Comparison of CYP2D6 Content and Metoprolol Oxidation Between Microsomes Isolated from Human Livers and Small Intestines

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Purpose. To assess the role of intestinal CYP2D6 in oral first-pass drug clearance by comparing the enzyme content and catalytic activity of a prototype CYP2D6 substrate, metoprolol, between microsomes prepared from human intestinal mucosa and from human livers.

Methods. Microsomes were prepared from a panel of 31 human livers and 19 human intestinal jejunal mucosa. Microsomes were also obtained from the jejunum, duodenum and ileum of four other human intestines to assess regional distribution of intestinal CYP2D6. CYP2D6 content (pmole/mg microsomal protein) was determined by Western blot. CYP2D6 activity was measured by α-hydroxylation and O-demethylation of metoprolol.

Results. Kinetic studies with microsomes from select livers (n = 6) and jejunal mucosa (n = 5) yielded K_M estimates of 26 ± 9 μM and 44 ± 17 μM, respectively. The mean V_{max} (per mg protein) for total formation of α-OH-M and ODM was 14-fold higher for the liver microsomes compared to the jejunal microsomes. Comparisons across intestinal regions showed that CYP2D6 protein content and catalytic activity were in the order of jejunum > duodenum > ileum. Excluding the poor metabolizer genotype donors, CYP2D6 content varied 13- and 100-fold across the panels of human livers (n = 31) and jejunal mucosa (n = 19), respectively. Metoprolol α-hydroxylation activity and CYP2D6 content were highly correlated in the liver microsomes ($r = 0.84$, $p < 0.001$) and jejunal microsomes ($r = 0.75$, $p < 0.05$). Using the well-stirred model, the mean microsomal intrinsic clearance (i.e., V_{max}/K_M) for the livers and jejunum were scaled to predict their respective in vivo organ intrinsic clearance and first-pass extraction ratio. Hepatic and intestinal first-pass extractions of metoprolol were predicted to be 48% and 0.85%, respectively.

Conclusions. A much lower abundance and activity of CYP2D6 are present in human intestinal mucosa than in human liver. Intestinal mucosal metabolism contributes minimally to the first-pass effect of orally administered CYP2D6 substrates, unless they have exceptionally high microsomal intrinsic clearances and/or long residence time in the intestinal epithelium.

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ABBREVIATIONS: CYP, cytochrome P450; S, substrate concentration; K_M, Michealis-Menten constant; V_{max}, maximum velocity of formation for a metabolite; α-OH-M, α-hydroxymetoprolol; ODM, O-demethylmetoprolol; $Cl_{int,mic}$, microsomal intrinsic clearance; $Cl_{int,org}$, organ intrinsic clearance; h, liver; g, small intestine; Q_h, hepatoportal blood flow; Q_{muc}, intestinal mucosal blood flow; f_u, free fraction of metoprolol in plasma; E_h, first-pass extraction by liver; E_g, first-pass extraction by small intestine; BCIP-NBT, 5-bromo-4-chloro-3-indoyl phosphate and nitroblue tetrazolium.

KEY WORDS: CYP2D6; first-pass metabolism; metoprolol oxidation; human intestine; human liver; *in vitro-in vivo* scaling.

INTRODUCTION

The ability of extrahepatic tissues to metabolize drug substrates has attracted much interest. Cytochrome P450-dependent mono-oxygenase activity is present in a variety of human tissues; the highest activities are observed in the liver and the intestinal mucosa. Immunoblot analysis using antibodies raised against the major members of human cytochrome P450 (CYP) enzymes showed that, second to CYP3A, the polymorphic CYP2D6 is the most abundant constitutive CYP enzyme in the human intestinal mucosa (1).

A wide range of therapeutically important drugs are metabolized by CYP2D6, notably β-blockers, opioid analgesics, and antidepressants. Many of these CYP2D6 substrates exhibit low and variable bioavailability upon oral administration due to extensive first-pass metabolism. Only a limited number of studies have investigated the relative contribution of the liver and the intestinal mucosa to the overall first-pass effect of CYP2D6 drug substrates.

Du Souich *et al.* (2) demonstrated CYP2D-mediated formation of 4-hydroxypropranolol in microsomes isolated from rabbit intestinal mucosa. *In vivo* studies further showed that the small intestine accounts for 43% of the first-pass extraction of an intra-duodenal dose of propranolol in the rabbit.

Prueksaritanont *et al.* (3,4) compared the 1′-hydroxylation of bufuralol (an *in vitro* CYP2D6 activity marker) in microsomes isolated from a single human intestine and two human livers. These investigators reported the K_M for the intestinal microsomes to be twice that for the liver microsomes (10 versus 5 μM). Further, the V_{max} values were about 20-fold lower for intestinal microsomes compared to liver microsomes (0.7 versus 23 pmole/min/mg). Because of the limited number of tissue samples in these studies, it is not possible to draw a firm conclusion on the differences between the expression and function of CYP2D6 in the human intestinal mucosa and liver.

