[CANCER RESEARCH 63, 6532–6536, October 1, 2003]

Human Cytochrome P450 3A7 Has a Distinct High Catalytic Activity for the 16α-Hydroxylation of Estrone but not 17β-Estradiol[1]

Anthony J. Lee, Allan H. Conney,[2] and Bao Ting Zhu[3]

Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of South Carolina, Columbia, South Carolina 29208 [A. J. L., B. T. Z.], and Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, College of Pharmacy, Rutgers–The State University of New Jersey, Piscataway, New Jersey 08854 [A. H. C.]

ABSTRACT

Like catechol estrogens, 16α-hydroxylated estrogens are hormonally active, chemically reactive, and potentially mutagenic. We report here our novel findings that human CYP3A7 has a distinct high catalytic activity for the NADPH-dependent 16α-hydroxylation of estrone (E_1; at 10 nM to 200 μM substrate concentrations) but not for the 16α-hydroxylation of 17β-estradiol (E_2). At a physiologically relevant low substrate concentration (10 nM), CYP3A7 had a strong catalytic activity for the 16α-hydroxylation of E_1, and the ratio of its 16α-hydroxylation to 2-hydroxylation was 107%. In addition to 16α-hydroxylation, CYP3A7 also had catalytic activity for the 2-, 4-, 6β-, and 16β-hydroxylation of E_1. However, when E_2 was the substrate, CYP3A7 had only very weak catalytic activity for its 16α-hydroxylation (<6% of E_1 16α-hydroxylation), and the ratio of its 16α-hydroxylation to 2-hydroxylation was 10–33%. Enzyme kinetic analysis showed that the maximal velocity and substrate-binding affinity ($1/K_m$) for CYP3A7-mediated 16α-hydroxylation of E_1 were both ~10 times higher than those for E_2, thereby giving the maximal velocity:K_m ratio of >100 times higher for the 16α-hydroxylation of E_1 than for E_2. Given the recent findings that human CYP3A7 is a polymorphic isoform also expressed in adult liver and certain extrahepatic tissues (in addition to fetal tissues), our data raise the possibility that CYP3A7 may be an important catalyst for the local and/or systemic formation of the procarcinogenic 16α-hydroxyestrone in women.

INTRODUCTION

Catechol estrogens (4-OH-E_2[4] in particular) and 16α-hydroxylated estrogens are two well-known groups of endogenous estrogen metabolites that have strong hormonal activity, high chemical reactivity, and also potential genotoxicity/mutagenicity (1–5). Because of their unique biological and chemical properties, these two groups of estrogen metabolites have been suggested to play an important role in the etiology of estrogen-induced cancers (2, 6). In the past decade or so, one of the notable efforts in this area of research is to identify hepatic and extrahepatic human CYP isoforms that have distinct catalytic activity for the formation of these bioactive estrogen metabolites. Whereas several human hepatic or extrahepatic CYP isoforms (such as CYP1A1, 1A2, and 3A4) were found to have dominant 2-hydroxylase activity, human CYP1B1 (an extrahepatic isoform) and CYP3A5 (mainly a hepatic isoform) were found to have distinct catalytic activity for the formation of 4-OH-E_2 and 4-OH-E_1 (7, 8). In comparison, much less is known about the catalytic activity of various human CYP isoforms for the 16α-hydroxylation of E_2 and E_1. It is of interest to note that it has been a long-held view that 16α-hydroxylation of estrogens in humans would only occur with E_1 as substrate but not with E_2 (9). However, when the 16α-hydroxylation of E_1 and E_2 was analyzed recently with 33 adult human liver microsomes (8, 10), we found that the average rates for their 16α-hydroxylation were very low, and no marked differences were observed between these two estrogen substrates for most of the liver microsomal preparations assayed. There is currently no published information available on possible CYP isoform(s) with selective catalytic activity for the 16α-hydroxylation of E_1 or E_2. We report here a novel finding that human CYP3A7 has a distinct high catalytic activity for the NAPDH-dependent 16α-hydroxylation of E_1 but not of E_2. The catalytic activity of CYP3A7 for estrogen 16α-hydroxylation was compared with that of 14 other human CYP isoforms.

