1521-009X/41/3/582–591$25.00
Drug Metabolism and Disposition
http://dx.doi.org/10.1124/dmd.112.049072
Drug Metab Dispos 41:582–591, March 2013

Regiospecificity and Stereospecificity of Human UDP-Glucuronosyltransferases in the Glucuronidation of Estriol, 16-Epiestriol, 17-Epiestriol, and 13-Epiestradiol[S]

Nina Sneitz, Mikko Vahermo, Johanna Mosorin, Liisa Laakkonen, Donald Poirier, and Moshe Finel

Centre for Drug Research (N.S., J.M., L.L., M.F.) and Division of Pharmaceutical Chemistry (N.S., M.V.), Faculty of Pharmacy, University of Helsinki, Finland; and CHUQ-CHUL Research Center and Laval University, Quebec City, Québec, Canada (D.P.)

Received September 15, 2012; accepted January 3, 2013

ABSTRACT

The glucuronidation of estriol, 16-epiestriol, and 17-epiestriol by the human UDP-glucuronosyltransferases (UGTs) of subfamilies 1A, 2A, and 2B was examined. UGT1A10 is highly active in the conjugation of the 3-OH in all these estriols, whereas UGT2B7 is the most active UGT toward one of the ring D hydroxyls, the 16-OH in estriol and 16-epiestriol, but the 17-OH in 17-epiestriol. Kinetic analyses indicated that the 17-OH configuration plays a major role in the affinity of UGT2B7 for estrogens. The glucuronidation of the different estriols by the human liver and intestine microsomes reflects the activity of UGT1A10 and UGT2B7 in combination with the tissues' difference in UGT1A10 expression. The UGT1A10 mutant 1A10-F93G exhibited much higher V_{max} values than UGT1A10 in estriol and 17-epiestriol glucuronidation, but a significantly lower value in 16-epiestriol glucuronidation. To this study on estriol glucuronidation we have added experiments with 13-epiestradiol, a synthetic estradiol in which the spatial arrangement of the methyl on C18 and the hydroxyl on C17 is significantly different than in other estrogens. In comparison with estradiol glucuronidation, the C13 configuration change decreases the turnover of UGTs that conjugate the 3-OH, but increases it in UGTs that primarily conjugate the 17-OH. Unexpectedly, UGT2B17 exhibited similar conjugation rates of both the 17-OH and 3-OH of 13-espiestradiol. The combined results reveal the strong preference of UGT1A10 for the 3-OH of physiologic estrogens and the equivalently strong preference of UGT2B7 and UGT2B17 for the hydroxyls on ring D of such steroid hormones.

Introduction

The UDP-glucuronosyltransferases (UGTs) are membrane enzymes of the endoplasmic reticulum that catalyze the conjugation of different aglycone substrates that carry suitable nucleophilic group or groups, such as hydroxyl or amine, with glucuronic acid from the cosubstrate UDP-glucuronic acid (Radominska-Pandya et al., 1999; King et al., 2000; Tukey and Strassburg, 2000).

Glucuronidation of endogenous estrogens was probably first reported already in 1936 (Cohen and Marrian, 1936), and since then the glucuronidation of estrogens has been extensively studied, both in vitro and in vivo. We have examined the regioselectivity and stereoselectivity of the 19 human UGTs of subfamilies 1A, 2A, and 2B in the glucuronidation of 17β-estradiol (the natural estradiol), 17α-estradiol (17-epiestradiol), and enantiomeric β-estradiol (Itäaho et al., 2008; Sneitz et al., 2011). One outcome of these studies was that as long as the steroids contain a phenolic A ring (Fig. 1), UGTs 1A1, 1A3, 1A7, 1A8, and 1A10, mainly or exclusively, catalyze the glucuronidation of the 3-OH of the estrogens (at variable rates), whereas the 17-OH of these steroid isomers is mainly conjugated by one or more UGTs of subfamily 2B. In addition, we have recently shown that in the case of UGT1A10, the most active human enzyme in estradiol glucuronidation, replacing phenylalanine 93 (F93) with glycine (generating mutant 1A10-F93G) stimulates the estrogen glucuronidation rate of UGT1A10 (Hoglund et al., 2011). These findings prompted us to further investigate estrogen glucuronidation by the human UGTs.

Estriol (16α,17β-estriol) is one of the three major endogenous estrogens, along with estradiol and estrone (Fig. 1). There are two naturally occurring stereoisomers of estriol, 16α,17α-estriol (17-epiestriol) and 16β,17β-estriol (16-epiestriol) (Eliassen et al., 2009) (Fig. 1). Endogenously, estriol is mainly generated from estradiol and estrone, but it can also arise from 16-hydroxyandrostenedione, or from 16-hydroxydehydroepiandrosterone. The stereoisomer 17-epiestriol is formed by the reduction of 16α-hydroxyestrone and 16-epiestriol in the oxidation and reduction of estriol first to 16-ketoestradiol and subsequently 16-epiestriol (Longcope, 1984; Lappano et al., 2010) (Fig. 1).

Estriol has lower estrogenic potency than estradiol or estrone, and its circulating levels are relatively low in comparison with estradiol and estrone (Ruggiero and Likis, 2002). During pregnancy, however, estriol levels can rise considerably due to its production in the placenta from 16-hydroxyandrostenedione and 16-hydroxydehydroepiandrosterone (Ruggiero and Likis, 2002; Lappano et al., 2010). Estriol can bind to and activate estrogen receptors, but it also exerts antiestrogenic effects, probably due to competitive binding that lowers the binding of the more potent activator, estradiol to estrogen receptors (Melamed et al., 1997).

The physiologic properties of the estriol diastereomers 16- and 17-epiestriol are poorly known. It has been reported that 17-epiestriol

Financial support was obtained from the Sigrid Juselius Foundation; the Magnus Ehrnrooth Foundation; and the Academy of Finland [Grant 260010].
dx.doi.org/10.1124/dmd.112.049072.
[S]This article has supplemental material available at dmd.aspetjournals.org.

ABBREVIATIONS: DMSO, dimethylsulfoxide; HIM, human intestine microsomes; HLM, human liver microsomes; HPLC, high-performance liquid chromatography; UDPGA, uridine 5′-diphosphoglucuronic acid-glucuronic acid; UGT, UDP-glucuronosyltransferase.

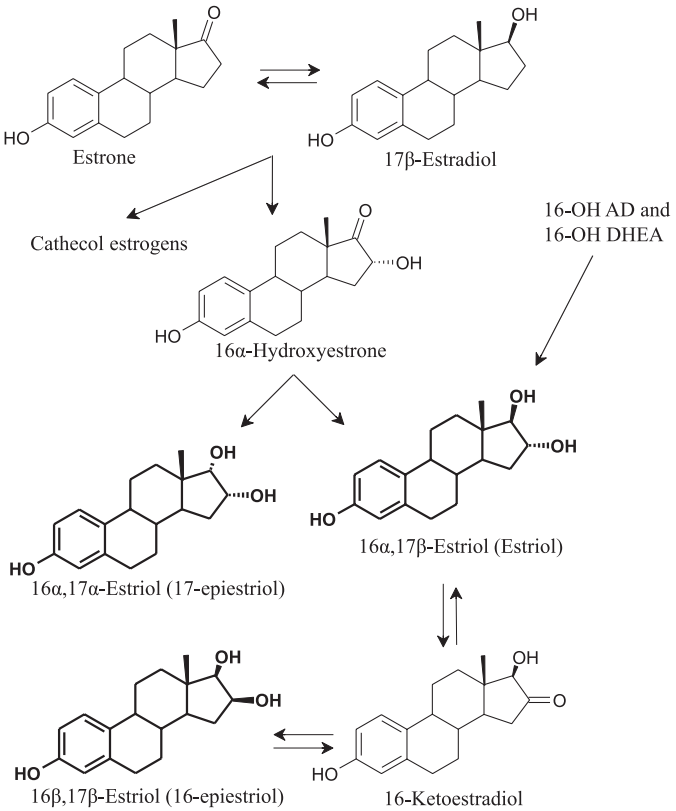

Fig. 1. Estriols structure and biosynthesis routes. 16-OH AD, 16-hydroxyandrostenedione; 16-OH DHEA, 16-hydroxydehydroepiandrosterone.

suppresses the expression of tumor necrosis factor α-induced vascular cell adhesion molecule 1 (Mukherjee et al., 2003). In addition, the urinary concentration of 17-epiestriol was found to positively correlate with the risk of breast cancer (Eliassen et al., 2012).

The human UGTs 1A1, 1A7, 1A9, 1A10, and 2B7 were previously shown to catalyze estriol glucuronidation (Gall et al., 1999; Starlard-Davenport et al., 2007). A comprehensive study on estriol glucuronidation, one that identifies the conjugation position in estriol, or the type(s) of estriol glucuronide(s) that is/are generated by each of the human UGTs, has yet to be performed, however; even less is known about the glucuronidation of 16-epiestriol and 17-epiestriol. The latter estrogen, 17-epiestriol, was reported to be a substrate for UGT2B4 and UGT2B7, but that study did not include other human UGTs, making it difficult to draw any conclusion about the UGT specificity of this steroid (Barre et al., 2007). No reports on 16-epiestriol glucuronidation have been published thus far.

