COMPARISON OF INHIBITORY EFFECTS OF THE PROTON PUMP-INHIBITING DRUGS OMEPRAZOLE, ESOMEPRAZOLE, LANSOPRAZOLE, PANTOPRAZOLE, AND RABEPRAZOLE ON HUMAN CYTOCHROME P450 ACTIVITIES

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ABSTRACT:

The human clearance of proton pump inhibitors (PPIs) of the substituted benzimidazole class is conducted primarily by the hepatic cytochrome P450 (P450) system. To compare the potency and specificity of the currently used PPIs (i.e., omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole) as inhibitors of four cytochrome P450 enzymes (CYP2C9, 2C19, 2D6, and 3A4), we performed in vitro studies using human liver microsomal preparations and recombinant CYP2C19. Sample analysis was done using selected reaction monitoring liquid chromatography/tandem mass spectometry. With several systems for CYP2C19 activity (two marker reactions, S-mephenytoin 4′-hydroxylation and R-omeprazole 5-hydroxylation, tested in either human liver microsomes or recombinant CYP2C19), the five PPIs showed competitive inhibition of CYP2C19 activity with K_i of 0.4 to 1.5 μM for lansoprazole, 2 to 6 μM for omeprazole, ~8 μM for esomeprazole, 14 to 69 μM for pantoprazole, and 17 to 21 μM for rabeprazole. Pantoprazole was a competitive inhibitor of both CYP2C9-catalyzed diclofenac 4′-hydr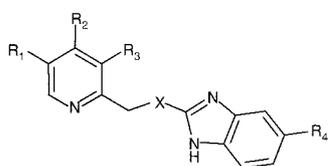oxylation and CYP3A4-catalyzed midazolam 1′-hydroxyla-tion (K_i of 6 and 22 μM, respectively), which were at least 2 times more potent than the other PPIs. All PPIs were poor inhibitors of CYP2D6-mediated bufuralol 1′-hydroxylation with IC_{50} > 200 μM. The inhibitory potency of a nonenzymatically formed product of rabeprazole, rabeprazole thioether, was also investigated and showed potent, competitive inhibition with K_i values of 6 μM for CYP2C9, 2 to 8 μM for CYP2C19, 12 μM for CYP2D6, and 15 μM for CYP3A4. The inhibitory potency of R-omeprazole on the four studied P450 enzymes was also studied and showed higher inhibitory potency than its S-isomer on CYP2C9 and 2C19 activities. Our data suggest that, although the inhibitory profiles of the five studied PPIs were similar, lansoprazole and pantoprazole are the most potent in vitro inhibitors of CYP2C19 and CYP2C9, respectively. Esomeprazole showed less inhibitory potency compared with omeprazole and its R-enantiomer. The inhibitory potency of rabe-prazole was relatively lower than the other PPIs, but its thioether analog showed potent inhibition on the P450 enzymes investigated, which may be clinically significant.

Knowledge of drug interactions with the liver microsomal mixed-function oxidase system is becoming increasingly important in the safety assessment of new compounds developed for clinical use. It is of particular importance during multidrug treatment, because the pharmacodynamics of coadministered drugs may be dramatically altered by the influence of one drug on the metabolism of another (Zumbrunnen and Jann, 1998). Drug-drug interaction (DDI) is mainly due to enzyme induction or interference with drug metabolism. The latter can be assessed by measuring the influence of the drug on the cytochrome P450 (P450) enzyme system, which is responsible for the metabolism of most drugs (Papp-Jambor et al., 2002). With this understanding, it has become essential to make educated predictions for new drugs in regards to the other drugs with which they are likely, or not likely, to interact (Tucker, 1994).

Proton pump inhibitors (PPIs) have been widely used as acid inhibitory agents for the treatment of disorders related to gastric acid secretion for about 15 years. Five such agents (i.e., omeprazole, lansoprazole, pantoprazole, rabeprazole, and the more recently introduced esomeprazole) are now available (Fig. 1). Each of these avail-able PPIs is a substituted benzimidazole, and they are all, with the exception of esomeprazole, marketed as the racemates. The rationale for developing esomeprazole (the S-enantiomer of omeprazole) was its higher metabolic stability (Äbelö et al., 2000), which leads to a higher bioavailability (Hassan-Alin et al., 2000) compared with the racemate. The resulting increase in the area under the plasma concentration-time curve (AUC) of esomeprazole provides more effective control of gastric acid secretion than that of all other PPIs given at their standard doses (Röhss et al., 2002; Lindberg et al., 2003). All of these PPIs have a common mechanism of action involving chemical rearrangement to a reactive sulfenamide that inhibits the ability of H^+,K^+-ATPase (the proton pump) to participate in gastric acid formation. As a result of structural and functional similarities, the PPIs share many pharmacokinetic features [e.g., similar rates of absorption, maximum plasma concentrations, and total drug absorption (Hassan-Alin et al., 2000; Andersson et al., 2001b; Vanderhoff and Tahboub, 2002)]. All PPIs are extensively metabolized in the liver via CYP2C19 and 3A4, and they all exhibit polymorphic metabolism in humans (Andersson et al., 1993; Yasuda et al., 1995; Sohn et al.,

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ABBREVIATIONS: DDI, drug-drug interaction; P450, cytochrome P450; PPI, proton pump inhibitor; AUC, area under the plasma concentration-time curve; PM, poor metabolizer; HLM, human liver microsomes; EM, extensive metabolizer; FWHM, full-width half maximum.

