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Review

Variability of human hepatic UDP-glucuronosyltransferase activity

Joanna M. Little[1], Roger Lester[2], Folkert Kuipers[3], Roel Vonk[4], Peter I. Mackenzie[5], Richard R. Drake[1], Lynn Frame[6] and Anna Radominska-Pandya[1]

[1]*Department of Biochemistry and Molecular Biology and the* [2]*Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, AR;* [3]*Center for Liver, Digestive and Metabolic Diseases, Academic Hospital Groningen, Groningen, The Netherlands;* [4]*Laboratory of Nutrition and Metabolism, University Hospital, Groningen, The Netherlands;* [5]*Department of Clinical Pharmacology, Flinders Medical Centre, Bedford Park, Australia, and* [6]*Texas Tech University, Institute of Environmental and Human Health, Lubbock, TX, U.S.A.*

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The availability of a unique series of liver samples from human subjects, both control patients (9) and those with liver disease (6; biliary atresia (2), retransplant, chronic tyrosinemia type I, tyrosinemia, Wilson's disease) allowed us to characterize human hepatic UDP-glucuronosyltransferases using photoaffinity labeling, immunoblotting and enzymatic assays. There was wide inter-individual variation in photoincorporation of the photoaffinity analogs, [^{32}P]5-azido-UDP-glucuronic acid and [^{32}P]5-azido-UDP-glucose and enzymatic glucuronidation of substrates specific to the two subfamilies of UDP-glucuronosyltransferases. However, the largest differences were between subjects with liver disease. Glucuronidation activities toward one substrate from each of the UDP-glucuronosyltransferases subfamilies, 1A and 2B, for control and liver disease, respectively, were 1.7–4.5 *vs* 0.4–4.7 nmol/mg × min for hyodeoxycholic acid (2B substrate) and 9.2–27.9 *vs* 8.1–75 nmol/mg × min

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✉Address correspondence to: Anna Radominska-Pandya, Ph.D., Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, 4301 W. Markham, Slot 516, Little Rock, AR 72205, U.S.A.; Tel: (501) 686 5414; Fax: (501) 603 1146; e-mail: radominskaanna@exchange.uams.edu

Abbreviations: [^{32}P]5N$_3$UDP-GlcUA, [β-^{32}P]5-azido-UDP-glucuronic acid; [^{32}P]5N$_3$UDP-Glc, [β-^{32}P]5-azido-UDP-glucose; UGT, UDP-glucuronosyltransferase; UDP-GlcUA, UDP-glucuronic acid; UDP-Glc, UDP-glucose; HDCA, hyodeoxycholic acid (3α,6α-dihydroxy-5β-cholanoic acid); pNP, 4-nitrophenol; BR, bilirubin; A, androsterone; T, testosterone; PCMX, *para*-chloro-*meta*-xylenol; GPDS, Glc-P-Dol synthase or UDP-glucose:dolichylphosphate-glucosyltransferase.

for *p*-chloro-*m*-xylenol (1A substrate). Microsomes from a patient with chronic tyrosinemia (HL32) photoincorporated [^{32}P]5-azido-UDP-glucuronic acid at a level 1.5 times higher than the other samples, was intensely photolabeled by [^{32}P]5-azido-UDP-glucose and had significantly higher enzymatic activity toward *p*-chloro-*m*-xylenol. Immunoblot analysis using anti-UDP-glucuronosyltransferase antibodies demonstrated wide inter-individual variations in UDP-glucuronosyltransferase protein with increased UDP-glucuronosyltransferase protein in HL32 microsomes, corresponding to one of the bands photolabeled by both probes. Detailed investigation of substrate specificity, using substrates representative of both the 1A (bilirubin, 4-nitrophenol) and 2B (androsterone, testosterone) families was carried out with HL32, HL38 (age and sex matched control) and HL18 (older control). Strikingly increased (5–8-fold) glucuronidation activity was seen in comparison to HL18 only with the phenolic substrates. The results indicate that one or more phenol-specific UDP-glucuronosyltransferase 1A isoforms are expressed at above normal levels in this tyrosinemic subject.

The UDP-glucuronosyltransferases (UGTs, EC 2.4.1.17) are a family of enzymes, located in the endoplasmic reticulum (ER), which catalyze the glucuronidation of a wide variety of both endogenous (e.g., bile acids, neutral steroids, bilirubin) and exogenous (e.g., drugs, xenobiotics) compounds. These substrates for the UGTs may be glucuronidated at almost any position bearing a hydroxyl, carboxyl, amino or thiol group [1, 2]. Glucuronidation represents a major detoxification pathway in mammalian species in that the products of the reaction are generally less metabolically active, more water soluble and more readily excreted in bile and/or urine than the parent compounds.

The data on native human UGTs is somewhat limited, owing to the limited availability of human tissue samples and the extreme lability of the human enzymes during purification. At present, only three human UGTs have been purified to apparent homogeneity [3, 4]. It is known that human microsomal UGTs, like those of the rat, demonstrate heterogeneity in terms of substrate specificity and also appear to have differential developmental and induction patterns [5]. Cloning and expression of human UGT cDNAs has proven to be productive: at least eleven human UGTs have been cloned and expressed [6, 7] and substrate specificity has been extensively studied with recombinant proteins [7–9].