A new estradiol diastereomer, 13-epiestradiol (previously called 18-epiestradiol), has been synthesized, and its estrogenic activity has been evaluated (Ayan et al., 2011). It was added to the current study because it allows, for the first time, testing of how the configuration of C13 and the spatial location of the methyl group $18CH_3$ affect estrogen glucuronidation by individual UGTs.

The present study obtains significant new knowledge on the glucuronidation of estrogens and further insights into the substrate specificity of the human UGTs, mainly their regio- and stereochemical preferences in estrogen glucuronidation.

Materials and Methods

Estriol (3,16α,17β), 17-epiestriol (3,16α,17α), 17β-estradiol, estriol 3-β-D-glucuronide, estriol 16α-β-D-glucuronide, estriol 17β-β-D-glucuronide, uridine 5′-diphosphoglucuronic acid (UDPGA) triammonium salt, UDPGA trisodium salt, and alamethicin were purchased from Sigma-Aldrich (St. Louis, MO). We obtained 16-epiestriol (3,16β,17β) from Steraloids (Newport, RI). Deuterated methanol (d_4) was from Sigma-Aldrich. The synthesis of 13-epiestradiol (18-epiestradiol) was recently described elsewhere (Ayan et al., 2011).

Recombinant UGTs were expressed, as C-terminally-His-tagged proteins, in baculovirus-infected Sf9 insect cells, and their relative expression levels were determined as previously described elsewhere (Kurkela et al., 2003; Kurkela et al., 2007). The F93G mutant of UGT1A10 was prepared as previously described elsewhere (Hoglund et al., 2011). Most enzyme activity results in this study, including the V_{max} values, are presented as normalized values, meaning that the measured rates were corrected according to the relative expression levels of each UGT. Among the UGTs batches that were used in our study, UGT1A3 had the lowest UGT expression level per mg of total protein in the sample, and it was selected as the reference value, meaning a relative expression level of 1.0. The relative expression levels of other UGT enzymes were: UGT1A1, 1.6; UGT1A4, 3.7; UGT1A7, 15.5; UGT1A8, 1.4; UGT1A9, 1.9; UGT1A10, 2.8; UGT1A10F93G, 2.3; UGT2A1, 2.4; UGT2A2, 6.7; UGT2B4, 1.6; UGT2B7, 2.2 and 4.8; and UGT2B17, 1.7 and 2.4 (two different batches of UGT2B7 and UGT2B17 were used). The only UGT for which normalized rates could not be determined is UGT2B15, because in this case an enzyme from a commercial supplier (BD Biosciences, Woburn, MA) that lacks a C-terminal His-tag was used. Human liver microsomes (pool of 30 donors) and human intestine microsomes (pool of 6 donors) were also purchased from BD Biosciences.

Glucuronidation Assays with the Different Estrogens and Glucuronides Chromatography. The glucuronidation assays were done in triplicate; negative controls, namely, assays without UDPGA, were included in each set of experiments. The substrate consumption during the different assays did not exceed 10%. The results from the enzyme kinetics assays were analyzed with GraphPad Prism 5 for Windows (GraphPad Software, San Diego, CA).

Estriol, 16- and 17-epiestriol, estradiol, and 13-epiestradiol incubation mixtures contained 5 or 50 μg of UGT-enriched insect cell membranes or 0.5 or 10 μg of microsomes from either human liver (HLM) or intestine (HIM), 50 mM phosphate buffer pH 7.4, 10 mM $MgCl_2$, and 5 mM UDPGA (triammonium salt). Reaction mixtures with HLM and HIM also contained alamethicin, 5% of the total protein concentration. In the screening experiments, performed at a single substrate concentration, the substrate concentration was 200 μM in each case. In the enzyme kinetic assays, the aglycone substrate concentration ranged from 0.2 to 300 μM. The substrates were dissolved in dimethylsulfoxide (DMSO). The final DMSO concentration in the reaction was 10%, and the total reaction volume was 100 μl. The incubation times ranged from 15 to 60 minutes at 37°C, and the reactions were terminated by the addition of 10 μl 4 M perchloric acid, transfer to ice, and subsequent protein sedimentation by centrifugation (16,000g, 5 minutes). The supernatants were analyzed by high-performance liquid chromatography (HPLC), using a Shimadzu LC-10 model (Shimadzu Corporation, Kyoto, Japan).

The three estriol glucuronides were separated using a Zorbax Eclipse Plus 4.6 × 150 mm, 5 μm column (Agilent Technologies, Palo Alto, CA) at 40°C. The eluents were 50 mM phosphate buffer pH 3.0 (A) and 1:1 (v/v) mixture of acetonitrile/methanol (B). The following gradient was used: 0–37 minutes, 21% B; 37–38 minutes, B concentration increase to 50% B; 38–45 minutes 50% B; 45–50 minutes, B concentration decrease to 21%, at a flow rate of 1.4 ml/min throughout. The detection of glucuronides and unconjugated estriols was performed by use of a fluorescence detector at 280 nm (excitation) and 305 nm (emission). The retention times for the 3, 17, and 16 monoglucuronides of estriol were 7.4, 32.6, and 34.1 minutes, respectively. They were identified and quantified by using authentic glucuronide standards.

The glucuronides of 17-epiestriol were also separated with Zorbax Eclipse Plus 4.6 × 150 mm 5 μm column at 40°C but using an isocratic chromatography with mobile phase of 55% 50 mM phosphate buffer pH 3.0 and 45% methanol, at a flow rate of 1.3 ml/min flow. The detection was done as in the case of estriol, and the retention times for the 3, 16, and 17 monoglucuronides were 3.9, 6.8, and 11.5 minutes, respectively. The identity of the 17-epiestriol 17-glucuronide was determined by NMR spectroscopy, and

the glucuronides were quantified by use of an external standard curve generated with estriol glucuronides.

The glucuronides of 16-epiestriol were separated using a Poroshell 120 EC-C18 4.6 × 100 mm, 2.7 μM column (Agilent Technologies) at 40°C. The isocratic mobile phase was composed of 55% 50 mM phosphate buffer pH 3.0 and 45% of 1:1 acetonitrile/methanol, at a flow rate of 0.8 ml/min. The detection was performed the same as with estriol, and the retention times for the 3, 17, and 16 monoglucuronides of 16-epiestriol were 3.7, 5.5, and 7.1 minutes, respectively. The identity of 16-epiestriol 16-glucuronide was determined by NMR, and the glucuronides were quantified by use of an external standard curve generated with estriol glucuronides.

Estradiol glucuronides were separated and quantified as previously described elsewhere (Itäaho et al., 2008). The glucuronides of 13-epiestradiol were separated using a Chromolith SpeedRod RP18e column (50 × 4.6 mm) (Merck, Darmstadt, Germany) at 40°C. The mobile phase consisted of 45% 50 mM phosphate buffer pH 3.0 and 55% MeOH, and the flow rate was 1.5 ml/min. Fluorescence detection was used (excitation 216 nm, emission 316 nm), and the retention times of 13-epiestradiol glucuronides were 2.2 and 6.1 minutes for the 3-glucuronide and 17-glucuronide of 13-epiestradiol, respectively.

Biosynthesis and NMR Spectroscopy of Selected Estriol Glucuronides. The reaction mixtures for the biosyntheses contained 50 mM phosphate buffer pH 7.4, 10 mM $MgCl_2$, either 4 mg of 17-epiestriol or 8 mg of 16-epiestriol, 50 mM UDPGA (trisodium salt), 10% DMSO, and 7 ml of freshly prepared homogenates of insect cells containing approximately 500 mg (total protein) of UGT2B7-enriched insect cells in 25 ml. The biosynthesis reactions were performed for 5 hours under continuous stirring at 37°C. The reactions were terminated by the addition of 2.5 ml of ice-cold 4 M perchloric acid and transfer to ice. Proteins were subsequently sedimented from the reaction mixture by centrifugation (16,000*g*, 10 minutes), and the supernatants were fractionated using an Agilent 1100 series HPLC (Agilent Technologies). The chromatography methods for fractioning the resulting 16-epiestriol or 17-epiestriol glucuronides were as described earlier for the screening analyses, except that phosphate buffer was replaced with 0.1% HCOOH in water and the detection was by ultraviolet-detector at 280 nm. The glucuronide fractions were finally concentrated using a rotary evaporator and lyophilized. The lyophilized glucuronides were later redissolved in deuterated methanol and subjected to NMR spectroscopy on a Varian 300 MHz MercuryPlus spectrometer (Varian, Inc., Palo Alto, CA). The chemical shifts of protons 16 and 17 of 16-epiestriol and 17-epiestriol glucuronides were assigned from ^{1}H-NMR spectra and the respective carbons from heteronuclear single quantum coherence spectra. The chemical shifts of the anomeric proton and carbon of the glucuronic acid moiety were similarly assigned. The site of glucuronidation was determined using heteronuclear multiple-bond correlation spectra.

