Roles of Cytochromes P450 1A2 and 3A4 in the Oxidation of Estradiol and Estrone in Human Liver Microsomes

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Of seven cDNA-expressed human cytochrome P450 (P450) enzymes (P450s 1A2, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4) examined, P450 1A2 was the most active in catalyzing 2- and 4-hydroxylations of estradiol and estrone. P450 3A4 and P450 2C9 also catalyzed these reactions although to lesser extents than P450 1A2. P450 1A2 also efficiently oxidized estradiol at the 16α-position but was less active in estrone 16α-hydroxylation; the latter reaction and also estradiol 16α-hydroxylation were catalyzed by P450 3A4 at significant levels. Anti-P450 1A2 antibodies inhibited 2- and 4-hydroxylations of these two estrogens catalyzed by liver microsomes of some of the human samples examined. Estradiol 16α-hydroxylation was inhibited by both anti-P450 1A2 and anti-P450 3A4, while estrone 16α-hydroxylation was significantly suppressed by anti-P450 3A4 in human liver microsomes. Fluvoxamine efficiently inhibited the estrogen hydroxylations in human liver samples that contained high levels of P450 1A2, while ketoconazole affected these activities in human samples in which P450 3A4 levels were high. α-Naphthoflavone either stimulated or had no effect on estradiol hydroxylation catalyzed by liver microsomes; the intensity of this effect depended on the human samples and their P450s. Interestingly, in the presence of anti-P450 3A4 antibodies, α-naphthoflavone was found to be able to inhibit estradiol and estrone 2-hydroxylations catalyzed by human liver microsomes. The results suggest that both P450s 1A2 and 3A4 have major roles in oxidations of estradiol and estrone in human liver and that the contents of these two P450 forms in liver microsomes determine which P450 enzymes are most important in hepatic estrogen hydroxylation by individual humans. P450 3A4 may be expected to play a more important role for some of the estrogen hydroxylation reactions than P450 1A2. Knowledge of roles of individual P450s in these estrogen hydroxylations has relevance to current controversies in hormonal carcinogenesis [Service, R. F. (1998) *Science* **279**, 1631−1633].

Introduction

Cytochrome P450 (P450) comprises a superfamily of enzymes that catalyze oxidation of a great number of xenobiotic chemicals such as drugs, toxic chemicals, and carcinogens as well as endobiotic chemicals including steroids, fatty acids, prostaglandins, and lipid-soluble vitamins (*1*−*3*). Several steroid hormones have been shown to be catalyzed by P450 enzymes localized in endoplasmic reticulum (microsomal) membranes of mammalian livers (*4*, *5*). Testosterone and progesterone have been shown to be oxidized mainly by the P450 3A subfamily of enzymes to 6β-, 2β-, and 15β-hydroxytestosterone and to 6β-, 16α-, and 2β-hydroxyprogesterone, respectively, in human liver microsomes (*5*−*7*), and we have recently shown that hydroxylation of testosterone at the 17-position (to form androstenedione) and hydroxylation of progesterone at the 21-, 16α-, and 17α-positions are catalyzed mainly by P450 2C19 and to lesser extents by P450 2C9 (*8*).

Estradiol and estrone, two major estrogens that are distributed widely in mammalian tissues, have been shown to be oxidized at several positions by different P450 enzymes (*9*, *10*). Catechol estrogens, the products of estrogens formed by 2- and 4-hydroxylations by P450 enzymes, have been implicated as important in breast cancer. These metabolites can serve as intermediates for the generation of more reactive semiquinones and quinones (*11*−*13*). Hydroxylation of estradiol by P450 enzymes at the 16α-position has also been postulated to be important in mammary carcinogenesis because 16α-hydroxyestradiol can bind covalently to estrogen receptors (*11*, *14*−*16*). Exactly which of these pathways is most important to estrogen-induced cancers remains controversial.

Studies have been done in several laboratories to identify which P450 enzymes are involved in the oxidation of estrogens in humans (*9*, *10*, *17*, *18*). P450 enzymes in mammalian tissues have generally been recognized to be more active in catalyzing estrone and estradiol at the C-2 than the C-4 position; in humans, the former reactions are reported to be catalyzed by several P450 enzymes including P450s 1A1, 1A2, and 3A4 (*17*−*19*).

