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Differential glucuronidation of bile acids, androgens and estrogens by human UGT1A3 and 2B7

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Abstract

In this work, UDP-glucuronosyltransferases (UGTs), UGT1A3, 2B7(H268) and 2B7(Y268), stably expressed in human embryonic kidney cells (HK293) were used to assess glucuronidation activities with a variety of steroid hormone and bile acid substrates. The rate of synthesis of carboxyl- and hydroxyl-linked glucuronides was determined under optimal reaction conditions. Expressed UGT1A3 catalyzed bile acid glucuronidation at high rates exclusively at the carboxyl moiety for all compounds tested. In contrast, UGT1A4 catalyzed bile acid glucuronidation at very low rates exclusively at the 3α-hydroxyl function. Both UGT2B7 allelic variants glucuronidated the bile acid substrates at both carboxyl and hydroxyl moieties, however, the 3α-hydroxyl position was preferentially conjugated compared to the carboxyl function. Similarly, androsterone, a 3α-hydroxylated androgenic steroid, was glucuronidated at very high rates by expressed UGT2B7. Of the estrogenic compounds tested, UGT2B7 catalyzed the glucuronidation of estriol at rates comparable to those determined for androsterone. Other structural discrimination was found with UGT2B7 which had activity toward estriol and estradiol exclusively at the 17β-OH position, yielding the cholestatic steroid D-ring glucuronides. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: UDP-glucuronosyltransferase; Glucuronidation; Bile acids; Androgens and estrogens

1. Introduction

UDP-glucuronosyltransferases (UGTs) (E.C. 2.4.1. 17), which are anchored in the membrane of the endoplasmic reticulum, catalyze the conjugation of endobiotics and xenobiotics with D-glucuronic acid derived from UDP-glucuronic acid. The polar conjugated products of this reaction are readily excreted into urine and/or bile [1]. The superfamily of UGT enzymes is divided into 3 families, based on evolutionary divergence. Three of the gene families have been shown to be expressed in humans, UGT1, UGT2 and UGT8 [2]. The UGT1A gene is localized on chromosome 2 and encodes proteins with unique N-terminal domains and identical C-terminal domains which are formed from alternate mRNA splicing of unique first exons with common exons 2–5 [3]. In contrast, the UGT2B genes are clustered on chromosome 4 and individual UGT proteins are encoded by unique genes having six exons [2].

Glucuronidation normally is considered a detoxification process, however, under pathophysiological conditions, steroid D-ring glucuronides (e.g. testosterone-

Abbreviations: UDP-GlcUA, UDP-glucuronic acid; UGTs, UDP-glucuronosyltransferases; HK293 cells, human embryonic kidney 293 cells; BA, bile acids; LA, lithocholic acid; HDCA, hyodeoxycholic acid; C20αβ, 3α-hydroxyetianic acid; C20ββ, 3β-hydroxyetianic acid.

