AN ASSESSMENT OF UDP-GLUCURONOSYLTRANSFERASE INDUCTION USING PRIMARY HUMAN HEPATOCYTES

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ABSTRACT:
Uridine diphosphate glucuronosyltransferases (UGTs) catalyze the glucuronidation of a wide range of xenobiotics and endogenous substrates. However, there is a lack of information concerning the response of human UGTs to inducers, and this observation prompted the current investigation. The glucuronidation of estradiol (3- and 17-positions), naphthol, propofol, and morphine (3- and 6-positions) was assessed against a battery of recombinant human UGTs to determine selective glucuronidation reactions for induction studies. The potential induction of the glucuronidation of estradiol at the 3-position, naphthol, propofol, and morphine at the 3-position was subsequently investigated in cultured primary human hepatocytes against a range of prototypic inducers including dexamethasone, 3-methylcholanthrene (3-MC), phenobarbital, rifampicin, and omeprazole. Treatment with 3-MC induced estradiol-3-glucuronidation (up to 2.5-fold) in four of five donors investigated. Statistically significant increases in naphthol glucuronidation (up to 1.7-fold) were observed following treatment with carbamazepine. UGT1A9-mediated propofol glucuronidation was induced by phenobarbital (up to 2.2-fold) and rifampicin (up to 1.7-fold). However, treatment with α-naphthoflavone and tanganetin resulted in a decrease in propofol glucuronidation (30% of control values). Statistically significant induction of morphine-3-glucuronidation was observed in at least three donors following treatment with phenobarbital, rifampicin, and carbamazepine. Each UGT isoform investigated displayed a distinct induction profile. Although statistically significant increases in glucuronidation were observed for each reaction studied, the level of induction was less than that observed for CYP1A2 or CYP3A4 and exhibited a large interdonor variability. The clinical relevance of the induction responses obtained in this study is unclear.

The potential for drug-drug interactions to affect the pharmacokinetic profile of new chemical entities has necessitated a thorough investigation into possible common causes, of which one is enzyme induction. The majority of the investigations on induction have thus far focused on the regulation of the cytochromes P450 (P4502), due to the large number of drugs that are metabolized by this family of enzymes (Bertz and Granneman, 1997). Perhaps the most studied mechanism of induction to date is that of the aryl hydrocarbon (Ah) receptor, responsible for CYP1A induction (Okino and Whitlock, 2000). The roles of nuclear receptors such as the pregnane X receptor (PXR), constitutive androstane receptor (CAR), and the peroxisome proliferator-activated receptor (PPAR) in the induction of drug-metabolizing enzymes have recently been elucidated (Waxman, 1999; Honkakoski and Negishi, 2000). However, investigations into the regulation of expression of the phase II enzymes such as the uridine diphosphate glucuronosyltransferases (UGTs) have been more limited.

UGTs are a superfamily of membrane-bound enzymes that detoxify a large variety of xenobiotics and endogenous substrates via the addition of a sugar moiety (Dutton, 1980; Clarke and Burchell, 1994). To date, 15 functionally active human UGTs have been characterized and classified into two separate families (UGT1 and UGT2) based on amino acid sequence similarity (Mackenzie et al., 1997).

Interestingly, the number of reports of clinically significant drug interactions as a result of UGT induction has been relatively sparse compared with those reported for P450 induction. Gallicano et al. (1999) observed an increase in zidovudine glucuronidation in patients taking the antituberculosis drug rifampicin. Several groups have noted an increased clearance of acetaminophen (via glucuronidation) in patients receiving anticonvulsants (phenytoin/carbamazepine) or rifampicin (Prescott et al., 1981; Miners et al., 1984). Mitchell et al. (1983) also observed an induction of acetaminophen glucuronidation in women taking contraceptive steroids. Further studies have been performed to examine the effect of dietary constituents on the metabolism of acetaminophen. Pantuck et al. (1984) showed that individuals fed a diet of cruciferous vegetables glucuronidated acetaminophen more rapidly than those on a control diet. However, the glucuronidation of oxazepam remained unaltered. Furthermore, heavy smoking...
(40 high-tar cigarettes per day) has also been shown to induce acetaminophen glucuronidation (Bock et al., 1987).