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There are also complicated results suggesting that several human P450 enzymes can be involved in the 16α-hydroxylation of estrogens, although definitive studies to indicate which P450 enzymes are primarily involved in the oxidations of these estrogens are lacking (*17*, *18*). Recently estradiol 4-hydroxylation has been reported to be catalyzed by P450 1B1 (*20*). Other studies have shown that estradiol 4-hydroxylation is catalyzed mainly by P450 3A enzymes in human liver, since anti-P450 3A4 antibodies can inhibit the microsome-catalyzed 4-hydroxylation of estradiol by 60−80% (*9*, *17*, *19*, *21*).

The present study was, therefore, undertaken to determine which P450 enzymes are most active in the hydroxylations of estradiol and estrone at different positions. Initial studies were done using recombinant human P450 enzymes, and further studies were done in the context of human liver microsomes in order to put the work with recombinant enzymes into perspective.

Experimental Procedures

Chemicals. Estrone, estradiol, and their hydroxylated metabolites were purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals and reagents used in this study were obtained from sources described previously or were of highest quality commercially available (*8*, *22*, *23*).

Enzyme Preparations. Recombinant P450s 2C9, 2C19, 2D6, and 3A4 expressed in microsomes of *Trichoplusia ni* cells infected with a baculovirus containing human P450 and the human NADPH−P450 reductase cDNA inserts were prepared as described (*24*, *25*). Other recombinant P450 enzymes including P450s 1A2, 2B6, and 2E1 in a baculovirus system coexpressing human NADPH−P450 reductase were obtained from Gentest Co. (Woburn, MA); the P450 contents in these latter systems were used as described in the data sheets provided by the manufacturer.

Human liver samples were obtained from organ donors or patients undergoing liver resection as described previously (*26*, *27*). Liver microsomes were suspended in 10 mM Tris-Cl buffer (pH 7.4) containing 1.0 mM EDTA and 20% glycerol (v/v) (*28*).

Human P450s 1A2, 2C9, and 3A4 were purified to electrophoretic homogeneity from liver microsomes as described (*9*, *29*, *30*). Rabbit anti-human P450 1A2, 2C9, and 3A4 antibodies were prepared as described (*30*, *31*).

Enzyme Assays. Estradiol and estrone hydroxylation activities were determined as described (*17*−*19*), with slight modifications. The standard incubation mixture (final volume of 0.25 mL) contained liver microsomes (1.0 μM P450) or recombinant P450 enzymes (0.2 μM), 50 mM potassium phosphate buffer (pH 7.4), an NADPH-generating system (*32*), and estradiol or estrone (100 μM). Incubations were carried out at 37 °C for 30 min and terminated by adding 1.5 mL of CH_2Cl_2 and 0.3 M NaCl. The organic phase was taken to dryness under a nitrogen stream at 37 °C. The residue was dissolved in a mixture of 33% CH_3CN (v/v) in 1.0% acetic acid (v/v). Product formation was determined by HPLC with a C_{18} (5 μm) analytical column (4.6 × 150 mm; Kanto Chemical, Tokyo). The elution was conducted with a mixture of 33% CH_3CN (v/v) in 1.0% acetic acid (v/v) at a flow rate of 1.0 mL/min, and detection was by UV absorbance at 280 nm. We did not add ascorbic acid in the HPLC mobile phase nor in the incubation mixture, since no difference in the hydroxylated metabolites of estrogens was found during assays in this system (*17*). Most assays were done in duplicate and are representative of other confirmatory studies.

Other Assays. Concentrations of P450 and protein were estimated spectrally by the methods of Omura and Sato (*33*) and Lowry et al. (*34*), respectively. The contents of human P450 proteins in liver microsomes were estimated by coupled sodium dodecyl sulfate−polyacrylamide gel electrophoresis/immunochemical development ("Western blotting") (*35*).

Kinetic parameters for substrate oxidations by recombinant P450 enzymes and human liver microsomes were estimated using a computer program (KaleidaGraph program from Synergy Software, Reading, PA) designed for nonlinear regression analysis.