Large inter-individual variation is characteristic of *in vivo*, first-pass extraction of CYP2D6 substrates (5). This inter-individual variability is consistent with the known variable CYP2D6 expression in human livers (6). Variability of CYP2D6 expression in human intestinal epithelium has not been assessed in a sufficient number of tissues.

Our study sought to characterize the expression of human intestinal CYP2D6 by measuring CYP2D6 protein content and *in vitro* α-hydroxylation and O-demethylation of racemic metoprolol, a moderately high turnover CYP2D6 marker, in 31 human livers and 19 human intestines. As part of the variability assessment, we measured the regional distribution of CYP2D6 along the length of four human small intestines. Finally, in an attempt to evaluate *in vivo* significance, the *in vitro* catalytic data were scaled to predict the *in vivo* first-pass extraction ratios of metoprolol across the human intestinal mucosa and liver.

MATERIALS AND METHODS

Chemicals

(±)-α-Hydroxymetoprolol (α-OH-M) and (±)-O-demethylmetoprolol (ODM) were gifts from Astra Hässle AB (Mölndal, Sweden). (±)-Metoprolol tartrate, atenolol and anti-rabbit

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IgG alkaline phosphatase conjugate were purchased from Sigma Co. (St. Louis, MO). Mouse monoclonal anti-CYP2D6 IgG was obtained from Gentest Co. (Woburn, MA). All other chemicals and solvents used were of the highest purity grade available, and were used without further purification.

Human Tissue and Microsomal Preparations

The University of Washington Human Subject Review Board approved the use of human donor liver and intestinal tissues for the purpose of research. The details on organ procurement and microsomal preparation have been described earlier (7). The small intestine was cut into one-foot sections. Based on a previous study of the physical characteristics (i.e., weight and length) of human small intestine (8), we assigned the first one-foot section as the duodenum, sections 2 through 9 as the jejunum, and the remaining sections as the ileum.

Protein concentration was measured according to the method of Lowry *et al.* (9) using bovine serum albumin as the reference standard. Total P450 concentrations in hepatic and intestinal microsomes were measured spectrophotometrically by the method of Omura and Sato (10) using an extinction coefficient of 91 cm^{-1} mM^{-1}.

CYP2D6 protein content was determined by Western blot using purified cDNA-expressed CYP2D6 (a gift from Dr. Robert L. Haining at the University of Washington) as calibration standard. The amount of intestinal and liver microsomal proteins loaded onto the gel was 100 and 20 μg, respectively. Monocolonal anti-CYP2D6 IgG was used as the primary antibody and anti-rabbit IgG alkaline phosphatase conjugate was used as the secondary antibody. BCIP-NBT solution was used to develop the protein bands on the nitrocellulose sheet. The CYP2D6 band was quantified by computerized densitometry. Linear calibration curves were obtained over the CYP2D6 concentration range of 0.05 pmole to 1 pmole.

Microsomal Incubation

The final incubate of 1-ml volume consisted of either 0.25 mg/ml of liver microsomal protein or 0.5 mg/ml of intestinal microsomal protein (i.e., protein concentrations appropriate for initial velocity measurements), desired concentration of metoprolol, and 1 mM NADPH in 0.1 M phosphate buffer (pH = 7.4). The incubate was placed in 15 ml conical polypropylene tubes, and the reaction was initiated with NADPH and stopped with 100 μl of 2 M NaOH. Incubation times were 10 minutes for liver microsomes and 20 to 30 minutes for intestinal microsomes. Previous studies indicated that the formation rate of metoprolol metabolites remains linear for at least 30 minutes (11). Incubations at each substrate concentration were performed in triplicate.

Metoprolol Metabolite Assay

Fifty microliters of 1 μg/ml of internal standard (atenolol) in water was added to each inactivated microsomal sample and calibration standards. Microsomal samples were extracted with 5-ml mixture of diethyl ether and methylene chloride (3:2, v/v). The organic phase was transferred to a fresh 15-ml tube, containing 300 μl of 1.5% phosphoric acid, vortexed for 5 minutes, and centrifuged to separate the layers. The bottom acid layer was transferred to an auto-injector vial, and a 200 μl aliquot was injected onto the HPLC column.

Chromatographic separation of α-OH-M and ODM was achieved on a 5 μm, 4.6 mm × 25 cm, C18 reversed-phase column. Mobile phase consisted of 0.1 M phosphate buffer, 10% methanol, and 5% tetrahydrofuran. The effluent was monitored by a fluorescence detector, with the excitation wavelength set at 227 nm and the emission wavelength at 304 nm. Retention times for atenolol, α-OH-M and ODM were 3.6, 4.6 and 5.3 minutes, respectively. Standard curve ranged from 10 to 200 pmole for α-OH-M, and 20 to 1000 pmole for ODM. The lower limit of quantitation was 5 pmole for α-OH-M and 10 pmole for ODM. Inter-batch coefficients of variation were 5 and 3% for α-OH-M and ODM, respectively.

