HUMAN UDP-GLUCURONOSYLTRANSFERASE, UGT1A8, GLUCURONIDATES DIHYDROTESTOSTERONE TO A MONOGLUCURONIDE AND FURTHER TO A STRUCTURALLY NOVEL DIGLUCURONIDE

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ABSTRACT:

We identified human UDP-glucuronosyltransferase (UGT) isoforms responsible for producing dihydrotestosterone (DHT) diglucuronide, a novel glucuronide in which the second glucuronosyl moiety is attached at the C2’ position of the first glucuronosyl moiety, leading to diglucuronosyl conjugation of a single hydroxyl group of DHT at the C17 position. Incubation of the DHT monoglucuronide with 12 cDNA-expressed recombinant human UGT isoforms and uridine 5’-diphosphoglucuronic acid resulted in a low but measurable DHT diglucuronidation activity primarily with UGT1A8, a gastrointestinal UGT, and to a lesser extent with UGT1A1 and UGT1A9. In contrast, the activity of DHT monoglucuronidation was high and was found in UGT2B17, UGT2B15, UGT1A8, and UGT1A4 in descending order. Among the 12 UGT isoforms tested, only UGT1A8 was capable of producing DHT diglucuronide from DHT. The kinetics of DHT diglucuronidation by microsomes from human liver and intestine fitted the Michaelis-Menten model, and the $V_{\text{max}}/K_m$ value for the intestinal microsomes was approximately 4 times greater than that for the liver microsomes.

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Glucuronidation is one of the most important phase II metabolic reactions not only for xenobiotics but also for endogenous substances such as steroids and bile acids (Burchell and Coughtrie, 1989; Radominska-Pandya et al., 1999). This biotransformation is catalyzed by a superfamily of enzymes, named UDP-glucuronosyltransferase (UGT), which is located in the inner membrane of endoplasmic reticulum. UGTs are classified into either of two families, UGT1 or UGT2, based on the evolutionary divergence (Mackenzie et al., 1997).

To date, nucleotide sequences encoding 18 human UGT proteins have been identified (Miners et al., 2004). UGT catalyzes the transfer of the glucuronosyl moiety from the cofactor uridine 5’-diphosphoglucuronic acid (UDPGA) to a suitable functional group, such as an amino, carboxyl, hydroxyl, or sulfhydryl group, as well as an acidic carbon on a lipophilic substrate (Miners et al., 2004). As a result of a hydrophilic glucuronosyl moiety being introduced into a drug molecule, glucuronides are generally less toxic, polar compounds and are readily excreted into the bile and/or urine. However, in some instances, when glucuronides are not hydrophilic enough, they can undergo subsequent glucuronidation to form a bisglucuronide or diglucuronide. In this paper, we define “bisglucuronide” as a glucuronide in which two separate functional groups on the molecule are conjugated at the same time. Bisglucuronides are relatively common phase II metabolites of both endogenous and exogenous substances. Examples include the bisglucuronides of bilirubin (Gordon et al., 1976), morphine (Yeh et al., 1977), benzo[a]pyrene (Bevan and Sadler, 1992; Bock et al., 1992), chrysene (Bock et al., 1992), diosmetin (Boutin et al., 1993), phenolphthalein (Griffin et al., 1998), and octylgallate (Soars et al., 2002). Conversely, we define “diglucuronide” as a particular glucuronide in which a single functional group on the molecule is repeatedly conjugated by two glucuronosyl groups in tandem (Murai et al., 2005).