	R₁	R₂	R₃	R₄	X
Omeprazole	CH₃	OCH₃	CH₃	OCH₃	
Lansoprazole	H	OCH₂CF₃	CH₃	H	
Pantoprazole	H	OCH₃	OCH₃	OCHF₂	
Rabeprazole	H	OCH₂CH₂CH₂OCH₃	CH₃	H	
Rabeprazole thioether	H	OCH₂CH₂CH₂OCH₃	CH₃	H	
Esomeprazole	CH₃	OCH₃	CH₃	OCH₃	
R-omeprazole	CH₃	OCH₃	CH₃	OCH₃	

FIG. 1. Structures of proton pump inhibitors discussed.

1997; Tanaka et al., 1997; McColl and Kennerley, 2002) (Table 1). The poor metabolizer (PM) phenotype of the PPIs is highly correlated with the PM phenotype of *S*-mephenytoin (CYP2C19), and the in vivo DDI potential of omeprazole, for example, has been investigated widely; however, the data of recently marketed PPIs, such as rabeprazole, are still not complete. Although only omeprazole has demonstrated interactions with the metabolism of other drugs in vivo, albeit regarded to be not clinically relevant, the other PPIs also show a certain potential for interacting with drugs metabolized by P450s according to previous in vitro inhibition studies (VandenBranden et al., 1996; Ko et al., 1997). In addition to P450-mediated metabolism, rabeprazole is also converted nonenzymatically to its thioether analog. Considering the relatively higher lipophilic character of this product and its relatively longer half-life in vivo compared with its parent (Yasuda et al., 1995; Shirai et al., 2001), it was also included in this study.

Based on a Medline search (1966 through August 2003), the in vitro inhibition studies of the PPIs omeprazole, lansoprazole, pantoprazole, rabeprazole, and esomeprazole on human P450 enzymes have not been described or compared thoroughly. So far, most, if not all, of the direct comparative studies referring to omeprazole have been performed with only one comparator PPI (VandenBranden et al., 1996; Ko et al., 1997; Pichard-Garcia et al., 2000). In addition, a significant variation exists between the reported inhibition studies from lab to lab, which might be caused by different marker reactions used for specific P450 enzymes, the microsome sources, and analytical methods (Ko et al., 1997; Furuta et al., 2001). To our knowledge, there are still no published data regarding the inhibition potency of pantoprazole in vitro.

Based on the above-mentioned lack of consistent data, we investigated the potency and specificity of these PPIs as inhibitors of the four principal P450 enzymes (CYP2C9, 2C19, 3A4, and 2D6) present in human liver microsomes (HLM), which are involved in the metabolism of most drugs and also might be influenced by PPIs as reported previously to get comparable information. Other P450 enzymes were not included in this study because of the low possibility of DDIs with regard to reported data [e.g., no inhibition of omeprazole and lansoprazole on CYP1A2 activity (Ko et al., 1997)] and because other P450 enzymes (e.g., CYP2A6, 2C8, and 2E1) are not the major enzymes involved in the metabolism of the PPIs (Andersson et al., 1993; Pearce et al., 1996; VandenBranden et al., 1996). Furthermore, due to the fact that CYP2C19 is the major enzyme involved in the metabolism of all

the PPIs, the inhibition potential of the PPIs on CYP2C19 activity was investigated in detail in this study with two marker reactions and using recombinant CYP2C19 as well.

Materials and Methods

Chemicals and Reagents. Bufuralol hydrochloride, 1′-hydroxybufuralol maleate, 4′-hydroxymephenytoin, 1′-hydroxymidazolam, *S*-mephenytoin, and sulfaphenazole were purchased from Ultrafine Chemicals (Manchester, UK). Midazolam and 4′-hydroxydiclofenac were purchased from BD Gentest (Woburn, MA). Diclofenac sodium, tinidazole (internal standard), quinidine, ketoconazole, and reduced NADPH were purchased from Sigma-Aldrich (St. Louis, MO). Ticlopidine hydrochloride was purchased from MP Biomedicals (Irvine, CA). Omeprazole sodium was obtained from AstraZeneca Bulk Production (Södertälje, Sweden). Esomeprazole sodium, *R*-omeprazole sodium, lansoprazole, and pantoprazole sodium sesquihydrate were obtained from AstraZeneca Process R&D (Södertälje, Sweden). 5-Hydroxyomeprazole and rabeprazole thioether were obtained from Synthelec AB (Lund, Sweden), and rabeprazole was obtained from Medicinal Chemistry (AstraZeneca R&D, Mölndal, Sweden). All other chemicals and reagents were of the highest commercially available quality.

