THE INTERACTIONS OF A SELECTIVE PROTEIN KINASE C BETA INHIBITOR WITH THE HUMAN CYTOCHROMES P450

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ABSTRACT:

Studies were performed to determine the cytochromes P450 (P450s) responsible for the biotransformation of (S)-[dimethylamino]-methy]-10,11,14,15-tetrahydro-4,9;16,21-dimetheno-1H, 13H-dibenzo[e,k]pyrrolo[3,4-h][1,4,13]oxadiazacyclohexadecene-1,3(2H)-dione (LY333531) to its equipotent metabolite, N-desmethyl LY333531, and to examine the ability of these two compounds to inhibit P450-mediated metabolism. Kinetic studies indicated that a single enzyme in human liver microsomes was able to form N-desmethyl LY333531 with an apparent \( K_m \) value of approximately 1 \( \mu \)M. The formation rate of N-desmethyl LY333531 was correlated with markers of nine P450s in a bank of 20 human liver microsomes. The only significant correlation observed was with the form-selective activity for CYP3A. Of the nine cDNA-expressed P450s examined, only CYP3A4 and CYP2D6 formed N-desmethyl LY333531. However, CYP3A4 formed N-desmethyl LY333531 at a rate 57-fold greater than that observed with CYP2D6. In incubations with human liver microsomes, quinidine, an inhibitor of CYP2D6, demonstrated little inhibition of metabolite formation while ketoconazole, an inhibitor of CYP3A, demonstrated almost complete inhibition. Thus, CYP3A is responsible for the formation of N-desmethyl LY333531. LY333531 and N-desmethyl LY333531 were also examined for their ability to inhibit metabolism mediated by CYP2D6, CYP2C9, CYP3A, and CYP1A2. LY333531 and N-desmethyl LY333531 were found to competitively inhibit CYP2D6 with calculated \( K_i \) values of 0.17 and 1.0 \( \mu \)M, respectively. Less potent inhibition by these compounds of metabolism mediated by the other three P450s examined was observed. In conclusion, CYP3A is primarily responsible for forming N-desmethyl LY333531. Therefore, alterations in the activity of this enzyme have the potential to affect LY333531 clearance. In addition, LY333531 and its metabolite are predicted to be potential inhibitors of CYP2D6-mediated reactions in vivo.

Over the last several years, evidence has accumulated that implicates the hyperglycemia-induced activation of protein kinase C (PKC) as one of the mechanisms responsible for the development and/or progression of chronic complications of diabetes. Hyperglycemia-induced increases in diacylglycerol, a physiological activator of PKC, have been demonstrated in the organs that are susceptible to the progression of chronic complications of diabetes. Hyperglycemia-induced activation of protein kinase C (PKC) as one of the mechanisms responsible for the development and/or progression of chronic complications of diabetes. Hyperglycemia-induced increases in diacylglycerol, a physiological activator of PKC, have been demonstrated in the organs that are susceptible to the progression of chronic complications of diabetes.

In vitro methodologies using human liver tissue have been developed to aid in the prediction of possible variation in metabolic clearance in vivo and drug-drug interactions for a new molecular entity. The studies described herein used these in vitro techniques to identify the P450(s) responsible for the formation of the major (and equipotent) metabolite of LY333531, N-desmethyl LY333531. The initial step in the identification of these enzymes was a kinetic analysis of the formation of N-desmethyl LY333531 following incubations of the drug with human liver microsomes. The identification of the enzymes involved in the formation of N-desmethyl LY333531 was then accomplished by correlating the rate of formation of the metabolite with immunoquantitated levels and/or the associated form-selective catalytic activities for the drug-metabolizing enzymes by a bank of human liver microsomes. The ability of specific cDNA-expressed cytochromes P450 (P450s) to form N-desmethyl LY333531 was used to corroborate the results of the correlation studies. Finally, P450-selective inhibitors were used to examine their effect on the formation of the metabolite in question.