Of particular interest are the drug-drug interactions initially believed to be the result of inductions of P450s, which upon thorough investigation were found to be the result of the induction of phase II enzymes. For example, rifampicin coadministration was thought to increase ethinyl estradiol clearance as a result of induction of CYP3A4 leading to unwanted pregnancies. However, Li et al. (1999) clearly demonstrated that induction of the phase II enzymes was actually the cause of the increased clearance of ethinyl estradiol. Thus, there is considerable need to develop tools to better investigate the induction of UGTs and other phase II enzymes in humans.

Historically, many of the studies investigating the potential induction of UGTs have been performed in the rat (Lilienblum et al., 1982; Coughtrie et al., 1987). Recent studies have used Caco-2 cells for the study of UGT induction by antioxidant-type inducers (Bock et al., 2000) and, more specifically, flavonoids (Galijatovic et al., 2001; Walle and Walle, 2002). However, perhaps the most accepted method of investigating the potential of an agent to cause enzyme induction in humans (particularly for P450s) has been to use primary cultures of hepatocytes (Strom et al., 1996). To date, the use of primary human hepatocytes to study UGT induction has been limited. Li et al. (1999) showed that 3-methylcholanthrene (3-MC), phenobarbital, dexamethasone, and rifampicin did not induce UGT1A1-mediated ethinyl estradiol-3-glucuronidation. However, Ritter et al. (1999) observed a 3-fold increase in both UGT1A1 protein and bilirubin glucuronidation activity (a specific substrate of UGT1A1) following treatment with 3-MC. Thus, the response of human UGTs in primary cultures of human hepatocytes to inducers has not been extensively examined, and where it has been investigated, there is conflicting information.

The aims of the current study were to determine the selectivity of estradiol, naphthol, propofol, and morphine as “probe” substrates for individual human UGT isofoms, and to use the identified selective substrates to investigate the effects of several known prototype P450 inducers on the enzyme activities of UGTs using a human hepatocyte induction model.

Materials and Methods

Materials. Unless stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Propofol was obtained from Aldrich Chemical Co. (Milwaukee, WI), and tangeretin was purchased from Indofine Chemical Co. (Belle Mead, NJ). Morphine and omeprazole were purchased from Sigma/RBI (St. Louis, MO). Morphine and omeprazole were purchased from Sigma/RBI (St. Louis, MO). Propofol was obtained from Aldrich Chemical Co. (Milwaukee, WI), and tangeretin was purchased from Indofine Chemical Co. (Belle Mead, NJ). Morphine and omeprazole were purchased from Sigma/RBI (St. Louis, MO). Propofol was obtained from Aldrich Chemical Co. (Milwaukee, WI), and tangeretin was purchased from Indofine Chemical Co. (Belle Mead, NJ). Morphine and omeprazole were purchased from Sigma/RBI (St. Louis, MO). Propofol was obtained from Aldrich Chemical Co. (Milwaukee, WI), and tangeretin was purchased from Indofine Chemical Co. (Belle Mead, NJ). Morphine and omeprazole were purchased from Sigma/RBI (St. Louis, MO). Propofol was obtained from Aldrich Chemical Co. (Milwaukee, WI), and tangeretin was purchased from Indofine Chemical Co. (Belle Mead, NJ). Morphine and omeprazole were purchased from Sigma/RBI (St. Louis, MO).
Glucuronides were resolved using the following gradient:

\[ t_{	ext{H11005}} / B_{	ext{H11005}} \times 100 \% \]

CYP3A-mediated metabolism of testosterone to estradiol (performed in negative ion mode monitoring: estradiol-3- and -17-glucuronides). Glucuronides of naphthol and propofol were analyzed using a 3-ground gradient. Estradiol glucuronides were resolved using the massie blue assay (Bradford, 1976).

