Hepatic UDP-Glucuronosyltransferase Is Responsible for Eslicarbazepine Glucuronidation

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ABSTRACT:

Eslicarbazepine acetate (ESL) is a once-daily novel antiepileptic drug approved in Europe for use as adjunctive therapy for refractory partial-onset seizures with or without secondary generalization. Metabolism of ESL consists primarily of hydrolysis to eslicarbazepine, which is then subject to glucuronidation followed by renal excretion. In this study, we have identified that human liver microsomes (HLM) enriched with uridine 5′-diphosphoglucuronic acid give origin to a single Escherichia coli β-glucuronidase-sensitive eslicarbazepine glucuronide (most likely the O-glucuronide). The kinetics of eslicarbazepine glucuronidation in HLM was investigated in the presence and absence of bovine serum albumin (BSA). The apparent Km were 412.2 ± 63.8 and 349.7 ± 74.3 μM in the presence and absence of BSA, respectively. Incubations with recombinant human UDP glucuronosyltransferases (UGTs) indicated that UGT1A4, UGT1A9, UGT2B4, UGT2B7, and UGT2B17 appear to be involved in eslicarbazepine conjugation. The UGT with the highest affinity for conjugation was UGT2B4 (Km = 157.0 ± 31.2 and 28.7 ± 10.1 μM, in the absence and presence of BSA, respectively). There was a significant correlation between eslicarbazepine glucuronidation and trifluoperazine glucuronidation, a typical UGT1A4 substrate; however, no correlation was found with typical substrates for UGT1A1 and UGT1A9. Diclofenac inhibited eslicarbazepine glucuronidation in HLM with an IC50 value of 17 μM. In conclusion, glucuronidation of eslicarbazepine results from the contribution of UGT1A4, UGT1A9, UGT2B4, UGT2B7, and UGT2B17, but the high-affinity component of the UGT2B4 isozyme may play a major role at therapeutic plasma concentrations of unbound eslicarbazepine.

Introduction

Eslicarbazepine acetate [(S)-(−)-10-acetoxy-10,11, dihydro-5H-dibenz[b][azepine-5-carboxamide; ESL] is a novel once-daily central nervous system active compound (Almeida and Soares-da-Silva, 2007) that completed the phase III clinical trials as an adjunctive therapy in partial epilepsy refractory to standard antiepileptic drugs (Elger et al., 2009; Gil-Nagel et al., 2009; Ben-Menachem et al., 2010). ESL shares with carbamazepine and oxcarbazepine (OXC) the dibenzazepine nucleus bearing the 5-carboxamide substituent, but it is structurally different at the 10,11-position (Almeida and Soares-da-Silva, 2007). This molecular variation results in differences in metabolism, namely by preventing the formation of toxic epoxide metabolites such as carbamazepine-10,11 epoxide (Hainzl et al., 2001; Almeida et al., 2009). In humans, ESL is rapidly absorbed and undergoes extensive first-pass hydrolysis to eslicarbazepine (Almeida and Soares-da-Silva, 2007). After an oral dosing of 800 mg, ESL, R-lcarbazepine, OXC, and its glucuronocojugates were clearly minor metabolites (Almeida et al., 2009). The major circulating metabolites were eslicarbazepine and eslicarbazepine glucuronide, with Cmax of 48.3 and 4.3 μM, respectively (Almeida et al., 2008; Maia et al., 2008). The major metabolites recovered in urine were eslicarbazepine and eslicarbazepine glucuronide, the sum of which corresponded to approximately 93% of all metabolites recovered in urine (Maia et al., 2008). However, the metabolism of ESL is strongly dependent on the species, and ESL metabolism may originate different proportions of R-lcarbazepine and OXC as minor metabolites (Hainzl et al., 2001).

