Characterization of the UDP Glucuronosyltransferase Activity of Human Liver Microsomes Genotyped for the UGT1A1*28 Polymorphism

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ABSTRACT:

The UGT1A1*28 polymorphism is known to correlate with altered clearance of bilirubin (Gilbert syndrome) and drugs such as 7-ethyl-10-[(4-[(1-piperidino)-1-piperidino] carboxyloxy camptothecin (CPT-11). Although this polymorphism is clinically relevant and leads to significant drug-related toxicity of CPT-11, in vitro tools to allow prediction of how it will affect the clearance of new chemical entities have not been completely developed. To allow a more complete assessment of whether new chemical entities will be affected by the UGT1A1*28 polymorphism, a panel of microsomes was prepared from 15 donor livers genotyped as UGT1A1*1/*1, UGT1A1*1/*28, and UGT1A1*28/*28 (five donors per genotype). The microsomes were phenotyped by measuring activities of a panel of substrates, both those reported to be conjugated specifically by UGT1A1 or by other UDP glucuronosyltransferase enzymes. Bilirubin, estradiol (3-OH), ethinyl estradiol (3-OH), and 7-ethyl-10-hydroxycamptothecin (SN-38) were found to show significantly lower rates of metabolism in the UGT1A1*28/*28 microsomes with no change in Km values. In addition, microsomes genotyped as UGT1A1*1/*28 showed intermediate rates of metabolism. Acetaminophen, 3′-azido-3′-deoxythymidine, muraglitazar, estradiol (17-OH), and ethinyl estradiol (17-OH) were all found to show similar rates of metabolism regardless of UGT1A1 genotype. Interestingly, muraglitazar (UGT1A3 substrate) showed an inverse correlation with glucuronidation of UGT1A1 substrates. These genotyped microsomes should provide a useful tool to allow a more comprehensive prediction of UGT1A1 metabolism of a new drug and gain insight into the effect of the UGT1A1*28 polymorphism.

The determination of the enzymes involved in the human metabolism of candidate drugs is a critical activity for the successful clinical development and commercialization of a new drug. The information derived from these studies is used to make decisions on drug-drug interaction studies as well as whether there will be subpopulations that will display altered pharmacokinetic parameters. One of the subpopulations potentially affected is individuals with allelic variants giving rise to change of function of a drug metabolizing enzyme important for the clearance of the new drug (Lesko et al., 2003; Huang et al., 2006). Early work in this area focused on the well characterized polymorphisms in the CYP2C19 and CYP2D6 enzymes. These polymorphisms have a functional consequence is polymorphisms of the UGT1A1 gene (Tukey et al., 2002). In many cases the functional significance of these polymorphisms has not been determined. One example of where a common polymorphism in a drug-metabolizing enzyme has been shown to have a functional consequence is polymorphisms of the UGT1A1 gene (Nagar and Blanchard, 2006; O’Dwyer and Catalano, 2006). The most common UGT1A1 polymorphism causing a functional consequence in many populations is the UGT1A1*28 variant. This genotype is associated with Gilbert syndrome, a mild nonhemolytic, unconjugated hyperbilirubinemia. The polymorphism is a change in the promoter region of the gene leading to a [A(TA)7TAA] sequence compared with a [A(TA)6TAA] in the wild-type gene (Bosma et al., 1995). Patients homozygous for the polymorphism display hyperbilirubinemia with an approximate 70% reduction in the transcriptional activity of the UGT1A1 gene (Tukey et al., 2002). Patients that are homo- or heterozygous for the UGT1A1*28 poly-

ABBREVIATIONS: UGT, UDP glucuronosyltransferase; APAP, acetaminophen; AZT, 3′-azido-3′-deoxythymidine; bp, base pair(s); CPT-11, 7-ethyl-10-[(4-[(1-piperidino)-1-piperidino] carboxyloxy camptothecin; EE, ethinyl estradiol; HLM, human liver microsome(s); LC/MS, liquid chromatography/mass spectrometry; LC/MS/MS, liquid chromatography/tandem mass spectrometry; P450, cytochrome P450; SN-38, 7-ethyl-10-hydroxycamptothecin; UDPGA, UDP-glucuronic acid; ESI, electrospray ionization; Gluc, glucuronide.

