Contribution of the Different UDP-Glucuronosyltransferase (UGT) Isoforms to Buprenorphine and Norbuprenorphine Metabolism and Relationship with the Main UGT Polymorphisms in a Bank of Human Liver Microsomes

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ABSTRACT:
The goal of this study was to evaluate the specific contribution of individual UDP-glucuronosyltransferase (UGT) isoforms in the metabolism of buprenorphine (BUP) and norbuprenorphine (Nor-BUP), as well as the impact of their genetic variations. The glucuronidation of BUP and Nor-BUP was examined using human liver microsomes (HLMs) and heterologously expressed UGTs. The individual contribution of UGT isoforms was estimated using enzyme kinetic experiments combined with the relative activity factor (RAF). Phenotype-genotype relationships were investigated in a bank of 52 HLMs. Among the six hepatic UGT isoforms tested, UGT1A1, UGT1A3, and UGT2B7 metabolized BUP and Nor-BUP. Using the RAF approach, we found that UGT1A1 and UGT2B7 accounted for approximately 10 and 41% of BUP glucuronidation, respectively. Nor-BUP glucuronidation involved predominantly UGT1A3 (approximately 63%) and UGT1A1 (34%), whereas UGT2B7 had only a minor role. The UGT1A1 promoter (TA)_{6/7}TAA mutation (UGT1A1*28) resulted in a 28% decrease of BUP glucuronidation V_{max} in pooled HLMs but was not statistically associated with glucuronidation rate in 52 individual HLMs. The presence of the UGT2B7 promoter (G–842A) mutation resulted in higher BUP glucuronidation V_{max} in pooled HLMs (+80% on average) and in a significant higher glucuronidation rate in noncarriers (but not in carriers) of the UGT1A1*28 allele (P = 0.0352). This study represents a functional basis for further clinical pharmacogenetic studies.

Buprenorphine (BUP) is a semisynthetic derivative of the morphine alkaloid thebaine with partial agonist properties on opioid receptors. It is used at low doses (ranging from 0.3–0.6 mg) in the treatment of moderate to severe pain by intravenous, intramuscular, and sublingual routes and at high doses by the sublingual route (up to 16 mg/day, in combination with naloxone in some countries) in opioid replacement therapy. In this indication, dose adjustment is critical because excessive doses can lead to adverse events (usually nonserious, but which may result in poor compliance), and alternatively inadequate doses usually result in treatment relapse. Although sublingual BUP has very low and variable bioavailability and overall variable pharmacokinetics (Kuhlman et al., 1996), dose adjustment relies mainly on clinical monitoring. It is thought that genetic polymorphisms in drug-metabolizing enzymes might contribute to the variability in BUP efficacy. Beside blood or urinary levels, patients’ genotyping may help improve a priori dose selection or a posteriori adjustment based on anticipated individual metabolic capacity.

The oxidative metabolism of BUP has been extensively studied. Rapid metabolism of BUP through N-dealkylation in the liver produces norbuprenorphine (Nor-BUP), an active metabolite (Huang et al., 2001). This pathway is mainly catalyzed by the cytochromes P450 (P450) 3A4 and 2C8 (Iribarne et al., 1997; Kobayashi et al., 1998; Picard et al., 2005a). Subsequently, BUP and Nor-BUP undergo extensive phase II metabolism, catalyzed by UDP-glucuronosyltransferases (UGTs) (Cone et al., 1984). This second pathway is mainly catalyzed by the cytochromes P450 3A4 and 2C8, although other UGTs may also contribute (Belfroid et al., 2000; Kobayashi et al., 1998; Rios and Tephly, 2002). In addition, it was shown that BUP and Nor-BUP are effectively glucuronidated by UGT2B7 and UGT1A3, respectively (Green et al., 1998; Rios and Tephly, 2002; Chang and Moody, 2009), Nor-BUP being a better substrate for UGT1A3 than BUP.

Interindividual variability in BUP or Nor-BUP conjugation in the liver may influence BUP pharmacokinetics. Coffman et al. (1998)
found, using human embryonic kidney (HEK) 293-transfected cells, that the C to T substitution at nucleotide 802 [reference single nucleotide polymorphism (SNP) number, rs7439366] of UGT2B7 giving rise to enzymes with either histidine (H) or tyrosine (Y) at the amino acid 268 position alters the affinity for BUP. The consequence of this polymorphism on BUP hepatic glucuronidation remains to be investigated.