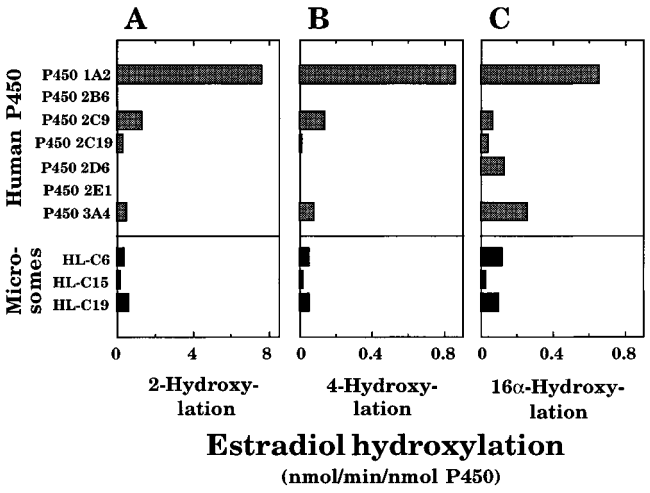

Figure 1. Estradiol hydroxylation by cDNA-expressed human P450 enzymes and by human liver microsomal samples HL-C6, HL-C15, and HL-C19. The substrate concentration used was 100 μM, and the P450 concentration was 0.2 μM for recombinant P450 enzymes and 1 μM for liver microsomes. Data are means of duplicate determinations.

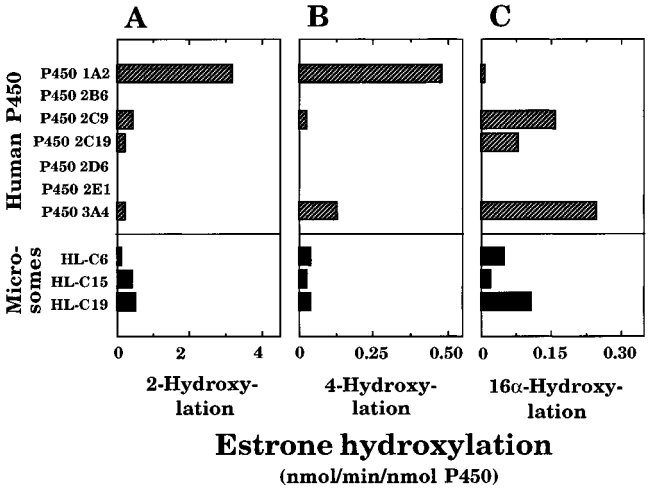

Figure 2. Estrone hydroxylation by cDNA-expressed human P450 enzymes and by liver microsomes of human samples HL-C6, HL-C15, and HL-C19. Other details are the same as in the legend to Figure 1.

Results

Estradiol and Estrone Hydroxylations by cDNA-Expressed Human P450 Enzymes. Seven forms of cDNA-expressed human P450 enzymes including P450 1A2, P450 2B6, P450 2C9, P450 2C19, P450 2D6, P450 2E1, and P450 3A4, prepared in baculovirus systems, were used to determine which P450 enzymes catalyze estradiol and estrone hydroxylations significantly (Figures 1 and 2). Estrogen oxidation activities by human liver microsomal samples HL-C6, HL-C15, and HL-C19 were also determined. These three microsomal preparations contained total spectrally determined P450 levels (nmol/mg of microsomal protein) of 0.66, 0.42, and 0.69,