Glucuronidation reactions are catalyzed by the enzymes UDP-glucuronosyltransferases (UGTs) expressed on the inner membrane of the endoplasmic reticulum. ESL exists as a superfamily of enzymes that exhibit distinct but overlapping substrate and inhibitor selectivity (Mackenzie et al., 2005). Seventeen human UGT proteins have been identified and classified into two families (UGT1 and UGT2) on the basis of sequence identity (Mackenzie et al., 2005). The contribution of different isoforms to glucuronidation is dependent on the relative expression levels in different tissues. UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, UGT2B4, and UGT2B17 have been detected in hepatic and extrahepatic tissues (Strassburg et al., 2000). UGT1A9 is one of the major drug glucuronidating enzymes (Ebner and Burchell, 1993) and is the

ABBREVIATIONS: ESL, eslicarbazepine acetate; OXC, oxcarbazepine; BSA, bovine serum albumin; HLM, human liver microsomes; MLM, mouse liver microsomes; ISTD, internal standard; HPLC, high performance liquid chromatography; UGT, UDP-glucuronosyltransferase; UDPGA, uridine 5′-diphosphoglucuronic acid; MS/MS, tandem mass spectrometry.
dominant isoform in the kidney (Strassburg et al., 2000). The most common human condition relating to UGT activity is Gilbert syndrome, which is related to loss of function of UGT1A1 (Strassburg et al., 2000). UGT2B7 is a very important human UGT isoform that appears to be expressed in many extrahepatic tissues and catalyzes the glucuronidation of a wide range of xenobiotics, including phenols, aliphatic alcohols, carboxylic acids, and tetrazoles (King et al., 2001).

To date, the identity of UGTs involved in eslicarbazepine glucuronidation have not been explored in detail. The study presented here reports on the enzymes involved in eslicarbazepine glucuronidation and characterizes their kinetic parameters using human liver microsomes (HLM) and recombinant UGTs.

**Materials and Methods**

**Chemicals.** ESL, eslicarbazepine, and $R$-licarbazepine were synthesized in Bial’s Laboratory of Chemical Research (Sao Mamede do Coronado, Portugal) with a purity $> 99.5\%$. OXC was synthesized and provided by Farchemia (Treviglio, Italy). Milli-Q (Millipore Corporation, Billerica, MA) water was used in all steps, and solvents were of high-performance liquid chromatography (HPLC) grade. All other reagents were obtained from Sigma-Aldrich (St. Louis, MO).

Pooled HLM (from 48 donors), mouse liver microsomes (MLM), and HLM single donor (HG3, HG95, HG64, HH13, and HG93) were purchased from BD Gentest (Woburn, MA). The protein contents were used as described in the data sheets provided by the manufacturers. Recombinant human UGTs expressed in baculovirus-infected insect cells were purchased from BD Gentest (Woburn, MA). The protein contents were used as described in the data sheets provided by the manufacturers. Recombinant human UGTs expressed in baculovirus-infected insect cells were purchased from PanVera Corp. (Madison, WI) (UGT1A1, 1A3, 1A6, 1A7, 1A10, and 2B7) and from BD Gentest (Woburn, MA; UGT1A4, 1A8, 1A9, 2B4, 2B17, and 2B15).

**Eslicarbazepine Glucuronidation in Pooled and Single-Donor HLM.**

Glucuronidation activity by HLM was measured using the following assay conditions: the incubation mixture (100 $\mu$L total volume) contained 0.5 mg/ml total protein, 10 mM MgCl$_2$, 2 mM uridine 5’-diphosphoglucuronic acid (UDPGA), 25 $\mu$L/ml of amethocinch, 5 mM saccharolactone in 50 mM phosphate buffer (pH 7.5), and 10 to 1000 $\mu$M eslicarbazepine. Drug was dissolved in dimethyl sulfoxide, and the final concentration of dimethyl sulfoxide in the reaction was below 0.5% (v/v). Experiments were performed in the presence and absence of bovine serum albumin (BSA; 2%). Reactions were preincubated for 5 min and were initiated with the drug. Reaction mixtures were incubated for up to 60 min and terminated by the addition of 400 $\mu$L of 10,11-dihydrocarbamazepine (ISTD) working solution (250 $\mu$L/ml in 0.1 M phosphate buffer, pH 5.6). Samples were subjected to solid-phase extraction and were injected on an HPLC mass spectrometer. All incubations were performed in a shaking water bath at 37°C. Linearity of product formation with time (0–120 min) was evaluated in HLM. All experiments were performed in duplicate.