The insertion of a TA dinucleotide in the TATA-box of UGT1A1 promoter (referred as UGT1A1*28) is associated with the most common inherited cause of unconjugated hyperbilirubinemia, known as Gilbert syndrome. This polymorphism results in a 20 to 80% reduction in gene expression (Bosma et al., 1995; Monaghan et al., 1996).

Materials and Methods

Chemicals and Reagents. BUP, etoposide (ETO), mycophenolic acid (MPA), UDP-glucuronic acid (UDPGA), Triton X-100, and dimethyl sulfoxide were obtained from Sigma-Aldrich (St. Louis, MO). Nor-BUP and BUP-glucuronide were purchased from Cerilliant (Molsheim, France). MPA acyl-glucuronide (AcMPAG) was a gift from F. Hoffman-La Roche (Basel, Switzerland). 3′-Azido-3′-deoxythymidine (AZT; zidovudine) was a gift from BUP. The consequence of this polymorphism on BUP hepatic single nucleotide polymorphism (SNP) number, rs7439366, of cells, that the C to T substitution at nucleotide 802 [reference Reactions included denaturation at 93°C for 8 min, followed by 35 cycles at 95°C for 15 s, 60°C for 15 s, and 72°C for 1 min. Extension products were purified using a standard ethanol/EDTA/sodium acetate precipitation procedure, and the nucleotide sequences were determined on an ABI 3100 automatic sequencer (Applied Biosystems).

Assay with Microsomes and Recombinant UGT Enzymes. The typical incubation mixture (250 μl) contained 0.2 mg/ml microsomal proteins (HLMs or Supersomes), 1 mM UDPGA, 10 mM MgCl₂, 5 μM BUP or Nor-BUP, and 0.1 M Tris·HCl buffer, pH 7.4. Microsomes were first detergent-activated by incubation with Triton X-100 during 30 min on ice at an optimized detergent-to-microsomal protein ratio of 0.4 (w/w). BUP and Nor-BUP were then preincubated with microsomes at 37°C for 5 min, and the reaction was initiated by addition of UDPGA. After 20-min incubation at 37°C, the reaction was stopped by addition of 100 μl of ice-cold acetone/titrile. Samples were centrifuged and stored at −20°C until analysis. Control incubations without microsomes or without UDPGA were performed in parallel in each experiment.

Enzyme Kinetic Experiments. In preliminary experiments with pooled HLMs, the linearity of metabolite formation with increasing microsomal protein concentration (0.1–1 mg/ml) and incubation time (10–60 min) was checked. Kinetic experiments were then performed by incubating increasing concentration of BUP (0.5–75 μM) for 20 min with 0.2 mg/ml microsomes (total pool and pools of specific genotype). Kinetic data were model-fitted using the Hill equation, and kinetic parameters were calculated by nonlinear regression analysis using Winreg 3.1 (http://www.unilim.fr/pages_perso/jean.debord/winreg/winreg1.htm). Maximal clearances (Clmax) were estimated using eq. 1 (Houston and Kenworthy, 2000) where Vmax is the maximal velocity, S0 the substrate concentration resulting in 50% of Vmax, and n is the Hill coefficient:

Because Nor-BUP was only available as a 100-μg/ml solution in methanol (Cerilliant), we could not prepare solutions at high concentrations, and kinetic experiments could not be performed with this compound.

Liquid Chromatography/Tandem Mass Spectrometry Analysis. BUP and Nor-BUP metabolites were determined using liquid chromatography/tandem mass spectrometry (LC/MS/MS). Sample preparation consisted of adding 10 μl of internal standard (glafenin, 2 mg/l) to 90 μl of incubation supernatants. Samples (10 μl) were then injected into the chromatographic system that consisted of a PerkinElmer Life and Analytical Sciences (Waltham, MA) Series 200 autosampler equipped with a Rhodyne model 7725 injection valve (Phenomenex, Torrance, CA) with a 5-μl internal loop, an Agilent Technologies (Santa Clara, CA) HP1100 high-pressure gradient pumping system, and a Waters (Milford, MA) Atlantis T3, 5-μm (150 × 2.1-mm) column maintained at 25°C. A linear gradient of mobile phase A (0.1 % formic acid in water) and mobile phase B (0.1% formic acid in methanol) was delivered at a constant flow rate of 200 μl/min and programmed as follows: 0 to 1.5 min, 3% B; 1.5 to 2.5 min, 3% to 55% B; 2.5 to 3.5 min, 55% to 60% B; 3.5 to 4.5 min, 60% to 90% B; 4.5 to 6.5 min, 90% B; 6.5 to 7 min, decrease from 90% to 3%; 7 to 9 min, 3% B.

