Effect of CYP3A5 Expression on Vincristine Metabolism with Human Liver Microsomes

Jennifer B. Dennison, David R. Jones, Jamie L. Renbarger, and Stephen D. Hall
Division of Clinical Pharmacology, Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana
Received December 12, 2006; accepted January 31, 2007

ABSTRACT
Vincristine is preferentially metabolized to a secondary amine, M1, by CYP3A5 with a 9- to 14-fold higher intrinsic clearance than CYP3A4 using cDNA-expressed enzymes. The genetically polymorphic expression of CYP3A5 may contribute to interindividual variability in vincristine efficacy and toxicity. The current study quantifies the contribution of cytochromes P450 to vincristine metabolism with a bank of human liver microsomes (HLMs). M1 was the major metabolite formed with HLMs, and selective chemical inhibition of P450s confirmed that CYP3A was the major metabolizing subfamily. The liver tissues were genotyped for low expression alleles, CYP3A5*3, *6, and *7, and the HLMs were phenotyped for CYP3A4 and CYP3A5 expression by Western blot. Testosterone 6β-hydroxylation and itraconazole hydroxylation were used to quantify CYP3A4 activity in the HLMs. For each CYP3A5 high expresser (n = 10), the rate of M1 formation from vincristine due to CYP3A5 was quantified by subtracting the CYP3A4 contribution as determined by linear regression with CYP3A5*3/*3 samples. For CYP3A5 high expressers, the contribution of CYP3A5 to the metabolism of vincristine was 54 to 95% of the total activity, and the rate of M1 formation mediated by CYP3A5 correlated with CYP3A5 protein content (r² = 0.95). Selective inhibition of CYP3A4 demonstrated that the M1 formation rate with CYP3A5 high expressers was differentially inhibited based on CYP3A4 activity. Using median values, the estimated hepatic clearances were 5-fold higher for CYP3A5 high expressers than low expressers. We conclude that polymorphic expression of CYP3A5 may be a major determinant in the P450-mediated clearance of vincristine.

Received December 12, 2006; accepted January 31, 2007

This study was funded by National Institutes of Health Grant 1 K23 RR019956-01 and a Pharmaceutical Research and Manufacturers of America Foundation Pharmacology predoctoral fellowship.

ABBREVIATIONS: P450, cytochrome P450; HLM, human liver microsome; VCR, vincristine; VLB, vinblastine; VRL, vinorelbine; DDC, diethyldithiocarbamic acid; TST, testosterone; ITZ, itraconazole; OH-ITZ, hydroxyitraconazole; MeO-ITZ, methoxyitraconazole; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS/MS, tandem mass spectrometry; CsA, cyclosporin A; CL, clearance.

553
maximal rate of M1 formation ($V_{\text{max}}$) of CYP3A5 was 7- to 9-fold higher than that of CYP3A4 (Dennison et al., 2006). The highly selective oxidation of vincristine by CYP3A5 is unexpected because with previously studied CYP3A substrates, CYP3A4 has a near equal or higher metabolic efficiency compared with CYP3A5 (Williams et al., 2002). Thus, for individuals that express the enzyme, CYP3A5 may be important in the metabolism of vincristine and a factor in the observed interindividual variation in vincristine exposure and response.

In theory, the contribution of CYP3A5 to the hepatic clearance of a CYP3A substrate can be estimated using recombinant enzyme kinetic parameters and the amounts of CYP3A4 and CYP3A5 protein in the liver (Rodrigues, 1999), but this approach has two major limitations. First, investigators often report dissimilar kinetic parameters for the same substrate using cDNA-expressed enzymes. For example, the metabolism of midazolam to 1′-hydroxy-midazolam with CYP3A4 is described by Michaelis-Menten kinetics with a $V_{\text{max}}$ of 320 nmol/min/nmol (Patki et al., 2003). However, in another report, a substrate inhibition model is required, and a $V_{\text{max}}$ of 35 nmol/min/nmol is estimated (Williams et al., 2002). The second limitation arises because the CYP3A5 protein content for individuals with the CYP3A*5 allele is not consistent between microsomal liver banks; Westlind- Johnsson et al. (2003) estimates that CYP3A5 is 13 to 27% of the total CYP3A, whereas Lin et al. (2002) estimates a 40 to 80% contribution. To avoid these inconsistencies, we quantify the CYP3A5 contribution to vincristine metabolism using CYP3A5-genotyped human liver microsomes (HLMs). Subsequent in vitro-in vivo scaling is used to estimate the potential impact of CYP3A5 expression on the hepatic clearance for vincristine.

### Materials and Methods

**Chemicals.** Vincristine sulfate (VCR), vinblastine sulfate (VBL), vinorelbine ditartrate (VRL), diethyldithiocarbamic acid (DDC), testosterone (TST), 6β-hydroxytestosterone, N-desmethyl diazepam, ketoconazole, furafylline, coumarin, orphenadrine citrate, sulfaphenazole, omeprazole, quinidine, itraconazole (ITZ), and NADPH were purchased from Sigma-Aldrich (St. Louis, MO). Hydroxyitraconazole (OH-ITZ) and methoxyitraconazole (MeO-ITZ) were gifts from Janssen Pharmaceuticals (Beersel, Belgium). All other reagents were of HPLC grade and were purchased from Fisher Scientific (Pittsburgh, PA).

**HLMs and cDNA-Expressed P450s.** HLMs ($n = 56$) were prepared from human liver tissues from the Clinical Pharmacology Liver Bank (Indiana University, Indianapolis, IN) as described previously (Gorski et al., 1994). Protein concentrations of the HLMs were determined using the Lowry method (Lowry et al., 1951). Supersomes containing cDNA-expressed P450s (CYP3A4 and CYP3A5) coexpressed with P450-reductase with and without coexpressed cytochrome $b_5$ were purchased from the BD Gentest (Woburn, MA). The manufacturer provided the P450-reductase activities, protein concentrations, and the P450 content of the Supersomes.

**CYP3A5 Genotyping.** The human liver bank was genotyped for the CYP3A*5/3 and CYP3A5*6 allelic variants using allele-specific polymerase chain reaction methods and primers described previously (Hiratsuka et al., 2002) with SYBR Green detection (Le et al., 2004). A TaqMan allelic discrimination assay (Applied Biosystems, Darmstadt, Germany) was used to determine the CYP3A5*7 allelic variant (Esp et al., 2004).