Experimental Protocols

The following four experiments were performed.

Michaelis-Menten Kinetics

Initial velocities of metoprolol oxidation catalyzed by human liver and intestinal microsomes were measured at substrate concentrations ranging from 5 to 100 μM. Six livers (HL-133, 134, 135, 137, 140, 148) spanning the range of CYP2D6 expression were chosen for the kinetic experiment. For the kinetic studies with microsomes from human intestinal mucosa, five intestinal mucosa with high to medium CYP2D6 activities (HI-31, 32, 35, 39, and 40) were chosen such that metabolites formed at the chosen substrate concentration were well within the quantification limit of the HPLC assay.

Regional Distribution

Regional variation in metoprolol oxidation activity and CYP2D6 content were measured in microsomes prepared from one-foot sections along the entire length of four human small intestines (HI-31, 32, 35 and 40) at 100 μM metoprolol.

Tissue Panel Correlation

Microsomal CYP2D6 content and activity were measured in a panel of human livers (n = 31, including the livers from the kinetic study) and human intestinal (jejunal) mucosa (n = 19, including intestines HI-31, 32 and 39 from the kinetic study). Metoprolol oxidation activities of the liver and jejunal microsomes were measured at 100 μM of metoprolol (i.e., under V_{max} condition).

Inhibitor Studies

The proportionate contributions of CYP2D6 and CYP3A in metoprolol oxidation activity were assessed by chemical inhibitor studies in two human livers and two jejunal microsomes. We performed inhibition studies with 2 μM quinidine (QD), a potent reversible CYP2D6-specific inhibitor, and 20 μM troleandomycin (TAO), a slowly reversible inhibitor of CYP3A. Metoprolol concentration was set at 20 μM, i.e., the K_M. Quinidine was co-incubated with metoprolol and reaction was initiated with the addition of NADPH. Troleandomycin was pre-incubated with the microsomes, in the presence of NADPH for 30 minutes, prior to starting the reaction by the

addition of metoprolol. The incubation condition for the measurement of metoprolol oxidation rates was the same as in the other experiments. Inhibitor experiments were conducted with microsomes from two livers (HL-133 and 143) and two jejunal mucosa (HI-35 and 39).

Kinetic Analysis

Initial formation rates of α-OH-M and ODM obtained from the kinetic studies were fitted simultaneously to a single enzyme Michealis-Menten model using the general purpose modeling program SAAM II (version 1.1). α-Hydroxylation and O-demethylation were assumed to have the same K_M, but different V_{max}'s. *In vitro* intrinsic clearance, $Cl_{int,mic}$, was calculated as V_{max}/K_M (μl/min/mg) for each pathway.

In Vitro-In Vivo Scaling

Mean microsomal intrinsic clearance for metoprolol per mg of microsomal protein ($\Sigma Cl_{int,mic}$) was computed by averaging the individual sums of $Cl_{int,mic}$ values for the two oxidative pathways of metoprolol from the kinetic studies in six human livers and five human intestines. Using CYP3A4 as the microsomal protein marker, Paine *et al.* (7) estimated the total microsomal proteins in an average-size human liver and human small intestine to be 78,750 and 2,977 mg, respectively. Accordingly, liver and small intestine intrinsic clearances ($Cl^h_{int,org}$, $Cl^g_{int,org}$) were scaled by multiplying $\Sigma Cl_{int,mic}$ by their respective organ content of microsomal protein. Using the well-stirred model for liver and intestinal clearance (12), we predicted the respective first-pass extraction ratio of metoprolol across the liver (E_h) and the gut mucosa (E_g) as follows.

$$E_h = \frac{f_u \cdot Cl^h_{int,org}}{f_u \cdot Cl^h_{int,org} + Q_h} \quad (1)$$

$$E_g = \frac{Cl^g_{int,org}}{Cl^g_{int,org} + Q_{muc}} \quad (2)$$

where Q_h was set at 1.5 L/min, Q_{muc} at 0.25 L/min (8,13), and f_u at 0.90 (14).

RESULTS AND DISCUSSION

Kinetics of Metoprolol Oxidation

Figure 1 shows a Lineweaver-Burke plot of α-OH-M and ODM formation kinetics in microsomes from six individual human livers. Acceptable regression fit of the data to a uni-enzyme Michealis-Menten model was observed for all the livers. Figure 2 presents the kinetic plots for microsomes isolated from the mucosa of five human jejuna. Preliminary analysis indicated that ODM formation rates at substrate concentrations >60 μM were consistently under-predicted, which suggested involvement of another low affinity CYP enzyme in the intestinal O-demethylation of metoprolol. An attempt to fit the data to a two-enzyme model failed to converge because the apparent K_M of the low affinity enzyme was much higher than 100 μM. Therefore, regression analysis of the intestinal kinetic data was limited to substrate concentrations ≤60 μM.