Detection was performed using an Applied Biosystems 4000 QTRAP LC/MS/MS system equipped with a Turbo V ionization source and controlled by Analyst 1.5 software. Positive ionization was performed with the following settings: ion spray voltage, 5500 V; curtain gas, 15; ion source gas flow rates 1 and 2 at 20 and 30 units, respectively; temperature set at 500°C.

Acquisitions were made in the multiple reaction monitoring mode using two transitions for BUP (m/z 468→55, m/z 468→83) and Nor-BUP (m/z 414→55, m/z 414→83), three for their respective glucuronides (BUP-glucuronide: m/z 644→468, m/z 644→55, m/z 644→83; Nor-BUP-glucuronide: m/z 590→414, m/z 590→55, m/z 590→83), and two for the internal standard glafenin (m/z 373→218, m/z 373→281). The limit of quantification was 5 μg/l for BUP, Nor-BUP, and BUP-glucuronide. Calibration curves obtained using quadratic regression from the limit of quantitation up to 5000 μg/l yielded r > 0.99. In the absence of pure Nor-BUP-glucuronide, the concentration of this metabolite was estimated as molar equivalent with respect to Nor-BUP calibration curves.

*UGT1A1 and UGT2B7 Relative Activity Factor Determination.* The relative activity factor (RAF) approach is proposed for scaling enzymatic activities obtained using cDNA-expressed enzymes to HLMs. RAFs are defined as the HLM/recombinant enzyme activity ratio of a particular isoform toward a probe substrate after experiments performed under identical conditions. The relative amount of specific substrate metabolization attributed to individual enzymes (contribution) is estimated by multiplying the rate of metabolism observed with this enzyme (V) by the corresponding RAF (eq. 2).

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\text{Contribution}_i = V_i \times \text{RAF}_i
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The RAF approach is well established for P450-dependant activity (Crespi and Miller, 1999). In this study, we calculated RAFAFs for UGT1A1 and UGT2B7 to scale velocities obtained using UGT Supersomes to HLMs. Based on incubation with a panel of UGT Supersomes, we first confirmed that, in our incubation conditions, ETO and AZT were selective substrates for UGT1A1 and UGT2B7, respectively (data not shown). MPA was used as a second probe substrate for UGT2B7 because this isoform catalyzes the production of AcMPAG in a specific manner (Picard et al., 2005b).

In the case of UGT1A3, the RAF could not be calculated as no adequate probe was identified. Incubations were performed as follows: HLMs (0.5 mg/ml) and recombinant UGT (0.25 mg/ml) were activated by preincubation with Triton X-100 as described above. Microsomes were then incubated with ETO (25 µM) and AZT (25 µM) at 37°C in 0.1 M Tris-HCl buffer, pH 7.4, containing 10 mM MgCl₂ and 2 mM UDPGA in a total volume of 125 µl. Incubations were carried out for 60 min and terminated by addition of 100 µl of ice-cold methanol containing glafenin (2.7 µM) as an internal standard. Samples were centrifuged at 10,000 g for 5 min at 4°C to pellet the precipitated protein. ETO and AZT glucuronides were determined using LC/MS/MS. The chromatographic and detection systems were similar to those described for BUP and Nor-BUP metabolite determinations. A linear gradient of mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in methanol) was delivered at a constant flow rate of 200 µl/min and programmed as follows: 0 to 1 min, 3% B; 1 to 2 min, 3 to 20% B; 2 to 4 min, 20 to 40% B; 4 to 5 min, 40 to 70% B; 5 to 10 min, 70 to 90% B; 10 to 12 min, 90% B; 12 to 13 min, decrease from 90% to 13 to 17 min, 3% B. The electrospray ionization source was operated in positive mode with the following settings: ion spray voltage, 5500 V; curtain gas, 20; ion source gas flow rates 1 and 2 at 15 and 35 units, respectively; and temperature set at 500°C. MS/MS detection was carried out in the multiple reaction monitoring mode, following transitions for ETO (m/z 256→110, m/z 268→127), three for the glucuronides of ETO (m/z 766→185, m/z 782→185, m/z 782→229) and of AZT (m/z 444→110, m/z 444→127, m/z 444→268), and two for the internal standard glafenin (m/z 373→218, m/z 373→281). For AZT-glucuronide quantification, a standard curve from 5 to 500 µg/l was constructed. Because ETO-glucuronide was not commercially available, its concentration was estimated as molar equivalent with respect to AcMPAG in a specific manner (Picard et al., 2005b).