**Eslicarbazepine Glucuronide Hydrolysis.** It was not possible to use the eslicarbazepine glucuronide standard; therefore, in its absence the compound was quantified by peak area ratio to ISTD. The identity of eslicarbazepine glucuronide was confirmed by hydrolysis with $\beta$-glucuronidase (from Escherichia coli) using HLM and MLM. In brief, eslicarbazepine glucuronide was obtained by incubating 100 $\mu$L eslicarbazepine with microsomes (1 $mg$/ml) in a final volume of 200 $\mu$L for 2 h under the conditions described under Eslicarbazepine Glucuronidation in Pooled and Single-Donor HLM. A control without UDPGA was also prepared. After incubation, the remaining eslicarbazepine was removed from the samples by liquid-liquid extraction with chloroform. The chloroform layer containing eslicarbazepine was discarded, and this process was repeated twice. The aqueous layer (100 $\mu$L) containing eslicarbazepine glucuronide was then incubated for 2 h with $\beta$-glucuronidase (100,000 units/ml) at 37°C, after adjusting pH to 6.8. The disappearance of eslicarbazepine glucuronide and the subsequent appearance of eslicarbazepine in the sample compared with an aliquot of the original sample that was not subject to $\beta$-glucuronidase hydrolysis identified the compound.

**Eslicarbazepine Glucuronidation Screening by Recombinant UGTs.** Glucuronidation by recombinant UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17 was evaluated with 1000 $\mu$L eslicarbazepine and 0.5 mg/ml total protein using the conditions described under Eslicarbazepine Glucuronidation in Pooled and Single-Donor HLM, with the exception that reactions did not contain saccharolactone and were performed over 120 min. Kinetics of eslicarbazepine glucuronidation by selected UGT were determined, as described under Eslicarbazepine Glucuronidation in Pooled and Single-Donor HLM, with eslicarbazepine concentrations ranging from 10 to 1000 $\mu$L and 60-min incubation time.

**Inhibition of Eslicarbazepine Glucuronide Formation by Typical Substrates for UGT Isoforms.** In experiments designed to evaluate the inhibition of eslicarbazepine glucuronidation, pooled HLM were incubated for 60 min with 100 $\mu$L eslicarbazepine in the presence and absence of the following substrates: propofol (substrate for UGT1A9; Hanioka et al., 2001), imipramine (substrate for UGT1A4; Nakajima et al., 2002), diclofenac (substrate for UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B15, and UGT2B17; Uchaipichat et al., 2006a), and testosterone (substrate for UGT2B17, UGT2B17, and UGT2B15; Bowalgaha et al., 2007) at 10, 50, 100, 500, and 1000 $\mu$L, respectively.

Experiments to determine the inhibition specificity of UGTs were performed by incubation of 500 $\mu$L eslicarbazepine with recombinant UGTs and 500 $\mu$L propofol, imipramine, diclofenac, or testosterone for 60 min at 37°C.

**Extraction of Eslicarbazepine Glucuronides.** The samples with ISTD were placed on an automatic liquid handler (ASPEC-XL4; Gilson, Villier Le Bel, France) for solid-phase extraction. The solid-phase extraction...
cartridges (Oasis HLB, 30 mg, 1 ml; Waters, Milford, MA) were conditioned with 1 ml of acetonitrile and then washed twice with 1 ml of water. Samples (400 μl) were loaded onto the cartridges, and the cartridges were washed twice with 1 ml of water. After the second wash, the cartridges were flushed with an air push of 10 ml at 6 ml/min. The cartridges were eluted twice with 200 μl of methanol with an air push of 2 ml at 6 ml/min. To the eluted sample, 200 μl of water were added and mixed twice with aspiring dispensing cycles. The eluted samples were injected (5 μl) into an HPLC mass spectrometer.