MATERIALS AND METHODS

Chemical Reagents and Human CYP Isoforms. E_1, E_2, 16α-OH-E_2, NADPH, and ascorbic acid were purchased from the Sigma Chemical Co. (St. Louis, MO). 16β-OH-E_1 was biosynthetically prepared in our laboratory from 16β-OH-E_2 through incubations with human liver microsomes in the presence of NAD^+ as cofactor. The product was extracted with ethyl acetate and then separated by the HPLC (described later). The reference compounds for all of the other estrogen metabolites used in the present study were obtained from Steraloids, Inc. (Newport, RI). *N*,*O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane was obtained from Pierce Chemical Co. (Rockford, IL). [2,4,6,7,16,17-^{3}H]E_2 and [2,4,6,7-^{3}H]E_1 (numerically labeled, specific radioactivity of 110 and 65.5 Ci/mmol, respectively) were purchased from Perkin-Elmer Life Sciences (Boston, MA).

Fifteen selectively expressed human CYP isoforms were obtained from BD Gentest Co. (Woburn, MA). These human CYP isoforms were expressed in insect cells that were selectively transfected with a baculovirus expression system containing the cDNA for each of the desired human CYP isoforms.

Assay of the NADPH-dependent Metabolism of [^{3}H]]E_2 or [^{3}H]]E_1 by Human CYP Isoforms. It is of note that all of the glass test tubes used in the present study were silanized with 5% (v/v) dimethyldichlorosilane to reduce physical adsorption of hydroxylated estrogen metabolites to the test tubes. The reaction mixture for the *in vitro* metabolism of estrogens consisted of microsomes (at 70 pmol of CYP/ml), a desired concentration of [^{3}H]E_1 or [^{3}H]E_2, 2 mM NADPH, and 5 mM ascorbic acid in a final volume of 0.5 ml of a buffer solution (pH 7.4). The presence of 5 mM ascorbic acid in the incubation mixture has been shown previously to protect catechol estrogen metabolites from oxidative degradation without significantly altering the enzyme activity. The enzymatic reaction was initiated by addition of microsomes, and the incubations were carried out at 37°C for 20 min with mild shaking. The microsomal reaction was arrested by placing test tubes on ice and then immediately extracted with 4 ml of ethyl acetate. The organic supernatants were transferred to another set of test tubes and dried under a stream of nitrogen. The resulting residues were redissolved in 60 μl of methanol, and an aliquot (50 μl) was injected into the HPLC for analysis of estrogen metabolite composition with in-line UV and radioactivity detections as described earlier (8, 10). The calculation of the amount of each estrogen metabolite formed was based on the amount of radioactivity detected for each corresponding metabolite peak. Here it should also be noted that CYP isoform-mediated formation of hydroxylated or keto metabolites of [^{3}H]E_2 or [^{3}H]E_1 at any of their [^{3}H]-labeled positions (namely, 2, 4, 6, 7, 16, and 17 for [^{3}H]E_2 and 2, 4, 6, and 7 for [^{3}H]E_1) was known to remove tritium from the substrate, resulting in the formation of [^{3}H]H_2O. Therefore, in the present study the calculated final rates

Received 4/16/03; revised 7/22/03; accepted 7/22/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by Grant CA74787 from the NIH.

[2] William M. and Myrle W. Garbe Professor of Cancer and Leukemia Research.

[3] To whom requests for reprints should be addressed, at Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of South Carolina, Room 617 of Coker Life Sciences Building, 700 Sumter Street, Columbia, SC 29208. Phone: (803) 777-4802; Fax: (803) 777-8356; E-mail: BTZhu@cop.sc.edu.

[4] The abbreviations used are: E_2, 17β-estradiol; E_1, estrone; OH, hydroxy; CYP, cytochrome P450; HPLC, high performance liquid chromatography; GC/MS, gas chromatography/mass spectrometry.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/63/19/6532/2508220/ch1903006532.pdf by guest on 13 June 2024

for the formation of hydroxylated metabolites at the [^{3}H]-labeled positions were adjusted according to the estimated loss of radioactivity in each of these products.

Structural Identification of E_2 or E_1 Metabolites. The identity of E_2 or E_1 metabolites formed by CYP3A4 was confirmed through comparisons of their HPLC retention times, GC/MS retention times, and mass fragmentation spectra with all of the authentic reference compounds. For the purpose of comparison, the mass spectrum for each trimethylsilylated reference compound was obtained using our GC/MS system under the same analytical conditions for metabolically formed estrogen metabolites. The method for the GC/MS analysis of estrogen metabolites was described in our recent studies (8, 10).