To predict interactions that may occur in the clinical setting between LY333531 and coadministered drugs, the ability of the PKC beta inhibitor, LY333531, and its N-desmethyl metabolite to inhibit metabolism mediated by CYP3A, CYP2D6, CYP1A2, and CYP2C9 was examined. Using in vitro metabolism of specific form-selective substrates as probes of metabolism, CYP2D6, CYP2C9, CYP1A2,
and CYP3A were selected for examination since they are responsible for the metabolism of the vast majority of drugs or, in the case of CYP2D6, display genetic polymorphism (Ring and Wrighton, 2000).

Materials and Methods

LY333531 mesylate monohydrate, N-desmethyl LY333531 hydrochloride, and internal standard (350942) were synthesized by Eli Lilly and Company (Indianapolis, IN). Quinidine, diclofenac, phencinet, fluimizopropan, and NADPH were obtained from Sigma-Aldrich (St. Louis, MO). Ketoconazole was a gift from Janssen Pharmaceutical, Inc. (Beershe, Belgium). Midazolam and 1'-'hydroxy midazolam were obtained from F. Hoffmann-La Roche (Nutley, NJ). and 4'-'hydroxy diclofenac was obtained from BD Gentest Corp. (Woburn, MA). Bufuralol and 1'-'hydroxy bufuralol were purchased from Ultrafine Ltd. (Manchester, UK). Acetaminophen was obtained from Eastman Kodak (Rochester, NY). Meclofenamate was obtained from Cyma Chemical (Ann Arbor, MI).

Human liver samples designated HLA through HLT were obtained from the Medical College of Wisconsin (Milwaukee, WI), Medical College of Virginia (Richmond, VA), or Indiana University School of Medicine (Indianapolis, IN), under protocols approved by the appropriate committee for the conduct of human research. Hepatic microsomes were prepared by differential centrifugation (van der Hoeven and Coon, 1974) and characterized for their relative levels of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 via immunooquantification or through the use of form-selective catalytic activities (Ring et al., 2001). A mixture of equal protein concentrations of microsomes from HLB, HLH, HLM, and HLP was prepared and used in the studies examining the ability of LY333531 to inhibit CYP1A2-, CYP2C9-, CYP2D6-, and CYP3A-mediated reactions. Microsomes prepared from human β-lactamase cells engineered to express CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4 were obtained from BD Gentest.

To examine the conversion of LY333531 to N-desmethyl LY333531 by human liver microsomes, 500 μl incubations were performed under initial rate conditions at 37°C and contained LY333531, microsomes, and NADPH (1 mM) in 100 mM sodium phosphate buffer, pH 7.4. For those studies examining the effect of P450 inhibitors on this transformation, quinidine (5 μM) or ketoconazole (5 μM) (Newton et al., 1995) were added to the reaction. Concentrations of LY333531 of 0.1, 0.25, 0.5, 1, 2.5, 5, 10, and 25 μM were used in the enzyme kinetic studies and equaled 1 μM in the correlation and inhibition studies. After 2 min, reactions were stopped with the addition of 1500 μl 50:50 methanol/acetonitrile containing internal standard. The denatured protein was removed by centrifugation, and the supernatant was analyzed by high-performance liquid chromatography as described above with 0.25 mg/ml protein. For studies examining LY333531 as inhibitor, diclofenac concentrations of 2.5, 5, 10, and 25 μM were used with 0, 2, 7, 12, and 19 μM LY333531 as the potential inhibitor. A Km diclofenac concentration of 2.5 μM was used to examine the potential inhibition by 0, 0.13, 0.25, 0.5, 1, 5, 10, and 20 μM N-desmethyl LY333531.