morphism have altered pharmacokinetic profiles and are more susceptible to the dose-limiting toxicities displayed after administration of the anticancer agent irinotecan (CPT-11), that is, severe diarrhea (Iyer et al., 2002; Tukey et al., 2002; Nagar and Blanchard, 2006). Although the exact mechanism for this has not been determined, it is generally accepted that decreased UGT1A1 activity leads to elevated levels of SN-38, the active form of CPT-11, through a decrease in clearance via glucuronide conjugate formation. In vitro studies of microsomes genotyped for the *28 polymorphism show a decrease in catalytic activity toward SN-38 and other UGT1A1 substrates (Iyer et al., 1998, 1999; Fisher et al., 2000; Gagné et al., 2002; Peterkin et al., 2007). The observation of the correlation between genotype and clinical outcome has led to the recommendation that patients are genotyped for the UGT1A1*28 polymorphism and dose adjustments made before treatment with CPT-11 (http://www.accessdata.fda.gov/scripts/cder/drugsatfda).

Although the UGT1A1*28 genotype is fairly common and does lead to a clinically important phenotype, there are currently few options for rigorously predicting whether lower UGT1A1 enzyme levels will affect the clearance of a new chemical entity. This is because the tools that exist for P450 reaction phenotyping (i.e., specific chemical and antibody inhibitors and well characterized expressed enzymes) are not available for UGT enzymes. The most effective tools at present for determining substrate-specific UGT activity are: 1) expressed UGT enzymes (Ethell et al., 2001) and 2) correlation analysis of an activity known to be specific for a single UGT with conjugate formation of an unknown compound across a bank of human liver microsomes (Court, 2005). However, drawing conclusions from these data alone is problematic in the absence of other confirmatory methods.

As a means to better evaluate the potential for new candidate drugs to be selective substrates for UGT1A1, and thus have the potential for altered pharmacokinetics in patients with the UGT1A1*28 polymorphism, sets of liver microsomes were isolated from subjects genotyped as UGT1A1*1/*1 or hetero- or homozygous for UGT1A1*28. These sets of microsomes were used to collect data from various substrates previously determined to be likely UGT1A1 substrates or substrates thought to be primarily conjugated by other UGT enzymes.

Materials and Methods

Materials. Alamethicin, magnesium chloride, uridine diphosphoglucuronic acid (UDPGA), APAP, APAP glucuronide, 3‘-azido-3‘-deoxythymidine (AZT), AZT glucuronide, bilirubin, estradiol, estradiol-3-glucuronide, estradiol-17-glucuronide, ethinyl estradiol (EE), EE-3-glucuronide, and EE-17-glucuronide were obtained from Sigma-Aldrich (St. Louis, MO). Sodium phosphate and trifluoroacetic acid were obtained from EM Science (Gibbstown, NJ). Lorazepam and lorazepam glucuronide were purchased from Alltech-Applied Science Labs (State College, PA). Muraglitazar, muraglitazar glucuronide, and BMS-AS (a compound from the same chemical series as muraglitazar) were synthesized at Bristol-Myers Squibb Co. (Hopewell, NJ). The structures and UGT-catalyzed reactions of these selected substrates are shown in Fig. 1. Atenonitrile was purchased from Allied Signal Inc. (Muskegon, MI). Pooled human liver microsomes (HLM; prepared from 22 donors), membranes from insect cells transfected with baculovirus containing cDNA of human UGT enzymes (UGT1A1, -1A3, -1A4, -1A6, -1A8, -1A9, -1A10, -2B4, and -2B7), were purchased from BD Gentest Co. (Hopewell, NJ). The structures and UGT-catalyzed reactions of these baculovirus containing cDNA of human UGT enzymes (UGT1A1, -1A3, and -1A9) was prepared by dissolving 21 mg in 1.6 ml of water. Sodium phosphate and trifluoroacetic acid were used in the polymerase chain reaction. Primers were used to generate a 194-bp product for the (TA)7 (*28) allele. The presence of both the 194-bp and 194-bp fragments represented a heterozygote sample. The polymerase chain reaction products were gel-electrophoresed on high-resolution gels and photographed under ultraviolet light.

