Identification of Human Cytochrome P450 and Flavin-Containing Monooxygenase Enzymes Involved in the Metabolism of Lorcaserin, a Novel Selective Human 5-Hydroxytryptamine 2C Agonist

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ABSTRACT:
Lorcaserin, a selective serotonin 5-hydroxytryptamine 2C receptor agonist, is being developed for weight management. The oxidative metabolism of lorcaserin, mediated by recombinant human cytochrome P450 (P450) and flavin-containing monooxygenase (FMO) enzymes, was examined in vitro to identify the enzymes involved in the generation of its primary oxidative metabolites, N-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin. Human CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, CYP3A4, and FMO1 are major enzymes involved in N-hydroxylorcaserin; CYP2D6 and CYP3A4 are enzymes involved in 7-hydroxylorcaserin; CYP1A1, CYP1A2, CYP2D6, and CYP3A4 are enzymes involved in 5-hydroxylorcaserin; and CYP3A4 is an enzyme involved in 1-hydroxylorcaserin formation. In 16 individual human liver microsomal preparations (HLM), formation of N-hydroxylorcaserin was correlated with CYP2B6, 7-hydroxylorcaserin was correlated with CYP2D6, 5-hydroxylorcaserin was correlated with CYP1A2 and CYP3A4, and 1-hydroxylorcaserin was correlated with CYP3A4 activity at 10.0 µM lorcaserin. No correlation was observed for N-hydroxylorcaserin with any P450 marker substrate activity at 1.0 µM lorcaserin. N-Hydroxylorcaserin formation was not inhibited by CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, and CYP3A4 inhibitors at the highest concentration tested. Furafylline, quinidine, and ketoconazole, selective inhibitors of CYP1A2, CYP2D6, and CYP3A4, respectively, inhibited 5-hydroxylorcaserin (IC50 = 1.914 µM), 7-hydroxylorcaserin (IC50 = 0.213 µM), and 1-hydroxylorcaserin formation (IC50 = 0.281 µM), respectively. N-Hydroxylorcaserin showed low and high Km components in HLM and 7-hydroxylorcaserin showed lower Km than 5-hydroxylorcaserin and 1-hydroxylorcaserin in HLM. The highest intrinsic clearance was observed for N-hydroxylorcaserin, followed by 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin in HLM. Multiple human P450 and FMO enzymes catalyze the formation of four primary oxidative metabolites of lorcaserin, suggesting that lorcaserin has a low probability of drug-drug interactions by concomitant medications.

Introduction

The cytochrome P450 (P450) monooxygenase system is composed of a superfamily of heme-containing enzymes expressed in many mammalian tissues, with the highest levels found in liver, and is capable of catalyzing the metabolism of a wide range of both endogenous and exogenous substrates (Nelson et al., 1996). The mammalian flavin-containing monooxygenases (FMOs), although not catalytically or structurally as diverse as the P450 superfamily, are important phase I enzymes that are responsible for the conversion of lipophilic xenobiotics to more hydrophilic metabolites. The FMO family is a complementary enzyme system to the P450 family and oxidizes at the nitrogen, sulfur, selenium, and phosphorus centers of xenobiotic compounds (Ziegler, 1991; Sadeque et al., 1992; Cashman, 2000; Rawden et al., 2000; Hodgson and Goldstein, 2001). In general, the oxidative metabolism of xenobiotics is primarily carried out by P450 and to a lesser extent by FMO.

In the adult human liver, members of the CYP3A, CYP2C, and CYP1A subfamilies are predominant P450 enzymes (Shimada et al., 1994; Guengerich, 1995). Among five mammalian FMOs (FMO1, FMO2, FMO3, FMO4, and FMO5), FMO3 is the most abundantly expressed enzyme in adult human liver (Sadeque et al., 1993; Lawton et al., 1994; Phillips et al., 1995), whereas FMO1 is predominantly expressed in adult human kidney, with some expression in intestinal tissues (Yeung et al., 2000). FMO1 is also a primary constitutive enzyme found in human fetal livers (Dolphin et al., 1996).

Lorcaserin [(1R)-8-chloro-2,3,4,5-tetrahydro-1-methyl-1H-3-benzazepine] is a selective 5-hydroxytryptamine 2C receptor agonist and a potential therapeutic agent for weight management (Smith et al., 2008, 2010). For most commonly used drugs biotransformation is the major path of elimination, and changes in the activity of P450 en-

ABBREVIATIONS: P450, cytochrome P450; 1-ABT, 1-aminobenzotriazole; HLM, human liver microsomes; HRM, human renal microsomes; FMO, flavin-containing monooxygenase; LC-MS/MS, liquid chromatography/tandem mass spectrometry; %TNR, total normalized rates; FAD, flavin adenine dinucleotide.
zymes is the major cause of drug-drug interactions. Therefore, it is relevant to assess the relative contribution of these oxidative enzymes to the overall clearance of lorcaserin and to identify the P450 and FMO enzymes responsible for lorcaserin metabolism. Human liver microsomes catalyze the metabolism of lorcaserin to N-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin, its four primary oxidative metabolites (Fig. 1). The objective of this in vitro study was to identify the predominant human P450 and FMO enzymes involved in the metabolism of lorcaserin using human liver and renal microsomes as well as human recombinant enzymes. For the screening, inhibition, and correlation experiments, two concentrations of lorcaserin were used. Lorcaserin concentration of 1.0 μM was closer to the clinically relevant plasma concentration (Smith et al., 2010), and 10.0 μM was 10-fold higher of the initial 1.0 μM concentration.