Glucuronides of naphthol and propofol were analyzed using a 3-ground gradient. Estradiol glucuronides were resolved using the massie blue assay (Bradford, 1976).

The testosterone (B) and naphthol (A) substrates were harvested into 1.0 ml of 100 mM potassium phosphate buffer, pH 7.4, and a 100 mM potassium phosphate buffer, pH 7.4, was added before analysis. Samples were injected onto a 50-mm monochrome HPLC column (Phenomenex, Torrance, CA) and a SecurityGuard column. All other glucuronides were resolved using a 5-µm, 150 × 3 mm Prodigy ODS (3) HPLC column (Phenomenex, Torrance, CA) and a SecurityGuard column. The mobile phase (solvent A) consisted of 10 mM ammonium acetate, whereas the organic phase (solvent B) consisted of 10% (v/v) 10 mM ammonium acetate and 90% (v/v) acetonitrile. Several HPLC gradients were used in this study. Estradiol glucuronides were resolved using the following gradient: $t = 0 \text{ min}, \%B = 15, t = 8 \text{ min}, \%B = 31, t = 8.1 \text{ min}, \%B = 100, t = 9.1 \text{ min}, \%B = 100, t = 9.2 \text{ min}, \%B = 15$, and a total run time of 15 min. Glucuronides of naphthol and propofol were analyzed using the following gradient: $t = 0 \text{ min}, \%B = 15, t = 3 \text{ min}, \%B = 100, t = 4 \text{ min}, \%B = 100, t = 4.1 \text{ min}, \%B = 15$, and a total run time of 11 min. Morphine glucuronides were resolved using the following gradient: $t = 0 \text{ min}, \%B = 10, t = 11 \text{ min}, \%B = 100, t = 12 \text{ min}, \%B = 100, t = 12.1 \text{ min}, \%B = 10$, and a total run time of 18 min. The flow rate for all HPLC methods was 0.25 ml/min. Metabolite formation was quantitated by comparing peak area ratios (metabolite/internal standard) in incubations with ratios obtained from a standard curve containing known amounts of glucuronide.

Chromatographic data were collected and analyzed using Analyst version 1.2 (Applied Biosystems/MDS Sciex). Samples were injected onto a 50-mm monochrome C18 HPLC column with a 2-mm internal diameter (Varian, Inc.). Mobile phase A consisted of 2 mM ammonium formate with 5% isopropyl alcohol, 95% water, 0.2% formic acid (v/v). Mobile phase B consisted of 2 mM ammonium formate with 95% methanol, 5% isopropyl alcohol, 0.2% formic acid (v/v). Following a fast gradient elution ($t = 0 \text{ min}, \%B = 10, t = 0.4 \text{ min}, \%B = 10, t = 2.0 \text{ min}, \%B = 95, t = 3.6 \text{ min}, \%B = 95, t = 3.7 \text{ min}, \%B = 10$, total run time = 5.0 min, flow rate = 0.2 ml/min), the analyte and internal standard were eluted and detected by MS/MS and quantitated using a seven-point standard curve.

**Data analysis.** Triplicate results for each preparation were averaged and activities expressed as picomoles of metabolite formed per minute per milliogram of glucuronide.

