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Human Bilirubin UDP-Glucuronosyltransferase Catalyzes the Glucuronidation of Ethinylestradiol

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SUMMARY

The synthetic estrogen ethinylestradiol is extensively eliminated as glucuronide metabolites in humans, but the UDP-glucuronosyltransferases (UGTs) catalyzing this reaction have not been identified. Therefore, ethinylestradiol was tested as a substrate for cloned human UGTs stably expressed in V79 cell lines. Two cloned expressed human enzymes, a bilirubin UGT and a phenol UGT, were observed to catalyze the glucuronidation of ethinylestradiol. High performance liquid chromatographic analysis of the products formed revealed that the expressed bilirubin UGT specifically produced ethinylestradiol-3-glucuronide. In human liver microsomes the ratio of 3-glucuronide/17-glucuronide was 97:3. Subsequent study of the cloned expressed enzymes and human liver microsomes from Crigler-Najjar patients by kinetic analysis and by substrate inhibition strongly indicated that a human liver bilirubin UGT was largely responsible for glucuronidation of ethinylestradiol. These results may provide an explanation for jaundice caused by ethinylestradiol in certain susceptible individuals.

Ethinylestradiol is a synthetic estrogen that is widely used as the estrogenic component in oral contraceptives. In humans it is extensively metabolized by both phase I and phase II metabolic reactions. The major biliary and urinary metabolites, however, are arylsulfates and glucuronides of ethinylestradiol and conjugates of its main phase I metabolites, 2- and 4-hydroxyethinylestradiol (1). In addition to hepatic metabolism, there is also evidence for the conjugation of ethinylestradiol by the human gut and jejunal mucosa (2, 3).

In vitro studies with human liver microsomes have demonstrated that the aromatic hydroxylation of ethinylestradiol is the principal phase I metabolic reaction. Direct glucuronidation and sulfation of the parent compund have also been shown in human liver microsomes (4–6). These studies revealed a marked variability of both conjugation reactions. Ethinylestradiol glucuronidation varied by 19-fold in human liver microsomes obtained from 110 subjects (7) and was also found to be phenobarbital inducible (4).

The glucuronidation of endogenous as well as xenobiotic compounds and drugs is catalyzed by microsomal UGTs (8). This enzyme family shows an extensive heterogeneity, as expected of a multigene family (9). In addition, UGT isozymes frequently exhibit broad and sometimes overlapping substrate specificities towards xenobiotic substrates (10). Many factors such as genetic identity, age, disease, drug treatment, or exposure to other xenobiotic inducers can influence the level of UGT isoenzyme expression in different organs (10). It is, therefore, difficult to identify and characterize the catalytic activity of single UGT isozymes for a given drug on the basis of *in vitro* incubations with microsome preparations or by differential induction *in vivo*. Recently, several human UGT-encoding cDNA clones have been isolated and characterized. Subsequently, cell lines stably expressing a defined UGT isozyme have been established and used for investigations on the glucuronidation of a variety of substrates (11, 12).

Here we report on the glucuronidation of ethinylestradiol by two different UGTs of the human *UGT1* gene family. HP3 is an isozyme that catalyzes the glucuronidation of bilirubin, whereas HP4 is capable of catalyzing the glucuronidation of a variety of complex and simple phenols but not bilirubin. *In vitro* experiments were performed by using Chinese hamster fibroblast (V79) cell lines stably expressing single UGTs as well as liver and kidney microsomes from different individuals. In addition, the relationship of ethinylestradiol glucuronidation to the well established genetic defect of bilirubin glucuronidation was examined.

Materials and Methods

Liver samples used in this study have been described in detail elsewhere (13, 14). Liver 5 was kindly donated by Dr. D. Burke,

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ABBREVIATIONS: UGT, UDP-glucuronosyltransferase; UDPGA, UDP-glucuronic acid; HPLC, high performance liquid chromatography.