**CYP3A Activity Assays for HLMs.** Testosterone $6\beta$-hydroxylation. TST in methanol was added to incubation buffer (100 mM Na$_2$HPO$_4$ with 5 mM MgCl$_2$, pH 7.4) for a final concentration of 200 $\mu$M, 0.3% (v/v) MeOH. HLMs (0.1 mg/ml) were preincubated at 37°C, and the reaction was initiated with NADPH (1 mM final concentration) for a total volume of 1 ml. Incubations without NADPH were used as negative controls. After 10 min, the reaction was quenched with an equal volume of chilled ethyl acetate and extracted with an additional 4 ml of ethyl acetate. N-Desmethyl diazepam was used as an internal standard. The solvent was evaporated at room temperature, and the residue was reconstituted in the HPLC mobile phase, 20 mM ammonium acetate, pH 6.4/methanol [40:60 (v/v)]. Chromatographic separation of 6β-hydroxytestosterone from TST and other related metabolites was performed on a C18 column (Luna, 4.6 × 150 mm, 5-μm particle size; Phenomenex, Torrance, CA) at a flow rate of 1 ml/min with UV detection at 254 nm. The limit of quantitation for 6β-hydroxytestosterone was 330 pmol/ml. The bank of HLMs ($n = 56$) was assayed in triplicate, and the coefficients of variation for the replicates were on average <5% with a maximum of 14%.

**Itraconazole hydroxylation.** ITZ was chosen as a substrate because it was reported to be a CYP3A4-specific substrate (Isoherranen et al., 2004). To confirm these results, cDNA-expressed CYP3A4 (10 pmol/ml; 2-min incubation) and CYP3A5 (100 pmol/ml; 15-min incubation) with coexpressed cytochrome $b_5$ were assayed for hydroxylase activity at 200 nM ITZ and compared with an NADPH-negative control. A higher protein concentration and longer incubation time were required for CYP3A5 because of its lower catalytic activity compared with CYP3A4. The rate of 6-hydroxylation at 200 nM ITZ with recombinant enzymes was 300-fold higher for CYP3A4 (1.5 pmol/min/pmol) versus CYP3A5 (5.3 × 10$^{-3}$ pmol/min/pmol). For HLM incubations, ITZ was added to incubation buffer (100 nM Na$_2$HPO$_4$ with 5 mM MgCl$_2$, pH 7.4) for a final concentration of 1 μM, 0.2% (v/v) MeOH. The HLMs (0.04–0.10 mg/ml) were preincubated at 37°C, and the reactions were initiated with NADPH (1 mM final concentration) in a total volume of 1 ml. After 2 min, the reactions were quenched with an equal volume of ethyl acetate/hexane [50:50 (v/v)] and 40 μl of 5% NaOH. A time of 2 min was chosen to ensure linear conditions because extended incubations cause the formation of secondary metabolites from ITZ as described previously (Isoherranen et al., 2004). To estimate the maximal velocity, the ITZ concentrations were chosen higher than the reported apparent $K_m$, 44 nM, for Supersomes (Isoherranen et al., 2004). The apparent $K_m$ for HLMs, approximately 50 nM, was estimated in a separate experiment (data not shown). Incubations without NADPH were used as negative controls. MeO-ITZ was added as an internal standard, and the solution was extracted with an additional 3 ml of ethyl acetate/hexane [50:50 (v/v)]. The organic layer was evaporated at room temperature, and the residue was reconstituted in the HPLC mobile phase, 20 mM ammonium acetate/40 mM ammonium formate (20:80 v/v) MeOH. Chromatographic separations of OH-ITZ were performed on a C18 column (Luna, 4.6 × 150 mm, 5-μm particle size; Phenomenex) at a flow rate of 1 ml/min. LC/atmospheric pressure chemical ionization-mass spectrometry (Finnigan Navigator; Thermo Electron Corporation, Waltham, MA) was used to monitor the following ions: m/z 721 (OH-ITZ), 705 (ITZ), and 733 (MeO-ITZ). The limit of quantitation for hydroxyitraconazole was 1.6 pmol/ml. Select HLMs ($n = 22$) were assayed in triplicate, and the coefficients of variation were on average <10% with a maximum of 18%.

**Western Blotting Analysis.** Immunodetectable CYP3A4 was quantified in select HLMs ($n = 22$), and purified CYP3A4 (14 nmol/mg protein; Invitrogen, Carlsbad, CA) was used as a standard. CYP3A4 standards at three concentrations (0.3–1.2 pmol or 0.75–3.0 pmol) and protein from typically four unknown HLMs were loaded on the same 15-lane gel. All samples were separated by at least three lanes, and lanes 1 and 15 were used for standards. To account for matrix effects, IUL-39, an HLM preparation in which CYP3A4 could not be detected, was added to the standards to give the same protein content as the unknown samples. Protein (20–50 μg) from HLMs was separated on a 9.6% Tris-HCl polyacrylamide gel (Bio-Rad Trans-blot apparatus; Bio-Rad, Hercules, CA), transferred to nitro-
cellulose, blotted with a rabbit anti-CYP3A4 primary antibody (WB-3A4; BD Gentest) and with a goat anti-rabbit secondary antibody (BD Gentest), and then developed using 5-bromo-4-chloro-3-indoyl phosphate and nitro blue tetrazolium. For each gel, standards and unknown samples were assayed at least in duplicate and quantified by densitometry (Kodak ID: Eastman Kodak, Rochester, NY). The standard curve for each blot was established using linear regression with the band intensities of the six standards, and for all standard curves, the linearity was maintained at the highest CYP3A4 concentration, confirming that the nitrocellulose was not overdeveloped. The band intensities of the unknown HLMs were between the values of the high and low standards on the blot. All unknown duplicates were within 15% of each other, or for more than two values, the coefficient of variation was no more than 15%. Likewise, the CYP3A5 content was determined for all high expressers of CYP3A5 (n = 10) in duplicate using a rabbit anti-CYP3A5 primary antibody (WB-3A5; BD Gentest) with 20 μg of loaded protein. CYP3A5 Supersomes (lot 22), loaded with protein from IUL-52 (an HLM with no detectable CYP3A5), were used for the standard curve (0.23–1.2 or 0.5–2.0 pmol). Supersomes can contain apoprotein not included in the P450 concentration provided by the manufacturer, so the purity of CYP3A5, lot 22, was directly compared with purified CYP3A4 using a nonspecific, monoclonal CYP3A antibody (WB-MAB-3A; BD Gentest). The immunoreactivity of CYP3A4 and CYP3A5 were equivalent. Thus, CYP3A5, lot 22, was used as the standard enzyme without correction. To verify low expression, CYP3A5 content at 150 μg of loaded protein was estimated for each of the CYP3A5*3/*3 HLMs. IUL-57 and IUL-71 exhibited low CYP3A5 expression contrary to genotype, and they were assayed on multiple gels to confirm that CYP3A5 levels were equivalent to CYP3A5 expression in CYP3A5*3/*3 HLMs.

