprazole was clearly stereoselective on incubation of human liver microsomal preparations (Fig. 1). The formation rates of both metabolites from $S$-lansoprazole were significantly higher than those from $R$- and racemic lansoprazole. Although Eadie-Hofstee plots for the formation of hydroxylansoprazole appeared to be nonlinear (data not shown), suggesting the involvement of more than one enzyme, fitting of the data to the Michaelis-Menten expression of a two-enzyme model did not significantly improve the regression, as compared with fitting of the data to the one-enzyme model. Therefore, enzyme kinetic parameters for hydroxylation metabolism were estimated according to the one-enzyme model. Sulfone metabolite formation was best explained by the sigmoid $V_{\text{max}}$ model, and the average sigmoidicity factor $\gamma$ was less than 1.6 (Table 1). The estimated $V_{\text{max}}$ values of $S$-lansoprazole for the formation of both hydroxy and sulfone metabolites were higher, and $K_m$ values were lower, than those of the $R$-form, which resulted in 4.9- and 2.4-fold higher formation $Cl_{\text{int}}$ values of both metabolites from the $S$-form, as compared with those from the $R$-form. The sums of the formation $Cl_{\text{int}}$ of both metabolites were 15.3 and 57.3 $\mu l/min/mg$ protein for $R$- and $S$-lansoprazole, respectively, indicating stereoselectivity of metabolism.

The $Cl_{\text{int}}$ of $R$-lansoprazole was almost the same for the formation of hydroxy and sulfone metabolites (8.1 $\pm$ 3.6 versus 7.2 $\pm$ 3.1 $\mu l/min/mg$ protein), whereas the $Cl_{\text{int}}$ of the $S$-form indicated that hydroxy metabolite formation contributes to 70% of $S$-lansoprazole metabolism and sulfone metabolism to 30% (40.3 $\pm$ 3.3 versus 17.0 $\pm$ 8.4 $\mu l/min/mg$ protein, respectively). In the case of omeprazole, hydroxy metabolite formation has been reported to account for 94% of $R$-omeprazole metabolism, whereas $Cl_{\text{int}}$ values for the formation of sulfone, 5-O-desmethyl, and hydroxy metabolites from the $S$-form were 27%, 46%, and 27% of the total $Cl_{\text{int}}$ of $S$-omeprazole in human liver microsomes, respectively (Abelo et al., 2000a).

A P450 isoform-selective inhibition study was performed to evaluate which P450 isoforms are involved in the enantiocselective metabolism of lansoprazole in human liver microsomes. Among the five inhibitors tested, sulfone metabolite formation was inhibited only by the CYP3A4-selective inhibitor ketoconazole (1 $\mu M$) (Fig. 2). After treatment with 1 $\mu M$ ketoconazole, the level of lansoprazole sulfone formation from the $S$-form (5 $\mu M$) markedly decreased to 4% of that in controls, as compared with 12% from the $R$-form, indicating the stereoselective inhibitory effect of ketoconazole on the formation of lansoprazole sulfone. Hydroxy metabolite formation, however, was inhibited by sulfaphenazoide and ketoconazole in addition to $S$-benzylirvanol and $S$-mephenytoin, suggesting that the stereoselective hydroxylation of 5 $\mu M$ lansoprazole is mediated by more than one enzyme.

Hydroxy and sulfone metabolite formation from lansoprazole enantiomers were also studied using the human cDNA-expressed P450 isoforms CYP1A2, CYP3A4, CYP2C9, CYP2C19, and CYP2D6. The sulfone metabolite formation rate from the $S$-enantiomer was significantly greater than that from the $R$-form at 1, 10, and 100 $\mu M$ lansoprazole (Fig. 3). Interestingly, at a substrate concentration of 1 $\mu M$, the hydroxy metabolite formation rate from $S$-lansoprazole was significantly higher than that from the $R$-form on incubation with cDNA-expressed CYP2C19 (Fig. 3A), whereas at higher concentrations (10 and 100 $\mu M$) of lansoprazole enantiomers, the CYP2C19-catalyzed formation rate of hydroxy metabolite from the $R$-form was significantly greater than that from the $S$-enantiomer, whereas the formation rate of sulfone metabolite from the $R$-enantiomer was significantly lower than that from the $S$-enantiomer at all substrate concentrations (10 and 100 $\mu M$) of lansoprazole enantiomers, the CYP2C19-catalyzed formation rate of hydroxy metabolite from the $R$-form was significantly greater than that from the $S$-enantiomer.