Bufuralol transformation to 1'-OH bufuralol was used as a marker of CYP2D6 activity (Ring et al., 1996). Incubations with human liver microsomes (0.1 mg/ml protein) were performed in the presence of 1 mM NADPH and 5, 10, 25, 50, and 100 μM bufuralol with 0, 0.05, 0.1, 0.3, and 0.7 μM LY333531 or 0, 0.63, 1.3, 2.5, and 5 μM N-desmethyl LY333531 as potential inhibitors. Finally, the 1'-hydroxylation of midazolam was used as a marker of CYP3A activity as described by Ring et al. (1999). For studies examining LY333531 as inhibitor, a Ki midazolam concentration of 5 μM was used with 0, 10, 200, and 300 μM LY333531 as the potential inhibitor. Midazolam concentrations of 5, 10, 25, 50, and 100 μM were used to examine the potential inhibition by 0, 1, 5, 10, and 20 μM N-desmethyl LY333531. An YMC basic column (5 μm, 4.6 × 150 mm; YMC Inc., Wilmington, NC) was used for the high-performance liquid chromatography analysis of samples which included LY333531, and a Capcell Pak column (5 μm, 4.6 × 150 mm; Shiseido, Tokyo, Japan) was used for N-desmethyl LY333531 analysis.

Samples were analyzed for N-desmethyl LY333531 and internal standard using a three pump high-performance liquid chromatography system equipped with an autosampler, a 6-port valve, an online extraction column (ExSil SCX 4 × 20 mm; Thermo Hypersil, Keystone Scientific, Bellefonte, PA), and an analytical column (Columbus C8, 5 μm, 100 angstrom, 3 × 150 mm; Phenomenex, Torrance, CA). Each sample was injected onto the analytical column in which the analytes were retained. The extraction column was washed at 1.5 ml/min with acetonitrile/water/85% concentrated phosphoric acid (300:700:5). After approximately 1.5 min, the analytes were back flushed from the extraction column onto the analytical column using mobile phase A [acetonitrile/water/2.5 M KCl/500 mM potassium phosphate buffer, pH 7 (20:65:10:5)]. The analytes were separated and eluted from the analytical column using a gradient of mobile phase B and mobile phase B [acetonitrile/water/500 mM potassium phosphate buffer, pH 5 (65:30:5)]. Following transfer of sample onto the analytical column, a 4-min gradient was used in which 100% A was ramped to a 45:55 A/B mixture. This step was followed by a ramp to 100% B over the next 2 min. Mobile phase B was allowed to flow isocratically for 2 min before a switch back to 100% A. The column was equilibrated for 2 min with A before the next sample sequence began. Analytes eluted from the column were detected using an UV detector set at 240 nm.

Kinetic analyses of the formation of N-desmethyl LY333531 were initially evaluated by visual examination of Eadie-Hofstee plots to assess whether one or more enzymes were involved in its formation. Enzyme kinetic parameters were then determined following fit of the data to the Michaelis-Menten model of enzyme kinetics (Segel, 1975) using nonlinear regression analysis (Win-Nonlin, version 1.5; Statistical Consultants, Inc., Cary, NC) (Ring et al., 2001). An intrinsic clearance (Clint) was determined by the formula Vmax/Km. For the inhibition studies, the apparent kinetic parameters of KM, Vmax, and Ki and the standard error of the estimated parameter were determined by using conventional relationships for inhibition (Segel, 1975; Ring et al., 1996).

Correlation analyses were performed (JMP, version 3.2.1, SAS Institute, Inc., Cary, NC) between the rate of N-desmethyl LY333531 formation and enzymatic activities or immunoquantified levels of various oxidative enzymes in a human liver microsomal bank of up to 20 samples as described previously (Ring et al., 2001). Form-selective catalytic activities for CYP1A2 (phenacetin O-deethylation), CYP2A6 (cumarin 7-hydroxylation), CYP2C8 (taxol 6-hydroxylation), CYP2C9 (diclofenac 4'-hydroxylation), CYP2C19 (S-mephyton 4'-hydroxylation), CYP2D6 (bufuralol 1'-hydroxylation), CYP2E1 (chlorzoxazone 6-hydroxylation), CYP3A (midazolam 1'-hydroxylation), and flavin containing monooxygenase (trans-(S)-nicotine-N'-1' hydroxylation) and immunoquantified levels of CYP2B6 were used as possible coregessors. The predicted in vivo inhibition by LY333531 and N-desmethyl LY333531 of the catalytic activities of the examined P450s was calculated as follows (Segel, 1975):