Regression estimates of the kinetic parameters for the six livers and five jejunal mucosa are summarized in Tables 1 and

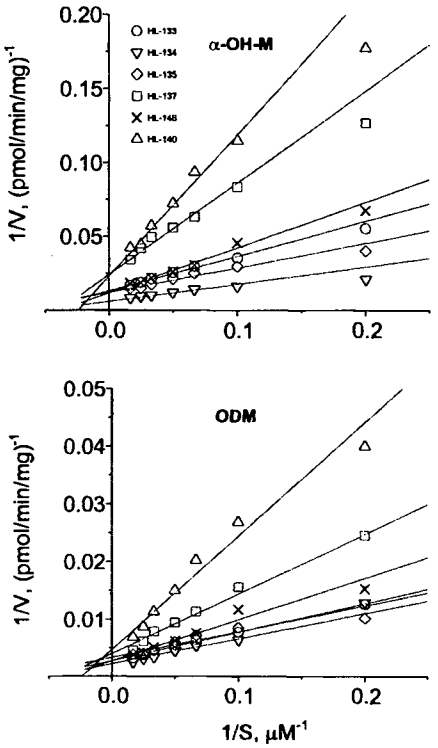

Fig. 1. Lineweaver-Burke plot of α-hydroxymetoprolol (top panel) and O-demethylmetoprolol (bottom panel) formation kinetics in six individual human liver microsomes. The lines represent predictions based on nonlinear regression estimates.

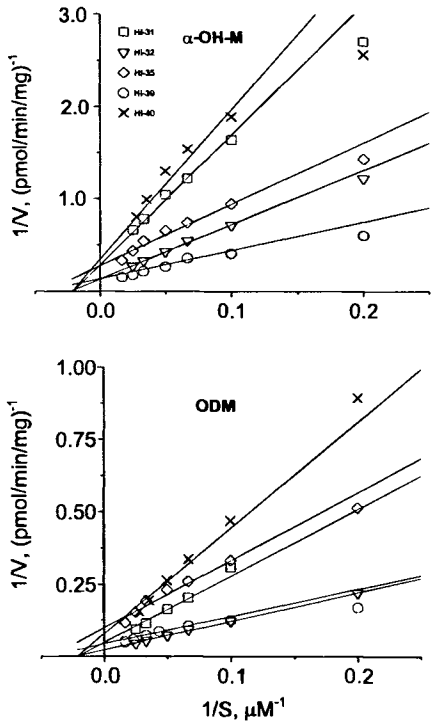

Fig. 2. Lineweaver-Burke plot of α-hydroxymetoprolol (top panel) and O-demethylmetoprolol (bottom panel) formation kinetics in five individual human intestinal microsomes. The lines represent predictions based on nonlinear regression estimates.

Table 1. Nonlinear Regression Estimates of Enzyme Kinetic Parameters for α-OH-M and ODM Formation Catalyzed by Human Liver Microsomes

Human liver #	K_M μM	V_{max} pmole/min/mg		$Cl_{int,mic}$ μl/min/mg		V_{max} ratio (ODM/α-OH-M)
		α-OH-M	ODM	α-OH-M	ODM	
133	23 (12)[a]	86 (6)	421 (7)	3.7	18.3	4.9
134	19 (12)	156 (6)	445 (7)	8.4	24.1	2.9
135	14 (13)	81 (5)	305 (6)	6.0	23.0	3.8
137	34 (12)	48 (7)	308 (8)	1.4	9.1	5.3
140	42 (8)	44 (5)	213 (27)	1.1	5.1	4.8
148	24 (13)	80 (7)	350 (8)	3.3	14.6	4.4
Mean	26	83	340	4.0	15.7	4.4
SD[b]	9	37	78	2.6	6.9	0.9

[a] Parenthetical value represents % coefficient of variation for the mean regression estimate.
[b] Standard deviation.

2. The average K_M value for metoprolol oxidation in liver microsomes was 26 ± 9 μM, a value similar to that reported by Kim *et al.* (11). The average V_{max} was 83 ± 37 pmole/min/mg protein for α-OH-M and 340 ± 78 pmole/min/mg protein for ODM. In comparison, a slightly higher average K_M of 44 ± 17 μM was estimated for the jejunal mucosal microsomes. The mean V_{max} value for mucosal microsomes was, on the other hand, much lower than liver microsomes; i.e., 5.5 ± 3.1 pmole/min/mg protein for α-OH-M and 25 ± 15 pmole/min/mg protein for ODM formation. The average ODM to α-OH-M ratio of V_{max} was comparable between the liver and intestinal microsomes (4.4 ± 0.9 versus 3.50 ± 0.8), indicating similar regioselectivity in metoprolol oxidation. Given the large difference in the V_{max} between the two tissues, *in vitro* intrinsic clearances (V_{max}/K_M) were about 30-fold greater in the liver than intestinal microsomes.

