16α-Hydroxylation of estrone by human cytochrome P4503A4/5

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The cytochrome P450 (P450) enzymes that catalyse metabolism of the estrogen, estrone (E₁), to the putative carcinogen 16α-hydroxy E₁ (16α-OH E₁) in humans were determined. The potential of the most abundant circulating form of estrogen, estrone 3-sulfate (E₁S), to be the substrate was also investigated. Human liver microsomal sulfatases convert E₁S to E₁, an essential prerequisite for formation of 16α-OH E₁ from added E₁S in this system. E₁ metabolism to 16α-OH E₁ in a panel of 15 human liver microsomal preparations correlated with total P450 concentrations (r² = 0.63) and with activities associated with P450 forms CYP3A4 and 3A5 (r² = 0.72). E₁ 16α-hydroxylase activity in human liver microsomes was inhibited by 75% by monoclonal anti human CYP3A4/S antibodies at 4 mg antibody/nmol total P450, and by troleandomycin, a specific CYP3A4/S inhibitor. Rates of E₁ metabolism to 16α-OH E₁ were 1.6-fold higher when E₁ was generated in situ from E₁S than when E₁ was added. Microsomal preparations of cDNA expressed CYP3A4 or 3A5, with NADPH-P450-reductase co-expressed, both metabolized E₁ to 16α-OH E₁, and added cytochrome b₅ increased the rates 5.1- and 7.5-fold, respectively. In these systems rates of E₁ metabolism to 16α-OH E₁ were 2.8-fold higher when E₁ was generated in situ from E₁S than when E₁ was added. Kinetic values for E₁ metabolism to 16α-OH E₁ by human liver microsomes and for the expressed CYP3A4 system were Kₘ 154 and 172 µM, respectively, and Vₘₙax 238 pmol/min/nmol total P450 and 1050 pmol/min/nmol CYP3A4, respectively. Thus, formation of the putative carcinogen 16α-OH E₁ is catalysed by CYP3A4 and 3A5 and stimulated by cytochrome b₅. E₁S is not a substrate but formation of E₁ in E₁S in situ stimulates formation of 16α-OH E₁, possibly because E₁S is more water soluble and in situ generation of E₁ provides for facilitated exposure of E₁ to the P450 substrate binding sites. Blocking of the pathway of E₁ to 16α-OH E₁ could provide a therapeutic approach for diminishing the risk of estrogen dependent breast cancer.

Introduction

Levels of circulating estrogen hormone and the duration of exposure to the hormone have been clearly established as risk factors for human breast cancer (1). There is extensive evidence that supports a role for oxidative metabolism of estrogen in modulating the carcinogenic potential of the hormone (2). 17β-Estradiol (E₂*) undergoes oxidative metabolism at C-17 to yield estrone (E₁) which can be hydroxylated to yield 16α-hydroxyestrone (16α-OH E₁; Figure 1A). Reduction of this metabolite by 17β-hydroxysteroid dehydrogenase yields estriol (E₃) (3). Several lines of evidence implicate 16α-hydroxylated estrogens in breast carcinogenesis. In breast cancer patients there is an elevated extent of 16α-hydroxylation of estrogen (4). Additionally 16α-OH E₁ induces genotoxic DNA damage, by binding covalently to DNA (5) and aberrant hyperproliferation, and it promotes expression of transformed phenotype (2,6). Estrogens are also 2- or 4-hydroxylated to yield 2-hydroxyestrone (2-OH E₁) and 4-hydroxyestrone (4-OH E₁) in the case of E₁ (3), which in turn can be converted to the corresponding methoxy metabolites by catechol-O-methyltransferase. 4-OH E₁ and 4-hydroxy-17β-estradiol (4-OH E₂), because of their potential to participate in metabolic redox cycling (7), have also been implicated in estrogen-mediated carcinogenicity (8).

