Identification of Human UDP-Glucuronosyltransferases Involved in N-Carbamoyl Glucuronidation of Lorcaserin

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Received October 20, 2011; accepted January 17, 2012

ABSTRACT:

Lorcaserin, a selective serotonin 5-HT2c receptor agonist, is a weight management agent in clinical development. Lorcaserin N-carbamoyl glucuronidation governs the predominant excretory pathway of lorcaserin in humans. Human UDP-glucuronosyltransferases (UGTs) responsible for lorcaserin N-carbamoyl glucuronidation are identified herein. Lorcaserin N-carbamoyl glucurononide formation was characterized by the following approaches: metabolic screening using human tissues (liver, kidney, intestine, and lung) and recombinant enzymes, kinetic analyses, and inhibition studies. Whereas microsomes from all human tissues studied herein were found to be catalytically active for lorcaserin N-carbamoyl glucuronidation, liver microsomes were the most efficient. With recombinant UGT enzymes, lorcaserin N-carbamoyl glucuronidation was predominantly catalyzed by three UGT2Bs (UGT2B7, UGT2B15, and UGT2B17), whereas two UGT1As (UGT1A6 and UGT1A9) played a minor role. UGT2B15 was most efficient, with an apparent $K_m$ value of $51.6 \pm 1.9 \mu M$ and $V_{\text{max}}$ value of $237.4 \pm 2.8 \text{ pmol/mg protein/min}$. The rank order of catalytic efficiency of human UGT enzymes for lorcaserin N-carbamoyl glucuronidation was UGT2B15 > UGT2B7 > UGT2B17 > UGT1A9 > UGT1A6. Inhibition of lorcaserin N-carbamoyl glucuronidation activities of UGT2B7, UGT2B15, and UGT2B17 in human liver microsomes by mfenamic acid, bisphenol A, and eugenol further substantiated the involvement of these UGT2B isoforms. In conclusion, multiple human UGT enzymes catalyze N-carbamoyl glucuronidation of lorcaserin; therefore, it is unlikely that inhibition of any one of these UGT activities will lead to significant inhibition of the lorcaserin N-carbamoyl glucuronidation pathway. Thus, the potential for drug-drug interaction by concomitant administration of a drug(s) that is metabolized by any of these UGTs is remote.

Introduction

UDP-glucuronosyltransferases (UGTs) compose a superfamily of endoplasmic reticulum membrane-bound enzymes (Mackenzie et al., 1997; Meech and Mackenzie, 2010). These enzymes are expressed in liver and in extrahepatic tissues such as kidney, intestine, brain, skin, breast, uterus, and prostate (Mackenzie et al., 1997; Tukey and Straussburg, 2000, 2001). UGTs glucuronidate many endobiotics and xenobiotics by transferring glucuronic acid from its cofactor uridine 5’-diphosphoglucuronic acid (UDPGA) to lipophilic substrates, thereby transforming them into hydrophilic glucuronides and facilitating their subsequent elimination via the biliary or renal routes. Consequently, glucuronidation is recognized as one of the major metabolic pathways for many drugs (Tukey and Strassburg, 2000; Bock, 2002). The majority of glucuronidation reactions in humans result in O- and N-glucuronides through conjugation of alcohols, carboxylic acids, and amines (Miners and Mackenzie, 1991; Tukey and Strassburg, 2000; Bock, 2002). Although rare, S-glucuronidation (Ethell et al., 2003) and C-glucuronidation (Kerdpin et al., 2006) have also been reported in humans. Another class of glucuronidation reactions, the N-carbamoyl glucuronidation of primary and secondary amines, is being reported more frequently in the literature (Straub et al., 1988; Tremaine et al., 1989; Schaefer, 1992, 2006). In recent years, drugs such as sertraline (Obach et al., 2005), varenicline (Obach et al., 2006), and a novel dipeptidyl peptidase-4 inhibitor (Gunduz et al., 2010) were shown to form N-carbamoyl glucuronide metabolites by UGT enzymes. Herein, N-carbamoyl glucuronidation of lorcaserin by human UGTs is reported.