RESULTS

We compared the catalytic activity for the 16α-hydroxylation of E_1 and E_2 by 15 human CYP isozymes from several families. The rates of their 16α-hydroxylation (at a representative 20 μM substrate concentration) and the ratios of their 16α-hydroxylation to 2-hydroxylation were summarized in Table 1. Among all of the 15 CYP isoforms analyzed, CYP1A1, 2C8, 3A4, and 3A5 showed a detectable catalytic activity for the 16α-hydroxylation of E_1 and E_2. In general, E_1 was somewhat more prone to be hydroxylated at the 16α-position by these CYP isoforms than was E_2. Surprisingly, CYP3A7 had a distinct high catalytic activity for the 16α-hydroxylation of E_1, but its catalytic activity for the 16α-hydroxylation of E_2 was very low, $<$10% of its activity for E_1.

With this interesting initial observation, we then further characterized the profiles of various estrogen metabolites formed by human CYP3A7 using many E_1 and E_2 substrate concentrations (from 10 nM to 200 μM), with a focus on the formation of 16α-hydroxylated metabolites. At physiologically relevant low concentrations (such as 10 nM) of E_1, 16α-OH-E_1 became the major metabolite formed by CYP3A7, which accounted for 30–50% of the total activity for the oxidative metabolism of E_1. The ratio of E_1 16α-hydroxylation:2-hydroxylation was 107% at a 10 nM substrate concentration, and the ratio was decreased to 65% when E_1 substrate concentration was increased to 25 μM. Representative HPLC metabolite traces at different E_1 substrate concentrations were shown in Fig. 1.

In addition to the formation of 16α-OH-E_1, CYP3A7 also catalyzed the conversion of E_1 to 2-OH-E_1 plus a small amount of 4-OH-E_1 (Fig. 1). 2-OH-E_1 was formed in comparable amounts as 16α-OH-E_1 at low E_1 substrate concentrations, but its formation was slightly more than 16α-OH-E_1 at higher E_1 concentrations (Fig. 1). To confirm the structural identities of the major metabolite peaks (namely, 16α-OH-E_1, 2-OH-E_1, and 4-OH-E_1), we collected the eluents of these peaks from the HPLC, and then subjected them to GC/MS analyses. Notably, very small amounts of 6β-OH-E_1 and 16β-OH-E_1 (which were coeluted with 16α-OH-E_1 on the HPLC) were also found to be formed by CYP3A7 when E_1 was the substrate. On the basis of selective monitoring of the most abundant ions (m/z) for these metabolites (namely, m/z 286 for 16α- and 16β-OH-E_1; m/z 340 for 6β-OH-E_1), we found that the ratios among 6β-OH-E_1, 16α-OH-E_1, and 16β-OH-E_1 were ~2:92:6 at 5 and 50 μM E_1 concentrations (data not shown).

In comparison, when E_2 was the substrate, 2-OH-E_2 was the major metabolite, and smaller amounts of 4-OH-E_2 and 6β-OH-E_2 were also formed by CYP3A7. 16α-Hydroxylation was only a very minor metabolic pathway, with the ratio of its 16α-hydroxylation:2-hydroxylation ~10% (Fig. 2). The overall catalytic activity of CYP3A7 for the oxidative metabolism of E_2 was lower than its activity for the metabolism of E_1, and the rate of E_2 16α-hydroxylation was $<$6% of the rate of E_1 16α-hydroxylation.

We also estimated the kinetic parameters (K_M and V_{max}) for CYP3A7-mediated 16α-hydroxylation of E_1 and E_2, as well as for the CYP3A7-mediated 2- and 4-hydroxylation. As shown in Fig. 3, the CYP3A7-mediated formation of each of these estrogen metabolites followed the typical Michaelis-Menten's curve patterns. CYP3A7 had a high capacity (V_{max} = 1423 pmol/nmol of CYP/min) and a relatively high affinity ($1/K_M$ = 0.1 μM^{-1}) for the 16α-hydroxylation of E_1. In comparison, the V_{max} for E_2 16α-hydroxylation was ~1/10 of the V_{max} for E_1 16α-hydroxylation, and the K_M for E_2 was ~10 times higher than that for E_1 (Fig. 3). Accordingly, the V_{max}:K_M ratio of CYP3A7-mediated 16α-hydroxylation of E_1 was $>$100-fold higher than that for the 16α-hydroxylation of E_2.