Materials and Methods

Chemicals. Lorcaserin was provided by Cilag AG (Schaffhausen, Switzerland). N-Hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin were synthesized by SAFC Pharma (Manchester, UK). 7-Hydroxylorcaserin was synthesized at Arena Pharmaceuticals, Inc. (San Diego, CA). Dextrophan-d6 was purchased from BD Biosciences (Woburn, MA). Other reagents used in this study, which include EDTA, MgCl2, potassium phosphate monobasic, potassium phosphate dibasic, dimethyl sulfoxide, tranylcypromine, thioTEPA, quinidine, ketocozazole, 1-aminobenzotriazole (1-ABT), N-benzylimidazole, methimazole, and β-NADPH, were purchased from Sigma-Aldrich (St. Louis, MO). Furafylline and N-benzylirvanol were purchased from BD Biosciences. High-purity grade acetonitrile and water were purchased from Honeywell Burdick & Jackson (Muskegon, MI). All reagents were of high-purity grade.

Enzyme Sources. Mixed sex-pooled human liver microsomal preparations (HLM) (pooled from 50 donors) and mixed sex-pooled human renal microsomal preparations (HRM) (pooled from eight donors) were purchased from XenoTech, LLC (Lexena, KS). Individual donor human liver microsomal preparations, membranes from insect cells (BTI-TN-5BI-4) transfected with baculovirus containing cDNA of human P450 enzymes (CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C18, CYP2C19, CYP2D6, CYP2D6*10, CYP2E1, CYP3A4, CYP3A5, CYP3A7, and CYP4A11) and FMO (FMO1, FMO3, and FMO5) were incubated with 1.0 or 10.0 μM lorcaserin in 100 mM potassium phosphate buffer containing 3 mM MgCl2 and 1 mM EDTA, pH 7.4. Parallel control reactions were performed with S9 insect cells (cells without P450 expression), under identical conditions.

Screening with Recombinant P450 and FMO. Metabolic activity assays designed to screen human recombinant P450 and FMO enzymes for lorcaserin were performed. In brief, 50 pmol/ml P450 (CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C18, CYP2C19, CYP2D6, CYP2D6*10, CYP2E1, CYP3A4, CYP3A5, CYP3A7, and CYP4A11) and FMO (FMO1, FMO3, and FMO5) were incubated with 1.0 or 10.0 μM lorcaserin in 100 mM potassium phosphate buffer containing 3 mM MgCl2, and 1 mM EDTA, pH 7.4. For P450-selective inhibitors, incubation mixtures contained lorcaserin (1.0 or 10.0 μM), liver microsomal protein (0.25 mg/ml), and 100 mM potassium phosphate buffer containing 3 mM MgCl2, and 1 mM EDTA, pH 7.4. For FMO-selective inhibitors, incubation mixtures contained lorcaserin (1.0 or 10.0 μM), individual P450-specific inhibitors, microsomal protein (0.25 mg/ml), and 100 mM potassium phosphate buffer containing 3 mM MgCl2, and 1 mM EDTA, pH 7.4. For P450-selective inhibitors, incubation mixtures contained lorcaserin (1.0 or 10.0 μM), individual P450-specific inhibitors, microsomal protein (0.25 mg/ml), and 100 mM potassium phosphate buffer containing 3 mM MgCl2, and 1 mM EDTA, pH 7.4. For FMO-selective inhibitors, furafylline (0.12–7.50 μM), and furafylline (0.02–1.28 μM), thioTEPA (0.937–30 μM), N-benzylirvanol (0.039–5.0 μM), quinidine (0.004–2.0 μM), and ketocozazole (0.059–3.75 μM) were used to investigate the involvement of CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, and CYP3A4, respectively. For the control incubations, chemical inhibitors were replaced with appropriate amount of solvent. For CYP1A2, furafylline was preincubated for 20 min with human liver microsomal protein in the presence of β-NADPH before the addition of lorcaserin.

Inhibition of FMO and P450 in Renal Microsomes. Additional experiments were designed to evaluate the contribution of FMO and P450 enzymes in HRM for lorcaserin metabolism. (described below) were prewarmed at 37°C for 5 min, after which the reactions were initiated by the addition of β-NADPH (1 mM) with gentle mixing and incubated at 37°C in a water bath for 20 min. All incubations were conducted in triplicate unless otherwise stated. A typical incubation mixture for each of the assays is described in the sections below with the final reaction concentrations given in parenthesis. All reactions were terminated by the addition of an equal reaction volume (0.5 ml) of ice-cold acetonitrile containing internal standard dextrophan-d6. After 10 min of centrifugation at 3700 rpm (2572g), 100 μl of supernatant was transferred to another 96-well plate containing 100 μl of 100 mM potassium phosphate buffer with 3 mM MgCl2, and 1 mM EDTA, pH 7.4, to avoid the high amount of organic content in the samples. Samples for all reference standard curves were processed in a similar way as the samples for metabolic incubations and analyzed by LC-MS/MS.
Inhibition of FMO-mediated metabolism. To investigate the contribution of FMO enzymes in HRM, three sets of incubations were conducted: set 1—control (without treatment)—the incubation mixture consisted of HRM (0.25 mg/ml protein), lorcaserin (1.0 or 10.0 μM), and 25 mM glycine/100 mM potassium pyrophosphate buffer, pH 8.5; set 2—methimazole treatment—the incubation mixture consisted of HRM (0.25 mg/ml protein), lorcaserin (1.0 or 10.0 μM), general FMO inhibitor methimazole (25 μM), and 25 mM glycine/100 mM potassium pyrophosphate buffer, pH 8.5; and set 3—heat treatment—HRM were preheated at 45°C for a total of 3 min in a water bath to inactivate FMO enzymes before the incubation. The incubation with preheated micromoles was then conducted under identical conditions as described above (set 1, control).