The gene products of the cytochrome P450 (P450) superfamily play a predominant role in estrogen metabolism. In humans >30 forms of P450 have been identified and sequenced (9), and the roles of specific forms in the formation of some estrogen metabolites have been resolved. Several specific human P450s (CYP) have been implicated in the 2- and 4-hydroxylations of E₂ in the liver including CYP1A2, 3A4, and 3A5 (10,11). In human MCF-7 breast cancer cells, after induction by 2,3,7,8-tetrachlorodibenzo-p-dioxin, formation of 2-hydroxy-17β-estradiol (2-OH E₂) is catalysed primarily by CYP1A1 and 4-OH E₂ by CYP1B1 (12). In a recent paper (13) CYP3A4 and CYP3A5 have been shown to play a role in the 16α-hydroxylation of estrogens in humans.

Several factors have implicated estrogen-3-sulfate as the substrates for 16α-hydroxylases in humans. Early studies indicated that human fetal liver microsomal preparations 16α-hydroxylated 17β-estradiol-3-sulfate (E₂S), but not E₂ (14), although estrone 3-sulfate (E₁S) and E₁ were 16α-hydroxylated at equivalent rates by human adult liver microsomal preparations (15). Subsequently, reconstituted purified CYP3A7 was shown to catalyse the 16α-hydroxylation of dehydroepiandrosterone-3-sulfate (16). The possibility that estrogen sulfates are substrates for 16α-hydroxylase is consistent with the fact that E₁S is the predominant circulating form of estrogen in women.

In this study we have investigated which form(s) of P450 are capable of catalyzing the 16α-hydroxylation of E₁S and E₁ in an effort to determine how such metabolism can ultimately be blocked, and thereby possibly prevent estrogen-dependent breast cancer.

*Abbreviations: E₂, 17β-estradiol; E₁, estrone; E₃, estriol; E₁S, estrone 3-sulfate; 16α-OH E₁, 16α-hydroxyestrone; 2-OH E₁, 2-hydroxyestrone; 4-OH E₁, 4-hydroxyestrone; 4-OH E₂, 4-hydroxy-17β-estradiol; P450, cytochrome P450 (also termed heme-thiolate protein P450 by the Enzyme Commission, EC 1.14.14.1); CYP, a specific form of cytochrome P450; 2-OH E₂, 2-hydroxy-17β-estradiol; 17β-estradiol, 3-sulfate.

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Materials and methods

Chemicals

The estrogen standards, E1, E2, E3, 16α-OHE1, 6α-OHE2, 2-OHE2, 4-OHE2, 2OH-E1 and 4-OHE1, were purchased from Steraloids Inc. (Wilton, NH) and Sigma (St Louis, MO) and were used without further purification in methanolic solution. Ascorbic acid was added to catechol estrogen solutions at a final concentration of 2 mM to prevent oxidation. The substrate E1 S was also purchased from Sigma (St Louis, MO) with Tris-HCl added as a stabilizer. Furafylline, a potent CYP1A2-specific inhibitor, was kindly provided by Dr Wolfgang Pfeiderer (Universitut Konstanz, Germany). All other chemicals, including troleandomycin, SKF 525A, sulfaphenazole, and quinidine, were purchased from Sigma (St Louis, MO).

Enzymes and antibodies

Microsomes were prepared from the liver of a 55-year-old male as described previously (17). Microsomal protein concentration was determined using the method of Lowry et al. (20). Consequently, to determine whether conversion of E1 S to E1 is a prerequisite for the hydroxylation activity, E1 S was pre-incubated with the microsomal preparation for varying periods prior to initiation of metabolic rates were determined after incubation for 30 min at 37°C with gentle shaking. Reactions were terminated by adding 1 ml ice-cold deionized water and 0.25 µM 6α-OHE for internal standard. This compound was not formed under the reaction conditions used. Reaction mixtures were solid-phase-extracted following loading onto Extrelut QE columns (EM Science, Gibbstown, NJ) and elution with ethyl acetate (12 ml). The organic solvents were removed under a stream of nitrogen and the residues were dissolved in methanol (45 µl) containing 2 mM ascorbic acid, and 20 µl was injected onto the HPLC column. Enzyme assays