The development of the photoaffinity analogs, [^{32}P]5-azido-UDP-glucuronic acid ([^{32}P]5N$_3$UDP-GlcUA) and [^{32}P]5-azido-UDP-glucose ([^{32}P]5N$_3$UDP-Glc) [10–12], has provided an effective means for characterizing microsomal UGTs as a group. Recent studies have shown that both rat and human liver microsomal UGTs photoincorporated both [^{32}P]5N$_3$UDP-GlcUA and [^{32}P]5N$_3$UDP-Glc in a concentration dependent manner and that photoincorporation of either probe was inhibited by both unlabeled UDP-GlcUA and UDP-Glc [11–13]. In addition, a new enzymatic activity, UDP-Glc-dependent 6-O-glucosylation of hyodeoxycholic acid, has been found in and appears to be unique to human liver [14].

This report represents a preliminary study of human hepatic UGTs which may lead to a better understanding of the role of these enzymes in the biotransformation of endogenous compounds as well as drugs. The purpose of the present study was to apply photoaffinity labeling to the initial screening and characterization of human liver UGTs in a series of microsomal preparations obtained from both normal and diseased livers. We believe that characterization of the samples by photoaffinity labeling, followed by additional biochemical and molecular characterization, will provide valuable information that can be used clinically. Photoaffinity labeling will also help to quickly identify samples useful for studies on human UGT polymorphism and

those aimed at recognizing potential inducers of human UGTs, an area where little information is available.

MATERIALS AND METHODS

[^{32}P]5N$_3$UDP-GlcUA and [^{32}P]5N$_3$UDP-Glc were synthesized and purified as previously described [10, 11]. [^{32}P]P$_i$ was obtained from ICN and Brij 58, sugar nucleotides, saccharolactone, unlabeled hyodeoxycholic acid (HDCA; 3α,6α-dihydroxy-5β-cholanoic acid) and [1-^{14}C]4-nitrophenol (pNP) were from Sigma Chemical. Tritium-labeled HDCA was synthesized as described in [15, 16] and checked for chemical purity by thin-layer chromatography (TLC) and gas-liquid chromatography. [^{3}H]Androsterone, [^{14}C]UDP-GlcUA and [^{3}H]UDP-Glc were purchased from New England Nuclear. Human liver tissue was obtained from the University of Groningen (Groningen, The Netherlands) and relevant details for the individual donors are given in Table 1. Human liver microsomes were prepared as previously described [16].

Photoaffinity labeling. Human liver microsomes (50 μg protein) were incubated for 10 min, on ice, in the presence of 0.05% Triton X-100, in 100 mM Hepes, pH 7.0, and 5 mM $MgCl_2$ in a total volume of 20 μl. Either [^{32}P]5N$_3$UDP-GlcUA or [^{32}P]5N$_3$UDP-Glc (200 mM; 2–5 mCi/mmol) was added to a final concentration of 40 μM (standard conditions) and allowed to equilibrate for 20 s, followed by UV-irradiation with a hand-held lamp (UVP-11, 254 nm, Ultraviolet Products, Inc.) at a distance of 4 cm from the sample for 90 s at room temperature. For competition experiments, the appropriate unlabeled competing nucleotide was added to the reaction mixture just before addition of the photoprobe. Reactions were terminated and processed for electrophoresis as previously described [11].

Table 1. Clinical details of liver donors

Control livers			
Sample No.	Age	Sex	Cause of death
15	56 year	male	cerebral bleeding
18	13 year	female	brain damage
22	32 year	female	brain damage
25	12 year	female	brain damage
26	63 year	male	brain damage
27	32 year	female	brain tumor
28	46 year	female	meningoendotheliomatous meningeoma
29	22 year	male	multi-trauma
36	31 year	male	coma diabetica due to bronchopneumonia
38	4 year	male	thorax trauma
Diseased liver			
Sample No.	Age	Sex	Disease
30	10 month	male	biliary atresia
31	20 month	female	retransplant
32	4 year	male	chronic tyrosinemia type I
33	4 year	male	biliary atresia
34	10 year	male	Wilson's disease
35	2.5 year	male	tyrosinemia

Proteins were separated on 10% SDS/polyacrylamide gels [17], followed by autoradiography for 1–2 days. In some cases, the separated proteins were transferred from the gel to nitrocellulose by electroblotting and Western blot analysis was performed by the method of Towbin *et al.* [18]. Blotted proteins were examined using anti-rat pNP-UGT antiserum (a gift from Dr. A. Dannenberg, The New York Hospital, Cornell Medical Center), anti-rat androsterone (A) and testosterone (T) UGT antibodies (gifts from M. Green and Dr. T. Tephly, University of Iowa) and anti-human UGT1A6 (naphthol-UGT) and UGT2B4 (HDCA-UGT) (gifts from Drs. J. Magdalou and S. Fournel-Gigleux, URA CNRS 1288, Nancy, France).