**TABLE 2**

Substrate selectivity of nine UDP-glucuronosyltransferases against four probe substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>UGT1A1</th>
<th>UGT1A3</th>
<th>UGT1A4</th>
<th>UGT1A6</th>
<th>UGT1A8</th>
<th>UGT1A9</th>
<th>UGT1A10</th>
<th>UGT2B7</th>
<th>UGT2B15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol-3</td>
<td>210 ± 76</td>
<td>66 ± 5.6</td>
<td>ND</td>
<td>ND</td>
<td>310 ± 14</td>
<td>ND</td>
<td>310 ± 7.8</td>
<td>2.3 ± 2.6</td>
<td>6.3 ± 1.3</td>
</tr>
<tr>
<td>Estradiol-17</td>
<td>ND</td>
<td>21 ± 1.0</td>
<td>44 ± 2.2</td>
<td>ND</td>
<td>13 ± 3.2</td>
<td>ND</td>
<td>6.3 ± 1.0</td>
<td>39 ± 4.6</td>
<td>ND</td>
</tr>
<tr>
<td>Naphthol</td>
<td>25 ± 25</td>
<td>ND</td>
<td>ND</td>
<td>3700 ± 1000</td>
<td>240 ± 80</td>
<td>87 ± 4.7</td>
<td>82 ± 21</td>
<td>150 ± 60</td>
<td>140 ± 56</td>
</tr>
<tr>
<td>Propofol</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>120 ± 11</td>
<td>1900 ± 67</td>
<td>21 ± 4.4</td>
<td>2.5 ± 2.8</td>
<td>2.5 ± 2.8</td>
</tr>
<tr>
<td>Morphine-3</td>
<td>ND</td>
<td>15 ± 4.3</td>
<td>ND</td>
<td>ND</td>
<td>7.8 ± 1.0</td>
<td>18 ± 1.3</td>
<td>4.8 ± 2.9</td>
<td>670 ± 200</td>
<td>19 ± 5.5</td>
</tr>
<tr>
<td>Morphine-6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>110 ± 40</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, below the level of detection.

**HPLC analysis** of 6β-hydroxytestosterone formation. Analysis of the CYP3A-mediated metabolism of testosterone to 6β-hydroxytestosterone by human hepatocytes was adapted from the method of Fayer et al. (2001). In all cases, chromatographic data were collected and analyzed using the Liggly global chromatography system (Eli Lilly & Co., Indianapolis, IN). The internal standard 11β-hydroxytestosterone was added before analysis. Samples were injected onto a Metachem Polaris C18A, 150 × 2 mm, 5 µm column (Varian, Inc., Palo Alto, CA) at 50°C. Mobile phase A consisted of acetonitrile/water/formic acid (100:900:0.5, v/v/v). Mobile phase B consisted of acetonitrile/water/formic acid (900:100:0.15, v/v/v). Following a gradient elution ($t = 0 \text{ min}, \%B = 15, t = 3.5 \text{ min}, \%B = 40, t = 3.6 \text{ min}, \%B = 100, t = 5 \text{ min}, \%B = 1000, t = 5.1 \text{ min}, \%B = 15$, total run time = 10 min, flow rate = 0.5 ml/min), the analyte and internal standard were detected by UV absorbance at 245 nm and quantitated using a 10-point standard curve.

**LCMS analysis** of resorufin formation from ethoxyresorufin. Detection of resorufin was conducted using a Perkin Elmer Sciex API 3000 tandem mass spectrometer (PerkinElmerSciex Instruments, Boston, MA) equipped with a TurboIonSpray source (Applied Biosystems/MDS Sciex, Foster City, CA) operating in the positive ion mode. The analyte, resorufin, was quantified using the transition m/z 214 → m/z 186, and the internal standard (metoprolol) was detected using the transition m/z 268 → m/z 133. Chromatographic data were collected and analyzed with Analyst version 1.2 (Applied Biosystems/MDS Sciex). Samples were injected onto a 50-mm monochrome C18 HPLC column with a 2-mm internal diameter (Varian, Inc.). Mobile phase A consisted of 2 mM ammonium formate with 5% isopropyl alcohol, 95% water, 0.2% formic acid (v/v). Mobile phase B consisted of 2 mM ammonium formate with 95% methanol, 5% isopropyl alcohol, 0.2% formic acid (v/v). Following a fast gradient elution ($t = 0 \text{ min}, \%B = 10, t = 0.4 \text{ min}, \%B = 10, t = 2.0 \text{ min}, \%B = 95, t = 3.6 \text{ min}, \%B = 95, t = 3.7 \text{ min}, \%B = 10$, total run time = 5.0 min, flow rate = 0.2 ml/min), the analyte and internal standard were eluted and detected by MS/MS and quantitated using a seven-point standard curve.