HLM and Recombinant Cytochromes P450 (rCYP). Human liver samples (excess material removed during surgery on the liver) were obtained from the Department of Surgery 1, Sahlgrenska Hospital, Göteborg, Sweden. Pooled HLM were prepared with a mixture of seven liver samples of male and female patients according to the method of Ernster et al. (1962). Recombinant human CYP2C19 was heterologously expressed in *Saccharomyces cerevisiae* obtained from AstraZeneca Biotech Laboratory (Södertälje, Sweden). Microsomal protein concentration was measured according to Lowry et al. (1951), using bovine serum albumin as standard. The microsomal preparations were stored at −80°C until use.

High-Performance Liquid Chromatography Conditions. The high-performance liquid chromatography system used included a Surveyor MS pump, a built-in degasser, a Surveyor PDA detector (Thermo Finnigan, San Jose, CA), and a CTC HTS autosampler (CTC Analytics, Zwingen, Switzerland). Chromatography was performed on a Zorbax SB C₁₈ column (2.1 × 50 mm, 3.5 μm; Agilent Technologies, Palo Alto, CA) with an Eclipse XDB-C₈ guard column (2.1 × 12.5 mm, 5 μm). The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile, which increased linearly from 5% of solvent B to 95% B during 3.5 min at a flow rate of 0.3 ml/min. During the investigation of the effects of rabeprazole thioether on bufuralol 1′-hydroxylase (CYP2D6) and omeprazole 5-hydroxylase (CYP2C19) activities, mobile phase of (A) 5 mM ammonium acetate (pH 7) in water and (B) acetonitrile was used to avoid the analytical interference by the inhibitor and its metabolites. In the latter case, for the analysis of 1′-hydroxybufuralol, a slower gradient profile of 5% B to 60% B in 3.5 min was used at 0.3 ml/min. The Surveyor PDA detector was set at 302 nm for monitoring of the consumption of the PPIs, which was less than 20% of the initial concentrations after incubation with HLM.

Mass Spectrometric Conditions. The mass spectrometric analyses were performed using a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer (ThermoFinnigan). The mass spectrometer was operated in the positive ionization electrospray mode. The spray voltage was set to 4.0 kV, heated capillary temperature to 325°C and its offset to 35 V, and the source collision-induced dissociation to off (except for the analysis of 4′-hydroxymephenytoin, in which a source collision-induced dissociation of 15 V was applied to assist ion desolvation, thus reducing the formation of acetonitrile adducts). Nitrogen was used as the sheath and auxiliary gas and set to 30 and 5 (arbitrary units), respectively. The argon collision gas pressure was set to 1.5 mTorr. The mass spectrometer was operated in the selected reaction monitoring mode, with the resolution of Q1 set at 0.5 Da FWHM and that of Q3 set at 0.7 Da FWHM. The parameters of the selected reaction monitoring transitions for the $[M + H]^+$ precursor ions to selected product ions were optimized with the following typical values for the analytes and internal standard (each at their optimum collision energy): 4′-hydroxydiclofenac *m/z* 312.0 to 230.0; 4′-hydroxymephenytoin *m/z* 235.0 to 150.1; 5-hydroxyomeprazole *m/z* 362.1 to 214.0; 1′-hydroxybufuralol *m/z* 278.0 to 186.1; 1′-hydroxymidazolam *m/z* 342.0 to 324.1; and the internal standard tinidazole *m/z* 248.0 to 121.0.

TABLE 1

Pharmacokinetic characteristics of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole following repeated oral doses in healthy subjects

The recommended daily doses for these five PPIs are 20 mg for omeprazole, 40 mg for esomeprazole, 30 mg for lansoprazole, 40 mg for pantoprazole, and 20 mg for rabeprazole.