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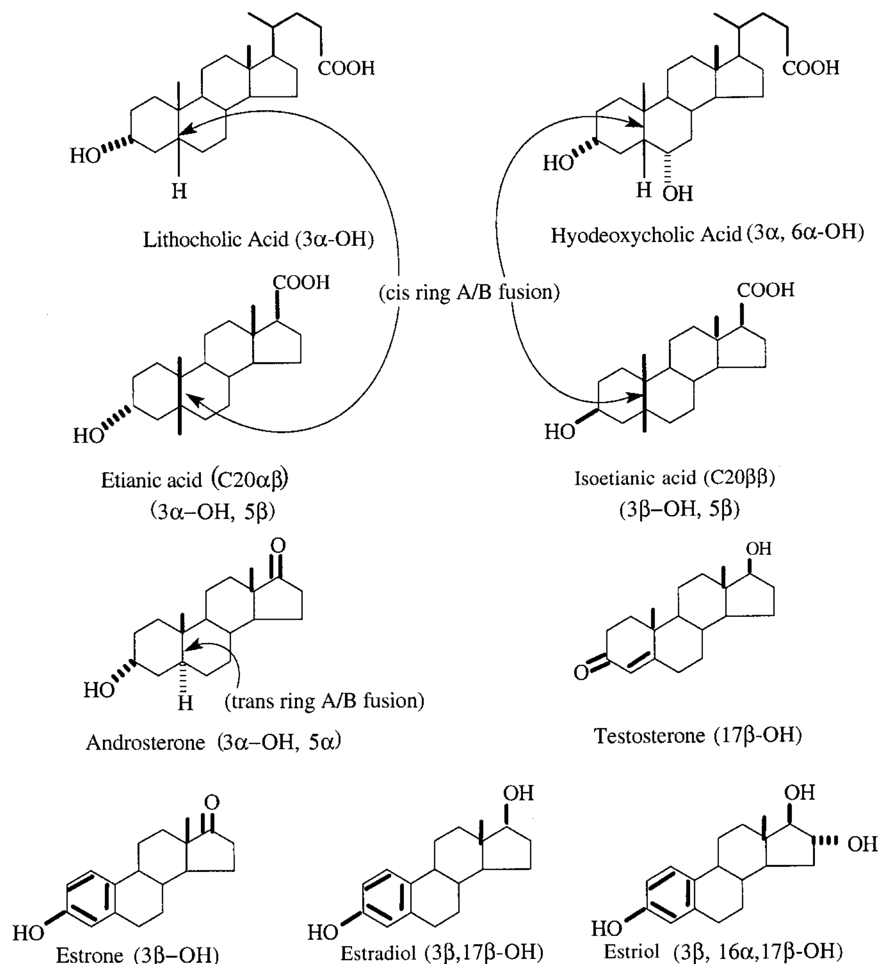

Fig. 1. Structure of substrates involved in glucuronidation: bile acids, lithocholic acid (LA) and hyodeoxycholic acid (HDCA); short chain bile acids, etianic acid (C20αβ) and isoetianic acid (C20ββ); steroid hormones, androsterone, testosterone, estrone, estradiol and estriol.

17β-(β-glucuronide), dihydrotestosterone-17β-(β-glucuronide), estradiol-17β-(β-glucuronide), estriol-16α-(β-glucuronide) and estriol-17β-(β-glucuronide)) are considered toxic metabolites which cause cholestasis [4]. Cholestasis can be described as the stagnation of bile flow, thus interfering with the efficient excretion of metabolites from endogenous and exogenous sources and secretion of bile acids (BA). The naturally occurring BA, lithocholic acid, certain estrogens and anabolic steroids are known to induce cholestasis [5]. The potent cholestatic nature of the D-ring glucuronides of estradiol, estriol and ethynylestradiol in animal models has been suggested as a potential cause of hepatic toxicity seen in pregnancy and with oral contraceptive use [6]. On the other hand, steroids with the glucuronic acid moiety on the A-ring, such as estradiol-3β-(β-glucuronide) and estriol-3β-(β-glucuronide), are choleretic rather than cholestatic in experimental animals [7,8]. Therefore, characterization of the reactivity of UGTs with these compounds is important.

Many cloned and expressed human UGTs have been shown to catalyze glucuronidation of steroids and BA, as well as numerous xenobiotics. The two allelic variants of UGT2B7 have been shown to be involved in the glucuronidation of a large variety of endogenous and exogenous substrates including BA, estrogens, androgens, opioids, NSAIDs and fibrates [9–11]. Expressed UGT2B15 was shown to glucuronidate testosterone and dihydrotestosterone, but had low glucuronidation activity toward catechol estrogens and did not conjugate androsterone or BA [12,13]. Expressed human UGT1A4 has been shown to glucuronidate androgens at very low rates and it has been suggested that progestins may be the preferred substrates for this isoform [14]. Most recently, human UGT1A3 and 1A8 have been shown to catalyze glucuronidation of estrone and estrogen catechols, however, the ability of these isoforms to glucuronidate BA has not been examined [15,16]. With the exception of expressed UGT2B4, which was shown to glucuronidate hyodeoxycholate at the 6α-hydroxyl position [17], the specific site of glucuronidation of steroids and BA by

other expressed human UGTs has not been demonstrated.