**Data analysis.** Triplicate results for each preparation were averaged and activities expressed as picomoles of metabolite formed per minute per milligram of glucuronide.

**Fig. 1.** The effects of omeprazole treatment on CYP1A2-mediated ethoxyresorufin de-ethylation (A) and rifampin treatment on CYP3A4-mediated testosterone 6β-hydroxylation (B) in 14 human hepatocyte preparations.

Human hepatocytes were treated for 72 h at 37°C under a 95% air/5% CO2 atmosphere with HMM5 containing either 50 µM omeprazole (A) or 10 µM rifampicin (B). Following an aspiration of medium, cells were incubated for 10 min with fresh HMM5 before incubating for 30 min with HMM5 containing either 2 µM ethoxyresorufin (A) or 100 µM testosterone (B). Analysis was performed by LCMS or HPLC as stated under Materials and Methods.
Results

Substrate Specificity of Probe UGT Substrates. Several recent reviews have suggested the need for a number of aglycones as probe substrates for particular UGT isoforms (Burchell et al., 1995, 2001; King et al., 2000). However, to date the majority of these substrates have not been characterized thoroughly in a single laboratory against the battery of recombinant human UGT isoforms currently available. The glucuronidation of estradiol (3- and 17-positions), naphthol, propofol, and morphine (3- and 6-positions) was investigated using recombinant cell lines expressing human UGT isoforms (1A1, 1A3, 1A4, 1A6, 1A8, 1A9, 1A10, 2B7, and 2B15). Estradiol-3-glucuronidation was primarily catalyzed by UGT1A8, UGT1A10, UGT1A1, and UGT1A3, although glucuronidation was also observed with UGT2B7 and UGT2B15, albeit at considerably slower rates (Table 2). In contrast, the glucuronidation of estradiol at the 17-position was catalyzed by UGT1A4, UGT2B7, and UGT1A3, whereas UGT1A8 and UGT1A10 had significantly lower activities (Table 2). The glucuronidation of naphthol was catalyzed by all the UGT isoforms except UGT1A3 and UGT1A4. However, it is important to note that UGT1A6-mediated glucuronidation (3700 pmol/min/mg) was over 15-fold greater than the activity observed with any of the other UGT isoforms (Table 2). Propofol glucuronidation was primarily catalyzed by UGT1A9 (1900 pmol/min/mg), although lower levels of activity were also detected with UGT1A8, UGT1A10, UGT2B7, and UGT2B15. UGT2B7 glucuronidated morphine extensively at the 3-position, whereas only low levels of activity were observed using UGT1A3, UGT1A8, UGT1A9, UGT1A10, and UGT2B15. Morphine-6-glucuronidation was catalyzed specifically by UGT2B7 with no activity observed using any of the other human UGT isoforms (Table 2). Although relatively low substrate concentrations have been used in this study (30 μM, except morphine which was 2 mM) in an effort to determine selectivity, a greater level of activity observed for one UGT isoform over another may also be related to differences in the expression level of the various UGTs in the recombinant systems.

Induction of CYP1A2 and CYP3A4 Using Human Hepatocytes. To ensure that each hepatocyte preparation was capable of responding to inducers, the induction of CYP1A2 by omeprazole and CYP3A4 by rifampicin was assessed using ethoxyresorufin de-ethylation and testosterone 6β-hydroxylation, respectively. A ≥2-fold induction of both CYP1A2 and CYP3A4 was required to classify a preparation as acceptable. Induction of CYP1A2 after treatment with omeprazole varied considerably between preparations with an 8-fold induction observed in HH1012, whereas a 241-fold induction was observed in HH1033 (Fig. 1). Induction of CYP3A4 by testosterone treatment was also variable, ranging from a 2-fold induction in preparation HH1019 to a 17-fold induction in HH993 (Fig. 1).

