INTERACTION OF DELAVIRDINE WITH HUMAN LIVER MICROSONAL CYTOCHROME P450: INHIBITION OF CYP2C9, CYP2C19, AND CYP2D6

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ABSTRACT:
Delavirdine, a non-nucleoside inhibitor of HIV-1 reverse transcriptase, is metabolized primarily through desalkylation catalyzed by CYP3A4 and CYP2D6 and by pyridine hydroxylation catalyzed by CYP3A4. It is also an irreversible inhibitor of CYP3A4. The interaction of delavirdine with CYP3A4 was examined with pooled human liver microsomes using diclofenac 4'-hydroxylation as a reporter of CYP2C9 catalytic activity. As delavirdine concentration was increased from 0 to 100 μM, the K_M for diclofenac metabolism rose from 4.5 ± 0.5 to 21 ± 6 μM, and V_max declined from 4.2 ± 0.1 to 0.54 ± 0.08 nmol/min/mg of protein, characteristic of mixed-type inhibition. Nonlinear regression analysis revealed an apparent K_i of 2.6 ± 0.4 μM. There was no evidence for bioactivation as prerequisite to inhibition of CYP2C9. Desalkyl delavirdine, the major circulating metabolite of delavirdine, had no apparent effect on microsomal CYP2C9 activity at concentrations up to 20 μM. Several analogs of delavirdine showed similar inhibition of CYP2C9. Delavirdine significantly inhibited cDNA-expressed CYP2C19-catalyzed (S)-mephenytoin 4'-hydroxylation in a noncompetitive manner, with an apparent K_i of 24 ± 3 μM. Delavirdine at concentrations up to 100 μM did not inhibit the activity of CYP1A2 or -2E1. Delavirdine competitively inhibited recombinant CYP2D6 activity with a K_i of 12.8 ± 1.8 μM, similar to the observed K_i for delavirdine desalkylation. These results, along with previously reported experiments, indicate that delavirdine can partially inhibit CYP2C9, -2C19, -2D6, and -3A4, although the degree of inhibition in vivo would be subject to a variety of additional factors.

The reverse transcriptase of HIV-1 catalyzes the transcription of viral RNA to proviral DNA, an essential step in the life cycle of HIV-1 and the progression to AIDS in humans. Delavirdine is a potent, specific non-nucleoside inhibitor of HIV-1 reverse transcriptase and is currently approved for the treatment of AIDS in combination with other antiretroviral agents.

The in vitro metabolism of delavirdine was examined using liver microsomes from several species (Voorman et al., 1998a). Microsomal metabolite profiles among all species, including human, differed only by the relative concentrations of the major metabolites, desalkyl delavirdine and 6'-pyridinol delavirdine. The K_M for delavirdine desalkylation ranged from 4.4 to 12.6 μM across several species. Delavirdine metabolism by microsomes pooled from several human livers was characterized by an apparent K_M of 6.8 μM and V_max of 0.44 nmol/min/mg. Delavirdine was metabolized primarily by CYP3A, which catalyzed both delavirdine desalkylation and 6'-hydroxylation. The apparent K_M for desalkylation by cDNA-expressed CYP3A4 was 5.4 μM. Delavirdine was also metabolized by CYP2D6, which catalyzed only desalkylation, was characterized by an apparent K_M of 10.9 μM, and was probably a lower capacity pathway than CYP3A. Delavirdine appeared to inactivate CYP3A in rats and monkeys as evidenced by diminished CYP3A activity in microsomes isolated from animals treated with delavirdine (Voorman et al., 1998b), by diminished plasma clearance and increased half-life of delavirdine in rats (Adams et al., 1996), and by reduced clearance in humans as shown with the erythromycin breath test (Cheng et al., 1997). Delavirdine appeared to be metabolically activated by CYP3A, forming a reactive but unidentified metabolite capable of irreversible interaction with microsomal protein.

In this report, we show that delavirdine does not inhibit CYP1A2 or CYP2E1 but can inhibit CYP2C9, CYP2C19, and CYP2D6, and we show that the core structure of delavirdine is involved in its inhibition of CYP2C9.
(HL-14, 17, 18, and 23), blending the homogenates, and completing the microsomal isolation with the blended homogenates. Microsomes were prepared essentially as described (Lu and Levin, 1972) and stored in 0.25 M sucrose at -80°C.