HPLC-Mass Spectrometry Analysis. The analysis of samples extracts was performed using an HPLC mass spectrometer (AP-ESI 1100 Series; Agilent Technologies, Santa Clara, CA) with positive ion detection. In brief, separation was performed on a Waters Symmetry C8, 3.5 μm, 4.6 cm × 150 mm using mobile phase A: water containing 1% formic acid (v/v) and mobile phase B: acetonitrile with gradient conditions of 80% of A and 20% of B at 1 min, 60% of A: 40% of B at 10 min, and 80% of A: 20% of B at 10.1 min. Selected ion monitoring with the detection of \( m/z \) 431 for eslicarbazepine glucuronide was used for quantification. For maximal sensitivity, the fragment energy was set to 120 V, and further settings were 3500 eV for the capillary voltage, 350°C for nebulizer gas temperature, and 40 psi for nebulizer pressure.

Liquid Chromatography-MS/MS Glucuronide Identification. The analysis of the extracted samples to identify eslicarbazepine glucuronide was performed using liquid chromatography (1290 Infinity; Agilent Technologies) coupled with an MS/MS detector (6460 Triple Quad LC/MS; Agilent Technologies). Electrospray ionization was used in positive ion detection with a fragmentor of 120 V and collision energy of 20 eV. The multiple reaction monitoring pair was \( m/z \) 431.3 → 237.0 and 431.3 → 194 m/z. The source parameters were as follows: gas temperature of 200°C; gas flow of 10 l/min; nebulizer of 30 psi; sheath gas temperature of 350°C; sheath gas flow of 11 l/min; capillary of 3500 V; and nozzle voltage of 300 V.

Data Analysis. Kinetic parameters of glucuronidation were obtained by fitting velocity data to the Michaelis-Menten equation with GraphPad Prism software (GraphPad Software, Inc., San Diego, CA).

Results

Eslicarbazepine Glucuronidation by HLM. Incubation of eslicarbazepine with HLM in the presence of UDPGA resulted in the appearance of one peak with an \([M - H]^{+}\) ion at \( m/z \) 431 \([M + H-176]^{+}\) corresponding to the \( m/z \) of eslicarbazepine glucuronide (Fig. 1). The disappearance of this peak with β-glucuronidase treatment and its absence in control experiments performed without UDPGA (data not shown) suggest that this peak corresponded to eslicarbazepine glucuronide. Furthermore, the product ion spectra from precursor \( m/z \) 431 contained an ion at \( m/z \) 237.2 for the aglycone (from loss of the neutral glucuronic acid) with an additional peak at \( m/z \) of 194.2 for the glucuronic acid. The neutral loss of a glucuronide moiety of 176 Da yielded a peak corresponding to \( m/z \) 431 (Fig. 2). In addition, the fact that this compound formation is time- and protein dependent further corroborated its identity as eslicarbazepine glucuronide. When incubation was performed with MLM in the presence of UDPGA, two peaks with an \([M - H]^{+}\) ion at \( m/z \) 431 were detected, but only one disappeared with β-glucuronidase treatment (Fig. 3), suggesting that in MLM two eslicarbazepine glucuronides were

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**Fig. 2.** MS/MS spectra for eslicarbazepine glucuronide. a, representative product ion spectrum for \( m/z \) of 431; b, neutral loss spectra for the 176-Da glucuronide moiety. The incubation mixture contains pooled HLM and eslicarbazepine in the presence of UDPGA.
formed (N-glucuronide and O-glucuronide), but only one was hydrolyzed by β-glucuronidase from *E. coli*.

Kinetic analysis of eslicarbazepine glucuronidation was performed in pooled HLM in the presence and absence of BSA. As shown in Fig. 4, eslicarbazepine glucuronidation displayed typical hyperbolic kinetics; however, the Eadie-Hofstee plot of the data in the presence of BSA was biphasic, suggesting the involvement of more than one enzyme in the reaction (Nakajima et al., 2002). The apparent $K_m$ and the $V_{max}$ derived from these curves fitted to the Michaelis-Menten equation are listed in Table 1. The apparent $K_m$ values were similar for liver microsomes irrespective of the presence of BSA, but the $V_{max}$ was significantly higher in the presence of BSA.

