PRELIMINARY REPORT

Role of Human Cytochrome P450 1A1, 1A2, 1B1, and 3A4 in the 2-, 4-, and 16α-Hydroxylation of 17β-Estradiol

Alaa F. Badawi, Ercole L. Cavalieri, and Eleanor G. Rogan

The steady-state kinetics and specific activity of 2-, 4-, and 16α-hydroxylation of 17β-estradiol (E_2) were evaluated for human cytochrome P450 (CYP) 1A1, 1A2, 1B1, and 3A4 enzymes, using complementary DNA–expressed CYP isoforms. CYP1A2 showed the highest 2-hydroxylation activity, followed by CYP1A1, 1B1, and 3A4. CYP1B1 had the highest 4-hydroxylation activity, followed by CYP1A2, 1A1, and 3A4. The 16α-hydroxylation reaction was catalyzed mainly by CYP1A2 and, to a similar, slightly lower extent, CYP3A4 and 1A1, with a lesser contribution by CYP1B1. The E_2 2-, 4-, and 16α-hydroxylation activities of human liver microsomes were 1.3 ± 0.3, 0.5 ± 0.06, and 0.3 ± 0.05 nmol metabolite/min/nmol P450, respectively. The contribution of CYP1A1 and 1B1 (mainly extrahepatic) to the E_2 hydroxylation reactions, relative to CYP1A2 and 3A4 (predominantly hepatic), may be relevant to understanding the process of hormonal carcinogenesis both in liver and in extrahepatic tissues.

ESTROGENS HAVE been implicated in the etiology of a variety of cancers, both in humans and in animal models.[1] The carcinogenic potential of estrogens can be attributed, at least partially, to the DNA modification caused by derivatives formed during metabolism. 17β-estradiol (E_2) is metabolized via two major pathways: formation of the 2- and 4-catechol estrogens (CEs, 2- and 4-OHE_2) and 16α-hydroxylation.[2] Unless detoxified, CEs may be oxidized to electrophilic metabolites, catechol estrogen quinones (CE-Qs), that can react with DNA to form depurinating and stable adducts. These adducts can lead to oncogenic mutations and may subsequently initiate many human cancers.[3] As to the possible role of 16α-hydroxylation in cancer, E_2 16α-hydroxylase activity was found to be directly correlated with susceptibility of mice to spontaneous mammary tumors.[4] Moreover, 16α-OHE_1 (estrone) can covalently bind to estrogen receptors and may cause genotoxic damage in mouse epithelial cells.[5]

Metabolism of estrogens is mediated by cytochrome P450 (CYP) enzymes, a superfamily of hemoproteins that catalyze NADPH-dependent monooxygenation of structurally diverse lipophilic substrates to yield more polar derivatives. Studies aimed at identifying the principal CYP isoforms involved in E_2 hydroxylation show that CYP1A2 and CYP3A4, compared with CYP2B6, 2C9, 2C19, 2D6, and 2E1, are the 2 major CYP isoforms involved in E_2 2-, 4-, and 16α-hydroxylation.[6,7] However, separate studies indicate that CYP1B1 catalyzes E_2 hydroxylation primarily at the C_4 position (with some 2-hydroxylation activity),[8,9] and CYP1A1 mainly mediates the 2-hydroxylation pathway with minor 4-hydroxylation capacity.[2] The contribution of CYP1A1 and 1B1 (mainly extrahepatic CYP) to the E_2 hydroxylation, relative to CYP1A2 and 3A4 (predominantly hepatic), has not been clearly defined. The present study examines the role of human CYP1A1, 1A2, 1B1, and 3A4 to the NADPH-dependent 2-, 4-, and 16α-hydroxylation of E_2. This information may provide a basis for understanding the complex pattern of CYP-linked metabolic pathways of estrogens, which may in turn be critical for elucidating the mechanisms of estrogen carcinogenesis.

MATERIALS AND METHODS

Enzymes and Human Liver Samples

Human liver microsomes (HLM, n = 3) and the recombinant human CYP1A1, 1A2, 1B1, and 3A4 , expressed in microsomes of insect cells infected with a baculovirus system expressing human CYP and NADPH-CYP reductase, were obtained from Gentest Corp (Woburn, MA). The total CYP contents in microsomes expressing CYPs and in HLM were used as described by the manufacturer. Microsomes from insect cells infected with wild-type baculovirus were used as a control that contained no detectable levels of CYP.

17β-Estradiol Metabolism

The E_2 hydroxylation activities of CYP1A1, 1A2, 1B1, and 3A4 and HLM were determined as previously described.[7] Briefly, recombinant CYP enzymes (0.2 μmol/L CYP) or HLM (1.0 μmol/L CYP) were incubated, in a final volume of 250 μL, with 100 μmol/L E_2, 50 μmol/L potassium phosphate buffer (pH 7.4), 1.4 μmol/L NADPH, and 5 μmol/L $MgCl_2$. For kinetic analysis, substrate concentration varied between 5 and 150 μmol/L, with CYP levels between 0.05 (CYP1A2) and 0.2 (CYP1A1, 1B1, and 3A4) μmol/L. Ascorbic acid (2 μmol/L) was added to the reaction mixture to protect E_2 metabolites from oxidative degradation.[10] After 15 minutes of incubation at 37°C, reactions were terminated by extracting the metabolites twice in 1 volume of ethyl acetate.[9] Extracts were combined, evaporated under argon, reconstituted in 100 μL of methanol:50 μmol/L ammonium

Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE.