Lorcaserin is a novel and selective serotonin 5-HT2c receptor agonist in clinical development that has been shown to be an effective agent for weight management in phase III studies (Smith et al., 2010; Fidler et al., 2011). Lorcaserin, (R)-8-chloro-1-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (Fig. 1A), is a secondary amine containing benzazepine. As with other benzazepines (Straub et al., 1988; Shaffer et al., 2009), we also observed that lorcaserin undergoes N-carbamoyl glucuronidation in vitro as well as in vivo. In humans, the N-carbamoyl glucuronidation was found to be the major clearance pathway of lorcaserin after oral administration (Chen et al., 2008; Morgan et al., 2011; Chen et al., 2008; Morgan et al., 2011).
were prepared in incubation buffer (sodium bicarbonate buffer, pH 7.4). As prepared microsomes. UGT2B7, UGT2B15, and UGT2B17. These recombinant UGTs were supplied UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B4, UGT2B7, UGT2B15, and UGT2B17). Incubation mixtures contained human liver microsomes (0.25 mg microsomal protein/ml) or recombinant UGT (0.125 mg protein/ml), saccharolactone (2 mM), magnesium chloride (10 mM), alamethicin (25 µg/ml), and 100 mM sodium bicarbonate buffer, pH 7.4. The incubation mixtures were kept on ice for 15 min, followed by the addition of lorcaserin (100 µM). All incubations were prewarmed at 37°C for 5 min and initiated by the addition of UDPGA (3 mM), bringing the total reaction volume to 500 µl. After 20 min of incubation at 37°C, the reaction was terminated by adding 500 µl of ice-cold acetonitrile containing the internal standard, lorcaserin sulfamate-Δ6 (25 ng/ml).

Assays with Microsomes from Liver, Kidney, Intestine, and Recombinant UGTs. Incubations for initial screening were carried out to determine the extent of lorcaserin N-carbamoyl glucuronide formation in various human tissue microsomes (liver, kidney, intestine, and lung) and in recombinant human UGTs (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B15, and UGT2B17). Incubation mixtures were prepared by combining human liver microsomes (0.25 mg microsomal protein/ml) or recombinant UGT (0.125 mg protein/ml), saccharolactone (2 mM), magnesium chloride (10 mM), alamethicin (25 µg/ml), and 100 mM sodium bicarbonate buffer, pH 7.4. Samples were kept on ice for 15 min, followed by the addition of varying concentrations of lorcaserin, ranging from 0.78 to 2000 µM depending on the enzyme system. All incubations were prewarmed at 37°C for 5 min, and reactions were initiated by the addition of UDPGA (3 mM), bringing the total reaction volume to 500 µl. After a 20-min incubation at 37°C, the reactions were quenched by adding 500 µl of ice-cold acetonitrile containing the internal standard, lorcaserin sulfamate-Δ6 (25 ng/ml).

Effect of Chemical Inhibitors on Human Liver UGT Activity. High-affinity UGT substrates such as mefenamic acid (Gaganis et al., 2007; Mano et al., 2007a,b), bisphenol A (Hanioka et al., 2008), and eugenol (Turgeon et al., 2003) were used as inhibitors to block UGT2B7-, UGT2B15-, and UGT2B17-catalyzed lorcaserin N-carbamoyl glucuronidation activity in HLMs, respectively. Two concentrations of lorcaserin, 1 µM (clinically relevant) and 100 µM (in the proximity of the Kₘ value of 128 µM in HLMs), were used during inhibition studies. Two concentrations of mefenamic acid (5 and 25 µM), bisphenol A (5 and 25 µM), and eugenol (20 and 100 µM) were used to inhibit UGT2B7-, UGT2B15-, and UGT2B17-mediated lorcaserin N-carbamoyl glucuronidation in HLMs. The concentrations of mefenamic acid, bisphenol A, and eugenol, selected to inhibit the reaction, were approximately equal to (low) or five times greater than (high) their respective Kₘ values, so that the maximum inhibitory effect of these compounds might be observed while maintaining UGT selectivity. In addition, two mixtures of mefenamic acid, bisphenol A, and eugenol together, one at 30 µM (mixture of three low concentrations of three inhibitors) and another at 150 µM (mixture of three high concentrations of three inhibitors), were used to inhibit lorcaserin N-carbamoyl glucuronidation in HLMs.