Lastly, it is of note that copresence of higher levels of cytochrome

Table 1 *The rate of estrogen 16α-hydroxylation and the ratio of estrogen 16α-hydroxylation: 2-hydroxylation by 15 selectively expressed human CYP isoforms*

The CYP enzymes were selectively expressed in insect cells infected with a Baculovirus expression system containing the desired cDNA (purchased from BD Gentest). The incubation mixture consisted of 20 μM of [^{3}H]E_1 or [^{3}H]E_2, 70 or 140 pmol of P450/ml, 2 mM NADPH and 5 mM ascorbic acid in a final volume of 0.5 ml of the reaction buffer. The incubation was at 37°C for 20 min with mild shaking. The rate of 16α-hydroxylation was expressed as average ± SD from triplicated determination. The method for the HPLC separation of the estrogen metabolites was described in "Materials and Methods."

	20 μM E_1 as substrate		20 μM E_2 as substrate	
CYP isoforms	Rates of 16α-hydroxylation (pmol/nmol of CYP/min)	Ratio of 16α-: 2-hydroxylation	Rates of 16α-hydroxylation (pmol/nmol of CYP/min)	Ratio of 16α-: 2-hydroxylation
CYP1A1	100.5 ± 5.8	5%	48.3 ± 1.6	2%
CYP1A2	—[a]	—	—	—
CYP1B1	—	—	—	—
CYP2A6	—	—	—	—
CYP2B6	—	—	—	—
CYP2C8	47.9 ± 3.4	107%	26.2 ± 3.1	51%
CYP2C9	—	—	—	—
CYP2C18	—	—	—	—
CYP2C19	—	—	—	—
CYP2D6	—	—	—	—
CYP2E1	—	—	—	—
CYP3A4	39.0 ± 4.9	13%	23.6 ± 2.0	7%
CYP3A4 + b_6	329.5 ± 25.4	12%	113.8 ± 5.6	4%
CYP3A5	31.0 ± 3.7	46%	24.9 ± 0.8	20%
CYP3A7 + b_5 [1][b]	288.0 ± 2.5	86%	48.3 ± 4.7	33%
CYP3A7 + b_5 [2][b]	954.0 ± 10.0	65%	23.1 ± 5.0	10%
CYP4A11	—	—	—	—

[a] —, denotes that the rate of estrogen 16α-hydroxylation was too low to be precisely quantified.

[b] Two different batches of CYP3A7 were assayed, which had different overall catalytic activity (based on testosterone 6β-hydroxylase activity) and different cytochrome b_5 content. Batch [1] contained the testosterone 6β-hydroxylase activity of 0.84 pmol/pmol of CYP/min and the cytochrome b_5 content of 170 pmol/mg of protein, and batch [2] contained 2.5 pmol/pmol of CYP/min and 220 pmol/mg of protein, respectively.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/63/19/6532/2508220/ch1903006532.pdf by guest on 13 June 2024