University of Aberdeen, and originated from a patient who was receiving treatment with phenytoin. The human kidney sample originated from a patient treated with thioridazine and was a gift of Dr. J. Dick, University of Dundee. Ethinylestradiol, ethinylestradiol-17β-glucuronide, bovine liver β-glucuronidase (type B-1), saccharo-1,4-lactone, UDPGA, Lubrol PX, and other compounds used as substrates were purchased from Sigma (Gillingham, Dorset, UK), 17α-[6,7-^{3}H]Ethinylestradiol (44.5 mCi/μmol) was obtained from NEN (NEN DuPont UK Ltd., Stevenhage, Hertfordshire, UK). Chinese hamster fibroblasts (V79) stably expressing HP1, HP3, and HP4 and Swiss mouse fibroblasts (NIH3T3) stably expressing H6 (UGT2B7) have been described elsewhere (15–18). Cells were grown in Dulbecco's modified Eagle's medium (GIBCO, Life Technologies, Paisley, UK) supplemented with 10% fetal bovine serum (GIBCO), 100 units/ml penicillin, and 0.1 mg/ml streptomycin. Cell homogenates of cultured cell lines and human liver and kidney microsomes were prepared as described (19). Protein concentrations were determined by the method of Lowry *et al.* (20).

Cells containing expressed UGT isozymes were scraped from tissue culture dishes, lysed by addition of distilled water, and subjected to three freeze-thaw cycles to ensure cell lysis. Ethinylestradiol and bilirubin glucuronidation in microsomes were assayed by methods described previously (4, 21). Incubations with cell homogenates or microsome preparations were performed as follows: 150 mg of microsomes or tissue culture cell homogenates were incubated in 100 μM Tris·HCl buffer (pH 7.5) containing 20 μM $MgCl_2$, 5 μM UDPGA, and 0.1–0.4 mCi of 17α-[6,7-^{3}H]ethinylestradiol, in a total volume of 200 μl, for 30 min at 37°. The concentration of ethinylestradiol ranged from 10 to 1000 μM, added in 20 μl of methanol/propylene glycol (1:1). Microsomes were preincubated for 15 min on ice with an optimally activating concentration of Lubrol PX (typically 0.1–0.2 mg/mg of protein). Reactions were then started by transfer to the 37° water bath and addition of UDPGA after 2 min of temperature equilibration.

Seven hundred micrograms of microsomal protein or tissue culture cells were used for assays of bilirubin glucuronidation. The incubation time was 60 min at 37° and the bilirubin concentration in inhibition experiments was 50 μM. An equal volume of the solvent used to dissolve ethinylestradiol was added to control incubations. Glucuronidation of 2,6-diisopropylphenol was measured by a thin layer chromatographic method (22, 23); plates were scanned for radioactive product spots in a Berthold digital autoradiograph (Berthold, Wildbad, Germany). Estriol and 1-naphthol glucuronidation was assayed as described previously (24, 25). Because of small tissue sample size, enzyme kinetic parameter and inhibition experiments could not be performed with all 12 liver samples. For the same reason enzyme kinetic parameters for ethinylestradiol glucuronidation by kidney microsomes were calculated from a single set of incubations. V_{max} and K_m were calculated by a computerized iterative curve-fitting program (26). A one-enzyme model was assumed for the calculation of enzyme kinetic parameters.

Regioselectivity for the glucuronidation of ethinylestradiol at positions 3-OH and 17-OH was determined by reverse phase HPLC. [^{3}H] Ethinylestradiol was incubated with HP3 cell homogenates and a human liver microsomal sample as described above, with the following modifications. Incubations (250 μl) contained 2 mg of protein/ml and were conducted for 45 min at 37°. Reactions were stopped by the addition of 100 μl of trichloroacetic acid/glycine buffer and were centrifuged at 13,000 × *g* for 2 min. The supernatant (300 μl) was loaded onto a 100-mg C18 Bond-Elut solid-phase extraction column (Varian, Harbor City, CA) that had been previously washed with 1.0 ml of methanol and 1.0 ml of H_2O. After elution of the supernatant, the column was washed with 1.0 ml of H_2O and then >99% of the radioactivity was eluted with 1.0 ml of methanol. The methanol fraction was evaporated to dryness under N_2 at 40° and was reconstituted in 100 μl of HPLC mobile phase (acetonitrile/0.05 M ammonium acetate buffer adjusted to pH 4.5 with acetic acid, 1:3). An aliquot (50 μl) was injected onto a 5-μm Spherisorb C8 column (250 × 4.6 mm; Phase Separations, Ltd., Clwyd, UK), and 0.5-min fractions (0.6 ml) were collected and counted in 4 ml of Emusifier-Safe cocktail (Packard, Groningen, Netherlands) by using a Beckman LS 700 liquid scintillation counter.

