Glucuronidation of arachidonic and linoleic acid metabolites by human UDP-glucuronosyltransferases

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Abstract Arachidonic acids (AA) and linoleic acids (LAs) are metabolized, in several tissues, to hydroxylated metabolites that are important mediators of many physiological and pathophysiological processes. The conjugation of leukotriene B_4 (LTB_4), 5-hydroxyeicosatetraenoic acid (HETE), 12-HETE, 15-HETE, and 13-hydroxyoctadecadienoic acid (HODE) by the human UDP-glucuronosyltransferase (UGT) enzymes was investigated. All substrates tested were efficiently conjugated by human liver microsomes to polar derivatives containing the glucuronyl moiety as assessed by mass spectrometry. The screening analyses with stably expressed UGT enzymes in HK293 showed that glucuronidation of LTB_4 was observed with UGT1A1, UGT1A3, UGT1A8, and UGT2B7, whereas UGT1A1, UGT1A3, UGT1A4, and UGT1A9 also conjugated most of the HETEs and 13-HODE. LA and AA metabolites also appear to be good substrates for the UGT2B subfamily members, especially for UGT2B4 and UGT2B7 that conjugate all HETE and 13-HODE. Interestingly, UGT2B10 and UGT2B11, which are considered as orphan enzymes since no conjugation activity has so far been demonstrated with these enzymes, conjugated 12-HETE, 15-HETE, and 13-HODE. In summary, our data showed that several members of UGT1A and UGT2B families are capable of converting LA and AA metabolites into glucuronide derivatives, which is considered an irreversible step to inactivation and elimination of endogenous substances from the body.—Turgeon, D., S. Chouinard, P. Bélanger, S. Picard, J-F. Labbé, P. Borgeat, and A. Bélanger. **Glucuronidation of arachidonic and linoleic acid metabolites by human UDP-glucuronosyltransferases. *J. Lipid Res.* 2003.** 44: **1182–1191.**

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Linoleic acid (LA), an 18-carbon fatty acid, is an abundant polyunsaturated fatty acid (PUFA) in phospholipids, the major component of the lipid bilayer of cell membranes (1). Arachidonic acid (AA), a 20-carbon fatty acid, is also present in substantial amounts in cellular membranes and is produced from LA by chain elongation and desaturation (1, 2). These two PUFAs are further converted into *a*) prostaglandins or thromboxanes by cyclooxygenases and/or *b*) hydroxyeicosatetraenoic acids (HETEs), leukotrienes, or 13-hydroxyoctadecadieneoic acid (13-HODE) by lipoxygenases (**Fig. 1**) (3).

The hydroxylated metabolites of LA and AA are important mediators of many physiological and pathophysiological processes (3). For instance, prostaglandins, the products of cyclooxygenases, regulate vascular homeostasis, kidney function, ovulation, and parturition (4). Although the physiological functions of HETE, the hydroxylated metabolites of AA, are not fully characterized, accumulating evidence indicates that they are involved in a wide spectrum of biological activities, such as platelet activation and aggregation, immune system activity, cell differentiation, and apoptosis (5–7). The products of cyclooxygenases and lipoxygenases have also been implicated in several forms of epithelial cell cancers, especially in prostate cancer, for which recent results indicate that they are important angiogenic and mitogenic factors (3, 8–11).

Although the catabolism of these PUFAs involves several P450 enzymes, the significant contribution of glucuronidation in their clearance has been recently established (12–16). Indeed, several authors have reported the presence in the urine of glucuronidated PUFAs (17–20). This conjugation reaction is catalyzed by UDP-glucuronosyltransferases (UGTs), a family of endoplasmic reticulum membrane-bound enzymes that transfer the glucuronic moiety from the UDP-glucuronic acid (UDPGA) to a wide variety of small lipophilic molecules carrying a functional group containing oxygen, nitrogen, or sulfur (21). Conjugation by UGT enzymes is also an important pathway of elimination for several endogenous compounds, namely steroids, thyroid hormones, retinoic acids, and bilirubin.

Based on amino acid sequence homology, UGT enzymes have been classified into two families, UGT1 and UGT2 (22). In humans, the UGT1 family is encoded by a

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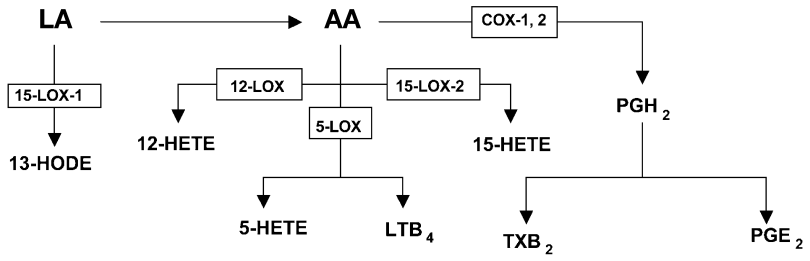


Fig. 1. Schematic representation of the various enzymes and products involved in the major pathways of linoleic acids (LAs) and arachidonic acids (AAs) metabolism. COX-1, cyclooxygenase type 1; COX-2, cyclooxygenase type 2; 5-Lox, 5-lipoxygenase; 12-Lox, 12-lipoxygenase; 15-Lox-1, 15-lipoxygenase type 1; 15-Lox-2, 15-lipoxygenase type 2; 13-HODE, 13-hydroxyoctadecadieneoic acid; 5-HETE, 5-hydroxyeicosatetraenoic acid; 12-HETE, 12-hydroxyeicosatetraenoic acid; 15-HETE, 15-hydroxyeicosatetraenoic acid; LTB_4, leukotriene B_4; TXB_2, thromboxane B_2; PGH_2, prostaglandin H_2; PGE_2, prostaglandin E_2.