PPI	Oral Dose	CYP2C19	Genotype	C_{max}	AUC	$t_{1/2\beta}$	Reference
	mg			*μM*	*$\mu mol \cdot h/l$*	*h*	
Omeprazole	20 (7th day)	EM	Mean ± S.D.	1.6 ± 1.0	2.9 ± 2.8	0.8 ± 0.4	Yasuda et al., 1995
		PM	Mean ± S.D.	3.1 ± 0.9	12.7 ± 7.2	2.1 ± 0.6	
	20 (7th day)	EM	Mean ± S.D.	1.1 ± 0.6	2.2 ± 1.9	0.9 ± 0.2	Andersson et al., 1998
		PM	Mean	3.2	12.0	2.3	
	20 (8th day)	EM	Mean ± S.E.M.		3.1 ± 0.6		Shirai et al., 2001
		PM	Mean ± S.E.M.		20.7 ± 1.9		
Esomeprazole	40 (5th day)	EM	Mean	5.2	14.1		AstraZeneca, 2002, data on file
		PM	Mean	6.7	19.7		
Lansoprazole	30 (7th day)	EM	Mean ± S.D.	2.2 ± 0.7	4.9 ± 2.1	1.1 ± 0.3	Andersson et al., 1998
		PM	Mean	4.8	28.8	4.8	
	30 (8th day)	EM	Mean ± S.E.M.	2.0 ± 0.3	4.6 ± 0.8	1.2 ± 0.1	Ieiri et al., 2001
		PM	Mean ± S.E.M.	3.8 ± 1.1	17.9 ± 2.4	3.9 ± 0.2	
Pantoprazole	40 (7th day)	EM	Mean ± S.D.	5.4 ± 1.4	9.5 ± 3.5	1.3 ± 0.3	Andersson et al., 1998
		PM	Mean	9.7	61.7	6.3	
	80 (7th day)	EM	Mean ± S.D.	12.8 ± 2.8	27.2 ± 6.5	1.2 ± 0.2	Tanaka et al., 1996
		PM	Mean	30.2	279.2	8.7	
Rabeprazole	20 (7th day)	EM	Mean ± S.D.	1.2 ± 0.7	2.0 ± 0.9	1.0 ± 0.6	Yasuda et al., 1995
		PM	Mean ± S.D.	1.7 ± 0.9	3.9 ± 1.1	1.6 ± 0.4	
	20 (8th day)	EM	Mean ± S.E.M.		1.3 ± 0.3		Shirai et al., 2001
		PM	Mean ± S.E.M.		6.8 ± 0.5		
	10 (8th day)	EM	Mean ± S.E.M.	0.6 ± 0.3	1.1 ± 0.3	0.9 ± 0.3	Ieiri et al., 2001
		PM	Mean ± S.E.M.	1.4 ± 0.3	4.1 ± 0.5	2.1 ± 0.1	
Rabeprazole Thioether	20 (7th day)	EM	Mean ± S.D.	0.3 ± 0.2	2.4 ± 1.6	2.8 ± 0.7	Yasuda et al., 1995
		PM	Mean ± S.D.	0.6 ± 0.3	4.2 ± 3.2	3.0 ± 1.4	
	20 (8th day)	EM	Mean ± S.E.M.		1.7 ± 0.5		Shirai et al., 2001
		PM	Mean ± S.E.M.		4.9 ± 0.9		
	10 (8th day)	EM	Mean ± S.E.M.	0.2 ± 0.06	0.8 ± 0.2	2.0 ± 0.2	Ieiri et al., 2001
		PM	Mean ± S.E.M.	0.26 ± 0.03	2.1 ± 0.3	6.2 ± 1.0	

C_{max}, peak plasma drug concentration; $t_{1/2\beta}$, terminal elimination half-life.

Instrument control, data acquisition, and data evaluation were performed using Xcalibur software version 1.3 (Thermo Finnigan). The lower limit of quantification was 10 nM for all five hydroxylated marker metabolites in the present study.

In Vitro Inhibition of P450 Enzyme-Specific Assays by PPIs. To measure the inhibition effects of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on the activities of CYP2C9, 2C19, 2D6, and 3A4, the following marker substrates with high specificity for P450 enzymes were selected: diclofenac 4′-hydroxylation for CYP2C9; *S*-mephenytoin 4′-hydroxylation for CYP2C19; bufuralol 1′-hydroxylation for CYP2D6; and midazolam 1′-hydroxylation for CYP3A4. *R*-omeprazole 5-hydroxylation was also used to reflect the activity of CYP2C19 (Äbelö et al., 2000). The effects of *R*-omeprazole and the nonenzymatically formed degradation product of rabeprazole (i.e., rabeprazole thioether) were also investigated under the same conditions. Sulfaphenazole, ticlopidine, quinidine, and ketoconazole were chosen as model inhibitors of CYP2C9, 2C19, 2D6, and 3A4, respectively.

All reactions were performed in 96-well plates. Each reaction mixture consisted of the enzyme, the substrate, and 1 mM NADPH in 0.1 M Tris-hydrochloride buffer (pH 7.4) with or without test compound in a final volume of 200 μl. The initial rate of each enzyme reaction was linear with time and protein concentration. The incubation conditions used for CYP2C9, 2D6, and 3A4 activities were of 0.2 mg/ml HLM with an incubation time of 15 min, and those for CYP2C19 activity were of 0.3 mg/ml HLM (or 10 pmol rCYP2C19/200 μl) with an incubation time of 20 min. The reactions were started by the addition of NADPH after a preincubation of 5 min at 37°C. A 100-μl aliquot of ice-cold acetonitrile (containing 1.5 μM internal standard) was added to stop the reaction. After centrifugation at 4,500g for 20 min, 20 μl of supernatant was injected into the liquid chromatography/tandem mass spectrometry system. Samples were quantified by monitoring the ratio between the hydroxylated metabolite of the marker substance and the internal standard in each sample and in calibration curves. All the incubations throughout the study were carried out in duplicate. For each incubation, the test compound was dissolved in methanol and sequentially diluted with 40% methanol in 0.1 M Tris-hydrochloride buffer (pH 9) [except rabeprazole thioether, which was diluted with 70% methanol in 0.1 M Tris-hydrochloride buffer (pH 9)]. The

final pH value of the incubation mixture was kept at 7.4, and the methanol concentration was less than 1%.