Ethinylestradiol, ethinylestradiol-3β-glucuronide, and ethinylestradiol-17β-glucuronide were separated by gradient elution as follows. Initial conditions (25% acetonitrile/75% 0.05 M ammonium acetate, pH 4.5) were held for 5 min, followed by a linear gradient from 25% to 60% acetonitrile over 25 min. Initial conditions were reestablished after a 5-min hold at 60% acetonitrile. Unlabeled standards for ethinylestradiol and ethinylestradiol-17β-glucuronide eluted at 26 min and 9.9 min, respectively, and were detected at 280 nm.

Results

Glucuronidation of ethinylestradiol by cloned and expressed human liver UGTs. Ethinylestradiol glucuronidation was assayed with four different cell homogenates (HP1, HP3, HP4, and H6) containing stably expressed human UGT isozymes at a fixed ethinylestradiol concentration of 500 mM. HP1 and H6 isozymes did not catalyze ethinylestradiol glucuronidation; however, ethinylestradiol glucuronide was formed in incubations with stably expressed HP3 or HP4 UGTs (Table 1). Thus, two liver-derived UGT enzymes recognized as specifically catalyzing the glucuronidation of bilirubin (HP3) and an enzyme catalyzing the glucuronidation of bulky phenols (HP4), such as 2,6-diisopropylphenol (propofol), also catalyzed the glucuronidation of ethinylestradiol.

HP3-catalyzed glucuronidation of ethinylestradiol was regiospecific for the 3-position. As shown in Fig. 1, a peak that eluted at 6.2 min was observed only in complete incubations containing 5 mM UDPGA. Control incubations lacking UDPGA failed to produce this peak. This peak (6.2 min) was presumed to be the 3-glucuronide of ethinylestradiol, based on the following observations. 1) Radioactivity produced from [^{3}H] ethinylestradiol incubated with homogenates from V79 cells containing expressed HP3 coeluted with the 6.2-min peak. 2) Incubation of beef liver β-glucuronidase (500 units/ml) and a solid-phase extract from an [^{3}H]ethinylestradiol incubation, for 16 hr in pH 4.5 acetate buffer, resulted in complete loss of the 6.2-min peak (and associated radioactivity). Incubation of the extract with β-glucuronidase plus 10 μM saccharo-1,4-lactone (a β-glucuronidase inhibitor) did not result in hydrolysis. 3) The radioactivity produced at 6.2 min eluted before authentic ethinylestradiol-17β-glucuronide (9.9 min). Literature citations concerning the separation of steroidal glucuronides reported that 3-glucuronides elute before 17-glucuronides on reverse phase HPLC columns (27, 28). Upon incubation of [^{3}H]ethinylestradiol with HP3 cell homogenates and UDPGA, no radioactivity was observed to elute at a retention time that

TABLE 1

Enzyme activities of different cloned and expressed human UGTs

Incubation was at fixed substrate concentrations, shown in parentheses. All other assay details are described in Materials and Methods.

Substrate	UGT isozyme activities			
	HP1	HP3	HP4	HP6
	nmol/min/mg of protein			
1-Naphthol (0.5 mM)	3.2	0.3	0.2	ND[a]
Bilirubin (0.122 mM)	ND	0.4	ND	ND
2,6-Diisopropylphenol (0.5 mM)	ND	ND	0.9	ND
Estriol (0.5 mM)	ND	0.05	ND	0.06
Ethinylestradiol (0.5 mM)	ND	0.35	0.14	ND

[a] ND, not detected.