Microsomal Incubations. Incubation in triplicate containing 50 mM Tris (pH 7.4), 0.5 mg/ml microsome protein, 5 mM MgCl2, 25 μg/ml alamethicin, 5 mM saccharic acid 1,4-lactone, and 2 mM UDPGA in a final concentration of 200 μM was conducted in 96 deep-well plates at 37°C. Preparation of substrate stock solutions, incubation times, and reaction quenching are shown in Table 1. For determination of glucuronidation activities of individual human liver microsomes, the substrate concentrations in incubations were based on literature-reported Km values for each substrate: 2000 μM for APAP by UGT1A6 and UGT1A7 (Court et al., 2001); 500 μM for AZT by UGT2B7 (Court et al., 2003); 5 to 10, 30, and 15 μM by UGT1A1 for bilirubin (Bosma et al., 1994; Zhang et al., 2005), estradiol (Senafi et al., 1994; Soars et al., 2003), and ethinylestadiol (Ehner et al., 1993); 3 μM for muraglitazar by UGT1A1, UGT1A3, and UGT1A9 (Zhang et al., 2007); 20 μM for SN-38 by UGT1A1, UGT1A7, and UGT1A9 (Iyer et al., 1998; Tallman et al., 2005); and 10 μM for Lorazepan (Court, 2005). Human cDNA-expressed UGT1A1, -1A3, -1A6, -1A7, -1A8, -1A9, -1A10, -2B4, and -2B7 were incubated at 0.5 mg of protein/ml with each substrate. For substrate concentration-dependent glucuronidation activities of selected human liver microsomes (HH19 and HH138, two homozygous; HH69, a heterozygous; and HH74, a wild-type), the substrate concentrations were 0.52, 1.563, 3.125, 6.25, 12.5, 25, 50, and 100 μM for bilirubin and ethinyl estradiol; 2.08, 6.25, 12.5, 25, 50, 100, 200, and 300 μM for estradiol and SN-38; and 0.234, 0.466, 0.938, 1.875, 3.75, 7.5, 15, and 30 μM for SN-38 and muraglitazar. The protein concentration was 0.5, 0.5, 0.25, and 0.125 mg/ml for HH19, HH138, HH69, and HH74 in incubations with ethinyl estradiol, estradiol, and bilirubin, respectively, and 0.5 mg/ml with muraglitazar and SN-38.

Analytical Methods. Because SN-38 glucuronide metabolite was not available as a standard to develop a quantification method, the relative amount of each metabolite formed in an incubation was calculated from the peak area ratio of the metabolite to the internal standard in selective reaction monitoring analyses by LC/MS/MS. Peak area ratios were then used to measure the relative amount of metabolite formation between different incubations. Detailed analytical methods, including detection methods, columns, high-performance liquid chromatography, gradients and elution flow rates, retention times of glucuronide products and internal standards, mass transitions, and standard concentration ranges are shown in Table 2. In each case, the substrate, glucuronide metabolite, and the internal standard were well separated with minimal or no cross-interference by LC/MS/MS or UV detection. The standard curves were fitted to a 1× weighted quadratic regression model.

Data Analysis. The glucuronidation rates at substrate concentrations near
FIG. 1. Glucuronidation reactions investigated in this study.
their $K_m$ values were analyzed by Pearson correlation in Excel based on the following equation:

$$r = \frac{n(\sum XY) - (\sum X)(\sum Y)}{\sqrt{[n(\sum X^2) - 1][n(\sum Y^2) - 1]}}$$

The $K_m$ and $V_{max}$ values of each enzyme were estimated by fitting the glucuronidation activities to the Michaelis-Menten equation using a nonlinear regression analysis in SigmaPlot as recommended in the literature (Bjornsson et al., 2003; $V_i = V_{max}[S]/[K_m + [S]]$).