Before full K_i determination was done, the concentration of the PPIs that caused 50% inhibition of the marker reaction (IC$_{50}$) was determined at a substrate concentration close to K_m. The inhibition mechanism was determined, and the apparent inhibition constants (K_i) for the respective P450 enzymes were performed on the test compounds, for which the IC$_{50}$ values were below 200 μM. In general, the test compounds and model inhibitors were studied at four different concentrations that were chosen based on the IC$_{50}$ values obtained with each P450 enzyme (approximately ½ × IC$_{50}$, IC$_{50}$, 2 × IC$_{50}$, and 4 × IC$_{50}$). The concentrations of marker substrates were chosen (approximately ½ K_m, K_m, 3 K_m, and 5 K_m) with regard to their Michaelis-Menten kinetics (K_m and V_{max}) (Table 2). The vehicle controls were analyzed in parallel. The K_i values were calculated by nonlinear regression analysis by fitting different models of enzyme inhibition to the kinetic data using SigmaPlot Enzyme Kinetics Module for Windows 7.0 (SPSS Inc., Chicago, IL). The inhibition mechanism was established by comparing the statistical results including the Akaike's information criterion values of different inhibition models and selecting the one with the best fit.

Results

The inhibitory effects of the five PPIs, as well as *R*-omeprazole and rabeprazole thioether, on four human P450 enzymes (CYP2C9, 2C19, 2D6, and 3A4) were investigated. The apparent inhibition constants, K_i values, were calculated using the inhibition model, which provided the best fit, and the final results are summarized in Table 3.

CYP2C9 Activity. Diclofenac 4′-hydroxylation was used as a marker reaction for CYP2C9 activity in HLM. All PPIs showed inhibitory effects on the formation of the 4′-hydroxylated metabolite of diclofenac. The mechanism of inhibition for all PPIs seemed to be competitive except for esomeprazole, which was best fit in a mixed inhibition model. The respective K_i values were 6, 16, 21, and 51 μM for pantoprazole, omeprazole, lansoprazole, and rabeprazole, respec-

TABLE 2

Enzyme kinetic parameters of marker reactions of CYP2C9, 2C19, 2D6, and 3A4 in HLM

Marker Reaction	P450	K_m	V_{max}
		μM	*pmol/min/mg protein*
Diclofenac 4′-hydroxylation	2C9	5.3	1396
S-Mephenytoin 4′-hydroxylation	2C19	20	30
R-Omeprazole 5-hydroxylation	2C19	1.1*	29
Bufuralol 1′-hydroxylation	2D6	23	70
Midazolam 1′-hydroxylation	3A4	4.1	636

* *R*-Omeprazole 5-hydroxylation was catalyzed by two enzyme systems in HLM: a high-affinity enzyme system with K_{m1} = 1.1 μM and a low-affinity enzyme system with K_{m2} = 14 μM, respectively.

tively. Compared with omeprazole and its *R*-enantiomer, the inhibitory effect of esomeprazole was significantly lower on diclofenac 4′-hydroxylation, with a K_i of 82 μM. The inhibitory effect of rabeprazole thioether was about 9-fold stronger (K_i = 6 μM) than that of its parent drug (51 μM).

CYP2C19 Activity. The inhibitions of CYP2C19 by the PPIs were measured in both HLM and rCYP2C19 using *S*-mephenytoin 4′-hydroxylation as a marker reaction. Representative Dixon plots for the inhibitory effects of omeprazole and lansoprazole on *S*-mephenytoin 4′-hydroxylase activity in HLM are shown in Fig. 2. In addition, the inhibition of *R*-omeprazole 5-hydroxylation by pantoprazole, lansoprazole, rabeprazole, and its thioether metabolite was also studied. The inhibition kinetics could best be described by applying a competitive inhibition model. The inhibition constants derived from the various systems and marker reactions were consistent except for pantoprazole, which showed a higher K_i value (69 μM) in HLM using *S*-mephenytoin as substrate than in the other systems, where the K_i varied between 14 and 17 μM. Lansoprazole was the most potent CYP2C19 inhibitor, exhibiting a K_i around 1 μM or lower depending on which system was used. *R*-omeprazole, esomeprazole, and omeprazole all showed K_i values between 2 and 9 μM, whereas rabeprazole showed K_i values between 17 and 21 μM. Again, the inhibitory effects of rabeprazole thioether (K_i = 2–8 μM) on CYP2C19-mediated reactions were stronger than that of the parent compound.

Pantoprazole showed higher K_i values in HLM using *S*-mephenytoin as substrate than in other systems measuring CYP2C19 inhibition. The enzyme kinetics in HLM could be influenced by many factors (e.g., substrate used, the metabolites formed from either substrate or inhibitor, and the enzymes involved in the metabolism of the compound). In fact, the data at low *S*-mephenytoin concentrations (10 and 20 μM) clearly indicated a potent inhibitory effect on *S*-mephenytoin 4′-hydroxylation by pantoprazole in HLM, leading to a much lower K_i (19 μM) than the one obtained when all tested concentrations were included in the calculation. Because of these problems in data analysis, the effect of pantoprazole on *S*-mephenytoin 4′-hydroxylation in HLM should be considered as a gross effect, not only as an effect of a parent drug to one P450 enzyme. Consequently, the rCYP2C19 incubation provided a much simpler environment for the enzyme kinetic study, and the results obtained might better represent the inhibitory effect of pantoprazole on the specific P450 enzyme activity than that in HLM. Considering the results of the two CYP2C19 marker reactions used in HLM and rCYP2C19, a K_i value around 15 μM seems to be more likely for the inhibitory potency of pantoprazole to CYP2C19 activity.