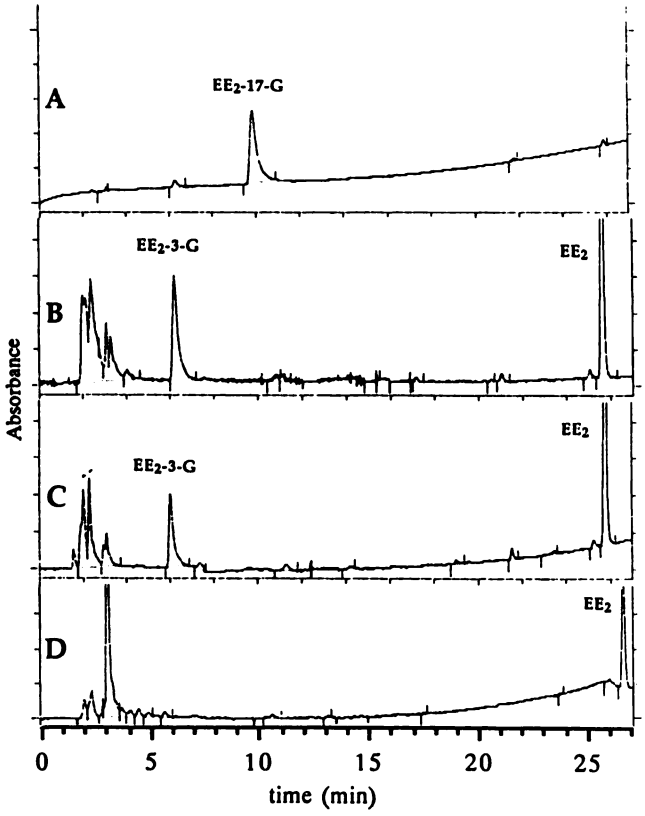

Fig. 1. HPLC traces for solid-phase extracts from incubations of ethinylestradiol (*EE*$_2$) with V79 cell homogenate containing expressed HP3 and with human liver microsomes. A, Authentic standard of ethinylestradiol-17β-glucuronide (*EE*$_2$-17-G; retention time, 9.9 min); B, extract of incubation of ethinylestradiol with cell homogenate containing expressed HP3 (*EE*$_2$-3-G, ethinylestradiol-3β-glucuronide; retention time, 6.2 min); C, extract of incubation of ethinylestradiol with human liver microsomes; D, extract of incubation of ethinylestradiol with human liver microsomes, treated with β-glucuronidase.

corresponded to authentic ethinylestradiol-17β-glucuronide. In a human liver microsomal incubation, a small amount of radioactivity eluted at a retention time corresponding to the 17-glucuronide (9.9 min), but the ratio of 3-glucuronide to 17-glucuronide production was 97:3 at 200 μM ethinylestradiol. These experiments demonstrated that the exclusive product generated by UGT HP3 and the predominant product formed in incubations with human liver microsomes was the 3-glucuronide. The apparent kinetic parameters towards ethinylestradiol were determined in recombinant cells and a variety of human samples in a further assessment of the relative contributions of HP3 and HP4 towards glucuronidation of this substrate.

Kinetic analysis of ethinylestradiol glucuronidation by recombinant cells and human liver microsomes. UGT enzyme kinetic parameters towards ethinylestradiol were determined from incubations with UGT HP3 and HP4 cell homogenates, with 10 different human liver microsomal samples, and with one kidney microsomal fraction. The ethinylestradiol concentration was varied between 10 and 1000 μM for the determination of enzyme kinetic parameters in detergent-treated microsomes. The optimal Lubrol PX concentration was individually determined for each liver sample to achieve full enzyme activation. Moderate increases in enzyme activities (1.05–1.5-fold) were observed with most microsome samples and optimal Lubrol PX concentrations were 0.1–0.2 mg/mg of protein. However, some microsomal samples (samples 1, 5, and Crigler 1) showed no activation, and addition of Lubrol PX led to a reduction of enzyme activity in these samples. The data were used to calculate kinetic parameters on the basis of a one-enzyme model and are presented as representative Eadie-Hofstee plots in Fig. 2, with a summary of all the data in Table 2.

Linear kinetics were observed, suggesting a major role for a single enzyme in all tissue preparations examined. The V_{max}/K_m was 3.2-fold higher with HP3 cell homogenate than with HP4 cell homogenate, suggesting that the major enzyme responsible for glucuronidation of ethinylestradiol may be the bilirubin UGT (HP3). This calculation assumes that HP3 and HP4 are expressed to a similar extent in cells (see Discussion).

A correlation of the rates of glucuronidation of ethinylestradiol and bilirubin was performed in a panel of seven "normal" liver microsomal samples. A highly significant correlation ($r^2 = 0.94$, $p < 0.001$) of the two glucuronidation reactions was observed (Fig. 3). The correlation was still highly significant ($r^2 = 0.83$) when the highest activity sample (human liver 5) was excluded from regression analysis. Four liver microsomal samples from Crigler-Najjar patients were also examined. Bilirubin glucuronidation was not detected in these liver microsomes and the ethinylestradiol glucuronidation was considerably reduced (Table 2).

