ABSTRACT:
In vitro studies were conducted to identify the hepatic cytochrome P-450 (CYP) enzymes responsible for the oxidative metabolism of the individual enantiomers of reboxetine. In human liver microsomes, each reboxetine enantiomer was metabolized to one primary metabolite, O-desethylreboxetine, and three minor metabolites, two arising via oxidation of the ethoxy aromatic ring and a third yet unidentified metabolite. Over a concentration range of 2 to 200 μM, the rate O-desethylreboxetine formation for either enantiomer conformed to monophasic Michaelis-Menten kinetics. Evidence for a principal role of CYP3A in the formation of O-desethylreboxetine for (S,S)-reboxetine and (R,R)-reboxetine was based on the results of the following studies: 1) inhibition of CYP3A activity by ketoconazole markedly decreased the formation of O-desethylreboxetine, whereas inhibitors selective for other CYP enzymes did not inhibit reboxetine metabolism, 2) formation of O-desethylreboxetine correlated (r² = 0.99; p < .001) with CYP3A-selective testosterone 6β-hydroxylase activity across a population of human livers (n = 14). Consistent with inhibition and correlation data, O-desethylreboxetine formation was only detectable in incubations using microsomes prepared from a Baculovirus-insect cell line expressing CYP3A4. Furthermore, the apparent Kᵢₜ for the O-desethylolation of reboxetine in cDNA CYP3A4 microsomes was similar to the affinity constants determined in human liver microsomes. In addition, (S,S)-reboxetine and (R,R)-reboxetine were found to be competitive inhibitors of CYP2D6 and CYP3A4 (Kᵢ = 2.5 and 11 μM, respectively). Based on the results of the study, it is concluded that the metabolism of both reboxetine enantiomers in humans is principally mediated via CYP3A.

Depression is a common, chronic medical condition believed to be a result of a retardation of psychomotor and cognitive functions associated with either the noradrenaline or serotonin neurotransmitter systems. Historically, drug therapies (e.g., tricyclic antidepressants) to treat depression were associated with a high incidence of untoward side effects that, in many instances, mimicked the symptoms of the disease (Hindmarsh, 1997). In contrast, newer noradrenergic agents (NARIs), such as reboxetine (Vestra; (RS)-2[(RS)-α-(2-ethoxyphenoxo)y]benzyl)morpholine methanesulfonate) demonstrate significant improvements with respect to the incidence and severity of side effects, in particular effects upon psychomotor function (Dubini et al., 1997; Mucci, 1997), and as a result may be beneficial in the treatment of depression in ambulant patients (Montgomery, 1997).

Reboxetine (Fig. 1) possesses two chiral centers; however, as a result of regio- and stereochemical constraints associated with key reactions in its synthesis, reboxetine exists as only the (R,R)- and (S,S)-enantiomers (Melloni et al., 1985). The in vivo pharmacokinetics and metabolism of reboxetine is relatively well documented (Dottage et al., 1997). In humans, reboxetine is rapidly absorbed (Tₘₐₓ ~2 h) and possesses a terminal half-life of elimination (T½ₑ) of 13 h, which allows for twice-daily administration (Edwards et al., 1995). In addition, reboxetine undergoes extensive hepatic oxidative metabolism, and the subsequent metabolites are excreted into the urine primarily as the glucuronic acid conjugates (Cocchiara et al., 1991).