Typical incubation mixtures for UGT inhibition assays contained HLM (0.25 mg microsomal protein/ml), saccharolactone (2 mM), magnesium chloride (10 mM), alamethicin (25 µg/ml), and 100 mM sodium bicarbonate buffer, pH 7.4. Samples were kept on ice for 15 min, followed by the addition of lorcaserin (1 or 100 µM) and UGT inhibitors (mefenamic acid, bisphenol A, or eugenol, as mentioned above). For the control activity, no inhibitors but appropriate volume of solvents were included in the incubation mixtures. All incubations were prewarmed at 37°C for 5 min, and reactions were then

Materials and Methods

Chemicals and Enzyme Sources. Lorcaserin hydrochloride hemihydrate was provided by Cilag AG (Schaffhausen, Switzerland). Lorcaserin N-carbamoyl glucuronide was supplied by SAFC Pharma (Manchester, UK). Lorcaserin sulfamate-Δ6, used as an internal standard, was synthesized at Arena Pharmaceu-
cal, Inc. (San Diego, CA). Alamethicin, magnesium chloride, dimethyl sulfoxide, saccharolactone, UDPGA, mefenamic acid, bisphenol A, and eugenol were purchased from Sigma-Aldrich (St. Louis, MO). High-purity, high-performance liquid chromatography-grade acetoneitrile was purchased from Honeywell Burdick & Jackson (Muskegon, MI). All reagents were of high-purity grade. Microsomes from human liver, kidney, intestine, and lung (smoker and nonsmoker) were purchased from XenoTech, LLC (Lexena, KS). The following human recombinant UGTs expressed in insect cells were purchased from BD Biosciences (Woburn, MA): UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17. These recombinant UGTs were supplied as prepared microsomes.

Preparation of Stock Solutions. Lorcaserin, saccharolactone, and UDPGA were prepared in incubation buffer (sodium bicarbonate buffer, pH 7.4).

Eugenol and bisphenol A were dissolved in acetonitrile and water mixture (1:1, v/v). Mefenamic acid was dissolved in acetonitrile and dimethyl sulfoxide mixture (1:1, v/v). Alamethicin was dissolved in methanol.

Metabolic Assays. All metabolic assays were performed using modifications of a previously reported method (Schafer, 1992). In our assay, we did not observe enhancement of lorcaserin N-carbamoyl glucuronide production by adding carbon dioxide gas to the sodium bicarbonate buffer, and 100 mM sodium bicarbonate buffer alone was found to be adequate as a source of carbon dioxide for the reaction. Therefore, all metabolic reactions were conducted in 100 mM sodium bicarbonate buffer along with other necessary reagents (Schafer, 1992; Kirsch et al., 2000). All metabolic assays, including enzyme kinetics assays, were performed in triplicate.

Assays with Microsomes from Liver, Kidney, Intestine, and Recombinant UGTs. Incubations for initial screening were carried out to determine the extent of lorcaserin N-carbamoyl glucuronide formation in various human tissue microsomes (liver, kidney, intestine, and lung) and in recombinant human UGTs (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B15, and UGT2B17). Incuba-
initiated by adding UDPGA (3.0 mM), bringing the total reaction volume to 500 μl. After 20 min of incubation at 37°C, the reactions were quenched with the addition of 500 μl of ice-cold acetonitrile containing the internal standard, lorcanerin sulfamate-d₆ (25.0 ng/μl).