The V_{max}/K_m values in Crigler-Najjar microsomes were significantly lower than those calculated for normal liver samples ($p < 0.05$; $p < 0.03$ when data obtained for liver 1 were omitted;

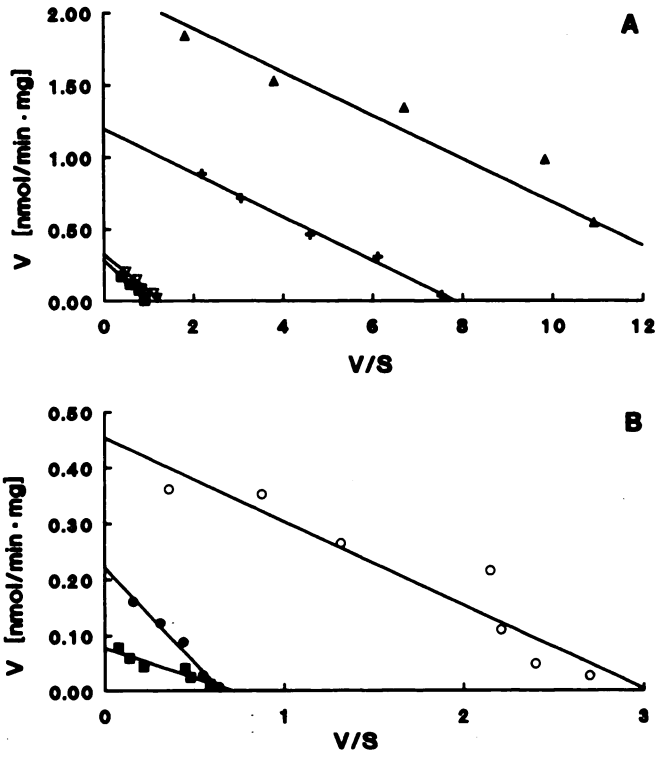

Fig. 2. Eadie-Hofstee plots of ethinylestradiol glucuronidation by expressed human UGT isoenzymes and microsomes from human liver and kidney. The assay was performed as described in the text, with a range of ethinylestradiol concentrations from 10 to 100 μM and 5 mM UDPGA. *Lines* were generated by linear regression analysis. A, Human liver microsomal samples 1 (■), 2 (+), and 5 (▲) and human kidney microsomes (▽). B, Human UGTs HP3 (○) and HP4 (●) and a representative microsomal sample from a Crigler-Najjar patient (■).

TABLE 2

Enzyme kinetic analysis of UGT activities towards ethinylestradiol in recombinant cells, human liver and kidney microsomal preparations

The numbers of experiments are indicated in parentheses. K_m, V_{max}, and standard errors were calculated by computerized curve fitting of mean values of multiple sets of incubations.

Liver sample/ cell line	V_{max}	K_m	V_{max}/K_m
	pmol/min/mg	*μM*	*ml/min/mg*
Cri[a] 1 (4)	77 ± 6.9	115 ± 24	0.67
Cri 2 (2)	173 ± 7.4	167 ± 16	1.04
Cri 3 (2)	113 ± 10	93 ± 18	1.22
Cri 4 (3)	36 ± 1.5	93 ± 13	0.39
1 (3)	348 ± 18	278 ± 27	1.25
2 (2)	1342 ± 135	172 ± 32	7.80
3 (2)	718 ± 62	179 ± 34	4.01
4 (3)	1163 ± 20	128 ± 77	9.09
5 (3)	2058 ± 115	121 ± 17	17.01
6 (3)	547 ± 19	158 ± 16	3.46
Kidney (1)	289 ± 14	281 ± 27	1.03
HP3 (3)	297 ± 34	130 ± 23	2.28
HP4 (3)	207 ± 10	284 ± 29	0.73

[a] Cri, Crigler-Najjar.