The somewhat higher K_M value for intestinal mucosa could reflect a difference in the non-specific binding of metoprolol to microsomal proteins/lipids. Since a 2-fold higher microsomal protein concentration was used in the gut microsomal incubations, metoprolol binding to the microsomal protein could be higher, and the free substrate concentration available to CYP2D6 could be lower than compared to liver microsomal incubations. By means of equilibrium dialysis, we determined that there was negligible binding of metoprolol to the microsomes at either 0.25 or 0.5 mg/ml of microsomal protein (data not shown). The difference in the K_M values probably reflects a small functional difference between hepatic and intestinal CYP2D6, possibly due to the difference in their microenvironments. A similar difference in K_M was previously observed for bufurolol 1′-hydroxylation, where the microsomal K_M value for the intestinal mucosa was twice that for the liver (3).

The average V_{max} values for the intestinal microsomes were only about 7% or 1/14th of those for the liver microsomes. This difference most likely reflects a much lower CYP2D6 content per mg microsomal protein in the intestinal mucosa. The mean CYP2D6 content for the jejunal microsomes that were used in the kinetics studies was about 6% or 1/16th of that for the liver microsomes; i.e., 1.11 ± 0.95 versus 17.5 ± 8.4 pmole/mg protein (see later section for more data from the full tissue panels). A difference in P450 reductase content (relative to CYP2D6) could also contribute towards the low catalytic activity of CYP2D6 in the small intestine. A recent study from our laboratory (7) indicates that, on average, P450 reductase activity per mg protein in mucosal microsomes from human small intestines was approximately 1/3 that of human liver microsomes (63 ± 50 versus 182 ± 52 nmol cytochrome c reduced/min/mg protein). Since CYP2D6 content per mg microsomal protein in the intestinal microsomes is so much lower (<1/16) than that of human liver microsomes, P450 reductase does not appear to be a relevant factor in the observed differences in the catalytic activity of CYP2D6 between the small intestine and the liver.

Table 2. Nonlinear Regression Estimates of Enzymes Kinetic Parameters for α-OH-M and ODM Formation Catalyzed by Human Intestinal Microsomes

Human intestine #	K_M μM	V_{max} pmole/min/mg		$Cl_{int,mic}$ μl/min/mg		V_{max} ratio (ODM/α-OH-M)
		α-OH-M	ODM	α-OH-M	ODM	
31	49 (27)[a]	3.5 (20)	22 (20)	0.07	0.44	6.3
32	45 (23)	7.6 (17)	45 (17)	0.17	1.01	5.9
35	18 (19)	3.1 (11)	9 (11)	0.20	0.50	2.8
39	47 (17)	9.7 (11)	31 (11)	0.21	0.67	3.2
40	41 (23)	2.6 (16)	12 (17)	0.06	0.28	4.4
Mean	44	5.5	25	0.15	0.47	3.5
SD[b]	17	3.1	15	0.07	0.20	0.8

[a] Parenthetical value represents % coefficient of variation for the mean regression estimate.
[b] Standard deviation.

Regional Variation

Oxidative activities of CYP2D6 as measured by the formation of α-OH-M and ODM at 100 μM metoprolol showed a modest variation (<2-fold) along the length of four intestines (data not shown). Activity tended to peak in the proximate jejunal sections and decreased toward the distal segment of the ileum. CYP2D6 protein followed a similar distribution along the small intestine. This pattern is not peculiar to CYP2D6. A similar regional distribution has been observed with the activities of other CYP enzymes (1,2,7,15).

Correlation of Activity and CYP2D6 Content

Western blots of liver and jejunal microsomes from a representative selection of donors are shown in Fig. 3 to illustrate the spectrum of CYP2D6 content.

Top portion of Fig. 4 shows the variation in microsomal CYP2D6 content across the entire panel of human livers (n = 31). Two of the 31 livers showed no detectable CYP2D6 protein. There was a 13-fold variation in CYP2D6 content among the other 28 livers. The median specific content of 13 pmole CYP2D6/mg microsomal protein in the liver panel constituted 4.3% of total spectral cytochrome P450 content (median = 300 pmole P450/mg protein).

The bottom portion of Fig. 4 shows the variation in CYP2D6 content in jejunal microsomes across the panel of human intestines (n = 19). CYP2D6 protein was not detected