The purpose of the current in vitro study was to characterize the primary hepatic drug-metabolizing enzymes responsible for the metabolism of the individual enantiomers of reboxetine in humans as a means to predict potential factors (e.g., drug-drug interactions) that may result in interindividual variability across a patient population. To this end, four types of in vitro experiments were conducted: 1) determination of the sample-to-sample variation in the metabolism of the individual reboxetine enantiomers across a bank of human liver microsomes followed by correlation analysis with variations in the catalytic activities associated with the major drug-metabolizing CYP enzymes expressed in human liver microsomes; 2) investigation of the effect of coinubcation with chemicals that selectively inhibit the activity of specific CYP enzymes on the human liver microsomal metabolism of each reboxetine enantiomer; 3) characterization of the Michaelis-Menten kinetic parameters associated with the formation of the major metabolite associated with each reboxetine enantiomer in the presence of human liver microsomes; and 4) investigation into the metabolism of reboxetine enantiomers using recombinant CYP enzymes. In addition, (S,S)-reboxetine and (R,R)-reboxetine were evaluated as possible catalytic inhibitors of the activities for CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. Based on the degree of inhibition, interactions judged to be significant were further evaluated to more fully characterize the magnitude of the drug-drug interaction.
**Materials.** Each reboxetine enantiomer (Fig. 1) synthesized with a uniform carbon-14 radiolabel; (R,R)-reboxetine (46.54 mCi/mmol) and (S,S)-reboxetine (46.54 mCi/mmol) in the morpholine moiety of the molecule was obtained from Pharmacia & Upjohn (Kalamazoo, MI). The radiochemical purity of each optically pure [14C]reboxetine enantiomer was >98% as determined by HPLC with radiochemical detection. [14C]Delavirdine was obtained from Pharmacia & Upjohn. [14C](S)-mephenytoin, [14C]diclofenac, and [14C]chlorzoxazone were purchased from Amersham Corp (Arlington Heights, IL); [14C]testosterone was obtained from DuPont-NEC (Boston, MA); [14C]para-nitrophenol, 1-aminobenzotriazole (ABT), coumarin (COUM), orphenadrine (ORPH), sulfaephazone (SULF), para-nitrophenol (NTR), quinidine (QUIN), papaverine (PAPV), ketokonazole (KETO), and NADPH were purchased from Sigma (St. Louis, MO). Furafylline (FURF) and (S)-mephenytoin (MEPF) were gifts from Dr. K. L. Kunze and Dr. W. F. Trager, Department of Medicinal Chemistry, University of Washington (Seattle, WA). UltimaFlo M liquid scintillant was purchased from Packard Instrument Company (Downers Grove, IL). MAB-3A4 (monoclonal antibody inhibitory to CYP3A4) was purchased from Gentest (Woburn, MA). All other reagents and solvents were of analytical grade.

**Microsomes.** Human livers were acquired from the International Institute for the Advancement of Medicine (IIAM; Exton, PA). Liver microsomal protein isolation and the specific catalytic activity of individual isoforms of P-450 were determined as described previously (Wienkers et al., 1996). Microsomes from a Baculovirus-insect cell line expressing CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 were purchased from Gentest (Woburn, MA).

**Incubation Conditions.** A typical incubation (final volume 0.2 ml) consisted of 0.1 mg of microsomal protein in 100 mM potassium phosphate buffer (pH 7.4). Stock solutions of the individual enantiomers of reboxetine were prepared in methanol (final concentration of methanol was less than 0.3% v/v) by combining appropriate amounts of radiolabeled (approximately 0.1 μCi/ incubation) and nonradiolabeled drug. The drug, buffer, and microsomes were mixed and preincubated at 37°C for 4 min. Incubations were started by the addition of the NADPH, and incubation was conducted at 37°C for 30 min. For control incubations, NADPH was omitted. Reactions were terminated upon addition of 200 μl of acetonitrile, after which samples were vortex mixed and centrifuged for 15 min at 14,000g. The subsequent supernatants were transferred to an HPLC autosampler vial and capped, and the samples were kept refrigerated until radio-HPLC or mass spectrometric analysis.

**Radio-HPLC.** Analytical separation of reboxetine enantiomers and their metabolites was accomplished using a binary gradient HPLC system equipped with a Perkin-Elmer Series 200 pump and autosampler (Perkin-Elmer, Norwalk, CT) equipped with a chilled sample tray maintained at 4°C. The analytical column was a reversed phase Zorbax SB-CN (250 × 4.6 mm, 5-μm particle size; Mac-Mod Analytical, Chadds Ford, PA). The mobile phase consisted of solvent A (90.0%:10.0%:0.2%, water/methanol/acetic acid) and solvent B (10.0%:90.0%:0.2%, water/methanol/acetic acid). Initial mobile-phase conditions (100% A) at a rate of 1.0 ml/min were held for 5.0 min, followed by a step gradient to 40% B in 10.0 min, then by a second step gradient to 90% B in 5.0 min. The final conditions were held for 5.0 min, then returned to the original starting conditions. Quantitation of (R,R)- and (S,S)-reboxetine and their metabolites were detected using a flow-through radioactivity detector (FLO-ONE; Beta Series AS50; Packard/Radiomatic, Meriden, CT), and peak areas were integrated with Windows-based Radio-HPLC Workstation software (FLO-ONE/Data for Windows). UltimaFlo M liquid scintillant was introduced post column at a rate of 3.0 ml/min. The fractional contribution of each metabolite to total radioactivity was used to calculate the rates of metabolite formation.