### Results

**Analytical Methods.** Glucuronide metabolites formed in various incubations were quantitated with authentic metabolite standards where available and generally fell within the linear range of their respective standard curves. Bilirubin was used as a standard for bilirubin glucuronide because the glucuronide standard was not available; this method has been successfully used in previous studies (Zhang et al., 2005). In addition, SN-38 glucuronide standard was not available, and a relative LC/MS quantification method (Zhang et al., 2007) was used to quantify this metabolite. In this method, stable isotope-labeled testosterone was added as an internal standard after incubations. SN-38 glucuronide metabolite was analyzed by LC/MS/MS selective reaction monitoring after chromatographic separation. The peak area ratio of metabolite to internal standard was obtained. The peak area ratios were compared between samples within the same series of incubations. Different volumes of the metabolite mixtures at low and high substrate concentration were injected to ensure the linear LC/MS/MS conditions. The relative reaction velocity (peak area ratio/min/mg protein) was calculated and plotted against substrate concentration. The $K_m$ and relative $V_{max}$ values were then obtained by using the Michaelis-Menten equation.

**Glucuronidation by Human cDNA-Expressed UGT Enzymes.** The probe substrates were incubated with a panel of expressed human UGT enzymes (Fig. 2). The results demonstrate that, as expected from literature studies, UGT1A1 was the primary hepatic enzyme capable of catalyzing the glucuronidation of bilirubin and the 3-OH conjugates of estradiol and EE. SN-38 was primarily glucuronidated by UGT1A1; however, it was also metabolized by expressed UGT1A8, UGT1A9, and UGT1A10 (Fig. 2), as well as UGT1A7 (data not shown). Multiple UGT enzymes catalyzed the glucuronidation of APAP. UGT2B7 was the only enzyme-catalyzing AZT and lorazepam glucuronidation and was the most active enzyme toward the 17-OH conjugation of estradiol and EE. UGT1A3 as well as UGT1A1 and UGT1A9 catalyzed the glucuronidation of muraglitazar.

**Glucuronidation by UGT1A1*28 Polymorphic Human Liver Microsomes.** Eleven UGT-catalyzed reactions were monitored in the individual donor human liver microsome samples genotyped for UGT1A1 and in a human liver microsome pool (Fig. 3). There was an apparent trend toward increasing glucuronidation activity for bilirubin (Fig. 3C), estradiol-3-OH conjugate (Fig. 3D), EE-3-OH conjugate (Fig. 3F), and SN-38 (Fig. 3J) in microsomes from subjects genotyped as homozygous (HH19, HH138, HH130, HH81, and HH90), heterozygous (HH50, HH124, HH69, HH71, and HH79), and wild-type (HH48, HH55, HH191, HH57, and HH74) for the UGT1A1*28 mutation. The mean values of glucuronidation activities of these four substrates (Table 3) further support this activity trend. One homozygous donor sample (HH19) demonstrated a relatively high concentration of bilirubin glucuronide formation, which was later found to be due to bilirubin diglucuronide and bilirubin present in the sample. There did not seem to be any pattern relative to genotype for glucuronidation of APAP (Fig. 3A), AZT (Fig. 3B), estradiol-17-hydroxyl (Fig. 3E), ethinyl estradiol-17 hydroxyl (Fig. 3G), or lorazepam (Fig. 3H). Interestingly, muraglitazar glucuronidation seemed to follow an inverse trend (Fig. 3I) from that of bilirubin, estradiol 3-OH, EE, 3-OH, and SN-38 glucuronidation activity.