The purpose of the present study was to examine the ability of expressed human UGTs to catalyze glucuronidation of certain steroids and BA and to elucidate the functional groups on BA and steroids that are the sites of conjugation. Two novel approaches were undertaken: (1) expression of UGTs in the same cell line and optimization of glucuronidation conditions, (2) substrate structure/activity relationship studies of the UGTs with a variety of substrates possessing 3α/3β, 16α and/or 17β hydroxyl groups, carboxyl groups, aromatic rings and 5α/β A/B ring junctions. Our studies were designed to examine the glucuronidation activity catalyzed by expressed UGTs on steroid hormone (androgens and estrogens) and various BA substrates in order to determine similarities and differences between the two UGT families in regard to structural conjugation specificity. The results show that certain UGT isoforms are very specific for their ability to conjugate specific sites on steroids and BA (see structures in Fig. 1).

2. Materials and methods

[^{3}H]-Hyodeoxycholic acid (HDCA; 3α,6α-dihydroxy-5β-cholanoic acid) was synthesized as described in [18,19] and was checked for chemical purity by thin-layer chromatography (TLC) and gas-liquid chromatography. [^{3}H]-3α-hydroxyetianic acid (C20αβ; 3α-hydroxy-5β-androstan-17β-carboxylic acid) and [^{3}H]-3β-hydroxyetianic acid (C20ββ; 3β-hydroxy-5β-androstan-17β-carboxylic acid) were synthesized as described previously [20]. Unlabeled C20αβ and C20ββ were purchased from Steraloids (Newport, RI). [^{3}H]-Androsterone, [^{3}H]-testosterone and [^{3}H]estradiol were from Dupont-New England Nuclear (Boston, MA), [^{14}C]-lithocholic acid (LA; 3α-hydroxy-5β-cholanoic acid) and [^{14}C]-estrone were from Amersham (Arlington Heights, IL) and [^{14}C]UDP-GlcUA was purchased from American Radiolabel Chemicals (St. Louis, MO). Brij 58, UDP-glucuronic acid (UDP-GlcUA), saccharolactone, unlabeled HDCA, LA, androsterone (A), testosterone (T), estrone (E_1), estradiol (E_2), estriol (E_3) and standards β-estradiol 17-(β-D-glucuronide), estriol 3-(β-D-glucuronide), estriol 16α-(β-D-glucuronide), estriol 17β-(β-D-glucuronide) were purchased from Sigma (St. Louis, MO). All other reagents were of the highest grade commercially available.

2.1. Human liver microsomes

The human liver microsomes used in these studies (HL15, from a 56 year old male who died from cerebral bleeding) were obtained from the University of Groningen, Groningen, The Netherlands. This liver sample was chosen because previous studies had shown that BA and steroid glucuronidation activities obtained using this sample were typical of the 'average' results obtained using many liver samples. Human liver microsomes served as a control for the glucuronidation assays on expressed proteins and as a basis of comparison for the metabolites formed.

2.2. Expression of cDNAs in HK293 cells

The development of HK293 cell lines stably expressing human UGT1A1, 1A4, 2B7(H268) and 2B7(Y268) have been described previously [11,14,21,22]. HK293 cell lines stably expressing these isoforms were grown in media containing 700 μg geneticin/ml. UGT1A3 was transiently expressed in HK293 cells as described by Green et al. (1998) [23]. For all UGTs, enriched membrane fractions of endoplasmic reticulum were obtained according to the protocol described by Battaglia et al. (1994) [24]. Membrane fractions were stored at −80°C in 5 mM Hepes, 0.25 M sucrose, 20 mM $MgCl_2$ (pH 7.4). No decrease in the enzymatic activity of the recombinant UGT protein was observed for up to 6 months under these conditions.

