Correlation between the UDP-Glucuronosyltransferase (UGT1A1) TATAA Box Polymorphism and Carcinogen Detoxification Phenotype: Significantly Decreased Glucuronidating Activity against Benzo(a)pyrene-7,8-dihydriodil(−) in Liver Microsomes from Subjects with the UGT1A1*28 Variant

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Abstract

Of the hepatic UDP-glucuronosyltransferases (UGTs), only UGT1A1 and UGT1A9 exhibit activity against benzo(a)pyrene-trans-7R,8R-dihydriodil [BPD(−)], precursor to the highly mutagenic anti-(+)-benzo(a)pyrene-7R,8S-dihydriodil-9S,10R-epoxide. The UGT1A1*28 allelic variant contains an additional (TA) dinucleotide repeat in the “TATAA” box [(TA)6> (TA)8] of the UGT1A1 promoter that has been linked to decreased expression of the UGT1A1 gene and decreased bilirubin conjugation, leading to the relatively nondebilitating condition known as Gilbert’s syndrome. To determine whether the UGT1A1 TATAA box polymorphism may play a role in the overall glucuronidation of BPD(−) in humans, we compared UGT1A1 TATAA box genotype with BPD(−) glucuronidating activity in normal liver microsomes. Significant decreases in UGT1A1 protein (P < 0.005) and bilirubin conjugation activity (P < 0.001) were observed in liver microsomes from subjects homozygous for the UGT1A1*28 allelic variant compared with subjects homozygous for the wild-type UGT1A1*1 allele. Significant decreases in BPD(−) glucuronidation activity (P < 0.02) were observed in subjects with the UGT1A1[*28*/28] genotype compared with subjects having the wild-type UGT1A1[*1*/1] genotype in assays of liver microsomes that included 0.1 mM α-naphthylamine, a competitive inhibitor of UGT1A9 and not UGT1A1. Similar phenotype:genotype correlations were observed when we compared subjects with the UGT1A1[*28*/28] genotype with subjects having the UGT1A1[*1*/28] genotype. In assays with α-naphthylamine, the Kₘ of liver microsomes against BPD(−) was similar to that reported for UGT1A1-overexpressing baculosomes (319 μM versus 290 μM; Fang et al., Cancer Res., 62: 1978–1986, 2002). These data suggest that the UGT1A1 TATAA box polymorphism plays a role in an individual’s overall ability to detoxify benzo(a)pyrene and in cancer risk.

Introduction

The UDP-glucuronosyltransferase (UGT) superfamily of enzymes catalyze the glucuronidation of various compounds, including endogenous compounds such as bilirubin and steroid hormones, as well as xenobiotics, including drugs and environmental carcinogens (1–4). On the basis of structural as well as sequence homology, UGTs are classified into several families and subfamilies, each containing several highly homologous UGT genes (5). The entire UGT1A family is derived from a single locus in chromosome 2 coding for nine functional proteins that differ only in their amino terminus as a result of alternate splicing of independent exon 1 regions to a shared carboxy terminus encoded by exons 2–5 (6). In contrast to the UGT1A family, the UGT2B family is composed of several independent genes, all located on chromosome 4 (7–11).

In addition to being the major enzyme involved in the metabolism and detoxification of bilirubin (12), UGT1A1 is one of several UGTs that glucuronidate metabolites of tobacco carcinogens, including benzo(a)pyrene (BaP). Of the hepatic UGTs, only UGT1A1 and UGT1A9 exhibit significant activity against benzo(a)pyrene-trans-7R,8R-dihydriodil [BPD(−)] (13), precursor to the highly mutagenic anti-(+)-BaP-7R,8S-dihydriodil-9S,10R-epoxide. UGTs 1A7, 1A8, and 1A10 also exhibit glucuronidating activity against BPD(−) (13), but these are extrahaepatic enzymes located primarily in the alimentary tract (14–18).

The UGT1A1*28 allelic variant contains an additional (TA) dinucleotide repeat in the “TATAA” box [(TA)6> (TA)8] of the UGT1A1 promoter that has been linked to decreased expression of the UGT1A1 gene (19, 20) and decreased bilirubin conjugation (19, 21, 22), leading to a nondebilitating condition known as Gilbert’s syndrome (19, 21–23). To determine whether the UGT1A1 TATAA box polymorphism potentially

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plays a role in differential glucuronidation and detoxification of carcinogens such as BPD(−), subjects were screened comparing UGT1A1 TATAA box genotype with normal liver UGT1A1 expression and BPD(−) glucuronidating activity in normal liver microsomes. Significant correlations between UGT1A1 genotype and both UGT1A1 expression and BPD(−) glucuronidating activity were demonstrated, implicating this polymorphism as a potentially important risk factor for BaP-induced carcinogenesis.