Inhibition of P450-mediated metabolism. Similar to FMO, three sets of experiments were conducted to evaluate the contribution of P450 in HRM. The incubations were performed in potassium phosphate buffer at pH 7.4 instead of 25 mM glycine/100 mM potassium pyrophosphate buffer, pH 8.5. For set 1 (control, without treatment), incubations were conducted with HRM (0.25 mg/ml protein), lorcaserin (1.0 or 10.0 μM), and 100 mM potassium phosphate buffer containing 3 mM MgCl₂ and 1 mM EDTA, pH 7.4. For set 2 (methimazole treatment), incubations were performed under identical conditions as for set 1 (control), with the exception that 1-ABT (1.0 mM), a general P450 inhibitor, was added to the incubation mixture before the addition of lorcaserin. For set 3 (heat treatment), incubations were conducted under the same conditions as described for set 1 (control), with the exception that the HRM were preheated for 3 min at 45°C as described above for FMO.

Inhibition of FMO in Human Liver Microsomes. Separate sets of experiments were designed to evaluate the contribution of FMO enzymes in HLM for lorcaserin metabolism. For set 1 (control, without treatment), the incubation mixture consisted of HLM (0.25 mg/ml protein), lorcaserin (10.0 μM), and 25 mM glycine/100 mM potassium pyrophosphate buffer, pH 8.5. For set 2 (methimazole treatment), the incubation mixture consisted of HLM (0.25 mg/ml protein), lorcaserin (10.0 μM), general FMO inhibitor methimazole (25 μM), and 25 mM glycine/100 mM potassium pyrophosphate buffer, pH 8.5.

Enzyme Kinetic Studies. For the kinetic studies, the incubation mixtures consisted of HLM (0.25 mg/ml protein) or human recombinant P450 (CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4) (25 pmol P450/ml) or human recombinant FMO1 (25 pmol FAD/ml); lorcaserin (0–10 μM) as a substrate, and 100 mM potassium phosphate buffer containing 3 mM MgCl₂ and 1 mM EDTA, pH 7.4. After preincubation for 5 min in a 37°C water bath, reactions were initiated by the addition of β-NADPH (1 mM) with gentle mixing. The reaction mixtures were then incubated for an additional 20 min for HLM and 10 min for recombinant P450 and FMO enzymes in a 37°C water bath. Samples for all reference standard curves (0.00121–2.5 μM) were prepared in a similar way as the samples for metabolic incubations. The assays for the kinetic studies with HLM (0.25 mg/ml protein) were conducted in similar way as described above for HLM, with the exception that 25 mM glycine/100 mM potassium pyrophosphate buffer, pH 8.5, was used.

Sample Analysis by LC-MS/MS. After sample processing as described above, analyses of lorcaserin metabolites N-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin were performed by LC-MS/MS. A 10-μl sample was introduced for chromatographic separation using a Mac Mod HALO column (2.7 μm, 3 × 30 mm) (MAC-MOD Analytical, Inc., Chadds Ford, PA) at a flow rate of 0.60 ml/min. A binary gradient was applied using water containing 0.1% formic acid (mobile phase A) and acetonitrile containing 0.1% formic acid (mobile phase B) as follows: initial 2% mobile phase B for 5 s, 2 to 30% mobile phase B over 120 s, and then 30 to 80% mobile phase B over 30 s. The column was washed next with 80% mobile phase B for 15 s followed by a gradient change from 80% to 2% mobile phase B over 5 s. Finally, the column was re-equilibrated with 2% B for 35 s. The total analysis time was 3.5 min. Mass spectrometric detection was achieved with an MSD Sciex API-3000 triple quadruple mass spectrometer (Applied Biosystems/MSD Sciex, Foster City, CA) equipped with an IonSpray LC/MS interface operated in positive ion mode and using MRM. The following Q1/Q3 transitions were monitored: m/z 212.1–152.2 for N-hydroxylorcaserin, m/z 212.1–160.3 for 7-hydroxylorcaserin, m/z 212.1–194.1 for 5-hydroxylorcaserin, m/z 212.1–194.2 for 1-hydroxylorcaserin, and m/z 261.2–157.2 for dextrophan-d₄. The MRM transitions for 5-hydroxylorcaserin and 1-hydroxylorcaserin are identical; however, 5-hydroxylorcaserin and 1-hydroxylorcaserin are chromatographically separated. Quantification was performed with regression analysis generated from calibration standards.

Data Analysis. Enzyme kinetic parameters Kᵣ and Vₘₐₓ were calculated using the SigmaPlot software (Systat Software, Inc., Richmond, CA), which generated a nonlinear least-square fit to the Michaelis-Menten equation. The intrinsic clearance (CLᵣₐᵣ) was calculated as CLᵣₐᵣ = Vₘₐₓ/Kᵣ (Segel, 1976).

The percentage of total normalized rates (%TNR) were determined as described by Rodrigues (1999a). The normalized rate was derived by multiplying the rate of hydroxylation (nanomole per nanomole P450 per minute) of each isoform by the nominal specific content (nanomole P450 per milligram of protein) of the corresponding P450 isoform in native human liver microsomes. The nominal specific content utilized in these determinations was derived from a pool of liver microsomes (n = 12) phenotyped by BD Biosciences as reported by Rodrigues (1999b) for all P450s, with the exception of CYP2B6.

Since BD Biosciences data reported by Rodrigues (1999b) indicated that CYP2B6 levels may have been high in this set of population and the high variability of expression (~39-fold) (Ogilvie et al., 2008), we used a median average value of 0.0207 mmol/mg protein derived from a different pool of liver microsomes (n = 12) phenotyped by BD Biosciences. The normalized rate values obtained were then summed, and the %TNRs were determined for each isoform.

Correlation coefficients (r²) were obtained by plotting the formation rate of each lorcaserin metabolites versus the probe substrate activity for each P450 enzyme using Microsoft Excel. Percentage of control activity of the enzyme (remaining enzyme activity after the inhibition) for each concentration of inhibitor was calculated by dividing the enzyme activity in the presence of inhibitor by the enzyme activity in the absence of inhibitor, and the resulting ratio was multiplied by 100. IC₅₀ values were calculated by plotting the percentage of control activity versus log[I], inhibitor concentration, using GraphPad Prism 4.3 (GraphPad Software, Inc., San Diego, CA). Statistical analysis was performed using Student’s t test (GraphPad Software, Inc.).