2.3. Enzyme assays

UGT activity was measured with radioactive and unlabeled BA and steroid hormones as aglycones and UDP-GlcUA as the sugar donor, as previously described [18,19]. All BA and steroid substrates were prepared in the form of mixed micelles with Brij 58 (final concentration of detergent in the reaction mixture, 0.05%), as described in detail in [18]. For the assays, microsomal fractions and recombinant UGT proteins (approximately 50 μg protein) and the BA or steroid substrates **(0.1 mM final concentration)** were incubated in a total volume of 60 μl including the following reaction components: 100 mM HEPES-NaOH buffer, pH 7.5 (pH 6.5 for C20αβ and pH 8.0 for steroids), 5 mM $MgCl_2$, 5 mM saccharolactone, 0.05% Brij 58. Reactions were started by adding 50 mM UDP-GlcUA (4.17 mM final concentration) for radioactive substrates or 20 mM [^{14}C]-UDP-GlcUA (3.33 mM final concentration) for unlabeled substrates. The reactions were incubated at 37°C for 10–30 min. The reactions were stopped by adding 20 μl ethanol, vortexing and putting on ice. 60 μl of the reaction mix was then applied directly to the preadsorbent layer of 19-channeled silica gel TLC plates (Baker 250Si-PA (19C); VWR Scientific), dried and developed twice in chloroform/methanol/glacial acetic acid/water (65:25:2:4, v/v) or in ethanol/ethyl acetate/concentrated ammonium hydroxide (45:45:15, v/v). Hydroxyl- and

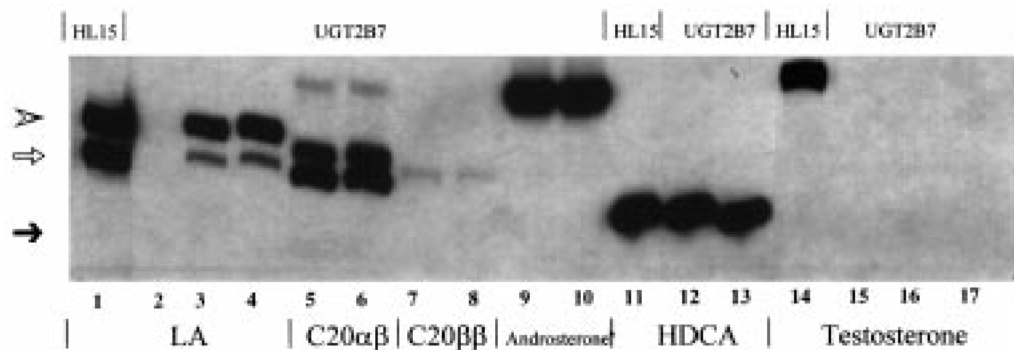

Fig. 2. Glucuronidation of ^{14}C-Lithocholic acid (lanes 1–4), ^{3}H-C20αβ (lanes 5 and 6), ^{3}H-C20ββ (lanes 7 and 8), ^{3}H-Androsterone (lanes 9 and 10), ^{3}H-HDCA (lanes 11–13), ^{3}H-Testosterone (lanes 14–17) by human microsomes (HL15) and UGT2B7: lane 1: HL15 with UDPGA, lane 2: UGT2B7 without UDPGA, lanes 3–10: UGT2B7 with UDPGA, lane 11: HL15 with UDPGA, lanes 12 and 13: UGT2B7 with UDPGA, lane 14: HL15 with UDPGA, lane 15: UGT2B7 without UDPGA, lane 16 and 17: UGT2B7 with UDPGA.

carboxyl-linked glucuronides were clearly separated under the former TLC conditions and carboxyl-linked glucuronides are generally hydrolyzed in the latter system as described previously [18]. In addition, some glucuronides were further analyzed to establish the exact position of the hydroxyl-linked glucuronide by comparison of labeled products to commercially available glucuronide standards detected on TLC using Krowicki's reagent [25]. After development, plates were dried and subjected to autoradiography for 3–7 days at −80°C. Plates using tritium labeled substrates were sprayed with EN^{3}HANCE (DuPont-New England Nuclear, Boston, MA) before autoradiography. Silica gel containing labeled metabolites (glucuronide bands were localized using the autoradiographs) and that from corresponding areas in control lanes was scraped into vials and radioactivity was determined by scintillation counting (LKB RackBeta 1214; Wallac Inc, Gaithersburg, MD). Specific activities of enzymes are expressed as pmol or nmol/min × mg protein; where applicable, means ± S.D. are reported.