Results

Identification of the Glucuronides of the Different Estriols and Estradiols. Estriols have three hydroxyl groups, and each of them may be conjugated but by different UGTs and at different rates. Identifying the different glucuronides that are generated by the tested human UGTs from estriol (17β,16α-estriol) is relatively simple because commercial glucuronide samples for each of them are available. On the other hand, achieving a good separation between the estriol-16- and 17-glucuronide peaks (G3 and G2, respectively, in Fig. 2A) by HPLC was challenging and required a long chromatography method.

In contrast to the situation with estriol, no authentic standards are currently available for any of the 16-epiestriol and 17-epiestriol glucuronides. Based on our results with estriol (Fig. 2) and the previous results with estradiol and 17-epiestradiol (also called 17α-estradiol or just epiestradiol in Itäaho et al., 2008), it is highly likely that the first eluting glucuronide of both 16-epiestriol and 17-epiestriol (G1 in Fig. 2, B and C), the main glucuronide products of UGT1A10 in both cases (Fig. 2), are the 3-glucuronide of each of these estriol stereoisomers. For the 16-glucuronide and 17-glucuronide identification, however, we had no grounds to make corresponding assumptions. To resolve this question and determine the identity of the glucuronide in one of the later-eluting glucuronide peaks, we turned to NMR spectroscopy. For the production of either the 16-glucuronide or the 17-glucuronide of each of the two estriol stereoisomers, we have employed UGT2B7. This enzyme was selected because preliminary experiments revealed that incubation of UGT2B7 with either 16-epiestriol or 17-epiestriol yields a large amount of a single, or nearly a single, glucuronide in each case (G3 in Fig. 2, B and C). These glucuronides were collected, purified, and subjected to structure determination by NMR (see *Materials and Methods* for technical details).

Heteronuclear multiple-bond correlation NMR spectrum of the purified glucuronide that UGT2B7 generates from 17-epiestriol exhibited a clear correlation between the proton at C17 and the anomeric carbon of the glucuronic acid moiety, as well as correlation between the anomeric proton and C17 of 17-epiestriol. These results indicate that when the substrate was 17-epiestriol, the UGT2B7-produced glucuronide (G3 in Fig. 2C) is 17-epiestriol-17-glucuronide.

In the case of 16-epiestriol, NMR analysis of the main glucuronide produced by UGT2B7 detected only a single correlation, between the anomeric proton and C16 of 16-epiestriol (Table 1). Based on this, the main glucuronide that UGT2B7 generates from 16-epiestriol was identified as 16-epiestriol-16-glucuronide (G3 in Fig. 2B). Having obtained these results, we concluded that the third glucuronide peak, the one produced by UGT2B17 alongside 16-epiestriol-16-glucuronide (Fig. 3B), is 16-epiestriol-17-glucuronide. Similarly, it was concluded that the small peak seen in 17-epiestriol glucuronidation by UGT2B7, the one that is the main peak in the case of 17-epiestriol glucuronidation by UGT2A1 (Fig. 3C) is 17-epiestriol-17-glucuronide.

Estriol Glucuronidation. Estriol glucuronidation by the different human UGTs, as well as the glucuronidation of 16-epiestriol and 17-epiestriol, was first investigated using a screening-type analysis in which all the different UGTs were incubated with a single and rather high-substrate concentration, 200 μM. The control experiments were performed in the absence of either the aglycone substrate, estriol, or the cosubstrate UDPGA. The determined glucuronidation rates for each of the UGTs, with the exception of UGT2B15 (see *Materials and Methods*), were corrected (normalized) according to their relative expression levels (see *Materials and Methods*).

The results of the estriol glucuronidation screening assays are presented in Fig. 3A. Numerical values (Supplemental Table 1) revealed that UGT1A10 and UGT2B7 are the most active enzymes toward this substrate, catalyzing its glucuronidation (at different hydroxyls) at as high a rate as 840 and 1200 pmol/min/mg, respectively (under the screening assays conditions). UGT1A10 catalyzed estriol glucuronidation at the 3-OH, whereas UGT2B7 conjugated it exclusively at the 16-OH. UGTs 1A1 and 1A8 also catalyzed estriol glucuronidation at the 3-OH, but only at low rates. UGTs 1A4, 2A1, and 2B4 catalyzed estriol conjugation at the 16-OH, but at low rates: 4–16 pmol/min/mg. UGT2B17 was the only human UGT that catalyzed estriol glucuronidation at the 17-hydroxyl. The glucuronidation rate of estriol by UGT2B17 was low, about 4 pmol/min/mg, but its unique regioselectivity and/or stereoselectivity are of interest.

Enzyme kinetic assays were performed with the two most active UGTs in estriol glucuronidation, 1A10 and 2B7, and the kinetic curves for both enzymes fitted best to the Michaelis-Menten equation (Fig. 4). UGT2B7 exhibited only a slightly higher V_{max} value than UGT1A10, apparently due to the high K_m value of UGT1A10 (Table 2).

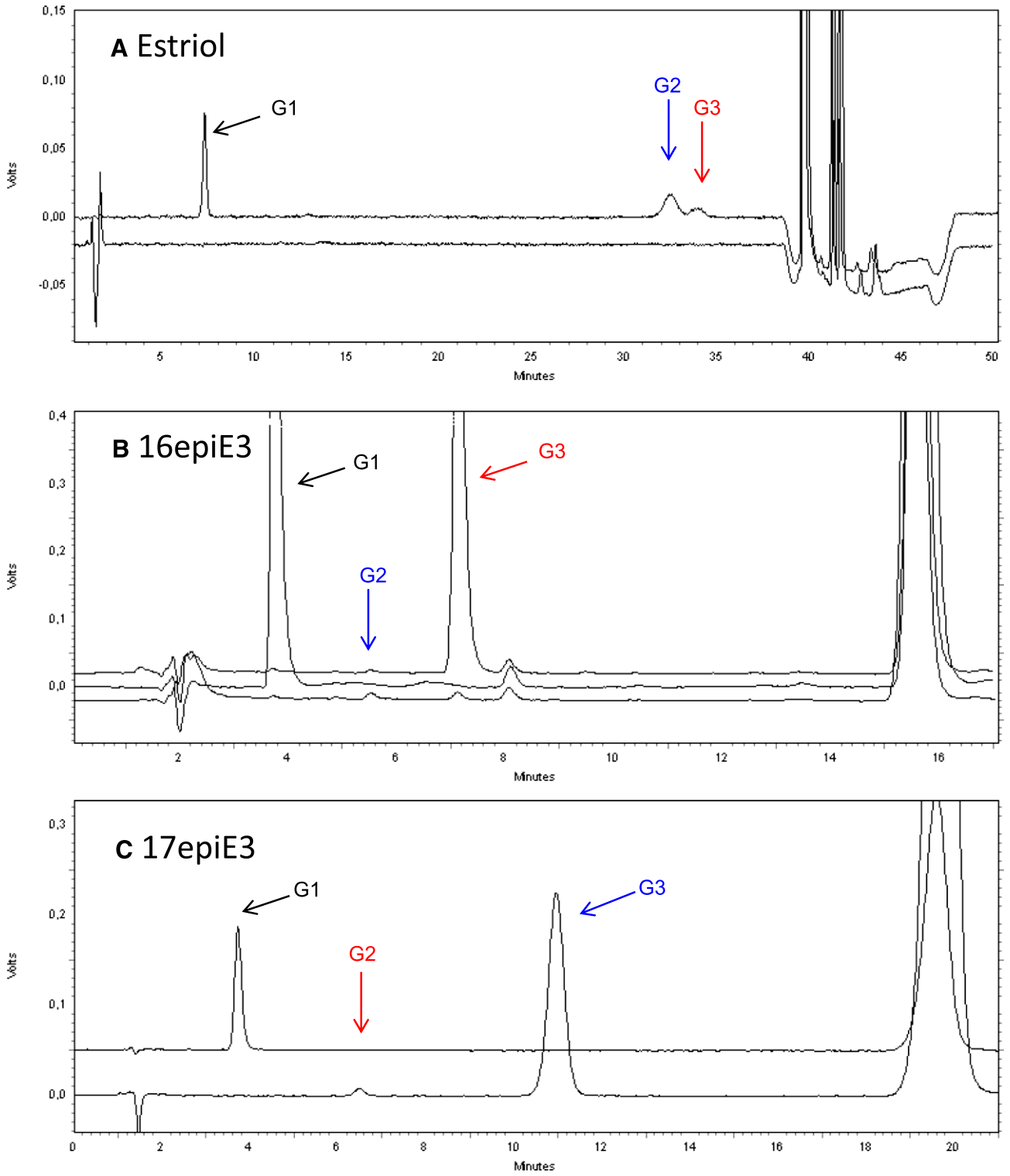

Fig. 2. Chromatographic separation of the different glucuronides of the three estriol stereoisomers. The glucuronide peaks are (A) authentic standards (estriol) or (B) generated from incubation with UGT1A10, 2B7, and 2B17 (16-epiestriol) or (C) UGT1A10 and 2B7 (17-epiestriol). A negative control, from the incubation of estriol with UGT1A10 in the absence of UGPGA, is presented at the bottom of panel A. The detected glucuronides, G1–G3, are numbered according to their elution order and are color-coded according to the glucuronidation site. Black represents 3-glucuronides, blue 17-glucuronides, and red 16-glucuronides. The numeric results of the screening assays are presented in Supplemental Table 1.