**Table 1**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>$R$-Enantiomer</th>
<th>Racemate</th>
<th>$S$-Enantiomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxy</td>
<td>$V_{\text{max}}$</td>
<td>263.3 ± 99.9</td>
<td>318.8 ± 84.8</td>
</tr>
<tr>
<td>$K_m$</td>
<td>37.3 ± 19.2</td>
<td>16.8 ± 7.9</td>
<td>11.1 ± 1.9</td>
</tr>
<tr>
<td>$Cl_{\text{int}}$</td>
<td>8.1 ± 3.6</td>
<td>21.0 ± 7.3</td>
<td>40.3 ± 3.3</td>
</tr>
<tr>
<td>Sulfone</td>
<td>$V_{\text{max}}$</td>
<td>931.5 ± 392.8</td>
<td>1387.5 ± 484.6</td>
</tr>
<tr>
<td>$K_m$</td>
<td>137.1 ± 40.2</td>
<td>136.2 ± 3.9</td>
<td>108.5 ± 40.6</td>
</tr>
<tr>
<td>$Cl_{\text{int}}$</td>
<td>7.2 ± 3.1</td>
<td>10.3 ± 3.8</td>
<td>17.0 ± 8.4</td>
</tr>
<tr>
<td>Sum $Cl_{\text{int}}$</td>
<td>15.3</td>
<td>31.3</td>
<td>57.3</td>
</tr>
</tbody>
</table>

$^a$ $V_{\text{max}}$ is expressed as pmol/min/mg of microsomal protein, $K_m$ as $\mu M$, and $Cl_{\text{int}}$ as $V_{\text{max}}/K_m$ ($\mu l/min/mg$ of microsomal protein).
significantly greater than that from the S-form (Fig. 3, B and C). The cDNA-expressed CYP2C9 also catalyzed lansoprazole hydroxylation with a higher formation rate from the S-enantiomer than from the R-form at the lowest concentration used (1 μM), and CYP3A4 was shown to be involved in hydroxy metabolite formation at higher concentrations of lansoprazole enantiomers (10 μM), albeit to a much lesser extent, as compared with CYP2C19. The levels of involvement of other recombinant P450 isoforms in the formation of lansoprazole metabolites were negligible.