Preliminary experiments established the optimal conditions for the glucuronidation reaction in terms of protein concentration, time of reaction and substrate concentration. No substrate inhibition was observed up to concentrations of 0.25 mM. The experiments to assess kinetic parameters were done using the same basic method over a range of concentrations of steroid or bile acids (25–100 μM) at a constant concentration of UDP-GlcUA (4 mM). The data were analyzed and apparent K_m and V_{max} determined using the program EnzymeKinetics (Trinity Software Compton, NH).

3. Results

3.1. *Enzymatic glucuronidation of bile acids and steroids*

Optimal conditions for the glucuronidation of ster-

Table 1
Glucuronidation of bile acid substrates by recombinant UGTs and liver microsomes. Human liver microsomes (HL-15) and recombinant proteins, UGT1A1, UGT1A3, UGT1A4, UGT2B7 (H268) and UGT2B7 (Y268), all were incubated with [^{14}C]-lithocholic acid, [^{3}H]-hyodeoxycholic acid, [^{3}H]-C20αβ and [^{3}H]-C20ββ as described in the Materials and methods. The results are the mean ± S.D. for at least two separate duplicate determinations

Substrate	Enzymatic Activity (pmol/mg × min)					
	UGT1A1	UGT1A3	UGT1A4	UGT2B7 (H268)	UGT2B7 (Y268)	HL-15
LA (carboxyl glucuronide)	nd[a]	207 ± 60	nd	25 ± 4	10 ± 6	387 ± 216
LA (hydroxyl glucuronide)	nd	nd	4 ± 0	160 ± 47	43 ± 24	378 ± 155
HDCA (carboxyl glucuronide)	nd	48 ± 7	nd	nd	nd	nd
HDCA (hydroxyl glucuronide)	nd	nd	1 ± 0	1431 ± 12	372 ± 99	1909 ± 571
C20αβ (carboxyl glucuronide)	nd	60	Nd	428 ± 53	118 ± 60	600
C20αβ (hydroxyl glucuronide)	nd	nd	6 ± 1	1040 ± 127	220 ± 13	1760
C20ββ (carboxyl glucuronide)	nd	28 ± 18	nd	155 ±7	33 ± 11	2280
C20ββ (hydroxyl glucuronide)	nd	nd	nd	nd	nd	nd

[a] nd = not detectable.

Table 2
Glucuronidation of steroid substrates by recombinant UGTs and liver microsomes. Human liver microsomes (HL-15) and recombinant proteins UGT1A3, UGT2B7 (H268) and UGT2B7 (Y268) were incubated as described in Materials and methods. The results are the mean ± S.D. for at least two separate duplicate determinations

Substrate	Enzymatic Activity (pmol/mg × min)			
	UGT1A3	UGT2B7 (H268)	UGT2B7 (Y268)	HL-15
Androsterone	10 ± 0	2190 ± 681	493 ± 122	2603
Testosterone	nd[a]	nd	nd	1418
Estrone	57 ± 5	nd	–[b]	140 ± 15
Estradiol (3β-OH)	45 ± 6	nd	nd	190 ± 68
Estradiol (17β-OH)	nd	32 ± 7	nd	192 ± 77
Estriol (3β-OH)	–	nd	–	nd
Estriol(16α/17β-OH)[c]	–	nd	–	2522 ± 507
Estriol (17β-OH)	–	2742 ± 211	–	nd

[a] nd = not detectable.
[b] no experiment done with that particular substrate.
[c] not resolved because of low rates of glucuronide formation.

oid substrates were elaborated in terms of protein concentration, time of incubation and, most significantly, substrate concentration. Routinely, 50 μg of membrane fractions, 0.1 mM concentration of substrate and either 15 min (for steroids) or 30 min (for BA) incubation times were used. For most of the substrates, the rate of glucuronidation was linear up to 0.1 mM and saturation was observed up to 0.25 mM; no visible substrate inhibition was demonstrated. For the evaluation of the K_m and V_{max} for androsterone and HDCA, 10 min incubations and a range of aglycone from 0.025–0.1 mM were used. The K_m values for androsterone and HDCA were 6 and 11 μmol/mg × min, respectively. The corresponding V_{max} values were 6.7 and 3.8 μmol/mg × min, respectively.