16-Epiestriol Glucuronidation. The initial screening experiment for 16-epiestriol glucuronidation demonstrated that, as with estriol glucuronidation, UGTs 1A10 (3-OH conjugation, see *Discussion*) and 2B7 (16-OH conjugation) are the most active enzymes (see earlier for the glucuronides identification). The 16-epiestriol glucuronidation rates of UGT1A10 and UGT2B7 were 3500 and 2700 pmol/min/mg, respectively. UGTs 1A1, 1A7, 1A8, and 2B15 catalyzed the formation of 16-epiestriol-3-glucuronide but at much lower rates, only 0.5–16.0

TABLE 1

Nuclear magnetic resonance analyses: 1H and 13C chemical shifts

	16-Epiestriol Glucuronide	17-Epiestriol Glucuronide
H16	4.18 (m)	4.48 (m)
H17	3.79 (d,J = 9.7 Hz)	3.79 (d,J = 5.1 Hz)
C16	80.5	73.3
C17	76.5	88.9
H (anomeric)	4.46 (d,J = 7.9 Hz)	4.42 (d,J = 7.5 Hz)
C (anomeric)	106.0	104.7

pmol/min/mg. Besides UGT2B7, UGTs 1A3, 2A1, 2B4, and 2B17 catalyzed the formation of 16-epiestriol-16-glucuronide. The 16-epiestriol-16-glucuronide formation by UGT1A4 was barely detectable, as was the 16-epiestriol-17-glucuronide formation by UGT2A1. UGT2A1 catalyzed a relatively high rate of 16-epiestriol glucuronidation, approximately 260 pmol/min/mg, whereas the activities of UGTs 1A3, 2B4, and 2B17 were significantly lower: 5-45 pmol/min/mg.

The enzyme kinetics of 16-epiestriol glucuronidation curves by UGT1A10 and UGT2B7 fitted best to the Michaelis-Menten equation with substrate inhibition (Fig. 4; Table 2). The V_{max} and K_m values for both these UGTs suggested low affinity for 16-epiestradiol but high turnover rates.

17-Epiestriol Glucuronidation. UGT2B7 exhibited the highest rate of 17-epiestriol glucuronidation, preferentially (but not exclusively) conjugating the 17-OH, not the 16-OH as in estriol and 16-epiestriol (Fig. 3). The other enzyme that catalyzes the conjugation of the 17-OH of 17-epiestriol is UGT2B4, an enzyme that often exhibits similar substrate selectivity to that of UGT2B7 but with lower turnover rates. UGT2A1 generated some 17-epiestradiol-17-glucuronide but at

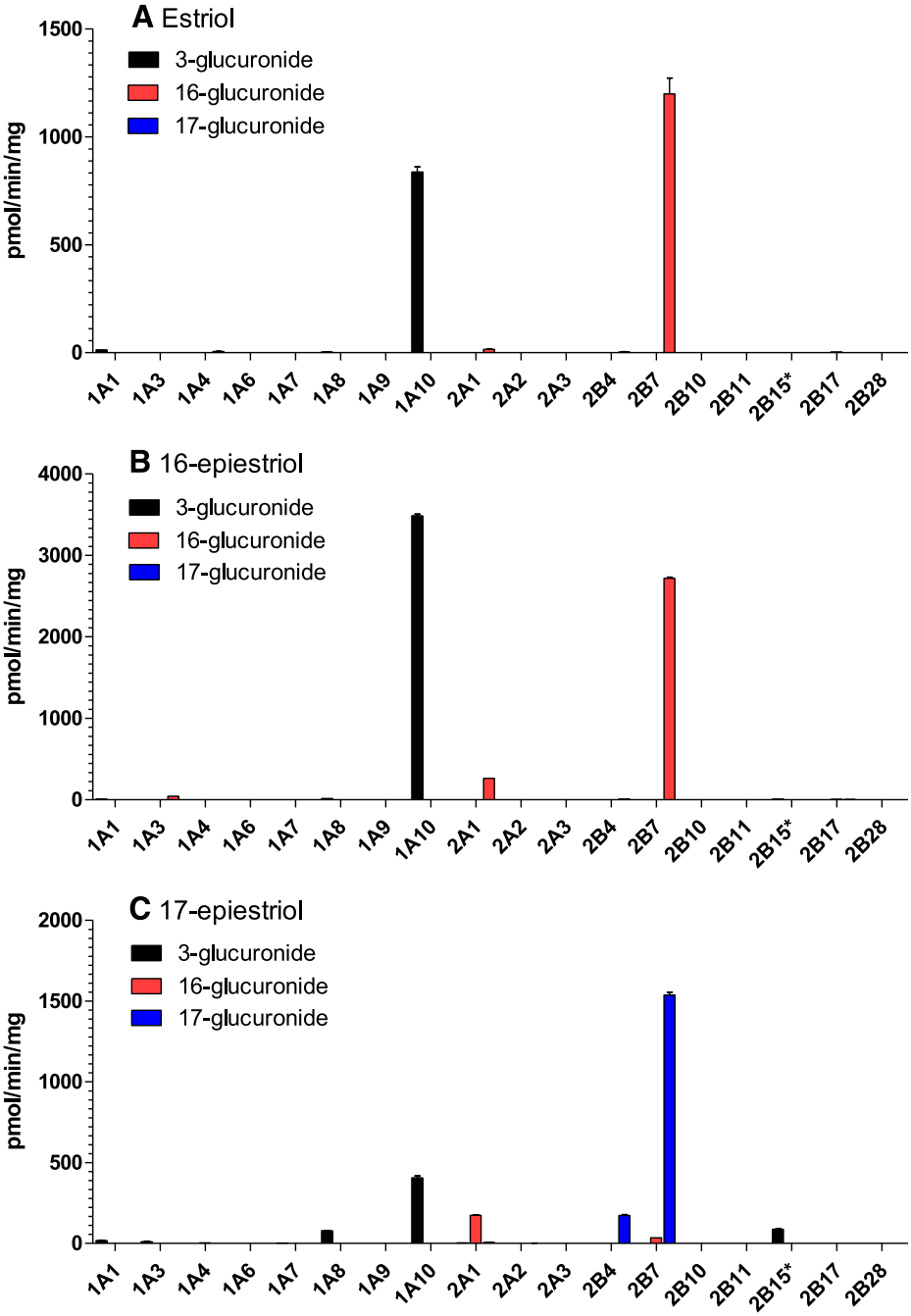

Fig. 3. Glucuronidation rates of the different estriol diastereomers (see Fig. 1 for structures) by the human UGTs at a single substrate concentration, 200 μM. The glucuronidation rate values for estriol (A), 16-epiestriol (B), and 17-epiestriol (C) are expressed as the mean of triplicate samples ± S.E. The glucuronidation rates of all the UGTs, with the exception of UGT2B15 (indicated by *), were corrected according to their relative expression values (normalized, see *Materials and Methods*).