**Eslicarbazepine Glucuronidation by Recombinant UGT.** Eleven commercially available UGT enzymes were used to evaluate their ability to conjugate eslicarbazepine (Fig. 5). To maximize product formation, a concentration of 1000 μM eslicarbazepine was used. From the tested UGTs, only UGT1A9, UGT1A4, UGT2B7, UGT2B4, and UGT2B17 produced significant amounts of eslicarbazepine glucuronide. No eslicar-
and UGT2B7 precluded full kinetic analysis. Each enzyme was incubated for 120 min with 1000 μM eslicarbazepine at 37°C. Values represent mean ± S.E.M. of two determinations. Results are expressed as relative units (peak area ratio).

Fig. 4. Kinetics of eslicarbazepine glucuronidation in human liver microsomal pools. Eslicarbazepine concentrations ranged from 10 to 1000 μM in the presence and absence of 2% BSA. A, the Michaelis-Menten equation was used to fit eslicarbazepine glucuronidation data. B, Eadie-Hofstee plots for the same data. Values represent means ± S.E.M. of duplicates. Velocities are expressed as relative units (peak area ratio/mg protein per minute).

Fig. 5. Eslicarbazepine glucuronidation catalyzed by recombinant human UGT enzymes. Each recombinant enzyme (total protein 0.5 mg/ml) was incubated for 120 min with 1000 μM eslicarbazepine at 37°C. Values represent mean ± S.E.M. of two determinations. Results are expressed as relative units (peak area ratio).

Fig. 6. Inhibitory Effect of Propofol, Imipramine, Diclofenac, and Testosterone on Eslicarbazepine Glucuronidation in HLM and Correlation Analysis. Eslicarbazepine glucuronidation was measured in the microsomal fraction from five different donors (HG3, HG93, HG95, HG64, and HH13) that were chosen to provide differences in their catalytic activities of the UGT1A1, UGT1A4, and UGT1A9 enzymes (Table 2). Eslicarbazepine conjugation activity in these individual HLM was significantly (r² = 0.65) correlated with trifluoperazine glucuronidase activity (Fig. 7).

Inhibitory Effect of Propofol, Imipramine, Diclofenac, and Testosterone on Eslicarbazepine Glucuronidation in HLM and UGTSs. The effect of propofol, imipramine, diclofenac, and testosterone (10, 50, 100, 500, and 1000 μM) on eslicarbazepine (100 μM) glucuronidation activities in pooled HLM was investigated. As shown in Fig. 8, diclofenac completely inhibited eslicarbazepine glucuronidation with an IC₅₀ of 17.0 μM. Imipramine and testosterone inhibited eslicarbazepine glucuronidation with IC₅₀ values of 642.2 and 694.2 μM, respectively. This effect of imipramine was abolished with 500 μM eslicarbazepine, and a reduction on the effect of testosterone was also observed. Propofol only had a slight inhibitory effect on eslicarbazepine glucuronidation at the eslicarbazepine concentration of 100 μM.

To evaluate the UGT isoform selective inhibition, we investigated the inhibitory effects on the eslicarbazepine glucuronidation in recombinant UGT isoforms by incubating 500 μM eslicarbazepine with
recombinant UGTs and 500 μM propofol, imipramine, diclofenac, or testosterone. Eslicarbazepine glucuronidation by recombinant UGT1A4, UGT2B4, UGT2B17, and UGT1A9 was completely inhibited by diclofenac. Testosterone completely inhibited the eslicarbazepine glucuronidation by UGT1A4 and UGT2B17, and it partially inhibited glucuronidation by UGT1A9 (50%) and UGT2B4 (50%). Propofol completely inhibited eslicarbazepine glucuronidation by UGT1A9 and also had an effect on the UGT2B4 (45%) and UGT2B17 (35%). Imipramine had no effect on eslicarbazepine glucuronidation at the eslicarbazepine concentration of 500 μM. The effect of inhibitors was not evaluated on UGT2B7 because of the low activity found in eslicarbazepine conjugation.