Enzyme assays. HDCA, pNP, A, and T glucuronyl- and glucosyltransferase activities were measured with radioactive substrates with UDP-GlcUA or UDP-Glc as the sugar donors [14–16]; reactions were started by addition of the appropriate UDP-sugar. The glucuronidated or glucosylated products and the unreacted substrate were separated by two TLC developments in chloroform/methanol/glacial acetic acid/water (65:25:2:4, by vol.). Bilirubin (BR) glucuronidation activities were measured with unlabeled BR and [^{14}C]UDP-GlcUA using a modification of the method of Bansal & Gessner [19]. Briefly, BR dissolved in bovine serum albumin (BSA; final concentration, 0.34 mM BR, 8 mg/ml BSA) was incubated in a total volume of 50 μl containing 20 mM Tris/Cl, pH 7.6, 5 mM $MgCl_2$, 5 mM saccharolactone, 0.05% Brij-58 and 50 μg of microsomal protein. Reactions were started by addition of 20 mM [^{14}C]UDP-GlcUA (0.07 μCi). After 30 min at 37°C, the reaction was stopped by addition of 30 μl of ethanol and 60 μl of the mixture was applied to the preabsorbent layer of channeled TLC plates (J.T. Baker, Inc.). The glucuronidated BR and unreacted substrate were separated by development in n-butanol/acetone/glacial acetic acid/ammonium hydroxide (30%)/water (70:50:18: 1.5:60, by vol.). In all assays, radioactive compounds were localized on TLC plates by autoradiography at -80°C. Zones corresponding to the glucuronide bands were scraped into scintillation vials and radioactivity was measured by liquid scintillation counting (Rackbeta Model 1214, Wallac). Activity towards *p*-chloro-*m*-xylenol (^{3}H-PCMX) was determined using the naphthol UGT assay system of Otani *et al.* [20] with methylene chloride substituted for chloroform as the extraction solvent.

RESULTS

Microsomes prepared from 14 different human livers (8 from control subjects and 6 from patients with various liver diseases; see Table 1) were screened for photoaffinity labeling, immunoreactivity and UGT enzymatic activity. At a later time, the studies were repeated to allow comparison of the most active tyrosinemia sample (HL32, Table 1) with an age- and sex-matched control sample which had become available. The results of the preliminary screening will be presented first, followed by those of the direct comparison between equivalent control and tyrosinemic livers.

Characterization of UGTs in human liver microsomes

Photoaffinity labeling

Figure 1 shows the autoradiographs made from Western blots of human liver microsomal proteins photolabeled in the presence of detergent with either [^{32}P]5N_3UDP-GlcUA Fig. 1A) or [^{32}P]5N_3UDP-Glc (Fig. 1B). There was a high degree of inter-individual variation even among control samples in the intensity of photolabeling by [^{32}P]5N_3UDP-GlcUA of proteins in the 50–56 kDa molecular mass range known to encompass the UGTs (Fig. 1A). In general, photolabeling of the mi-

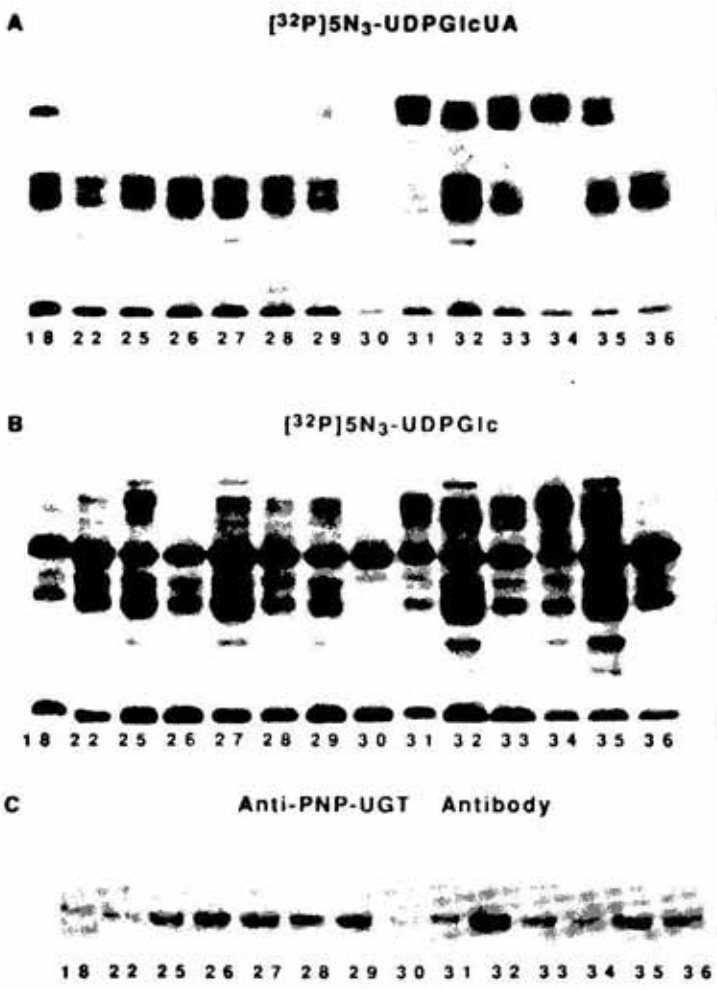

Figure 1. Photoaffinity labeling of microsomes prepared from 14 different human liver samples.