The first diglucuronide reported was nalmefene diglucuronide, and this metabolite of the narcotic antagonist was detected in dog urine (Dixon et al., 1989; Murthy et al., 1996). After the discovery of nalmefene diglucuronide in vivo, we were the first to report the in vitro production of the diglucuronide of 4-hydroxybiphenyl, a non-planar phenolic compound used commonly as a model substrate for glucuronidation, using dog liver microsomes (Murai et al., 2002). The diglucuronidation reaction was found to proceed in a stepwise manner, from 4-hydroxybiphenyl to 4-hydroxybiphenyl monoglucuronide and from the monoglucuronide to the diglucuronide. Rat, monkey, and human liver microsomes showed undetectable 4-hydroxybiphenyl diglucuronidation activities (Murai et al., 2002). Although the formation of this type of diglucuronide had been found solely for xenobiotics, we recently found that physiologically important endogenous sex steroids, namely, androsterone, dihydrotestosterone (DHT), estradiol, estriol, estrone, and testosterone, also undergo diglucuronidation in vitro (Murai et al., 2005). For the steroid diglucuronidation, dog liver microsomes again had the highest activity, although it was shown for the first time that monkey and human liver microsomes also have a low but still detectable activity. Of the six steroid substrates tested, only DHT was converted to the diglucuronide at a detectable level by human liver microsomes (Murai et al., 2005).

To date, human UGT isoforms that are capable of producing the

ABBREVIATIONS: UGT, UDP-glucuronosyltransferase; DHT, dihydrotestosterone; UDPGA, uridine 5’-diphosphoglucuronic acid; MS, mass spectrometry; MS/MS, tandem mass spectrometry; HPLC, high-performance liquid chromatography; LC/MS/MS, liquid chromatography-tandem mass spectrometry; ESI, electrospray ionization; COSY, correlated spectroscopy; HSQC, heteronuclear single quantum correlation spectroscopy; HMBC, heteronuclear multiple bond correlation spectroscopy; DMSO, dimethyl sulfoxide; DMSO-d$_6$, deuterated dimethyl sulfoxide.
diglucuronide have not been identified. Therefore, the objectives of the present study were 1) to identify the human UGT(s) responsible for producing the DHT diglucuronide from the DHT monoglucuronide and DHT monoglucuronide from DHT using a panel of 12 different recombinant human isoforms and 2) to determine enzyme kinetic parameters for DHT diglucuronidation for microsomes from human liver and intestine as well as recombinant human UGT. For these objectives, authentic standards of DHT monoglucuronide and diglucuronide were biosynthesized using rat and dog liver microsomes, respectively.

Materials and Methods

Materials. UDPGA, DHT, estrone 3-O-glucuronide, d-saccharic acid 1,4-lactone, and alamethicin were purchased from Sigma-Aldrich Co. (St. Louis, MO). d-Saccharic acid 1,4-lactone was used as a β-glucuronidase inhibitor. Alamethicin was used as a pore-forming peptide to enhance the access of substrates to the UGTs present in the inner membrane of the microsomal vesicles (Fisher et al., 2000). Deuterated dimethyl sulfoxide (DMSO-d$_6$, 99.9% D) was obtained from Isotec Inc. (Miamisburg, OH). Other reagents and solvents used were either of analytical or of HPLC grade. Microsomes prepared from baculovirus-infected insect cells expressing human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17 (Supersomes), as well as the control microsomal preparation, were obtained from BD Gentest (Woburn, MA). Pooled human intestinal microsomes prepared from the duodenum and jejunum sections of five donors, as well as pooled human liver microsomes prepared from 23 donors, were obtained from BD Gentest. Pooled rat liver microsomes were also obtained from BD Gentest. Dog liver microsomes used were prepared as described previously (Murai et al., 2002). All microsomes were stored frozen at approximately −80°C until use.