CYP2D6 Activity. No significant effects on the formation of 1′-hydroxylated bufuralol (CYP2D6) were found, indicating that none of the five PPIs inhibited CYP2D6 activity in vitro (IC$_{50}$ > 200 μM). However, rabeprazole thioether inhibited CYP2D6 activity with a K_i of 12 μM in HLM.

CYP3A4 Activity. The effects of the five PPIs on CYP3A4-mediated 1′-hydroxymidazolam formation were investigated in HLM. Pantoprazole showed a stronger inhibitory effect (K_i = 22 μM) on midazolam 1′-hydroxylation than omeprazole (K_i = 42 μM), esomeprazole (K_i = 47 μM), and rabeprazole (K_i = 51 μM). The IC$_{50}$ of lansoprazole was over 200 μM. The pattern of inhibition was established to be competitive by fitting the data to different inhibitory models. Omeprazole and its enantiomers showed similar inhibitory effects on the production of the 1′-hydroxylated metabolite of midazolam. Again, the inhibitory potency of rabeprazole thioether to CYP3A4 activity in HLM was about 3-fold higher than that of its parent compound.

Discussion

In this study, we investigated the inhibitory effects of the five currently available PPIs (i.e., omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole) on four human P450 enzymes (CYP2C9, 2C19, 2D6 and 3A4) using human liver microsomal preparations to assess their potential of interaction with coadministered drugs that are metabolized by these enzymes. Several pharmacokinetic studies have been performed on the five PPIs in healthy *S*-mephenytoin EMs and PMs (Table 1). After therapeutically repeated doses, the range of maximum plasma concentration of each PPI in EMs was 1 to 5 μM, with omeprazole and pantoprazole in the low and high ends, respectively. In PMs, the range was 2 to 10 μM, with rabeprazole and pantoprazole in the low and high ends, respectively. The ratio of the mean AUC values in PMs versus EMs was about 1- to 2-fold for esomeprazole, 2- to 5-fold for rabeprazole, and about 5-fold for omeprazole, lansoprazole, and pantoprazole, indicating considerable variation in drug exposure between EMs and PMs for the five compounds (Table 1). Because the K_i values determined in our study for certain P450 enzyme and PPI combinations are in the same order of magnitude as the plasma levels encountered in vivo, this might point to the potential of DDIs for any of the five PPIs during multidrug treatment. PPI drug-drug interactions in vivo have been reviewed thoroughly (Andersson, 1996; Steinijans et al., 1997; Jungnickel, 2000; Andersson et al., 2001a; Gerson and Triadafilopoulos, 2001; Fuhr and Jetter, 2002; Vanderhoff and Tahboub, 2002), and conclusions have been drawn that no clinically relevant DDIs exist for this class of compounds. However, the potential of possible clinical interactions is not fully explored for all PPIs [e.g., rabeprazole (Fuhr and Jetter, 2002)], and the population under study may also influence the conclusion of clinical studies (Chong and Ensom, 2003) (e.g., most of the rabeprazole studies were performed in Japanese men).

Several in vivo DDI studies claim a significant inhibition of CYP2C19 by omeprazole, [e.g., the ability of omeprazole to inhibit diazepam clearance in humans (Gugler and Jensen, 1985; Ishizaki et al., 1995)]. This seems to be due to the fact that CYP2C19 plays a major role in the primary metabolism of both omeprazole and diazepam. In a study on genotyped subjects (Andersson et al., 1990), the diazepam-omeprazole drug interaction could be attributed to

TABLE 3

K_i (μM) values[a] of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole for human P450 isoforms

	Marker Reaction	Microsomes	R-Omeprazole	Omeprazole	Esomeprazole	Lansoprazole	Pantoprazole	Rabeprazole	Rabeprazole Thioether	Model Inhibitor
CYP2C9	Diclofenac 4'-hydroxylation	HLM	5.3 ± 0.4	16.4 ± 3.0	81.5 ± 72.7[a]	20.8 ± 3.3	6.5 ± 1.0	51.0 ± 9.4	5.8 ± 0.6	Sulfaphenazole 0.15 ± 0.03
CYP2C19	S-Mephenytoin 4'-Hydroxylation	HLM	5.8 ± 0.6	6.2 ± 0.8	8.6 ± 1.0	0.45 ± 0.07	69.4 ± 9.2	21.3 ± 2.8	2.4 ± 0.1	Ticlopidine 0.31 ± 0.05
		rCYP2C19	1.7 ± 0.1	2.4 ± 0.05	7.9 ± 0.5	0.74 ± 0.09	15.3 ± 1.1	18.8 ± 1.3	2.8 ± 0.1	0.68 ± 0.04
	R-Omeprazole 5-Hydroxylation	HLM	NA	NA	NT	1.5 ± 0.2	17.4 ± 1.0	16.8 ± 1.9	3.1 ± 0.2	1.7 ± 0.2
		rCYP2C19	NA	NA	NT	0.91 ± 0.05	13.9 ± 0.9	17.3 ± 1.4	8.3 ± 0.8	0.86 ± 0.08
CYP2D6	Bufuralol 1'-Hydroxylation	HLM	NT	IC$_{50}$ > 200	IC$_{50}$ > 200	IC$_{50}$ > 200	IC$_{50}$ > 200	IC$_{50}$ > 200	12.4 ± 1.2	Quinidine 0.03 ± 0.004
CYP3A4	Midazolam 1'-hydroxylation	HLM	44.5 ± 5.0	41.9 ± 5.9	46.6 ± 6.8	IC$_{50}$ > 200	21.9 ± 2.7	50.7 ± 6.4	15.0 ± 1.8	Ketoconazole 0.0052 ± 0.0005