Next, we examined the enzyme kinetic parameters for the formation of 5-hydroxylansoprazole and lansoprazole sulfone from lansoprazole enantiomers and racemates (0–100 μM) on incubation with cDNA-expressed human CYP2C9, -2C19, and -3A4. The hydroxy metabolite was formed from both lansoprazole enantiomers by all three P450 isoforms, whereas sulfone formation was catalyzed only by CYP3A4 (Table 2). The total \( C_{\text{int}} \) values of all three P450 isoforms were consistently higher for S-lansoprazole than for the R-form. The CYP2C19-catalyzed hydroxylation of S-lansoprazole showed lower \( V_{\text{max}} \) and \( K_{\text{m}} \) than those of the R-enantiomer, resulting in a slightly higher formation \( C_{\text{int}} \) from the S-form than from the R-form (179.6 versus 143.3 μl/min/pmol of CYP2C19). CYP2C19-catalyzed hydroxylation of S-lansoprazole showed the lowest \( K_{\text{m}} \) (2.3 ± 0.2 μM), and R-lansoprazole hydroxylation by CYP2C19 showed the next lowest \( K_{\text{m}} \) (13.1 ± 2.5 μM), indicating that CYP2C19 is the high-affinity enzyme catalyzing the hydroxylation of lansoprazole, especially of S-lansoprazole. Although the formation \( C_{\text{int}} \) values of hydroxylansoprazole by cDNA-expressed CYP2C9 and CYP3A4 were small compared with those by CYP2C19, the total \( C_{\text{int}} \) value by CYP2C9 was 7.5-fold higher for S-lansoprazole than for the R-form (9.0 versus 1.2 μl/min/pmol of CYP2C9) due to its lower \( K_{\text{m}} \) and higher \( V_{\text{max}} \) values, whereas total \( C_{\text{int}} \) of hydroxylation by CYP3A4 was similar for both S- and R-enantiomers (2.7 versus 3.6 μl/min/pmol of CYP3A4) due to the similar \( V_{\text{max}} \) and \( K_{\text{m}} \) values for both lansoprazole enantiomers. The \( C_{\text{int}} \) value for sulfone formation by CYP3A4 was 7.1-fold higher for S-lansoprazole than for the R-form.
The present in vitro incubation studies using human liver microsomes and cDNA-expressed P450s clearly demonstrate the stereoselective metabolism of lansoprazole enantiomers in both hydroxylation and sulfone formation pathways. The Cl_{int} values of the S-enantiomer were 4.9-fold higher for hydroxy metabolite formation and 2.4-fold higher for sulfone metabolite formation than those of the R-form in the microsomal incubation study, resulting in a 3.8-fold higher total Cl_{int} of S-lansoprazole, as compared with the R-form (57.3 versus 15.3 μl/min/mg of protein). This stereoselective metabolism supports the in vivo disposition of lansoprazole enantiomers in humans. It has been reported that the clearance of S(-)-lansoprazole was significantly higher and the plasma concentrations and AUC of S(-)-lansoprazole were lower than those of the R(+)-enantiomer, after administration of racemic lansoprazole (Katsuki et al., 1996; Kim et al., 2002).

The differences in stereoselective disposition between lansoprazole and omeprazole seem to be largely due to their characteristic stereoselective metabolism. In the present study, the stereoselective metabolism of lansoprazole was mediated by three P450 isoforms, CYP3A4, CYP2C19, and CYP2C9, and this was confirmed by both studies of P450 isoform-selective inhibition and incubation of cDNA-expressed human P450 isoforms. Hydroxy metabolite was formed by all of these cDNA-expressed human P450 enzymes, but mainly by CYP2C19. In contrast, sulfone formation was catalyzed only by CYP3A4. The total formation Cl_{int} values of all three of these P450 isoforms were consistently higher for S-lansoprazole than for the R-form, but the difference in Cl_{int} for CYP2C19-catalyzed hydroxylation between S- and R-enantiomers was not significant (179.6 ± 13.5 versus 143.3 ± 15.7 μl/min/pmol of P450), in contrast to the difference in the total Cl_{int} for CYP3A4-catalyzed sulfoxidation and hydroxylation (79.2 versus 14.4 μl/min/pmol of P450). Moreover, the Cl_{int} values of lansoprazole enantiomers in human liver microsomes predicted from enzyme kinetic parameters for cDNA-expressed enzymes, estimated by correction of P450 content in liver microsomes, suggested that enantioselective metabolism of lansoprazole is largely determined by CYP3A4 rather than by CYP2C19 (Table 3). The Cl_{int} of CYP3A4 for S-lansoprazole (10.2 μl/min/mg) was extremely high compared with that of CYP2C19-catalyzed hydroxylation of S- and R-forms (1.65 and 1.32 μl/min/mg), and compared with Cl_{int} of CYP3A4 for the R-enantiomer (1.85 μl/min/mg). These observations support the findings of our previous in vivo study, in which the higher clearance of the S-enantiomer in CYP2C19 EM subjects did not show a switch in stereoselectivity to favor metabolism resulting in higher clearance of the R-form in CYP2C19 PM subjects (Kim et al., 2002). In the case of omeprazole and pantoprazole, however, it has been reported that higher clearance of the R-form in CYP2C19 EM subjects was switched to higher clearance and thus lower plasma concentrations of S-form in PM subjects, suggesting that stereoselective dispositions of these PPs are highly influenced by CYP2C19 genetic polymorphism (Tybring et al., 1997; Tanaka et al., 2001). These in vivo observations regarding omeprazole have been explained by its charac-