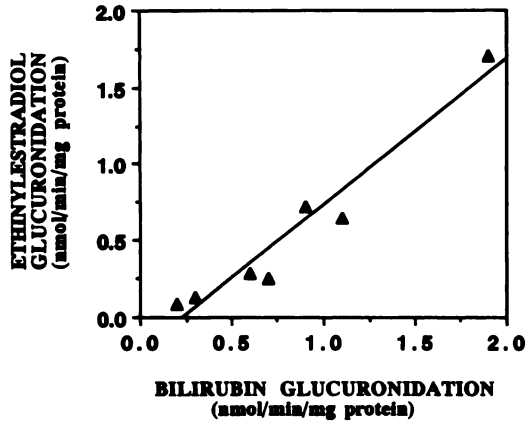

Fig. 3. Correlation of bilirubin glucuronidation with ethinylestradiol glucuronidation by human liver microsomes. Microsomal samples obtained from seven different normal human livers were assayed for the rate of glucuronidation, as described in Materials and Methods, with 500 μM ethinylestradiol and 120 μM bilirubin.

unpaired *t* test). Thus, data obtained from kinetic analyses and liver samples with a genetic defect in bilirubin glucuronidation strongly indicate that human bilirubin UGT is a major catalyst of ethinylestradiol glucuronidation.

Inhibition by ethinylestradiol of bilirubin glucuronidation. The effect of various concentrations of ethinylestradiol on the ability of human liver microsomes and HP3 cell homogenates to glucuronidate bilirubin was determined to further assess the role of bilirubin UGT as a potential catalyst of ethinylestradiol glucuronidation. Ethinylestradiol caused a marked inhibition of bilirubin glucuronidation, such that >50% of the activity was inhibited at equimolar concentrations of inhibitor and substrate. More than 80% of the bilirubin UGT activity was inhibited by 0.4 μM ethinylestradiol in three liver microsomal samples and in HP3 cell homogenates (Fig. 4). Microsomes from liver 1 exhibited only a slight inhibition of bilirubin UGT by ethinylestradiol. This exceptional result was not easily explained and is discussed below. Nonetheless, the data convincingly demonstrated that bilirubin UGT was dramatically inhibited by ethinylestradiol, providing further evidence that HP3 UGT is primarily responsible for glucuronidation of this synthetic steroid derivative.

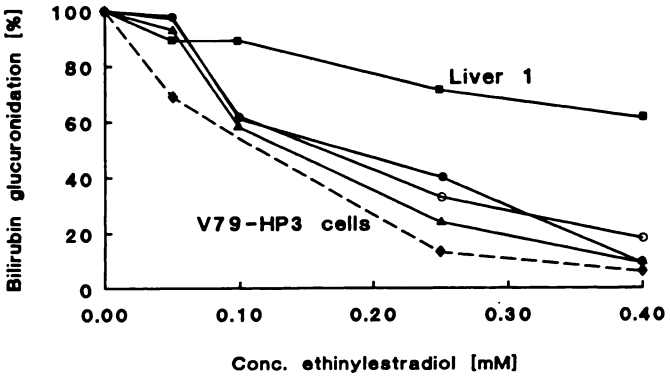

Fig. 4. Inhibition of human liver bilirubin glucuronidation by ethinylestradiol. Bilirubin glucuronidation by human liver microsomes and UGT HP3 homogenate was assayed in the presence of various levels of ethinylestradiol up to 0.4 mM, with 120 μM bilirubin as substrate and 5 mM UDPGA. Data using liver microsomal samples 1 (■), 2 (●), 3 (○), 4 (▲) and V79-HP3 cell (◆) homogenate are shown as indicated on the diagram.

Discussion

Our results with stably expressed UGTs indicate the involvement of at least two different isozymes, HP3 (a bilirubin UGT) and HP4 (a phenol UGT), in the glucuronidation of the synthetic steroid ethinylestradiol. Surprisingly, the steroid UGT H6 was not capable of catalyzing ethinylestradiol glucuronidation. Both HP3 and HP4 are members of the *UGT1* subfamily, a group of gene products that probably originate from alternative splicing of a single gene located on chromosome 2 (29, 30). HP3 is known to be one of the major enzymes catalyzing bilirubin glucuronidation (17, 31), whereas HP4 has been shown to glucuronidate a variety of compounds including both endogenous and xenobiotic substrates (12). Both enzymes were discovered by screening of human liver cDNA libraries by hybridization to ^{32}P-labeled HP1 and HP2 DNA probes, two other members of the *UGT1* gene family (16, 17). mRNA for HP3 and HP4 isozymes can also be detected in human liver by Northern blotting (14). Although both isozymes are present in normal human liver, examination of enzyme kinetic parameters determined in different liver samples did not reveal biphasic enzyme kinetics, in agreement with previously published work (4).