RAF values were calculated by dividing the mean activity of glucuronidation formation by that obtained in Super- somes (both expressed in picomoles per milligram of protein per minute). Contributions of UGT1A1 and UGT2B7 were calculated using eq. 2.

**Statistical Analysis.** The distributions of UGT1A1 and UGT2B7 alleles were compared with the Hardy-Weinberg theoretical distribution using the Fisher’s exact test. All the activities were measured at least in duplicate and averaged. For both genes, because of the limited number of samples, data obtained with microsomes of the homozygous variant and heterozygous genotypes were grouped for statistical analyses. Microsomal velocity rates between genotype groups were then compared using analysis of variance in the StatView program (version 5.0, SAS Institute, Cary, NC) with a level of significance set at 0.05. The effect of the UGT2B7 polymorphism was also investigated after stratification of the data by the UGT1A1 genotype (i.e., carriers and noncarriers of the UGT1A1*28 allele). In this case, the nonparametric Mann-Whitney test was used with P < 0.05 considered statistically significant.

**Results**

**UGT Isoforms Involved in BUP and Nor-BUP Glucuronidation.** As shown in Fig. 1, incubation of BUP or Nor-BUP with recombinant UGTs (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, and UGT2B7) revealed that only UGT1A1, UGT1A3, and UGT2B7 were able to produce BUP and Nor-BUP glucuronides in detectable amounts. However, their respective activities regarding BUP and Nor-BUP were different. Recombinant UGT1A1 metabolized BUP with the highest efficiency, whereas UGT1A3 was the most efficient for Nor-BUP. The activity of recombinant UGT2B7 toward Nor-BUP was very low compared with that of UGT1A1 and UGT1A3 (Fig. 1). In contrast, UGT2B7 showed the second highest activity in BUP metabolism.

**Kinetics of BUP-Glucuronidation Production by UGT1A1, UGT1A3, and UGT2B7.** The kinetic constants of BUP glucuronidation were calculated using the three competent recombinant enzymes. Sigmoidal kinetics was observed with the three UGTs, as well as with HLMs. As shown in Table 1, the affinity of UGT2B7 was 2- and 3.4-fold higher than that of UGT1A3 and UGT1A1, respectively. In contrast, the maximal velocity (Vmax) of BUP-glucuronidation production was much higher for UGT1A1 than for UGT1A3 and UGT2B7 (Table 1).

The velocity rates obtained with recombinant UGT1A1 and UGT2B7 were scaled to HLMs using the RAF calculated from the UGT1A1-mediated glucuronidation of ETO (RAF = 0.6) and the averaged RAF (= 0.68) obtained from the UGT2B7-mediated glucuronidation of MPA (RAF = 4.5) and AZT (RAF = 9.0). As can be seen in Table 1, the scaled Clmax of UGT2B7 was 4.1-fold higher than that of UGT1A1, each representing 41 and 10% of the Clmax of HLMs, respectively. This left approximately 49% of BUP-glucuronide production in HLM not attributable to UGT1A1 and UGT2B7.