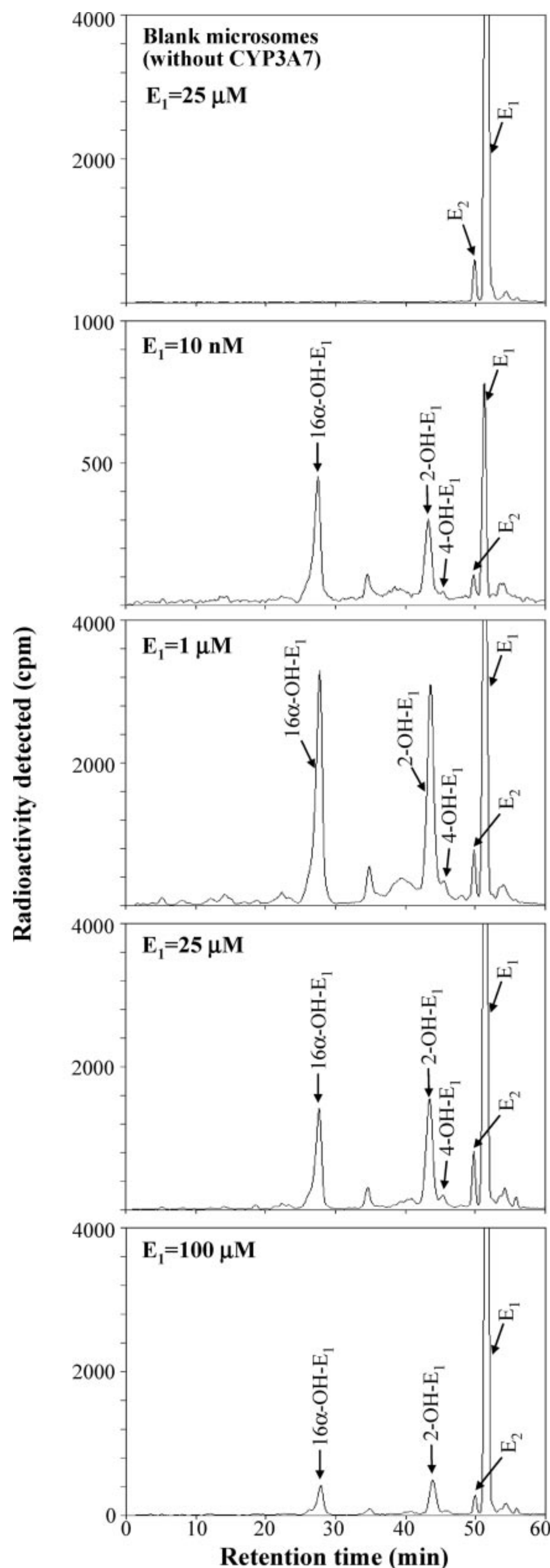


Fig. 1. Representative HPLC traces for the NADPH-dependent metabolism of different concentrations of $[^3H]E_1$ by human CYP3A7. CYP3A7 was selectively expressed in insect cells infected with a Baculovirus expression system containing the desired cDNA (purchased from BD Gentest). The incubation mixture consisted of 20 μM of $[^3H]E_1$ or $[^3H]E_2$, 70 pmol of P450/ml, 2 mM NADPH, and 5 mM ascorbic acid in a final volume of 0.5 ml of the reaction buffer. The incubation was at 37°C for 20 min with mild shaking. The method for the HPLC separation of the estrogen metabolites was described in "Materials and Methods." The catalytic activity and cytochrome b_5 content of the CYP3A7 microsomes were 2.5 pmol of 6β-hydroxytestosterone formed/pmol of CYP/min and 220 pmol/mg of protein, respectively.

b_5 (such as in the case of CYP3A4 *versus* CYP3A4 + b_5) nonselectively enhanced the overall catalytic activity of the CYP isoform toward the formation of various estrogen metabolites (data not shown). This activation likely is because of a nonspecific mechanism, such as the increased availability of the second electron that is required for the CYP-mediated reactions.

DISCUSSION

The results of our present study showed that among the 15 selectively expressed human CYP isoforms assayed, only CYP1A1, 2C8, 3A4, 3A5, and 3A7 showed detectable catalytic activity for the 16α-hydroxylation of E_1 and E_2. We report here, for the first time, that CYP3A7 had a distinct high catalytic activity for the 16α-hydroxylation of E_1 but not of E_2 (Table 1). Enzyme kinetic analysis showed that the V_{max} and substrate-binding affinity ($1/K_M$) for CYP3A7-mediated 16α-hydroxylation of E_1 were both ~10 times higher than those for E_2, thereby giving a $V_{max}:K_M$ ratio >100 times higher for the 16α-hydroxylation of E_1 than for E_2.

The differential rates of the 16α-hydroxylation of E_1 *versus* E_2 likely are determined by their different structures at the C17-position. We believe that the presence of a C17-keto group in the steroid is essential for it to be a suitable substrate for the 16α-hydroxylation by CYP3A7. In support of this suggestion, earlier studies have reported that whereas CYP3A7 was capable of catalyzing the 16α-hydroxylation of dehydroepiandrosterone and its 3-sulfate (both have a C17-keto group), it could not catalyze the 16α-hydroxylation of testosterone or cortisol (both lack a C17-keto group; Refs. 11, 12).

It is of great interest to point out that the long-held view that 16α-hydroxylation only occurred with E_1 as the substrate (9) appears to be true in the case of CYP3A7 as a catalyst. Notably, although CYP3A7 was originally found in human fetal liver where it accounted for 30–50% of total CYP contents (13, 14), studies have also suggested that CYP3A7 is expressed in human uterine endometrium, placenta, adrenal gland, and prostate (15, 16). In addition, the pres-