Results

Metabolism by Recombinant P450 Enzymes. Recombinant human cytochrome P450 enzymes CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C18, CYP2C19, CYP2D6, CYP2D6*10, CYP2E1, CYP3A4, CYP3A5, CYP3A7, and CYP4A11 were used to evaluate the involvement of P450s in lorcaserin metabolism. Incubations were conducted with two concentrations of lorcaserin, 1.0 and 10.0 μM, for metabolic screening. Formation of four primary metabolites, N-hydroxylorcaserin, 1-hydroxylorcaserin, 5-hydroxylorcaserin, and 7-hydroxylorcaserin, was observed with different rates of formation catalyzed by various P450s tested. However, the N-hydroxylorcaserin metabolism was predominantly formed. As shown in Fig. 2, incubation of 1.0 and 10.0 μM lorcaserin with human CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4 catalyzed the formation of N-hydroxylorcaserin with different extents of rates. Recombinant CYP2B6, CYP2C9, and CYP2D6 were the dominant P450 enzymes that catalyzed N-hydroxylorcaserin formation at both 1.0 and 10.0 μM lorcaserin. At a 1.0 μM lorcaserin concentration, the formation rate of N-hydroxylorcaserin was dominated by CYP2D6. In contrast, at a 10.0 μM lorcaserin concentration, N-hydroxylorcaserin was predominantly formed by CYP2B6 and exhibited by far the highest turnover rate catalyzed by any P450s tested. This may be attributed to the difference in kinetic properties of these enzymes (see kinetic determination below). Likewise, CYP2D6 and CYP3A4 were involved in the metabolism of 7-hydroxylorcaserin; CYP1A1, CYP1A2, CYP2D6, and CYP3A4 were involved in the metabolism of 5-hydroxylorcaserin; and CYP3A4 only was involved in 1-hydroxylorcaserin formation (Fig. 2, A and B).

Rate of metabolites formed by various recombinant P450s at 1.0 and 10.0 μM concentrations of lorcaserin was normalized with respect to the average expression of individual P450 isoforms in native HLM (Fig. 2, C and D). The normalized results showed that at 1.0 μM lorcaserin concentration, N-hydroxylorcaserin was predominantly catalyzed by both CYP2B6 and CYP2D6 with %TNR values of 25.9 and 36.2%.
30.0, respectively, whereas CYP1A2, CYP2A6, CYP2C19, CYP2E1, and CYP3A4 played an ancillary role with %TNR values of 4.54, 14.8, 8.34, 3.14, and 13.3, respectively (Fig. 2C). At a 10.0 μM lorcaserin concentration, N-hydroxylorcaserin was predominantly catalyzed by CYP2B6 with %TNR value of 43.6, whereas CYP1A2, CYP2A6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 played ancillary roles with %TNR values of 5.70, 7.04, 13.7, 7.83, 2.91, and 19.3, respectively (Fig. 2D). For CYP2D6-catalyzed 7-hydroxylorcaserin formation at 1.0 and 10.0 μM lorcaserin concentrations, the %TNR values were 38.8 and 52.3, respectively, whereas for CYP3A4, %TNR values were 61.2 and 47.7, respectively (Fig. 2, C and D). Normalized rate for 5-hydroxylorcaserin showed that at 1.0 μM lorcaserin concentration, it was predominantly catalyzed by CYP1A2 (%TNR = 63.4) and CYP2D6 (%TNR = 36.6), whereas at 10.0 μM lorcaserin concentration, CYP3A4 catalyzed 5-hydroxylorcaserin predominantly (%TNR = 80.1) (Fig. 2, C and D). CYP3A4 was the primary enzyme for 1-hydroxylorcaserin formation with both 1.0 and 10.0 μM lorcaserin concentrations.

Human Liver Microsomal Metabolism and Correlation Analysis. Metabolism by individual liver microsomes. Lorcaserin metabolism was evaluated in 16 individual human liver microsomal preparations using 1.0 and 10.0 μM lorcaserin. As shown in Fig. 3, incubation with 1.0 μM lorcaserin concentration, N-hydroxylorcaserin was predominantly catalyzed by CYP1A2 (%TNR = 43.6), whereas CYP1A2, CYP2A6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 played ancillary roles with %TNR values of 5.70, 7.04, 13.7, 7.83, 2.91, and 19.3, respectively (Fig. 2D). For CYP2D6-catalyzed 7-hydroxylorcaserin formation at 1.0 and 10.0 μM lorcaserin concentrations, the %TNR values were 38.8 and 52.3, respectively, whereas for CYP3A4, %TNR values were 61.2 and 47.7, respectively (Fig. 2, C and D). Likewise, the formation rate for 7-hydroxylorcaserin varied 16.9- and 9.47-fold with 1.0 and 10.0 μM concentrations of lorcaserin, respectively (Fig. 3, A and B). Further examination of the rate of metabolite formation between individual human male and female liver microsomes did not suggest any statistically significant sex-specific variability (% values for N-hydroxylorcaserin 0.88 and 0.11; 7-hydroxylorcaserin 0.94 and 0.76 at 1.0 and 10.0 μM concentrations of lorcaserin, respectively), indicating that the variability in metabolic rate might be attributed to the variation in the expression level of metabolizing enzyme(s) among the individual donors (Fig. 4, A and B). However, irrespective of the sex, all 16 individual HLM catalyzed lorcaserin metabolism.