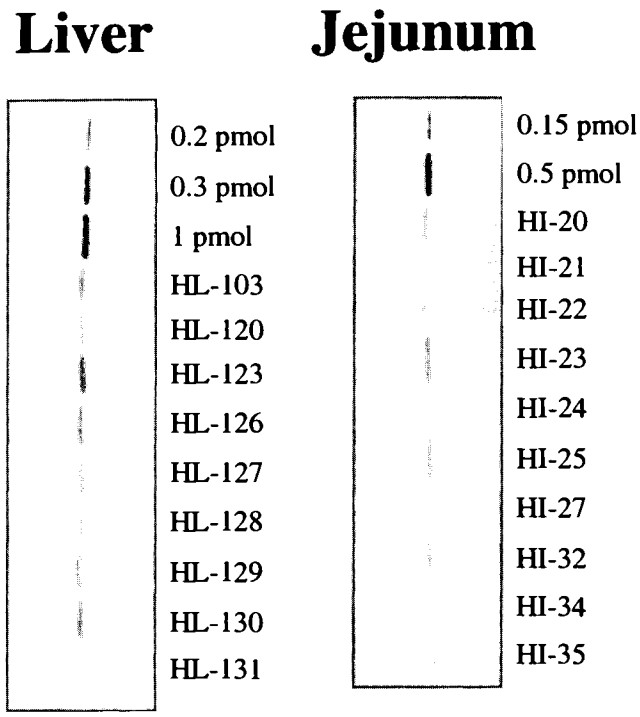

Fig. 3. Representative Western blots of microsomes prepared from a subset of human liver and jejunal mucosa along with purified cDNA-expressed CYP2D6 standards using a mouse monoclonal anti-CYP2D6 IgG.

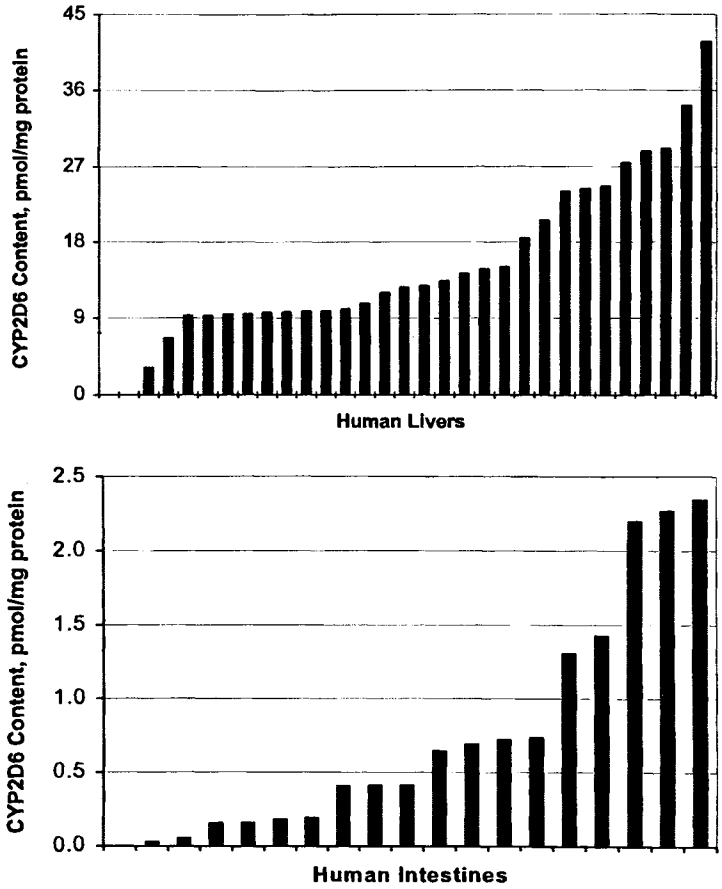

Fig. 4. Variation in CYP2D6 content across the panels of human liver microsomes (top), and human jejunal microsomes (bottom).

in one intestine. A nearly 100-fold variation in CYP2D6 content was observed in the remaining 18 intestines. The median content of 0.88 pmole CYP2D6/mg microsomal protein was only 1.75% of total P450 content, a much lower percentage than observed in the liver microsomes.

Absolute jejunal CYP2D6 content (median values) in the intestinal bank was 7% of that in the liver bank, which is much lower than the report of 20% by de Wazier *et al.* (1). This disagreement may be due to the small number of samples (8 intestines and 5 livers) and the pooling of duodena and jejuna in the earlier study.

Catalytic activities of CYP2D6 enzyme as measured by the formation rates of α-OH-M and ODM at 100 μM of metoprolol across the human liver and intestinal panels are shown in Fig. 5. At 100 μM, a concentration near the V_{max} regime, the median α-hydroxylation rate in the human liver bank was 43.6 pmole/min/mg, whereas the median O-demethylation formation was 242 pmole/min/mg. Variations in α-OH-M and ODM formation rate for the human liver bank were 20-fold and 12-fold, respectively (Fig. 5). α-Hydroxylation and O-demethylation rates across the panel of human livers were well correlated ($r = 0.89$, $p < 0.001$). The average metabolite formation ratio of ODM to α-OH-M was 4.9 ± 1.6. In comparison, the formation rates of α-OH-M and ODM across the intestinal microsomes varied by 56-fold and 68-fold, with respective medians of 2.02 and 24.6 pmole/min/mg. The intestinal metabolite formation ratio (ODM/α-OH-M) was, on average, three-fold higher than those for the panel of human livers (median of 12.7 versus 4.9). This appears to disagree with the results of our earlier kinetic study at lower substrate concentrations that showed comparable V_{max} ratios of the two metabolites in the few livers and intestines (Tables 2 and 3). Most likely, this discrepancy was the result of contribution of lower affinity, non-2D6 enzymes in the intestinal mucosa toward the formation of ODM at 100 μM of metoprolol (see below). A relatively modest correlation ($r = 0.67$, $p < 0.05$) existed between α-hydroxylation and O-demethylation rates across the panel of jejuna.