complex of genes localized on chromosome 2q37 (23). These genes share exons 2 to 5, which are coupled to specific exons 1, leading to the expression of different transcripts that encode nine functional proteins (24). The UGT1 members are known to catalyze the conjugation of bilirubins, phenols, and estrogen hormones (25, 26). The UGT2 family is divided into two subfamilies, UGT2A, which is expressed in olphactive epithelium, and UGT2B (27). The genes encoding the UGT2B members have been localized on chromosome 4q13, and each of them is composed of six specific exons (28). In humans, seven members of the UGT2B family have been characterized: UGT2B4 (29, 30), UGT2B7 (31, 32), UGT2B10 (33), UGT2B11 (31), UGT2B15 (28), UGT2B17 (34), and more recently UGT2B28 (35). All UGT2B cDNAs have been stably transfected in the HK293 cell line, and it was demonstrated that these enzymes and their allelic variants have conjugation activities toward carboxylic acids, bile acids, and steroid hormones, namely androgens and estrogens (35, 36). However, UGT2B10 and UGT2B11 did not demonstrate conjugating activity for over 100 substrates tested (33).

The objective of this study was to demonstrate the role of UGT enzymes in the conjugation of the AA and LA metabolites. All available human UGT1A and UGT2B enzymes stably transfected in the HK293 cell line, devoid of endogenous steroid transferase activity, have been tested. Our data demonstrate that several UGT enzymes conjugate AA metabolites 5-HETE, 12-HETE, 15-HETE, and leukotriene B_4 (LTB_4), and the LA metabolite 13-HODE.

EXPERIMENTAL PROCEDURES

Materials

UDPGA and eugenol were obtained from Sigma Chemical Co. (St. Louis, MO). LTB_4, 5-HETE, 12-HETE, 15-HETE, and 13-HODE were purchased from Cayman Chemicals (Ann Arbor, MI), and androsterone (ADT) was obtained from Steraloids Inc. (Wilton, NH). Ammonium formate was from Aldrich Chemical (Milwaukee, WI), and high-performance liquid chromatography (HPLC)-grade methanol was provided by VWR Canlab (Montreal, Canada). Human HK293 cells were obtained from the American Type Culture Collection (Rockville, MD), and human liver microsomes were obtained from the Human Cell Culture Center (Laurel, MD).

Stable expression of UGT2B enzymes

HK293 cells were grown in Dulbecco's modified Eagle medium containing 4.5 g/l glucose, 10 mM HEPES, 110 μg/ml sodium pyruvate, 100 IU of penicillin/ml, 100 μg/ml of streptomycin, and 10% fetal bovine serum under a humidified atmosphere containing 5% CO_2 at 37°C. HK293-UGT2B7 and HK293-UGT1A8 stable cell lines were kindly provided by Dr. Thomas R. Tephly (37). HK293-UGT2B4, HK293-UGT2B10, HK293-UGT2B11, HK293-UGT2B15, HK293-UGT2B17 and HK293-UGT2B28 stable cell lines were obtained as previously described (29). UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A9, and UGT1A10 cDNA were obtained by RT-PCR from kidney and liver total RNA, as previously reported (38).

Preparation of microsomal fractions

Microsomal preparations were obtained by differential centrifugation. HK293 cells stably expressing exogenous UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17, or UGT2B28 were homogenized in 4 mM K_2HPO_4, pH 7.0, 20% glycerol, 1 mM EDTA, and 0.5 mM dithiothreitol, and the homogenates were centrifuged at 12,000 *g* for 20 min to remove nuclei, unbroken cells, and mitochondria. Pellets were discarded, and supernatants were centrifuged at 105,000 *g* for 60 min to obtain the microsomal pellets, which were resuspended in homogenization buffer and stored at −20°C.

Glucuronidation assay using microsomal fractions

Enzymatic assays were conducted using 1 mM UDPGA, 30 μM to 200 μM of the different aglycons, and 5 μM to 40 μg of protein from microsome preparations in 50 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 100 μg/ml phosphatidylcholine, and 8.5 mM saccharolactone in a final volume of 100 μl. Assays were terminated by adding 100 μl of methanol and by centrifugation at 14,000 *g* for 1 min, as previously described (34). The screening for the reactivity of the various UGT proteins with fatty acid substrates was performed using 1 mM UDPGA for 16 h at 37°C. Compounds that demonstrated reactivity with UGT proteins were subsequently reassayed in the presence of 1 mM UDPGA for 1 h with all UGT enzymes, except for UGT2B17, which was incubated for 30 min at 37°C in order to obtain linear reaction rates. The microsomal fraction from HK293 cells, which do not express endogenous UGT enzymes, was used as a negative control for the glucuronidation reaction.

Liquid chromatography coupled with mass spectrometry

The products from the glucuronidation assays (25 μl) of the denaturated incubation media were separated by HPLC using an

Alliance 2690 system (Waters, Milford, MA). Chromatographic separation was achieved with a Phenomenex Colombus C18 column, 5 μm particles, 50 × 3.2 mm (Phenomenex, Torrance, CA) using a two-solvent gradient system: *A*) 1 mM ammonium formate in water; and *B*) 1 mM ammonium formate in methanol. A linear gradient from 15% to 95% B was run over 6 min, held 2 min and then reequilibrated to 15% of the second gradient system over 2 min at the constant flow rate of 0.7 ml/min. The HPLC column was coupled to a Finnigan LCQ ion trap mass spectrometer equipped with an electrospray ionization source in the negative ion mode. The mass spectrometer was operated in full scan mass spectrometry (MS) mode, except in fragmentation studies where tandem MS scan was used.

K_m determination

Determination of the K_m values of UGT1A3 and UGT2B7 for LTB_4 was performed by incubating 1 μM to 15 μM of the aglycon in the presence of 1 mM UDPGA during 1 h, as previously reported (34), whereas K_m values of 5-, 12-, and 15-HETE for the UGT2B7 enzyme were determined at concentrations of 5 μM to 25 μM of the different aglycons.