### Table 2

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>CYP2C9 (μl/min/mg)</th>
<th>CYP2C19 (μl/min/mg)</th>
<th>CYP3A4 (μl/min/mg)</th>
<th>Total Cl_{int} (μl/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-Form</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxy</td>
<td>0.1</td>
<td>1.32</td>
<td>0.46</td>
<td>1.88</td>
</tr>
<tr>
<td>Sulfone</td>
<td></td>
<td></td>
<td>1.39</td>
<td>1.39</td>
</tr>
<tr>
<td>Total Cl_{int}</td>
<td>0.1 (3.1)²</td>
<td>1.32 (40.4)</td>
<td>1.85 (56.6)</td>
<td>3.27 (100)</td>
</tr>
<tr>
<td>S-Form</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxy</td>
<td>0.75</td>
<td>1.65</td>
<td>0.35</td>
<td>2.75</td>
</tr>
<tr>
<td>Sulfone</td>
<td></td>
<td></td>
<td>9.86</td>
<td>9.86</td>
</tr>
<tr>
<td>Total Cl_{int}</td>
<td>0.75 (6.0)</td>
<td>1.65 (13.1)</td>
<td>10.21 (81.0)</td>
<td>12.61 (100)</td>
</tr>
</tbody>
</table>

² – indicates that no metabolite was detected.

### Table 3

**Prediction of Cl_{int} in human liver microsomes from enzyme kinetic parameters of lansoprazole enantiomers from cDNA-expressed P450 enzymes**

The predicted Cl_{int} was estimated from the nmol/mg of protein content of each P450 enzyme and the enzyme kinetic parameters shown in Table 2, obtained by incubation of expressed P450, as described under Materials and Methods.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>CYP2C9 (μl/min/mg)</th>
<th>CYP2C19 (μl/min/mg)</th>
<th>CYP3A4 (μl/min/mg)</th>
<th>Sum Cl_{int} (μl/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-Form</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Downloaded from dmd.aspetjournals.org at ASPEJournals on June 25, 2017
teristic metabolism in which the hydroxylation pathway mediated mainly by CYP2C19 is dominant for the R-form (94% in the microsomal incubation study); however, this metabolic pathway is not the only dominant elimination route of the S-form (Abelo et al., 2000a). For S-omeprazole, the formation Cl_{int} values of three major omeprazole metabolites, i.e., sulfone, O-desmethyl, and hydroxy omeprazole, were equally important for its metabolic elimination in the microsomal incubation study, and the total Cl_{int} of CYP2C19 was predicted to be comprised of 40% of total Cl_{int} of S-omeprazole (cf. 57% by CYP3A4) based on the results of the incubation study of cDNA-expressed P450s. In contrast, for R-omeprazole, the total Cl_{int} of CYP2C19 was predicted to account for 87% of the total Cl_{int}.

Although three P450 isoforms, CYP2C19, CYP2C9, and CYP3A4, were involved in the stereoselective hydroxylation of lansoprazole, the \( K_m \) values estimated by incubation with cDNA-expressed enzymes indicated that the S-form had a considerably higher affinity to CYP2C19 (mean \( K_m = 2.3 \mu M \), compared with the affinity of the R-form to CYP2C19 and to that of both enantiomers to CYP2C9 and CYP3A4 (mean \( K_m = 13.1-66.4 \mu M \)). Based on the \( K_m \) and \( V_{max} \) values calculated from the expressed enzymes, hydroxylation of the R-form by CYP2C19 appears to be a lower-affinity, but higher-capacity, metabolic pathway (5.7- and 4.6-fold higher \( K_m \) and \( V_{max} \), respectively), compared with hydroxylation of the S-form by CYP2C19. These kinetic characteristics resulted in the observed concentration-dependent formation of lansoprazole enantiomers on incubation with the expressed CYP2C19 enzyme (Fig. 3). The formation rate of hydroxy metabolite from S-form was significantly higher than that from R-form at 1 \( \mu M \) lansoprazole; i.e., the formation rate of hydroxy metabolite from R-lansoprazole was significantly higher than that from S-lansoprazole. This finding explains the discrepancy between our findings and those of Katsuki et al. (2001), who reported that hydroxylation by cDNA-expressed CYP2C19 from R(-)-lansoprazole was greater than that from S(-)-lansoprazole. Our observations suggest that this is not always the case over all substrate concentration ranges. Our results