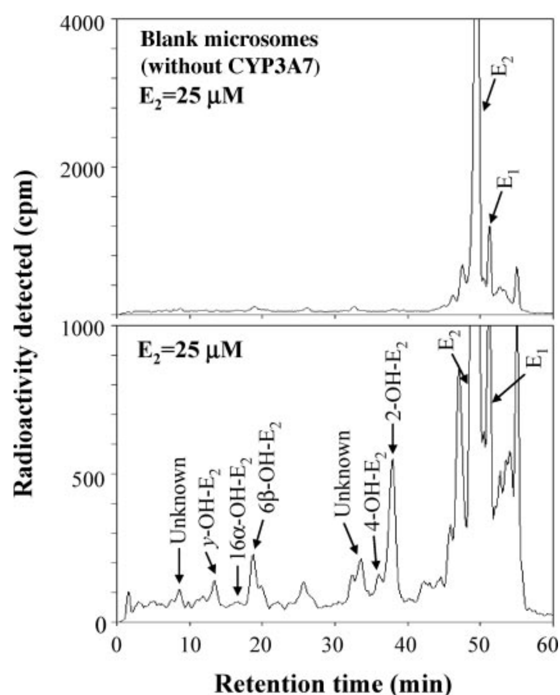
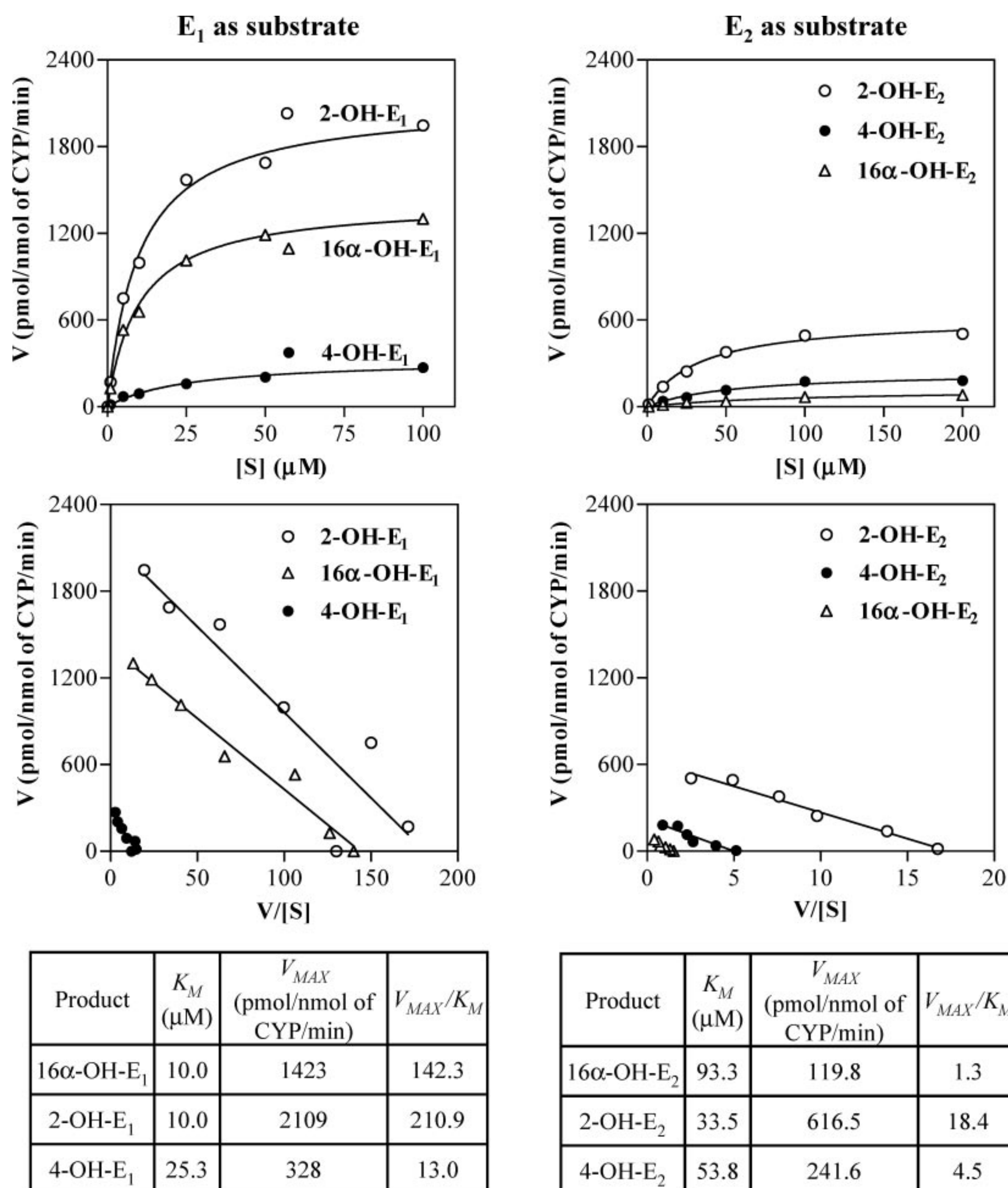