Materials and Methods

Chemicals and Materials. BPD(−) was obtained from the National Cancer Institute Chemical Carcinogen Repository (synthesized and characterized at Midwest Research Institute, Kansas City, MO), dissolved in DMSO, and stored protected from light at −70°C. Bilirubin was purchased from Fluka Chemicals (Ronkonkoma, NY). UDP-glucuronic acid (UDPGA; D.L-2-lysocephatidyl choline palmital C16:0; α-naphthylamine; Escherichia coli β-glucuronidase; and monoclonal anti-β-actin antibody were purchased from Sigma (St. Louis, MO). 14C-labeled UDPGA (specific activity, 326 Ci/mol), [γ-32P]-ATP (specific activity, 3000 Ci/mmol), and [α-35S]-dATP (specific activity, 1250 Ci/mmol) were obtained from NEN Life Scientific Products (Boston, MA). Taq DNA polymerase was purchased from Boehringer Mannheim (Indianapolis, IN), a 10-bp DNA ladder was purchased from Invitrogen (Carlsbad, CA), the QIAEX II gel extraction kit was purchased from Qiagen (Valencia, CA), and the UGT1A1 Western blotting kit and baculosomes overexpressing UGT1A1 or UGT1A9 were purchased from Gentest (Woburn, MA). HPLC-grade solvents were provided by various suppliers and used after further purification. All other chemicals were of analytical grade and used without further purification.

Tissues. Genomic DNA was extracted from adjacent pathologically confirmed normal liver specimens from 95 Caucasian individuals undergoing surgery for excision of hepatocellular carcinoma at the H. Lee Moffitt Cancer Center. All DNA specimens were provided by the Tissue Procurement Facility at the H. Lee Moffitt Cancer Center, and demographic data were provided for all subjects. Normal liver specimens from 60 of these 95 normal human liver samples were obtained from the same facility and were confirmed to be histologically normal by the H. Lee Moffitt Pathology Department. Tissue microsomes were prepared from the 60 normal liver specimens through differential centrifugation as described previously (24) and were stored (10–20 mg protein/ml) at −70°C in 100-µl aliquots, with total protein concentrations measured with the BCA assay (Pierce Corporation, Rockford, IL).

All protocols involving the analysis of tissue specimens were approved by the Institutional Review Board at the University of South Florida and were in accordance with assurances filed with and approved by the United States Department of Health and Human Services. Assurances were given by the Tissue Procurement Facility at the H. Lee Moffitt Cancer Center that all samples were isolated and quick-frozen at −70°C within 2 h post surgery.

UGT1A1 PCR Amplifications and Genotyping Analysis. The region spanning the UGT1A1 TATAA box promoter element was PCR-amplified (PCR product, 98–100 bp) as described previously (25) with sense (1A1S, 5'-GTACGTTGA-CACAGTCAAC-3') and antisense (1A1AS, 5'-TTTGGCTCC-TGCCAGAGGT-3') primers corresponding to nucleotides −104 through −85 and −25 through −7, respectively, relative to the translation start site in UGT1A1 exon 1 (GenBank accession no. AF297093). PCR amplifications were routinely performed in a 50-µl reaction volume containing 150 ng of purified genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, 0.2 mM each of deoxynucleotide triphosphate, 20 pmol of both sense and antisense UGT1A1 primers, 5 µCi [α-32P]-dATP, and 2.5 units of Taq DNA polymerase. The reaction mixture underwent the following protocol: a GeneAmp 9700 thermocycler (Perkin-Elmer Corp., Foster City, CA): 1 cycle of 94°C for 5 min; 41 cycles of 94°C for 40 s, 61°C for 40 s, and 72°C for 30 s; followed by a final cycle of 10 min at 72°C. Four µl of the final PCR product were mixed with in 4 µl of 95% formamide-20 mM EDTA (pH 8.0)-0.05% bromphenol blue-0.05% xylene cyanol FF and denatured by heating at 95°C for 5 min before loading on a 6% denaturing polyacrylamide gel. Samples were subjected to electrophoresis at 50 V/cm for 4 h; gels were dried and subsequently exposed for autoradiography for 1 week. Bands of either 98 or 100 bp, representing the six or seven (TA) repeats of UGT1A1*1 or UGT1A1*28, respectively, were visualized, and their size were determined by comparison with a 5'-end 32P-labeled 10-bp DNA ladder.