In Vitro Biosynthesis of DHT Monoglucuronide. DHT monoglucuronide, which is not commercially available, was biosynthesized using rat liver microsomes. For this purpose, we did not use the dog liver microsomes to avoid risk of contamination of DHT monoglucuronide with DHT diglucuronide. The incubation mixture contained Tris-HCl buffer (100 mM, pH 7.5), magnesium chloride (10 mM), d-saccharic acid 1,4-lactone (5 mM), DHT (2 mM), rat liver microsomes (0.5 mg protein/ml), and UDPGA (5 mM). DHT was added to the incubation mixture as a solution in dimethyl sulfoxide (DMSO; final, 1% DMSO). Before starting the incubation, microsomes were treated with alamethicin (50 µg/mg protein) on ice for 15 min. The glucuronidation reaction was started by the addition of UDPGA to make a total incubation volume of 60 ml. After incubation for 24 h at 37°C, the reaction was terminated by adding 3 ml of formic acid, and the mixture was placed on ice for 15 min. After centrifugation of the acidified mixture, the supernatant was collected and applied to a solid phase extraction column (Varian Mega Bond Elut C18, 10 g; Varian, Inc., Palo Alto, CA), which had been washed successively with 50 ml of acetonitrile containing 0.1% formic acid (solvent B) and 10 ml of distilled water containing 0.1% acetic acid (solvent A) for pre-conditioning. After application of the sample, the column was washed with 50 ml of solvent A, followed by elution with 50 ml of solvent B. The column eluate was lyophilized, and the residue was reconstituted in a mixture of solvent A and solvent B (80:20) for the purification and isolation of DHT monoglucuronide using the same HPLC system used for the isolation of DHT monoglucuronide. The gradient program for HPLC, starting with a mixture of solvent A and solvent B (80:20) and increasing solvent B from 20% to 44% linearly over a period of 6 min, was used for isolating DHT diglucuronide (eluted at 3.8 min). After lyophilization of the fraction containing DHT diglucuronide, DHT diglucuronide (approximately 2 mg) was obtained as a white amorphous powder. For obtaining authentic standard to generate the standard curve for the quantification of DHT diglucuronide by LC/MS/MS, a portion of the DHT diglucuronide was again subjected to a solid phase extraction using two Mega Bond Elut C18 columns, which had been preconditioned in the same manner as described in the preceding section. Each column was eluted with 50 ml of solvent B, and the eluates obtained were combined, lyophilized, and reconstituted in a mixture of solvent A and solvent B (80:20) for the purification and isolation of DHT diglucuronide using the same HPLC system used for the isolation of DHT monoglucuronide. For generating the standard curve for the quantification of DHT diglucuronide by LC/MS/MS in UGT assays, a portion of the DHT diglucuronide was again reconstituted in the initial mobile phase for HPLC, and the solution was subjected to purification and isolation. The authentic standard of DHT diglucuronide for quantification was obtained as a white amorphous powder (approximately 0.6 mg) and its purity was more than 95% by UV absorption at 220 and 280 nm.

Structure Determination of DHT Glucuronides. The DHT monoglucuronide and DHT diglucuronide isolated as above were each dissolved in a mixture of acetonitrile and water (50:50) and analyzed by a Waters Q-ToF Ultima mass spectrometer (Waters, Milford, MA) operated in negative ion electrospray ionization (ESI) mode. A capillary voltage of −2800 V and a cone voltage of −80 V were used. For the ESI/MS/MS analysis, argon gas was used as the collision gas and collision energy was set at 25 eV.

DHT monoglucuronide and DHT diglucuronide, each dissolved in DMSO-d$_6$, were subjected to NMR analysis. One-dimensional 1H NMR, correlated spectroscopy (COSY), heteronuclear single quantum correlation spectroscopy (HSQC), and heteronuclear multiple bond correlation (HMBC) spectra were obtained using a Unity Inova 500 NMR spectrometer (Varian Inc.), operating at 500 MHz for 1H and 125 MHz for 13C, equipped with a Varian nano probe. All measurements were carried out at 25°C. Chemical shifts were referred to the signals of the residual DMSO in DMSO-d$_6$ at 2.50 ppm for 1H and 39.5 ppm for 13C.