Discussion
In this study, we have identified the main UGT isozymes responsible for eslicarbazepine glucuronidation in the human liver by first investigating this reaction in pooled HLM, in individual HLM, and using recombinant UGTs. Eslicarbazepine is conjugated into one glucuronide in human liver microsomes identified by the fragmentation ion at \( m/z \) 431 [M + H-176], the neutral loss of a glucuronide moiety of 176 Da in the positive ion mode, the product ion spectrum from precursor \( m/z \) 431, and the enzymatic hydrolysis of conjugation product. In opposition to HLM, in MLM two glucuronides of eslicarbazepine were found; however, only one was hydrolyzed by \( E. \ coli \) β-glucuronidase, an enzyme that preferentially hydrolyzes O-glucuronides over N-glucuronides (Zenser et al., 1999). The difficulty to obtain standards of eslicarbazepine glucuronides precluded the distinction between the N- and O-glucuronide of eslicarbazepine; however, the selectivity reported for \( E. \ coli \) β-glucuronidase and the fact that OXC undergoes O-glucuronidation (Flesch, 2004) point to the conjugation of eslicarbazepine in the hydroxyl group (O-glucuronide).

The Eadie-Hofstee plots show that eslicarbazepine glucuronidation in pooled HLM follows typical Michaelis-Menten kinetics with high- and low-affinity components (Fig. 4). According to the Eadie-Hofstee plot of the data in the presence of BSA, more than one enzyme is involved in eslicarbazepine glucuronidation. All UGT enzymes are capable of forming O-linked glucuronides (Mackenzie et al., 2005); however, N-glucuronidation of amines was only described for UGT1A4 and to a lesser extent for UGT1A3 (Green et al., 1998). UGT2B7 has been described to be involved in the N-glucuronidation of perfluorooctanesulfonamide (Xu et al., 2006) and of carbamazepine primary amine (Staines et al., 2004). The hydroxylated metabolites of carbamazepine are subject to O-glucuronidation; however, the enzymes involved were not yet characterized (Maggis et al., 1997). UGT1A1, UGT1A3, UGT1A4, and UGT1A9 were reported to be involved in the N-glucuronidation of retigabine (Borlak et al., 2006), and UGT1A4, UGT1A1, UGT1A3, UGT1A6, UGT2B7, and UGT1A7 were reported to be involved in the N-glucuronidation of lamotrigine (Rowland et al., 2006).

Glucuronidation of eslicarbazepine was detected in preparations of UGT1A4, UGT1A9, UGT2B4, UGT2B7, and UGT2B17. UGT2B7 and UGT2B4 have been reported to be involved in O-glucuronidation of 1'-hydroxymidazolam, a metabolite of midazolam, whereas 1'-hydroxymidazolam N-glucuronidation is catalyzed by UGT1A4 (Zhu et al., 2008). O-glucuronidation of several coumarins, anthraquinones, and flavonoids was observed with UGT2B17 (Turgeon et al., 2003). There is no information in the literature regarding the enzymes involved in O-glucuronidation of OXC.

The kinetic analysis of eslicarbazepine glucuronidation by HLM showed that the presence of 2% BSA does not significantly change the affinity of conjugation; however, the capacity was largely increased. The mechanism by which BSA affects the glucuronidation process has been evaluated in several studies (Rowland et al., 2006, 2007; Uchaipichat et al., 2006a). It has been suggested that BSA is involved in the sequestration of inhibitory long-chain
unsaturable fatty acids, particularly linoleic and arachidonic acids released from microsome membranes during the course of incubation (Rowland et al., 2007). In the presence of BSA, the magnitude of inhibitory interactions of fluconazol-zidovudine and valproic acid-lamotrigine in vitro correctly predicted the in vivo interactions, improving the predictive capacity of the models used. Results herein suggest that adding BSA to the incubation medium may trigger UGTs involved in eslicarbazepine conjugation. It should be noted that in in vivo experimental conditions, approximately 30% of eslicarbazepine is bound to plasma proteins (Almeida et al., 2009), and therefore the kinetic constants obtained in the work presented here are apparent constants.