**Correlation of Glucuronidation of Different Substrates by Individual Human Liver Microsomes.** Table 4 shows Pearson correlation coefficients ($r$) for the 10 glucuronidation reactions monitored in this study in the 15 individual donor liver microsomes genotyped for the UGT1A1*28 polymorphism. The average glucuronidation activity in four of the homozygous microsomal samples, HH138, HH130, HH81, and HH90, was used for the bilirubin reaction calculations, because the HH19 microsomal sample was found to contain significant endogenous bilirubin glucuronide. There was an excellent correlation (coefficient value of $0.78$) between the rate of glucuronidation of bilirubin, estradiol (3-OH), EE (3-OH), and SN-38 across the panel of microsomes. APAP glucuronidation was modestly correlated to EE-17-glucuronidation and AZT. Lorazepam did not seem to correlate with the activities of classic UGT1A1 or UGT2B7 substrates. As mentioned above, there was a strong negative correlation of the rate of muraglitazar glucuronidation relative to the rates found for bilirubin, estradiol, EE, and SN-38.

**Kinetic Determination for Glucuronidation of UGT1A1 Substrates by Selected Human Liver Microsomes.** The concentration-dependent kinetics of glucuronide formation were explored in representative HLM samples from the homozygous (HH19 and HH138), heterozygous (HH69), and wild-type (HH74) sets of microsome samples (Fig. 4; Table 5). HH19 was not used in the kinetic experiments for bilirubin glucuronidation because it was contaminated with bilirubin diglucuronide. The $V_{max}$ values for bilirubin, estradiol 3-OH, EE 3-OH, and SN-38 glucuronidation all decreased in the following order: wild-type, heterozygous, and homozygous. However, the $K_m$
TABLE 2

Analytical methods for quantification of glucuronide metabolites in microsome incubations