Autoradiographs of Western blots of human liver microsomal proteins photolabeled with [^{32}P]5N$_3$UDP-GlcUA **(A)** and [^{32}P]5N$_3$UDP-Glc **(B)** and a representative Western blot developed with an anti-rat pNP-UGT antibody **(C)** as described in "Materials and Methods". The results shown are representative of a total of three experiments. Microsomes were incubated in the presence of detergent for 10 min at room temperature before photolabeling with 40 μM [^{32}P]5N$_3$UDP-GlcUA or [^{32}P]5N$_3$UDP-Glc. Each lane is labeled with the number of the corresponding liver sample (see Table 1 for details). In both Fig. 1A and B, the 37 kDa protein photolabeled by both probes has been previously identified as the cytoplasmically oriented UDP-Glc:Dol-P-glucosyltransferase (or Glc-P-Dol synthase (GPDS)) [11, 12, 21]. Additionally, in Fig. 1B, the 62 kDa protein labeled by [^{32}P]5N$_3$UDP-Glc even in the absence of UV irradiation has been shown to be phosphoglucomutase [22].

crosomal UGT proteins from diseased livers by [^{32}P]5N$_3$UDP-GlcUA was at or below the level seen in control microsomes. However, HL32 proteins (from a patient with chronic type I tyrosinemia) were more strongly labeled than those of any other sample. Densitometric quantitation of the autoradiographs (Fig. 2) showed that [^{32}P]5N$_3$UDP-GlcUA photolabeling of HL32 UGTs was, on average, 2.3 times greater than that in control livers and 6.5 times higher in comparison to other diseased livers.

Photolabeling of the 50–56 kDa proteins with [^{32}P]5N$_3$UDP-Glc (Fig. 1B) followed the same general pattern seen with [^{32}P]5N$_3$-UDP-GlcUA. Inter-individual variability of photoincorporation was similar and HL32 proteins were as intensely photolabeled with [^{32}P]5N$_3$UDP-Glc as they had been with [^{32}P]5N$_3$UDP-GlcUA. Densitometric quantitation of the 50–56 kDa proteins photolabeled with [^{32}P]5N$_3$UDP-Glc (Fig. 2) showed that HL32 labeling was increased 2.3 and 5.1 times as compared with control and diseased livers, respectively, with the exception of HL35. HL35 microsomes, also from a patient with tyrosinemia, photoincorporated [^{32}P]-5N$_3$UDP-Glc to an extent equivalent to that of HL32 proteins but photolabeling by [^{32}P]-5N$_3$UDP-GlcUA was 2.6 times less in HL35 as compared with HL32.

In both Fig. 1A and B, the 37 kDa protein photolabeled by both probes has been previously identified as the cytoplasmically oriented UDP-Glc:Dol-P-glucosyltransfease (or Glc-P-Dol synthase (GPDS)) [11, 12, 21]. Additionally, in Fig. 1B, the 62 kDa protein labeled by [^{32}P]5N$_3$UDP-Glc even in the absence of

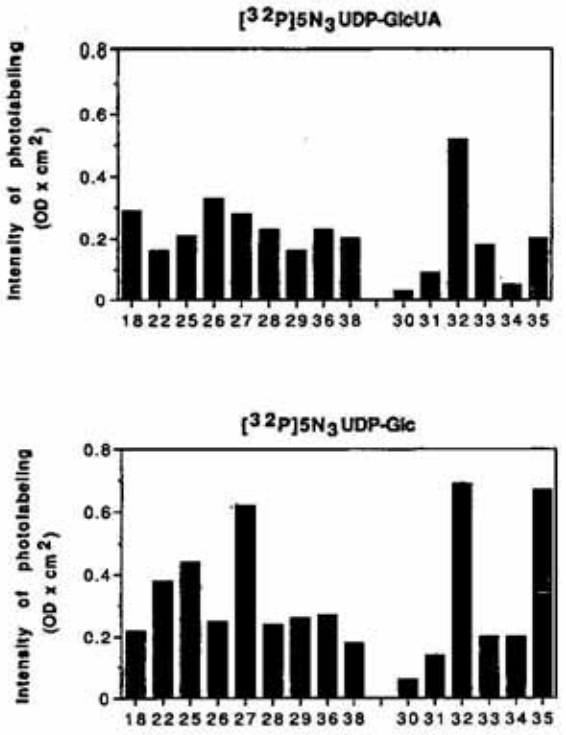

Figure 2. Quantitation of UGT photolabeling.