Correlation between lorcaserin metabolite formation and P450-specific activity. Results from the 16 individual human liver microsomal incubations at concentrations of 1.0 and 10.0 μM lorcaserin were used to perform a correlation analysis for lorcaserin metabolite formation of these metabolites among the 16 individual HLM. The formation rate for N-hydroxylorcaserin was significantly higher than that of the other metabolites in individual liver microsomal incubations with 10.0 μM lorcaserin (Fig. 3B), whereas for incubations with 1.0 μM lorcaserin, the trend varied for N-hydroxylorcaserin and 7-hydroxylorcaserin formation (Fig. 3A). Among 16 individual HLM, a 3.72- and 3.84-fold difference in N-hydroxylorcaserin formation rate was observed with 1.0 and 10.0 μM lorcaserin, respectively (Fig. 3, A and B). Likewise, the formation rate for 7-hydroxylorcaserin varied 16.9- and 9.47-fold with 1.0 and 10.0 μM concentrations of lorcaserin, respectively (Fig. 3, A and B). Further examination of the rate of metabolite formation between individual human male and female liver microsomes did not suggest any statistically significant sex-specific variability (P values for N-hydroxylorcaserin 0.88 and 0.11; 7-hydroxylorcaserin 0.94 and 0.76 at 1.0 and 10.0 μM concentrations of lorcaserin, respectively), indicating that the variability in metabolic rate might be attributed to the variation in the expression level of metabolizing enzyme(s) among the individual donors (Fig. 4, A and B). However, irrespective of the sex, all 16 individual HLM catalyzed lorcaserin metabolism.
suggests that CYP2D6 was the major contributor of 7-hydroxylation (Fig. 5A). This is in agreement with the findings that multiple P450 enzymes are involved in the formation of 7-hydroxylorcaserin near the origin for any P450 activities, indicating that more than one (Table 2; Fig. 5A). The regression line did not pass through or appear correlation with any of the P450 enzymes at this concentration (Table 1).

Inhibition of CYP2B6. As demonstrated in Fig. 7A, increasing concentrations of furafylline showed an inhibitory effect on 5-hydroxylorcaserin formation with an IC50 value of 1.914 μM. This is consistent with Fig. 2 showing that formation of 5-hydroxylorcaserin was catalyzed by multiple recombinant P450s, including CYP1A2. Furafylline showed a moderate effect on 1-hydroxylorcaserin formation without reaching 50% inhibition; however, its effect on the formation of N-hydroxylorcaserin and 7-hydroxylorcaserin was not significant (Fig. 7A).

Inhibition of microsomal CYP2A6. The contribution of recombinant CYP2A6 on the metabolism of lorcaserin was minor in that it catalyzed only the formation of N-hydroxylorcaserin with a limited rate of formation (Fig. 2). The addition of varying concentrations of tranylcypromine, a CYP2A6 inhibitor, did not show a substantial inhibitory effect on liver microsomal metabolism of lorcaserin (data not shown).

Inhibition of microsomal CYP2B6. Recombinant CYP2B6 catalyzed the formation of N-hydroxylorcaserin with a higher rate at 10.0 μM lorcaserin concentration compared with other recombinant P450 enzymes (Fig. 2). However, CYP2B6 inhibitor thioTEPA showed minimal inhibitory effects on N-hydroxylorcaserin formation at concentrations of 10.0 (Fig. 7B) and 1.0 μM lorcaserin (data not shown). This might be attributed to the fact that the N-hydroxylation pathway is catalyzed by multiple human P450 enzymes.
enzymes (Fig. 2) and that the inhibition of one individual P450 may not affect the overall metabolic pathway of this metabolite in HLM. The inhibitory effect of thioTEPA on the formation of the three other lorcaner metabolites was also minimal (Fig. 7B).

**Inhibition of microsomal CYP2C19.** Recombinant CYP2C19 catalyzed the formation of N-hydroxylorcaner (Fig. 2). However, N-benzylvinarnanol, a potent CYP2C19 inhibitor, did not inhibit the formation of N-hydroxylorcaner or other metabolites at relevant concentrations (Fig. 7C).

**Inhibition of microsomal CYP2D6.** The inhibitory effect of quinidine, a potent CYP2D6 inhibitor, on the formation of 7-hydroxylorcaner was prominent, with an IC₅₀ value of 0.213 μM (Fig. 7D) at a concentration of 10.0 μM lorcaserin. As expected, by use of 1.0 μM lorcaserin concentration, the quinidine exerted much stronger inhibitory effect (~10-fold) on 7-hydroxylorcaner formation with an IC₅₀ value of 0.024 μM (Fig. 7E) compared with 10.0 μM lorcaserin concentration (Fig. 7D). These results are in agreement with the finding that recombinant CYP2D6 is the main contributor of 7-hydroxylorcaner formation (Fig. 2). Nevertheless, quinidine did not inhibit N-hydroxylorcaner formation at either concentration (1.0 or 10.0 μM) of lorcaserin in HLM (Fig. 7, D and E). Formation of 5-hydroxylorcaner and 1-hydroxylorcaner was also unaffected by quinidine as well (Fig. 7D).