Representative samples exhibiting each of the UGT1A1 TATAA box genotype patterns observed by electrophoresis were examined by dideoxy sequencing, with PCR products purified after electrophoresis in 2% agarose using the QIAEX II gel extraction kit and sequenced at the DNA Sequencing Facility at the Department of Genetics at the University of Pennsylvania Medical Center using the same sense and antisense primers as were used for PCR amplification. Because the prevalences of non-UGT1A1*28 variant alleles are low in the population (26, 27), all UGT1A1 genotyping for the Gilbert’s syndrome polymorphism that exhibited a pattern consistent with the wild-type A(TA)6 TAA promoter region were considered to be UGT1A1*1.

Analysis of UGT1A1 Protein Levels. UGT1A1 protein levels were determined with the UGT1A1 Western blotting kit provided by Gentest, whereas β-actin protein levels were assayed with the monoclonal anti-β-actin antibody provided by Sigma. Western blots were performed with 20 µg of liver microsomal protein by standard procedures (28). UGT1A1 levels were quantitated against a known amount of human UGT1A1 protein (10 ng; supplied in the Western blotting kit provided by Gentest) by densitometric analysis of Western blots, with quantitation made relative to the levels of β-actin observed in each lane (also quantitated by densitometric analysis of Western blots).

Analysis of Bilirubin Glucuronidating Activity. Bilirubin glucuronidation was assayed in a 100-µl reaction volume using 0.25 mg of liver microsomes incubated for 1 h at 37°C in 0.7 mM bilirubin, 4 mM UDPGA, 10 mM MgCl2, 50 mM sodium citrate (pH 7.4), and α,β-2-lysocephatidyl choline palmital C16:0 (10 µg/100 µg of protein). Where indicated, α-naphthylamine (0.01–1 mM) was added to individual reactions. Reactions were terminated by the addition of an equal volume (100 µl) of 2% ascorbic acid in ethanol. Precipitates were removed by centrifugation (5 min at 14,000 × g), and supernatants were filtered (0.22 µm) and analyzed for bilirubin glucuronides by high-performance liquid chromatography (HPLC) using a Waters Associates binary pump (model 1525) HPLC system equipped with a dual λ absorbance detector operated at 450 nm, an automatic injector (model WISP 710B) and a β-RAM radioactive flow detector (IN/US, Tampa, FL). Samples were injected onto a Waters µBondapak (3.9 × 300
mm) 5 μ C18 column. HPLC separations were performed with the following linear gradient conditions: 0–20 min, 10–90% solvent A (0.01% trifluoroacetic acid in acetoneitrile); 20–25 min, 90–100% A, where solvent A was diluted in solvent B (0.01% trifluoroacetic acid in water). The HPLC flow rate was 1 ml/min, whereas the scintillation fluid flow rate was 4 ml/min. The column was routinely washed with 100% A for at least 20 min. Absorbance (450 nm) and 14C-labeled peaks corresponding to glucuronide conjugates of bilirubin were tentatively identified by relative retention times, and then confirmed by sensitivity to E. coli β-glucuronidase treatment (1000 units; overnight incubation at 37°C), using HPLC as described above.

**Analysis of BPD(−) Glucuronidation Activity.** BPD(−) glucuronidation assays were performed essentially as described previously (13), with liver microsomes (0.25 mg of protein) or baculosomes (0.2 mg of protein) incubated with 1 mM BPD(−) and 4 mM UDPGA in 10 mM MgCl2, 50 mM Tris-HCl (pH 7.4), and Na2-2-lysophosphatidyl choline palmital C16:0 (10 μg/100 μg of protein) in a total reaction volume of 100 μl for 1 h at 37°C. Where indicated, α-naphthylamine (0.05–0.5 mM) was added to individual reactions. All reactions were initiated by the addition of UDPGA and terminated by the addition of an equal volume of ice-cold acetoniitrile. Precipitates were removed by centrifugation (5 min at 14,000 × g), and supernatants were filtered (0.22 μm) and analyzed for glucuronidated BPD(−) isomers by HPLC as described previously (13). For reactions including α-naphthylamine, HPLC was performed with the following linear gradient conditions: 0–40 min, 20% solvent C (100% acetonitrite); 40–60 min, 20–30% C; 60–65 min, 30% C, where solvent C was diluted in solvent D (20 mM ammonium acetate, pH 6.5).