**HLM Incubations with Vincristine.** All HLMs with at least one CYP3A5*1 allele (n = 12) and select HLMs with CYP3A5*3/*3 genotype (n = 10) were used to determine the Michaelis-Menten parameters associated with M1 formation from vincristine. The HLMs with CYP3A5*3/*3 genotype were chosen to include a range of CYP3A5 activities as determined by testosterone 6β-hydroxylation (700–11,900 pmol/mg/min). VCR (5, 10, 15, 25, 50, and 100 μM) dissolved in 100 mM Na2HPO4 with 5 mM MgCl2, pH 7.4, was preincubated with select HLMs (0.7–3.0 mg/ml) at 37°C. The reaction was initiated with the addition of 0.5 mM NADPH for a final incubation volume of 250 μl. Incubations without NADPH were used as negative controls. The incubation times (4–20 min) were chosen so that the initial substrate concentration was not reduced by more than 20%. In addition, the incubation conditions for the HLMs were within the linear range for protein concentration and incubation time. The supernatant (50 μl) was directly assayed by HPLC with UV detection at 254 nm. The Vmax and Km values were estimated for each HLM with average coefficients of variation of 4.2 and 11.1%, respectively.

**HPLC Analysis of M1.** An HPLC system (Agilent 1100 Series; Agilent Technologies, Santa Clara, CA) was used with UV detection (Hewlett Packard 1050 Series; Hewlett Packard, Palo Alto, CA) at a wavelength of 254 nm. Chromatographic separation of M1, VCR, and VRL (internal standard) was achieved with a C18 column (Inertsil ODS3, 3.0 × 150 mm, 5-μm particle size; MetaChem Technologies Inc., Torrance, CA) at a flow rate of 0.4 ml/min. The mobile phase consisted of 0.2% formic acid (mobile phase A) and methanol (mobile phase B). Analytes were eluted using a series of linear gradients: 0 min/20% B, 7 min/20% B, 42 min/56% B, 42.1 min/80% B, 52 min/80% B, and 52.1 min/20% B. M1 is not available as a standard, so a VCR standard curve was used to quantify M1 as described previously (Dennison et al., 2006). The vincristine sulfate starting material was not available, so the M1 content was determined by an epoxide impurity (M5) that did not increase during incubations with or without NADPH (LC-MS/MS data not shown). The epoxide content in the starting material was subtracted from the M1 area to determine the metabolite concentration.

**Contribution of Drug-Metabolizing P450s to M1 Formation.** Selective chemical inhibitors were used to quantify the contribution of human drug-metabolizing P450s to the formation of M1 from vincristine using HLMs (Rodrigues, 1999). Vinca alkaloids that are known CYP3A substrates, VLB and VRL, were used as additional positive controls (Rahmani and Zhou, 1993). HLMs (n = 5) were pooled by combining equal amounts of protein and used as the enzyme source in the chemical inhibitor studies. Two of the five pooled HLMs contained high levels of CYP3A5. VCR at 15 μM was incubated with furafylline (CYP1A2; 20 μM), coumarin (CYP2A6; 20 μM), orphenadrine (CYP2B6; 100 μM), sulfaphenazole (CYP2C9; 10 μM), omeprazole (CYP2C19; 10 μM), quinidine (CYP2D6; 5 μM), DDC (CYP2E1; 50 μM), ketoconazole (CYP3A; 1 μM), VB (10 μM), and VRL (10 μM). All chemical inhibitors were dissolved in methanol and evaporated to dryness. For competitive inhibitors, pooled HLMs (2 mg/ml), VCR dissolved in 100 mM Na2HPO4 with 5 mM MgCl2, pH 7.4, and 0.5 mM NADPH were added at 37°C for a final volume of 250 μl. The incubation was quenched with acetonitrile after 15 min, and VRL (or VB if an inhibitor coeluted with VRL) was used as an internal standard. VCR without inhibitor was used to determine the noninhibited rate of M1 formation. For mechanism-based inhibitors (DDC, furafylline, and orphenadrine), the inhibitor was preincubated with the HLM pool (2 mg/ml) and 0.5 mM NADPH for 15 min before adding 9.2 μl of VCR in water for a total volume of 250 μl. The reaction was then quenched after 15 min. The HLM pool incubated without inhibitor was used as the control. Sample preparation and HPLC/UV analysis of M1 were the same as described above for VCR metabolism. No interfering peaks from the inhibitors were detected for M1.

**Cyclosporin A Inhibition of CYP3A4 and CYP3A5.** The inhibitory effects of CsA on M1 formation at 20 μM VCR with CYP3A4, CYP3A5, and HLMs (three high and three low expressers of CYP3A5) were quantified. The concentration of CsA was varied from 0.4 to 25 μM at 0.3% MeOH (v/v). VCR in 100 mM Na2HPO4 with 5 mM MgCl2, pH 7.4, CsA, and enzyme (CYP3A4 or CYP3A5 at 30 pmol/ml or HLMs at 1.0–2.0 mg/ml) were coincubated at 37°C. The incubations were initiated with 0.5 mM NADPH in a total volume of 667 μl and quenched after 15 min (recombinant enzymes only) or 20 min. The vehicle controls contained 0.3% MeOH but no CsA, and the negative controls lacked NADPH. The fraction of activity not inhibited by CsA (f) and the inhibition dissociation constant (Kf) was estimated using a modified competitive inhibition Michaelis-Menten equation:

\[
V = (1 - f) \left( \frac{V_{\text{max}} \times S}{K_m + f \times K_i + S} + f \frac{V_{\text{max}} \times S}{K_m + S} \right)
\]

The maximal rates of M1 formation (Vmax) were determined from the positive control, and the Km values were determined from incubations without inhibitors. The substrate concentration (S) was a constant, 20 μM, and the inhibitor concentration (I) was the concentration of CsA. For IUL-79, the highest concentration of CsA was used to estimate the uninhibited fraction because the model did not fit the data.