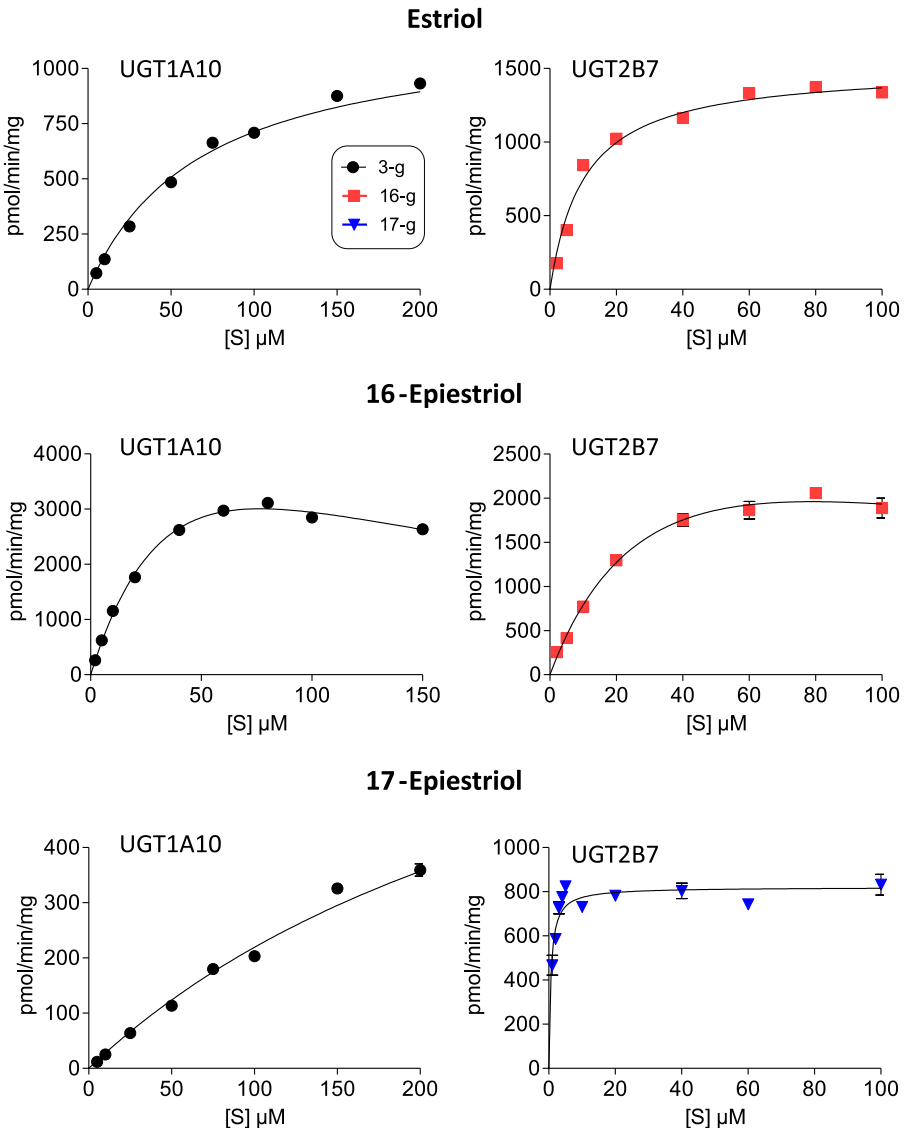

Fig. 4. Enzyme kinetics of the glucuronidation of estriol, 16-epiestriol, and 17-epiestriol by UGT1A10 and UGT2B7. The analyses were performed in triplicate, and the rates were normalized according to the relative expression levels of the UGTs. The derived kinetic constants are presented in Table 2. The Eadie-Hofstee transformations of the curves are shown in Supplemental Fig. 1.

a far lower rate than it generated 17-epiestradiol-16-glucuronide, while UGT2A2 produced trace amounts of 17-epiestradiol-17-glucuronide (Fig. 3; Supplemental Table 1).

TABLE 2

Enzyme kinetic parameters for the glucuronidation of estriol, 16-epiestriol, and 17-epiestriol by UGTs 1A10 and 2B7 as well as the mutant 1A10-F93G

Formation of the three glucuronides (g) from the different substrates was assayed in the cases of UGT1A10 and the mutant 1A10-F93G, whereas formation of 16-glucuronide by UGT2B7 was assayed when the substrate was estriol or 16-epiestriol; formation of 17-glucuronide by UGT2B7 was assayed when the substrate was 17-epiestriol.

	UGT1A10 (3-g)	1A10-F93G (3-g)	UGT2B7 (16-g or 17-g)
Estriol			(16-g)
V_{max} (pmol/min/mg)	1200 ± 28.1	7535 ± 618	1509 ± 27.7
K_m (μM)	68.4 ± 4.7	230 ± 29.7	10.3 ± 0.8
16-epiestriol			(16-g)
V_{max} (pmol/min/mg)	7700 ± 740	2249 ± 201	3866 ± 788
K_m (μM)	59.8 ± 8.0	186 ± 28.0	38.4 ± 11.4
K_i (μM)[a]	98.1 ± 16.6		162 ± 86.4
17-epiestriol			(17-g)
V_{max} (pmol/min/mg)	958 ± 75.0	5712 ± 216	820 ± 18.3
K_m (μM)	337 ± 42.1	51.1 ± 5.4	0.6 ± 0.1

UGTs, UDP-glucuronosyltransferases.

[a] K_i values are indicated when the kinetic curves fit best to the Michaelis-Menten with substrate inhibition model.

UGT2B7 also catalyzed 17-epiestradiol-16-glucuronide formation but at a much lower rate than 17-epiestriol-17-glucuronide formation. The only UGT that catalyzed rather high rates of 17-epiestriol-16-glucuronide formation was the extrahepatic UGT2A1. UGT1A4 produced some 17-estriol-16-glucuronide but at a very low rate (Fig. 3; Supplemental Table 1).

UGT1A10 exhibited the highest rate of 17-epiestriol-3-glucuronide formation (see Discussion), even if this rate was much lower than the rate at which UGT1A10 catalyzes the conjugation of the 3-OH in either estriol or 16-epiestriol (Fig. 3). The other UGTs that exhibit detectable activity in the glucuronidation of 17-epiestriol at the 3-OH were 1A1 (low), 1A3 (low), 1A7 (traces), 1A8, 2A1 (traces), and 2B15 (Fig. 3; Supplemental Table 1).

Enzyme kinetic assays of 17-epiestriol glucuronidation were performed with UGT1A10 and UGT2B7. The kinetic curves fitted best to the Michaelis-Menten equation in both cases (Fig. 4), even if the low affinity of UGT1A10 for this substrate, in combination with the limited water solubility of estrogens, makes it difficult get a fully reliable analysis for this enzyme. The V_{max} values of both enzymes were rather similar while their K_m values differed fundamentally (Table 2). It is particularly worth noting the apparent K_m value of UGT2B7 for this substrate, only 0.6

6 0.1 μM, which is rather uncommonly low among the human UGTs.

Estriol Diastereomers Glucuronidation by Human Liver and Intestine Microsomes. Estriol stereoisomer glucuronidation by HLM and HIM was examined as well. The results (Fig. 5) are in good agreement with the results for individual UGTs when the tissue-specific expression pattern of the UGTs, particularly UGT2B7, UGT1A10, and UGT2B4, is taken into account, as will be discussed.

Effect of the F93G Mutation on the Glucuronidation of Estriol Diastereomers by UGT1A10. In a recent study we found that replacing phenylalanine 93 with a glycine residue, the amino acid with no side chain, strongly stimulated the estriol and ethinylestradiol glucuronidation activity of UGT1A10 (Höglund et al., 2011). We have now examined the activity and kinetics of the 1A10-F93G mutant in the glucuronidation of 16-epiestriol and 17-estriol, in comparison with the wild-type UGT1A10 and with the estriol glucuronidation activity.

The strong stimulation of the estriol glucuronidation rate by the 1A10-F93G mutant (Supplemental Fig. 3) was nearly the same as in the previous study (Höglund et al., 2011), and it stems mainly from the increased V_{max} value of the mutant, not from a decreased K_m value (Table 2). The results with the two epiestriols, however, were somewhat unexpected (Supplemental Fig. 3; Table 2). They revealed that the glucuronidation activities of this mutant are strongly affected by the configuration of C16 and C17 of estriol, but in different ways than wild-type UGT1A10 was affected by them. In the case of 16-epiestriol, the mutant exhibited much lower V_{max} and higher K_m values than wild-type UGT1A10, whereas in the case of 17-epiestriol the mutant's activity was very high due to both lower K_m and higher V_{max} values those of the wild-type enzyme (Supplemental Fig. 3; Table 2).

13-Epiestradiol Glucuronidation. Our previous and current results have suggested that the configuration of the D-ring carbons C16 and C17 strongly affect the glucuronidation of estrogens by the different human UGTs. Nevertheless, a closer inspection of the results, particularly the conjugation site by UGT2B7, raises the possibility that the spatial relations between the 17-OH and the methyl group on C13, a carbon that belongs to both ring C and ring D, also affects the results. In particular, the only case where UGT2B7 exhibited high glucuronidation rates and high substrate affinity was 17-epiestriol, the