3.2. Glucuronidation of bile acid substrates

The separation of BA hydroxyl glucuronides from carboxyl glucuronides by TLC is illustrated in Fig. 2. Lithocholic acid (LA) was glucuronidated by human liver microsomes (HL-15) in roughly equal proportions at the carboxyl and hydroxyl function (Table 1). Among the recombinant proteins, UGT1A3 glucuronidated LA with the highest activity and the site of glucuronide conjugation is exclusively at the carboxyl function. In contrast, the allelic forms of UGT2B7 catalyzed glucuronidation of LA at lower rates at both the hydroxyl function and the carboxyl function. Approximately 4–6 times more hydroxyl-linked LA glucuronide is formed by UGT2B7 than carboxyl-linked glucuronide. These results suggest that UGT1A3 or other UGTs contribute significantly to LA glucuronidation in humans because the amount of LA carboxyl-linked glucuronide formed in liver microsomes cannot be accounted for solely by LA conjugation catalyzed by UGT2B7. The HK293 cell lines expressing UGT2B7(H268) exhibited about 3–4 times higher glucuronidation activities for LA compared to those determined using cells expressing UGT2B7(Y268). These results are comparable to those determined by Rios et al. (unpublished observations) who have shown that the UGT2B7(H268) cell line expresses 3–3.5 times more of the enzyme compared to the UGT2B7(Y268) cell line. Human UGT1A1 did not catalyze glucuronidation of LA, or any of the BA tested and UGT1A4 glucuronidated LA and other BA, at very low rates exclusively at the hydroxyl moiety.

Hyodeoxycholic acid (HDCA) was glucuronidated exclusively at the hydroxyl functions (3α- and 6α-OH glucuronides were not separated under these experimental conditions) by the human liver microsomes and recombinant proteins, with UGT1A3 being the sole exception. UGT1A3 glucuronidated HDCA only at the carboxyl function with an activity of 48 pmol/mg × min., HL-15 and UGT2B7(H268) glucuronidated HDCA with activities of 1.9 and 1.4 nmol/mg × min, respectively. UGT2B7(Y268) was shown to have enzymatic activity of 372 pmol/mg × min toward HDCA. Given the difference in protein expression of the allelic forms of UGT2B7 in the HK293 cell lines, differences do appear to be evident between the forms for HDCA glucuronidation.

C20αβ and C20ββ were the short-chain BA substrates tested. These saturated C20 acids are acidic catabolites of steroids with 20 carbon atoms [26]. C20αβ and C20ββ are short-chain homologues of lithocholic and isolithocholic acid, respectively, and have been shown to be substrates for glucuronidation [27]. C20αβ was glucuronidated most effectively by human liver microsomes and recombinant UGTs at the hydroxyl position (with the exception of UGT1A3), whereas C20ββ was glucuronidated only at the carboxyl position. Similar to LA, C20αβ glucuronidation rates were