Standard kinetic experiments could not be performed for Nor-BUP because no powder formulation of Nor-BUP was commercially available, preventing us from preparing solutions at high enough concentrations. As a surrogate approach, the velocity of Nor-BUP glucuronidation obtained with recombinant enzymes and HLMs at a low single concentration of substrate (5 µM) was compared. The scaled velocity of Nor-BUP glucuronidation obtained with UGT1A1 (17.4 ± 1.2 pmol/mg protein/min) represented 34% of the velocity obtained in HLMs (51.8 ± 1.8 pmol/mg protein/min). UGT2B7 activity was barely detectable (i.e., the metabolic rate was lower than the limit of quantitation of the assay). The activity observed for UGT1A3 (32.5 ±
Influence of UGT1A*28 and UGT2B7 G–842A Genotypes on BUP Glucuronidation in HLMs. Genotype distribution among the banked microsomes. UGT1A1 and UGT2B7 genotype distributions were in Hardy-Weinberg equilibrium and similar to those reported previously (Iyer et al., 1999; Djebli et al., 2007). UGT1A1*28 allelic frequency was 0.37, with 21 microsomal preparations (40%) carrying the TA3/TA3 (wild-type) genotype, 24 (46%) the heterozygous genotype, and 7 (13%) the TA3/TA7 (variant) genotype. UGT2B7 G–842A polymorphism frequency was 0.53, with 11 (21%), 26 (50%), and 15 (29%) preparations of the wild-type (GG-842), heterozygous, and variant genotype, respectively.

BUP glucuronidation by genotyped microsomes. BUP was incubated with each of the 52 microsomal preparations at a single concentration of 5 μM. Velocities varied up to 3.4-fold (mean ± S.D., 2.1 ± 0.6-fold). Using two-way analysis of variance, a trend was found for UGT2B7 G–842A (P = 0.0722), whereas no significant difference in BUP glucuronidation was found for UGT1A1 polymorphism (P = 0.3214). However, as there was an interaction between the two SNPs (P = 0.0199), we investigated the effect of the UGT2B7 polymorphism within each UGT1A1 genotypic group, showing a significant effect of UGT2B7 on BUP glucuronidation in the UGT1A1 homozygous wild-type group (P = 0.0352) and none in UGT1A1*28 allele carriers (P = 0.4532) (Fig. 2).

Kinetics of BUP glucuronidation by pools of genotyped HLMs. Three microsome pools were constituted using the following genotype: GG–842 (n = 5), G–842A (n = 10), and −842AA (n = 6). The microsomes used in these three pools were selected to be non-carriers of the UGT1A1*28 allele. Another pool was prepared from HLM homozygous for the UGT1A1*28 allele (n = 7), comprising one, four, and two preparations of the UGT2B7 wild-type, heterozygous, and variant genotype, respectively. Thus, the distributions of the UGT2B7 variant allele in the UGT1A1*1 wild-type and mutated groups were similar (allelic frequency = 0.52 versus 0.57; Fisher exact P > 0.999).

BUP glucuronidation kinetic parameters were estimated in each pool (Table 2; Fig. 3). All the preparations displayed comparable S50 values, similar to that obtained with the pool of 52 HLMs. The Vmax obtained with the pool of mutated microsomes for UGT1A1 was 1.4-fold lower than the average Vmax in wild-type preparations. This resulted in a 1.3-fold lower Clmax.

The effect of the UGT2B7 G–842A polymorphism was assessed with a pool of microsomes wild-type for UGT1A1. The pool of heterozygous microsomes for UGT2B7 G–842A showed approximately the same Vmax as the homozygous mutated one, both being
approximately 1.7-fold higher than that of the homozygous wild-type microsomes.

**Discussion**

We performed experiments with human recombinant enzymes and confirmed that among the six hepatic UGT tested only UGT1A1, UGT2B7, and UGT1A3 were involved in BUP and Nor-BUP metabolism. Kinetic experiments showed that their respective contribution was, in decreasing order: UGT1A3 > UGT2B7 > UGT1A1 for BUP and UGT1A3 > UGT1A1 > UGT2B7 for Nor-BUP. The \( \text{UGT1A1} \) promoter polymorphism \((\text{TAA}_n \text{TAA}) (\text{UGT1A1}*28)\) had a moderate effect on BUP glucuronidation \( V_{\text{max}} \) in pooled HLMs, but it was not statistically associated with BUP glucuronidation in 52 individual HLMs. The presence of the \( \text{UGT2B7} \) promoter \((\text{G–842A})\) mutant allele resulted in higher \( V_{\text{max}} \) in pooled HLMs and significantly higher BUP glucuronidation in individual microsome noncarriers (but not in carriers) of the \( \text{UGT1A1}*28 \) allele.