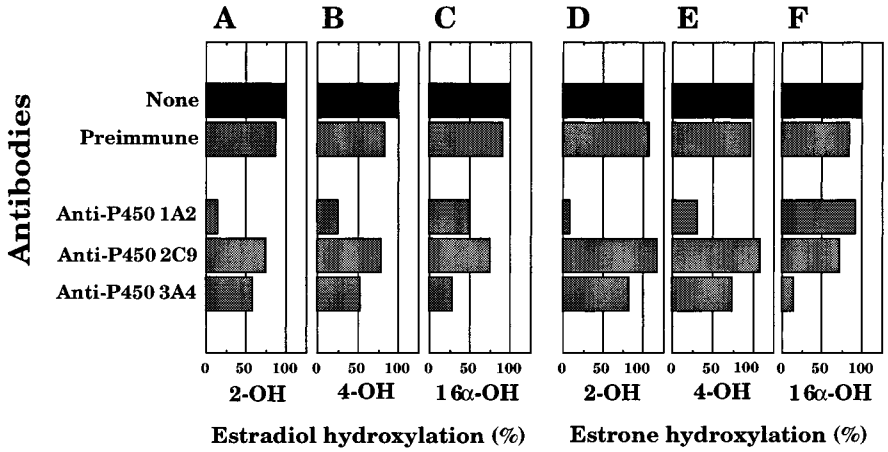

Figure 3. Effects of preimmune IgG and anti-P450 antibodies (5 mg of IgG/nmol of microsomal P450) on oxidations of estradiol (A, B, and C) and estrone (D, E, and F) catalyzed by human liver microsomes (sample HL-C19). Data are means of duplicate determinations. Control activities (nmol of products formed/min/nmol of microsomal P450) in the absence of antibodies were 0.72, 0.051, and 0.069 for 2-, 4-, and 16α-hydroxylations of estradiol and 0.52, 0.045, and 0.075 for 2-, 4-, and 16α-hydroxylations of estrone, respectively. Abbreviations used in the figure: 2-OH, 2-hydroxylation; 4-OH, 4-hydroxylation; 16α-OH, 16α-hydroxylation.

respectively. Microsomal sample HL-C6 had P450 1A2, P450 2C9, and P450 3A4 levels of 5%, 12%, and 73% total P450, respectively, as judged by immunoblotting analysis. Sample HL-C15 contained P450 1A2, 2C9, and 3A4 levels of 23%, 21%, and 14%, respectively, and sample HL-C19 had levels of 25%, 16%, and 46% total P450, respectively.

Of the seven cDNA-expressed human P450 enzymes examined, the P450 1A2 system was the most active in catalyzing estradiol 2-, 4-, and 16α-hydroxylations, when determined at a substrate concentration of 100 μM (Figure 1). P450s 2C9 and 3A4 catalyzed estradiol 2- and 4-hydroxylations at lower rates than did P450 1A2. Several P450 enzymes including P450s 1A2, 2C9, 2C19, 2D6, and 3A4 oxidized estradiol at the 16α-position. Human liver microsomal activities for estradiol hydroxylations were lower than those catalyzed by P450 1A2.

Similar patterns of activities of estradiol hydroxylations by recombinant human P450 enzymes were observed when estrone was used for the substrate (at 100 μM), except that estrone 16α-hydroxylation activities were highest with P450 3A4 followed by P450s 2C9 and 2C19 (Figure 2). The catalytic activity of P450 1A2 for estrone 16α-hydroxylation was very low.

Concentration Dependence of Oxidation of Estradiol and Estrone by Human P450 Enzymes. Steady-state kinetic analysis was done for hydroxylations of estradiol and estrone by recombinant P450 1A2 and P450 3A4 systems (Table 1). In all cases, the K_m values were in the range of 10−120 μM, so most of the subsequent experiments were done at the single substrate concentration of 100 μM. With P450 1A2, the rank order of V_{max} for estradiol and estrone hydroxylation was 2 > 4 ∼ 16α. With P450 3A4, the rank order was 2 ∼ 4 ∼ 16α for estrone. The substrate concentrations used in many of the incubations were 100 μM (both estradiol and estrone); the levels are much higher than those seen in the physiological state. However, the K_m values for the three hydroxylations are relatively high for P450s 1A2 and 3A4 (Table 1), and even a concentration of 100 μM is not saturating.

Effects of Anti-P450 Antibodies on Hydroxylations of Estradiol and Estrone by Human Liver Microsomes. Antibodies raised against P450 1A2, P450 2C9, and P450 3A4 were used to inhibit estradiol and estrone hydroxylations catalyzed by human microsomal HL-C19 (Figure 3). Anti-P450 1A2 significantly inhibited the 2-hydroxylation of estradiol and estrone, 4-hydroxylation of estradiol and estrone, and 16α-hydroxylation of estradiol. In contrast, estrone 16α-hydroxylation was markedly suppressed by anti-P450 3A4; the antibody also inhibited 2-, 4-, and 16α-hydroxylations of estradiol. Effects of anti-P450 2C9 antibodies were less than those of anti-P450 1A2 and anti-P450 3A4 in the estrogen hydroxylations catalyzed by liver microsomes.