**LC/ESI/MS and Metabolite Confirmation.** The identity of the primary in vitro reboxetine metabolites was confirmed using a Finnigan LCQ ion-trap (San Jose, CA) operated in positive-ion electrospray ionization mode. The ESI spray voltage was 4.5 kV, and nitrogen (99.9% pure; AGA, Maumee, OH) was used as a drying gas at a sheath pressure of 80 p.s.i. and auxiliary flow rate of 20 ml/min. The heated capillary was set at 250°C. Analytical separation was accomplished using the HPLC conditions described above. Under these conditions, authentic standards of desethylreboxetine, and the two phenolic metabolites (A and B), were characterized by retention time and molecular ion (M + H+).

**Kinetic Analysis.** Kinetics parameters (apparent K_M and V_max) for the O-desethylation of each reboxetine enantiomer (2–200 μM) were determined in vitro in conditions, which were linear with respect to protein concentration and time of incubation. The untransformed data were fitted to both a one- and a two-enzyme model using least-squares nonlinear regression analysis (SYSTAT, Evanston, IL). Initial kinetic parameters for O-desethylyreboxetine formation for each reboxetine enantiomer were obtained by the method of sum-of-squares nonlinear regression analysis using the graphical/statistical program Prism 2.01 (GraphPad, San Diego, CA).

**Correlation Analysis.** The rates of formation of the primary metabolites for each reboxetine enantiomer (100 μM) were determined in a panel of liver microsomes prepared from 14 different human organ donors. The rates of formation of the primary metabolites for each reboxetine enantiomer were compared to the catalytic activities previously characterized for specific P-450 substrates (Wienkers et al., 1996). Incubations and sample workup were carried out as described above. Correlation of determination (r^2) for enzyme activities were determined by linear regression analysis using the statistical program SYSTAT.

**Chemical Inhibition Experiments.** The individual enantiomers of reboxetine were incubated in pooled human liver microsomes in the presence of a panel of compounds that interacted selectively with various P-450 enzymes. The following P-450 enzyme substrates/inhibitors were examined for their ability to inhibit the microsomal metabolism of (R,R)-reboxetine and (S,S)-reboxetine: ABT (10 μM), FURF (60 μM), COUM (100 μM), ORPH (10 μM), SULF (10 μM), MEPH (300 μM), QUIN (5 μM), NTR (100 μM), PAPV (100 μM), MAB-3A4 (10 μl/0.1 mg protein), and KETO (5 μM). All the inhibitors were dissolved in methanol and were added to the incubations such that the final amount of methanol was 1%. Control incubations (minus inhibitor) also contained 1% methanol. The mechanism-based inhibitors, ABT and FURF, and the anti-CYP3A4 antibody, MAB-3A4, were preincubated with microsomes and NADPH for 10 min before the addition of substrate.

**Metabolism by cDNA-Expressed Microsomes.** The metabolism of (R,R)-reboxetine and (S,S)-reboxetine was examined in microsomes prepared from a Baculovirus-insect cell line expressing CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. The incubations were conducted in a manner essentially as described above with 50 μM of either [14C](R,R)-reboxetine or [14C](S,S)-reboxetine and equivalent protein concentrations (1 mg/ml; 40–120 pmol of CYP/ml) of each P-450 isoform in 100 mM potassium phosphate buffer (pH 7.4).