Although UGT1A1, UGT1A3, and UGT2B7 were already highlighted as competent isoforms for BUP (King et al., 1996, 1997; Coffman et al., 1997, 1998; Cheng et al., 1998; Green et al., 1998; Rios and Tephy, 2002; Soars et al., 2003) and Nor-BUP glucuronidation (King et al., 1997), their specific contributions were unclear. Simultaneously investigating the metabolism of BUP and its primary phase I metabolite Nor-BUP allowed us to compare the UGT activity toward the two compounds. The same isoforms metabolized BUP and Nor-BUP but with different relative activities, consistent with results recently published by Chang and Moody (2009) showing that UGT1A1 and UGT1A3 are preferentially involved in Nor-BUP metabolism. We found here that UGT1A3 and UGT2B7 are predominantly involved for BUP, whereas UGT1A1 only plays a minor role. Concerning UGT1A3 more specifically, we found, as already reported by Green et al. (1998), that it had a higher activity for Nor-BUP than for BUP.

The major limitation of recombinant enzymes or transfected cells in drug metabolism studies is that extrapolation of the results to humans requires taking into account specific factors such as differences in membrane compositions between expression models and hepatocytes, the absence of competing enzymes, and above all the relative expression level of enzyme isoforms in the liver. To overcome this limitation, an extrapolation method has been proposed for P450 enzymes (Crespi and Miller, 1999, Venkatakrishnan et al., 2000), consisting of RAFs, which allow extrapolation of recombinant enzyme formation rates to native human liver enzyme activity. Toide et al. (2004) previously proposed an RAF determination method for UGT enzymes. Because UGTs exhibit overlapping substrate affinities and probe substrates had not been identified for each UGT, the authors calculated RAF using the activity of different recombinant UGTs toward selective and nonselective substrates using a system of equations.

In this study, we used the RAF approach proposed for P450 by Crespi and Miller (1999) to quantitatively evaluate the role of UGT1A1 and UGT2B7 in BUP and Nor-BUP metabolism. RAFs were calculated from the metabolic rates obtained by incubating ETO, AZT, and MPA with recombinant enzymes and with a large pool of HLM preparations. The choice of these substrates was supported by the literature (Barbier et al., 2000; Watanabe et al., 2003; Picard et al., 2005b) and experiments performed herein showing that ETO and AZT were specific of UGT1A1 and UGT2B7, respectively. RAF values obtained from UGT2B7-mediated glucuronidation of AZT (RAF = 9.0) and MPA (RAF = 4.5) showed that the activity of heterologously expressed UGT2B7 was lower than that of native hepatic UGT2B7. In contrast, the RAF value obtained from UGT1A1-mediated glucuronidation of ETO (RAF = 0.6) was less than unity, showing that recombinant UGT1A1 has a much higher activity than native hepatic UGT1A1, which is in accordance with the RAF value of 0.31 found by Toide et al. (2004) using UGT1A1 Supersomes and bilirubin as a probe substrate.

Using the RAF approach, we found that UGT1A1 accounted for approximately 10 and 30% of BUP and Nor-BUP glucuronidation, respectively, whereas UGT2B7 would be responsible for at least 40% of BUP glucuronidation, with a very limited role in Nor-BUP glucuronidation. Because of the absence of RAF for UGT1A3, we can only hypothesize that this isozyme would account for the remaining part of the metabolism of BUP (approximately 50%) and Nor-BUP (approximately 65%) in HLMs.

We cannot exclude that other isoforms can contribute to BUP metabolism. Chang and Moody (2009) recently showed that UGT2B17 is a competent isozyme in BUP metabolism. This isoform glucuronidates a large number of xenobiotics (Beaulieu et al., 1996; Turgeon et al., 2003; Lazarus et al., 2005), and its expression was found in various tissues, including liver, kidney, placenta, uterus, small intestine, and colon (Beaulieu et al., 1996; Ohno and Nakajin, 2009). However, the expression level of UGT2B17 in the liver is approximately 5% that of UGT2B7 (Ohno and Nakajin, 2009), suggesting a minor role in BUP hepatic metabolism.