Table 1. Concentration Dependence of Estradiol and Estrone Hydroxylations by Recombinant P450 1A2 and P450 3A4 Expressed in Baculovirus Systems[a]

	2-hydroxylation		4-hydroxylation		16α-hydroxylation	
	K_m (μM)	V_{max} (min^{-1})	K_m (μM)	V_{max} (min^{-1})	K_m (μM)	V_{max} (min^{-1})
Estradiol Hydroxylation						
P450 1A2	20 ± 6	11 ± 2	28 ± 6	0.9 ± 0.1	58 ± 4	0.7 ± 0.2
P450 3A4	54 ± 11	0.8 ± 0.2	111 ± 42	0.3 ± 0.2	75 ± 18	0.4 ± 0.2
Estrone Hydroxylation						
P450 1A2	19 ± 6	9.2 ± 2.1	17 ± 5	2.0 ± 0.3		<0.1
P450 3A4	102 ± 30	0.7 ± 0.4	78 ± 26	0.6 ± 0.3	64 ± 8	0.5 ± 0.2

[a] Kinetic parameters were determined at substrate concentrations between 10 and 150 μM and P450 concentrations of 0.04 μM for P450 1A2 and 0.2 μM for P450 3A4. Incubation time used for P450 1A2 and 3A4 was 15 and 30 min, respectively. Results are presented as means ± SE.

Effects of Chemical P450 Modifiers on Hydroxylations of Estradiol and Estrone by Human Liver Microsomes. The results obtained using cDNA-expressed P450s suggest that several P450 enzymes including P450s 1A2, 3A4, and 2C9 are involved in the hydroxylations of estradiol and estrone by human liver microsomes. However, these systems may not be directly comparable, and in addition, the results must be considered in terms of the expression levels of individual P450s in liver. Therefore, we utilized several human liver microsomal samples to further investigate P450 roles. Ketoconazole (inhibitor of P450 3A4), sulfaphenazole (inhibitor of P450 2C9), fluvoxamine (inhibitor of P450 1A2), and α-naphthoflavone (inhibitor of P450 1A2 and sometimes activator of P450 3A4) were examined for their effects on estrogen hydroxylation in human liver microsomes.

When liver microsomal sample HL-C6 (a sample high in P450 3A4) was used, ketoconazole caused very sig-

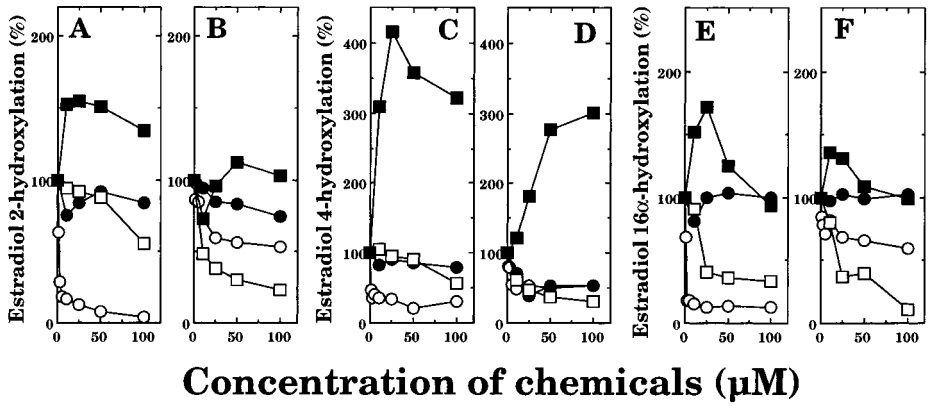

Figure 4. Effects of ketoconazole (○), sulfaphenazole (●), fluvoxamine (□), and α-naphthoflavone (■) on estradiol 2-hydroxylation (A and B), 4-hydroxylation (C and D), and 16α-hydroxylation (E and F) catalyzed by liver microsomes of human samples HL-C6 (A, C, and E) and HL-C19 (B, D, and F). Different concentrations of chemicals were added to the incubation mixtures as indicated. Data are means of duplicate determinations. Control activities (nmol of products formed/min/nmol of microsomal P450) of 2-, 4-, and 16α-hydroxylations of estradiol in the absence of chemicals were 0.53, 0.049, and 0.076 for liver microsomal preparation HL-C6 and 0.56, 0.048, and 0.069 for sample HL-C19, respectively.