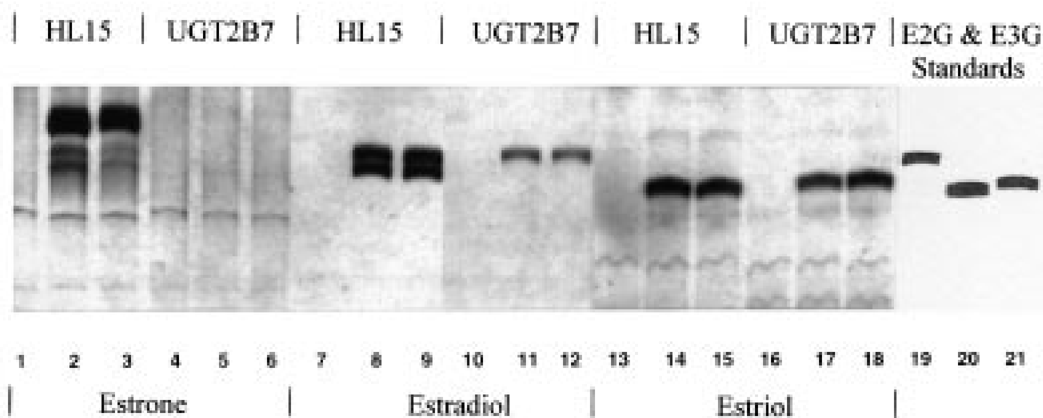

Fig. 3. Glucuronidation of ^{14}C-Estrone (lanes 1–6), ^{3}H-Estradiol (lanes 7–12) and Estriol (lanes13–18) by human liver microsomes (HL15) and UGT2B7: lane 1: HL15 without UDPGA, lanes 2 and 3: HL15 with UDPGA, lane 4: UGT2B7 without UDPGA, lanes 5 and 6: UGT2B7 with UDPGA, lane 7: HL15 without UDPGA, lanes 8 and 9: HL15 with UDPGA, lane 10: UGT2B7 without UDPGA, lanes 11 and 12: UGT2B7 with UDPGA, lane 13: HL15 without estriol, lanes 14 and 15: HL15 with estriol, lane 16: UGT2B7 without estriol, lanes 17 and 18: UGT2B7 with estriol, lane 19: 17β-estradiol-(β-D-glucuronide) standard (E2G), lane 20: estriol 16α-(β-D-glucuronide) standard (E3G), lane 21: estriol 17β-(β-D-;glucuronide) standard (E3G).

in the nanomolar level at the hydroxyl position while activities at the carboxyl position were lower using expressed UGT2B7. The ratio of C20αβ hydroxyl/carboxyl glucuronide was approximately 2:1 for both expressed UGT2B7 allelic forms. Expressed UGT1A3 glucuronidated C20αβ and C20ββ exclusively at the carboxyl moiety with activities of 60 and 28 pmol/mg × min, respectively. Human liver microsomes showed high activities for C20ββ, only at the carboxyl function.

3.3. Glucuronidation of steroid hormones

The steroid hormones used as substrates were androsterone, testosterone, estradiol and estriol. HL-15 and UGT2B7(H268) glucuronidated androsterone at similar levels (Table 2). UGT2B7(Y268) exhibited high enzymatic activity toward androsterone with a value of 493 pmol/mg × min. UGT1A3 had minimal activity toward androsterone with a value of 10 pmol/mg × min. Testosterone was not glucuronidated by any of the recombinant proteins, although significant activity was observed with HL-15 (1.4 nmol/mg × min). Estrone was only glucuronidated by HL-15 and UGT1A3 with activities of 140 and 57 pmol/mg × min, respectively. Estradiol was glucuronidated by HL-15 at the aromatic 3-OH and 17β-OH position in equal proportions (Fig. 2). Among the recombinant proteins, UGT2B7(H268) and UGT1A3 were the only ones that exhibited detectable activity toward estradiol with a value of 32 and 45 pmol/ mg × min, respectively. UGT2B7(H268) yielded the 17β-OH glucuronide and UGT1A3 yielded the 3-OH glucuronide. Estradiol glucuronidation catalyzed by UGT2B7(Y268) was probably not detectable due to the lower protein expression levels in this cell line. In regard to estriol, HL-15 and UGT2B7 were the only ones to show activity, with similar rates of 2.3 and 2.8 nmol/mg × min, respectively. Human liver microsomes were found to glucuronidate estriol at the 16α-OH and/or 17β-OH position and UGT2B7 was shown to glucuronidate estriol at the 17β-OH position (Fig. 3).

4. Discussion

The UGT1A subfamily is recognized as the group of enzymes responsible for primarily glucuronidating phenol substrates, such as *p*-nitrophenol and bilirubin. Certain UGT1 proteins (e.g. UGT1A1, 1A3, 1A4 and 1A8) are also known to conjugate certain steroids and drugs, such as morphine [14–16,21,23]. BA are not substrates, or are very poor substrates, for UGT1A1 and UGT1A4.