\[
\% \text{ inhibition} = \left(1 - \left(\frac{I}{I_0 + K_i}\right)\right) \times 100
\]

The Ki values generated in this study were used in this formula. The inhibitor concentration (I) used was the plasma concentrations of LY333531 or its N-desmethyl metabolite determined in a clinical study after dosing 32 mg LY333531/day for 7 days. In this study, the Cmax on day seven was 0.13 and 0.14 μM for LY333531 and N-desmethyl LY333531, respectively (J. Burkey, Eli Lilly and Co.,
values for these microsomal samples ranged from 323 to 533 pmol/min/mg.

formation of N-desmethyl LY333531 by any of the other seven enzymes examined. N-desmethyl was formed by expressed CYP2D6 but to a much lesser extent (31 pmol/min/mmol P450). In addition, LY333531 was formed to the greatest extent by expressed CYP3A4.

The apparent kinetic parameters for LY333531 Formation. Upon fitting the data to the Michaelis-Menten equation, apparent values were calculated for HLB, HLG, and HLQ, respectively (Table 1). The calculated Cl int (Vmax/KM) values for these microsomal samples ranged from 323 to 533 µmol/min/mg (Table 1).

The rates of formation of N-desmethyl LY333531 were determined in a P450-characterized bank of 20 human liver microsomal samples (Ringe et al., 2001) at a substrate concentration (1 µM) reflective of the KM value for this biotransformation (Table 2). The formation rates of N-desmethyl LY333531 by the microsomal samples were monophasic in nature (data not shown), consistent with one enzyme producing the metabolite. Upon fitting the data to the Michaelis-Menten equation, apparent KM values of 1.0, 3.2, and 1.1 µM were calculated for HLB, HLG, and HLQ, respectively (Table 1). The calculated Cl int (Vmax/KM) values for these microsomal samples ranged from 323 to 533 µmol/min/mg (Table 1).

Inhibition of CYP1A2, CYP2C9, CYP2D6, and CYP3A by LY333531 and N-desmethyl LY333531. The inhibition by LY333531 of 1'-hydroxy bufuralol formation by CYP2D6 and 4'-hydroxy diclofenac by CYP2C9 was found to model best to competitive inhibition yielding apparent Ki values of 0.17 and 49 µM, respectively (Table 3). Using these Ki values and a total plasma concentration of 0.13 µM in the formula for inhibition of catalytic activity in vivo yielded a conservative estimate of predicted inhibition of CYP2D6-mediated metabolism of 43%. Due to the 95% protein binding of LY333531, the free concentration of LY333531 (0.0065 µM) was also used in the calculation which yielded an estimate of inhibition of CYP2D6-mediated metabolism of 4%. The large Ki value obtained for the inhibition of CYP2C9 metabolism resulted in a prediction of essentially no inhibition of CYP2C9-mediated metabolism. Inhibition (to a maximum of 42%) of the 1'-hydroxylation by CYP3A of midazolam at a KM concentration (5 µM) was observed by LY333531 at concentrations up to 300 µM. The CYP1A2 biotransformation of phenacetin to acetaminophen following incubation with a KM concentration of phenacetin (12.5 µM; Belle et al., 2000) was not inhibited to a great extent (maximum 13%) by LY333531 concentrations ranging from 5 to 145 µM.