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Analytical Methods</th>
<th>Column</th>
<th>Gradient, Flow</th>
<th>( T_R ), min</th>
<th>HPLC-MS or UV</th>
<th>Internal Standard</th>
<th>Mass Transition</th>
<th>Standard</th>
<th>Standard Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>LC/MS/MS (ESI−)</td>
<td>ODS-AQ 5 μ C18, 50 × 2.0 mm</td>
<td>5–55% B in 1.5’, hold for 0.8’; 0.3</td>
<td>1.35; IS: 1.97</td>
<td>Shimadzu; API3000</td>
<td>Naphthyl glucuronide (1000 ng/ml)</td>
<td>326→150 IS: 314→138</td>
<td>Acetaminophen glucuronide</td>
<td>1000, 800, 500, 250, 100, 50, 20, 10 ng/ml</td>
</tr>
<tr>
<td>AZT</td>
<td>LC/MS/MS (ESI−)</td>
<td>ODS-AQ 5 μ C18, 50 × 2.0 mm</td>
<td>5–55% B in 1.5’, hold for 0.8’; 0.3</td>
<td>1.82; IS: 1.97</td>
<td>Shimadzu; API3000</td>
<td>Naphthyl glucuronide (1000 ng/ml)</td>
<td>442→125 IS: 314→138</td>
<td>AZT glucuronide</td>
<td>1000, 800, 500, 250, 100, 50, 20, 10 ng/ml</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>LC/UV (445 nm)</td>
<td>Sunfire 3 μ C18, 150 × 2.1 mm</td>
<td>20–98% B in 18’, hold 98% B for 8’; 0.3 (50 μl injection)</td>
<td>Di-G9.1, mon-G/12.3; IS: 6.0</td>
<td>Alliance; Finnigan PDA</td>
<td>Naphthyl glucuronide (1000 ng/ml)</td>
<td>442→125 IS: 314→138</td>
<td>Bilirubin</td>
<td>0.167, 0.333, 0.667, 1.667, 3.333, 13.333 μM; ( \rho = 0.997 )</td>
</tr>
<tr>
<td>Estradiol</td>
<td>LC/MS/MS (ESI−)</td>
<td>Acquity UPLC BEH C18 1.7 μ 2.1 × 50 mm</td>
<td>5–30% B in 0.5’, to 40% in 0.5’, to 95% in 0.2’, hold for 0.3’; 0.6</td>
<td>ESQ0.93, E17Q0.98; IS: 0.86</td>
<td>ULC-Qtrapi</td>
<td>Naphthyl glucuronide (5 μM)</td>
<td>447→113 IS: 319→113</td>
<td>Estradiol-3-glucuronide; estradiol-17-glucuronide</td>
<td>10,000, 7500, 5000, 1000, 500, 100, 50, 10, 5, 1 nM</td>
</tr>
<tr>
<td>EE</td>
<td>LC/MS/MS (ESI−)</td>
<td>Atlantis 5 μ C18, 50 × 2.1 mm</td>
<td>2’ at 20% B, 20–40% B in 2 min; 1.0</td>
<td>3-G/5.27, 17-G/3.61; IS: 2.84</td>
<td>Shimadzu; Qtrapi</td>
<td>D5-trime (250 ng/ml)</td>
<td>471→295 IS: 447→297</td>
<td>EE 3-glucuronide; EE 17-glucuronide</td>
<td>5000, 1000, 100, 10, 1 for 17-G; 5000, 100, 10, 1 for 3-G</td>
</tr>
<tr>
<td>Lorazepan</td>
<td>LC/MS/MS (ESI−)</td>
<td>Atlantis 5 μ C18, 50 × 2.1 mm</td>
<td>0.5’ at 10% B in 2’; 10–40% B in 2’, 40–80% in 0.5’; 1</td>
<td>2.43; IS: 2.97</td>
<td>Shimadzu; Ultima</td>
<td>TFMU (250 ng/ml)</td>
<td>497→283 IS: 229→201</td>
<td>Lorazepan glucuronide</td>
<td>1000, 500, 200, 100, 50, 10, 5 ng/ml for 3-G</td>
</tr>
<tr>
<td>Muraglitazar</td>
<td>LC/MS/MS (ESI+ )</td>
<td>Luna 3 μ C18, 50 × 4.6 mm</td>
<td>35% A’, 65% B; 0.6</td>
<td>6.2; IS: 12.24</td>
<td>Alliance; Quantum</td>
<td>BMU-426707 (100 ng/ml)</td>
<td>693→292 IS: 531→306</td>
<td>Muraglitazar glucuronide</td>
<td>2000, 1000, 500, 100, 50, 25, 10, 5 ng/ml</td>
</tr>
<tr>
<td>SN-38</td>
<td>LC/MS/MS (ESI+)</td>
<td>ODS-AQ 5 μ C18, 50 × 2.0 mm</td>
<td>0.5’ at 10% B in 2’; 10–95% B in 2.5’, hold for 1.5’; 0.3</td>
<td>2.42; IS: 2.70</td>
<td>Shimadzu; Qtrapi</td>
<td>D3-testosterone (100 ng/ml)</td>
<td>569.1→393 IS: 308.2→272</td>
<td>No</td>
<td>Multiple volumes injected to ensure the linearity of MS response</td>
</tr>
</tbody>
</table>

IS, internal standard; HPLC, high-performance liquid chromatography; PDA, photodiode array; UPLC, ultra-performance liquid chromatography; N.A., not applicable; TFMU, trifluoromethyl umbelliferone; ACMA, 9-amino-6-chloro-2-methoxyacridine.

A: 0.1% TFA in water; B: 0.1% TFA in acetonitrile.

B: 0.05% TFA in water; C: 0.05% TFA in 1:1 (v/v) methanol:acetonitrile.

A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile.

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Fig. 2. Glucuronidation of ten substrates catalyzed by cDNA-expressed UGT enzymes.
values for all substrates were similar regardless of UGT1A1*28 polymorphism. In contrast, there were no significant differences for all substrates were similar regardless of UGT1A1*28 polymorphism. This is because several polymorphisms, the most common of which is UGT1A1*28, lead to decreased expression of the UGT protein and, thus, decreased clearence of UGT1A1 substrates. The effect of this polymorphism on the clearance of SN-38 is well documented and has led to the recommendation that patients be genotyped for the polymorphism before treatment (http://www.accessdata.fda.gov/scripts/cder/drugsatfda).