**Analyses for Lorcanerin N-Carbamoyl Glucuronide.** The quenched samples were centrifuged for 15 min at 2572 g (3700 rpm, Allegra 25-R centrifuge; Beckman Coulter, Fullerton, CA), and 150 μl of supernatant was transferred to another 96-well plate. Lorcanerin N-carbamoyl glucuronide was analyzed by liquid chromatography/tandem mass spectrometry using lorcanerin sulfamate-d₆ as an internal standard. A 10-μl sample was introduced for chromatographic separation using a HALO C18 (2.7 μm, 3 × 30 mm) column (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 methanol (mobile phase B). Initially, 50% of mobile phase B was applied for 30 s and then switched to 95% B over 60 s. The column was then washed with 95% B for 15 s, followed by a gradient change from 95% to 50% B over 5 s. Finally, the column was re-equilibrated with 50% B for 70 s. The total analysis time was approximately 3 min. Mass spectrometric detection was achieved with an A/M Sciex API-4000 (Applied Biosystems/MDS Sciex, Foster City, CA) operated in negative ion mode and using multiple reaction monitoring. Lorcanerin N-carbamoyl glucuronide (analyte) and lorcanerin sulfamate-d₆ (internal standard) were monitored with Q1/Q3 transitions of m/z 214.1/193.8 and 280.0/79.8, respectively. Quantification was performed with regression analysis generated from calibration standards.

**Enzyme Kinetic Analysis.** Kinetic parameters were estimated using SigmaPlot software (Systat Software, Inc., San Jose, CA) designed for a nonlinear least-square fit to the standard Michaelis-Menten equation: \( v = V_{\text{max}} \frac{[S]}{K_m + [S]} \), where \( v \) is the rate of reaction, \( V_{\text{max}} \) is the maximum reaction velocity, \( K_m \) is Michaelis constant (substrate concentration at 0.5 of \( V_{\text{max}} \)), and \( [S] \) is the substrate concentration (Segel, 1976). Goodness of fit for the Michaelis-Menten kinetic model was assessed from the S.E., 95% confidence interval, and \( r^2 \) values. The kinetic constants reported as the mean ± S.E. and the \( r^2 \) values are shown in Table 1.

### Results

**Lorcanerin N-Carbamoyl Glucuronide Formation in Human Tissue Microsomes.** Incubation of lorcanerin (100 μM) with liver, kidney, intestine, and lung microsomes generated the N-carbamoyl glucuronide metabolite of lorcanerin (Table 2). HLMS generated substantially greater amounts of lorcanerin N-carbamoyl glucuronide than microsomes from other tissues. The liver microsomes produced approximately 6- and 8-fold more lorcanerin N-carbamoyl glucuronide than the kidney and intestine microsomes, respectively. Lorcanerin N-carbamoyl glucuronide production in liver microsomes was approximately 900- and 2000-fold higher than lung microsomes of smokers and nonsmokers, respectively. The rank order of the lorcanerin N-carbamoyl glucuronide formation rate in these tissue microsomes was liver (1028.0 ± 21.6 pmol/mg protein/min) > kidney (185.9 ± 5.2 pmol/mg protein/min) > intestine (133.1 ± 0.3 pmol/mg protein/min) > lung (1.116 ± 0.060 pmol/mg protein/min for smokers and 0.493 ± 0.070 pmol/mg protein/min for nonsmokers). Because liver tissue was the most efficient in the formation of the lorcanerin N-carbamoyl glucuronide, additional kinetic and inhibition studies were performed with microsomes from human liver only.

**Lorcanerin N-Carbamoyl Glucuronide Formation in Recombinant UGTs.** Twelve recombinant human UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17) expressed in insect cells were used to determine their relative ability to form lorcanerin N-carbamoyl glucuronide (Fig. 2). UGT1A6, UGT1A9, UGT2B7, UGT2B15, and UGT2B17 catalyzed lorcanerin N-carbamoyl glucuronidation (Fig. 1). The rank order of the catalytic activity was UGT2B15 > UGT2B7 > UGT2B17 > UGT1A9 > UGT1A6. UGT1A6 was excluded from further kinetic studies because of its negligible catalytic activity.