NT, not tested; NA, not applicable.
[a] All the K_i values were calculated with the competitive inhibition model except esomeprazole to CYP2C9-mediated diclofenac 4'-hydroxylation, which showed the best fit to the mixed inhibition model.

CYP2C19, since no DDI was seen in PMs lacking CYP2C19 activity. This inhibitory potency has been confirmed in our present study and by others (Furuta et al., 2001). In our study, esomeprazole showed a slightly weaker inhibitory potency on CYP2C19 than omeprazole (K_i value of 8 versus 2 μM in rCYP2C19). One might expect that this would be reflected in favor of esomeprazole in DDI studies in vivo for, for example, typical CYP2C19 substrates, such as diazepam, phenytoin, and R-warfarin. However, the interaction potential for esomeprazole seems to be similar to that of omeprazole in the clinical situation. This can be explained by the higher standard dose used for esomeprazole (40 mg) and its lower clearance compared with omeprazole (20 mg) resulting in higher plasma levels and extended exposure for esomeprazole. Comparing the inhibitory potential of omeprazole and its R- and S-enantiomers, it seems that the contribution to inhibition of CYP2C19 activity by omeprazole was higher from R-omeprazole than from esomeprazole, which probably is due to the higher affinity of R-omeprazole to this enzyme (Äbelö et al., 2000).

Observations that lansoprazole had no significant effect on diazepam (Lefebvre et al., 1992) or warfarin clearance in vivo (Cavanaugh et al., 1991) lead these authors to claim that lansoprazole has less significant drug interactions than omeprazole. However, later it was shown that CYP2C19 was the primary metabolizing enzyme at pharmacologically relevant concentrations of lansoprazole (1–5 μM) (Pearce et al., 1996; Table 1). The clinical correlate of these data were presented by Sohn et al. (1996), who reported in Korean subjects that PMs of S-mephenytoin are also PMs of lansoprazole. Our study clearly demonstrates that lansoprazole exhibits a strong competitive inhibition of CYP2C19 activity, with an average K_i of about 1 μM. Thus, lanzoprazole has the potential to be an equally or more potent inhibitor than omeprazole or esomeprazole. This result is also consistent with the work of Ko et al. (1997) (K_i = 3 μM for lansoprazole). Interestingly, this higher inhibitory potential of lansoprazole is not reflected in vivo, which suggests that the in vivo DDI studies may not have been performed under optimum conditions. Unless complementary clinical DDI studies are performed, it is difficult to conclude whether lansoprazole has clinically important DDIs with drugs such as mephenytoin and some benzodiazepine derivatives or tricyclic antidepressants, which are mainly metabolized by CYP2C19 (Zumbrunnen and Jann, 1998).

Pantoprazole showed the highest inhibition potency on CYP2C9 (K_i, 6 μM) of the five PPIs, although drug interactions with substrates for this enzyme have not been shown in vivo. Still, there is a potential for DDIs by pantoprazole upon coadministration with drugs mainly metabolized by CYP2C9, at least in CYP2C19 PM patients who are subjected to elevated plasma concentrations of pantoprazole. Thus, DDI studies in CYP2C19 PMs using, for example, pantoprazole and S-warfarin, which have a narrow therapeutic index, would be important to exclude serious effects on S-warfarin pharmacodynamics. A potential metabolic interaction with diazepam would be the most reasonable prediction with respect to pantoprazole because both are metabolized mainly by CYP2C19. The interaction study performed with diazepam and pantoprazole indicated that the plasma concentrations of diazepam were unchanged during pantoprazole treatment, but the plasma elimination half-life of diazepam was shorter (9%), and the apparent volume of distribution was decreased (8%) (Gugler et al., 1996). However, pantoprazole was administered intravenously, which would have avoided a potential influence on the liver enzymes by the first pass metabolism of pantoprazole.