Fig. 2. Representative HPLC traces for the NADPH-dependent metabolism of $[^3H]E_2$ by human CYP3A7. The experimental procedures were the same as described in the legend to Fig. 1.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/63/19/6532/2508220/ch1903006532.pdf by guest on 13 June 2024



Product	K_M (μM)	V_{MAX} (pmol/nmol of CYP/min)	V_{MAX}/K_M
16α-OH-E_1	10.0	1423	142.3
2-OH-E_1	10.0	2109	210.9
4-OH-E_1	25.3	328	13.0

Product	K_M (μM)	V_{MAX} (pmol/nmol of CYP/min)	V_{MAX}/K_M
16α-OH-E_2	93.3	119.8	1.3
2-OH-E_2	33.5	616.5	18.4
4-OH-E_2	53.8	241.6	4.5

Fig. 3. Michaelis-Menten curves (*top panels*), Eadie-Hofstee plots (*middle panels*), and the calculated kinetic parameters (*bottom panels*) for the 2-, 4-, and 16α-hydroxylation of E_1 (*left panels*) or of E_2 (*right panels*) by human CYP3A7. The experimental procedures were the same as described in the legend to Fig. 1. The K_M and V_{max} values were obtained by nonlinear regression using Prism software (GraphPad Software, Inc., San Diego, CA).

ence of constitutive or induced expression of CYP3A7 in adult human liver has also been suggested (17, 18), and its expression in adult liver and intestine appears to have a polymorphic distribution, with an estimated ~10% of Caucasians belonging to a distinct subgroup of high expression phenotype (19). In light of this information, the findings of our present study raise the possibility that human CYP3A7 may be an important catalyst for the local and/or systemic formation of 16α-OH-E_1 in humans. It is important to note that when the 16α-hydroxylation of E_1 or E_2 was recently analyzed with 33 adult human liver microsomes (8, 10), the average rates for the 16α-hydroxylation of these two estrogens were found to be similarly low for most of the liver microsomal preparations. This observation indicates that the contribution of CYP3A7 to hepatic estrogen 16α-hydroxylation in most adult human liver samples likely is rather minimal.

It is well known that very large amounts of 16α-OH-E_2 (estriol) are present in blood and urine of pregnant women. It has been suggested that dehydroepiandrosterone sulfate (synthesized in the fetal adrenal glands) is metabolically converted to 16α-hydroxydehydroepiandrosterone sulfate in the adrenal glands and liver, which is then further aromatized to form 16α-OH-E_2 in the placenta. It is believed that the fetus is the source of ~90% of the precursor for 16α-OH-E_2, because of the presence of high levels of CYP3A7 in fetal tissues. However, on the basis of the findings of our present study, it appears that the CYP3A7-mediated 16α-hydroxylation of E_1 in the fetal liver, coupled with C17-reduction by 17β-hydroxysteroid dehydrogenase, could be another potential pathway for the formation of 16α-OH-E_1 and 16α-OH-E_2 in a pregnant woman.

In summary, human CYP3A7 has a distinct high catalytic activity for the NAPDH-dependent 16α-hydroxylation of E_1, but not of E_2. Additional studies are warranted to determine whether the CYP3A7 expression levels correlate with the tissue or circulating levels of

Downloaded from http://aacrjournals.org/cancerres/article-pdf/63/19/6532/2508220/ch1903006532.pdf by guest on 13 June 2024

16α-OH-E_1 and also with the risk of human breast or endometrial cancer.