Kinetic analysis in liver microsomes was performed as described above using 0.05–1 mM BPD(−) and 0.1 mM α-naphthylamine at a maximal incubation time (1 h) when the rate of BPD(−) glucuronide formation was still linear. The Kₘ values for the glucuronidation of BPD(−) by human liver microsomes were calculated after linear regression analysis of Lineweaver–Burk plots.

Glucuronidation activities against both BPD(−) and bilirubin were calculated based on radioflow detection and quantification of dpm within glucuronidated BPD(−) or bilirubin-specific HPLC peaks as determined with the IN/US radioactivity detection program.

**Statistical Analyses.** The two-tailed Student’s t test was used to compare UGT1A1 protein levels as well as bilirubin and BPD(−) glucuronidating activities in subjects with different UGT1A1 genotypes.

**Results**

To screen whether the UGT1A1 TATAA box [(TA)₆>TAA] polymorphism plays a role in the glucuronidation and detoxification of BPD(−) in humans, we screened a cohort of 95 subjects from whom pathologically normal liver specimens could potentially be obtained for UGT1A1 genotype. This cohort was 59% male with a mean age of 63 years. Efficient separation of wild-type (98-bp) versus polymorphic (100-bp) PCR amplimers was obtained with the denaturing gel system.
used for UGT1A1 genotyping (Fig. 1A). Sequencing analysis of individual PCR amplimers confirmed data obtained from genotyping analysis in all samples examined (Fig. 1B). As shown in Table 1, 12% of these subjects had the homozygous polymorphic UGT1A1(*28/*28) genotype, which was similar to the genotype prevalence observed previously for Caucasians (26). The prevalence of the UGT1A1*28 allele was 0.37 in this cohort, and it followed Hardy–Weinberg equilibrium.

The UGT1A1*28 allelic variant has been previously linked to decreased expression of the UGT1A1 gene and decreased bilirubin conjugation (19–23). To demonstrate a potential link between UGT1A1 TATAA box genotype and carcinogen-metabolizing phenotype, we obtained liver specimens from 60 of the 95 subjects screened for UGT1A1 genotype. This included liver specimens from 24 subjects with the homozygous wild-type UGT1A1(*1/*1) genotype, 27 subjects with the heterozygous UGT1A1(*1/*28) genotype, and 9 subjects with the homozygous polymorphic UGT1A1(*28/*28) genotype. Microsomal fractions were prepared for all liver specimens and used for all biochemical analysis. As shown in Fig. 2A, Western blotting for the UGT1A1 enzyme using an anti-UGT1A1-specific antibody demonstrated lower levels of microsomal UGT1A1 protein in liver microsomes from subjects who exhibited either the homozygous polymorphic UGT1A1(*28/*28) genotype or the heterozygous polymorphic UGT1A1(*1/*28) genotype compared with microsomes from subjects with the UGT1A1(*28/*28) genotype (Fig. 2B). A similar increase of 1.7-fold (P < 0.05) was observed when we compared liver microsomes from subjects with the heterozygous UGT1A1(*1/*28) genotype with microsomes from subjects with the UGT1A1(*28/*28) genotype. No decrease in UGT1A1 protein was observed when we compared subjects who were heterozygous for the UGT1A1*28 variant with individuals who were homozygous for UGT1A1*1.

To assess the effect of genotype-associated decreases in UGT1A1 protein levels on liver glucuronidation activity of a UGT1A1 substrate, we assessed the rates of bilirubin glucuronide formation in the same 60 liver microsomal specimens by HPLC. Several peaks corresponding to potential bilirubin glucuronides were observed in this HPLC assay (Fig. 3A). Bilirubin glucuronides were confirmed by sensitivity to β-glucuronidase (Fig. 3B). Consistent with the results for actual UGT1A1 protein levels, we observed significantly lower rates of bilirubin glucuronide formation for liver microsomes from subjects with the homozygous polymorphic UGT1A1(*28/*28) genotype compared with subjects with either the wild-type UGT1A1(*1/*1) (P < 0.001) or heterozygous UGT1A1(*1/*28) (P < 0.002) genotypes (Fig. 4A). Similar results were obtained when data were analyzed per mg of total liver microsome protein (Fig. 4A) or when values were normalized to levels of microsomal β-actin (results not shown).