It has been suggested that rabeprazole has significantly less drug interactions than other PPIs, and the main reason is claimed to be its nonenzyme-catalyzed degradation, which forms a thioether product
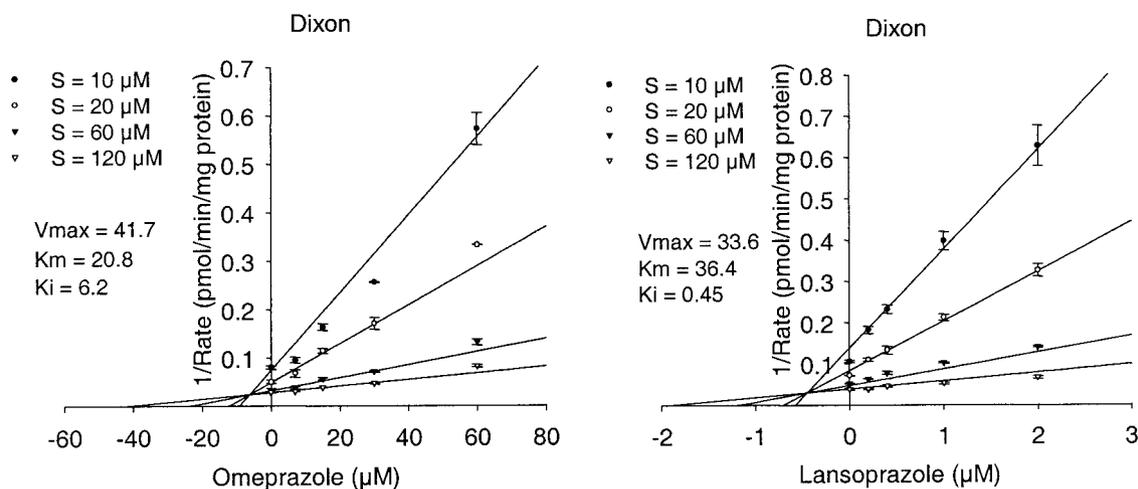
Dixon

Dixon plots showing 1/Rate (pmol/min/mg protein) versus Omeprazole (μM).

- S = 10 μM
- S = 20 μM
- S = 60 μM
- S = 120 μM

Vmax = 41.7
Km = 20.8
Ki = 6.2

Dixon

Dixon plots showing 1/Rate (pmol/min/mg protein) versus Lansoprazole (μM).

- S = 10 μM
- S = 20 μM
- S = 60 μM
- S = 120 μM

Vmax = 33.6
Km = 36.4
Ki = 0.45

FIG. 2. Dixon plots of omeprazole (left) and lansoprazole (right) on CYP2C19-catalyzed S-mephenytoin 4′-hydroxylation in pooled human liver microsomes. A competitive inhibition model was used, which showed the best fit compared with other inhibition types. Data were analyzed using SigmaPlot Enzyme Kinetics Module software.

(Fuhr and Jetter, 2002). Our results showed that this thioether product by itself exhibited inhibitory potency to all the four major P450 enzymes (K_i = 2–8 μM for 2C19, 6 μM for CYP2C9, 12 μM for CYP2D6, and 15 μM for CYP3A4). Now that this information on the affinity and thus inhibitory potential of the thioether metabolite is available, this further accentuates the need for DDI studies with rabeprazole and substrates for CYP2D6 as well as CYP3A4, which today are lacking. Ishizaki et al. (1995) reported a study on the effects of rabeprazole and omeprazole on diazepam metabolism in CYP2C19 PMs and EMs. Interestingly, rabeprazole significantly increased the AUC (0–16 days) of desmethyldiazepam in the PMs compared with omeprazole or placebo ($p < 0.05$). The authors suggested that, since CYP3A is involved in both 3-hydroxylation of desmethyldiazepam and O-demethylation of rabeprazole, the role of CYP3A in the metabolism of desmethyldiazepam might be more dominant in PMs than in EMs; however, the reason why omeprazole did not show the same effect was not discussed. Our present results suggest that omeprazole and rabeprazole have similar affinity to CYP3A4, but the nonenzyme-catalyzed product rabeprazole thioether showed a K_i (15 μM) that indicates a higher potential for inhibition of CYP3A4 activity. Thus, inhibition by rabeprazole thioether may be the reason of the increased AUC of desmethyldiazepam after diazepam and rabeprazole coadministration in S-mephenytoin PMs. Considering the relatively higher lipophilicity and lower in vivo clearance of rabeprazole thioether than its parent drug (Shirai et al., 2001), its influence on the pharmacokinetics of coadministered drugs may be worth studying further [e.g., on typical CYP2C9 and 2C19 substrates (the K_i values of rabeprazole thioether on CYP2C9 and 2C19 being 6 and 2–8 μM, respectively)]. On the other hand, omeprazole has been shown to have no inhibitory potential of CYP3A in vivo (Andersson, 1991), which is the major reason why this effect on desmethyldiazepam was not observed on omeprazole.

In conclusion, we have studied the inhibitory properties of the five currently marketed PPIs on P450 activities. Although the in vitro inhibitory properties of some of these compounds have been studied previously, this is the first complete study of all five compounds carried out and analyzed under the exact same experimental conditions. Due to the fact that these compounds are fully developed, approved by the regulatory authorities, found to have acceptable drug metabolism and pharmacokinetic properties, and are available for treatment of acid-related gastrointestinal disorders, the in vivo corre-

late of our in vitro data can at least partly be found in the public domain. However, the apparent lack of correlation between the in vivo DDIs reported and the predicted interaction potential from our in vitro studies may at least partly be explained by nonoptimal clinical study design or the simple fact that sufficient clinical data are not yet available. Thus, further studies are required to fully assess whether this in vitro inhibition of P450 enzymes could be a cause of concern during multidrug treatment.

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