**Protein Binding.** The fraction unbound of VCR with HLMs was quantified using ultrafiltration (Centrifree YM-30; Millipore Corporation, Bedford, MA). Pooled HLMs were assayed at 5 μM VCR in 100 mM Na2HPO4 with 5 mM MgCl2, pH 7.4, at 0.7, 1.2, 2.0, and 3.0 mg/ml protein. After the VCR solution was incubated at 37°C for 30 min, 800-μl aliquots were added in triplicate to the filtration devices and the control tubes to determine initial concentrations. Centrifugation conditions were chosen (37°C; 4 min; 1400g) so that less than 15% of the total volume was filtered. All samples used VRL as an internal standard and an equal volume of acetonitrile to precipitate protein. VCR and VRL were assayed by HPLC/UV as described above. The apparent fraction unbound (fapp) was determined as the
ratio of the unbound concentration in the filtrate to the total concentration. A partition approach assuming nonsaturable binding of VCR to protein and the ultrafiltration apparatus (Schuhmacher et al., 2000) was used to calculate the fraction unbound caused by nonspecific binding to the ultrafiltration device, \( f_{u,nob} \), and the fraction unbound at any protein concentration, \( f_u \), using \( f_{u,nob} \). The binding parameter, \( K \), and \( f_{u,nob} \) were estimated by nonlinear regression (WinNonlin 4.0; Pharsight, Mountain View, CA) for the pooled HLMs using the following relationship:

\[
f_{u,nob} = f_u = f_{u,nob} \left( \frac{1}{1 + (C_p \times K)} \right)
\]

(2)

**Hepatic Clearance.** For each HLM, a corresponding hepatic blood clearance of vincristine (\( CL_{H} \)) was estimated using the well-stirred model (Rowland et al., 1973):

\[
CL_H = Q_0 \left( \frac{f_{u,p} \times CL_{int}}{Q_H + f_{u,p} \times CL_{int}} \right)
\]

(3)

A plasma fraction unbound (\( f_{u,p} \)) of 0.51 (Mayer and St-Onge, 1995) and a blood-to-plasma ratio (\( \rho \)) of 1.2 (Bender et al., 1977) were used. Hepatic blood flow (\( Q_H \)) of 1500 ml/min for a 70-kg man was assumed. Intrinsic clearance (\( CL_{int} \)) was calculated from the microsomal Michaelis-Menten parameters \( V_{max}/K_m \) assuming a liver mass of 1.5 kg and a microsomal mass of 45 mg per g of liver (Houston and Carlile, 1997).

**Data Analysis.** For incubations with HLMs and VCR, the Michaelis-Menten constants, \( K_m \) and \( V_{max} \), were determined by fitting the data to a one-enzyme model. WinNonlin 4.0 (Pharsight) was used for all nonlinear least square regression analyses. The 95% prediction intervals for CYP3A5 low expressers were calculated using SigmaPlot 8.0 (Systat Software, Inc., Point Richmond, CA). The reported \( p \) values were determined using the Student’s \( t \) test or one-way analysis of variance for multiple comparisons.

**TABLE 1**

Characterization of human liver microsomes by CYP3A5 genotype, CYP3A4 and CYP3A5 protein content, and \( V_{max} \) with VCR, TST, and ITZ as substrates

<table>
<thead>
<tr>
<th>Liver</th>
<th>CYP3A5 Genotype</th>
<th>CYP3A4 Protein Content</th>
<th>CYP3A5 Protein Content</th>
<th>( V_{max} ) (ITZ-OH/ITZ)</th>
<th>( V_{max} ) (TST-6a-OH)</th>
<th>VCR (M1)</th>
<th>( K_m^a )</th>
<th>( CL_{int}^b )</th>
<th>( CL_{tot}^c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A5 high expressers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IUL-40</td>
<td>*1/*1</td>
<td>103.8</td>
<td>89.4</td>
<td>121.3</td>
<td>7244</td>
<td>1262</td>
<td>14.0</td>
<td>6070</td>
<td>823</td>
</tr>
<tr>
<td>IUL-32</td>
<td>*1/*2</td>
<td>20.9</td>
<td>18.2</td>
<td>18.1</td>
<td>1021</td>
<td>172</td>
<td>18.2</td>
<td>636</td>
<td>169</td>
</tr>
<tr>
<td>IUL-41</td>
<td>*1/*3</td>
<td>14.7</td>
<td>20.5</td>
<td>52.5</td>
<td>2886</td>
<td>467</td>
<td>26.2</td>
<td>1290</td>
<td>291</td>
</tr>
<tr>
<td>IUL-42</td>
<td>*1/*3</td>
<td>23.7</td>
<td>16.1</td>
<td>26.4</td>
<td>1518</td>
<td>302</td>
<td>25.1</td>
<td>813</td>
<td>210</td>
</tr>
<tr>
<td>IUL-59</td>
<td>*1/*3</td>
<td>36.3</td>
<td>14.7</td>
<td>51.6</td>
<td>3274</td>
<td>302</td>
<td>14.6</td>
<td>1400</td>
<td>328</td>
</tr>
<tr>
<td>IUL-66</td>
<td>*1/*3</td>
<td>24.4</td>
<td>20.6</td>
<td>35.7</td>
<td>2223</td>
<td>327</td>
<td>17.0</td>
<td>1300</td>
<td>310</td>
</tr>
<tr>
<td>IUL-73</td>
<td>*1/*3</td>
<td>96.1</td>
<td>20.1</td>
<td>147.0</td>
<td>6564</td>
<td>521</td>
<td>15.9</td>
<td>2220</td>
<td>461</td>
</tr>
<tr>
<td>IUL-74</td>
<td>*1/*6</td>
<td>10.7</td>
<td>26.2</td>
<td>13.5</td>
<td>1958</td>
<td>470</td>
<td>16.1</td>
<td>1970</td>
<td>424</td>
</tr>
<tr>
<td>IUL-79</td>
<td>*1/*3</td>
<td>11.2</td>
<td>13.6</td>
<td>15.4</td>
<td>1291</td>
<td>201</td>
<td>17.0</td>
<td>803</td>
<td>208</td>
</tr>
<tr>
<td>IUL-85</td>
<td>*1/*3</td>
<td>85.5</td>
<td>24.8</td>
<td>39.4</td>
<td>4013</td>
<td>393</td>
<td>20.7</td>
<td>1280</td>
<td>305</td>
</tr>
<tr>
<td>CYP3A5 low expressers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IUL-6</td>
<td>*3/*3</td>
<td>19.0</td>
<td>0.8</td>
<td>33.8</td>
<td>3082</td>
<td>80</td>
<td>22.2</td>
<td>244</td>
<td>70</td>
</tr>
<tr>
<td>IUL-49</td>
<td>*3/*3</td>
<td>48.8</td>
<td>0.8</td>
<td>52.3</td>
<td>2511</td>
<td>68</td>
<td>23.8</td>
<td>193</td>
<td>56</td>
</tr>
<tr>
<td>IUL-52</td>
<td>*3/*3</td>
<td>14.8</td>
<td>0.0</td>
<td>8.4</td>
<td>1337</td>
<td>9</td>
<td>11.4</td>
<td>52</td>
<td>16</td>
</tr>
<tr>
<td>IUL-55</td>
<td>*3/*3</td>
<td>130.4</td>
<td>0.8</td>
<td>136.2</td>
<td>7260</td>
<td>245</td>
<td>29.8</td>
<td>555</td>
<td>150</td>
</tr>
<tr>
<td>IUL-57</td>
<td>*3/*3</td>
<td>40.6</td>
<td>0.8</td>
<td>41.1</td>
<td>3947</td>
<td>104</td>
<td>31.2</td>
<td>225</td>
<td>65</td>
</tr>
<tr>
<td>IUL-65</td>
<td>*3/*3</td>
<td>15.6</td>
<td>0.6</td>
<td>14.7</td>
<td>1139</td>
<td>18</td>
<td>10.7</td>
<td>115</td>
<td>34</td>
</tr>
<tr>
<td>IUL-71</td>
<td>*1/*7</td>
<td>10.9</td>
<td>1.1</td>
<td>41.9</td>
<td>2950</td>
<td>50</td>
<td>15.8</td>
<td>213</td>
<td>61</td>
</tr>
<tr>
<td>IUL-72</td>
<td>*1/*7</td>
<td>25.3</td>
<td>0.9</td>
<td>27.1</td>
<td>2100</td>
<td>40</td>
<td>20.6</td>
<td>132</td>
<td>38</td>
</tr>
<tr>
<td>IUL-75</td>
<td>*3/*3</td>
<td>78.2</td>
<td>2.3</td>
<td>108.2</td>
<td>4588</td>
<td>145</td>
<td>15.6</td>
<td>609</td>
<td>165</td>
</tr>
<tr>
<td>IUL-78</td>
<td>*3/*3</td>
<td>109.5</td>
<td>3.1</td>
<td>148.7</td>
<td>6993</td>
<td>198</td>
<td>19.2</td>
<td>695</td>
<td>183</td>
</tr>
<tr>
<td>IUL-81</td>
<td>*3/*3</td>
<td>71.7</td>
<td>2.0</td>
<td>65.2</td>
<td>3424</td>
<td>132</td>
<td>22.5</td>
<td>395</td>
<td>110</td>
</tr>
<tr>
<td>IUL-86</td>
<td>*3/*3</td>
<td>77.8</td>
<td>2.1</td>
<td>160.0</td>
<td>8851</td>
<td>288</td>
<td>23.0</td>
<td>844</td>
<td>217</td>
</tr>
</tbody>
</table>