Autoradiographs from representative experiments were quantitated by densitometry. The results are expressed as the spot volumes (OD × cm^2) of proteins in the 50–56 kDa molecular mass range photolabeled by [^{32}P]5N_3UDP-GlcUA and [^{32}P]5N_3UDP-Glc.

UV irradiation has been shown to be phosphoglucomutase [22].

Immunoreactivity of photolabeled UGTs

As would be expected, the pattern of immunoreactive protein detected with an anti-pNP-UGT antibody was the same on Western blots of microsomes labeled with either [^{32}P]5N_3UDP-GlcUA or [^{32}P]5N_3UDP-Glc and this pattern is shown in Fig. 1C. Inter-individual variability of UGT protein content similar to that observed with photoincorporation was detected with the antibody. Additionally, increased UGT protein levels were observed in HL32 which could be superimposed on the heavily photolabeled, approximately 52 kDa, bands in each of the autoradiographs (Fig. 1A and B). The results of densitometric analysis of the intensity of staining on this blot and on others probed with anti-A UGT and anti-T UGT antibodies (blots not shown) are tabulated in Table 2. Increased levels of UGT protein were detected in HL32 microsomes with all three antibodies. With the anti-pNP antibody, HL32 UGT protein was, on average, 3.8 times higher than in control livers and 7.9 times higher than in diseased livers. The same values were 1.8 and 2.6 (for control and diseased livers, respectively) with the anti-A antibody and 2.9 and 11.9 for the anti-T antibody. With respect to HL35, which photoincorporated [^{32}P]5N_3UDP-Glc at a level equivalent to that of HL32, UGT protein detected by the anti-pNP and anti-T antibodies were significantly higher in HL32 than in HL35 (3.1 and 5.8 fold, respectively); with the anti-A antibody, roughly equivalent levels of UGT protein were detected for HL32 and HL35.

Enzymatic activity assays

In the preliminary screening, the microsomes from control and diseased livers used for photolabeling were also assayed for enzymatic glucuronidation activity toward HDCA and PCMX. The results of these assays, shown in Fig. 3A (HDCA) and 3B (PCMX), again demonstrated a high degree of variability among individuals, even within the control group. The HDCA glucuronidation activity of HL32 microsomes (Fig. 3A), while high (4.51 nmol/min × mg), was not outside the range of activities of the other samples (0.41–4.54 nmol/min × mg). With PCMX as substrate (Fig. 3B), however, the activity of HL32 microsomes was 3–8 times higher than that found in the other samples even though the results were obtained under conditions optimized for the control samples and the activity for HL32 microsomes was falsely low due to substrate depletion. When PCMX activity was determined under optimal condition for HL32 microsomes, formation of PCMX glucuronide was 10 times higher in HL32 as compared with the mean activity of the other sam-

ples (172 nmol/min × mg for HL32 *vs* 17.3 ± 3.0 nmol/min × mg for the other livers).

Comparison of UGTs in microsomes from tyrosinemic liver and matched control liver

Photoaffinity labeling

Microsomes from HL32 and HL38, an age- and sex-matched control liver, were photolabeled with [^{32}P]5N$_3$UDP-GlcUA and [^{32}P]-5N$_3$UDP-Glc as described above (autoradiographs not shown). Densitometric analysis of the autoradiographs showed that photoincorporation of both probes into the 50–56 kDa proteins was three times greater for HL32 than HL38 (Fig. 2).

Immunoreactivity

The Western blots shown in Fig. 4 are from new preparations of microsomal proteins of HL32, HL38 (the age- and sex-matched control) and HL18 (included for comparison to the original blots, discussed above) probed with the same three anti-rat UGT antibodies used above, anti-pNP UGT (A), anti-A UGT (B) and anti-T UGT (C) as well as two anti-human UGT antibodies, anti-UGT1A6 (D) and anti-UGT2B4 (E). The UGT proteins detected with each of the five antibodies were increased in HL32 relative to those of the other two samples and, in all cases, the increased protein was of the same approximate 51 kDa molecular mass as the HL32 protein photolabeled by [^{32}P]5N$_3$UDP-GlcUA and [^{32}P]-5N$_3$UDP-Glc. The results of densitometry tabulated in Table 2 quantitate a significant increase in UGT protein levels in HL32, with the biggest difference being detected by the anti-T and anti-UGT1A6 antibodies.

Enzymatic activity assays

The results of a more detailed examination of the substrate specificity of HL32 microsomal glucuronidation activity in comparison to