**Instrumentation.** The HPLC systems consisted of a Perkin-Elmer Series 410 LC pump, either an ISS 100 or ISS 200 autosampler (Perkin-Elmer, Norwalk, CT), and a Perkin-Elmer LC-235 diode array detector, Spectroflow 783 UV detector (Kratos, Ramsey, NJ), or Waters model 470 spectrophotometer (Waters, Milford, MA). For assay of (S)-mephénytoin, chloroxazone, and 4-nitrophenol metabolism, the autosampler tray was maintained at 4°C. For radiochemical detection, a Radiomatic model A-515, A-500, or A-200 flow-through detector (Packard Instrument Co., Meriden, CT) was used, equipped with a 0.5-ml flow cell. Unless otherwise noted, the HPLC eluate was blended 1:3 (v/v) with Ultima-Flo M (Packard) scintillant. The radiochemical signal was stored and processed by the Radiomatic detector.

**Diclofenac 4'-Hydroxylation.** Microsomal metabolism of diclofenac was measured based on a previously reported method (Leemann et al., 1993). The reaction mixture consisted of pooled human liver microsomes (0.1 mg/ml), 0.1 M Tris HCI (pH 7.5), NADPH-generating system (1 mM β-NADP⁺, 5 mM trisodium isocitrate, 5 mM magnesium chloride, and 0.4 units/ml isocitrate dehydrogenase), and diclofenac concentrations of 4, 6, 10, 20, and 40 μM. Delavirdine was added from stock solutions in methanol (diluted 1:100) to achieve final concentrations of 3, 10, 30, and 100 μM. Desalkyl delavirdine was added from stock solutions in acetone for final concentrations of 2, 4, 10, and 20 μM. The inhibitory effect of delavirdine analogs was tested by incubating the compounds with cDNA-expressed CYP2C9 (15 pmol/ml) at concentrations ranging from 0.1 to 50 μM and using a diclofenac concentration of 6 μM. Reactions were terminated by addition of 0.5 volumes of acetonitrile/glacial acetic acid (94:6, v/v), followed by addition of 50 μl of 0.1 M mfenamic acid as internal standard and centrifugation to pellet protein. Quantitative analysis of diclofenac 4'-hydroxylation was accomplished using HPLC with UV detection at 280 nm. Sample components were separated on a 4.6-mm i.d. × 25-cm YMC basic 5-μ column (YMC Inc., Wilmington, NC) coupled to a 4.0-mm i.d. × 2.3-cm YMC basic guard column. The mobile phase (1 ml/min) consisted of a linear gradient from 38% acetonitrile to 75% acetonitrile over 24 min and was balanced to 100% with 0.1 M ammonium acetate (pH 4.0) containing 3% acetonitrile. For the enzyme assays described in this report, reactions were linear with time and protein concentration in the range of the incubation conditions.

**S-Mephénytoin 4'-Hydroxylation.** Interaction of delavirdine with CYP2C19 was evaluated by measuring (S)-mephénytoin 4'-hydroxylation using cDNA-expressed CYP2C19. Initially, the experiment was set up with quadruplicate incubations, each containing 0.3 mg of microsomal protein, 1 mM NADPH, 20 μM 3H(S)-mephénytoin, and 0, 10, or 100 μM delavirdine in a final volume of 0.3 ml of 100 mM potassium phosphate buffer, pH 7.4. Samples were preincubated for 4 min at 37°C, and reactions were initiated by NADPH addition. Incubations were allowed to proceed for 40 min and were then terminated by addition of 20 μl of 25% (v/v) perchloric acid. Sample tubes were vortex mixed for 10 s and centrifuged at 14,000g for 15 min at 27°C. Supernatants (300 μl) were individually transferred to HPLC autoinjector vials, capped, and assayed by HPLC using flow-through radiochemical detection. Sample components were separated on a Zorbax SB-C8 column (4.6-mm i.d. × 25 cm; Mac-Mod Analytical, Chadds Ford, PA). The mobile phase (1 ml/min) consisted of solvent A (90:10, water/methanol containing 0.5% acetic acid) and solvent B (10:90, water/methanol containing 0.5% acetic acid). An analytical run consisted of 10% solvent B for 1 min followed by a linear gradient to 75% solvent B for 40 min and holding for 5 min. Flowscint II scintillation cocktail was introduced post-column at a rate of 1.5 ml/min. Retention times for (S)-mephénytoin and its 4'-hydroxy metabolite were approximately 32 and 24 min, respectively.