* Corrected for microsomal binding to vincristine.
* Intrinsic clearance = \( CL_{int} = V_{max}/1500 \) g liver \cdot 45 mg microsomal protein/g liver \cdot \( K_m \) for a 70-kg man.
* Hepatic clearance = \( CL_H = f_{u,p} \times CL_{int}(Q + f_{u,p} \times CL_{int}) \), where \( f_{u,p} = 0.51, \rho = 1.2, \) and \( Q = 1500 \) ml/min for a 70-kg man.
* HLMs not selected randomly.

**Results**

**CYP3A5 Expression for HLMs.** The bank of HLMs (\( n = 56 \)) was genotyped for CYP3A5*1, *6, and *7, alleles previously associated with low expression of CYP3A5. One liver, IUL-40, was homozygous, and 11 livers were heterozygous for CYP3A5*1 (Table 1). CYP3A5 expression in these livers was quantified by Western blot (Table 1), and all but two livers (IUL-57 and IUL-71) showed high levels of CYP3A5 expression. The CYP3A5 expression in IUL-57 and IUL-71 was comparable with other livers with the CYP3A5*3/*3 genotype (Fig. 1). General enzyme degradation was eliminated as a possible explanation for the low CYP3A5 expression because CYP3A4 activities as measured by ITZ hydroxylase activity and CYP3A4 protein contents of IUL-57 and IUL-71 were typical of other CYP3A5 high expressers (Table 1). Thus, IUL-57 and IUL-71 were classified as CYP3A5 low expressers contrary to genotype (Table 1).

**CYP3A4 Expression and Activity for HLMs.** The testosterone 6b-hydroxylase activity, itraconazole hydroxylase activity, and CYP3A4 content by Western blot were determined for select HLMs with low and high CYP3A5 expression (Table 1). The testosterone 6b-hydroxylase activity was correlated to CYP3A4 content and itraconazole activity \( (r^2 = 0.77 \) and 0.98, respectively), but it did not differ between CYP3A5 high and low expressers (Fig. 2). Thus, no contribution from CYP3A5 to testosterone 6b-hydroxylation was evident. For CYP3A5 high expressers with only one CYP3A5*1 allele, the CYP3A4 and testosterone 6β-hydroxylated protein contents (Table 1) were not correlated \( (r^2 = 0.07) \).
Formation of M1 with HLMs. The formation of VCR metabolites, including compounds described previously from recombinant enzyme incubations (M1, M2, M3, and M4), was quantified for select HLMs (n = 22) (Dennison et al., 2006). M1 was identified as the major metabolite by comparing the chromatograms to controls lacking NADPH. M4 was a minor metabolite with a rate of formation 10% or less than M1 for HLMs with low and high CYP3A5 expression. Representative chromatograms for incubations with IUL-73 and the corresponding negative control are shown in Fig. 3. The chemical identification of M1, a secondary amine formed by oxidation of the dihydro-hydroxycatharanthine unit of vincristine, and M4 was confirmed by LC-MS/MS (data not shown) as discussed previously (Dennison et al., 2006). The V\(_{\text{max}}\) and K\(_{\text{m}}\) values, corrected by microsomal protein binding, of M1 formation for the HLMs are listed in Table 1. The V\(_{\text{max}}\) values for the CYP3A5 high and low expresser groups were not directly compared because the CYP3A5 low expressers were not selected randomly and therefore did not represent the population. An alternative approach (see Effect of CYP3A4 and CYP3A5 Expression on M1 Kinetics) was used to compare V\(_{\text{max}}\) values between the groups. The K\(_{\text{m}}\) values between the groups were directly compared because the K\(_{\text{m}}\) values were not correlated to V\(_{\text{max}}\) values (r\(^2\) = 0.26) in the
CYP3A5 low expresser group. The \( K_m \) values for CYP3A5 high and low expressers (averages of 18.4 and 20.5 \( \mu \)M, respectively) were not statistically different (\( p = 0.49 \)). The \( K_m \) values of M1 formation using HLMs and cDNA-expressed CYP3A5 and CYP3A4 with coexpressed cytochrome \( b_5 \) (16.7 and 19.9 \( \mu \)M, respectively; Dennison et al., 2006) were in good agreement. The \( K_m \) values of the recombinant enzymes were not corrected for nonspecific binding because the incubations had low protein concentrations (\( C_p \leq 0.55 \) mg/ml) corresponding to \( f_o \geq 0.92 \) using eq. 2 and the estimated parameter \( K \) from HLMs.