Because UGT enzymes exhibit distinct but overlapping substrate selectivity (Wen et al., 2007), further correlation analysis and chemical inhibition studies were performed to determine the UGT isoforms responsible for eslicarbazepine conjugation. For the eslicarbazepine glucuronidation concentrations of 1000 μM, the glucuronidation correlates well with trifluoperazine glucuronidation, which is catalyzed mainly by UGT1A4. There was no significant correlation with propofol and trifluoperazine glucuronidation, which is catalyzed mainly by UGT2B4- and UGT2B17-mediated eslicarbazepine glucuronidation. Therefore, the UGT high-affinity component of the UGT isozyme may play a major role in eslicarbazepine glucuronidation. On the basis of the results of this study, ESL metabolism is not expected to be altered in patients with Gilbert syndrome, which is related to a loss of function of UGT1A1 (Strassburg et al., 2000).

Antiepileptic drugs are frequently administered in combination with other anticonvulsants (Riva et al., 1996); therefore, the potential for the drug-drug interaction in the glucuronidation pathway frequently exists among medications that are extensively glucuronidated. Pharmacokinetic interactions have been observed between ESL and phenytoin (Bial data on file), but no significant interaction was observed with topiramate (Nunes et al., 2010), carbamazepine, valproate, and levetiracetam (Bial data on file; see also CHMP, 2009). In a study of healthy subjects (Bial data on file, clinical trial BIA-2093-121; see also CHMP, 2009), concomitant administration of ESL (1200 mg) and phenytoin (300 mg) resulted in an average decrease of 31 to 33% in exposure to eslicarbazepine, most likely caused by an induction of glucuronidation, and an average increase of 31 to 35% in exposure to phenytoin, most likely resulting from inhibition of CYP2C19, which is known to be involved in the first-pass metabolism of phenytoin (Nakajima et al., 2007). A population pharmacokinetics analysis of phase III studies in epileptic adult patients indicated that carbamazepine increases eslicarbazepine clearance (Almeida et al., 2009), with the probable mechanism being a dose-dependent induction of glucuronidation (CHMP, 2009). Phenytoin has been shown to be involved in the inhibition of UGTs (UGT1A6, UGT1A9, and UGT2B15) responsible for detoxifying of acetaminophen through the glucuronidation pathway (Kostrubsky et al., 2005), and valproate increased lamotrigine concentration by UGT2B7 inhibition (Rowland et al., 2006). Interaction of valproate with carbamazepine glucuronidation has also been described (Bernus et al., 1997). Glucuronidation of valproate (30–70% of which is eliminated via glucuronidation) has been reported to be carried out by UGT1A3, UGT1A6, UGT1A9, UGT2B7, and UGT2B15 (Ethell et al., 2003), and carbamazepine N-glucuronidation is mediated by UGT2B7 (Staines et al., 2004). However, UGTs involved in glucuronidation of the 13 hydroxylated metabolites of carbamazepine are not yet described.

Lamotrigine glucuronidation by UGT1A4 and UGT2B7 accounts for approximately 70% of compound elimination (Rowland et al., 2006); however, no significant pharmacokinetic interaction between ESL and lamotrigine was observed (Almeida et al., 2010), which indicates that eslicarbazepine does not interfere with lamotrigine glucuronidation. In contrast, lamotrigine interacts with retigabine, which undergoes predominantly N-glucuronidation by UGT1A1, UGT1A3, UGT1A4, and UGT1A9, and its renal excretion (Hermann et al., 2003).