Biphasic enzyme kinetics in microsomal samples have been interpreted as support for the existence of two different isozymes for substrates such as naphthol and morphine (11), especially when there are large differences in the apparent K_m values. Apparent linear kinetics may be observed when the V_{max}/K_m ratios are similar or if one isozyme is expressed at much higher levels in tissue, thus masking a biphasic reaction.

Because the kinetic parameters were based on an assay that measured the rate of production of total radioactive glucuronides (nonextractable radioactivity), the regioselectivity of human liver microsomes and HP3 for positions 3-OH and 17-OH of ethinylestradiol was determined by reverse phase HPLC. The only product formed by HP3 and the predominant product from human liver microsomes was ethinylestradiol-3β-glucuronide, representing conjugation at the phenolic hydroxyl as opposed to the aliphatic 17-OH position. Thus, kinetic param-

eters determined by the widely used extraction assay method appear to reflect the rate of 3-glucuronidation in human liver samples. Examinations of the metabolic profile of ethinylestradiol in the urine of animals and humans have generally measured the production of ethinylestradiol by difference, i.e., after enzymatic hydrolysis. Separation of conjugate fractions in urine by open-column Sephadex LH-20 chromatography suggested that both the 17β- and 3β-glucuronides of ethinylestradiol were present in human urine (32). In baboon plasma, only ethinylestradiol-3β-glucuronide was found after administration of [^{3}H]ethinylestradiol (28). Our data for human liver microsomes suggest that the 17-glucuronide may be a minor metabolite of ethinylestradiol and that this reaction is catalyzed by a UGT other than HP3.

One interpretation of our results is that a single bilirubin UGT (HP3) is largely responsible for glucuronidation of ethinylestradiol. HP3 is a major hepatic UGT enzyme with an important role in the detoxication of bilirubin. Measurement of mRNA levels for two human bilirubin UGTs (HP2 and HP3) and two human phenol UGTs (HP1 and HP4) showed that HP3 mRNA was expressed at a higher level than was HP2 mRNA and was inducible by phenytoin and phenobarbital (14). Further, HP4 mRNA was poorly expressed in human liver but highly expressed in human kidney. HP2 and HP3 mRNAs were not detectable in human kidney (14).

This independent analysis indicates that HP3 UGT is a predominant enzyme present in human liver. This cloned expressed UGT catalyzed the glucuronidation of ethinylestradiol. The strong correlation between hepatic microsomal ethinylestradiol glucuronidation and bilirubin glucuronidation supports the hypothesis that HP3 UGT plays a predominant role in hepatic ethinylestradiol glucuronidation. Further, inhibition by ethinylestradiol of hepatic microsomal bilirubin glucuronidation in V79 HP3 cell homogenate was similar to results with three of the four liver samples examined.

Results obtained with liver sample 5, which originated from a patient undergoing phenytoin treatment, showed that ethinylestradiol glucuronidation was inducible by phenobarbital-type inducers in humans, as indicated previously (4), and induction correlated with the induction of bilirubin UGT and HP3 mRNA in this liver sample (14).

UGT HP3 exhibited a 2-fold higher affinity for ethinylestradiol than did UGT HP4, and the catalytic potential (V_{max}/K_m) was >3-fold greater in the HP3-expressing cell line. Comparison of the measured velocities in V79 cell homogenates containing HP3 and HP4 was based on the assumption that the level of UGT expression per mg of cell homogenate was equivalent for the two cell lines. This assumption was supported by a previous Western blotting experiment with a well characterized anti-rat liver UGT antibody, which indicated a similar level of expression of immunoreactive protein in the two cell lines (17). This antibody preparation has a broad specificity and recognizes multiple rat and human UGTs in liver and kidney. Furthermore, immunoinhibition experiments have revealed that this antibody inhibits the glucuronidation of a variety of substrates to a similar extent (33), suggesting that the epitopes recognized by this polyclonal antibody are in the highly conserved constant region of UGTs. Because HP3 and HP4 are members of the *UGT1* gene family whose substrate-specific exons are spliced to the same constant region exon at the RNA level (29, 30), an antibody directed to the constant regions should cross-react with HP3 and HP4 to equal extents. Based on this evidence of similar expression in V79 cells, the higher catalytic potential of HP3, combined with a higher level of HP3 mRNA expression in liver, indicates that UGT HP3 is the predominant enzyme involved in 3-glucuronidation of ethinylestradiol in human liver.