**P450 Chemical Inhibition Screen.** To quantify the contribution of hepatic drug-metabolizing P450s in VCR metabolism, the rates of M1 formation were determined with selective chemical inhibitors in pooled HLMs at 15 \( \mu \)M VCR (Fig. 4). The rates of M1 formation in incubations with omeprazole, quinidine, ketoconazole, VLB, and VRL were statistically lower than the control incubations (\( p < 0.05 \)). Only known CYP3A substrates VLB and VRL and the CYP3A inhibitor ketoconazole inhibited the M1 formation rate by more than 25%. The rate of VCR disappearance was only significantly reduced by ketoconazole, VLB, and VRL (\( p > 0.05 \)).

**Effect of CYP3A4 and CYP3A5 Expression on M1 Kinetics.** The \( V_{max} \) values of M1 formation from VCR for CYP3A5 low expressers were correlated with CYP3A4 activity and protein content: testosterone 6\( \beta \)-hydroxylase activity (\( r^2 = 0.89 \); Fig. 5A), itraconazole hydroxylase activity (\( r^2 = 0.91 \); Fig. 5B), and CYP3A4 protein content (\( r^2 = 0.75 \); Fig. 5C). The CYP3A5 low expressers were assumed to have an insignificant amount of CYP3A5 activity, compared with CYP3A4 activity, in the formation of M1. For the HLMs with CYP3A5 low expression, regression lines and corresponding 95% prediction intervals for the M1 \( V_{max} \) values were calculated using each measure of CYP3A4 (activity or protein content; Fig. 5). Using these prediction intervals, the \( V_{max} \) values from the CYP3A5 high expressers were compared with the CYP3A5 low expressers. Regardless of CYP3A4 measure, the \( V_{max} \) values of all the CYP3A5 high expressers were above the 95% prediction intervals established by the CYP3A5 low expressers. To determine the specific contribution of CYP3A5 to M1 formation at \( V_{max} \) for CYP3A5 high expressers, the CYP3A4 contributions were subtracted from the \( V_{max} \) values. For each CYP3A5 high expresser, the CYP3A4 contribution was the corresponding best-fit \( V_{max} \) value of the CYP3A5 low expressers at a specific CYP3A4 measurement (activity or protein content). The resulting CYP3A5 contributions (percentage of total activity; Table 2) for the CYP3A5 high expressers were in good agreement with each other, regardless of the CYP3A4 value (activity or protein content) used to determine the CYP3A4 contribution to M1 formation. The average contribution of CYP3A5 to the formation of M1 for CYP3A5 high expressers at \( V_{max} \) was 81% of the total activity. Thus, VCR metabolism to M1 was primarily mediated by CYP3A5 in CYP3A5 high expressers at \( V_{max} \). The CYP3A5 contributions to the rate of M1 formation using intrinsic clearances, rather than \( V_{max} \) values, were also quantified because therapeutic concentrations of VCR are less than 1 \( \mu \)M, well below the \( K_m \). However, as indicated earlier, the \( K_m \) values of M1 formation for CYP3A5 high and low expressers were not statistically different (\( p = 0.49 \)). As a result, the calculated CYP3A5 contributions for CYP3A5 high expressers using \( V_{max} \) values were similar to the CYP3A5 contributions using intrinsic clearances (data not shown). The CYP3A5 activities of M1 formation for the CYP3A5 high expressers, calculated by subtraction of the CYP3A4 contribution using ITZ hydroxylase activities, were correlated to the CYP3A5 protein content (\( r^2 = 0.95 \); Fig. 6). The correlation was highly influenced by IUL-40 because its CYP3A5 protein content was much higher than those of the other HLMs (Table 1); yet, even without including IUL-40 in the analysis, the CYP3A5 activities were correlated to the CYP3A5 protein content (\( r^2 = 0.63 \)). The CYP3A5 M1 activities using testosterone 6\( \beta \)-hydroxylation to calculate the CYP3A4 contribution were almost identical to the CYP3A5 activities calculated using itraconazole hydroxylation as a CYP3A4 standard (Table 2). Thus, the CYP3A5 activities derived using testosterone 6\( \beta \)-hydroxylation were also corre-
lated to CYP3A5 protein content ($r^2 = 0.96$). The specific activities of M1 formation for CYP3A4 and CYP3A5 in HLMs were calculated using the protein contents as quantified by Western blot and the maximal rates of M1 formation. For the CYP3A5 high expressers, the ITZ hydroxylase activity was used to calculate the CYP3A4 contribution to the rate of M1 formation as described above. The specific activities were 7-fold higher for CYP3A5 (Fig. 7) and consistent with the previously reported values obtained using cDNA-expressed CYP3A4 and CYP3A5 with coexpressed cytochrome $b_5$ (Dennison et al., 2006).

**CsA Inhibition of HLMs.** The potential for CsA to exert a selective, competitive inhibition of CYP3A4 activity was tested using the rate of M1 formation with cDNA-expressed CYP3A4 and CYP3A5 (Fig. 8A). CsA was a potent inhibitor of M1 formation using CYP3A4, whereas the CYP3A5 activity was only reduced 15%. Thus, CsA was used as a CYP3A4 selective inhibitor, and the inhibition of M1 formation by CsA was determined for HLMs with low (Fig. 8B) and high expression of CYP3A5 (Fig. 8C). The M1 rate of formation for CYP3A5 low expressers was not abolished by CsA, and the remaining activity as estimated by the modified Michaelis-Menten equation (eq. 1) was 27% for IUL-6 and less than 10% for IUL-78 and IUL-55. The percentages of activity remaining after CsA inhibition for the CYP3A5 high expressers, IUL-79, -59, and -73, were in the same rank order as the previously estimated CYP3A5 percentage of contributions (Table 2). The percentage of contributions of CYP3A5 as determined by CsA inhibition was probably underestimated because of CYP3A5 nonselective inhibition by CsA using cDNA-expressed CYP3A5 (Fig. 8A).