Microsomal assays for P450-mediated metabolism were typically performed in a total volume of 1 ml containing 1.0 mg microsomal protein, 1.2 µmol NADPH, and variable amounts of E1 S in a Tris-Mg2+ buffer system (50 mM Tris-HCL, pH 7.4, and 15 mM MgCl2). In those studies with E1 S as substrate it was added at 150 µM in methanol. Arylsulfatase was added to those reaction mixtures containing microsomes deficient in estrone sulfatase activity. Rabbit cytochrome b5 (1 mM) was also included in some reaction mixtures to test its role in the metabolism. Reaction mixtures were pre-incubated for 10 min at 37°C in the absence of NADPH to permit sulfatase activity to proceed. P450-mediated reactions were initiated by the addition of NADPH, and metabolic rates were determined after incubation for 30 min at 37°C with gentle shaking. Reactions were terminated by adding 1 ml ice-cold deionized water and 0.25 µM 6α-OHE as internal standard. This compound was not formed under the reaction conditions used. Reaction mixtures were solid-phase-extracted following loading onto Extrelut QE columns (EM Science, Gibbstown, NJ) and elution with ethyl acetate (12 ml). The organic solvents were removed under a stream of nitrogen and the residues were dissolved in methanol (45 µl) containing 2 mM ascorbic acid, and 20 µl was injected onto the HPLC column. Inhibition assays

In studies to assess metabolic inhibition, inhibitors were added to the reaction mixtures described above. For troleandomycin, furafylline and diethylthiocarbamate, the reaction mixtures were pre-incubated with the inhibitors and NADPH for 30 min at 37°C before the addition of substrate and for sulfaphenazole, SKF 525A and quinidine pre-incubation was without NADPH for 10 min. For antibody inhibition studies, the microsomal protein concentration was determined spectrophotometrically as described by Omura and Sato (18). Microsomes were prepared from the liver of a 55-year-old male as described previously (17). Microsomal protein concentration was determined using the method of Lowry et al. (20). Consequently, to determine whether conversion of E1 S to E1 is a prerequisite for the hydroxylation activity, E1 S was pre-incubated with the microsomal preparation for varying periods prior to initiation of metabolic rates were determined after incubation for 30 min at 37°C with gentle shaking. Reactions were terminated by adding 1 ml ice-cold deionized water and 0.25 µM 6α-OHE as internal standard. This compound was not formed under the reaction conditions used. Reaction mixtures were solid-phase-extracted following loading onto Extrelut QE columns (EM Science, Gibbstown, NJ) and elution with ethyl acetate (12 ml). The organic solvents were removed under a stream of nitrogen and the residues were dissolved in methanol (45 µl) containing 2 mM ascorbic acid, and 20 µl was injected onto the HPLC column. HPLC analyses were conducted with a system from Millipore Corp. (Milford, MA), comprising a multisolvent delivery unit (Waters 717) and a photodiode array detector (Waters 996). The system was controlled with Millennium software V2.10. A Waters 8VNC18 4 µm column was used with 90% acetonitrile (A) and deionized water (B) as solvents using a linear gradient beginning at 20% A and increasing to 60% A over 20 min at a flow rate of 2 ml/min. Retention times for the estrogen standards were 6α-OHE (IS), 7.61 min; E1, 8.69 min; 16α-OHE, 12.00 min; 2/4-OHE2, 13.79 min; 2-OHE, 15.79 min; 4-OHE, 16.54 min; E2, 17.19 min and E3, 19.74 min (Figure 1B). Metabolites were identified by matching both the retention time and the spectra of the standards, which were pre-stored in the software library. Data analysis

All the data analysis was performed with Sigmaplot and SigmaStat software (Jandel Scientific, San Rafael, CA). Enzyme kinetic parameters (apparent Km and Vmax) were determined using linear regression analysis of Lineweaver–Burk plots.