Assay of Glucuronidation of DHT and DHT Monoglucuronide Using Recombinant Human UGT Isoforms. Activities of DHT monoglucuronidation (glucuronidation of DHT to DHT monoglucuronide) and DHT diglucuronidation (glucuronidation of DHT monoglucuronide to DHT diglucuronide) were measured using 12 cDNA-expressed recombinant human UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17). Microsomes from insect cells without cDNA were used as the negative control. The incubation mixture, prepared in triplicate for each UGT isoform, contained Tris-HCl buffer (100 mM, pH 7.5), magnesium chloride (10 mM), d-saccharic acid 1,4-lactone (5 mM), d-saccharic acid 1,4-lactone (10 mM), d-saccharic acid 1,4-lactone (5 mM), d-saccharic acid 1,4-lactone (5 mM), d-saccharic acid 1,4-lactone (10 mM), d-saccharic acid 1,4-lactone (10 mM), d-saccharic acid 1,4-lactone (10 mM), and UDPGA (5 mM), in a final volume of 100 µl. The substrate, DHT or DHT monoglucuronide, was added as a solution in DMSO (final: 1% DMSO). Before starting the incubation, the
micromones were treated with alamethicin (50 μg/mg protein) on ice for 15 min. According to preliminary experiments, monoglucuronidation and diglucuronidation formation was linear up to 30 min and 360 min, respectively. The reaction was started by the addition of UDPGA, allowed to proceed for 30 min (monoglucuronidation assay) or 240 min (diglucuronidation assay) at 37°C on a shaking incubator, and terminated by the addition of 100 μl of acetonitrile. After addition of 10 μl of the internal standard solution (5000 ng/ml estrone glucuronide in water), the samples were placed on ice. After centrifugation, the supernatants were transferred to other tubes and concentrated using a centrifugal evaporator. The residue was reconstituted in 1000 μl of water, the samples were placed on ice. After centrifugation, the supernatant was subjected to LC/MS/MS quantification of the DHT monoglucuronide or DHT diglucuronide.

Furthermore, to investigate whether each single UGT isoform could produce DHT diglucuronide from DHT, we incubated DHT (100 μM) with recombinant human UGT isoform (1 mg protein/ml) in the presence of UDPGA for 24 h, and the DHT diglucuronide produced was determined by LC/MS/MS.

**Quantification of DHT Glucuronides by LC/MS/MS.** For the quantification of DHT monoglucuronide and DHT diglucuronide by LC/MS/MS, a Waters Alliance 2795 separations module was used as the HPLC system. Chromatographic separation was carried out using an Inertsil ODS-3 column (2.1 × 75 mm, 5 μm in particle size; GL Sciences, Inc., Tokyo Japan), which was maintained at 35°C. Two mobile phases, 10 mM ammonium formate in a mixture of water and methanol (50:50; solvent 1) and 10 mM ammonium formate in a mixture of water and methanol (10:90; solvent 2), were used at a flow rate of 200 μl/min. A linear gradient elution program, starting with solvent 1 as the initial mobile phase, increasing solvent 2 linearly from 0% to 100% over a period of 3 min, and holding 100% solvent 2 for 9 min, was used.

The detection was performed by a Micromass Quattro II triple quadrupole mass spectrometer (Micromass, Manchester, UK) with an ESI interface operated in the negative ion mode. The quantification was accomplished in the negative ion mode using a transition pair of m/z 245 → 85 for DHT monoglucuronide, m/z 641 → 351 for DHT diglucuronide, and m/z 445 → 175 for estrone glucuronide (internal standard). Argon was used as the collision gas. The MS operating conditions were optimized as follows: capillary voltage, −2500 V; source temperature, 100°C; and desolvation temperature, 400°C. Cone voltage and collision energy were set at −55 V and 40 eV for DHT monoglucuronide, −55 V and 25 eV for DHT diglucuronide, and −40 V and 16 eV for estrone glucuronide, respectively.