![Figure 4. Abundance-adjusted simulations of the relative contributions of CYP2C9 (△), CYP2C19 (●), and CYP3A4 (■) to lansoprazole S-hydroxylation in relation to R- and S-lansoprazole concentrations.](image)

Table 4: Comparison of regioselective metabolism of lansoprazole, omeprazole, and its analog H259/31

<table>
<thead>
<tr>
<th></th>
<th>CYP3A4</th>
<th>CYP2C19</th>
</tr>
</thead>
<tbody>
<tr>
<td>H259/31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lansoprazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omeprazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( Cl_{int} )</td>
<td>S&gt;R</td>
<td>S&gt;R</td>
</tr>
<tr>
<td>(present study)</td>
<td>( 76.8 \pm 10.8 )</td>
<td>( 179.8 \pm 143.3 )</td>
</tr>
<tr>
<td>( Cl_{int} )</td>
<td>S&lt;R</td>
<td>S&gt;R</td>
</tr>
<tr>
<td>(Abelo et al., 2000a)</td>
<td>( 160 \pm 15.7 )</td>
<td>( 1479 \pm 133 )</td>
</tr>
</tbody>
</table>

\( Cl_{int} \) values estimated from the incubation study of cDNA-expressed CYP3A4 and CYP2C19 were compared between PPI enantiomers. Each arrow indicates the favored hydroxylation site of PPI, which is mediated by CYP2C19.
suggest that lansoprazole shows concentration-dependent stereoselectivity in CYP2C19-catalyzed hydroxylation, resulting in a higher formation rate of the S-form at lower concentrations around the \( K_m \) value (2.3 \( \mu M \)), but a lower rate of S-form formation at higher concentrations (>10 \( \mu M \)).

To determine the relative contributions of three P450 isoforms to hydroxylation metabolism at different concentrations of both enantiomers in human liver microsomes, we estimated the percentage of the net reaction rate from the abundance-adjusted simulation of each P450 isoform in human liver microsomes (Fig. 4). For this, we normalized the metabolic rates of the expressed P450 isoforms according to the abundance of the respective isoforms in the human liver, as described by Rodrigues (1999). Briefly, reaction rates measured with individual cDNA-expressed P450 isoforms were normalized with respect to the nominal specific content of the corresponding P450 in native human liver microsomes. In this study, we adapted the data of immunologically determined P450 isoform liver contents reported by Shimada et al. (1994) and Yamazaki et al. (1997); i.e., 2% for CYP2C19, 18% for CYP2C9, and 28% for CYP3A4. In turn, the normalized rates for each cDNA-expressed P450 were summed, yielding a “total normalized rate” (TNR = \( S_f \cdot V_i \)), and the normalized rate for each P450 isoform (\( = f_i \cdot V_i \)) was expressed as a percentage of the net reaction rate (\( = 100 \times f_i \cdot V_i / V_f \), where \( f_i \) indicates the fraction of each P450 isoform content in the human liver, and \( V_i = V_{\text{max},i} \cdot [S_i] / K_{\text{m},i} + [S_i] \)). This simulation shows that CYP2C19 is the major P450 isoform responsible for hydroxy metabolite formation from R-lansoprazole over the whole concentration range tested (0–100 \( \mu M \)). For S-lansoprazole, the percentage of the net reaction by CYP2C19 (around 60%) decreased rapidly with increasing substrate concentration and reached 20% at 100 \( \mu M \). Therefore, the percentage of net reaction accounted for by CYP2C19 was higher than that by CYP2C9 and CYP3A4 only at low concentrations of S-lansoprazole (less than about 6 \( \mu M \)).