**Kinetics Studies with Human UGTs.** Kinetic analyses for the formation of lorcanerin N-carbamoyl glucuronide were performed with human liver microsomes using a wide range of concentrations (0.78–1000 μM) of lorcanerin. The reactions were saturated at ~400 μM, and no substrate inhibition was observed with increased lorcanerin concentration up to 1000 μM in the kinetic reactions (Fig. 3). The \( K_m \) and \( V_{\text{max}} \) values calculated using a nonlinear least-square fit to the standard Michaelis-Menten equation were 128.1 ± 5.6 μM and 2379.2 ± 28.5 μl/mg protein/min, respectively. The intrinsic clearance (\( CL_{\text{int}} = V_{\text{max}}/K_m \)) in human liver microsomes was 18.57 μl/min/μg protein (Table 1).

**Kinetics Studies with Recombinant Human UGTs.** Kinetic analysis for the determination of \( K_m \) and \( V_{\text{max}} \) values were performed with UGT1A9, UGT2B7, UGT2B15, and UGT2B17 using a wide range (0.78–2000 μM) of lorcanerin concentrations, depending on the enzyme system. Like human liver microsomes, no substrate inhibition

![Fig. 2. Lorcanerin N-carbamoyl glucuronidation activity of various recombinant human UGT enzymes. The enzymatic assay contained 0.125 mg/ml recombinant protein and 100 μM lorcanerin, UDPGA, saccharolactone, and alamethicin in sodium bicarbonate buffer, pH 7.4. Detailed incubations were described under Materials and Methods.](image-url)
Inhibition of UGT Activity in HLMs. As shown in Fig. 5A, a low concentration of mefenamic acid (5 μM), bisphenol A (5 μM), or eugenol (20 μM), added either separately or combined (total inhibitor concentration of 30 μM, designated as combined-low) to the HLM incubation containing 1 μM lorcaserin (substrate), inhibited ≤5% of lorcaserin N-carbamoyl glucuronidation. However, increased concentrations of mefenamic acid (25 μM), bisphenol A (25 μM), and eugenol (100 μM) inhibited 23, 37, and 18% of lorcaserin N-carbamoyl glucuronidation, respectively (Fig. 5A). The addition of a mixture containing the increased concentrations of these inhibitors to the incubation (total inhibitor concentration of 150 μM, designated as combined-high) resulted in 66% inhibition of lorcaserin N-carbamoyl glucuronidation in liver microsomes (Fig. 5A), which is similar to the summed effect (23 + 37 + 18% = 78%) exerted by the individual inhibitors.

When the lorcaserin (substrate) concentration was increased to 100 μM, mefenamic acid (25.0 μM), bisphenol A (25.0 μM), and eugenol (100 μM) inhibited 15, 30, and 11% of lorcaserin N-carbamoyl glucuronidation, respectively, under identical conditions (Fig. 5B). The addition of a mixture of these three inhibitors (total inhibitor concentration, 150 μM) caused 53% inhibition of lorcaserin N-carbamoyl glucuronidation in liver microsomal incubation (Fig. 5B), which is close to the combined effect (15 + 30 + 11% = 56%) exerted by individual inhibitors under identical substrate conditions (100 μM lorcaserin). No inhibitory effect was observed with low inhibitor concentrations (5 and 20 μM) incubated with 100 μM lorcaserin concentration in HLMs (Fig. 5B).

Discussion

Although N-carbamoyl glucuronidation is considered to be rare, it represents another important metabolic pathway that has increasingly drawn attention from drug metabolism researchers in recent years. Although a number of publications investigated its possible mechanism (Schaefer, 1992, 2006; Shaffer et al., 2005), few studies report the identification of UGT enzymes that catalyze the N-carbamoyl glucuronidation pathway (Obach et al., 2005, 2006; Gunduz et al., 2010). In this study, we identified the human UGT enzymes involved in N-carbamoyl glucuronidation of lorcaserin.