Results

With E1 S as substrate the human liver microsomal preparation yielded 16α-OHE2 and 2-OHE2 in NADPH-dependent reactions (Figure 1C). We previously demonstrated that the microsomal preparation possesses E1 S sulfatase activity with biphasic kinetics yielding Km 14.3 µM and 1.5 µM, and Vmax 0.5 and 21.5 nmol/min/mg protein (20). Consequently, to determine whether conversion of E1 S to E1 is a prerequisite for the hydroxylation activity, E1 S was pre-incubated with the microsomal preparation for varying periods prior to initiation of hydroxylation with NADPH. Rates of 16α-OHE2 formation increased with increased pre-incubation time up to 30 min before plateauing off (Figure 2), suggesting that hydrolysis of...
Table I. Correlation coefficients ($r^2$) for estrone (E$_1$) 16α- (16α-OH) and 2-hydroxylation (2-OH) activities with various P450 activities and P450 contents in a set of 15 human liver microsomal preparations.

<table>
<thead>
<tr>
<th>P450</th>
<th>P450 activity$^b$</th>
<th>E$_1$ concentration (µM)</th>
<th>16α-OH$^a$</th>
<th>2-OH$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P450 Content</td>
<td></td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>7-Ethoxyresorufin O-dealkylation</td>
<td>0.63*</td>
<td>0.84**</td>
<td>0.70**</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Coumarin 7-hydroxylation</td>
<td>0.38</td>
<td>0.27</td>
<td>0.39</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Tolbutamide methyl-hydroxylation</td>
<td>0.36</td>
<td>0.60*</td>
<td>0.49</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-Mephenytoin 4’-hydroxylation</td>
<td>0.09</td>
<td>0.34</td>
<td>0.23</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Dextromethorphan O-demethylation</td>
<td>0.09</td>
<td>0.34</td>
<td>0.23</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Chlorozaxone 6-hydroxylation</td>
<td>0.19</td>
<td>0.29</td>
<td>0.24</td>
</tr>
<tr>
<td>CYP3A4/5</td>
<td>Testosterone 6β-hydroxylation</td>
<td>0.72**</td>
<td>0.82**</td>
<td>0.77**</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Dextromethorphan N-demethylation</td>
<td>0.09*</td>
<td>0.71**</td>
<td>0.70**</td>
</tr>
<tr>
<td>CYP4A9/11</td>
<td>Lauric acid 12-hydroxylation</td>
<td>0.15</td>
<td>0.35</td>
<td>0.06</td>
</tr>
</tbody>
</table>

$^a$The rates of the estrone 16α- and 2-hydroxylation activities were determined as described in the experimental procedures at 100 and 1000 µM substrate concentrations.

$^b$Rates of P450 activities and concentrations were provided by XenoTech LLC Inc.

$^*P < 0.05; **P < 0.01.$

Figure 2. Rates of estrone (E$_1$) 16α-hydroxylation catalysed by human hepatic microsomes as a function of pre-incubation time prior to initiation of hydroxylase activity with NADPH. The substrate was estrone-3-sulfate (E$_1$S) at 100 µM, and pre-incubation permits the estrone sulfatase in the microsomal preparation to convert E$_1$S to E$_1$ prior to 16α-hydroxylation. E$_1$S to E$_1$ is required prior to 16α-hydroxylation of E$_1$, and that E$_1$ is the substrate for the 16α-hydroxylase.

The rates of 16α-OHE$_1$ and 2-OHE$_1$ formation from E$_1$S at 100 and 1000 µM, after 10 min pre-incubations with 15 human liver microsomal preparations, were determined, and correlated with the total P450 concentrations and P450 activities provided with the microsomal preparations. The correlation coefficients ($r^2$) are reported in Table I and Figure 3 and the rates of 16α-OHE$_1$ formation in Figure 3. The 16α-OHE$_1$ formation rates correlated statistically significantly with total P450 contents and with CYP3A4 and CYP3A5 catalysed activities at both E$_1$S concentrations, and additionally with CYP2A6 catalysed activity at 1000 µM E$_1$S. 2-OHE$_1$ formation rates exhibited similar correlations (Table I). These results suggest that, at 100 µM substrate concentrations, E$_1$ 16α- and 2-hydroxylations are primarily catalysed by CYP3A4 or CYP3A5, but at lower concentrations in human liver, other P450s may be involved.