Effect of ADT on LTB_4 glucuronidation

Microsomal proteins from HK293 cells stably expressing UGT2B7 were incubated in the presence of 100 μM LTB_4 and concentrations of ADT ranging from 0.1 μM to 100 μM, which represent 0.01- to 10-fold the K_m value of UGT2B7 for this substrate (37). Assays were incubated for 1 h at 37°C and glucuronidation products were subsequently analyzed by liquid chromatography (LC)-MS.

RESULTS

Glucuronidation of arachidonic and LA metabolites by human liver microsomes

To ascertain the experimental conditions used for incubation of human liver microsomal preparations with LA and AA metabolites and the system of quantification by liquid chromatography-mass spectrometry (LC-MS), incubations were first conducted with LTB_4 and its 20-COOH metabolite, for which the formation of conjugated products by human liver microsomes has already been demonstrated (12). As illustrated in **Fig. 2A**, LTB_4 was detected in the supernatant at the retention time of 6.41 min when the detector was set at *m/z* 335–337, whereas two major metabolites corresponding to the monoglucuronide derivatives of LTB_4 (*m/z* 511–513) eluted at retention times of 5.14 and 5.86 min (Fig. 2B). Our data indicate that the LTB_4-monoglucuronide molecules were fragmented into the parental LTB_4 molecule and a glucuronic acid (*m/z* 176) (data not shown), and that the formation of di- or triglu-

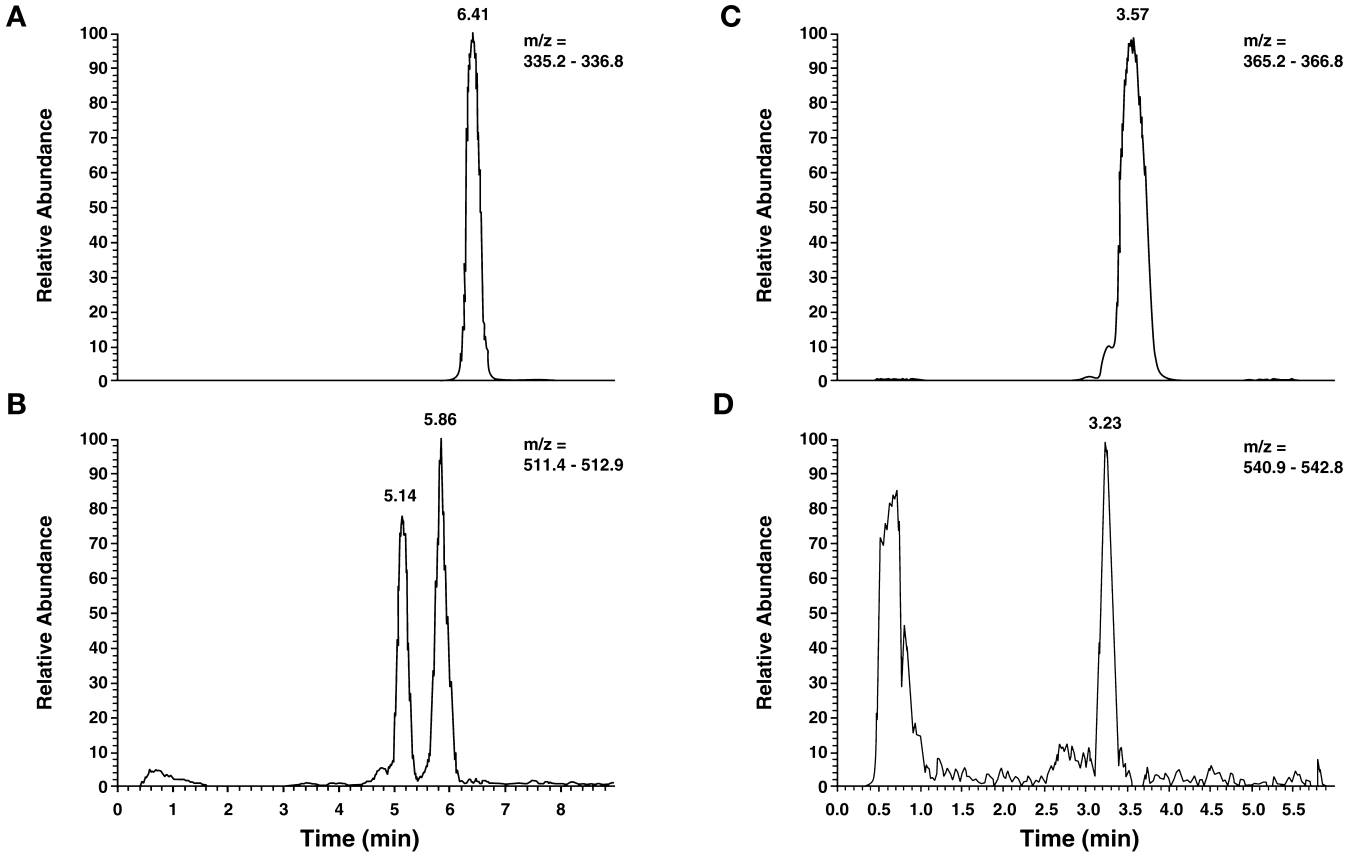


Fig. 2. Mass chromatograms of LTB_4 and 20-COOH-LTB_4 after incubation with human liver microsomal proteins. Microsomal proteins (40 μg) were incubated with 500 μM LTB_4 or 20-COOH-LTB_4 in the presence of 2 mM UDP-glucuronic acid (UDPGA) for 3 h at 37°C. Reactions were stopped by adding methanol, and products were analyzed by liquid chromatography-mass spectrometry (LC-MS). Mass chromatograms at *m/z* 335–337 and *m/z* 365–367 allowed detection of LTB_4 and 20-COOH-LTB_4, respectively (A and C). Additional mass chromatograms at *m/z* 511–513 and *m/z* 541–543 allowed detection of monoglucuronides of the two substrates (B and D).