First, we examined the formation of lorcaserin N-carbamoyl glucuronide in microsomes prepared from various human tissues, which include liver, kidney, intestine, and lung. Among these organs, human liver was, by a substantial margin, the most efficient for lorcaserin N-carbamoyl glucuronidation. We also identified that UGT2B7, UGT2B15, and UGT2B17 are the primary enzymes involved in lorcaserin N-carbamoyl glucuronidation. These UGT enzymes are abundantly expressed in liver compared with other tissues (Turgeon et al., 2003; Ohno and Nakajin, 2009). For example, quantitative estimation showed that UGT2B7 is 2-fold and UGT2B15 is 25-fold higher in liver compared with the small intestine (Ohno and Nakajin, 2009). UGT2B15 has the highest intrinsic clearance, followed by UGT2B7 for lorcaserin N-carbamoyl glucuronidation. Thus, human liver tissues have a high pool of catalytic UGT2B enzymes involved in lorcaserin metabolism. Therefore, the high intrinsic clearance for lorcaserin N-carbamoyl glucuronidation by liver microsomes seems to be associated with higher expression of these enzymes in the liver, which is clearly in accordance with these literature reports (Turgeon et al., 2003; Ohno and Nakajin, 2009).

Among extrahepatic tissues, kidney was most efficient at producing the N-carbamoyl glucuronide, followed by intestine and lung. These findings suggest that both hepatic and extrahepatic tissues are involved in lorcaserin N-carbamoyl glucuronidation in humans. Human liver microsomes also showed a much higher turnover rate than any individual recombinant UGT enzyme examined. This finding is similar to the observation previously reported for oxazepam glucuronidation in HLMs compared with recombinant UGTs, where HLMs produced more oxazepam glucuronide than individual recombinant enzymes (Court et al., 2002). Formation of lorcaserin N-carbamoyl glucuronide in the microsomes of lung was the least efficient compared with microsomes from other organs, consistent with observations for 4-(methyleneamino)-1-(3-pyridyl)-1-butanol glucuronidation (Ren et al., 2000). Ulrich et al. (1997) reported that lung microsomes from smokers have approximately 2-fold higher glucuronidation activity compared with nonsmokers, suggesting a functional increase in UGT activity in the lung of smokers. UGT2B7 (Ren et al., 2000) and UGT2B17 (Beaulieu et al., 1996; Ohno and Nakajin, 2009) are constitutively expressed in human lung. Several studies have also suggested the induction of UGT2B7 and UGT2B17 in tobacco smokers’ lung (Ren et al., 2000; Carmella et al., 2002; Gallagher et al., 2007). In line with these literature reports, a likely explanation for the 2-fold increased formation of lorcaserin N-carbamoyl glucuronidation in lung microsomes from smokers is the induction of one or more of these UGTs (UGT2B7 and UGT2B17), which are involved in this metabolic pathway of lorcaserin along with UGT2B15.

Furthermore, lorcaserin is rapidly and highly absorbed after oral administration in humans. It is metabolized mainly in the liver. On the basis of total radioactivity, >90% of lorcaserin dose is excreted in the urine, and ~33% of dose is accounted for N-carbamoyl glucuronidation (Chen et al., 2008; Morgan et al., 2008). It is conceivable that the sum of activity of each UGT isoform expressed in human liver microsomes involved with lorcaserin N-carbamoyl glucuronidation is equal to the UGT activity of human liver microsomes. Therefore, the contribution of UGT1A9, UGT2B7, UGT2B15, and UGT2B17 to the observed activity in human liver microsomes (V_{max,UGT}/V_{max, HLM}) may be estimated as 4.3, 7.8, 10.0, and 6.5%, respectively. It is noteworthy that the sum of these estimated fractions (~29%) is close to the fraction accounted for N-carbamoyl glucuronidation in human
urine (~33%). Thus, multiple UGTs are involved in this pathway and the contribution of each individual UGT seemed not to be >10%.