**TABLE 1**

<table>
<thead>
<tr>
<th>P450 Marker Reaction</th>
<th>N-Hydroxylorcaner</th>
<th>7-Hydroxylorcaner</th>
<th>5-Hydroxylorcaner</th>
<th>1-Hydroxylorcaner</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2  Phenacetin O-deethylation</td>
<td>0.001</td>
<td>0.000</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>2A6  Coumarin 7-hydroxylation</td>
<td>0.193</td>
<td>0.039</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>2B6  (S)-Mephentoin N-demethylation</td>
<td>0.007</td>
<td>0.002</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>2C8  Paclitaxel 6α-hydroxylation</td>
<td>0.034</td>
<td>0.091</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>2C9  Tolfbutamide 4′-hydroxylation</td>
<td>0.000</td>
<td>0.003</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>2C19 (S)-Mephentoin 4′-hydroxylation</td>
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<td>0.006</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>2D6  Bufuralol 1′-hydroxylation</td>
<td>0.074</td>
<td>0.696</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
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<td>3A4  Testosterone 6β-hydroxylation</td>
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<td>0.000</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>4A11 Lauric acid 12-hydroxylation</td>
<td>0.008</td>
<td>0.000</td>
<td>n.d.</td>
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n.d., not detected.
Inhibition of microsomal CYP3A4. Ketoconazole, a potent CYP3A4 inhibitor, significantly inhibited the formation of 1-hydroxylorcaserin, with an IC₅₀ value of 0.281 μM (Fig. 7F). Consistent with Fig. 2, only recombinant CYP3A4 catalyzed the formation of 1-hydroxylorcaserin. The inhibitory effect of ketoconazole on the formation of N-hydroxy- lorcaserin, 7-hydroxylorcaserin, and 5-hydroxylorcaserin was minimal (Fig. 7F). 

Metabolism by Human Renal Microsomes and Recombinant FMO Enzymes. Among the four lorcaserin metabolites, HRM produced only N-hydroxylorcaserin. Human recombinant FMO1, FMO3, and FMO5 were incubated with two lorcaserin concentrations, 1.0 and 10.0 μM. Only FMO1 contributed to lorcaserin metabolism through oxidation on the nitrogen-center, resulting in N-hydroxylorcaserin formation (Fig. 8). Neither human renal microsomes nor human recombinant FMO enzymes formed 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin metabolites (data not shown).

Relative Contribution of FMO and P450 Enzymes in HRM and HLM on Lorcaserin Metabolism. As depicted in Fig. 9, heating of renal microsomes for 3 min at 45°C before the incubation with lorcaserin (glycine buffer, pH 8.4) substantially decreased (>95%) the formation of N-hydroxylorcaserin. Likewise, the addition of methimazole, a competitive inhibitor of FMO enzymes, also significantly inhibited N-hydroxylorcaserin formation (>80%) in HRM (Fig. 9A), suggesting that human FMO (FMO1, which is expressed in human renal tissues) catalyzed the formation of N-hydroxylorcaserin in HRM. When HRM at pH 7.4 in potassium phosphate buffer (P450 assay condition) were treated with 1-ABT, a general inhibitor of P450 enzymes, N-hydroxylorcaserin formation was not inhibited, suggesting that P450 enzymes are not involved in N-hydroxylorcaserin pathway in HRM (Fig. 9B). However, heat treatment of HRM under this condition, pH 7.4, demonstrated >95% inhibition of N-hydroxylorcaserin formation (Fig. 9B). Furthermore, when HLM incubations were

### Table 2

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<th>N-Hydroxylorcaserin</th>
<th>7-Hydroxylorcaserin</th>
<th>5-Hydroxylorcaserin</th>
<th>1-Hydroxylorcaserin</th>
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<td>1A2</td>
<td>Phenacetin O-deethylation</td>
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<td>0.819</td>
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<td>0.680&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>4A11</td>
<td>Lauric acid 12-hydroxylation</td>
<td>0.055</td>
<td>0.002</td>
<td>0.000</td>
<td>0.000</td>
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</tbody>
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<sup>a</sup> Only 3 of 16 individual HLM formed 1-hydroxylorcaserin.

---

**FIG. 5.** Correlation analyses of the formation of lorcaserin metabolites in 16 individual human liver microsomal preparations. A, N-hydroxylorcaserin versus CYP2B6 activity; B, 7-hydroxylorcaserin versus CYP2D6 activity; C, 5-hydroxylorcaserin versus CYP1A2 activity; and D, 5-hydroxylorcaserin versus CYP3A4 activity.
treated with methimazole (glycine buffer, pH 8.4, FMO condition), no inhibition of the formation of any of the lorcaserin metabolites was observed, which ruled out a possible contribution of FMO enzymes in these lorcaserin metabolic pathways in HLM (Fig. 10). These findings are in agreement with the demonstration that only recombinant human FMO1 (Fig. 8), and not FMO3 or FMO5, catalyzed the formation of N-hydroxylorcaserin.

Enzyme Kinetic Studies. Human liver microsomes and recombinant P450 enzymes. Table 3 represents the kinetic parameters ($K_m$, $V_{max}$, and $CL_{int}$) for the formation of lorcaserin metabolites N-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin by the general human cytochrome P450 inhibitors 1-ABT and N-benzylimidazole in human liver microsomal preparations using potassium phosphate buffer, pH 7.4.

In vitro drug metabolism studies, such as reaction phenotyping and P450 inhibition, are valuable tools to predict the potential for drug-drug interactions in vivo as well as polymorphic impact on drug disposition ( Bjornsson et al., 2003; Venkatakrishnan et al., 2003 ). The objective of this study was to identify human P450 and FMO enzymes responsible for the primary oxidative metabolism of lorcaserin. The following three basic approaches were used for the enzyme identification: 1) use of a set of human recombinant P450 and FMO enzymes, 2) evaluation of lorcaserin metabolism in 16 individual HLM to determine the correlation coefficient for lorcaserin metabolism versus P450 probe substrate activities, and 3) inhibition of lorcaserin metabolism using P450-specific chemical inhibitors. The study was further substantiated by determining the kinetic parameters, $K_m$, $V_{max}$, and $CL_{int}$, of the four primary oxidative metabolites of lorcaserin, using HLM as well as human recombinant P450 and FMO enzymes.

The results of this study demonstrated that multiple P450 enzymes catalyzed the metabolism of lorcaserin into the four primary oxidative metabolites, N-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin.