Subsequently, assay methodology was changed to improve sensitivity. Similar incubation conditions were used, but delavirdine concentrations were 0, 3, 9, and 27 μM (selected based on simulation model), and (S)-mephénytoin 4'-hydroxylation was measured by API mass-spectrometric detection, as previously described (Wienkers et al., 1996). The HPLC system was equipped with a Hewlett Packard 1050 Series pump and autosampler (Hewlett Packard, Naperville, IL) and a Thar two-position valve actuator (Thar Designs Inc., Pittsburgh, PA). The HPLC conditions were the same as previously described.

Mass spectrometric analysis was performed on a Finnigan-MAT TSQ 7000 triple quadrupole mass spectrometer (Finnigan-MAT, San Jose, CA) directly coupled to the HPLC system via a Finnigan API source operated in the atmospheric pressure chemical ionization mode. Data were collected on a DEC 3000 model 300X computer running OSF/1 version 2.0 as the operating system. The mass spectrometer was controlled using Instrument Control Language version 8.0, and the data were processed using Interactive Chemical Information System version 8.1.1 software (Finnigan-MAT). Quantitation of 4'-hydroxymephénytoin was accomplished while operating the MS in precursor ion mode for the transition m/z 235 to m/z 150 [(4'-hydroxymephénytoin) and m/z 238 to m/z 150 [trideuterio-4'-hydroxymephénytoin (internal standard)] at a scan rate of 0.45 amu/s. Argon (99.999% pure; AGA, Maumee, OH) was used as the collision gas with a collision cell pressure of 2.2 mtorr and offset of -30 eV. The conversion dyneon and electron multiplier were set to 15 kV and 1500 V, respectively. The capillary was operated at 250°C. The corona current was set to 5 μA, and nitrogen was used as a drying gas at a sheath pressure of 70 psi.

**Chloroxazone 6-Hydroxylation.** Chloroxazone 6-hydroxylation, an index of CYP1A2 activity (Ono et al., 1995), was measured in CDNA-expressed CYP1A2 microsomes at 10 or 100 μM delavirdine. Samples were run in quadruplicate, with each incubation containing microsomal protein (0.1 mg), NADPH (1 mM), [14C]chloroxazone (8.5 μM), and delavirdine (0, 10, and 100 μM) in a final volume of 0.3 ml of potassium phosphate buffer (100 mM, pH 7.4). Samples were preincubated for 4 min at 37°C, and reactions were initiated by the addition of NADPH. Incubation reactions were allowed to proceed for 30 min and then terminated by addition of 20 μl of a 25% (v/v) perchloric acid solution. Sample tubes were vortex mixed for 10 s, then centrifuged at 14,000g for 15 min at 27°C, and the subsequent incubation supernatant (300 μl) was transferred to HPLC autoinjector vials and capped. Formation of 6-hydroxylchloroxazone was quantitated by HPLC using radiochemical detection, as described above, except that the mobile phase consisted of solvent A (90:10, ammonium acetate, pH 3.8/acetonitrile) and solvent B (10:90, ammonium acetate, pH 3.8/acetonitrile). Initial mobile phase conditions (100%) at a rate of 1.0 ml/min were held for 5 min followed by a linear gradient to 100% B in 20 min, and the final conditions were held for 3 min. Retention times for chloroxazone and 6-hydroxylchloroxazone were approximately 19 and 14 min, respectively.