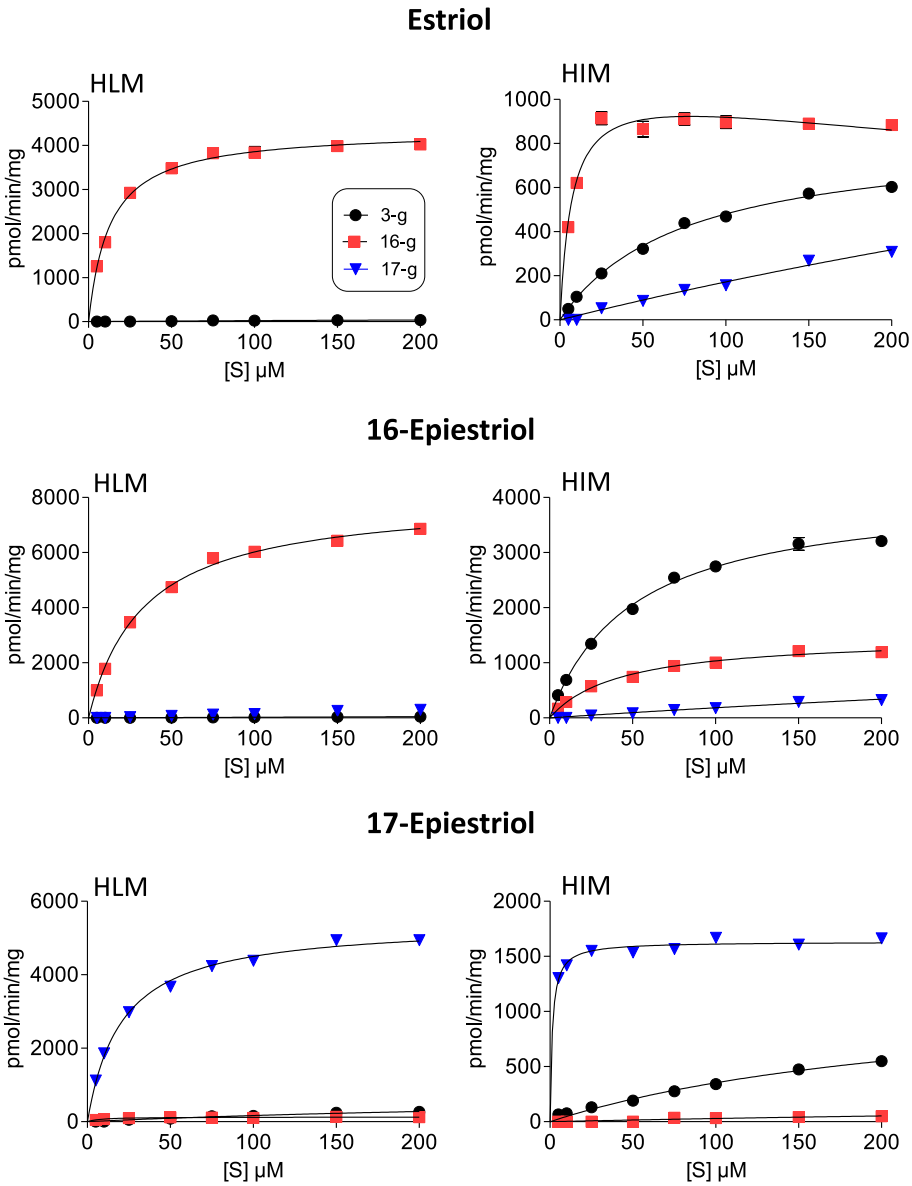

Fig. 5. Enzyme kinetics of estriol, 16-epiestriol, and 17-epiestriol glucuronidation by HLM and HIM. ●, 3-glucuronide; ■, 16-glucuronide; ▼, 17-glucuronide. The Eadie-Hofstee transformations of the curves are shown in Supplemental Fig. 2.

only substrate in this study in which C13 and C17, which are bonded to each other in ring D (Fig. 6), are in opposite configurations. Due to this, when the synthetic estradiol isomer 13-epiestradiol (Fig. 6) became available (Ayan et al., 2011), we included it in this study and screened the UGTs for the glucuronidation of this estrogen. The results with 13-epiestradiol were compared with the glucuronidation of estradiol (17β-estradiol) using the very same batches of the different recombinant UGTs.

The two different glucuronides of 17β-estradiol, the main physiologic estradiol isomer, were identified using authentic standards. No authentic glucuronide standards were available for 13-epiestradiol, however. As noted earlier, our previous results indicated that the first eluting glucuronide, the produced by UGT1A10 and UGT1A1, is the 3-glucuronide. It follows that the second eluting glucuronide of 13-epiestradiol, the main product of 13-epiestradiol glucuronidation by UGT2A1 and the only product of UGT2B7 in this case, is 13-epiestradiol-17-glucuronide.

The screening results suggest that the α-configuration of C13 significantly lowers the rates of the UGT1A10 catalyzed glucuronidation reaction as well as the activity of the other UGTs of subfamily 1A that mainly catalyze estradiol conjugation at the 3-OH, namely, UGTs 1A1, 1A3, and 1A8. (Fig. 7). On the other hand, the change in the configuration of C13 from β in the regular estradiol to α in 13-epiestradiol appears to stimulate or even change the activities of many UGTs of subfamilies 2A and 2B. In addition, it also stimulates somewhat the low activity of UGT1A4, the only member of the UGT1A subfamily that specifically conjugates estrogens at the 17-OH, even if at low rates (Fig. 7).

Both UGTs 2A1 and 2A2 catalyzed the formation of the two 13-epiestradiol glucuronides at much higher respective rates than the formation of the corresponding glucuronides of 17β-estradiol (Fig. 7). The rates of both the 3- and 17-glucuronide formation from 13-epiestradiol by UGT2B15 were also much higher than the corresponding rates when the substrate was 17β-estradiol (Fig. 7). In the case of UGT2B7, only the formation of the 13-epiestradiol-17-glucuronide was observed, but the rate of this activity was increased in comparison with 17β-estradiol. Moreover, although no 17β-estradiol glucuronidation by UGT2B4 was observed, it catalyzed measurable rates of 13-epiestradiol glucuronidation at the 17-OH (Fig. 7). Perhaps the most significant change between the glucuronidation of the two estradiols that were tested here was with UGT2B17, an enzyme that is mostly specific for the 17-OH in 17β-estradiol and in all the estrogens in which the C17 is in the β configuration (Itäaho et al., 2008; Sneitz et al., 2011). In the case of 13-epiestradiol, however, UGT2B17 catalyzes the conjugation of the 3-OH at somewhat faster rate than the conjugation of the 17-OH (Fig. 7).

One more unexpected result with 13-epiestradiol was the formation of some glucuronides, let alone both glucuronides, even by UGT1A9, an enzyme that has not exhibited estrogen glucuronidation before even if we have demonstrated that it binds estradiol (Itäaho et al., 2008). We have also tested estradiol and 13-epiestradiol glucuronidation by the F93G mutant of UGT1A10. In agreement with previous results (Höglund et al., 2011) and in contrast to the estriol glucuronidation activity, the F93G mutation did not stimulate estradiol glucuronidation by UGT1A10. On the other hand, the 13-epiestradiol glucuronidation rate of the 1A10-F93G mutant was about 4-fold higher than the corresponding activity of wild-type UGT1A10 (Fig. 7).

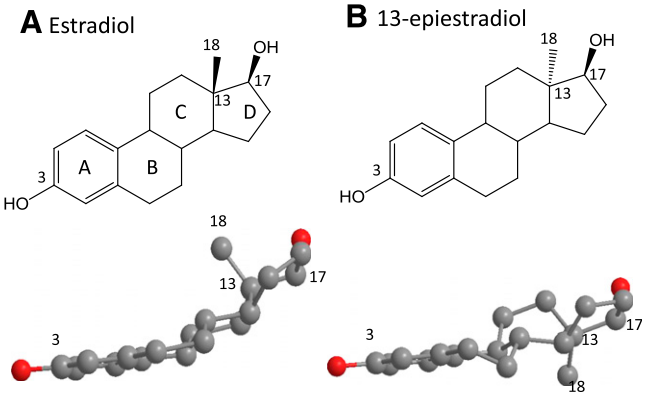

Fig. 6. Structures of estradiol (A) and 13-epiestradiol (B) in both two-dimensional (upper panels) and space-filling modes (lower panels). The different rings of the steroids' backbone are indicated in the two-dimensional presentation (upper panels), along with carbons 3, 13, 17, and 18 (both upper and lower panels). The oxygen atoms are shown in red in the space-filling models.

Discussion

Estrogens and androgens regulate important functions and gene expression in the body (Katzenellenbogen, 1996). From the point of view of UGTs research, steroids are also an interesting group of compounds for the study of substrate specificity due to their rigid backbone structure and the availability of several regio-isomers and stereo-isomers. Estriol, 16-epiestriol, and 17-epiestriol are all built of four rings, and the main difference between them is in ring D (Fig. 1). To extract information on the substrate specificity of the different UGTs, one has to identify the site of glucuronidation in each case, an important but sometimes challenging objective. In this study, we have partly overcome this challenge by purification of two glucuronides and identification of the conjugation site in them by use of NMR (Table 1).

Our results demonstrate that UGT1A10 is the most active enzyme in the conjugation of the 3-OH in each of these estriol diastereomers. It may be noted that the assignment of the conjugation site of UGT1A10 in estriol was based on the migration of the authentic estriol-3-glucuronide standard. No such standards are available for the two epiestriols, but there are good reasons to assume that the glucuronides generated by UGT1A10 from either 16-epiestriol or 17-epiestriol, the glucuronide that is eluted first under our chromatography conditions, is the corresponding 3-glucuronide This assignment is based on the similarity of epiestriols in physicochemical properties to estriol (for which authentic standards are available) and the similarity of such properties among their 3-glucuronides (the differences among the 16- and 17-glucuronides are likely to be much larger). The second reason for the suggested assignment is the tendency of UGT1A10 to mainly conjugate the phenolic hydroxyl in estrogens, the 3-OH (or other phenolic hydroxyls in ring A, if present), rather than the 16-OH or the 17-OH that are secondary hydroxyls (Fig. 3A of this study for estriol; Itäaho et al., 2008 for estradiol; Lepine et al., 2004 for estrone, estradiol, and hydroxyestradiols). These reasons and the NMR results for the ring D glucuronide that is generated by UGT2B7 (Table 1) give strong support to the assignment of the glucuronidation sites within the estrogens that were tested in this study.