**Protein Binding.** The fraction unbound at the lowest tested concentration of VCR, 5 $\mu$M, in HLM preparations was estimated by ultrafiltration. For the negative controls in buffer without protein, the VCR fraction unbound was consistently lower than in samples with HLMs and plasma. Thus, the negative controls in buffer could not be used to correct for nonspecific binding of VCR to the ultrafiltration apparatus. Pretreatment of the device with $1\%$ bovine serum albumin, as described previously (Mayer and St-Onge, 1995), improved the recovery of the buffer control to 77 versus 66%, but it did not completely remove the nonspecific binding of VCR to the apparatus. An alternative approach (eq. 2) was used to estimate the fraction unbound caused by nonspecific binding ($f_{unab}$) and the parameter $K$ ($0.161 \pm 0.05 \text{ ml/mg}$) with nonlinear regression. These values were used to calculate the fraction unbound ($f_u$) at any protein concentration ($C_p$) and then to correct the $K_m$ values for the HLMs ($f_u = 0.67–0.90$; Table 1).

**Estimation of Clearance.** The hepatic clearance of VCR was estimated for each HLM using the well stirred model (eq. 3). M1 was assumed to be the only metabolite formed from VCR, and the rate of M1 formation was assumed to be equal to the rate of VCR disappearance. The testosterone 6$\beta$-hydroxylase activities of CYP3A5 low expressers were correlated to the rate of M1 formation (Fig. 5A). Thus, for any HLM that was not incubated with VCR, all CYP3A5 low expressers, the M1 maximal rate of formation from VCR was estimated by linear regression using the testosterone 6$\beta$-hydroxylase activity. The average $K_m$ (20.5 $\mu$M) of the CYP3A5 low expressers was used for each HLM not incubated with VCR. The intrinsic and hepatic clearances of the

---

**Fig. 5.** Maximal rate of M1 formation with human liver microsomes versus testosterone 6$\beta$-hydroxylase activity (A), itraconazole hydroxylase activity (B), and CYP3A4 protein content (C). Microsomes are separated by CYP3A5 expression: $\blacktriangledown$, low expression; $\odot$, heterozygous high expression (CYP3A5*1/*0); and $\bullet$, homozygous high expression (CYP3A5*1/*1). Two microsomes (IUL-57 and IUL-71) with a genotype of CYP3A5*1/*0 are plotted as CYP3A5 low expressers.
from both groups was not normally distributed. The role of the genetically polymorphic CYP3A5 in the metabolism of CYP3A substrates is unclear. In clinical studies, the presence of at least one CYP3A5*1 allele is correlated to higher clearance of tacrolimus, a common immunosuppressant (MacPhee et al., 2002). However, no association between clearance and CYP3A5 genotype is consistently observed for other CYP3A substrates, such as midazolam, despite the fact that in vitro estimated intrinsic clearances using cDNA-expressed enzymes predict higher clearances for individuals with the CYP3A5*1 allele (Williams et al., 2002). CYP3A5 expression, in theory, may increase the clearance of midazolam, but in vitro data with human liver microsomes show that CYP3A5 expression increases midazolam 1'-hydroxylase activity only modestly (1.4-fold higher intrinsic clearance; Huang et al., 2004) or has no effect on midazolam 1'-hydroxylase activity (He et al., 2006). Midazolam is a CYP3A substrate without selectivity for CYP3A5 (Williams et al., 2002); therefore, compared with other variables such as CYP3A4 expression, the CYP3A5 genotype may play a minor role in the overall interindividual variability of drug clearance for midazolam. Unlike midazolam, vincristine is preferentially metabolized by CYP3A5 in vitro (9- to 14-fold higher intrinsic clearances than CYP3A4; Dennison et al., 2006). In the current study with vincristine, in vitro data from human liver microsomes is used to predict in vivo intrinsic and hepatic clearances and to evaluate the impact of CYP3A5 expression on drug disposition. For heterozygous CYP3A5*1 individuals with high CYP3A5 expression, the median intrinsic and hepatic clearances of vincristine are 7- and 5-fold higher, respectively, compared with CYP3A5 low expressers. For the CYP3A5*1/*1 individual in the liver bank, the estimated intrinsic clearance is 33-fold higher than the median value of the CYP3A5 low expressers. These differences may be clinically relevant if hepatic P450 metabolism is responsible for the majority of the drug clearance. In vivo, an extensive metabolizer of vincristine may be underdosed, and a poor metabolizer, especially an individual with low CYP3A4 activity, may be at a higher risk for vincristine-mediated neurotoxicity. In the case of a coadministered CYP3A4-selective inhibitor, CYP3A5 expression may also protect patients from adverse events associated with vincristine overdose. A similar protective effect of CYP3A5 expression on platelet aggregation is described with clopidogrel treatment and the coadministration of itraconazole (Suh et al., 2006).

Discussion

The genotypic/phenotypic relationship for CYP3A5*3,*6, and *7 alleles and low expression of CYP3A5 is well established (Hustert et al., 2001; Kuehl et al., 2001). From the liver bank in this study, two HLMs (IUL-57 and IUL-71) with at least one CYP3A5*1 allele (n = 12) express low levels of CYP3A5 by Western blot. The CYP3A5 phenotype of these two HLMs is additionally confirmed by metabolism of vincristine to M1. As shown by the linear regression plots (Fig. 5), the V_{max} values of IUL-57 and IUL-71 are typical of the M1 formation by CYP3A5*3/*3 HLMs at their measured CYP3A4 activity. The cause of the discrepancy between CYP3A5 genotype and phenotype for these HLMs is unknown. Enzyme degradation does not seem to be a factor.
because the activities of other P450 enzymes, including CYP3A4, for IUL-57 and IUL-71 are within the normal range of the other HLMs. A rare CYP3A5 mutation may be responsible for the low expression of CYP3A5, and the CYP3A5 genes of IUL-57 and IUL-71 will be sequenced to test this possibility.