**4-Nitrophenol Hydroxylation.** The potential for delavirdine interaction with CYP2E1 was determined by measuring rates of [14C]4-nitrophenol hydroxylation (Tassaneeyakul et al., 1993) by cDNA-expressed CYP2E1 microsomes across a 10-fold concentration range of delavirdine. Samples were incubated in quadruplicate, with each incubation containing microsomal protein (0.07 mg), NADPH (1 mM), [14C]4-nitrophenol (20 μM), and delavirdine (0, 10, and 100 μM) in a final volume of 0.3 ml of potassium phosphate buffer (100 mM, pH 7.4). Samples were preincubated for 4 min at 37°C, and reactions were initiated by the addition of NADPH. Incubation reactions were allowed to proceed for 30 min and were then terminated by addition of 20 μl of a 25% (v/v) perchloric acid solution. Sample tubes were vortex mixed for 10 s and then centrifuged at 14,000g for 15 min at 27°C. The incubation supernatants (300 μl) were transferred to HPLC autoinjector vials and capped. Formation of 4-nitrocatechol was quantitated by HPLC with radiochemical detection. Ultima-Flo M scintillation cocktail was introduced post-column at a rate of 2.0 ml/min. Analytical separations were performed on a Zorbax SB-C8 column (4.6-mm i.d. × 25 cm; Mac-Mod Analytical). The mobile phase consisted of solvent A (90:10, water/methanol containing 0.5% acetic acid) and solvent B (90:10, water/methanol containing 0.5% acetic acid)

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<th>Enzyme</th>
<th>Activity</th>
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<tr>
<td></td>
<td></td>
<td>10 μM</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>Chloroxazone 6-hydroxylation</td>
<td>6.9 14</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>4-Nitrophenol hydroxylation</td>
<td>7.5 1.3</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>(S)-Mephénytoin 4'-hydroxylation</td>
<td>31 81</td>
</tr>
</tbody>
</table>
Effect of delavirdine on kinetic parameters for diclofenac 4'-hydroxylation by human liver microsomes

<table>
<thead>
<tr>
<th>Delavirdine (µM)</th>
<th>K_M (µM)</th>
<th>V_max (nmol/min/mg)</th>
<th>K_i (µM)</th>
<th>α (Not applicable)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>4.5 ± 0.5</td>
<td>4.2 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 ± 2</td>
<td>3.8 ± 0.3</td>
<td></td>
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</tr>
<tr>
<td>10</td>
<td>12 ± 3</td>
<td>2.4 ± 0.2</td>
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<tr>
<td>30</td>
<td>15 ± 4</td>
<td>1.3 ± 0.2</td>
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<tr>
<td>100</td>
<td>21 ± 6</td>
<td>0.54 ± 0.08</td>
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<td>Combined</td>
<td>4.7 ± 0.4</td>
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<td>2.6 ± 0.4</td>
<td>6.1 ± 1.8</td>
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</table>

*Estimated by fitting data to eq. 3.

FIG. 1. Effect of delavirdine on diclofenac 4'-hydroxylation.

Pooled human liver microsomes were incubated with diclofenac and increasing concentrations of delavirdine (upper left). Graphical analysis of inhibition showing Lineweaver-Burk plot (upper right) and secondary plots for graphical illustration of K_i (lower left) and α (lower right).

FIG. 2. Characterization of diclofenac 4'-hydroxylation inhibition by delavirdine.

Diclofenac 4'-hydroxylation following a 10-min incubation of human liver microsomes with delavirdine (upper panel). Time-dependent diclofenac 4'-hydroxylation in the presence of delavirdine (lower panel).
Results

Interaction with CYP1A2 and -2E1. Delavirdine had no apparent effect on the activity of cDNA-expressed CYP1A2 as measured by chlorzoxazone hydroxylation and using delavirdine concentrations of 10 or 100 μM. Likewise, no effect was observed for 10 or 100 μM delavirdine on cDNA-expressed CYP2E1 as measured by 4-nitrophenol hydroxylation (Table 1). Results of CYP2C19 inhibition will be addressed below.