To further evaluate the role of CYP3A4/5 in E$_1$ 16α- and 2-hydroxylations, the effect of varying concentrations of anti-human CYP3A4 monoclonal antibody on the hepatic microsomal mediated reactions of E$_1$S was investigated following hydrolysis of E$_1$S to E$_1$. Both hydroxylase activities were inhibited in a dose-dependent manner, with the 16α-hydroxylase activity being inhibited by 60% and the 2-hydroxylase activity by 75% at 4 mg antibody per nmol total P450 (Figure 4). The rate of formation of E$_2$ was enhanced by the antibodies, apparently as a consequence of the inhibition of the competing hydroxylation reactions.

A number of specific P450 inhibitors were also used to gather...
Fig. 4. Inhibition of human hepatic microsomal 17β-estradiol (●) formation and 2- (▲) and 16α-hydroxylation activity (■) by anti-CYP3A4 monoclonal antibody with estrone (E1) as substrate. Human liver microsomes were pre-incubated with antibodies on ice for 20 min before addition of remaining reaction components. The concentration of the added substrate, estrone-3-sulfate (E1S) was 100 µM. E1S was hydrolyzed to E1 prior to initiation of hydroxylase activity with NADPH. Rates of 2- and 16α-hydroxylation were determined as described in Materials and methods. Each data point represents the mean of duplicate determinations.

Fig. 5. Inhibition of human hepatic microsomal estrone 2- (□) and 16α-hydroxylation (■) by P450-specific inhibitors. The concentration of added substrate, estrone-3-sulfate (E1S) was 100 µM. E1S was hydrolysed to E1 prior to initiation of hydroxylase activity with NADPH. Rates were determined from triplicate tests as described in Materials and methods. Abbreviations: CONT, control; SKF525A, 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride; FURAF, furafylline (CYP1A2 inhibitor); DDC, diethyldithiocarbamate (CYP2A6 and 2E1 inhibitors); SULF, sulfaphenazole (CYP2C9 inhibitor); QUINID, quinidine (CYP2D6 inhibitors); and TAO, troleandomycin (CYP3A4/5 inhibitor).

Fig. 6. Rates of estrone (E1) 2- (▲) and 16α-hydroxylation (□) catalysed by microsomal preparations with cDNA-expressed human P450 enzymes, CYP1A1, 1A2, 1B1, 2A6, 2C9-Arg444, 2C19, 2D6, 2E1, 3A4-b5 and 3A5. All of these preparations had NADPH-P450 reductase co-expressed, and in the case of CYP3A4, cytochrome b5 was also co-expressed. The added substrate was estrone sulfate (E1S) at 150 µM. Reaction mixtures were pre-incubated with arylsulfatase prior to initiation of hydroxylation with NADPH because of lack of sulfatase activities in these microsomes.

supporting evidence for the proposed roles of CYP3A4/5 in 16α-OHE1 and 2-OHE1 formation with human liver. The results are presented in Figure 5, and indicate that SKF525A, a general P450 inhibitor, and troleandomycin, a CYP3A4/5-specific inhibitor, inhibited both activities markedly, with the 2-hydroxylase activity being most extensively inhibited. Sulfaphenazole, a CYP2C9-specific inhibitor, also slightly inhibited both reactions, but furafylline, quinidine and diethyl-dithiocarbamate, which are CYP1A2, CYP2D6, and CYP2A6 and CYP2E1 inhibitors, respectively, inhibited neither reaction (Figure 5).