Second, it was shown that the UGT2B family, namely UGT2B7, UGT2B15, and UGT2B17, predominantly catalyzed lorcaserin N-carbamoyl glucuronidation and that UGT2B15 was the most efficient UGT, followed by UGT2B7 and UGT2B17. It is noteworthy that UGT2B15 and UGT2B17 share high amino acid sequence identity (92%), tissue distribution, and specificity for substrates, such as steroids (Hum et al., 1999; Tukey and Strassburg, 2000; Turgeon et al., 2001). In contrast, UGT2B7 shares less amino acid sequence identity (78%) with UGT2B15 and UGT2B17 and has a much broader substrate specificity, as it is involved in the metabolism of drugs from all major classes, including opioids, cancer drugs, and steroids (Tukey and Strassburg, 2000; Innocenti et al., 2001; Turgeon et al., 2003). Despite their differences in sequence similarity, specificity, and catalytic rate, UGTs show a great deal of substrate redundancy (Miners and Mackenzie, 1991; Tukey and Strassburg, 2000). For example, UGT2B7, UGT2B15, and UGT2B17 all catalyze glucuronidation of testosterone, dihydrotestosterone, and androstane-3α,17β-diol (Turgeon et al., 2001). We observed that lorcaserin N-carbamoyl glucuronidation is also catalyzed by these three steroid-metabolizing enzymes, with comparable rates. Among these three UGTs, UGT2B7 was shown to be the only UGT involved in N-carbamoyl glucuronidation of varenicline (Obach et al., 2006) and was the major UGT for sertraline (Obach et al., 2005); both of these drugs are secondary amines. In contrast, UGT2B7 plays a very minor role in N-carbamoyl glucuronidation of a primary amine, dipeptidyl peptidase-4 inhibitor (Gunduz et al., 2010). We observed, for the first time, that UGT2B15 and UGT2B17, in addition to UGT2B7, are involved in N-carbamoyl glucuronidation of a secondary amine, lorcaserin. These examples suggest that carbamic acids of secondary amines may be a preferred or at least a good substrate for UGT2B7-mediated N-carbamoyl glucuronidation.

Third, because of the substrate overlap among UGTs and the lack of specific chemical inhibitors of UGTs, known high-affinity UGT substrates are often used to inhibit UGT activity toward other substrates, assuming the competitive nature of substrate specificity and binding (Williams et al., 2004). A mixture of three UGT substrates, bisphenol A (UGT2B15), mefenamic acid (UGT2B7), and eugenol (UGT2B17), decreased N-carbamoyl glucuronidation activity by 66 and 53% with 1 and 100 μM of lorcaserin (substrate), respectively, suggesting that at either concentration of substrate, lorcaserin N-car-

![Fig. 4. Michaelis-Menten kinetic plots for lorcaserin N-carbamoyl glucuronide formation catalyzed by human recombinant UGTs UGT1A9 (A), UGT2B7 (B), UGT2B15 (C), and UGT2B17 (D). The enzymatic assays were conducted with 0.125 mg/ml recombinant protein and a wide range of lorcaserin concentration (up to 2000 μM depending on the system), UDPGA, saccharolactone, and alamethacine in sodium bicarbonate buffer, pH 7.4. The values are the average of triplicate (±S.E.) incubations. Details of incubations were described under Materials and Methods.](https://dmd.aspetjournals.org/)

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![A](https://dmd.aspetjournals.org/)
![B](https://dmd.aspetjournals.org/)

![C](https://dmd.aspetjournals.org/)

![D](https://dmd.aspetjournals.org/)
In conclusion, this in vitro study demonstrated that multiple human UGT2B isoforms, such as UGT2B7, UGT2B15, and UGT2B17, predominantly catalyze lorcaserin N-carbamoyl glucuronidation. UGT1A isoforms such as UGT1A6 and UGT1A9 exhibit a minor contribution to this pathway. Considering the fraction of lorcaserin cleared as N-carbamoyl glucuronide, the involvement of multiple UGTs, and contribution from other phase I enzymes in lorcaserin metabolism, it is unlikely that inhibition of any of the UGTs will cause a significant impact on lorcaserin clearance in humans. Thus, drug-drug interaction through inhibition of the N-carbamoyl glucuronidation pathway is unlikely for lorcaserin when taken with concomitant medication.

Acknowledgments

We are grateful to Michael Ma, Forest Flodin, Salma Sarwary, Jane Angeles, and Yong Tang from Arena Bioanalytical Chemistry Group for providing liquid chromatography/tandem mass spectrometry analytical support. We also thank Wim D’Haese for critical reading and formatting of this manuscript.

Authorship Contributions

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

Conducted experiments: Usmani and Palamar.

Performed data analysis: Sadeque, Usmani, and Palamar.

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

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