Diclofenac 4’-Hydroxylation (CYP2C9). Estimation of apparent \( K_a \) and \( V_{max} \) for diclofenac 4’-hydroxylation in the presence of increasing concentrations of delavirdine showed an apparent \( K_M \) rising from 4.5 ± 0.5 to 21 ± 6 μM and \( V_{max} \) declining from 4.2 ± 0.1 to 0.54 ± 0.08 nmol/min/mg as the concentration of delavirdine rose from 0 to 100 μM (Table 2). An increase in apparent \( K_M \) and decline in \( V_{max} \) in the presence of increasing inhibitor concentration, are indicative of mixed-type inhibition. Results are shown graphically both as nontransformed substrate-velocity data and in the form of a Lineweaver-Burk plot (Fig. 1). Evaluation of the combined data by nonlinear regression analysis, using the equation as set forth by Segel (1975), revealed an apparent \( K_M \) of 4.7 ± 0.4 μM, \( V_{max} \) of 4.3 ± 0.1 nmol/min/mg, \( K_i \) of 2.6 ± 0.4 μM, and \( \alpha \) of 6.1 ± 1.8 μM. Graphical analyses of the inhibitor terms are shown in Fig. 1 and displayed simple linear dependence of 1/\( V \) and \( K_M/V_{max} \) on delavirdine concentration, the defining characteristics of linear mixed-type inhibition. The term \( \alpha \) represents the interaction of inhibitor with the enzyme substrate complex and substrate with the enzyme inhibitor complex.

Since delavirdine was known to be an irreversible inhibitor of CYP3A4 and was not thought to be a substrate for CYP2C9 (Voormann et al., 1998a), two experiments were completed to determine whether or not delavirdine was catalytically activated to form an irreversible complex with CYP2C9. Measurement of diclofenac 4’-hydroxylation following preincubation with delavirdine (10 μM, complete system) yielded a reaction rate that was 44% of control, whereas addition of delavirdine at the start of the diclofenac assay resulted in a reaction rate that was 43% of control. Likewise, measurement of diclofenac 4’-hydroxylation in the presence of delavirdine displayed a diminished but linear reaction rate over a 30-min incubation period (Fig. 2), indicating that delavirdine is not subject to bioactivation, either by CYP2C9 or other isozymes, as a prerequisite for inhibition of CYP2C9.

Desalkyl delavirdine had no apparent effect on microsomal diclofenac 4’-hydroxylation at inhibitor concentrations up to 20 μM (Fig. 3). The solubility of desalkyl delavirdine limited the highest concentration to 20 μM. The mean apparent \( K_M \) was 5.2 ± 0.5 μM, and the \( V_{max} \) was 4.7 ± 0.3 nmol/min/mg of protein.

Effect of Delavirdine Analogs on Diclofenac Metabolism. An experiment was conducted to evaluate the significance of the sulfonamide, piperazine, or alkyl substituents on interaction with CYP2C9. However, the delavirdine analogs, including compounds that lacked the corresponding methyl sulfonamide group, inhibited diclofenac metabolism similar to delavirdine (Fig. 4).

(S)-Mephentoin 4’-Hydroxylation (CYP2C19). Measurement of [14C](S)-mephentoin 4’-hydroxylation by cDNA-expressed CYP2C19 in the presence of 10 or 100 μM delavirdine showed 31 or 81% decline, respectively, in CYP2C19 activity (Table 1). To adequately assess the inhibition at low reaction rates, the experiment was repeated using a narrower range of inhibitor concentrations and using API-MS detection for improved sensitivity. As delavirdine concentration increased from 3 to 27 μM, the apparent \( V_{max} \) declined, but \( K_M \) remained unchanged (Table 3). This was reflected in the double reciprocal and untransformed plots of concentration-dependent initial rates, indicative of noncompetitive inhibition (Fig. 5). Analysis of the data by nonlinear regression revealed an apparent \( K_i \) of 24 ± 3 μM.

Dextromethorphan O-Demethylation (CYP2D6). The effect of delavirdine on cDNA-expressed CYP2D6 activity was determined by measuring dextromethorphan O-demethylation in the presence of 0.5 to 100 μM delavirdine. Data were best fit to a competitive model and yielded a \( K_i \) of 12.8 ± 1.8 μM for delavirdine inhibition of dextromethorphan O-demethylation (Fig. 6). Kinetic parameters for dextromethorphan O-demethylation (\( K_M \), 4.54 ± 0.56 μM; \( V_{max} \), 3.79 ± 0.16 nmol/min/mg) were consistent with published parameters (Krohnbach et al., 1987).