In the panel of human livers, both α-hydroxylation and O-demethylation rates correlated significantly with CYP2D6 protein content at 100 μM; the respective correlation coefficients were 0.84 ($p < 0.001$) and 0.75 ($p < 0.001$). Likewise, in the human intestinal bank, the formation rate of α-hydroxy-metoprolol correlated significantly with CYP2D6 content ($r = 0.75$, $p < 0.05$). However, the correlation between ODM formation rates and CYP2D6 content was poor and statistically not significant ($r = 0.33$, $p = 0.23$).

Inhibitor Experiments

The lack of correlation between ODM formation rate and intestinal CYP2D6 level, and the discrepancy in regioselectivity of metoprolol oxidation between the liver and the intestine in the tissue panel study may be explained by the contribution of other non-CYP2D6 enzymes in O-demethylation at the relatively high metoprolol concentration of 100 μM. To investigate the specificity of CYP2D6 for the oxidation of metoprolol, we conducted co-incubation experiments with 2 μM quinidine, which is a selective inhibitor of CYP2D6. Prueksaritanont *et*

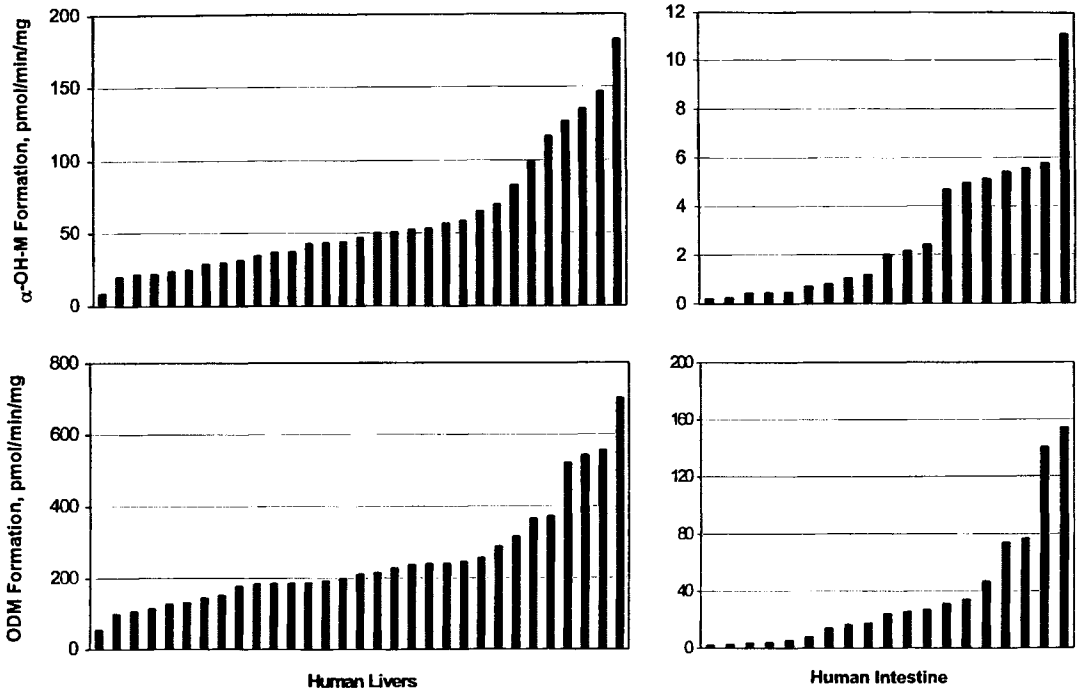

Fig. 5. Variation in microsomal formation rates of α-hydroxymetoprolol (α-OH-M, top panels) and O-demethylmetoprolol (ODM, bottom panels) at 100 μM of metoprolol across the panels of human livers (left) and human jejuna (right).

al. (3) have shown less complete inhibition of (+)-bufurolol oxidation by quinidine in human intestinal microsomes than in human liver microsomes (80% versus 96%). The same study also reports greater immuno-3A inhibition of (+)-bufurolol oxidation in intestinal microsomes than in liver microsomes (20% versus 10%). In view of these earlier data and since CYP3A's are the most abundant CYP isoforms in the intestinal mucosa, we also performed inhibition experiments with 20 μM troleandomycin (TAO), a selective inhibitor of CYP3A.

In microsomes from two human livers, α-hydroxymetoprolol formation was inhibited to an average extent of 96% and 3% by QD and TAO, respectively. Formation of ODM was inhibited to a lesser extent by quinidine (79%), and to a slightly larger extent by TAO (10%). The results confirm the CYP2D6 specificity in the oxidative metabolism of metoprolol in the liver. In an earlier study in human liver microsomes, Kim *et al.* (11) showed that low affinity enzyme(s) contribute to metoprolol hydroxylation and O-demethylation, but its contribution is only significant at substrate concentrations exceeding 500 μM.