**P-450 Inhibition Screen.** The ability of the individual reboxetine enantiomers to inhibit P-450 enzymes was investigated against six different cDNA-expressed human CYP enzyme systems (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4). Incubations were conducted in quadruplicate, and each incubation contained cDNA CYP microsomal protein (0.1–0.15 mg), NADPH (1 mM), [14C]CYP marker substrate ([S] = K_M), and one of the reboxetine enantiomers (0, 10, or 100 μM) in a final volume of 0.2 ml of 100 mM potassium phosphate buffer (pH 7.4). Incubation reactions, sample workup, and quantitation of CYP marker metabolite formation using HPLC/radiochemical detection was conducted as described previously (Wynda and Wienkers, 1997).
Reboxetine enantiomers were oxidized to four primary metabolites obtained from human liver microsomes indicate that both enantiomers (1 using diminished (i.e., biologically relevant) reboxetine concentrations (up to 0.5 mg/ml protein for 30 min) at a substrate concentration of 100 μM (results not shown). Incubations carried out using diminished (i.e., biologically relevant) reboxetine concentrations (1 μM) revealed a single metabolite, desethylreboxetine, as being the principle reboxetine metabolite for both enantiomers (Fig. 2). Using cochromatography with authentic standards and by mass spectrometry, three of the four metabolites were identified as being the previously described primary in vivo reboxetine oxidative metabolites (Cocchiara et al., 1991). The fourth primary in vitro metabolite, UK1, could not be determined by co-elution with authentic standards or by mass spectrometry. Formation of all four metabolites was dependent on addition of NADPH and was proportional with time (up to 30 min at 0.5 mg of protein) and protein concentration (up to 0.5 mg/ml protein for 30 min) at a substrate concentration of 100 μM (results not shown). Incubations carried out using diminished (i.e., biologically relevant) reboxetine concentrations (1 μM) revealed a single metabolite, desethylreboxetine, as being the principle reboxetine metabolite for both enantiomers (Fig. 3).

The effects of substrate concentration on the rate of O-dealkylation for each of the reboxetine enantiomers were determined in two human liver microsomal preparations and are listed in Table 1. For each enantiomer, formation of desethylreboxetine appeared to undergo saturable kinetics. Moreover, analysis of the kinetic data using Eadie-Hofstee graphical analysis (Fig. 4), revealed a linear relationship between V (the rate of desethylreboxetine formation) and V/[S] (the velocity divided by the substrate concentration), which suggests that a single enzyme or two enzymes with similar Km values were responsible for reboxetine O-dealkylation for either enantiomer. This observation was then substantiated through a comparison of goodness-of-fit values generated for desethylreboxetine velocity data modeled to single enzyme and multiple enzyme equations using sum-of-squares nonlinear regression analysis.

Correlation Studies. Sample-to-sample variation of rates of oxidation for each reboxetine enantiomer in human liver microsomes from 14 donors was determined with the intention of correlating these data with previously measured isoform-specific P-450 catalytic activities.

The formation of each (S,S)-reboxetine oxidative metabolites; UK1, Phenol A, Phenol B, and desethylreboxetine were highly correlated (r² = 0.87–0.98) (Table 2). Moreover, comparison of the relative rates of formation of (S,S)-reboxetine oxidative metabolites across the panel of human livers revealed that all four were highly correlated (r² > 0.93) with CYP3A4 (testosterone 6β-hydroxylase) activity and did not correlate with any of the other measured CYP activities within the panel of liver microsomes tested as presented in Table 2.

The rates of formation for three of the primary (R,R)-reboxetine metabolites, UK1, Phenol A, and desethylreboxetine were highly correlated (r² = 0.92–0.99); however, these metabolites did not correlate as strongly (r² = 0.76) with the rate of formation for Phenol B. Comparison of the formation of (R,R)-reboxetine oxidative metabolites across the panel of human livers revealed that metabolites, UK1, Phenol A, and desethylreboxetine were highly correlated (r² = 0.94–0.99) with CYP3A4 (testosterone 6β-hydroxylase) activity (Table 3). In contrast, Phenol B demonstrated only a weak correlation with CYP3A4 activity (r² = 0.73). The poor correlation between formation of phenol B and CYP3A4 activity may simply reflect the analytical difficulties associated with quantitating this minor metabolite. The correlation coefficients for (R,R)-reboxetine oxidative metabolites with other measured CYP activities are shown in Table 3.