UGT2B7 is clearly the most active enzyme in the conjugation of one of the two hydroxyls on ring D of the estriols (Fig. 3). In addition to the glucuronidation site, UGT1A10 and UGT2B7 also differ considerably from each other in their affinity toward the different estriols, particularly toward 17-epiestriol (Fig. 4). Nevertheless, both enzymes exhibited very high glucuronidation rates toward 16-epiestriol while the lowest rate of both enzymes was toward 17-epiestriol (Fig. 3; Table 2).

Considering that the expression levels of UGT1A10 and UGT2A1 in HLM are very low (Ohno and Nakajin, 2009; Sneitz et al., 2009;

TABLE 3

Enzyme kinetic parameters for the glucuronidation of estriol, 16-epiestriol, and 17-epiestriol by human liver microsomes (HLM) and human intestine microsomes (HIM)

	HLM			HIM		
	3-g	16-g	17-g	3-g	16-g	17-g
Estriol						
V_{max} (pmol/min/mg)	95 ± 45	4347 ± 50	—	839 ± 19	953 ± 21	2095 ± 947
K_m (μM)	313 ± 214	13 ± 0.7	—	75 ± 4	5.1 ± 0.7	1119 ± 575
16-epiestriol						
V_{max} (pmol/min/mg)	569 ± 1144	8011 ± 106	~8400[a]	4111 ± 94	1480 ± 35	2324 ± 1107
K_m (μM)	2375 ± 5094	33 ± 1	~5300[a]	50 ± 3	43 ± 3	1168 ± 629
17-epiestriol						
V_{max} (pmol/min/mg)	805 ± 157	128 ± 6	5425 ± 67	1239 ± 150	266 ± 394	1633 ± 13
K_m (μM)	380 ± 102	7.8 ± 2	21 ± 1	251 ± 47	820 ± 1438	1.3 ± 0.14

[a] An estimate, as accurate kinetic parameters could not be determined.

Court et al., 2012), the results with HLM (Fig. 5) suggest that UGT2B7 plays a major role in the glucuronidation of the three estriols in the liver. The kinetic results (Fig. 5) also suggest that another enzyme, perhaps UGT2B4 which is highly expressed in the liver but not in the intestine (Ohno and Nakajin, 2009; Court et al., 2012), contributes to 17-epiestriol glucuronidation on the 17-OH in the liver.

One of the main reason to undertake a detailed study on estriols glucuronidation by the human UGTs was a previous result with estradiol and 17-epiestradiol according to which the affinity of UGT2B7 for the estrogen substrate is strongly increased when the configuration of C17 is α rather than β (Itäaho et al., 2008). The new results on the kinetics of 17-epiestriol by UGT2B7 (Fig. 4) fully support this property of UGT2B7. The results with the other two estriols suggest that when the estriol's C17 is in the β configuration, UGT2B7 only conjugates the 16-OH, regardless of whether the C16 is in the α (estriol) or the β (16-epiestriol) configuration (Fig. 3). This suggests that the configuration of C17 in either estriol or estradiol plays a dominant role in determining the affinity of UGT2B7 for estrogens. The only case so far in which C17 of estradiol was in the α configuration and the UGT2B7 K_m for the substrate was not very low was enantio-estradiol glucuronidation (Sneitz et al., 2011), a result that may be due to the configuration of the other chiral centers in the substrate molecule. Hence, it might be suggested that the α configuration of C17 has such an effect on UGT2B7 because it

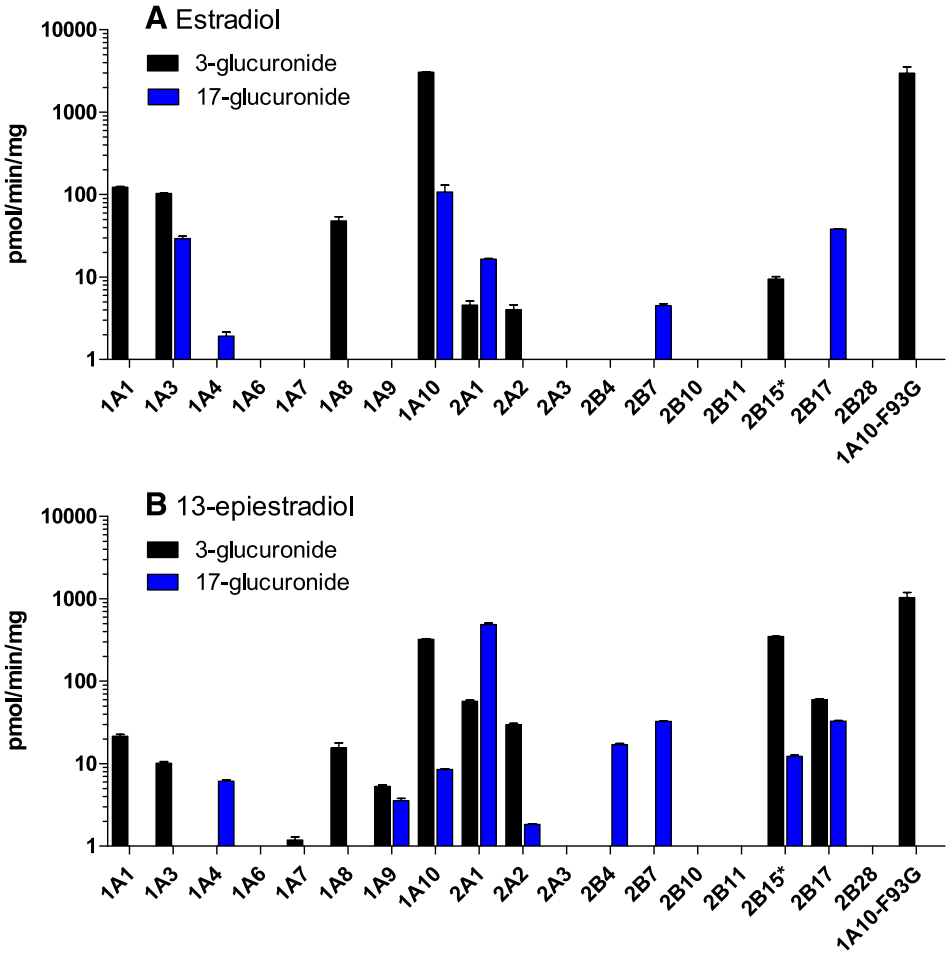

Fig. 7. Glucuronidation of estradiol (A) and 13-epiestradiol (B) (see Fig. 6 for structures) by the human UGTs. The screening assay was performed at a single substrate concentration, 200 μM. The rates, the mean of triplicate assays with S.E., were normalized according to the relative expression levels (see *Materials and Methods*) and are presented on a scale. The numeric results are presented in Supplemental Table 1. Note that the Y-axis values are presented on a logarithmic scale.

changes the spatial relations between 17-OH and the methyl group on C18. It was not easy to find a good substrate for testing the latter suggestion, and the reason 13-epiestradiol was added to the current study was because it was the only possible currently available estrogen in which the spatial organization of the 17-OH and 18-CH_3 is considerably different than in other estrogens. Nevertheless, even 13-epiestradiol does not provide a very good test compound because the conformational change in C13 leads to larger changes in the structure of the entire estrogen molecule (Fig. 6).

While the α configuration of C17 strongly increases the affinity of UGT2B7 for estrogens, in the case of UGT1A10 it lowers the enzyme's affinity for estrogens, as far as can be judged from the K_m values (Table 2). The configuration of the estriols' C17 and/or C16 also affects the activity of UGT2B15 in estrogen glucuronidation, except that the rates exhibited by this enzyme are relatively low and the effects are more complex. When C17 of estradiol (17α-estradiol, Itäaho et al., 2008, as well as enantio-estradiol, Sneitz et al., 2011) or estriol (17-epiestriol, our study) is in the α configuration, UGT2B15 conjugates the 3-OH, the only member of the UGTs subfamily 2B to do so, or at least this was our conclusion until testing the glucuronidation of 13-epiestradiol (Fig. 7). In the case of UGT1A3, the configuration change in C16 of estriol from α to β stimulates conjugation but at the 16-OH, not at the 3-OH as it does when the substrate is 17-epiestriol (Fig. 3B), estradiol, or 13-epiestradiol (Fig. 7).

UGT2B17, like UGT2B15, exhibited low rates in the glucuronidation of the different estriols but an interesting stereoselectivity. As with estradiols (Itäaho et al., 2008), UGT2B17 only conjugates the 17-OH of estrogens when it is located "above" the plane of ring D. UGT2B17 failed completely to glucuronidate 17-epiestriol, the variant in which both C17 and C16 are in the α configuration (Fig. 1). In the case of 16-epiestriol, where both the C16 and C17 are in the β configuration, UGT2B17 conjugated both of them at similar rates (Fig. 3).