**Enzyme Kinetics and Data Analysis.** Pooled human liver microsomes (1 mg protein/ml), pooled human intestinal microsomes (1 mg protein/ml), and human UGT1A8 Supersomes (1 mg protein/ml) were incubated with various concentrations of DHT monoglucuronide (50–2000 μM) in the presence of UDPGA. According to preliminary experiments, DHT diglucuronidation formation was linear up to 360 min. The incubation was performed in triplicate for 240 min at 37°C. The apparent enzyme kinetic parameters, V_{max} (maximum velocity), and K_{m} (substrate inhibition constant), were estimated by nonlinear regression analysis using WinNonlin Professional (Ver. 3.1; Pharsight Corporation, Mountain View, CA). The plots of the velocities (V) versus substrate concentrations ([S]) were fitted either to the Michaelis-Menten equation, \( V = V_{max} \times ([S]/K_{m} + [S]) \), or to the substrate inhibition equation, \( V = V_{max}/(1 + ([S]/K_{s})) + ([S]/K_{u}) \).

**Results**

**Chemical Structure of DHT Monoglucuronide.** The negative ion ESI/MS of DHT diglucuronide, biosynthesized using DHT and dog liver microsomes, exhibited deprotonated molecule [M − H]− at m/z 641, demonstrating that the molecular weight is greater than that of DHT by a mass of two glucuronosyl moieties. In the ESI/MS/MS spectrum of DHT diglucuronide, a characteristic fragment ion was detected at m/z 351, demonstrating that the two glucuronosyl moieties introduced were connected in tandem (Fig. 1). In the 1H NMR spectrum, two anionic protons were observed at 4.37 and 4.43 ppm. Detailed analysis of two-dimensional COSY, HSQC, and HMBC spectral data enabled unambiguous assignments of all protons and carbons in DHT diglucuronide (Table 2). The regiochemistry of the two glucuronosyl moieties was elucidated by HMBC correlations (Fig. 2). The first glucuronosyl moiety was attached to the C17 position of DHT, as confirmed by the key HMBC correlation peak from the H1 proton (3.7 ppm) to the C17 carbon (88.9 ppm). The second glucuronidation occurred at the C2' position of the first glucuronosyl moiety, which was determined by two key HMBC correlation peaks from the H1’ proton (4.43 ppm) to the C2' carbon (82.3 ppm) and from the H2' proton (3.25 ppm) to the C1’ carbon (104.5 ppm).

**DHT Monoglucuronidation Activities in Recombinant Human UGT Isoforms.** Activities of DHT monoglucuronidation (glucuronidation of DHT to DHT monoglucuronide) in 12 recombinant human UGT isoforms were examined using DHT (20 μM) as a substrate. Under the condition used, decrease of DHT monoglucuronidation was small enough to be negligible. As shown in Fig. 3A, UGT2B17 exhibited the highest DHT monoglucuronidation activity (1626 ± 51 pmol/min/mg protein). UGT2B15 (388 ± 10 pmol/min/mg protein), UGT1A8 (127 ± 2 pmol/min/mg protein), and UGT1A4 (21 ± 4 pmol/min/mg protein) also exhibited the monoglucuronidation activities, in descending order. All other isoforms (UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A9, UGT1A10, UGT2B4, and UGT2B7) had very low (<10 pmol/min/mg protein) monoglucuronidation activities.
DHT Diglucuronidation Activities in Recombinant Human UGT Isoforms. Activities of DHT diglucuronidation (glucuronidation of DHT monoglucuronide to DHT diglucuronide) in 12 recombinant human UGT isoforms were examined using the biosynthesized DHT monoglucuronide (500 \(\mu\)M) as a substrate. Among the UGT isoforms tested, primarily UGT1A8 (0.047 ± 0.004 pmol/min/mg protein), and secondarily UGT1A1 (0.014 ± 0.001 pmol/min/mg protein) and UGT1A9 (0.012 ± 0.003 pmol/min/mg protein) showed low but measurable activities for DHT diglucuronidation (Fig. 3B). All other isoforms (UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17) showed undetectable diglucuronidation activities under the conditions used.