Inhibition Studies. The effects of various P-450 substrates and/or inhibitors on the metabolism of (R,R)-reboxetine or (S,S)-reboxetine were investigated in human liver microsomes. Although some of the inhibitors used in this study interact with more than one P-450 isoform (Newton et al., 1995), they do so with differing enzyme affinities, such that, with appropriate inhibitor concentrations, it is possible to interact predominantly with the target CYP enzyme. Inhibitor concentrations chosen in the current study were selected to produce greater than 80% inhibition of total enzyme activity based on literature Km values for each chemical and the initial kinetic estimates determined for the desethylation of both reboxetine enantiomers. The data presented in Table 4 are expressed as a percentage of control (minus inhibitor) activity. Results are shown as the mean of triplicate determinations. The chemicals are listed with the concentrations used and categorized according to which P-450 isoform they inhibit. From the data presented in Table 4 it appears that preincubation with the general P-450 mechanism-based inhibitor, ABT (Xu et al., 1994), markedly inhibited the formation of desethylreboxetine for each enantiomer. Moreover, dealkylation of each reboxetine enantiomer was markedly inhibited upon co-incubation with anti-CYP3A4 inhibitory antibodies and with the CYP3A4 inhibitors KETO and PAPV.

Metabolism by Recombinant CYP Microsomes. Incubations with either reboxetine enantiomer (50 μM) in the presence of microsomal preparation containing cDNA-expressed CYP3A4 resulted in the formation of all four primary human liver microsomal reboxetine metabolites (data not shown). However, when either enantiomer was incubated with a greatly reduced reboxetine concentration (1 μM), only a single metabolite, desethylreboxetine, was detected (Fig. 3). Kinetic analysis of the rates of formation of desethylreboxetine in CYP3A4 microsomes for either enantiomer resulted in an apparent Km value that in both cases was similar to the Km values obtained in

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**Fig. 2. Proposed metabolic scheme of either reboxetine enantiomers in human liver microsomes.**

The heavy arrow indicates major in vitro metabolite; the lighter arrows reflect minor in vitro metabolites.

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2 The unambiguous identification of the unknown reboxetine in vitro metabolite, UK1, is currently under further investigation.
human liver microsomes (Table 1). No activity was observed in control (minus cDNA vector) microsomes. Similarly, incubations of either reboxetine enantiomer and microsomes containing cDNA-expressed CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP2E1 were devoid of any reboxetine oxidase activity.

**P-450 Inhibition Screen.** The selectivity of inhibition for the individual enantiomers of reboxetine on the activities of six human cytochrome P-450 enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) were evaluated using a simple in vitro inhibition screen (Table 5). Of the P-450 enzymes tested only CYP2D6 and CYP3A4 were potentially inhibited (35% inhibition at 10 μM inhibitor concentration) by each enantiomer. Further characterization of the nature and magnitude of the observed inhibition revealed that each enantiomer is a competitive inhibitor of CYP2D6 (IC\textsubscript{50} = 5.6 and 4.9 μM, for (R,R)- and (S,S)-reboxetine, respectively) and CYP3A4 (IC\textsubscript{50} = 14.3 and 21.3 μM, for (R,R)- and (S,S)-reboxetine, respectively).

**Discussion**

Results of the current study indicate that CYP3A4 is the principal human P-450 responsible for the metabolism of each reboxetine enantiomer in vitro. Moreover, the identified in vitro metabolites are consistent with the primary oxidative metabolites observed for reboxetine in vivo (Cocchiara et al., 1991). Although stereoselective metabolism is not uncommon for optically active compounds (Caldwell, 1995), the current results suggest a lack of stereoselectivity in the rates of CYP3A4-mediated O-desethylation for both reboxetine enantiomers (V/K\textsubscript{M} for (R,R)- and (S,S)-reboxetine were 0.051 and 0.043, respectively). These in vitro observations are consistent with existing in vivo data that suggest there is no statistically significant difference in any of the pharmacokinetic parameters associated with
**Experimental Procedures**

Points on graph represent triplicate determinations of human liver microsomes and metabolite formation was characterized as described in Experimental Procedures. Points on graph represent triplicate determinations of velocity for each reboxetine concentration tested.