Aside from the glucuronidation site, the results also show that the glucuronidation rate of the different estriols depends on the configuration of C16 and/or C17, even if the conjugation target is the 3-OH, as in UGT1A10 (Fig. 3). In this respect, it is interesting that a point mutation in UGT1A10, F93G, largely changes the relation between the structure of the estriol and its glucuronidation rate. The findings with mutant 1A10-F93G also suggest that the differences in the glucuronidation rate of the three estriols could not be assigned to a small difference in the physicochemical properties among them.

The configuration change at C13 of estradiol changes the spatial organization of the 17-OH and 18CH_3 without changing the location of the 17-OH with respect to the plane of ring D (Fig. 6). The glucuronidation results with estradiol and 13-epiestradiol suggest that, with the exception of UGT2B17, the large change in the estradiol molecule upon the configuration change at C13 does not affect the site at which the different UGTs conjugate estradiol, but it does change the reaction rate. The results with UGT2B17 were, however, surprising because it also conjugated the 3-OH on ring A, an observation that has never been reported before for UGT2B17 with any estrogen.

The results of this study shed new light on estrogen glucuronidation by the human UGTs, particularly the structural components within the estrogen substrate that, often differently, affect their glucuronidation by individual UGT enzymes. These findings are expected to assist in predicting the glucuronidation of drugs that resemble estrogens or carry estrogen-like structural elements, such as the phase I metabolite of exemestane (Sun et al., 2010). In addition, they will contribute to a better understanding of the complex structure of the substrate-binding site of the human UGTs and identification of the residues and other structural properties that allow them to glucuronidate many different substrates while retaining a considerable degree of specificity.

Authorship Contributions

Participated in research design: Sneitz, Finel.
Conducted experiments: Sneitz, Mosorin, Vahermo.
Contributed new reagents or analytic tools: Poirier, Vahermo.
Performed data analysis: Sneitz, Laakkonen, Vahermo.
Wrote or contributed to the writing of the manuscript: Sneitz, Finel.

References

Ayan D, Roy J, Maltais R, and Poirier D (2011) Impact of estradiol structural modifications (18-methyl and/or 17-hydroxy inversion of configuration) on the in vitro and in vivo estrogenic activity. *J Steroid Biochem Mol Biol* **127**:324–330.

Barre L, Fournel-Gigleux S, Finel M, Netter P, Magdalou J, and Ouzzine M (2007) Substrate specificity of the human UDP-glucuronosyltransferase UGT2B4 and UGT2B7. Identification of a critical aromatic amino acid residue at position 33. *FEBS J* **274**:1256–1264.

Cohen SL and Marrian GF (1936) The isolation and identification of a combined form of oestriol in human pregnancy urine. *Biochem J* **30**:57–65.

Court MH, Zhang X, Ding X, Yee KK, Hesse LM, and Finel M (2012) Quantitative distribution of mRNAs encoding the 19 human UDP-glucuronosyltransferase enzymes in 26 adult and 3 fetal tissues. *Xenobiotica* **42**:266–277.

Eliassen AH, Spiegelman D, Xu X, Keefer LK, Veenstra TD, Barbieri RL, Willett WC, Hankinson SE, and Ziegler RG (2012) Urinary estrogens and estrogen metabolites and subsequent risk of breast cancer among premenopausal women. *Cancer Res* **72**:696–706.

Eliassen AH, Ziegler RG, Rosner B, Veenstra TD, Roman JM, Xu X, and Hankinson SE (2009) Reproducibility of fifteen urinary estrogens and estrogen metabolites over a 2- to 3-year period in premenopausal women. *Cancer Epidemiol Biomarkers Prev* **18**:2860–2868.

Gall WE, Zawada G, Mojarrabi B, Tephly TR, Green MD, Coffman BL, Mackenzie PI, and Radominska-Pandya A (1999) Differential glucuronidation of bile acids, androgens and estrogens by human UGT1A3 and 2B7. *J Steroid Biochem Mol Biol* **70**:101–108.

Höglund C, Sneitz N, Radominska-Pandya A, Laakonen L, and Finel M (2011) Phenylalanine 93 of the human UGT1A10 plays a major role in the interactions of the enzyme with estrogens. *Steroids* **76**:1465–1473.

Itäaho K, Mackenzie PI, Ikushiro S, Miners JO, and Finel M (2008) The configuration of the 17-hydroxy group variably influences the glucuronidation of beta-estradiol and epiestradiol by human UDP-glucuronosyltransferases. *Drug Metab Dispos* **36**:2307–2315.

Katzenellenbogen BS (1996) Estrogen receptors: bioactivities and interactions with cell signaling pathways. *Biol Reprod* **54**:287–293.

King CD, Rios GR, Green MD, and Tephly TR (2000) UDP-glucuronosyltransferases. *Curr Drug Metab* **1**:143–161.

Kurkela M, García-Horsman JA, Luukkanen L, Mörsky S, Taskinen J, Baumann M, Kostiainen R, Hirvonen J, and Finel M (2003) Expression and characterization of recombinant human UDP-glucuronosyltransferases (UGTs). UGT1A9 is more resistant to detergent inhibition than other UGTs and was purified as an active dimeric enzyme. *J Biol Chem* **278**:3536–3544.

Kurkela M, Patana AS, Mackenzie PI, Court MH, Tate CG, Hirvonen J, Goldman A, and Finel M (2007) Interactions with other human UDP-glucuronosyltransferases attenuate the consequences of the Y485D mutation on the activity and substrate affinity of UGT1A6. *Pharmacogenet Genomics* **17**:115–126.

Lappano R, Rosano C, De Marco P, De Francesco EM, Pezzi V, and Maggiolini M (2010) Estriol acts as a GPR30 antagonist in estrogen receptor-negative breast cancer cells. *Mol Cell Endocrinol* **320**:162–170.

Lépine J, Bernard O, Plante M, Têtu B, Pelletier G, Labrie F, Bélanger A, and Guillemette C (2004) Specificity and regioselectivity of the conjugation of estradiol, estrone, and their catecholestrogen and methoxyestrogen metabolites by human uridine diphosphoglucuronosyltransferases expressed in endometrium. *J Clin Endocrinol Metab* **89**:5222–5232.

Longcope C (1984) Estriol production and metabolism in normal women. *J Steroid Biochem* **20** (4B):959–962.

Melamed M, Castaño E, Notides AC, and Sasson S (1997) Molecular and kinetic basis for the mixed agonist/antagonist activity of estriol. *Mol Endocrinol* **11**:1868–1878.

Mukherjee TK, Nathan L, Dinh H, Reddy ST, and Chaudhuri G (2003) 17-epiestriol, an estrogen metabolite, is more potent than estradiol in inhibiting vascular cell adhesion molecule 1 (VCAM-1) mRNA expression. *J Biol Chem* **278**:11746–11752.

Ohno S and Nakajin S (2009) Determination of mRNA expression of human UDP-glucuronosyltransferases and application for localization in various human tissues by real-time reverse transcriptase-polymerase chain reaction. *Drug Metab Dispos* **37**:32–40.

Radominska-Pandya A, Czernik PJ, Little JM, Battaglia E, and Mackenzie PI (1999) Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metab Rev* **31**:817–899.

Ruggiero RJ and Likis FE (2002) Estrogen: physiology, pharmacology, and formulations for replacement therapy. *J Midwifery Womens Health* **47**:130–138.

Sneitz N, Court MH, Zhang X, Laajanen K, Yee KK, Dalton P, Ding X, and Finel M (2009) Human UDP-glucuronosyltransferase UGT2A2: cDNA construction, expression, and functional characterization in comparison with UGT2A1 and UGT2A3. *Pharmacogenet Genomics* **19**:923–934.

Sneitz N, Krishnan K, Covey DF, and Finel M (2011) Glucuronidation of the steroid enantiomers ent-17β-estradiol, ent-androsterone and ent-etiocholanolone by the human UDP-glucuronosyltransferases. *J Steroid Biochem Mol Biol* **127**:282–288.

Starlard-Davenport A, Xiong Y, Bratton S, Gallus-Zawada A, Finel M, and Radominska-Pandya A (2007) Phenylalanine(90) and phenylalanine(93) are crucial amino acids within the estrogen binding site of the human UDP-glucuronosyltransferase 1A10. *Steroids* **72**:85–94.

Sun D, Chen G, Dellinger RW, Sharma AK, and Lazarus P (2010) Characterization of 17-dihydroexemestane glucuronidation: potential role of the UGT2B17 deletion in exemestane pharmacogenetics. *Pharmacogenet Genomics* **20**:575–585.

Tukey RH and Strassburg CP (2000) Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol* **40**:581–616.

Address correspondence to: Moshe Finel, CDR, Faculty of Pharmacy, P.O. Box 56 (Viikinkaari 5), FIN-00014 University of Helsinki, Finland. E-mail: moshe.finel@helsinki.fi