The inhibition of 1'-hydroxy bufuralol formation by CYP2D6 of N-desmethyl LY333531 formation was found to model best to competitive inhibition yielding an apparent Ki value of 1.0 µM (Table 3). The best-fit model describing the inhibition by N-desmethyl LY333531 of the formation of 1'-hydroxy midazolam by CYP3A was found to be noncompetitive, yielding an apparent Ki value of 14 µM (Table 3). Using a N-desmethyl LY333531 concentration of 0.14 µM, the calculated percent in vivo inhibition by N-desmethyl LY333531 was 12 and 1% for CYP2D6 and CYP3A, respectively. Inhibition to a maximum of 48% was observed by N-desmethyl LY333531 (0.13 to 20 µM) of 4'-hydroxy diclofenac formation following incubation with 2.5 µM diclofenac. N-Desmethyl LY333531 (0.5 to 20 µM) inhibited acetaminophen formation following incubation with 12.5 µM phenacetin to a maximum of 36%.

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>KM</th>
<th>Vmax</th>
<th>Cl int</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µM</td>
<td>pmol/min/mg</td>
<td>µmol/min/mg</td>
</tr>
<tr>
<td>Human Liver Microsomes</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>HLB</td>
<td>1.0 ± 0.1**</td>
<td>533 ± 30</td>
<td>533</td>
</tr>
<tr>
<td>HLG</td>
<td>3.2 ± 0.3</td>
<td>1033 ± 64</td>
<td>323</td>
</tr>
<tr>
<td>HLQ</td>
<td>1.1 ± 0.1</td>
<td>491 ± 26</td>
<td>446</td>
</tr>
</tbody>
</table>

**Parameter estimate ± standard error of the parameter estimate.

### Table 2

<table>
<thead>
<tr>
<th>Microsomal Sample</th>
<th>N-Desmethyl LY333531 pmol/min/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA</td>
<td>521</td>
</tr>
<tr>
<td>HLB</td>
<td>303</td>
</tr>
<tr>
<td>HLC</td>
<td>222</td>
</tr>
<tr>
<td>HLD</td>
<td>107</td>
</tr>
<tr>
<td>HLF</td>
<td>1158</td>
</tr>
<tr>
<td>HLG</td>
<td>715</td>
</tr>
<tr>
<td>HLH</td>
<td>425</td>
</tr>
<tr>
<td>HLI</td>
<td>323</td>
</tr>
<tr>
<td>HLM</td>
<td>193</td>
</tr>
<tr>
<td>HLN</td>
<td>536</td>
</tr>
<tr>
<td>HLO</td>
<td>1661</td>
</tr>
<tr>
<td>HLP</td>
<td>647</td>
</tr>
<tr>
<td>HLQ</td>
<td>288</td>
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<td>HLR</td>
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<tr>
<td>HLS</td>
<td>1068</td>
</tr>
<tr>
<td>HLT</td>
<td>1414</td>
</tr>
</tbody>
</table>

**Formation rates of N-desmethyl LY333531 are the average of duplicate sample analyses.

a Assays were performed as described under Materials and Methods.
The formation of N-desmethyl LY333531 in human liver microsomal samples exhibited Michaelis-Menten kinetics yielding apparent $K_M$ values ranging from 1 to 3 $\mu$M. Identification of the enzyme involved in the formation of N-desmethyl LY333531 was initiated via correlating the rate of its formation following incubation with a concentration of LY333531 approximating the $K_M$ value (1 $\mu$M) to immunoquantified levels and/or form-selective catalytic activities of specific drug-metabolizing enzymes in a bank of 20 human liver microsomes. The only catalytic activity to significantly correlate with formation of N-desmethyl LY333531 was that associated with CYP3A. Further corroborating the role of CYP3A in this biotransformation, formation of N-desmethyl LY333531 was observed by both expressed CYP3A4 and CYP2D6 but to a much greater extent by CYP3A4 (57-fold greater formation rate with CYP3A4 versus CYP2D6). To help delineate the role that either CYP3A or CYP2D6 plays in the formation of N-desmethyl LY333531, specific inhibitors of these enzymes were examined for their ability to decrease the formation of N-desmethyl LY333531 by human liver microsomes. The CYP2D6 inhibitor quinidine (Newton et al., 1995) had little effect on the formation of N-desmethyl LY333531 by microsomes from human liver samples containing a full complement of P450s. Further, with microsomes deficient in CYP2D6, the effect of quinidine was similar to that with microsomes containing CYP2D6, suggesting what little change in activity that was observed was not the result of CYP2D6 inhibition. These data clearly demonstrate that CYP2D6 does not play a significant role in the formation of this metabolite. In contrast, the potent CYP3A inhibitor ketoconazole (Newton et al., 1995) inhibited the formation of the N-desmethyl metabolite by 95 to 98%, confirming the conclusion that CYP3A is the major P450 responsible for the formation of this metabolite.