Efficient separation of BPD(−) diastereomers was obtained by HPLC in assays with liver microsomes (Fig. 3C) as well as UGT1A1-overexpressing baculosomes (Fig. 3D). BPD(−) glucuronides were confirmed by sensitivity to β-glucuronidase (Fig. 3E); based on known retention times for BPD(−) glucuronide diastereomers (13), peak 1 corresponded to BPD-7R-Gluc (retention time, 25.5 min), whereas peak 2 corresponded to BPD-8R-Gluc (retention time, 26.5 min). As observed previously for BPD(−) in liver microsomes (13), peak 1 was higher than peak 2 for all liver microsomes tested, suggesting that on exposure to BPD(−), BPD-7R-Gluc is preferentially formed in human liver regardless of potential variations in UGT expression or genotype. The BPD(−) glucuronide

<table>
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<th>Table 1</th>
<th>Genotypes and allelic prevalence of the UGT1A1 TATAA box promoter polymorphism</th>
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<td>UGT1A1 genotype</td>
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<td>120</td>
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<td>*28</td>
<td>70</td>
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* UGT1A1, UDP-glucuronosyl transferase 1A1.
isomer pattern of BPD-7R-Gluc > BPD-8R-Gluc for human liver microsomes (Fig. 3C) is similar to that observed for both UGT1A1- (Fig. 3D) and UGT1A9-overexpressing baculosomes (13).

After screening for BPD(−) glucuronidation phenotype in all 60 liver microsomes, similar levels of BPD(−) glucuronide formation were observed in subjects with different UGT1A1 TATAA box genotypes (Fig. 4B). Unlike that observed for bilirubin glucuronidation, no significant differences in BPD(−) glucuronide formation were observed when we compared subjects with the UGT1A1(*28/*28) genotype with either the UGT1A1(*1/*1) or UGT1A1(*1/*28) genotypes. Because UGT1A1 and UGT1A9 are the only two hepatic UGT enzymes that exhibit significant activity against BPD(−) (13), attempts were made to eliminate UGT1A9-induced BPD(−) glucuronidating activity as a potential confounder in these assays by identifying a substrate that could inhibit UGT1A9 but not UGT1A1. Of the compounds tested, α-naphthylamine inhibited BPD(−) glucuronide formation in UGT1A9-overexpressing baculosomes but not in baculosomes overexpressing UGT1A1, with the inhibition of UGT1A9 activity against BPD(−) approaching 97% in assays with 0.5 mM α-naphthylamine (Fig. 5, A and B). In addition, no significant inhibition of bilirubin glucuronide conjugation was observed in liver microsomes at α-naphthylamine concentrations up to 0.1 mM (Fig. 5C), which is equivalent to approximately 9 times the \( k_i \) for α-naphthylamine inhibition of BPD(−) glucuronide formation in UGT1A9-overexpressing baculosomes (11.3 ± 4.0 \( \mu M \)). Efficient separation of α-naphthylamine from BPD(−) glucuronide diastereomers was observed in the HPLC assays used, with α-naphthylamine eluting at 56 min and BPD-7R-Gluc and BPD-8R-Gluc eluting at 65 and 66 min, respectively (Fig. 6).
Together, these data suggest that 0.1 mM \( \alpha \)-naphthylamine can be used as a selective inhibitor of UGT1A9-induced glucuronidation of BPD(\(^{-}\)) in vitro in liver microsomes.