Since the initial studies with hepatic microsomes indicated that E1 rather than E1S was the substrate for the 16α- and 2-hydroxylase activities, the rates of hydroxylation of E1, when added as substrate in methanolic solution, by the human liver microsomal preparations were investigated. The rate for 16α-hydroxylation was 0.05 pmol/min/pmol total P450 and for 2-hydroxylation was 0.20 pmol/min/pmol total P450 at 150 µM E1.

To facilitate investigations of human metabolism of E1S the rates of NADPH-dependent metabolism by a series of cDNA expressed human P450s were investigated. The microsomal preparations from the P450 transfacted human B-lymphoblastoid or insect cells did not have E1S sulfatase activity (data not provided). E1S was incubated with CYP3A4 expressed microsomes and NADPH in an attempt to determine whether 16α- and 2-hydroxylation could be detected from this substrate. Since the putative products, 16α-OHE1S and 2-OHE1S, are not detectable in our analytical system, the microsomes were removed by filtration (0.2 µm Corning filters) from the reaction mixtures after incubation and sulfatase was then added. If any sulfate metabolites had formed during the first incubation, the sulfatase would then convert them to 16α-OHE1 and 2-OHE1, which are detectable in our assay systems. No metabolites were detected. In view of these results all studies of E1S metabolism using microsomes from P450 cDNA transfected cells had sulfatase added and were then pre-incubated for 10 min prior to addition of NADPH. The results are shown in Figure 6. All of the microsomal preparations had NADPH-P450 reductase coexpressed with the P450, and in the case of CYP3A4, cytochrome b5 was also coexpressed. At an E1S concentration of 150 µM the highest rate of 16α-hydroxylation was produced by CYP3A4, with CYP1A1 and CYP3A5...
exhibiting moderate activities, and CYP2C9 and CYP2C19 exhibiting very low activities (Figure 6). In contrast, the highest rate of 2-hydroxylation was produced by CYP1A2, with CYP3A4 and CYP1A1 also exhibiting moderate activities (Figure 6).

These studies supported a predominant role for CYP3A4 in human hepatic formation of 16α-OHE1. Although CYP3A4 was apparently more effective than CYP3A5 in catalysing the 16α-hydroxylation of E1 (Figure 6), this relationship could be influenced by cytochrome b5. To test this rabbit liver cytochrome b5 was added to microsomal preparations expressing CYP3A4 or CYP3A5, but without co-expressed cytochrome b5. The rates of 2- and 16α-hydroxylation with and without added cytochrome b5 are shown in Figure 7. Cytochrome b5 increases the rates of CYP3A4 mediated 2- and 16α-hydroxylation by 3.7- and 5.1-fold, respectively, and of CYP3A5 mediated 16α-hydroxylation by 7.5-fold.

When E1 was used as substrate, co-expressed CYP3A4 and NADPH-P450 reductase yielded 16α-OH-E1 at a rate of 0.15 pmol/min/pmol CYP3A4 at 150 µM E1, and 2-OHE1 at a rate of 0.43 pmol/min/pmol CYP3A4 at 150 µM E1.

The kinetic parameters for E1 16α-hydroxylation catalysed by human liver microsomes and by cDNA expressed CYP3A4 with cytochrome b5 coexpressed were determined using Lineweaver–Burk plots (Figure 8). The apparent K_m and V_max values for the liver microsomes were 154 µM and 238 pmol/min/nmol total P450, respectively, and for the expressed CYP3A4 were 172 µM and 1050 pmol/min/nmol CYP3A4.

**Discussion**

These studies have identified the forms of P450 that are the principal catalysts of the metabolism of E1 to 16α-OHE1 in humans. In view of the carcinogenic potential of 16α-OHE1 (1), the results presented here provide a basis for the design of therapeutic interventions against breast cancer. Although much of this study was conducted with human liver, which is more readily available in the quantities required for these studies, the relevance to breast cancer resides in the fact that the CYP3A4/5 enzymes are also expressed in human breast tissue (21). Thus it appears probable that 16α-OHE1 is generated within breast tissue cells where it is more likely to directly affect the processes of breast cancer.