To allow a prospective measure of effect of UGT1A1 on the formation of conjugates in HLM, a panel of human microsomes genotyped for the UGT1A1*28 polymorphism was generated. The panel consisted of 15 subjects, with 5 from each of the following genotypes: UGT1A1*1/UGT1A1*1, UGT1A1*1/UGT1A1*28, and UGT1A1*28/UGT1A1*28. Although the method was geared toward detertermination of the effect of the UGT1A1*28 allelic variant, it could be applied to the multiple other allelic variants that also affect UGT1A1 expression.

A good correlation of genotype with conjugation activity was found for bilirubin, estradiol (3-OH conjugate), EE (3-OH conjugate), and SN-38 glucuronidation, as has been noted in previous studies (Iyer et al., 1998, 1999; Fisher et al., 2000; Gagné et al., 2002; Peterkin et al., 2007). All substrates demonstrated a gene dosage effect with the heterozygous subjects falling between the *1/*1 and *28/*28 subjects. The first three reactions are well documented as being selective for UGT1A1 (Ebner et al., 1993; Bosma et al., 1994; Senafi et al., 1994; Tallman et al., 2002; Tallman et al., 2005). The data collected from these subjects do

![FIG. 3. Glucuronidation of ten substrates catalyzed by HLM genotyped for the UGT1A1*28 polymorphism. Samples included a random HLM pool and microsomes prepared from livers genotyped as UGT1A1*28/*28 (HH19, HH138, HH130, HH61, HH90), UGT1A1*1/*28 (HH50, HH124, HH69, HH71, HH79), and UGT1A1*1/*1 (HH48, HH55, HH91, HH57, HH74).](image-url)
support a significant role of UGT1A1 but do not rule out the involvement of other enzymes.

The substrates examined in this study that have been reported to be primarily metabolized by other UGT enzymes besides 1A1, that is, APAP (Court et al., 2001), AZT (Court et al., 2003), estradiol (17-OH conjugate) (Senafi et al., 1994; Soars et al., 2003), EE (17-OH conjugate) (Ebner et al., 1993), lorazepam (Court, 2005), and muraglitazar (Zhang et al., 2007), did not show any trends toward correlation in the same direction as the UGT1A1 substrates. There were some significant correlations of the activities measured for the non-UGT1A1 substrates across the panel with other non-UGT1A1 substrates. For instance, APAP glucuronidation showed a positive correlation \(r = 0.53\) with the glucu-
ronization of AZT, a classic UGT2B7 substrate, which suggests that UGT2B7 might play an important role for this reaction in HLM. Previous studies have shown that multiple UGT enzymes can catalyze APAP glucuronidation with the predominant enzyme in HLM being UGT1A9 (Court et al., 2001), although recent data have suggested an important role for UGT2B15 (Mutilb et al., 2006). Although UGT2B7 has been shown to catalyze the glucuronidation of APAP (Court et al., 2001), there have not been any previous results demonstrating that the enzyme plays an important overall role in the activity. The results found in this study correlating APAP and AZT glucuronidation activity would need to be studied in more depth before concluding that UGT2B7 played an important role in APAP glucuronidation. This would include study of the correlation over a broader range of APAP concentrations (2 mM was used in this study), as the kinetics of glucuronidation are concentration-dependent (Court et al., 2001).