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Address reprint requests to Alaa F. Badawi, PhD, Eppley Institute for Research in Cancer, 986805 University of Nebraska Medical Center, Omaha, NE 68198-6805. E-mail: abadawi@unmc.edu.

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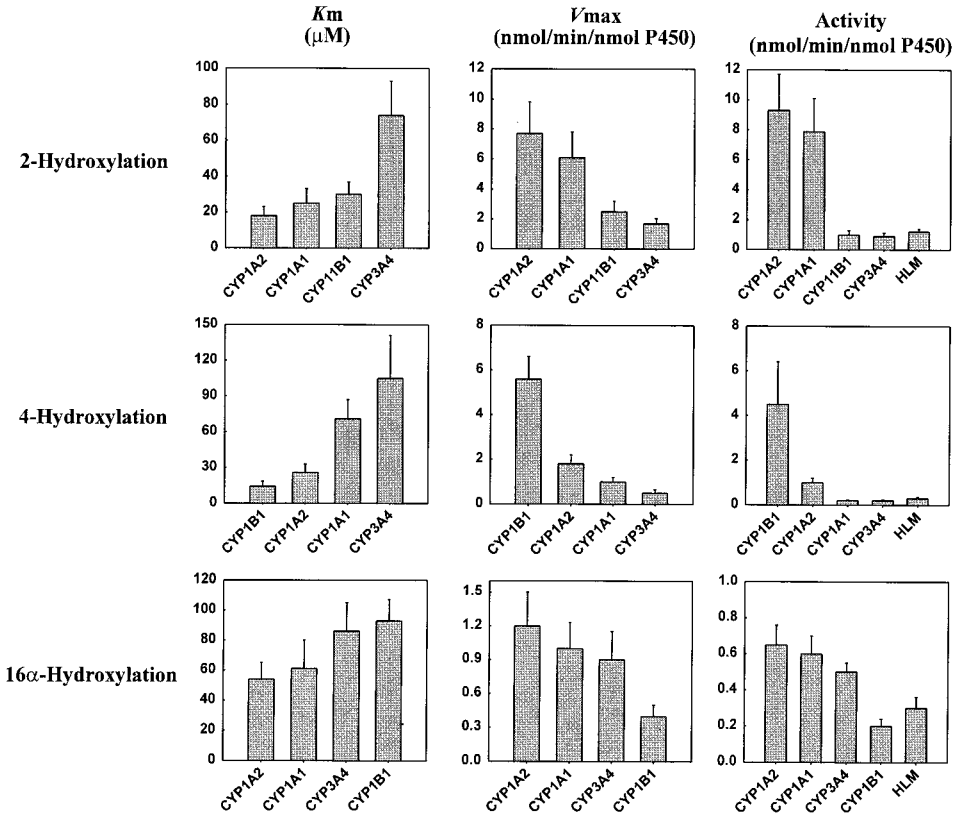

Fig 1. Kinetic parameters and specific activities of cDNA-expressed human CYPs and human liver microsomes for the metabolism of 17β-estradiol. Values are means ± SD of triplicates or 3 different HLM. Analysis of 2-, 4-, and 16α-hydroxylation was carried out by quantifying 2-OHE$_2$, 4-OHE$_2$, and 16α-OHE$_2$, respectively, using a high-performance liquid chromatography equipped with a 12-channel CoulArray detector as described.[10] Enzymes were ranked in the order of their contribution to the particular hydroxylation reaction and were followed by HLM in the activity panels.

acetate, pH 4.4 (4:1), and subjected to high-performance liquid chromatography (HLPC) analysis for 2-OHE$_2$, 4-OHE$_2$, and 16α-OHE$_2$ using a 12-channel CoulArray detector (ESA, Inc, Chelmsford, MA) as previously described.[10]

Kinetic parameters for E_2 hydroxylation by recombinant human CYP enzymes and HLM were estimated from the Lineweaver-Burk double reciprocal plot by linear regression analysis using SigmaStat and SigmaPlot software (Jandel Corp., Corte Madera, CA).