Induction of Estradiol Glucuronidation Using Human Hepatocytes. The induction of both estradiol-3- and -17-glucuronidation was investigated following 72 h of treatment with dexamethasone (10 μM), 3-MC (4 μM), phenobarbital (2 mM), rifampicin (10 μM), omeprazole (50 μM), α-naphthoflavone (80 μM), tamoxifen (10 μM), quercetin (25 μM), or tangeretin (25 μM). Treatment with dexamethasone, tamoxifen, and quercetin showed no effect on either estradiol-3- or -17-glucuronidation (Figs. 2 and 3). Interestingly, a

![Figure 2](https://example.com/figure2.png)
Fig. 3. The effect of nine treatments on estradiol-17-glucuronidation in five human hepatocyte preparations.

Human hepatocytes were treated for 72 h at 37°C under a 95% air/5% CO₂ atmosphere with HMM⁺ containing the indicated treatments. Following an aspiration of medium, cells were incubated for 10 min with fresh HMM⁺ before incubating for 30 min with HMM⁺ containing 30 μM estradiol. Analysis was performed by LC/MS as stated under Materials and Methods. *, statistically significant (α = 0.05). Control levels of estradiol-17-glucuronidation (pmol/min/mg of protein ± S.E.M.) for preparations HH993, HH994, HH1009, HH1012, and HH1013 were 12 ± 0.7, 381 ± 24, 178 ± 19, 47 ± 2, and 50 ± 3, respectively. PB, phenobarbital; alpha NP, α-napthoflavone.

Fig. 4. The effect of 10 treatments on naphthol glucuronidation in five human hepatocyte preparations.

Human hepatocytes were treated for 72 h at 37°C under a 95% air/5% CO₂ atmosphere with HMM⁺ containing the indicated treatments. Following an aspiration of medium, cells were incubated for 10 min with fresh HMM⁺ before incubating for 30 min with HMM⁺ containing 30 μM naphthol. Analysis was performed by LC/MS as stated under Materials and Methods. *, statistically significant (α = 0.05). Control levels of naphthol glucuronidation (pmol/min/mg of protein ± S.E.M.) for preparations HH1014, HH1019, HH1029, HH1031, and HH1033 were 10,988 ± 264, 12,447 ± 1004, 24,308 ± 1373, 14,044 ± 592, and 11,932 ± 1148, respectively. PB, phenobarbital; alpha NP, α-naphthoflavone.
statistically significant induction (up to 2.5-fold) of estradiol-3- and -17-glucuronidation was observed in four of the five preparations investigated following treatment with 3-MC. Treatment with phenobarbital also produced a statistically significant induction of estradiol-17-glucuronidation in three of the four preparations studied, whereas significant levels of induction of estradiol-3-glucuronidation were only observed in one of the preparations. Estradiol-17-glucuronidation was consistently induced following omeprazole treatment; however, the effects on estradiol-3-glucuronidation were more variable, leading to both increases and decreases in activity (Fig. 2). Rifampicin treatment also produced inconsistent effects on both estradiol-3- and -17-glucuronidation. (Figs. 2 and 3).

**Induction of Naphthol Glucuronidation Using Human Hepatocytes.** The induction of naphthol glucuronidation was investigated using the same nine treatments as for estradiol glucuronidation in addition to carbamazepine (100 μM). Most of the treatments studied did not consistently affect naphthol glucuronidation, with only one human hepatocyte preparation producing a significant induction for the majority of treatments (Fig. 4). In contrast, a small but statistically significant induction (up to 1.7-fold) was observed following treatment with carbamazepine in all four preparations investigated. Phenobarbital treatment also appeared to produce an induction in all five preparations studied; however, the increases in naphthol glucuronidation were only statistically significant in two cases. The remaining eight treatments resulted in inconsistent effects on naphthol glucuronidation in the human hepatocyte preparations.

**Induction of Propofol Glucuronidation Using Human Hepatocytes.** The induction of propofol glucuronidation was examined using the nine treatments used for estradiol glucuronidation. Phenobarbital treatment induced propofol glucuronidation in all five preparations investigated, although the increase was only statistically significant in three of the hepatocyte preparations (Fig. 5). Treatment with rifampicin and omeprazole also increased propofol glucuronidation; however, the effects were less consistent than those observed with phenobarbital. Interestingly, a statistically significant decrease in propofol activity (30% of control) was observed after treatment with α-naphthoflavone. Decreased propofol activity was also noted after treatment with 3-MC, tamoxifen, and tangeretin (Fig. 5).