Altogether, our results allow us to narrow down the investigation of candidate genetic polymorphisms with potential consequences on BUP metabolism. The UGT2B7 promoter \( G–842A \) SNP is a frequent polymorphism, described as functional. It is strongly linked to other promoter variants, including the A–1248G, A–268G, and T–102C SNPs, and is inversely linked to the C802T exonic SNP (Duguay et al., 2004; Djebli et al., 2007) known to alter UGT2B7 affinity for BUP (Coffman et al., 1998). As described in the introduction section, the \( \text{UGT1A1}*28 \) promoter polymorphism was also described to contribute to variability in UGT1A1 expression level and activity (Beutler et al., 1998; Lampe et al., 1999; Peterkin et al., 2007). However, its effect on BUP metabolism has not been reported to date. Kinetic experiments performed with pools of genotyped HLMs revealed that the presence of at least one mutated allele in \( \text{UGT2B7} \) increased the \( V_{\text{max}} \) 1.7-fold compared with homozygous wild-type microsomes, suggesting that the level of gene expression is modified by the \( G–842A \) regulatory SNP. These results are identical to those of Djebli et al. (2007) for MPA acyl-glucuronidation. The effect of \( \text{UGT2B7}*2 \) \((C802T)\) was already studied on opioids and other substrates using HEK293-transfected cells; Coffman et al. (1998) showed that the affinity of the variant protein UGT2B7 268H for BUP and morphine was altered. The present results suggest that the effect of the promoter SNP (in complete inverse linkage with \( \text{UGT1A1} \) promoter SNP) on UGT2B7 expression may be altered, as \( V_{\text{max}} \) was increased whereas affinity was not altered. Such an effect on enzyme expression could not be detected using HEK293-transfected cells.

For UGT1A1, the pool of microsomes carrying the \( \text{UGT1A1}*28 \) allele showed a modest decrease in \( V_{\text{max}} \), in agreement with most previous studies on bilirubin or SN-38 (the active metabolite of irinotecan) glucuronidation (Bosma et al., 1995; Monaghan et al., 1996; Iyer et al., 1999) and consistent with the modest implication of UGT1A1 in BUP glucuronidation found here (10% based on the RAF approach). To assess whether these changes in UGT1A1 and UGT2B7 velocity would translate into different BUP liver glucuronidation rates, BUP was incubated with 52 individual genotyped HLMs. The \( \text{UGT1A1}*28 \) allele was not associated with a significant difference in BUP glucuronidation, suggesting that Gilbert syndrome would only have a limited effect on BUP liver glucuronidation. It was previously shown that BUP glucuronidation was drastically decreased in microsomes prepared from Criqler-Najjar type I patients’ liver samples \((n = 2)\) compared with other patients’ liver microsomes \((n = 3)\) (King...
et al., 1996). However, the Crigler-Najjar syndrome which also concerns UGT1A1 is more severe (but much rarer) than the Gilbert syndrome as it results in a near (type I) or complete (type II) loss of enzyme activity.

In these 52 microsomes, the UGT2B7 G−842A polymorphism was associated with a trend to higher glucuronidation rates of BUP, an effect significant in noncarriers but not in carriers of the UGT1A1*28. We have no clear explanation for this apparent difference in UGT2B7 effect depending on the UGT1A1 status. It may only indicate that the effect of the UGT2B7 G−842A SNP is relatively modest with respect to the overall variability in UGT activity.

In summary, we have qualitatively and quantitatively evaluated the contribution of UGT hepatic isoforms in the metabolism of BUP and Nor-BUP and investigated the effect of their polymorphisms. We suggest that polymorphisms in UGT1A1 and UGT2B7 might not notably influence the disposition of BUP in humans. Because phase I metabolism is known to modulate the extent of phase II metabolism, it would be interesting to evaluate the simultaneous effect of P450 and UGT polymorphisms in BUP metabolism. For that, an integrated model such as genotyped HLMs and experimental conditions involving simultaneous incubation with phase I and phase II substrates such as that recently described by Kilford et al. (2009) might be particularly suited.

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References


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