The conclusion that CYP3A is responsible for the formation of N-desmethyl LY333531 is important considering that CYP3A participates, at least in part, in the metabolism of approximately 50% of xenobiotics for which the P450s responsible for their metabolism have been identified (Wrighton and Thummel, 2000). It is also the most abundant P450 found in the human liver accounting for, on average, 29% of the total P450 present (Shimada et al., 1994). There is high variability in the levels of hepatic CYP3A between individuals, which has been reported to range by at least 20-fold. In addition, CYP3A4 is the dominant P450 in the intestine and has been shown to play a significant role in the first pass metabolism of various drugs, but CYP3A4 is also highly variable in the population, varying by more than 20-fold (Wrighton and Thummel, 2000). In addition to these individual differences in the endogenous expression of CYP3A, it is known that many compounds both inhibit and induce CYP3A activity. Therefore, the identification of CYP3A as the primary enzyme involved in the formation of the N-desmethyl metabolite of LY333531 suggests that one would expect to observe variability in the metabolic clearance of LY333531 with alterations in CYP3A catalytic activity due to factors such as induction or inhibition by coadministered drugs.

In vitro drug interaction studies were also performed to determine whether the presence of the LY333531 or its N-desmethylated metabolite might inhibit the metabolism of a concurrently administered drug. Form-selective catalytic activities were used as models for metabolism mediated by the four major P450s involved in human drug metabolism (CYP3A, CYP2D6, CYP2C9, and CYP1A2). The inhibition of these catalytic activities by LY333531 and its N-desmethyl metabolite was used to determine the potential clinical significance of drug-drug interactions, keeping in mind several factors. First, the dependence of the particular route of metabolism that is inhibited on the overall clearance of the drug is important. For example, if clearance of the drug is less than 30% dependent on the route of metabolism inhibited, the overall effect of this interaction on total drug clearance would be in question regardless of the degree of inhibition since the alterations in overall clearance would likely be within the variability normally seen in the patient population (Tucker, 1992). Second, a consideration of drug and inhibitor concentration at the site of metabolism is important. This last factor is difficult to evaluate in vitro; however, the in vivo plasma concentration of the drug and/or inhibitor is used as a conservative estimate of the concentrations of both at the enzyme site.