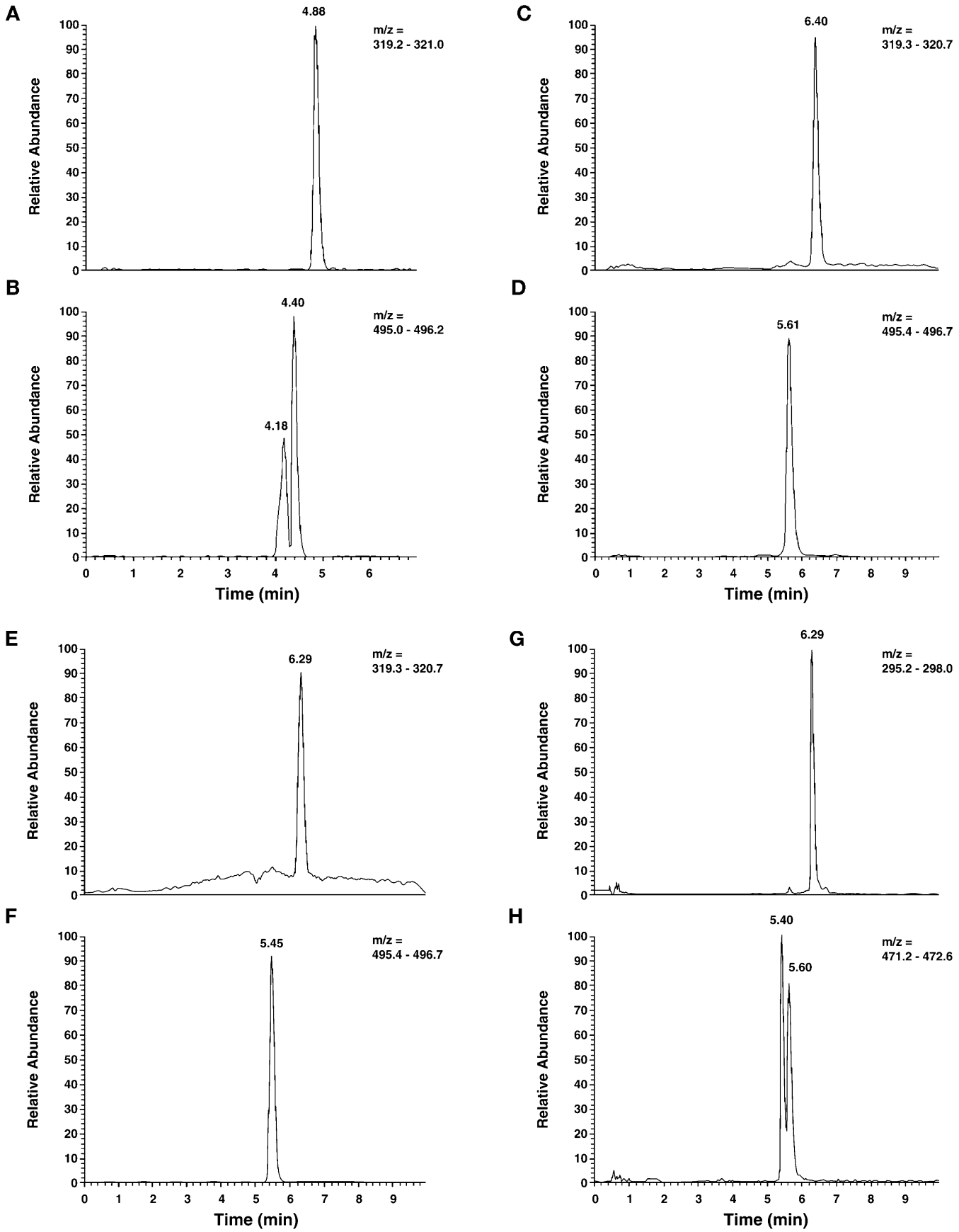


Fig. 3. Mass chromatograms of 5-HETE, 12-HETE, 15-HETE, and 13-HODE after incubation with human liver microsomal proteins. Microsomal proteins (40 μg) were incubated with 500 μM of aglycons in the presence of 2 mM UDPGA for 3 h at 37°C. Reactions were stopped by adding methanol, and products were analyzed by LC-MS. Mass chromatograms at *m/z* 319–321 and *m/z* 295–298 allowed detection of the three HETE substrates and 13-HODE, respectively (A, C, E, and G). Additional mass chromatograms at *m/z* 495–496 and *m/z* 471–473 allowed detection of monoglucuronides of the four substrates (B, D, F, and H).

TABLE 1. Screening analysis of the conversion of PUFA metabolites to glucuronide derivatives by microsomal proteins from human liver and HEK293 cells stably expressing human UGT proteins

Isoforms	LTB_4	20-COOH-LTB_4	5-HETE	12-HETE	15-HETE	13-HODE
Liver	+	+	+	+	+	+
UGT1A						
UGT1A1	+	−	−	+	+	+
UGT1A3	+	−	−	+	+	+
UGT1A4	−	−	−	+	+	+
UGT1A5	−	−	−	−	−	−
UGT1A6	−	−	−	−	−	+
UGT1A7	−	−	−	−	−	−
UGT1A8	+	−	−	−	+	+
UGT1A9	−	+	+	+	+	+
UGT1A10	−	−	−	−	−	−
UGT2B						
UGT2B4	−	−	+	+	+	+
UGT2B7	+	−	+	+	+	+
UGT2B10	−	−	+	+	+	+
UGT2B11	−	−	−	+	+	+
UGT2B15	−	−	−	+	−	−
UGT2B17	−	−	−	−	−	+
UGT2B28	−	−	−	−	−	−

HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; LTB_4, leukotriene B_4; UGT, UDP-glucuronosyltransferase. To determine which UGT isoenzyme was involved, microsomal proteins (40 μg) were incubated with 1 mM UDP-glucuronic acid, 200 μM of substrate for 16 h at 37°C. Enzymatic reactions were stopped by adding methanol, and the samples were centrifuged to remove proteins. The supernatants (25 μl) were analyzed by liquid chromatography-mass spectrometry. (+) indicates that glucuronidation activity was observed, whereas (–) indicates that no conjugation was observed.

curonide of LTB_4 was not be observed. The substrate 20-COOH-LTB_4 was also conjugated by hepatic UGT enzymes and, in this case, only one metabolite was observed at retention time of 3.23 min at *m/z* 540–543, also corresponding to a monoglucuronide derivative (Fig. 2C, D).

Glucuronidation reaction by human liver microsomes of 5-, 12-, and 15-HETE, and 13-HODE was also assessed and, as shown in **Fig. 3**. Glucuronide derivatives were detected by MS for each of the substrates tested. Except for 5-HETE and 13-HODE, which were conjugated into two distinct monoglucuronides, the two other fatty acids yielded only one product at the *m/z* corresponding to a monoglucuronide, and the formation of diglucuronide was not detected. Incubation of liver microsomes with 12-HETE, 15-HETE, and 13-HODE for 16 h resulted in the transformation of more than 95% of the substrates, whereas 5-HETE and LTB4 were converted by 53% and 17%, respectively.

Glucuronidation of AA and LA metabolites by stably transfected UGT enzymes in HK293

To determine which UGT enzymes are responsible for the glucuronidation of the substrates tested, incubations were performed with all recombinant UGT1 and UGT2 family members presently available (**Table 1**). Glucuronidation of LTB_4 was observed with UGT1A1, UGT1A3, UGT1A8, and UGT2B7. Screening for glucuronidation activity toward 20-COOH-LTB_4 demonstrated that UGT1A9 is the only isoform that catalyzes conjugation of this compound. Among the UGT1A family members, UGT1A9 demonstrated wide substrate specificity, conjugating the three HETEs and 13-HODE. UGT1A1, UGT1A3, and UGT1A4 also showed broad specificity toward PUFA, conjugating 12-HETE, 15-HETE, and 13-HODE. LA and AA metabolites also appear to be good substrates for the UGT2B subfamily members, especially for UGT2B4 and UGT2B7, which conjugate all HETEs and 13-HODE. Surprisingly, UGT2B15 and UGT2B17, which are known to conjugate androgens, glucuronidate 12-HETE, and 13-HODE, respectively. Most interestingly, UGT2B10 and UGT2B11, which are considered orphan enzymes since no conjugation activity has so far been demonstrated with these enzymes, both conjugated 12-HETE, 15-HETE, and 13-HODE, whereas UGT2B10 also glucuronidated 5-HETE. **Figure 4** illustrates the LC-MS analysis of the supernatant from incubation of UGT2B10 with 12-HETE, where a product at *m/z* corresponding to a monoglucuronide derivative of 12-HETE was detected. To ascertain that the absence of conjugation activity toward fatty acid derivatives observed with other UGT isoforms cannot be attributed to protein degradation, each UGT enzyme was incubated in the presence of eugenol. Glucuronidation toward this positive marker of glucuronidation was observed with all enzymes, with the exception of UGT2B10, UGT2B11, and UGT1A5, the latter having no identified substrate thus far (data not shown).

Kinetic analyses of AA and LA glucuronidation

To determine the extent of glucuronidation toward fatty acid metabolites, all isofoms that significantly reacted with PUFA were reassayed in linear conditions. Kinetic analyses performed with microsomal preparations of human liver and recombinant UGT enzymes demonstrated high levels of LTB_4 conjugation. The glucuronidation rates were 1,688, 117, 16, and 493 pmol·min^{-1}·mg protein^{-1} for liver, UGT1A3, UGT1A8, and UGT2B7 microsomes, respectively (**Fig. 5**). Although screening analyses demonstrated that UGT1A1 conjugates LTB_4 (Table 1), the low level of conjugation rate did not allow quantification under the conditions used for kinetic analysis. Determination of UGT affinities for LTB_4 was performed using microsomal preparations of stably transfected UGT1A3 and UGT2B7. As shown in **Fig. 6**, K_m values were 35 μM and 28 μM for UGT1A3 and UGT2B7, respectively, and the glucuronidation efficiency corresponding to the V_{max}/K_m ratio was 3.4 and 17.6 μl·min^{-1}·mg protein^{-1} for UGT1A3 and UGT2B7, respectively.

Because previous experiments suggested that UGT2B7 shows the broader substrate specificity and the higher velocity toward the majority of PUFAs, we retained this enzyme to investigate the glucuronidation capacities of UGT enzymes toward 5-HETE, 12-HETE, and 15-HETE. As observed with LTB_4, UGT2B7 is an excellent conjugator of these eicosanoids, with glucuronidation rates of 88, 855, and 591 pmol·min^{-1}·mg protein^{-1}, respectively (**Fig. 7**). K_m values of the UGT2B7 enzyme for 5-, 12-, and 15-HETE are, respectively, 80 μM, 71 μM, and 52 μM, leading to glucuronidation efficiencies of 1 μl, 12 μl, and 11 μl·min^{-1}·mg protein^{-1}.