Even CYP2C19 was expected to contribute to hydroxylation of S-lansoprazole at concentrations higher than 20 \( \mu M \) (Fig. 4). These results, however, suggest that CYP2C19 is the major enzyme involved in the hydroxylation of both lansoprazole enantiomers at concentrations in the usual therapeutic range. The maximum plasma concentration of lansoprazole has been shown to be less than 2 \( \mu g/ml \) (i.e., 5.6 \( \mu M \)) after administration of the usual therapeutic doses (Barclay et al., 1999; Kim et al., 2002).

Abelö et al. (2000a) proposed the regio- and stereoselective metabolism of omeprazole and other structural analogs. The present study also demonstrated the regioselectivity of stereoselective hydroxylation of lansoprazole by CYP2C19 based on comparison of intrinsic clearances of each enantiomer for lansoprazole with those for omeprazole (Abelö et al., 2000a) and H2S9/31, a structural analog of omeprazole (Abelö et al., 2000b). For the hydroxylation of H2S9/31 and O-desmethylation of omeprazole, the benzimidazole group is the favored target of drug metabolism by CYP2C19. The intrinsic clearances of these metabolic pathways were significantly greater for the S-isoform than for the R-form, respectively (Table 4). Similarly, lansoprazole hydroxylation also occurs at the benzimidazole group, and the intrinsic clearance of the S-form was greater than that of the R-form, although the difference was not as marked in comparison to those of H2S9/31 hydroxylation and omeprazole O-demethylation.

For omeprazole, on the other hand, hydroxylation occurs in the pyridine substituent, and the intrinsic clearance estimated from cDNA-expressed CYP2C19 was significantly greater for the R-form than for the S-form. These findings are expected to be useful for prediction of the stereoselective metabolism of CYP2C19-catalyzed hydroxylation of other PPI analogs, such as pantoprazole and rabeprazole, according to their hydroxylation sites, i.e., a benzimidazole or pyridine group.

In conclusion, our study clearly demonstrated stereoselectivity in the formation of hydroxy and sulfone metabolites from lansoprazole, and intrinsic clearances of both metabolic pathways were consistently and significantly higher for S-lansoprazole than those for the R-form in human liver microsomal fractions. On incubation with cDNA-expressed P450 enzymes, CYP2C9, CYP2C19, and CYP3A4 were found to catalyze the stereoselective hydroxylation of lansoprazole, but the relative contribution of these enzymes on stereoselective hydroxylation appeared to be highly concentration-dependent. In the usual therapeutic concentration range of lansoprazole, CYP2C19 is expected to have higher affinity to S-enantiomer compared with R-form, which may lead to greater elimination and thus lower plasma concentration of S-lansoprazole in vivo.

The CYP3A4 also contributes to the stereoselective disposition of lansoprazole mainly due to stereoselective sulfoxidation. The predicted \( \text{Cl}_{\text{int}} \) values for the formation of sulfone metabolite from S- and R-enantiomers by CYP3A4 were higher than those by CYP2C9 and CYP2C19, even though the contribution of this enzyme was expected to be less prominent in the usual therapeutic concentration due to its low affinity. These findings explain, at least in part, the previous in vivo data that the stereoselective disposition of lansoprazole enantiomers is not highly influenced by CYP2C19 genetic polymorphism and that the plasma concentrations of R-lansoprazole are consistently higher than those of S-form in both CYP2C19 EM and PM subjects when given as the racemate, in contrast to the case of omeprazole.

Acknowledgments. We thank Dr. Ichiro Ieiri of Tottori University (Yonago, Japan) and Dr. Takashi Ishizaki of Kumamoto University (Kumamoto, Japan) for providing the lansoprazole metabolites.

References