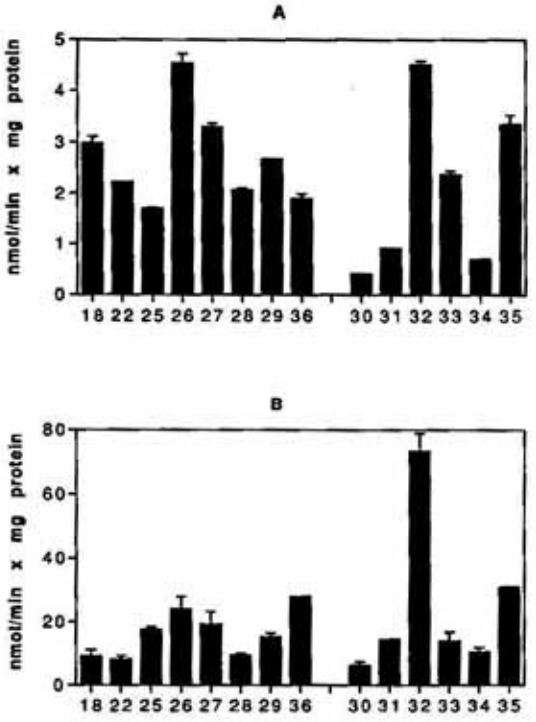

Figure 3. Enzymatic glucuronidation of representative UGT substrates by microsomes from different human liver samples.

UDP-GlcUA dependent glucuronidation of [^{3}H]HDCA **(A)** and [^{3}H]PCMX **(B)** was determined as described in "Materials and Methods" using 50 mg microsomal protein, 100 mM substrate and 4 mM UDP-GlcUA. The results are expressed as the mean ± S.D. of at least two separate duplicate determinations. Each bar is labeled with the number of the corresponding liver sample (see Table 1 for details).

HL38 and HL18 are tabulated in Table 3. For the endogenous substrates, HDCA, BR and A, the glucuronidation activities of HL32 microsomes were all approximately 3 times higher than those of HL38 microsomes; when compared to the older control, HL18, HL32 glucuronidation activities were 1.7 times higher for HDCA, the same for BR and 1.3 times higher for A. On the other hand, there was a much greater induction of HL32 glucuronidation activity towards the exogenous substrates, pNP and PCMX: pNP was glucuronidated at rates that were 10.6 and 5.4 times greater than those for HL38 and HL18, respectively, while the rates for PCMX were increased 12 and 8 fold relative to HL38 and HL18, respectively.

Table 2. Quantitation of UGT protein detected on Western blot.

The intensity of staining on representative Western blots was quantitated by densitometry. The results are expressed as the spot volumes (OD × cm^2) of proteins detected by various antibodies.

Control liver	Intensity of Western blot staining (OD × mm^2)				
Sample No.	Anti-PNP UGT	Anti-A UGT	Anti-T UGT	Anti-UGT1A6	Anti-UGT2B4
18	1.4	5.9	2.2	10.0	1.6
22	0.6	5.1	1.9		
25	0.6	5.8	1.5		
26	1.9	7.3	3.4		
27	1.4	6.5	1.2		
28	1.3	6.6	1.4		
29	1.1	6.8	1.6		
36	1.7	5.7	2.4		
38	1.6	5.7	1.2	7.1	1.6
Diseased liver	Intensity of Western blot staining (OD × mm^2)				
Sample No.	Anti-PNP UGT	Anti-A UGT	Anti-T UGT	Anti-UGT1A6	Anti-UGT2B4
30	1.4	2.8	0.3		
31	0.3	2.8	0.6		
32	4.6	11.3	5.0	31.0	5.6
33	0.8	6.3	1.3		
34	0.3	3.6	0.2		
35	1.3	8.4	0.9		

No activity towards T was found in any of the microsomal preparations.

These three microsomal preparations were also checked for enzymatic glucosidation activity towards HDCA, A, pNP and PCMX (Table 3). The glucosidation activity of HL32 microsomes towards all of the substrates was 2–3 times greater than that of HL38, but was equal to or less than the activity of HL18 microsomes.

DISCUSSION

Although human genetic polymorphism has been extensively studied with cytochrome P450s and sulfotransferases [23–26], the information in relation to glucuronidation of exogenous and endogenous compounds by human UGTs is very limited. The few papers devoted to variation in drug glucuronidation indicate that UGTs also exhibit polymorphism but that the variability can also be influenced by physiological factors, induction and drug interactions [23, 27]. Genetic factors in disorders such as Crigler-Najjar types I and II have been shown to result in polymorphism of bilirubin/phenol UGTs [23, 28, 29]. In this paper, devoted to the characterization of human UGTs in normal and diseased livers, significant individual variations in glucuronidation of drugs and endogenous compounds and in the amounts of UGT protein have been demonstrated. These studies are important to establish that such differences exist before more systematic investigations of polymorphism at the phenotype and genotype levels are undertaken.