Decreased BPD-glucuronide formation (3–6-fold) was observed when 0.1 mM \( \alpha \)-naphthylamine was added to BPD glucuronidation assays of human liver microsomes (Figs. 4B and 7), a decrease that was significant regardless of \( \text{UGT1A1} \) genotype (\( P < 0.001 \) for all genotypes). When 0.1 mM \( \alpha \)-naphthylamine was added to glucuronidation assays of liver microsomes, differences in the levels of BPD(\(^{-}\)) glucuronide formation were observed for subjects with different \( \text{UGT1A1} \) TATAAA box genotypes (Fig. 7). We observed a significantly lower rate of BPD(\(^{-}\)) glucuronide formation in subjects with the homozygous polymorphic \( \text{UGT1A1}^{*28/28} \) genotype compared with subjects who were either homozygous (\( P < 0.02 \)) or heterozygous (\( P < 0.02 \)) for the \( \text{UGT1A1}^{*1} \) allele. Similar results were obtained when data were analyzed per mg of total liver microsome protein (Fig. 7) or when results were normalized to levels of microsomal \( \beta \)-actin (results not shown).

No difference in BPD(\(^{-}\)) glucuronidation phenotype was observed in heterozygous subjects compared with subjects with the wild-type \( \text{UGT1A1}^{*1/*1} \) genotype. The \( K_m \) of human liver microsomes for BPD(\(^{-}\)) in assays with \( \alpha \)-naphthylamine (0.1 mM) was 319 ± 52 \( \mu \)M and was similar in subjects with various \( \text{UGT1A1} \) genotypes (\( *1/*1, *1/*28, \) or \( *28/*28 \); results not shown).

**Discussion**

In addition to being the major enzyme involved in the metabolism and detoxification of bilirubin, \( \text{UGT1A1} \) is one of several UGTs that glucuronidate carcinogens, including metabolites of BaP. The UGTs 1A1, 1A7, 1A8, 1A9, 1A10, and 2B7 all exhibit activity against the racemic form of BPD and, with the exception of \( \text{UGT2B7} \), the BPD(\(^{-}\)) isomer (13), precursor to the highly mutagenic \( \text{anti}^{-}\)-BaP-7,8,8,9,10,13-hexahydrodiol-8,9,10-ring-epoxide. Of the BPD(\(^{-}\))-glucuronidating UGTs, only \( \text{UGT1A1} \) and \( \text{UGT1A9} \) are expressed in liver (18), which indicates that both of these enzymes are the sole hepatic glucuronidators of this potent carcinogenic precursor. The mutual importance of both enzymes in the hepatic metabolism and detoxification of BPD(\(^{-}\)) is supported by the fact that the ratio of the two glucuronide isomers formed from BPD(\(^{-}\)), BPD-7R-\text{Gluc}, BPD-8R-\text{Gluc}, is similar for both enzymes in in vitro assays with BPD(\(^{-}\)) as substrate, with both enzymes exhibiting a \( K_m \) in the 0.2–0.3 mM range (13). In addition, the BPD-7R-\text{Gluc}: BPD-8R-\text{Gluc} ratio observed with both enzymes individually (1.0.3–1.0.4) is similar to that observed with liver microsomes (present study and Ref. 13). When we used \( \alpha \)-naphthylamine, 

![Fig. 5. Effects of \( \alpha \)-naphthylamine on UDP-glucuronosyltransferase 1A1 (\( \text{UGT1A1} \)), \( \text{UGT1A9} \), and human liver microsome-induced glucuronidation. Shown are bar graphs of the relative glucuronidation activities of \( \text{UGT1A1} \)- or \( \text{UGT1A9} \)-overexpressing baculosomes (0.2 mg of protein) in assays with 1 mM \( \alpha \)-naphthylamine as described in the "Materials and Methods." A, \( \text{UGT1A1} \)-overexpressing baculosomes (0.2 mg of protein) against 1 mM \( \text{BPD} \); B, \( \text{UGT1A9} \)-overexpressing baculosomes with 1 mM \( \text{BPD} \); C, human liver microsomes with 0.7 mM bilirubin.

![Fig. 6. High-performance liquid chromatographic analysis of benzo(\( \alpha \))pyrene-trans-7R,8R-diarylidiol (\( \text{BPD} \)) glucuronidation by human liver microsomes in the presence of \( \alpha \)-naphthylamine. Human liver microsomes (0.25 mg of protein) were incubated with 1 mM \( \text{BPD} \) and 0.1 mM \( \alpha \)-naphthylamine as described in "Materials and Methods." Glucuronidation formation is shown by UV detection (254 nm). A, 0.1 mM \( \alpha \)-naphthylamine alone; B, glucuronidation assays performed with 1 mM \( \text{BPD} \) without 0.1 mM \( \alpha \)-naphthylamine; C, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; D, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; E, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; F, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; G, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; H, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; I, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; J, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; K, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; L, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; M, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; N, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; O, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; P, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; Q, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; R, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; S, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; T, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; U, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; V, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; W, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; X, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; Y, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine; Z, glucuronidation assays performed with 1 mM \( \text{BPD} \) plus 0.1 mM \( \alpha \)-naphthylamine.