Discussion

Previously, we showed that delavirdine was metabolized by CYP3A4 and -2D6 and could partly block the catalytic activity of CYP3A4 through mechanism-based inactivation. Results of the present studies showed no interaction of delavirdine with CYP1A2 or -2E1, but showed that delavirdine inhibited CYP2C9 with an apparent \( K_i \) of 2.6 ± 0.4 μM and CYP2C19 with an apparent \( K_i \) of 24 ± 3 μM. Earlier work showed that delavirdine was not metabolized by CYP2C9 or CYP2C19. Delavirdine competitively inhibited CYP2D6 with a \( K_i \) of 12.8 ± 1.8 μM, consistent with the \( K_M \) (10.9 μM) for its metabolism by CYP2D6.

The chemical features of delavirdine that permit a good fit in the CYP2C9 active site are not apparent. Like sulfaphenazole and quinidine, delavirdine appears to be an inhibitor of an enzyme for which it is not an apparent substrate (Miners et al., 1988; Otton et al., 1988; Newton et al., 1995). Sulfaphenazole and quinidine inhibit CYP2C9 and -2D6, respectively, with inhibition constants in the range of 0.1 μM, indicative of likely ligand or coordinate bond interaction with heme iron of the enzyme (Jones et al., 1996a). It has been proposed that substrates and inhibitors of CYP2C9, like those of CYP2D6, interact with a specific electrostatic or acceptor residue in the active site of the enzyme which, in the context of other structural features, positions the molecule in proximity to the heme-oxo complex to facilitate the catalytic or inhibitory interaction (Jones et al., 1996a,b). For many CYP2C9 substrates, a potential hydrogen donor is present in the form of amido- or carboxyl-hydrogen, which is located approximately 7 Å from the site of hydroxylation and with the carbon backbone at an angle of approximately 130 degrees. The best examples of substrates fitting this description are tolbutamide, phenytoin,
and (S)-warfarin. In the case of sulfaphenazole, it is thought that enzyme inhibition occurs as a result of hydrogen bond formation from the sulfonamide hydrogen and ligation of the pyrazole nitrogen to heme iron (Jones et al., 1996a). By analogy of delavirdine to other inhibitors, it would seem like the sulfonamide functionality of delavirdine might be important in its interaction with CYP2C9. However, experiments with delavirdine analogs lacking the methylsulfonamide substituent demonstrated relatively strong inhibition of CYP2C9. Replacement of the piperazine ring with piperidine and isopropyl with t-butyl also did not diminish the inhibitory interaction. Thus, other core features of the template appear to moderate the fit of delavirdine in the active site of CYP2C9.

Delavirdine inhibition of CYP2C19 might be useful in understanding common features of the CYP2C9 and CYP2C19 active sites, although again, the structural characteristics of delavirdine that promote occupation of the active site are not apparent. Both enzymes are closely related in primary structure, and although they display distinct differences in substrate specificity, the discrimination might be a result of subtle differences in their active sites. For example, an examination of phenytoin (CYP2C9 substrate) and mephenytoin (CYP2C19 substrate) by computer modeling using an empirically derived active site model for CYP2C9 showed spatially similar sites of hydroxylation and that only differences in spatial arrangement within the active site led to reciprocal specificity (Jones et al., 1996a). Similarly, delavirdine probably fits the active sites of both enzymes, partially occupying the substrate binding surfaces but unable to react with the activated oxygen. It would be interesting to test for increased uncoupling in the presence of delavirdine. Since substrate binding and

**Fig. 4. Effect of several delavirdine analogs on inhibition of CYP2C9-catalyzed diclofenac 4'-hydroxylation.**

Compounds were incubated with CYP2C9 (15 pmol/ml) and diclofenac (6 μM) for a period of 15 min. Columns represent mean of duplicate determinations.
catalysis by CYP is not well understood, it is difficult to provide a meaningful explanation for the observations of mixed-type and non-competitive inhibition for CYP2C9 and CYP2C19, respectively.