Although there was no positive trend to the activity results from subjects with different UGT1A1 genotype and the non-UGT1A1 substrates, muraglitazar showed a significant trend in the opposite direction to the UGT1A1 substrates, with significant negative correlation coefficients found for each UGT1A1 substrate (Table 4). The conjugation of muraglitazar has been demonstrated to be carried out by expressed UGT1A1, UGT1A3, and UGT1A9, with UGT1A3 playing a predominant role (Zhang et al., 2007), so the lack of correlation with the UGT1A1 substrates is not surprising. However, the significant increase in activity upon decrease in UGT1A1 enzyme levels is an unexpected finding. The increased UGT1A3 activity in the HLM isolated from UGT1A1*28 donor tissue may be due to either increased UGT1A3 enzyme expression or increased UGT1A3 activity. The expression of UGT1A1 has been reported to be controlled by a number of important regulatory pathways including pregnane X receptor, constitutive androstane receptor, glucocorticoid receptor, aryl hydrocarbon receptor, and hepatic nuclear factor-1α (Chen et al., 2005; Sugatani et al., 2005; Ramirez et al., 2007). Pregnan X receptor has been reported to play a role in regulation of UGT1A3 expression (Gardner-Stephen et al., 2004), but more recent reports have indicated that hepatic nuclear factor-1α and liver X receptor may play a larger role (Verreault et al., 2006; Gardner-Stephen and Mackenzie, 2007). Because the genes do share the potential for coordinate regulation of expression, it is possible that, in the UGT1A1*28 livers, UGT1A3 expression is up-regulated due to a compensatory mechanism driven by low UGT1A1 expression. A second hypothesis would be the increased activity of the UGT1A3 enzyme due to lower levels of UGT1A1 enzyme via a direct enzyme-enzyme mediated inhibitory effect. A direct effect on the activity of UGT enzymes upon concomitant expression of multiple enzymes has been recently described and shown to affect the activity of the UGT1A family of enzymes (Fujisawa et al., 2007a,b).

The kinetic parameters of the UGT1A1 substrates were determined across the panel of microsomes, and the results suggest that: 1) the substrate had similar binding properties (similar Km values) between microsomes genotyped as UGT1A1*1/*1, UGT1A1*1/*28, UGT1A1*28/*28, or UGT1A1*28/*UGT1A1*28, and 2) the lower catalytic efficiencies for glucuronidation by homozygous or heterozygous subjects were due to decreases in Vmax. These conclusions are consistent with a lower expression of UGT1A1 enzyme due to the *28 promotor polymorphism and not due to changes of catalytic properties.

The formation of the 3-OH glucuronide conjugates of estradiol and EE has been well described to result from UGT1A1 activity (Ebner et al., 1993). In contrast, the identity of the major enzyme responsible for formation of the 17-OH conjugates has not been completely identified, and the only reports demonstrate that the conjugation is not a UGT1A1 reaction (Ebner et al., 1993) and is catalyzed by UGT2B7 (Gall et al., 1999; Williams et al., 2002). Across the panel of enzymes there were no significant differences found in the Vmax values for the rates of glucuronidation of estradiol-17-OH (Table 5; Fig. 4E) and EE-17-OH (data not shown) and no correlation to the activity of any of the UGT1A1 substrates. The data presented in this study support the conclusion that the major enzyme responsible for the 17-OH conjugate formation seems to be UGT2B7, both based on the expressed enzyme profiling (Fig. 2, E and G) and the significant correlation of both estradiol and EE 17-OH conjugate formation with AZT conjugate formation across the panel of microsomes (Table 4). AZT glucuronide has been demonstrated to be formed predominantly by UGT2B7 (Court et al., 2003; Court, 2005).

Gaining prospective information on the effect of genetic polymorphisms on the exposure of new chemical entities is an increasingly important part of drug development, especially for new drugs that have narrow therapeutic indices. The first step in determining whether polymorphisms may play a role in producing variable exposure is through a complete understanding of the reaction phenotype of important metabolic clearance pathways of the new compound. Reaction phenotyping of the UGT enzymes is not as robust as it is for P450 enzymes because of the limitations of reagents available. To gain a better understanding of the contribution of UGT1A1 to the metabo-
lism of a new chemical entity while simultaneously generating information on the effects of the UGT1A1*28 polymorphism, a panel of microsomes genotyped for UGT1A1*1 and UGT1A1*28 was generated. The panel was characterized for conjugation activity with both known UGT1A1 and non-UGT1A1 substrates and found to have properties as expected for microsomes from subjects with the *28 polymorphism, which is associated with lower UGT1A1 expression and Gilbert syndrome. This panel of microsomes will be useful in characterizing the role of UGT1A1 in the conjugation of a new chemical entity and in predicting the effect of the UGT1A1*28 polymorphism on drug exposure.

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