UGT Isoforms Capable of Producing DHT Diglucuronide from DHT. The capability of each UGT isoform to produce DHT diglucuronide from DHT (glucuronidation of DHT to DHT monoglucuronide, and further to DHT diglucuronide) was investigated using 12 recombinant human UGTs. The incubation of DHT (100 \(\mu\)M) with 12 recombinant human UGTs in the presence of UDPGA for 24 h resulted in the production of DHT diglucuronide (5.56 ± 0.34 ng/ml) exclusively in the sample after incubation with UGT1A8 (Fig. 3C), consistent with the data showing that only UGT1A8 had the activities of both DHT monoglucuronidation and diglucuronidation (Fig. 3, A and B). The \(y\)-axis unit in Fig. 3C represents DHT diglucuronide concentration instead of rate as in Fig. 3, A and B because 1) the result demonstrates the capacity of each UGT isoform to form DHT diglucuronide after long incubation where linearity is not verified, and 2) there are two enzymatic steps involved.

Kinetics of DHT Diglucuronidation in Pooled Human Liver Microsomes, Pooled Human Intestinal Microsomes, and Recombinant Human UGT1A8. Enzyme kinetic studies were performed by incubating DHT monoglucuronide (50–2000 \(\mu\)M) with pooled human liver microsomes, pooled human intestinal microsomes, and recombinant human UGT1A8. Over the substrate concentration range tested, the kinetics of DHT diglucuronidation by human liver microsomes and human intestinal microsomes fit Michaelis-Menten kinetics (Fig. 4, A and B). However, the kinetic data of UGT1A8, which showed decreasing velocity with increasing substrate concentration, were best described by a substrate inhibition kinetic model (Fig. 4C). Enzyme kinetic parameters, obtained by fitting data to the appropriate kinetic model, are summarized in Table 3. The apparent \(K_m\) values for human liver and intestinal microsomes were 527.9 ± 66.5 \(\mu\)M and 653.4 ± 69.0 \(\mu\)M, respectively, being comparable to each other. In contrast, the apparent \(V_{max}\) value for human intestinal microsomes (1.303 ± 0.056 pmol/min/mg protein) was approximately 5 times larger than that for human liver microsomes (0.256 ± 0.012 pmol/min/mg protein). The \(V_{max}/K_m\) value for human intestinal microsomes
(1.994 × 10⁻³ µl/min/mg protein) was approximately 4 times larger than that for human liver microsomes (0.486 × 10⁻³ µl/min/mg protein). In contrast, the $K_{me}$, $V_{max}$, $V_{max}/K_{me}$, and $K_{si}$ values for recombinant UGT1A8 were 382.3 ± 79.7 µM, 0.107 ± 0.015 pmol/min/mg protein, 0.279 × 10⁻³ µl/min/mg protein, and 964.4 ± 231.7 µM, respectively.