It is generally known that when multiple enzymes are involved in the metabolism of a particular metabolite or a metabolic pathway, the correlation studies with a panel of human liver microsomes and individual enzyme-specific inhibitor experiments provide results that are difficult to elucidate. In this situation, human recombinant enzymes were used to identify the enzymes involved in the metabolism of a drug. Upon screening with human recombinant enzymes, multiple P450 enzymes, CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, and CYP2E1, and CYP3A4, were found to be involved in the formation of N-hydroxylorcaserin. It was observed that recombinant CYP2B6, CYP2C19, and CYP2D6 enzymes played a dominant role in the formation of N-hydroxylorcaserin at both concentrations of 1.0 and 10.0 μM lorcaserin (Fig. 2, A and B). CYP2B6 played the predominant role for the formation of N-hydroxylorcaserin at high concentration of lorcaserin (10.0 μM), whereas at a low concentration (1.0 μM), the formation rate by CYP2D6 appeared to be higher than CYP2B6.

However, when comparing the normalized rate with respect to average P450 expression in human liver, the contribution by both CYP2B6 (%TNR = 25.9) and CYP2D6 (%TNR = 30.0) in the formation of N-hydroxylorcaserin appeared to be very similar at a
lower lorcaserin concentration (1.0 μM). Other P450s, such as CYP1A2, CYP2A6, CYP2C19, CYP2E1, and CYP3A4, were also involved in this pathway with their combined %TNR = ~40.0, which is greater than the individual %TNR of CYP2B6 and CYP2D6. Furthermore, a close examination of Fig. 3A showed that, of 16 individual human livers, a few of them (HH18, HH91, HG93, and HH74) primarily formed N-hydroxylorcaserin, but there was no or minimal formation of 7-hydroxylorcaserin (lower activity/expression of CYP2D6 was suggested by vendors supplied information and our data). We also observed that 7-hydroxylorcaserin formation at a 1.0 μM concentration of lorcaserin was completely inhibited by quinidine, a CYP2D6-selective inhibitor, whereas formation of N-hydroxylorcaserin remains unaffected by quinidine under identical conditions (Fig. 7E). These lines of evidence clearly suggested that, even though CYP2D6 played an important role in N-hydroxylorcaserin formation at lower lorcaserin concentration (1.0 μM). Other P450s, such as CYP1A2, CYP2A6, CYP2C19, CYP2E1, and CYP3A4, were also involved in this pathway with their combined %TNR = ~40.0, which is greater than the individual %TNR of CYP2B6 and CYP2D6. Furthermore, a close examination of Fig. 3A showed that, of 16 individual human livers, a few of them (HH18, HH91, HG93, and HH74) primarily formed N-hydroxylorcaserin, but there was no or minimal formation of 7-hydroxylorcaserin (lower activity/expression of CYP2D6 was suggested by vendors supplied information and our data). We also observed that 7-hydroxylorcaserin formation at a 1.0 μM concentration of lorcaserin was completely inhibited by quinidine, a CYP2D6-selective inhibitor, whereas formation of N-hydroxylorcaserin remains unaffected by quinidine under identical conditions (Fig. 7E). These lines of evidence clearly suggested that, even though CYP2D6 played an important role in N-hydroxylorcaserin formation at lower lorcaserin concentration, this pathway can be catalyzed without CYP2D6 participation due to either inhibition or low expression in HLM. At a concentration of 10.0 μM lorcaserin, N-hydroxylorcaserin was predominantly catalyzed by CYP2B6 (%TNR = 43.6); however, other P450s (CYP1A2, CYP2A6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) were also involved in this pathway with their combined %TNR = ~56.0. Regression analysis for N-hydroxylorcaserin showed a poor correlation with any particular P450 enzyme activity. Whereas no single P450-selective inhibitor alone was effective in the inhibition of N-hydroxylorcaserin formation, two general P450 inhibitors, 1-ABT and N-benzylimidazole (both are known to inhibit multiple P450 isoforms) individually inhibited N-hydroxylorcaserin to a greater extent (~75–95%) in HLM, suggesting the participation of multiple P450s in this pathway. Thus, these data clearly suggested that N-hydroxylorcaserin formation is not influenced by any single P450 either at concentrations of 1.0 or 10.0 μM lorcaserin but rather catalyzed by multiple P450 enzymes in human liver microsomes.

7-Hydroxylation of lorcaserin was catalyzed by both recombinant CYP2D6 and CYP3A4. 7-Hydroxylorcaserin formation showed a good correlation \( r^2 = 0.819 \) with microsomal CYP2D6 activity, and its formation was strongly inhibited by quinidine \( IC_{50} = 0.213 \) and 0.024 μM at 10.0 and 1.0 μM lorcaserin concentrations, respectively,
suggesting that CYP2D6 is the main P450 enzyme for 7-hydroxylo-
caserin formation. The rate for 7-hydroxylorcaserin formation by
recombinant CYP2D6 and CYP3A4 was further normalized with their
average expression level in human liver. For CYP2D6-catalyzed 7-hy-
droxylorcaserin formation at concentrations of 1.0 and 10.0 μM lorca-
serin, the %TNR values were 38.8 and 52.3, respectively, whereas for
CYP3A4, the %TNR values were 61.2 and 47.7, respectively. This
suggests that both CYP2D6 and CYP3A4 showed major contribution for
7-hydroxylorcaserin formation. Thus, at a clinically relevant concentra-
tion of 1.0 μM lorcaserin, both CYP2D6 and CYP3A4 play an important
role in the 7-hydroxylation pathway of lorcaserin.