REFERENCES

1. Liehr, J. G. Is estradiol a genotoxic mutagenic carcinogen? Endocr. Rev., *21:* 40–54, 2000.
2. Bradlow, H. L., Hershcopf, R. E., and Fishman, J. F. Oestradiol 16α-hydroxylase: a risk marker for breast cancer. Cancer Surv., *5:* 573–583, 1986.
3. Fishman, J., and Martucci, C. Biological properties of 16α-hydroxyestrone: implications in estrogen physiology and pathophysiology. J. Clin. Endocrinol. Metab, *51:* 611–615, 1980.
4. Martucci, C. P., and Fishman, J. P450 enzymes of estrogen metabolism. Pharmacol. Ther., *57:* 237–257, 1993.
5. Zhu, B. T., and Conney, A. H. Functional role of estrogen metabolism in target cells: review and perspectives. Carcinogenesis (Lond.), *19:* 1–27, 1998.
6. Fishman, J., Schneider, J., Hershcope, R. J., and Bradlow, H. L. Increased estrogen-16α-hydroxylase activity in women with breast and endometrial cancer. J. Steroid Biochem., *20:* 1077–1081, 1984.
7. Hayes, C. L., Spink, D. C., Spink, B. C., Cao, J. Q., Walker, N. J., and Sutter, T. R. 17β-Estradiol hydroxylation catalyzed by human cytochrome P450 1B1. Proc. Natl. Acad. Sci. USA, *93:* 9776–9781, 1996.
8. Lee, A. J., Mills, L. H., Kosh, J. W., Conney, A. H., and Zhu, B. T. NADPH-dependent metabolism of estrone by human liver microsomes. J. Pharmacol. Exp. Ther., *300:* 838–849, 2002.
9. Fishman, J. Aromatic hydroxylation of estrogens. Annu. Rev. Physiol, *45:* 61–72, 1983.
10. Lee, A. J., Kosh, J. W., Conney, A. H., and Zhu, B. T. Characterization of the NADPH-dependent metabolism of 17β-estradiol to multiple metabolites by human liver microsomes and selectively expressed human cytochrome P450 3A4 and 3A5. J. Pharmacol. Exp. Ther., *298:* 420–432, 2001.
11. Ohmori, S., Fujiki, N., Nakasa, H., Nakamura, H., Ishii, I., Itahashi, K., and Kitada, M. Steroid hydroxylation by human fetal CYP3A7 and human NADPH-cytochrome P450 reductase coexpressed in insect cells using baculovirus. Res. Commun. Mol. Pathol. Pharmacol., *100:* 15–28, 1998.
12. Kitada, M., Kamataki, T., Itahashi, K., Rikihisa, T., and Kanakubo, Y. P-450 HFLa, a form of cytochrome P-450 purified from human fetal livers, is the 16α-hydroxylase of dehydroepiandrosterone 3-sulfate. J. Biol. Chem., *262:* 13534–13537, 1987.
13. Wrighton, S. A., Molowa, D. T., and Guzelian, P. S. Identification of a cytochrome P-450 in human fetal liver related to glucocorticoid-inducible cytochrome P-450HLp in the adult. Biochem. Pharmacol., *37:* 3053–3055, 1988.
14. Shimada, T., Yamazaki, H., Mimura, M., Wakamiya, N., Ueng, Y. F., Guengerich, F. P., and Inui, Y. Characterization of microsomal cytochrome P450 enzymes involved in the oxidation of xenobiotic chemicals in human fetal liver and adult lungs. Drug Metab. Dispos., *24:* 515–522, 1996.
15. Schuetz, J. D., Kauma, S., and Guzelian, P. S. Identification of the fetal liver cytochrome CYP3A7 in human endometrium and placenta. J. Clin. Investig., *92:* 1018–1024, 1993.
16. Koch, I., Weil, R., Wolbold, R., Brockmoller, J., Hustert, E., Burk, O., Nuessler, A., Neuhaus, P., Eichelbaum, M., Zanger, U., and Wojnowski, L. Interindividual variability and tissue-specificity in the expression of cytochrome p450 3A mRNA. Drug Metab. Dispos., *30:* 1108–1114, 2002.
17. Schuetz, J. D., Beach, D. L., and Guzelian, P. S. Selective expression of cytochrome P450 CYP3A mRNAs in embryonic and adult human liver. Pharmacogenetics, *4:* 11–20, 1994.
18. Greuet, J., Pichard, L., Bonfils, C., Domergue, J., and Maurel, P. The fetal specific gene CYP3A7 is inducible by rifampicin in adult human hepatocytes in primary culture. Biochem. Biophys. Res. Commun., *225:* 689–694, 1996.
19. Burk, O., Tegude, H., Koch, I., Hustert, E., Wolbold, R., Glaeser, H., Klein, K., Fromm, M. F., Nuessler, A. K., Neuhaus, P., Zanger, U. M., Eichelbaum, M., and Wojnowski, L. Molecular mechanisms of polymorphic CYP3A7 expression in adult human liver and intestine. J. Biol. Chem., *277:* 24280–24288, 2002.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/63/19/6532/2508220/ch1903006532.pdf by guest on 13 June 2024