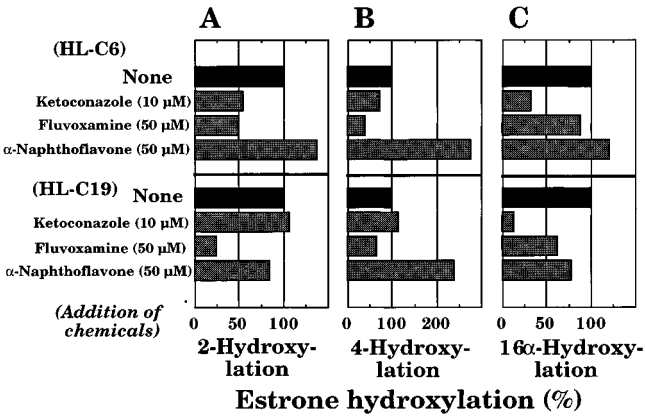

Figure 5. Effects of ketoconazole, fluvoxamine, and α-naphthoflavone on estrone 2-hydroxylation (A), 4-hydroxylation (B), and 16α-hydroxylation (C) catalyzed by human liver microsomal samples HL-C6 and HL-C19. Control activities (nmol of products/min/nmol of microsomal P450) of 2-, 4-, and 16α-hydroxylations of estrone in the absence of chemicals were 0.21, 0.015, and 0.043 for liver microsomal sample HL-C6 and 0.51, 0.018, and 0.11 for sample HL-C19, respectively.

nificant inhibition of the 2-, 4-, and 16α-hydroxylations of estradiol, and these reactions were all found to be enhanced by α-naphthoflavone (Figure 4). Estradiol 16α-hydroxylation, but not estradiol 2- and 4-hydroxylations, was inhibited by the P450 1A2 inhibitor fluvoxamine in HL-C6 liver microsomes. However, when liver microsomal sample HL-C19 (which had significant amounts of P450s 1A2, 2C9, and 3A4) was used, fluvoxamine significantly suppressed the estradiol hydroxylations, being more inhibitory than ketoconazole. In addition, α-naphthoflavone enhanced the 4- and 16α-hydroxylations, but not 2-hydroxylation, of estradiol by sample HL-C19 liver microsomes. Sulfaphenazole inhibited estradiol 4-hydroxylation catalyzed by human sample HL-C19 microsomes by 40−50%.

Effects of chemical P450 modifiers on estrone hydroxylations catalyzed by liver microsomal samples HL-C6 and HL-C19 were also determined (Figure 5). Fluvoxamine inhibited estrone 2- and 4-hydroxylations while 16α-hydroxylation of estrone was not affected significantly by fluvoxamine in human liver sample HL-C6 microsomes. In contrast, ketoconazole was very effective in inhibiting estrone 16α-hydroxylation by liver sample HL-C6 and HL-C19 microsomes, while it did not affect estrone 2- and 4-hydroxylations by liver sample HL-C19 microsomes. α-Naphthoflavone did not affect estrone 2- and 16α-hydroxylations but significantly enhanced estrone 4-hydroxylation catalyzed by liver microsomes.

Effects of Combinations of Anti-P450 Antibodies and Chemical P450 Modifiers on Hydroxylations of Estradiol and Estrone by Human Liver Microsomes. The effects of chemical P450 modifiers on the estrogen hydroxylations by human liver microsomes were examined in the presence or absence of anti-P450 1A2 and anti-P450 3A4. In the presence of anti-P450 1A2, estradiol hydroxylations were completely inhibited by ketoconazole and were stimulated by α-naphthoflavone in liver microsomes of a sample containing a high level of P450 3A4 (HL-C6) (Table 2). In the presence of anti-P450 3A4, 2-hydroxylations of estradiol and estrone catalyzed by liver microsomes (sample HL-C19) were significantly suppressed (but not enhanced) by α-naphthoflavone as well as fluvoxamine (Figure 6). In contrast, 16α-hydroxylation of estrone was not affected significantly by the addition of fluvoxamine or α-naphthoflavone.

Discussion

Recent studies have established that both P450s 1A2 and 3A4 are able to catalyze oxidations of estradiol and estrone in human liver microsomes and that other P450 enzymes such as P450s 2C9 and 2E1 may have some role in estrogen catabolism (*10*, *17*−*20*). It is, however, not precisely defined which P450 forms are actually involved in the estradiol and estrone hydroxylations at different positions in human livers. For example, although Guengerich et al. (*9*) first reported that P450 3A4 ($P450_{NF}$) is involved in 2- and 4-hydroxylations of estradiol by human liver microsomes, Ball et al. (*10*) reported that the major proteins involved in estradiol metabolism are in the P450 1A gene family and that members of the P450 2C and P450 2E gene subfamilies also have important roles. The results of Kerlan et al. (*19*) support the view that 2- and 4-hydroxylations of estradiol are catalyzed mainly by P450 3A enzymes in humans. There are also complicated results reporting that several human P450 enzymes are able to catalyze 16α-hydroxylation of estrogens in human liver microsomes, although definitive studies indicating which P450 enzymes are primarily