In microsomes from two human intestinal mucosa, α-OH-M formation was inhibited to an average extent of 81% and 23% by QD and TAO, respectively. The corresponding values for ODM formation were 59% and 11%. Thus, while CYP2D6 remains the major oxidative enzyme for α-hydroxylation of metoprolol in the human small intestine, other low affinity P450 enzymes including CYP3A are clearly involved in the intestinal O-demethylation of metoprolol.

In Vitro-In Vivo Scaling

The first step in our *in vitro-in vivo* scaling was the prediction of organ intrinsic clearance ($Cl_{int,org}$) for the liver and small intestine. Based on the kinetic data from Tables 1 and 2, we computed the total *in vitro* intrinsic clearance per mg microsomal protein for each donor tissue by summing $Cl_{int,mic}$ values of α-hydroxylation and O-demethylation of metoprolol. This yielded mean values of 19.7 μl/min/mg for liver microsomes (n = 6) and 0.72 μl/min/mg for intestinal mucosal microsomes (n = 5). Based on our Western blot data and previously determined organ/tissue content of microsomal protein, we estimated the respective CYP2D6 content of the human liver and small intestine to be 1000 nmole and 2.9 nmole. From these estimates, we predicted an average scaled $Cl_{int,org}$ of 1.55 L/min for the liver and 0.002 L/min for the small intestine. Based on these values and the assumption of a well-stirred model, we predicted an average hepatic extraction ratio (E_h) of 48%; the range based on the individual $Cl_{int,mic}$ varied from 22 to 61%. Not surprisingly, a very low average first-pass intestinal extraction ratio (E_g) of 0.85% was predicted for metoprolol, with a range of 0.40 to 1.4%. Assuming that CYP-mediated metabolism at the small intestinal mucosa and the liver constitute the only mechanisms of first-pass removal (i.e., excluding the possibility of efflux transport of metoprolol at the intestinal epithelium and lung extraction), the overall first-pass extraction of metoprolol would be ~48%. Clinical studies have shown that the gastrointestinal absorption of metoprolol is complete. Hence, its overall first-pass extraction ratio can be directly estimated from oral systemic availability (i.e., 1-F). Based on the systemic availability data reported by Lennard *et al.* (16) for a 100 mg oral dose of metoprolol in 12 healthy volunteers, the average first-pass extraction was estimated to be about 50%, with a range of 35% to 77% in extensive metabolizers of debrisoquine. Our prediction of metoprolol first-pass extraction agrees well with the *in vivo* data from this human study.

Our estimate of a minimal intestinal contribution to the first-pass metabolism of metoprolol is not surprising in view of the very low content of CYP2D6 (~3%) in the small intestine compared to that in the liver. First principle dictates that the extent of first-pass intestinal mucosal metabolism is a function of two factors: intrinsic clearance of the metabolizing enzymes, and the residence time of the drug in the metabolic compartment. Intrinsic clearance is a function of the amount of enzymes and their catalytic efficiencies. Residence time is governed by the pharmacokinetics of the substrate in the intestinal epithelium; the longer the residence time the higher the extent of drug metabolism at the intestinal mucosa. The most basic factor controlling the epithelial residence time is the mucosal membrane permeability characteristics of the substrate, i.e., the rates of diffusion into and out of the enterocytes (17). The well-stirred model assumed that diffusion and/or transport across epithelium is/are not rate-limiting. Given the high lipid solubility of metoprolol and the good agreement between our first-pass prediction and *in vivo* data, this appears to be a justifiable assumption. We recognize that in the event diffusion across the basolateral membrane of the enterocyte becomes rate-limiting, intestinal CYP2D6 could mediate a significant inactivation of orally administered drug during first-pass (17). There are additional ways by which residence time of drug molecules at the enzyme site may be increased. Drug molecules may be sequestered inside the enterocytes and subsequently redistribute to the enzyme compartment. Likewise futile cycling by epithelial efflux pumps may effectively increase the residence time of the drug to the enzymatic site and increase local metabolism (18). In our scaling exercise, we did not invoke these biological factors in the small intestine or liver. We recognize that consideration of these factors may lead to prediction of a significant intestinal extraction for some CYP2D6 substrates.

In summary, the present study shows similar catalytic characteristics for CYP2D6 in the intestinal mucosa and the liver. CYP2D6 expression in the human small intestine is very low compared to that in the human liver. Hence, unless a CYP2D6 substrate is sequestered in the mucosa or undergoes futile cycling via an efflux transporter, intestinal metabolism is not expected to contribute significantly to its first-pass removal. The low presence of CYP2D6 activity may, nevertheless, assume clinical importance in the event it mediates the local formation of a cytotoxic metabolite that could lead to mucosal damage. As a side note, standard CYP2D6 activity markers for the liver may be less specific in the intestinal microsomes because of the low CYP2D6 activity and the greater relative contributions of other intestinal P450 enzymes (e.g., CYP3As).

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