The contribution of UGT HP4 to ethinylestradiol metabolism might be assessed by examination of the enzyme activity data from human kidney and Crigler-Najjar livers, where bilirubin activities were not detectable. The kidney sample in this study, however, showed HP4 mRNA levels and UGT activity towards 2,6-diisopropylphenol that were higher than all the results obtained for liver samples (14). This indicates that the ethinylestradiol glucuronidation by kidney microsomes was catalyzed by highly expressed HP4.

The catalytic potential (V_{max}/K_m) of the kidney preparation for ethinylestradiol was approximately 1.0 μl/min/mg and was therefore approximately 6-fold less than the average catalytic potential of normal livers excluding liver 1 and liver 5. Thus, the estimated contribution of UGT HP4 in liver towards ethinylestradiol glucuronidation would be <20%. UGT activity towards 2,6- diisopropylphenol (HP4 enzyme) was very low or nondetectable in the four Crigler-Najjar liver preparations[2] and the average catalytic potential of these tissues was approximately 0.8, a value 8-fold lower than the normal liver catalytic potential. Therefore, because both HP4 and HP3 UGTs are essentially absent in Crigler-Najjar liver preparations, other UGTs could be considered to contribute to ethinylestradiol glucuronidation. This contribution should be estimated at <10%, because two of the Crigler-Najjar patients had been treated with barbiturates, which induced phenobarbital-responsive enzymes (e.g., estriol UGT) >2-fold above normal,[2] thereby overestimating this contribution.

Earlier studies with rat liver tissue (34) showed that bilirubin glucuronidation was inhibited by ethinylestradiol *in vitro*. Our data with human liver microsomes have extended and confirmed this observation. Inhibition by ethinylestradiol of bilirubin glucuronidation *in vivo* may be significant, especially in susceptible individuals exhibiting lower abilities to glucuronidate bilirubin, such as in Gilbert's disease. In fact, there are reports of elevated bilirubin plasma levels after treatment with ethinylestradiol (35). The work repeated here confirmed the wide variability of ethinylestradiol glucuronidation catalyzed by human liver microsomes observed in previous studies (7). In liver samples from individuals with low bilirubin conjugation activity one might expect that UGT HP4 would be more important for elimination of ethinylestradiol. This speculation may be illustrated by our observations with liver sample 1. Microsomes prepared from this liver sample exhibited impaired glucuronidation of bilirubin and ethinylestradiol. In contrast to the other normal livers, an elevated K_m value was observed for ethinylestradiol glucuronidation, similar to that found in HP4 cell homogenates. Furthermore, ethinylestradiol inhibited bilirubin glucuronidation to a smaller extent with liver sample 1. Glucuronidation of 1-naphthol, 4-methylumbelliferone, and 2,6-diisopropylphenol was not impaired (14), thereby excluding a general degradation of the microsome preparation. It is possible that this liver is deficient in functional HP3 protein. The inhibitory effects of ethinylestradiol *in vivo* are dependent on

[2] L. Sutherland, T. Ebner, and B. Burchell, unpublished observations.

the balance between the level of bilirubin UGT activity and the doses of ethinylestradiol.

We conclude that a bilirubin UGT (HP3) is a major human enzyme responsible for the 3-glucuronidation of ethinylestradiol. The type and amount of glucuronide metabolite formed may be important to our knowledge of the adverse drug reactions where glucuronides of ethinylestradiol have been implicated as cholestatic agents (35, 36). Vore *et al.* (36) have demonstrated that the 17β-glucuronide of ethinylestradiol is cholestatic, whereas the ethinylestradiol-3-glucuronide is inactive as a cholestatic agent. Further work will be required to identify the UGTs responsible for 17-glucuronidation and their possible role in cholestasis. In addition, future work on the interindividual variability of single UGT isozymes may provide an explanation of the marked variability of ethinylestradiol glucuronidation and perhaps allow one to predict the susceptibility of individuals to adverse drug reactions on treatment with ethinylestradiol.

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