### TABLE 2

<table>
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<tr>
<th>UK1</th>
<th>A</th>
<th>B</th>
<th>C</th>
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<th>2D6</th>
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### TABLE 3

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### TABLE 4

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<th>Inhibitor</th>
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<th>(R,R)-Desethylreboxetine</th>
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<td>MPh (200)</td>
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<td>23.2 (12.1)</td>
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**FIG. 4.** Representative Eadie-Hofstee plots for the formation of desethylreboxetine for each reboxetine enantiomer in human liver microsomes (HL39).

(R,R)-Reboxetine (□) or (S,S)-reboxetine (▲) (2–200 µM) were incubated with human liver microsomes and metabolite formation was characterized as described in Experimental Procedures. Points on graph represent triplicate determinations of the individual enantiomers of reboxetine (Strolin-Benedetti et al., 1994).

CYP3A4 is the most abundant human CYP isoform expressed in the liver (Shimada et al., 1994) and is responsible for the biotransformation of numerous clinically useful therapeutics (Wilkinson, 1996). Several factors may potentially influence CYP3A activity. For instance, CYP3A expression/activity can be markedly elevated by chemical inducers like phenytoin (Fleishaker et al., 1995) or significantly inhibited with drugs such as ketoconazole (Floren et al., 1997) as well as with other CYP3A substrates when given concomitantly with the liver microsomal system.

The chemicals are listed according to the CYP enzyme they inhibit. Incubation conditions were carried out as described in Experimental Procedures. Each data point represents the mean (± S.D.) of triplicate determinations.

(Wrighton et al., 1996). Thus, an understanding of the relative contribution of CYP3A to the overall metabolism of a prospective drug candidate is an important factor in the development process.

Confirmation that CYP3A4 is the predominant P-450 involved in human microsomal oxidation of both reboxetine enantiomers is provided by several lines of evidence. First, correlation analysis of reboxetine oxidation across 14 human liver microsomal preparations correlated with CYP3A4 (testosterone 6-β-hydroxylase) activity. In addition, the y intercepts for these correlations are near zero, which suggests that a single enzyme being primarily responsible for the formation of metabolites. Second, prototypic CYP3A inhibitors, ketoconazole and papaverine (Richard et al., 1996; Pearse et al., 1996), markedly inhibited the human liver microsomal formation of desethylreboxetine for each enantiomer. It has been demonstrated that ketoconazole is specific inhibitor of CYP3A4 activity (Newton et al., 1995). In contrast, the second CYP3A4 inhibitor, papaverine, does possess some weak CYP2A6 inhibitory activity (Draper et al., 1997). However, given under the incubations conditions used in the current study, papaverine is expected to be selective toward CYP3A4. Moreover, coincubation of either (S,S)-reboxetine or (R,R)-reboxetine with coumarin, a potent inhibitor of CYP2A6, did not substantially alter metabolite formation for either enantiomer (Table 4). To further substantiate the principal role of CYP3A4 as the reboxetine O-desethylase, inhibition studies were conducted using anti-human CYP3A4 antibodies (Gelboin et al., 1995). Coincubation of human liver microsomes with anti-CYP3A4 antibody markedly decreased desethylreboxetine formation (77 and 82%, for (S,S)-reboxetine and (R,R)-reboxetine, respectively) compared with control. Third, incubations of the individual enantiomers of reboxetine across a panel of cDNA-expressed P-450 enzymes revealed that only CYP3A4 microsomes were able to oxidize reboxetine. Finally, kinetic analysis of the determined Michaelis-Menten kinetic constants (Km) for reboxetine O-desethylase for each enantiomer in recombinant CYP3A4 microsomes were similar to the kinetic constants observed in human liver microsomes (apparent Km = 17 and 27 µM for CYP3A4 and human microsomes, respectively). The small discrepancy between the determined Km values for each microsomal system may in part reflect nonspecific substrate binding to microsomal proteins (Ludden et al., 1997; Obach, 1997). In the current studies, the ratio of absolute protein concentrations used in the human liver microsomal incubations was similar to those used with cDNA-expressed CYP3A4 microsomes.
was 5:1, which suggests that nonselective binding may be a plausible explanation for the slight differences in observed $K_i$ values.