CYP2C9 is subject to inhibition by several commonly used agents. For example, fluconazole increased the in vivo half-life of tolbutamide 1.8-fold and the half-life of \((S)\)-warfarin nearly 3-fold (Lazar and Wilner, 1990; Black et al., 1996). Allelic variants of CYP2C9 have been characterized and could be important in population differences in the metabolism of some CYP2C9 substrates. It has been shown that the variant CYP2C9(Arg144\(^{3}\)Cys 144) is nearly devoid of \((S)\)-warfarin 6- and 7-hydroxylation activity but catalyzes tolbutamide hydroxylation equivalent to the wild type (Rettie et al., 1994), although it’s been suggested that catalytic differences result from differential interaction of the allelic variants with cytochrome P450 reductase (Crespi and Miller, 1997). Although delavirdine could interfere with the metabolism of CYP2C9 substrates, its effect would be most acute on those substrates with a narrow therapeutic index and whose clearance is controlled by metabolism through CYP2C9. However, the ability of delavirdine to inhibit CYP2C9 should be placed in...

**TABLE 3**

<table>
<thead>
<tr>
<th>Delavirdine</th>
<th>(K_M)</th>
<th>(V_{max})</th>
<th>(K_i)</th>
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<td>pmol/min/mg</td>
<td>(\mu M)</td>
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</tr>
<tr>
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</tr>
<tr>
<td>9</td>
<td>61 ± 7</td>
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</tr>
<tr>
<td>27</td>
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<tr>
<td>Combined(^a)</td>
<td>62 ± 7</td>
<td>92 ± 4</td>
<td>24 ± 3</td>
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\(^a\) Estimated by fitting data to eq. 2.
the context of population variables and other factors, such as disease, which might affect the capacity of CYP2C9.

CYP2C19 controls the clearance of several clinically important drugs and is subject to polymorphic expression to the extent that about 3 to 5% of Caucasian and 20% of Asian populations appear to lack functional expression of the enzyme (Wilkinson et al., 1989). Nevertheless, there appear to be few adverse consequences associated with CYP2C19 deficiency (Tucker, 1994), indicating that inhibition of CYP2C19 is generally not a serious clinical drug interaction issue. Accordingly, clinically significant interaction of delavirdine with CYP2C19 seems unlikely.

The interaction of delavirdine with CYP2D6 appears to be simply a result of its metabolism by that isoform, with its Km approximating the observed Ki and demonstrating kinetics characteristic of competitive inhibition. Delavirdine contains chemical features that would likely make it a reasonable fit in the CYP2D6 active site (de Groot et al., 1999). CYP2D6 is polymorphically expressed and subject to inhibition by many drugs, including various antipsychotic agents (Shin et al., 1999). Interestingly, delavirdine, through its various metabolic and inhibitory interactions, demonstrates four types of inhibition: competitive, noncompetitive, mixed-type, and mechanism-based inhibition.

Administration of delavirdine to patients (400 mg, 3 times daily) produced average steady-state plasma concentrations in the range of 17 μM (n = 7) (Cheng et al., 1997). Although concentrations of delavirdine at the site of metabolism are not known and can be influenced by factors such as protein binding and partition coefficients, one could conservatively assume that hepatic concentrations of delavirdine might approximate plasma drug concentrations (Tucker, 1992; Houston, 1994). Accordingly, under the treatment regimen described above, delavirdine concentrations could exceed the estimated inhibition constants for CYP2C9 and CYP2D6, possibly resulting in partial inhibition of each isoform. Milder inhibition of CYP2C9 might be expected since the concentrations of delavirdine will likely be lower than the measured Ki for CYP2C19 inhibition. The degree to which delavirdine might interact with metabolism of concomitantly administered drugs, or conversely, how other drugs might affect delavirdine metabolism, depends on the nature of their interaction with cytochrome P450 and the relative affinity of each for a particular isoform.

Although inhibition of CYP can be a potentially complicating factor in therapeutic use of a drug, when placed in the context of other clinically used agents that are also inhibitors of CYP, the inhibitory characteristics of delavirdine appear moderate and clinically manageable. Indeed, inhibition of CYP has been used advantageously in the treatment of HIV-1 to enhance exposure to certain rapidly metabolized agents (Kempf et al., 1997).

References