Based on earlier reports that steroid sulfates could serve as substrates for human hydroxylases (14,15), and the major contribution of E1S to circulating estrogen levels, we used E1S as substrate in these studies. However, several pieces of evidence, most compelling of which was that in the absence of sulfatase activity CYP3A4 did not catalyse 16α-hydroxylation of E1S, indicated that E1 and not E1S was the substrate. In studies with human liver microsomes, which contain estrone sulfatases (20), and microsomal preparations of cDNA expressed P450s, where sulfatase was added, we pre-incubated the microsomes to permit conversion of the substrate, estrone-3-sulfate (E1S), to estrone prior to initiation of hydroxylase activity with NADPH. With P450 expressed microsomes, reaction mixtures were pre-incubated with arylsulfatase prior to initiation of hydroxylation with NADPH because of lack of sulfatase activities in these microsomes. Concentrations of the substrate, estrone-3-sulfate (E1S), were varied from 50 to 300 µM. Rates were determined as described in Materials and methods. Each point represents the mean of duplicate determinations.
with $E_S$ is that the water soluble $E_S$ is freely available to the sulfatase, where it is converted to $E_1$, which is then readily transported to CYP3A4 in the microsomal membrane. In contrast, $E_1$ is not soluble in water and its access to the membrane bound CYP3A4 is less facile. The relevance of this observation to in vivo metabolism is unknown.

Several approaches were applied to determine which forms of P450 catalyse the metabolism of $E_1$ to 16α-OHE1. The excellent correlation in a series of 15 human liver microsomal preparations of rates of 16α-hydroxylation of $E_1$ with rates of dextromethorphan N-demethylation and rates of testosterone 6-β-hydroxylation, both primarily CYP3A4/5 activities (22–24), clearly supports the role of CYP3A4/5 in $E_1$ 16α-hydroxylation. The high correlations reflect contributions of both CYP3A4 and CYP3A5, which probably are expressed at differing relative levels in each of the microsomal preparations, and probably contribute differently to the metabolism of each of the substrates. This variability could be expected to contribute unfavorably to the correlations. Additionally we demonstrated that, consistent with other studies with CYP3A4 and CYP3A5 (25), cytochrome $b_5$ contributes markedly to CYP3A4 and CYP3A5 $E_1$ 16α-hydroxylation activity levels. Since the cytochrome $b_5$ levels in the human liver preparations vary by 2-fold, this would be expected to differentially affect rates of CYP3A4/5 catalysed reactions and thus influence correlations between activities. However, there was no correlation between cytochrome $b_5$ levels and $E_1$ 16α-hydroxylase activities (data not provided), suggesting that differences in cytochrome $b_5$ levels contribute minimally to the correlations. Since there are no substrates available to permit differentiation between CYP3A4- and CYP3A5-mediated activities, our conclusion that both P450s are involved in $E_1$ 16α-hydroxylation is based on our results that individually expressed CYP3A4 and CYP3A5 preparations both catalysed $E_1$ 16α-hydroxylase activity. Other studies which supported the conclusion that CYP3A4/5 were the principal catalysts of $E_1$ 16α-hydroxylase activity involved inhibition by specific antibodies and chemical inhibitors. The poor correlation of CYP1A2 activities with 2-OH$E_2$ formation rates in a series of human liver microsomal preparations, despite our observation that CYP1A2 catalyses the metabolism of 2-OH$E_2$ formation, is surprising. Possibly CYP1A2 contributes to 2-hydroxylation only at higher estrogen concentrations.

In summary we have demonstrated that human CYP3A4 and CYP3A5 catalyse the 16α-hydroxylation of $E_1$, and that $E_S$ is not a substrate for this reaction. Since 16α-OHE$1$ is a putative breast carcinogen, knowledge of the enzymes involved in its synthesis provides a basis for blocking its synthesis in vivo. Since CYP3A4 and CYP3A5 are both expressed in human female breast tissue, but not in all individuals (21), these results also provide a basis for selection of potentially susceptible individuals.

References


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