**Induction of Morphine Glucuronidation Using Human Hepatocytes.** The induction of morphine glucuronidation was investigated using the 10 treatments used in studies with naphthol. Morphine-6-glucuronidation was not observed in any of the preparations or treatment conditions studied; however, morphine-3-glucuronidation was detected in all cases. A large interpreparation variability in response was observed for morphine-3-glucuronidation with all 10 treatments studied. That is, all treatments resulted in induction in hepatocyte preparation HH1031, whereas no increase in activity was observed with HH1029 after any treatment (Fig. 6). Statistically significant induction of morphine-3-glucuronidation (in at least three preparations) was observed after treatment with, phenobarbital, rifampicin, and carbamazepine.

**Discussion**

The apparent large degree of redundancy exhibited in the human UGT family with respect to overlapping substrate specificity has hindered the identification of form-selective substrates (Clarke and Burchell, 1994). The current study focused initially on the selectivity of nine UGTs for the formation of six different glucuronides. The goal was to identify substrate probes that were selective for at least a few of the human UGTs expressed in the liver.

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**Fig. 5.** The effect of nine treatments on propofol glucuronidation in five human hepatocyte preparations.

Human hepatocytes were treated for 72 h at 37°C under a 95% air/5% CO₂ atmosphere with HMM containing the indicated treatments. Following an aspiration of medium, cells were incubated for 10 min with fresh HMM before incubating for 30 min with HMM containing 30 μM propofol. Analysis was performed by LC/MS as stated under Materials and Methods. *, statistically significant (α = 0.05). Control levels of propofol glucuronidation (pmol/min/mg of protein ± S.E.M.) for preparations HH995, HH996, HH1002, HH1009, and HH1012 were 10,775 ± 684, 6826 ± 238, 7072 ± 473, 10,723 ± 514, and 6113 ± 204, respectively. PB, phenobarbital; alpha NP, α-naphthoflavone.
Several human UGTs including UGT1A8, UGT1A10, UGT1A1, and UGT1A3 significantly glucuronidated estradiol at the 3-position (Table 2). However, the role of UGT1A8 and UGT1A10 in the hepatic glucuronidation of estradiol would be negligible, since previous work has shown a lack of expression in the liver (Strassburg et al., 1997). Furthermore, Senafi et al. (1994) used microsomes from the livers of Crigler-Najjar patients, who are genetically devoid of UGT1A1, to demonstrate that the majority of estradiol-3-glucuronidation in human liver is catalyzed by UGT1A1. In contrast, estradiol-17-glucuronidation was catalyzed by several hepatic forms, UGT1A3, UGT1A4, and UGT2B7, to similar extents. Therefore, with no information regarding relative expression levels of these forms in the available expression systems, it is not possible to determine the individual role these forms play in the production of estradiol-17-glucuronide.

Naphthol glucuronidation has historically been used as a selective substrate for UGT1A6 in liver microsomes even though its glucuronidation has been shown to be catalyzed by a number of UGT isoforms (Burchell et al., 1995, 2001). This view is supported by the data in the current study (Table 2). Naphthol glucuronidation mediated by UGT1A6 was found to be over 24-fold greater than by any other UGT form known to be expressed in the liver. In addition to this extensive rate of glucuronidation, recent studies have illustrated that the UGT1A6-mediated glucuronidation of naphthol has a 4-fold greater affinity than that catalyzed by UGT2B7 (Soars et al., 2003a,b). Propofol glucuronidation was catalyzed by UGT1A9 in the current study at a rate over 90-fold greater than that observed by other hepatic UGT forms. Hence, propofol appears to be a suitable selective probe for hepatic UGT1A9 activity. Morphine glucuronidation has historically been used as a probe for UGT2B7 (Coffman et al., 1997; Burchell et al., 2001). Table 2 confirmed that the production of morphine-6-glucuronide was specific to UGT2B7 and that morphine-3-glucuronidation was also highly selective for this form (>35-fold more active than the next best form). Therefore, morphine glucuronidation to both the 3- and 6-glucuronides appear to be selectively catalyzed by hepatic UGT2B7. The reactions detailed above were then used to assess the potential effects of a range of treatments on individual UGT forms.