Table 2. Effects of Ketoconazole and α-Naphthoflavone on Estradiol Hydroxylation Activities by Human Liver Microsomal Sample HL-C6 in the Presence of Anti-P450 1A2 IgG[a]

system			estradiol hydroxylation activities (nmol of products/min/nmol of P450)		
anti-P450 1A2 (5 mg of IgG/nmol of P450)	ketoconazole (μM)	α-naphthoflavone (μM)	2-hydroxylation	4-hydroxylation	16α-hydroxylation
−	−	−	0.52 ± 0.06	0.041 ± 0.005	0.082 ± 0.011
+	−	−	0.17 ± 0.02	0.025 ± 0.010	0.065 ± 0.008
+	10	−	0.003 ± 0.002	0.002 ± 0.001	0.021 ± 0.011
+	100	−	<0.001	<0.001	0.018 ± 0.009
+	−	10	0.21 ± 0.04	0.027 ± 0.003	0.071 ± 0.007
+	−	100	0.42 ± 0.10	0.148 ± 0.020	0.121 ± 0.020

[a] Results are presented as means ± SD of triplicate determinations.

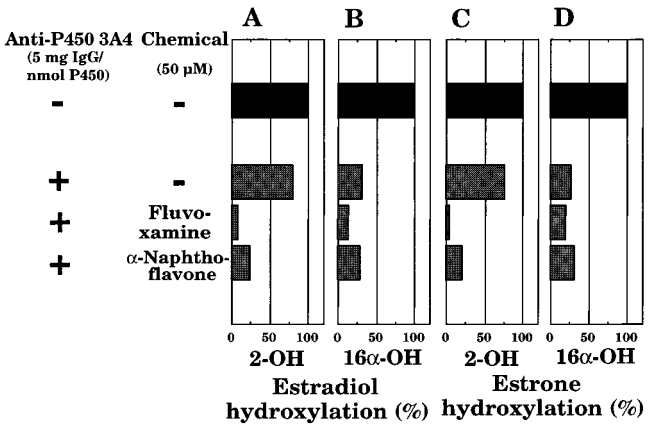

Figure 6. Effects of fluvoxamine and α-naphthoflavone (both 50 μM) on estradiol 2-hydroxylation (A) and 16α-hydroxylation (B) and on estrone 2-hydroxylation (C) and 16α-hydroxylation (D) catalyzed by human liver microsomal sample HL-C19 when anti-CYP3A4 antibodies (5 mg of IgG/nmol of microsomal P450) were present in the incubation mixture. Control activities (nmol of products formed/min/nmol of microsomal P450) in the absence of antibodies and chemicals were 0.68, 0.09, 0.55, and 0.11 for 2-hydroxylation and 16α-hydroxylation of estradiol and 2-hydroxylation and 16α-hydroxylation of estrone, respectively. Results are presented as means of duplicate determinations.

involved in the 16α-hydroxylation of estradiol and estrone are lacking (*17*, *18*).

Why have such complicated results been reported from several laboratories on the roles of P450 enzymes involved in the oxidation of estrogens by human liver microsomes? It has been shown that there are interindividual variations in the levels of individual P450 enzymes in humans (*36*−*40*). For example, average levels of P450 3A4 in human livers have been determined to be ∼30% of total P450 in 30 Japanese and 30 Caucasians examined; in some people this P450 level accounts for >60% of total P450, probably due to the induction of P450 3A4 by various chemical agents (*41*−*44*). It is also known that P450 1A2 is induced in human livers by several chemicals such as polycyclic aromatic hydrocarbons, arylamines, and chlorinated hydrocarbons through ingestion of charcoal-broiled foods, smoking, and other exposures (*3*, *42*, *44*). These results support the view that contributions of P450 enzymes in estrogen hydroxylation reactions in human livers may be altered by using different human liver samples whose compositions of various P450 enzymes differ.

Using different human samples that contain varying levels of individual P450 enzymes in the liver and also recombinant human P450 enzymes expressed in baculovirus systems, we obtained several pieces of evidence to support the view that different human P450 enzymes, particularly P450s 1A2 and 3A4, contribute significantly to the oxidations of estradiol and estrone at 2-, 4-, and 16α-positions in humans and that the roles of these P450 enzymes vary with the use of human samples used and with the positions of estrogen hydroxylations determined. The results obtained in this study can be summarized as follows.