In conclusion, glucuronidation of eslicarbazepine results from the contribution of UGT1A4, UGT1A9, UGT2B4, UGT2B7, and UGT2B17, but the high-affinity component of the UGT2B4 isozyme may play a major role at therapeutic plasma concentrations of unbound eslicarbazepine.

### TABLE 2

Activities of UGT in single-donor human liver microsomes

<table>
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<th>Enzyme activity</th>
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<th>HG93</th>
<th>HG95</th>
<th>HG64</th>
<th>HH13</th>
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<td>400</td>
<td>570</td>
<td>250</td>
</tr>
</tbody>
</table>

Assay HG3 HG93 HG95 HG64 HH13

Enzyme activity is expressed as picomoles of product per milligram of protein per minute. Data are from BD Biosciences (San Jose, CA). Because UGT enzymes exhibit distinct but overlapping substrate selectivity, further correlation analysis and chemical inhibition studies were performed to determine the UGT isoforms responsible for eslicarbazepine conjugation. For the eslicarbazepine glucuronidation concentrations of 1000 μM, the glucuronidation correlates well with trifluoperazine glucuronidation, which is catalyzed mainly by UGT1A4. There was no significant correlation with propofol and estradiol 3-glucuronide, which are typical substrates of UGT1A9 and UGT1A1, respectively. Imipramine, an UGT1A4 substrate (Nakajima et al., 2002), and testosterone, a substrate for UGT2B7, UGT2B17, and UGT2B15 (Bovalgaha et al., 2007), were equipotent in inhibiting eslicarbazepine glucuronidation (100 μM substrate concentration) in liver microsomes, whereas propofol had almost no effect. On the other hand, diclofenac was much more potent than imipramine and testosterone in inhibiting eslicarbazepine conjugation (IC₅₀ values of 17.0, 642.2, and 694.2 μM, respectively). Diclofenac at 500 μM was found to inhibit UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B15, and UGT2B17-catalyzed 4-methylumbelliferone glucuronidation (Uchaipichat et al., 2006b). In the study presented here, diclofenac markedly inhibited eslicarbazepine glucuronidation through UGT1A4, UGT1A9, UGT2B4, and UGT2B7. Inhibition of UGT1A4 and UGT2B17 and partial inhibition of UGT2B4 and UGT1A9 eslicarbazepine glucuronidation was also observed in the presence of testosterone. In addition, propofol was found to inhibit UGT1A9 and partially inhibit UGT2B4- and UGT2B17-mediated eslicarbazepine glucuronidation. Therefore, identification of specific UGTs was challenging because of the lack of isoform-specific probe substrates and inhibitors and overlapping substrate specificities (Uchaipichat et al., 2006b).

A previous study showed that the levels of therapeutic doses of eslicarbazepine ranged from 18.5 ± 15.37 μM for 800 mg/day to 35.1 ± 26.0 μM for 1200 mg/day (Brown and El-Mallakh, 2010). In conclusion, glucuronidation of eslicarbazepine results from the contribution of UGT1A4, UGT1A9, UGT2B4, UGT2B7, and UGT2B17, but the high-affinity component of the UGT2B4 isozyme may play a major role at therapeutic plasma concentrations of unbound eslicarbazepine.
FIG. 7. Correlation analysis between eslicarbazepine glucuronidation and specific substrate glucuronidation in single-donor human liver microsomes. Liver microsomes from five donors (HG3, HG93, HG95, HG64, and HH13) were incubated with 1000 µM eslicarbazepine for 60 min at 37°C. Values represent mean ± S.E.M. of duplicates. Results are expressed as relative units (peak area /H9262 M eslicarbazepine). Values represent mean ± S.E.M. of duplicates.

Authorship Contributions

Participated in research design: Loureiro, Bonifácio, Wright, and Soares-da-Silva.

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Contributed new reagents or analytic tools: Loureiro, Fernandes-Lopes, and Bonifácio.


Wrote or contributed to the writing of the manuscript: Loureiro, Fernandes-Lopes, Bonifácio, Wright, and Soares-da-Silva.

References