The 2.5-fold induction of estradiol-3-glucuronidation by 3-MC in this study (Fig. 2) agrees with that observed previously by Ritter et al. (1999). Other Ah receptor ligands such as omeprazole and α-naphthoflavone had a more variable response, ranging from a 2.5-fold increase in activity to a statistically significant decrease (Fig. 2). Historically, the Ah receptor has been hypothesized to be involved in the regulation of UGT1A1. The recent identification of a xenobiotic response element in the promoter of UGT1A1 that interacts directly with the Ah receptor complex has confirmed the involvement of this receptor system (Yueh et al., 2003). Despite this observation, the results reported here indicate that the induction of estradiol-3-glucuronidation in human hepatocytes is quite variable.

Treatment with phenobarbital also increased the appearance of estradiol-3-glucuronide, although the response was variable and not as large as that observed with 3-MC (Fig. 2). However, this increase may well be clinically significant as barbiturates such as phenobarbital have been shown to lower serum bilirubin levels in individuals with unconjugated hyperbilirubinemia (Yaffe et al., 1966). Indeed, the
identification of a phenobarbital response-enhancer module in the UGT1A1 gene by Sugatani et al. (2001) suggests that the nuclear receptor CAR may regulate this response. Thus, the current study using human hepatocytes supports the regulation of expression of UGT1A1 activity by both the Ah receptor and CAR systems, although the responses were generally small and variable.

In contrast to estradiol-3-glucuronidation, with the exception of carbamazepine treatment, very little induction by any treatment of naphthol glucuronidation was observed in the current study (Fig. 4). This is consistent with a high constitutive expression level of UGT1A6 in human liver as has been postulated for the lung (Munzel et al., 1998) and canine liver (Bock et al., 2002). However, the induction of naphthol glucuronidation by carbamazepine may explain the increased clearance of acetaminophen (a drug glucuronidated in part by UGT1A6) when patients were administered anticonvulsants such as carbamazepine (Prescott et al., 1981; Miners et al., 1984).

UGT1A6 has been shown to be inducible in both Caco-2 cells (Munzel et al., 1998; Bock et al., 2000) and rat liver (Lilienblum et al., 1982; Coughtrie et al., 1987), suggesting that the regulation of this form may be tissue-/species-dependent.

The increase in propofol glucuronidation after treatment with phenobarbital and rifampicin (Fig. 5) may suggest the involvement of CAR/PXR in the regulation of UGT1A9. A recent study by Barbier et al. (2003) has suggested that UGT1A9 may be a PPAR α and γ target gene; however, no PPAR ligands were used in the current study. Interestingly, a decrease in propofol glucuronidation was observed after treatment with 3-MC, α-naphthoflavone, tamoxifen, and tangerin, which suggests either a down-regulation of expression or inhibition of UGT1A9. Antibodies specific to UGT1A9 are currently not available, and thus a thorough investigation into potential mechanisms of the decrease in UGT1A9 activity was not possible.

It is difficult to draw definitive conclusions about the induction of UGT2B7 due to the large interindividual variability observed in response to treatments for morphine-3-glucuronidation (Fig. 6). Both genetic (polymorphisms have been identified in UGT2B7) and environmental factors (for example, diet and previous drug history) may influence the regulation of expression of the UGT2B7 gene; however, no PPAR ligands were used in the current study. Nevertheless, the induction of morphine-3-glucuronidation following rifampicin treatment may explain the decrease in the area under the curve of zidovudine, a drug primarily metabolized by UGT2B7, observed in patients taking this antituberculosis drug (Gallicano et al., 2001).

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