To gather some insight into the potential of reboxetine as an inhibitor of CYP enzymes, the individual enantiomers of reboxetine were screened for their ability to inhibit the catalytic activity of select P-450 enzymes using isomeric-specific probe reactions. The current studies revealed that each reboxetine enantiomer had an inhibitory effect upon the activities of CYP2D6 and CYP3A4. Based upon the factors governing the in vitro metabolism for each enantiomer, the interaction between reboxetine and CYP3A4 is not surprising. Moreover, the observed inhibition was competitive and the calculated inhibition constant was similar in magnitude to the $K_i$ determined for each enantiomer and CYP3A4. Interestingly, both reboxetine enantiomers inhibited CYP2D6 without appearing to be important substrates for this isoenzyme. This observation is hardly unique as drugs such as pimozide (Desta et al., 1998), halofantrine (Hindmarch I, 1997), and quinidine (Guengerich et al., 1986) are also inhibitors of CYP2D6 without being important in vitro substrates. The notion of a minor or nonexistent role of CYP2D6 in reboxetine metabolism is further supported in vivo, where coadministration of the potent CYP2D6 inhibitor quinidine did not significantly alter reboxetine pharmacokinetics (Rocchetti et al., 1995). Finally, each reboxetine enantiomer lacked any inhibitory effect on the activities of other CYP450 isoforms (CYP1A2, CYP2C9, CYP2C19, and CYP2E1) tested, even at high concentrations (>100 times greater than therapeutic plasma concentrations of reboxetine).

Assuming first order kinetics, liver drug metabolism is governed by the intrinsic enzyme catalytic capacity of individual hepatocytes and the availability of drug at the site of metabolism (Rane et al., 1977). In this light, the underlying determinants for predicting a drug's potential to inhibit a particular P-450 is its $I/K_i$ ratio, where $I$ is the concentration of the inhibitor at the site of metabolism and $K_i$ is the apparent inhibitory constant of the inhibitor (Bertz and Granneman, 1997). For instance, an $I/K_i$ ratio of unity would predict that the metabolism of a drug that is cleared via a single metabolic pathway would be decreased by one-half on the concomitant administration of a second drug that inhibits the same pathway. For the current study, each reboxetine enantiomer was found to be a competitive inhibitor of the catalytic activities for CYP3A4 and CYP2D6. In the case of competitive inhibition, under incubation conditions were $[S] = K_M$, the calculated $IC_{50}$ is equal to $2K_i$ (Cheng and Prusoff, 1973). Therefore, the calculated $K_i$ values for either reboxetine enantiomer toward CYP2D6 and CYP3A4 are about 2.5 and 11 $\mu$M, respectively.

Reboxetine is extensively bound to plasma proteins, in particular to $\alpha$-acid glycoprotein (Edwards et al., 1995). Administration of reboxetine as a single 4-mg tablet results in a plasma $C_{max}$ of 125 ng/ml at 1 h (Dostert et al., 1997). Therefore, the maximum concentration of reboxetine (protein bound and free) achieved in vivo will be about 0.4 $\mu$M. Using the $K_i$ values determined from the inhibition study, the $I/K_i$ ratio for the reboxetine with respect to CYP2D6 and CYP3A4 are approximately 0.15 and 0.04, respectively, which predicts a nominal interaction between reboxetine and either cytochrome P-450 enzyme. Thus, as long as the presence of reboxetine does not drastically alter the dispositional characteristics of a second drug (i.e., the concentration of drug available at the site of metabolism is not different in the presence or absence of reboxetine) and the enzyme affinity constant for drug is independent of inhibitor, the current data suggest that reboxetine should not substantially alter the metabolism of a second drug whose clearance is primarily mediated by the major hepatic P-450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4).

We have demonstrated for the first time that the individual enantiomers of reboxetine are metabolized in humans primarily via $O$-dealkylation and that this metabolic step is catalyzed primarily by human CYP3A4. Therefore, given the relatively low substrate binding affinity of reboxetine toward CYP3A4 and the submicromolar plasma concentrations of the drug achieved in humans, metabolism and clearance of reboxetine may be susceptible to inhibition upon coadministration with drugs (e.g., ketoconazole) that are known to inhibit the catalytic activity of CYP3A4.

References


Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (ki) and the concentration of inhibitor which causes 50% per cent inhibition IC50 of an enzymatic reaction. Biochem Pharmacol 22:3099–3108.