5-Hydroxylorcaserin was formed by recombinant CYP1A1,
CYP1A2, CYP2D6, and CYP3A4. Normalized rate for 5-hydroxylo-
caserin showed that, at a concentration of 1.0 μM lorcaserin, it was
predominantly catalyzed by CYP1A2 (%TNR = 63.4) and CYP2D6
(%TNR = 36.6), whereas at a 10.0 μM lorcaserin concentration,
CYP3A4 (%TNR = 80.1) contributed the most in this pathway (Fig.
2, C and D). Although 5-hydroxylorcaserin formation was only in-
hibited by furafylline (a CYP1A2 inhibitor) and only poorly, no good
correlation was observed with any microsomal P450 marker substrate
activity for the formation of this metabolite. These results suggested
that multiple P450 enzymes catalyze the formation of 5-hydroxylo-
caserin. CYP3A4 was the primary enzyme for 1-hydroxylorcaserin
formation with both 1.0 and 10.0 μM lorcaserin concentrations.

1-Hydroxylorcaserin formation was strongly inhibited by ketocona-
zole, a CYP3A4-selective inhibitor (Wrighton and Ring, 1994; New-
ton et al., 1995) in liver microsomes, suggesting that CYP3A4 cata-
ylates this pathway. However, a good correlation analysis was not
possible because of the involvement of only a small number (three
individuals) of liver microsomes in this pathway.

The human FMO are an important enzyme system that catalyzes the
oxidation of a variety of nitrogen and sulfur-containing drugs and
xenobiotics to more polar metabolites. FMO readily catalyzes the
drugs that contain primary, secondary, and tertiary nitrogen centers
(Krueger and Williams, 2005). Lorcaserin contains secondary nitro-
gen, which can be metabolized by FMO. FMO1 is a well expressed

![FIG. 8. Rate of formation of lorcaserin metabolites N-hydroxylorcaserin by human recombinant FMO1, FMO3, and FMO5 enzymes. The concentration of lorcaserin was 1.0 and 10.0 μM in the incubation mixture. n.d., not detected.](image)

![FIG. 9. Inhibitory effect of methimazole (A), a general FMO inhibitor, and heat on FMO and 1-ABT (B), a general P450 inhibitor, and heat on P450-mediated formation of N-hydroxylorcaserin in human renal microsomal incubations with 1.0 and 10.0 μM lorcaserin. Glycine buffer, pH 8.5, and potassium phosphate buffer, pH 7.4, were used, respectively, to support FMO- and P450-mediated N-hydroxylorcaserin formation in human renal microsomal preparations. Both methimazole and heat significantly inhibited N-hydroxylorcaserin formation, whereas 1-ABT had no inhibitory effect, consistent with the primacy of FMO1 in N-hydroxylorcaserin formation in renal microsomes.](image)

![FIG. 10. Inhibitory effect of methimazole, a general FMO inhibitor, on the formation of lorcaserin metabolites N-hydroxylorcaserin, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin in human liver microsomes was performed using glycine buffer, pH 8.5. There was no or minimal inhibitory effect of methimazole observed on the formation of these metabolites, consistent with the lack of FMO (FMO3) involvement.](image)

TABLE 3

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<th>Tissue</th>
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<th>Kinetic Constant</th>
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<td>K_m (μM)</td>
<td>V_max (pmol/mg protein/min)</td>
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<td>HLM</td>
<td>N-Hydroxylorcaserin</td>
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<td>HLM</td>
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<td>HLM</td>
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<td>HRM*a</td>
<td>N-Hydroxylorcaserin</td>
<td>768</td>
<td>2670</td>
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</table>

*a Three other metabolites, 7-hydroxylorcaserin, 5-hydroxylorcaserin, and 1-hydroxylorcaserin, were not observed in the HRM incubations.
isoform in adult human kidney, and FMO3 is the major liver isoform (Yeung et al., 2000). Only human recombinant FMO1 enzyme metabolized lorcaserin and catalyzed the formation of N-hydroxylorcaserin; FMO3 and FMO5 did not have detectable activity toward lorcaserin metabolism. Therefore, both P450 and FMO are involved in the metabolism of lorcaserin on the nitrogen-center, resulting in the formation of the N-hydroxylorcaserin metabolite. Further assessment of the contribution of P450 and FMO to the formation of N-hydroxylorcaserin in HRM, demonstrated that heat and methimazole treatments strongly inhibited N-hydroxylorcaserin formation, whereas 1-aminobenzotriazole, a general P450 inhibitor, did not. Furthermore, no inhibition was observed with methimazole treatment using HLM, which suggested that human FMO1 (expressed in human kidney), and not FMO3 (expressed in human liver), was involved in the lorcaserin N-hydroxylation pathway. However, kinetic studies with HRM as well as recombinant FMO1 showed lower activities in the in vitro metabolism of therapeutic agents by human liver microsomes. Drug Metab Dispos 23(4):54–58.

A schematic overview of the P450 and FMO enzymes responsible for the various metabolic pathways of lorcaserin is depicted in Fig. 1. It may be mentioned here that, based on the human mass balance radioactivity study, lorcaserin and its metabolites were primarily eliminated in human urine (>90% dose). These oxidative (phase I) metabolites reported here were found as conjugated (phase II) metabolites in human urine (Chen et al., 2008). In this in vitro study, by use of various approaches, we have characterized P450 and FMO enzymes associated with lorcaserin metabolism. We demonstrated that multiple human P450 enzymes, such as CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, CYP2E1, and CYP3A4, and human FMO1 enzyme are responsible for the oxidative metabolism of lorcaserin. Taken together, these results suggest that lorcaserin is metabolized by multiple enzymes and that its metabolism does not depend on any of the single metabolic pathway; thus, lorcaserin has a low probability of P450- and FMO-mediated interactions by concomitant medications.

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Authorship Contributions

Participated in research design: Usmani, Chen, and Sadeque.

Conducted experiments: Usmani.

References


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HUMAN P450 AND FMO INVOLVED IN LORCASERIN METABOLISM

TABLE 4

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<tr>
<th>Enzymes</th>
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<th>Kinetic Parameters</th>
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Performed data analysis: Usmani and Sadeque.

Wrote or contributed to the writing of the manuscript: Usmani and Sadeque.