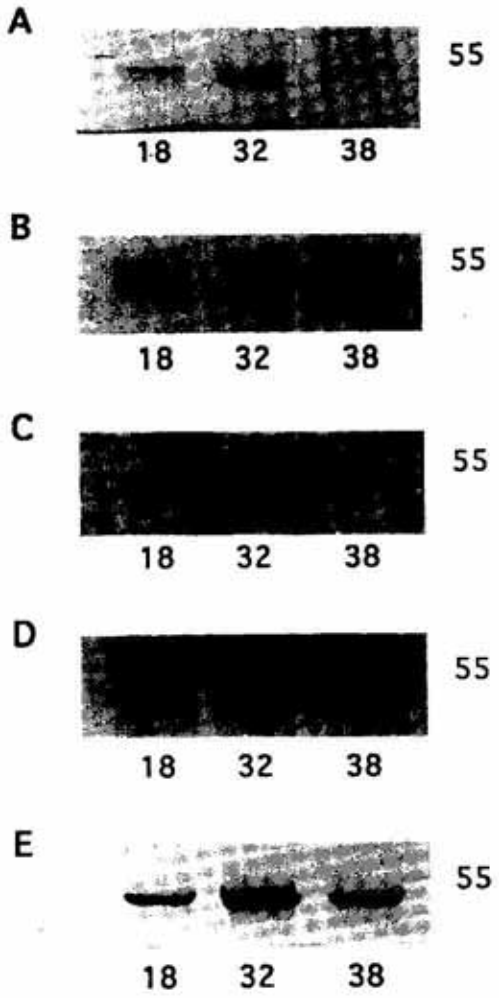

Figure 4. Immunoreactivity of microsomal proteins from tyrosinemic and control liver samples.

Western blots of microsomal proteins (50 μg) from the tyrosinemic liver (HL32), an age and sex matched control liver (HL38) and an older control liver (HL18) probed with five different anti-UGT antibodies. **(A)** Anti-rat pNP UGT; (B) anti-rat androsterone UGT; **(C)** anti-rat testosterone UGT; **(D)** anti-human UGT1A6 (phenol-specific); **(E)** anti-human UGT2B4 (HDCA-specific). The results shown are representative of at least two separate experiments. Each bar is labeled with the number of the corresponding liver sample (see Table 1 for details).

Photoaffinity labeling of microsomal and recombinant UGTs with [^{32}P]5N$_3$UDP-GlcUA and [^{32}P]5N$_3$UDP-Glc is a powerful tool for characterizing these enzymes as a group within their native membrane milieu. This technique has been successfully used to provide strong evidence for the presence in human liver microsomes of a UDP-glucose dependent transferase which catalyzes the 6-O-glucosidation of HDCA [14]. The present report extends the prior studies of human UGTs from a single subject [13, 14] to hepatic microsomes prepared from livers obtained from several different human subjects, both controls and those suffering from various forms of liver disease.

Photoaffinity labeling of this series of microsomes with [^{32}P]5N$_3$UDP-GlcUA, as shown in Fig. 1A, led to two immediately apparent observations. First, there was a wide variation in photoincorporation into the 50–56 kDa proteins of different individuals. Second, in spite of the variability of photolabeling with [^{32}P]5N$_3$UDP-GlcUA, microsomal UGTs of HL32, a subject with tyrosinemia, are more strongly labeled than those of any other sample. The intensity of [^{32}P]5N$_3$UDP-GlcUA photolabeling of 50–56 kDa proteins of HL32 microsomes was 1.6 to 3.2 times higher than that of control samples and 3 to 15 times higher than in other diseased livers (Figs. 1A and 2).

A similar degree of inter-individual variation was seen when the same proteins were photolabeled with [^{32}P]5N$_3$UDP-Glc (Figs. 1B and 2) although the pattern of labeling was somewhat different from that seen with [^{32}P]5N$_3$UDP-GlcUA. With this probe, 50–56 kDa proteins of both HL32 and HL35 were equally heavily labeled as was one of the control samples (HL27). Our recent finding of a UDP-Glc dependent, hyodeoxycholic acid specific 6-O-glucosylation activity in human microsomes [14] and a subsequent report providing strong indirect evidence that this activity is catalyzed by a distinct UDP-glucosyltransferase in the same molecular mass range as the UGTs [13] might explain the differences in the patterns of photoincorporation of [^{32}P]5N$_3$UDP-GlcUA and [^{32}P]5N$_3$UDP-Glc. Another contributing factor might be cross-reactivity of the [^{32}P]5N$_3$UDP-Glc with the UDP-GlcUA binding site which would lead to photoincorporation of [^{32}P]5N$_3$UDP-Glc into both UGTs and the glycosyltransferase.

Table 3. Substrate specificity of the UDP-glucuronosyltransferase and glucosyltransferase activity of HL32, HL38 and HL18 microsomes.

Human liver microsomes were incubated in the presence of detergent with UDP-GlcUA or UDP-Glc and various substrates under conditions described in the text. The results are expressed as the mean ± standard deviation of at least 2 separate duplicate determinations, except for the data on PCMX glucosidation which are the mean ± range of single duplicate determinations.

Sample No.	Specific activity (nmol/min × mg)					
	Glucuronidation					
	Substrate					
	HDCA	BR	A	T	PCMX	pNP
32	5.3 ± 0.3	0.5 ± 0.1	2.4 ± 0.3	ND	73.2 ± 5.8	94.5 ± 9.1
38	1.7 ± 0.6	0.2 ± 0.1	0.9 ± 0.3	ND	6.0 ± 0.4	8.9 ± 3.3
18	3.2 ± 0.8	0.5 ± 0.1	1.8 ± 0.5	ND	9.2 ± 1.9	17.4 ± 5.2
	Glucosidation					
Sample No.	Substrate					
	HDCA	A	PCMX	pNP		
32	3.2 ± 0.1	0.2 ± 0.03	1.2 ± 0.1	0.9 ± 0.1		
38	1.7 ± 0.05	0.1 ± 0.02	b	0.3 ± 0.1		
18	3.6 ± 0.02	0.2 ± 0.01	1.0 ± 0.1	2.4 ± 0.5		

ND, not detectable; b, not determined.