The P450 responsible for the genetic polymorphism of debrisoquine/sparteine metabolism is CYP2D6. The poor metabolizer phenotype is found in about 5 to 10% of the Caucasian population (Zanger and Eichelman, 2000). Both LY333531 and N-desmethyl LY333531 exhibited competitive inhibition of CYP2D6 catalyzed 1'-hydroxy bufuralol formation yielding apparent $K_i$ values of 0.17 and 1.0 $\mu$M, respectively (Table 3). For in vitro to in vivo extrapolation of inhibition, the formula listed in the Materials and Methods section was used with the caveat that the predicted inhibition by LY333531 and its metabolite, N-desmethyl LY333531, is additive. Since in vivo concentrations of LY333531 and N-desmethyl LY333531 have been observed to be 0.13 and 0.14 $\mu$M, respectively, the total calculated inhibition was 56%. However, this calculation represents a very conservative estimate since it uses, as the inhibitor concentration, the total plasma concentration of LY333531. The concentration of LY333531 available to interact with the enzyme is likely significantly less than 0.13 $\mu$M due to the 95% percent protein binding observed for LY333531. It would be anticipated that only the free concentration of drug (0.0065 $\mu$M) is able to access the enzyme. Therefore, if the free concentration of LY333531 was used in the calculation for percent inhibition, LY333531 would be predicted to inhibit CYP2D6-mediated metabolism by only 4%. Using this value for percent inhibition, the additive inhibition predicted for both LY333531 and N-desmethyl LY333531 is 16%, which would not be expected to yield clinically significant alterations in the clearance of coadministered substrates of CYP2D6.

As mentioned above, the human CYP3A subfamily has been implicated in the metabolism of a large number of drugs and is the major P450 expressed in the liver and small intestine (Shimada et al., 1994; Wrighton and Thummel, 2000). Although LY333531 was identified as a substrate of CYP3A, a at a $K_M$ concentration of midazolam (5 $\mu$M), only 42% inhibition of CYP3A-mediated metabolism was observed at
the highest concentration of LY333531 (300 μM) examined. These results suggest that LY333531 would be unlikely to significantly inhibit the metabolism of concomitantly administered CYP3A substrates. Further, noncompetitive inhibition of CYP3A-mediated metabolism by N-desmethyl LY333531 was observed, exhibiting an apparent $K_i$ value of 14 μM (Table 3). When this $K_i$ value was entered into the calculation for percent inhibition, assuming an inhibitor concentration of 0.14 μM, it was determined there would be negligible inhibition by N-desmethyl LY333531 of CYP3A-mediated metabolism.

Substrates for CYP2C9 include tolbutamide, phenytoin, nonsteroidal anti-inflammatory agents, and S-warfarin (Rettie et al., 2000) and those for CYP1A2 include phenacetin, tacrine, and theophylline (Minters and McKinnon, 2000). LY333531 and N-desmethyl LY333531 were not found to be significant inhibitors of CYP2C9 or CYP1A2 in vitro. Therefore, these results suggest that LY333531 and N-desmethyl LY333531 would be unlikely to significantly inhibit the metabolic clearance of concomitantly administered substrates of CYP2C9 or CYP1A2.

Overall, LY333531 exhibited the following rank order of inhibitory potential: CYP2D6 > CYP2C9 > CYP3A > CYP1A2. The inhibition potential of the N-desmethyl metabolite of LY333531 exhibited a rank order of CYP2D6 > CYP3A4 > CYP1A2 > CYP2C9. The results generated in these studies suggest that at known in vivo concentrations of LY333531 and N-desmethyl LY333531, these compounds reach a level that may affect the metabolic clearance of coadministered drugs metabolized by CYP2D6. However, the extent of this interaction is dependent on the amount of drug able to access the enzyme, which is most likely related to the circulating concentration of free (not protein bound) drug. The inhibition of metabolism observed by these compounds mediated by the P450s examined was less potent, ranging from a $K_i$ value of 14 μM to negligible inhibition. Therefore, it would be unlikely that metabolism of coadministered compounds metabolized by CYP1A2, CYP2C9, or CYP3A would be affected by coadministration of LY333531.

In conclusion, these studies indicated that CYP3A is responsible for the formation of N-desmethyl LY333531, the major metabolite of LY333531. Therefore, patient variability in clearance may be observed due to the large population variability in CYP3A levels in the gut and liver. In addition, LY333531 and its N-desmethyl metabolite were found to be possible inhibitors of CYP2D6-mediated reactions but exhibited little potential to inhibit metabolism mediated by CYP1A2, CYP2C9, or CYP3A.

References