To understand the role of CYP3A4 variability in vincristine metabolism, CYP3A activity of the liver bank was characterized using TST, a typical probe substrate, and ITZ, a substrate reported to be exclusively metabolized by CYP3A4, not CYP3A5 (Isoherranen et al., 2004). Our results confirm that OH-ITZ is selectively formed by CYP3A4 (300-fold higher Vmax) compared with CYP3A5. TST is known to have at least a 10-fold selectivity in the rate of 6β-hydroxylation with CYP3A4 versus CYP3A5 at 200 μM (Williams et al., 2002). For livers in this study, the ITZ and TST activities are highly correlated as expected, but the CYP3A5 high expressers are not distinguishable from the low expressers (Fig. 2B). Other investigators observe differences in the metabolism of ITZ and TST with HLMs but at lower TST concentrations (Huang et al., 2004). We conclude that ITZ and TST are selective probe substrates of CYP3A4 using the methods we describe.

Vincristine is metabolized by cDNA-expressed CYP3A4 and CYP3A5 to a primary metabolite, M1 (Dennison et al., 2006). Likewise, in the current study, M1 is the primary metabolite of HLMs, and only known CYP3A inhibitors, ketoconazole and other Vinca alkaloids (also CYP3A substrates), inhibit the formation of M1 more than 25% in pooled HLMs with moderate CYP3A5 expression. Two chemical inhibitors of CYP2C19 and CYP2D6 (omeprazole and quinidine) reduce the formation of M1 by 22 to 25%. However, previously presented data with cDNA-expressed enzymes (CYP2C19 and CYP2D6) support a minor role for the enzymes in vincristine depletion and M1 formation (Dennison et al., 2006). A Km value as low as 12 μM for 3-hydroxylation of omeprazole is reported for CYP3A4 (Li et al., 2005). Therefore, CYP3A3-nonselective effects may be in part responsible for the observed chemical inhibition with 10 μM omeprazole.

Based on published inhibitor concentrations, 5 μM quinidine seems unlikely to inhibit CYP3A4 (Obach et al., 2006), but as with omeprazole, the inhibition of CYP3A5 with quinidine is unknown.

Chemical inhibition of CYP3A in HLMs and the relative rates of M1 formation using cDNA-expressed enzymes provide evidence that CYP3A4 and CYP3A5 are the major enzymes in the metabolism of vincristine. Most CYP3A substrates are metabolized by both CYP3A4 and CYP3A5, so the exact contribution of CYP3A5 to the metabolism of a CYP3A substrate is difficult to determine when both enzymes are present, as with HLMs. The role of CYP3A5 in the metabolism of CYP3A substrates is evaluated by Huang et al. (2004) using pairs of HLMs with similar CYP3A4 protein content but different CYP3A5 expression. The pairing of liver samples by CYP3A4 content assumes that the CYP3A4 protein measured by Western blot is enzymatically equal for all HLMs. For the IU liver bank, CYP3A4 does correlate to CYP3A3 activity (r2 = 0.77), but the relationship is not fully explained by protein content. A highly selective CYP3A4 activity probe, itraconazole, is also used by Huang et al. (2004) to normalize the activities of CYP3A3 test substrates. For this type of analysis, statistical differences of the normalized activities between high and low CYP3A5-expressing groups can be calculated, but the technique cannot evaluate the contribution of CYP3A5 at different expression levels of CYP3A4. We use an alternative, general method that avoids these shortcomings. With vincristine as the test CYP3A substrate, CYP3A5 low expressers with a range of CYP3A4 activities are used to establish a baseline activity relationship, i.e., M1 Vmax versus 6β-OH TST Vmax (Fig. 5). As expected, the M1 rate of formation highly correlates to CYP3A4 activity and protein content for the CYP3A5 low expressers, so CYP3A4 is a major enzyme in the metabolism of vincristine for these livers. For CYP3A5 high-expressing livers, the rate of M1 formation due to CYP3A4 is calculated from the M1 Vmax versus CYP3A4 (activity or protein content) relationship obtained for CYP3A5 low expressers. The CYP3A4-mediated rate of M1 formation is then subtracted from the total rate to give the CYP3A5-catalyzed formation...
rate of M1. For all HLMs that express high levels of CYP3A5, the majority of the metabolism is mediated by CYP3A5 (54–95%), and the overlap of M1 activity between the high and low expressers of CYP3A5 is explained by CYP3A4 activity and expression. The M1 formation rate due to CYP3A5 correlates to CYP3A5 protein content as determined by Western blot. Consistent with the recombinant enzyme model, the rate of M1 formation in HLMs is highly selective for CYP3A5 (7-fold higher specific activity). The selective, competitive inhibition of CYP3A4 by CsA demonstrates the importance of CYP3A5 in the metabolism of VCR in HLMs with high CYP3A5 expression. In this case, the rates of M1 formation from HLMs with the highest CYP3A4/CYP3A5 protein ratios are most repressed using CsA as an inhibitor. Collectively, this evidence is the first to show major differences in vincristine metabolism between high and low expressers of CYP3A5 in human liver tissue.

To allow direct comparison with published pharmacokinetic parameters, the estimated blood clearances from the HLMs are converted to plasma clearances with the blood-to-plasma ratio (1.2) and normalized by mass (70 kg). The median values of 1.3 (low expressers) and 6.8 ml/min/kg (high expressers) are consistent with reported plasma clearances of 1.8 to 3.7 ml/min/kg in adult patients (Sethi et al., 1981; Nelson, 1982). However, comparison of the calculated
hepatic clearance values with this clinical data may have limited utility because the radioimmunoassays used in these studies cannot separate vincristine from related compounds (Nelson, 1982), possibly including vincristine metabolites or degradation products. A recent adult study that uses HPLC-MS/MS to specifically quantify vincristine reports a higher average plasma clearance of 7.1 ml/min/kg (Villikka et al., 1999). The disposition of vincristine and its metabolites in vivo is required to understand the importance of CYP3A5 genotype on drug clearance and ultimately to justify any individualized treatment.

Acknowledgments

We thank Robert J. Barbuch (Eli Lilly & Co., Indianapolis, IN) for M1 and M4 structure verification by LC-MS/MS.

References


Address correspondence to: Dr. Stephen D. Hall, Division of Clinical Pharmacology, Department of Medicine, Indiana University School of Medicine, 1001 West 10th St., W1723, Indianapolis, IN 46202. E-mail: sshall@iuui.edu