Western blot analysis of the photolabeled proteins, carried out using anti-pNP UGT, anti-A UGT and anti-T UGT antibodies, showed that the inter-individual variation in degree of photolabeling was reflected in the amount of UGT protein detected by immunostaining. The protein bands detected with the anti-pNP UGT antibody on the representative blot shown in Fig. 1C were superimposable on the photolabeled bands shown in the autoradiographs in Fig. 1A and 1B and the higher photoincorporation of the photoaffinity probes by HL32 correlated with the detection of larger amounts of immunoreactive protein in this sample than in any of the others. Blots probed with the other two anti-UGT antibodies are not shown but the results of densitometric analysis of all three blots, tabulated in Table 2, demonstrated that the results were similar.

After these preliminary screenings, which did not include a control subject whose age and sex matched those of HL32, were done, we obtained such a sample (HL38) and the photolabeling and Western blot analysis (using two highly specific anti-human UGT antibodies in addition to the three relatively non-specific anti-rat antibodies used above) were repeated with HL32, HL38 and HL18. Photoincorporation of both probes into HL32 proteins was consistently 3 times higher than into those of HL38 (Fig. 2). Depending on the antibody used, immunoreactive UGT protein was increased from 2- to 4-fold in HL32 as compared to both HL38 and HL18 (Fig. 4 and Table 2) with the highest relative levels of HL32 UGT protein being detected with the anti-human UGT1A6 antibody, suggesting specific induction of this isoform.

In the initial screening of all the liver samples for enzymatic activity, two substrates were used: HDCA which is a representative substrate for the steroid subfamily of UGTs and PCMX, a substrate for the phenol subfamily. As with photolabeling, there was wide inter-individual variation in enzymatic glucuronidation of both substrates (Fig. 3). With HDCA as substrate, the glucuronidation activ-

ity of HL32 microsomes, while higher than most of the other samples, was not outside the range of activity found in the control samples. On the other hand, glucuronidation of PCMX by HL32 microsomes, under non-optimal conditions in which there was significant substrate depletion in the HL32 assay, was 2.5 times higher than the most active control preparation.

A more detailed investigation of enzymatic activity was carried out with microsomes from HL32, HL38 (the age- and sex-matched control) and HL18 (from an older control subject) using HDCA, BR, A, T, PCMX and pNP as substrates with UDP-GlcUA as sugar donor (Table 3). With regard to glucuronidation of HDCA, BR and A, HL32 microsomes routinely showed an approximately 2-fold greater activity as compared with HL38 microsomes but demonstrated equal or only slightly increased activity relative to HL18 microsomes. In contrast, glucuronidation of pNP by HL32 microsomes was 10 and 5 times greater than that by HL38 and HL18 microsomes, respectively. Testosterone glucuronidation was not detectable in any of the microsomal preparations assayed. Glucosidation of HDCA, A, PCMX and pNP was also investigated and, despite the significantly increased photoincorporation of [^{32}P]5N$_3$UDP-Glc into the 50–56 kDa proteins by HL32, there was no significant increase in glucosidation of any of the substrates by HL32 microsomes (Table 3).

The phenol-specific UGT isoform, UGT1A6, has been shown to be differentially induced, on both the level of mRNA and of protein, in preneoplastic nodules in the rat [30, 31]. Since hepatocellular carcinoma is a common outcome in the chronic form of tyrosinemia [32], it is possible that UGT1A6 levels are increased in pre-cancerous hepatocyte foci and/or nodules in the human as they are in the rat [30, 31] and that the liver sample used here contained such areas. Additionally, the "lethal albino mouse" has multiple genetic deletions from chromosome 7, including the fumarylacetoacetate hydrolase gene responsible for the major enzyme deficiency in human tyrosinemia type I [32]. One of the biochemical alterations occurring in the homozygous lethal albino mouse model is a significant increase in glucuronidation activity towards pNP [33] and 4-methylumbelliferone [34], both of which are substrates for phenol-specific UGTs. Thus, the activation of the activity of one or more phenol-specific UGTs found in a sample of human tyrosinemic liver used in these studies has also been found in liver of a mouse model for human tyrosinemia.

From these results, it is apparent that there is considerable individual variation of UGT protein and activity in human liver. In addition, the significantly increased glucuronidation by HL32 microsomes relative to all control subjects seen with pNP and PCMX, substrates for the phenol-specific isoforms, and the high level of relative immunoreactivity HL32 protein with the highly specific anti-human UGT1A6 antibody suggest the induction of one or more phenol isoforms, including UGT1A6, in HL32.

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