Human UGT1A3 has been reported to glucuronidate estrone, 2-hydroxyestrone, *N*-hydroxy metabolites of 2-acetylaminofluorene (AAF) and 6-OH and 12-OH metabolites of benzo(α)pyrene [15]. Green *et al* (1998) [23] showed further that UGT1A3 catalyzes the OH-glucuronidation of opioids, flavonoids, coumarins, anthraquinones and phenols, as well as *N*-glucuronidation of primary, secondary and tertiary amines and glucuronidation of NSAIDs and other carboxylic acid containing compounds at the carboxyl moiety. Our studies show that UGT1A3 has exclusive specificity for the carboxyl function of BA substrates. The rank order of glucuronidation activity for the carboxyl function of these substrates is the following: LA > HDCA > C20αβ > C20ββ. The C_{20} acids involved were used to highlight not only the regio- and stereo-

selectivity of human liver microsomes and both allelic forms of UGT2B7, but also to reinforce UGT1A3's specificity for the carboxyl function of BA regardless of whether the hydroxyl function is in the α or β position.

Our results also suggest that hepatic UGT1A3 may contribute significantly to the formation of LA carboxyl-linked glucuronide in human liver microsomal preparations since the hydroxyl/carboxyl glucuronide ratio of 1:1 in liver cannot be accounted for solely by hepatic UGT2B7 which has a LA hydroxyl/carboxyl glucuronide ratio of 4:1. On the other hand, expressed UGT1A3 catalyzes glucuronidation of HDCA at the carboxyl moiety, however, this HDCA glucuronide is not formed in detectable amounts in human liver microsomal preparations. One possible explanation for this is that HDCA hydroxyl glucuronide formation catalyzed by UGT2B7 may be more kinetically favorable than HDCA carboxyl glucuronide formation catalyzed by UGT1A3. In addition, UGT2B4 is also expressed in human liver and also competes with UGT2B7 and UGT1A3 for HDCA glucuronidation.

Various UGT isoforms of the 2B subfamily play a significant role in steroid and BA glucuronidation. Androsterone is glucuronidated by both allelic forms of recombinant UGT2B7 [11]. These same isoforms catalyze estriol glucuronidation [28]. Human UGT2B4, 2B7, 2B8, 2B11, 2B15 and 2B17 [9,10,12,13,28–32] have all been reported to effectively glucuronidate certain C18, C19, or C21 hydroxysteroids. Our present data demonstrate that recombinant 2B7 catalyzes glucuronidation of HDCA, androsterone and estriol at very high rates. Jin *et al.* [10] found that UGT2B7 is primarily responsible for glucuronidating steroids at the 3α-OH position. In this study, our more detailed characterization of this isoform with a range of steroid substrates indicates that UGT2B7 is involved in glucuronidation of androsterone at the 3α-OH group, estradiol and estriol at the 17β-OH group and, finally, has no activity toward testosterone which has a 17β-OH but no 3-OH group. Interestingly enough, however, epitestosterone has been reported to be glucuronidated by UGT2B7 at the 17α-OH position [11].

The elucidation of the specificities of UGTs for various endogenous substrates is important due to the potential toxic nature of certain glucuronides formed. In addition to the estrogen D-ring glucuronides, for example, the 3-O glucuronide of LA is three to four times more potent as a cholestatic agent than its parent compound [33]. Our data show here that UGT1A3 exclusively catalyzes the biosynthesis of the noncholestatic carboxyl-linked LA glucuronide, whereas, UGT2B7 yields predominantly the cholestatic 3α-OH LA glucuronide. For the glucuronidation of estriol, human liver microsomes are shown to yield either the 16α-OH or 17β-OH glucuronide and UGT2B7 yields the 17β-OH glucuronide, both forming the toxic D-ring glucuronide. Vore and Slikker [6] have suggested that, due to the structural similarity between the steroid-D-ring glucuronides and the bile acids, these cholestatic agents may act as competitive inhibitors of a bile acid carrier.

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