First, estradiol and estrone 2-hydroxylations are mainly catalyzed by P450 1A2 in human livers because anti-P450 1A2 and fluvoxamine inhibited these reactions significantly and recombinant P450 1A2 had a higher V_{max}/K_m ratio than P450 3A4. However, minor but significant roles of P450 3A4 were found in the 2-hydroxylation of estradiol and estrone by human livers, since (in human samples which contained high levels of P450 3A4) both reactions were inhibited by anti-P450 3A4 and ketoconazole and were stimulated by α-naphthoflavone, a chemical known to enhance some P450 3A4-dependent xenobiotic oxidations (*45*, *46*).

Second, 4-hydroxylations of estradiol and estrone are also catalyzed principally by P450 1A2 in human liver microsomes, but the roles of P450 3A4 in the 4-hydroxylation reactions may be more significant than those seen in the 2-hydroxylations of estradiol and estrone. For example, when liver sample HL-C6 (which contained P450 3A4 at a high level) was used, anti-P450 1A2 inhibited estradiol 4-hydroxylation only ∼40% and the resultant activities were completely inhibited by ketoconazole and significantly enhanced by α-naphthoflavone. Such enhancements of 4-hydroxylation activities by α-naphthoflavone were also seen when estrone was incubated with human liver microsome sample HL-C19, as well as with HL-C6. It should, however, be mentioned that roles of P450 1A2 in the 4-hydroxylation of estradiol and estrone may be more significant than those by P450 3A4, because recombinant P450 1A2 had a higher V_{max}/K_m ratio than P450 3A4 and anti-P450 1A2 inhibited estradiol and estrone 4-hydroxylations more than anti-P450 3A4 in liver microsome HL-C19 sample, in which P450 1A2 and 3A4 levels in the liver were estimated to be 25% and 46%, respectively, of total P450.

Third, estradiol 16α-hydroxylation is suggested to be catalyzed primarily by both P450s 1A2 and 3A4 in human liver microsomes, although the roles of these two P450 enzymes in the reaction were affected by which human samples were used for the determinations. In contrast, estrone 16α-hydroxylation is found to be catalyzed most actively by P450 3A4, because recombinant P450 3A4 catalyzed this reaction at the highest level and anti-P450 3A4 was very effective in inhibiting the reaction. Roles of P450 1A2 in estrone 16α-hydroxylation may be very minor due to the fact that the recombinant enzyme catalyzed estrone 16α-hydroxylation at a very low level

and that anti-P450 1A2 poorly inhibited the reaction catalyzed by human liver microsomes.

P450 2C9 may also make some, but not a major, contribution to estrogen metabolism. Although recombinant P450 2C9 catalyzed oxidations of estradiol and estrone at several positions, anti-P450 2C9 and sulfaphenazole did not significantly affect estradiol and estrone hydroxylations catalyzed by human liver microsomes. Other P450 enzymes including P450s 2B6, 2D6, and 2E1 are also suggested to have only minor roles in estrogen catabolism in humans, since the recombinant enzymes had very low catalytic activities for the hydroxylation reactions.

In conclusion, our present results suggested that both P450s 1A2 and 3A4 are major P450 enzymes involved in the oxidations of estrogens in human livers. The roles of these two P450s vary depending on the contents of P450s 1A2 and 3A4 in livers. In general, P450 1A2 may be more active in catalyzing 2- and 4-hydroxylations of estradiol and estrone than P450 3A4, while the latter enzyme catalyzes the 16α-hydroxylation of estrone more than P450 1A2. Estradiol 16α-hydroxylation is catalyzed by several P450 enzymes including P450s 1A2 and 3A4. This information is important because human P450 1A2 is expressed only in liver but P450 3A4 is also expressed, at lower concentrations, in a number of extrahepatic tissues (*2*). Therefore, the P450 3A4-catalyzed hydroxylations may have special significance at these sites. P450 2C enzymes may make some contribution. This information about the roles of individual human P450s in estrogen 2-, 4-, and 16α-hydroxylations is of relevance in adhering current controversies of hormonal estrogen and "environmental estrogen" carcinogenesis (*47*, *48*).

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