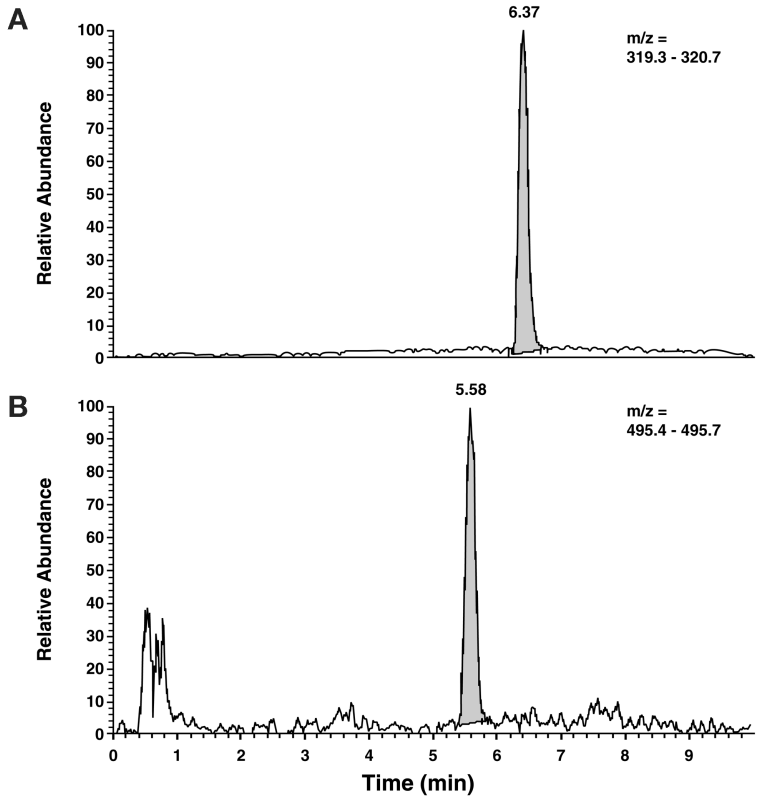


Fig. 4. Mass chromatograms of 12-HETE after incubation with UDP-glucuronosyltransferase (UGT)2B10 stably-transfected HK293 microsomal proteins. Microsomal proteins (40 μg) were incubated with 500 μM of aglycons in the presence of 2 mM UDPGA for 3 h at 37°C. Reactions were stopped by adding methanol, and products were analyzed by LC-MS. Mass chromatograms at *m/z* 319–321 and *m/z* 495–496 allowed detection of 12-HETE (A) and its glucuronide (B).

Effect of steroids on PUFA glucuronidation

To investigate the putative interaction between steroids and PUFAs for glucuronidation by UGT2B7 isoform, increasing concentrations of the major androgen metabolite, ADT, were incubated in the presence of 100 μM LTB_4. Interestingly, glucuronidation of LTB_4 at concentrations of ADT lower than 10 μM was increased, whereas a decrease of LTB_4 conversion was observed at concentrations above 15 μM (Fig. 8). Up to 50% inhibition was observed at the concentration of 100 μM ADT, which corresponds to 10-fold the K_m value of UGT2B7 for ADT (37).

DISCUSSION

The lipoxygenase enzymes constitute a family of enzymes that metabolize PUFAs such as LA and AA into a variety of biologically active products (3). These enzymes are expressed in platelets, leukocytes, and several tissues, such as the liver, lung, mammalian tissues, prostate, and colon, and recent data indicate that their products are implicated in several forms of epithelial cell cancers (3, 8–11, 39–41). It is generally believed that the inactivation of PUFA metabolites occurs in several tissues, including the liver, through oxidation processes and, as a phase II metabolism, by glucuronidation, a conversion that is also known to be present in the liver, to further facilitate their elimination from the body (17–20). Conjugation by UGT expressed in the liver as a catabolic pathway is considered extremely important for the elimination of exogenous compounds (27). Recent evidence also suggests that, for endogenous substances such as steroids, thyroid hormones, and retinoic acids, specific UGT enzymes are expressed, in addition to the liver, in several tissues including the skin, lung, mammary gland, uterus, adipocytes, and prostate (26). In the present study, glucuronidation of LTB4, 20-COOH-LTB_4, 5-HETE, 12-HETE, 15-HETE, and 13-HODE was investigated. The data showed that several UGT enzymes are capable of converting LA and AA metabolites into glucu-

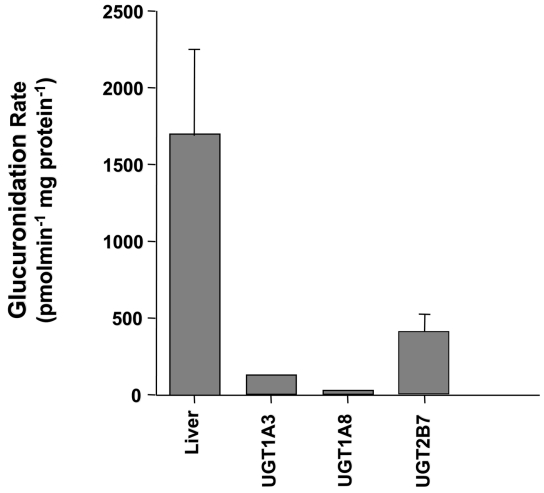


Fig. 5. Glucuronidation rates of LTB_4 by microsomal proteins from human liver and HK293 cells stably expressing UGT1A3, UGT1A8, or UGT2B7 in the presence of 15 μM LTB_4 and 1 mM UDPGA for 1 h at 37°C. Values represent the mean of three different experiments, each performed in duplicate, ± SD.