**Discussion**

First, in this study, authentic DHT monoglucuronide and DHT diglucuronide were biosynthesized using rat and dog liver microsomes, respectively, and their chemical structures were analyzed by mass spectrometry and nuclear magnetic resonance spectroscopy. The negative ion MS/MS spectrum of DHT diglucuronide clearly indicated that the two glucuronosyl moieties are linked in tandem because the characteristic fragment ion at m/z 351, which corresponds to a dehydrated diglucuronosyl anion, was observed in the spectrum (Fig. 1). The regiochemistry of the two glucuronosyl moieties in DHT diglucuronide, which was unable to be elucidated by analyzing the MS/MS spectrum, was determined using two-dimensional NMR analysis (Fig. 2). It was determined that the first glucuronosyl moiety in
DHT diglucuronidation was attached to the C17 position of DHT and that the second glucuronyl moiety was attached at the C2 position of the first glucuronyl moiety. Interestingly, the regiochemistry of the diglucuronidation in human liver microsomes is different from that in human intestinal microsomes. However, unlike human liver and intestinal microsomes, the kinetics of UGT1A8 was atypical, and was well described by the substrate inhibition model (Fig. 4C). By comparing the apparent kinetic parameters of UGT1A8 to those of intestinal microsomes, the $K_m$ value was within a 2-fold range, but there was a 7-fold difference in the intrinsic clearance, calculated as $V_{max}/K_m$ (Table 3). The higher diglucuronidation activity in the human intestinal microsomes compared with the human liver microsomes might be due to the higher expression level of intestinal DHT diglucuronidation enzyme, namely, either UGT1A8 or UGT1A1, or both, in intestinal microsomes. The kinetic data for UGT1A8, in terms of the substrate inhibition, differed from those for the intestinal microsomes. It is reported that UGT enzymes exist as UGT oligomers in the endoplasmic reticulum membrane and that these oligomers may act as cooperative multisubunit enzymes and thus affect the kinetics of UGT-catalyzed reactions (Miners et al., 2004). The UGT oligomers reported are not only homo-oligomers, consisting of single UGT enzymes (Meech and Mackenzie, 1997; Ghosh et al., 2001; Kurkela et al., 2003), but also hetero-oligomers, consisting of different UGT enzymes (Ikushiro et al., 1997; Ishii et al., 2001). It is even reported that hetero-oligomer formation of UGT with a cytochrome P450 altered kinetic parameters of the UGT reactions (Takeda et al., 2005). Based on this accumulating evidence, it is presumed that the production of DHT diglucuronide in human liver and intestinal microsomes is mostly catalyzed by UGT hetero-oligomers. The findings of these previous reports would explain the atypical kinetic behavior of UGT1A8 in DHT diglucuronidation.

In conclusion, we identified a structurally novel DHT diglucuronide and demonstrated that the formation of DHT diglucuronide from DHT monoglucuronide is catalyzed primarily by UGT1A8, a gastrointestinal UGT, and secondarily by UGT1A1 and UGT1A9. The DHT diglucuronidation was more efficiently catalyzed by human intestinal microsomes than by human liver microsomes, suggesting a large contribution of UGT1A8 to this reaction. Very low activities for DHT diglucuronidation in UGT isoforms compared with those for DHT monoglucuronidation (Fig. 3, A and B) indicate that further investigation should be conducted to determine whether or not this novel diglucuronidation reaction takes place under in vivo conditions.

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References


Table 3: Kinetic parameters for DHT diglucuronidation in human liver microsomes (HLM), human intestinal microsomes (HIM), and recombinant human UGT1A8.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol/min/mg protein)</th>
<th>$V_{max}/K_m$ (µl/min/mg protein)</th>
<th>$K_m^a$ (µM)</th>
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<tr>
<td>HLM</td>
<td>527.9 ± 66.5</td>
<td>0.256 ± 0.012</td>
<td>0.486 × 10⁻³</td>
<td>527.9</td>
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<tr>
<td>HIM</td>
<td>653.4 ± 60.0</td>
<td>1.303 ± 0.056</td>
<td>1.984 × 10⁻⁵</td>
<td>653.4</td>
</tr>
<tr>
<td>UGT1A8</td>
<td>382.3 ± 79.7</td>
<td>(0.107 ± 0.015)</td>
<td>(0.279 ± 10⁻⁵)</td>
<td>382.3</td>
</tr>
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*a* Substrate inhibition constant.

*b* Michaelis-Menten model.

*c* Substrate inhibition model.

According to the data obtained using 12 human UGT isoforms, DHT diglucuronidation was catalyzed by 3-methylcholanthrene-inducible phenol UDP-glucuronosyltransferase (UGT1A1).


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