RESULTS AND DISCUSSION

The steady-state kinetics and specific activity of 2-, 4-, and 16α-hydroxylation of E_2 were evaluated for human CYP1A1, 1A2, 1B1, and 3A4 enzymes, using cDNA-expressed CYP isoforms (Fig 1). The role of some of these enzymes in E_1 hydroxylation was previously reported.[7,11] CYP enzymes such as CYP 2B6, 2C9, 2C19, 2D6, and 2E1 were not examined because they have very low or no E_2 hydroxylation activity.[7] Among the 4 CYPs studied, CYP1A2 had the highest 2-hydroxylation activity, as evidenced by the lowest K_m and the highest V_{max} values and specific activity (Fig 1). The capacity of CYP1A2 for E_2 2-hydroxylation was followed, in order, by CYP1A1, 1B1, and 3A4. Data presented here for CYP1A2 and 3A4 are in a general agreement with a previous report showing that CYP1A2 is more active than 3A4 in catalyzing not only E_2 2-hydroxylation, but also 4- and 16α-hydroxylation.[7] CYP1A2 is also the major enzyme responsible for E_1 2-hydroxylation, with K_m (19 ± 6 μmol/L) and V_{max} (9.2 ± 2.1 nmol/min/nmol P450) values that were, respectively, lower and higher than the values obtained for CYP3A4 (102 ± 30 μmol/L and 0.7 ± 0.4 nmol/min/nmol P450).[7] Moreover, the E_1 2-hydroxylation activity of CYP1A2 is higher than that of CYP1B1, 1A1, and 3A4.[8] It is known that CYP1A2 and 3A4 together account for approximately 35% to 65% of the total hepatic CYP content, with levels of CYP3A4 that are approximately 4- to 14-fold higher than CYP1A2. This abundance delineates CYP3A4, followed by 1A2, as the major CYP isoforms responsible for hepatic metabolism of E_2.

CYP1B1 had the lowest K_m and the highest V_{max} and metabolic capacity for E_2 4-hydroxylation reaction (Fig 1) and was followed, in order, by CYP1A2, 1A1, and 3A4. In support, CYP1B1 had approximately 12-, 5-, and 9-fold higher E_1 4-hydroxylation activity than CYP1A1, 1A2, and 3A4, respectively.[8] The role of CYP1B1 in the formation of 4-CEs may be of special significance in extrahepatic tissues, eg, breast and kidney, where the enzyme is mainly expressed. In support of this idea, 4-CEs were found to be carcinogenic in the hamster kidney[12] and were associated with increased risk of human breast cancer.[13] Liehr and Ricci[14] reported that 4-hydroxylation of E_2 may be an important marker for human breast cancer. CYP1B1 was indeed overexpressed in 92% of human breast cancer tissue specimens compared with normal tissue.[15] Therefore, CYP1B1 induction in target organs may lead to elevated levels of in situ synthesis of 4-CEs and, consequently, high risk of cancer initiation.

The 16α-hydroxylation reaction was catalyzed by CYP1A2, 1A1, and 3A4 to varying extent, with a lesser contribution from CYP1B1 (Fig 1). The present study and 3 previous reports[6-8]

suggest that recombinant CYP1A1 and 1A2 are the principle sources of E_2 16α-hydroxylation. These results are in conflict with in vivo results from human, cell culture, and animal models (for review see Safe[20]) showing that CYP1A1 and 1A2 by administration of exogenous agents (eg, indol 3-carbinol and epigallocatechin gallate) only result in an increase in 2-OHE_1 with no change or a modest decrease in 16α-OHE_1. This discrepancy between isolated enzyme studies and observations in human, animal, and cell culture may be explained by the contribution of other CYP isoenzyymes in the in vivo E_2(E_1) 16α-hydroxylation. For example, CYP3A4 is known to be the most abundent CYP isoenzyme in vivo (see above), and its contribution to 16α-hydroxylation may be greater than that of CYP1A1 and 1A2. Moreover, although CYP1A2, as shown ealier[7] and in the present report, may be responsible for 16α-hydroxylation of E_2, CYP3A4 is the major enzyme responsible for E_1 16α-hydroxylation with a V_{max} value (0.5 ± 0.2 nmol/min/nmol P450) and specific activity (approximately 0.26 nmol/min/nmol P450) that were significantly higher than those obtained for CYP1A1 and 1A2 (<0.1 and <0.03, respectively).[7] Various evidence suggested that 16α-OHE_2(E_1) can be involved in the carcinogenic potential of E_2.[16,17] For example, E_2 metabolized into 16α-OHE_1 was higher in breast cancer patients than in controls.[18] Moreover, 16α-OHE_1 stimulated the proliferation of MCF-7 human breast cancer cells.[19] In the present study, HLM exhibited significantly higher E_2 4-hydroxylation capacity than the 16α-hydroxylation activity (0.5 ± 0.06 *v* 0.3 ± 0.05 nmol/min/nmol P450; $P < .05$), an observation that was supported by various earlier reports for both E_2[6,7] and E_1.[7,11] In general, the predictive utility of E_2 hydroxy metabolites as biomarkers of cancer risk (eg, breast cancer) is an interesting but controversial subject, and evidence that supports and contradicts such a use has been evaluated recently.[20]

The differences in E_2 metabolism between hepatic (mediated mainly by CYP1A2 and 3A4) and extrahepatic (catalyzed by CYP1B1 and 1A1) tissues may contribute to estrogen-associated carcinogenesis in target organs. Evidence has accumulated indicating that various E_2 metabolites play a role in the tumorigenic process in which reactive intermediates are generated and can contribute to cancer initiation.[2] Information about the role of individual CYP in E_2 hydroxylation may be relevant to understanding the process of hormonal carcinogenesis both in liver and in extrahepatic tissues.

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