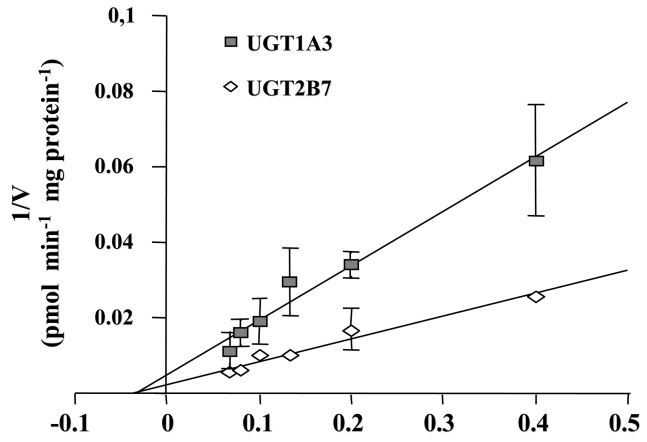
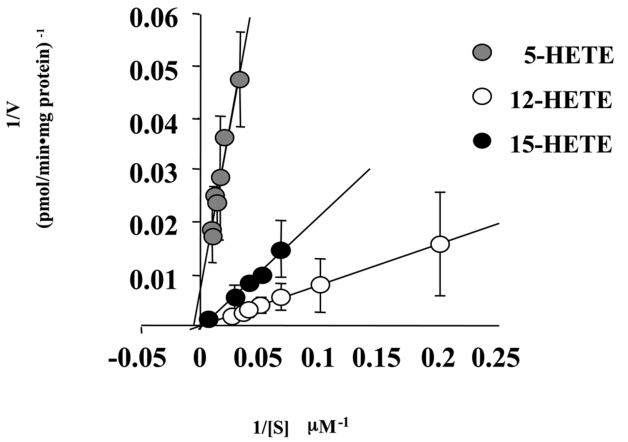


Fig. 6. Lineweaver-Burk plots of LTB_4 conjugation by recombinant human UGT enzymes. Microsomal proteins from HK293 cells stably expressing UGT1A3 or UGT2B7 were incubated in the presence of 1 μM to 15 μM LTB_4 and 1 mM UDPGA for one h at 37°C. Values represent the mean ± SD of three different experiments, each performed in duplicate.

Enzyme	Km (app) (μM)	Vmax (pmol min^{-1} $mg\ protein^{-1}$)	Vmax/Km (μl min^{-1} $mg\ protein^{-1}$)
UGT1A3	34.9 ± 11.5	117.3 ± 34.4	3.4 ± 1.0
UGT2B7	28.0 ± 16.8	493.0 ± 152.9	17.6 ± 5.5

ronide derivatives. Moreover, kinetic parameters of UGT enzymes toward PUFA metabolites suggest that the glucuronidation activity may be important in the pathway of PUFA catabolism. Interestingly, several of the UGT enzymes capable of conjugating PUFA are expressed in various tissues other than the liver (26, 36).

Glucuronidation of LA and AA metabolites was first ascertained using human liver microsomes, a tissue expressing most UGT enzymes, except UGT1A7, UGT1A8, and UGT1A10. All substrates tested were efficiently conjugated by liver microsomes to polar derivatives containing the glucuronyl moiety, as assessed by MS. Screening analyses demonstrated strong activities of liver UGT1A and UGT2B isoforms, since the 16 h incubation period leads



Substrate	Km (app) (μM)	Vmax (pmol•min^{-1} •mg $protein^{-1}$)	Vmax/Km (μliters•min^{-1} •mg $protein^{-1}$)
5-HETE	80.0 ± 8.3	88.1 ± 28.9	1.1 ± 0.4
12-HETE	71.1 ± 21.0	855.0 ± 168.1	12.0 ± 2.4
15-HETE	52.4 ± 8.6	591.9 ± 163.7	11.3 ± 3.1

Fig. 7. Lineweaver-Burk plots of 5-, 12-, and 15-HETE conjugation by the human UGT2B7 enzyme. Microsomal proteins from HK293 cells stably expressing recombinant UGT2B7 enzyme were incubated in the presence of 1 mM UDPGA and 5 μM to 25 μM 5-, 12-, or 15-HETE for 1 h at 37°C. Results represent the mean ± SD of three independent experiments, each performed in duplicate.

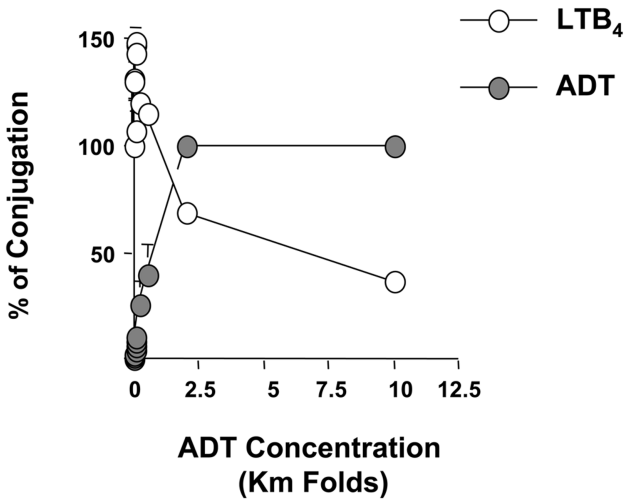


Fig. 8. Inhibition of LTB_4 glucuronidation by androsterone (ADT). Microsomes from recombinant UGT2B7 stably expressed in the HK293 cell line were incubated in the presence of 100 μM LTB_4 and ADT at concentrations from 0 μM to 100 μM for 1 h at 37°C. Values represent the mean ± SD of three independent experiments, each performed in duplicate.