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UGT1A1 Genotype and BaP Glucuronidation Phenotype

...an inhibitor of UGT1A9-induced glucuronidation of BPD(−), results from the present study suggest that ~30% of BPD(−) glucuronidation is catalyzed by UGT1A1, whereas ~70% is catalyzed by UGT1A9 in subjects homozygous for the wild-type UGT1A1*1 allele, a fact that is consistent with both enzymes playing major roles in the hepatic glucuronidation of BPD(−).

The studies described here are the first to directly examine glucuronidation activity against a major carcinogen in liver specimens from a large group of subjects. In assays focusing solely on UGT1A1-induced BPD(−) glucuronidation, significantly decreased levels of BPD(−) glucuronide were observed in liver microsomes from subjects with the UGT1A1(*28/*28) genotype compared with subjects with the homozygous wild-type UGT1A1(*1/*1) or heterozygous UGT1A1(*1/*28) genotypes. These results are consistent with the decreased expression observed in vitro with a reporter gene transcriptionally regulated by the UGT1A1 promoter with an additional (TA) dinucleotide repeat in its TATAA box [(TA)n > (TA)2; Refs. 19, 20]. The results of the present study also conclusively demonstrate that donors from subjects who are homozygous for the UGT1A1*28 allele exhibit significantly lower liver microsomal UGT1A1 protein and decreased liver microsomal glucuronidating activity against bilirubin. These results are consistent with the decreased levels of bilirubin glucuronide formation observed in liver microsomes from subjects homozygous for the variant TATAA box genotype in previous studies (29) and the decreased levels of bilirubin glucuronide observed in serum from subjects homozygous for the variant TATAA box genotype (19, 20) and in subjects clinically diagnosed with Gilbert’s syndrome (19, 21–23).

Together, these data suggest that the UGT1A1 TATA box polymorphism is associated with a decreased overall ability to glucuronidate an important metabolite [BPD(−)] within the BaP carcinogenic pathway. This suggests that individuals with the variant UGT1A1(*28/*28) genotype may be less able to detoxify BaP (and potentially other carcinogens metabolized by UGT1A1) than those who are wild-type for UGT1A1, further suggesting that individuals with the variant UGT1A1(*28/*28) genotype are at increased risk for certain cancers. Although the association between BPD(−) glucuronide formation and UGT1A1 genotype was not observed in assays where hepatic UGT1A9 activity was not inhibited, this was likely because hepatic UGT1A9-induced activity comprised a majority (~70%) of the total BPD(−) glucuronidation observed in human liver microsomes. The fact that significant differences in genotype-associated UGT1A1-induced BPD(−) glucuronidation formation were not detectable when UGT1A9-associated activities were not inhibited is consistent with the possibility that small but significant long-term decreases in the overall ability to detoxify a procarcinogen such BPD(−) may play an important role in cancer susceptibility. A potential role for UGT1A1 genotype in cancer risk has been examined in studies of breast cancer (30, 31), but studies have as yet to be performed for organs/tissues that are targets for BaP exposure (e.g., tobacco-related cancers). The effect of UGT1A1 genotype on cancer risk may be most pronounced for those organs/tissues where there is exposure to BaP and where UGT1A1 is expressed. Although previous studies have shown no significant levels of expression of UGT1A1 in either the aerodigestive tract or lung (17), a hepatic UGT1A1 genotype-associated effect on overall BPD(−) detoxification and cancer risk cannot be excluded for these organ/tissue sites. Further studies examining UGT1A1 genotype in relation to susceptibility for tobacco-related cancers (as well as other cancers where exposure to BaP is a risk factor) will be necessary to better evaluate the role of UGT1A1 genotype on cancer risk.

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References


Correlation between the UDP-Glucuronosyltransferase (UGT1A1) TATAA Box Polymorphism and Carcinogen Detoxification Phenotype: Significantly Decreased Glucuronidating Activity against Benzo(a)pyrene-7,8-dihydrodiol(−) in Liver Microsomes from Subjects with the UGT1A1*28 Variant

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