to the conjugation of more than 95% of 12- and 15-HETE, as well as of 13-HODE. Moreover, the screening analyses with stably expressed UGT enzymes in HK293 underlined the very wide substrate specificity of UGT enzymes for PUFA metabolites with substantially different chemical structures. Indeed, UGT enzymes reacted with both 18- and 20-carbon substrates carrying two and four double bonds, respectively; the apparent lack of specificity is also observed for the position of the hydroxyl groups, since 5-HETE, 12-HETE, 15-HETE, and 13-HODE were conjugated by nearly the same set of enzymes. Although there is a clear redundancy in conjugation of fatty acid metabolites by UGT enzymes, screening analyses underlined subtle substrate specificity for some conjugating enzymes. For instance, UGT2B15, which shows 95% homology with UGT2B17 (34), conjugated 12-HETE exclusively, whereas the latter enzyme only conjugated 13-HODE. As observed for steroid hormones, these results suggest that the same substrate can be conjugated by several UGT enzymes, whereas each enzyme has a specific panel of substrates (26, 36). The most surprising results were observed, however, with UGT2B10 and UGT2B11, which conjugate 13-HODE and almost all HETE substrates. These enzymes were screened over the past years on more than 100 substrates, including eugenol that is conjugated by all active UGT enzymes, but no substrate has so far been identified (33). Thus, we demonstrate herein for the first time that two UGT enzymes, UGT2B10 and UGT2B11, can catalyze the glucuronidation of LA and AA metabolites; in addition, several UGT enzymes that were previously considered highly specific for steroids may also be implicated in the glucuronidation of these products.

While screening analyses revealed the significant activity of UGT enzymes toward PUFA metabolites, the kinetic parameters of their conjugation support a role of UGT enzymes in their metabolism. Indeed, comparison of LTB_4 glucuronidation by UGT2B7 with that of other substrates showed high glucuronidation rates. For instance, the glucuronidation rate for LTB_4 exceeded by almost 4-fold the rate observed for ADT, the endogenous substrate of UGT2B7 with the highest glucuronidation rate observed to date (36). Interestingly, UGT2B7 glucuronidation rates for LTB_4 are similar to those of eugenol, which is generally used as a positive control for glucuronidation (36). Since kinetic analyses demonstrated that UGT2B7 has a 3-fold lower affinity for LTB_4 than for ADT (36, 37), the resulting LTB_4 glucuronidation efficiency, as expressed by the V_{max}-K_m ratio, was 17.6 $\mu l \cdot min^{-1} \cdot mg\ protein^{-1}$, which was in the range of the glucuronidation efficiency observed for steroids. Results obtained with UGT1A3 also demonstrated very high glucuronidation rates for LTB_4 and K_m values within the micromolar range. To gain more information on the glucuronidation rates of PUFA metabolites, UGT enzymes that showed the highest activity for 5-, 12-, and 15-HETE during screening analyses were investigated to determine the kinetic parameters of glucuronidation. As observed with LTB_4, glucuronidation rates were higher than that for steroid hormones, especially for 12- and 15-HETE, which have V_{max} values of 855 and 591 $pmol \cdot min^{-1} \cdot mg\ protein^{-1}$, respectively; however, affinity of the UGT2B7 enzyme for the tested substrates was relatively low; therefore, glucuronidation efficiencies were similar to those observed with steroid hormones. The levels of glucuronidation efficiencies observed in the present study are also in agreement with data reported previously by Jude et al. of LA conjugation by UGT2B7 (14).

In the human prostate, 5-HETE and 13-HODE promote cell proliferation, whereas 12-HETE and 15-HETE may regulate angiogenesis and prostate cell differentiation, respectively (3, 10, 11). Our previous studies revealed that UGT2B4, UGT2B10, UGT2B11, UGT2B15, and UGT2B17 are expressed in the prostate (36, 42); therefore the present data suggest that prostate tissue has the capacity to conjugate the lipoxygenase products. In addition, some of these UGT enzymes, namely UGT2B15 and UGT2B17, can also inactivate dihydrotestosterone and its metabolites formed in the prostate (26). As for some PUFA metabolites, androgens also play a determining role in prostate differentiation and development, and stimulate the growth of prostate cancer cells. Therefore, UGT enzymes expressed in the prostate could be implicated in the metabolism of two distinct classes of endogenous compounds. Our data further reinforce the concept that several tissues possess the enzymatic machinery to produce and inactivate biologically active products (26, 43, 44). In addition, since it was previously demonstrated that the expression of UGT enzymes in LNCaP cells could be modulated by several factors, such as androgens, growth factors, and cytokines, it is reasonable to believe that concentrations of PUFAs metabolites in tissues such as human prostate may also be altered by changes in inactivating enzyme activity (45, 46). The dual action of UGT enzymes on two classes of biologically-active products is further supported by competition analyses that demonstrated that both steroids and PUFAs compete in vitro for the same binding sites or two overlapping sites. In this experiment, it was also interesting to observe an increase of LTB_4 conjugation by UGT2B7 at low concentrations of ADT. A homotropic activation of glucuronidation activity at low concentrations of substrates was recently reported for estradiol conjugation at position 3 by UGT1A1 (47), whereas this effect was not observed for the glucuronidation of estradiol at position 17 by UGT2B7. Although the phenomenon may be an artifact of the in vitro system used, further studies will be needed to evaluate this conjugation in vivo.

The expression of UGT enzymes capable of conjugating leukotrienes in tissues such as the liver and lung also suggests a role of these enzymes in the catabolism of both systemic and locally produced leukotrienes, and a possible implication of UGT enzymes in pathologies such as asthma and allergic airway response through regulation of their levels. Although leukocytes, especially neutrophils, are known to efficiently catalyze the degradation and inactivation of LTB_4, the role of the liver would also be of major importance, since this tissue is also responsible for the elimination of LA and AA metabolites released into systemic circulation (48–50).

In summary, our data demonstrate that several UGT en-

zymes are capable of conjugating LA and AA metabolites. Since this conjugating activity is expressed in several tissues other than the liver, it is reasonable to believe that UGT enzymes may serve to maintain homeostasis of physiologically important endogenous substances and/or to protect from deleterious high concentrations.

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