Association of CYP1A1 Polymorphisms with Differential Metabolic Activation of 17β-Estradiol and Estrone

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Abstract

Several epidemiologic studies associate certain *CYP1A1* genotypes, alone or in combination, with an increased risk of estrogen-related cancers. To answer the question of whether genotype-dependent activation of estrogens by CYP1A1 could be the underlying mechanism, we studied the hydroxylation activity of the most common allelic variants of human CYP1A1 towards both endogenously occurring estrogens, 17β-estradiol (E2) and estrone (E1). We expressed and purified CYP1A1.1 (wild-type), CYP1A1.2 ($Ile^{462}Val$), and CYP1A1.4 ($Thr^{461}Asn$) and did enzymatic assays of NADPH-dependent estrogen hydroxylation in reconstituted CYP1A1 systems. All CYP1A1 variants catalyzed the formation of 2-, 4-, 6α-, and 15α-hydroxylated estrogen metabolites from E2 and E1, yet with varying catalytic efficiency and distinct regiospecificity. Whereas the variant CYP1A1.2 ($Ile^{462}Val$) had a significant higher catalytic activity for all hydroxylation sites and both substrates, it was most pronounced for 2-hydroxylation. Catalytic efficiencies for the formation of the major metabolites, 2-OH-E2 and 2-OH-E1, by CYP1A1.2 were 5.7- and 12-fold higher, respectively, compared with the wild-type enzyme. The catalytic efficiencies for hydroxylations catalyzed by CYP1A1.4 were roughly comparable with those of the wild-type enzyme. Enzyme kinetics showed that the superior activity of CYP1A1.2 ($Ile^{462}Val$) is mainly caused by a higher V_{max}, whereas K_m values of all variants were similar. The data suggest that risk of estrogen-induced cancers and cardiovascular diseases might be—at least partially—determined by the *CYP1A1* genotype. (Cancer Res 2005; 65(7): 2972-8)

Introduction

CYP1A1 has been generally considered as one of the major cytochrome P450 (CYP) isoforms responsible for the 2-hydroxylation of 17β-estradiol (E2) and estrone (E1) in extrahepatic tissues including breast (1). This reaction leads to the formation of the catechol estrogens 2-hydroxy-E2 (2-OH-E2) and 2-hydroxy-E1 (2-OH-E1). Subsequent metabolism of catechol estrogens involves *O*-methylation by catechol-*O*-methyltransferase (COMT; ref. 2) and their conjugation by other phase II enzymes. Under conditions of inadequate protection of catechol estrogens by phase II enzymes, they can undergo oxidation to their semiquinone and quinone derivatives. The methylation product 2-methoxyestradiol (2-MeO-E2) exhibited antitumorigenic and antiangiogenic effects (3) and its endogenous formation may protect against estrogen-induced cancers in target organs (reviewed in ref. 4) and shows protective activities in the cardiovascular and renal systems (reviewed in refs. 5–7). In contrast to the potentially beneficial roles of the CYP1A1-COMT pathway, the CYP-mediated oxidation of catechol estrogens to semiquinones and quinones has been postulated to be an initiating/promoting factor in estrogen-induced carcinogenesis (reviewed in refs. 4, 8). The quinones are reactive metabolites capable of forming DNA adducts (9). DNA damage may also result from the generation of reactive oxygen species during redox cycling between quinones and semiquinones (10–12). Whereas quinones from 2-hydroxylated estrogens produce less harmful, stable DNA adducts, the quinones derived from 4-hydroxylated estrogens lead to depurinating adducts that may initiate carcinogenesis (9). 4-OH-E2 is a minor product of CYP1A1—but the major product of CYP1B1-catalyzed E2 hydroxylation (13). 4-OH-E2, but not 2-OH-E2, induced cancer in the Syrian hamster (14) and the incidence of adenocarcinoma in mice was much higher after treatment with 4-OH-E2 compared with 2-OH-E2 (15). Additionally, a faster rate of metabolism by COMT of 2-OH-E2, and a more rapid clearance of 2-OH-E2 *in vivo*, as well as the antiproliferative effect of 2-MeO-E2 resulting from methylation of 2-OH-E2, may contribute to differences in the carcinogenic potential of 2- and 4-OH-E2 (4). Providing a further mechanism that may contribute to the differential effects of the metabolites on carcinogenesis, 4-OH-E2 but not 2-OH-E2 was found to induce the expression of hypoxia-inducible factor 1α and vascular endothelial growth factor A in human ovarian carcinoma cells (16).

The human gene for CYP1A1 is polymorphic. Apart from the wild-type (*CYP1A1*1*), 10 alleles have been described in different populations; however, several are very rare and of unknown functional significance (see http://www.imm.ki.se/CYPalleles/cyp1A1.htm). The most common alleles resulting in amino acid substitutions are *CYP1A1*2* ($Ile^{462}Val$) and *CYP1A1*4* ($Thr^{461}Asn$). Several epidemiologic studies discuss an association of *CYP1A1* genotypes with an increased risk of certain types of cancer. Besides lung cancer (17, 18), these include cancers possibly related to CYP1A1-mediated estrogen activation such as breast cancer (19, 20), prostate cancer (21, 22), and ovarian cancer (23). Interestingly, when the human body burden of polychlorinated biphenyls, known as potent inducers of CYP1A1, was high, an increased risk of breast cancer associated with the presence of the *CYP1A1*2* allele was reported (24). However, note that the same biphenyls could also induce CYP1B1; thus, the association is likely to have a more complex basis. Because CYP1A1 metabolizes not only endogenous compounds such as estrogens but also a variety of xenobiotics, including polycyclic aromatic hydrocarbons (25), the CYP1A1 polymorphism might affect the risk of cancer by modifying the activation of estrogens and/or of environmental carcinogens.

In vitro studies done with the recombinant CYP1A1 variants did not reveal dramatic effects of the amino acid exchanges present in CYP1A1.2 and CYP1A1.4 on the metabolism of xenobiotics such as benzo(*a*)pyrene and ethoxyresorufin (26–28). These results

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suggested that the Ile[462]Val and Thr[461]Asn polymorphisms are probably not simply related to enhanced activation of environmental carcinogens. However, the effects of these amino acid exchanges on the function of CYP1A1 may be dependent on the substrate, and we speculated that the CYP1A1 variants might differ from the wild-type in terms of their activity and regioselectivity towards estrogens. To address this question, we expressed and purified recombinant CYP1A1.1 (wild-type), CYP1A1.2 (Ile[462]Val), and CYP1A1.4 (Thr[461]Asn) proteins and compared their capacity to metabolize the two endogenously occurring estrogens, E2 and E1.

Materials and Methods

Chemicals. E2, E1, 2-OH-E2, 4-OH-E2, 6α-OH-E2, estriol (16α-OH-E2), 4-OH-E1, and 16α-OH-E1 were purchased from Sigma (Deisenhofen, Germany). [4-^{14}C]E2 (specific activity, 1,665 MBq/mmol) and [4-^{14}C]E1 (specific activity, 1,898.1 MBq/mmol) were purchased from Perkin-Elmer Life Sciences (Boston, MA). The other reference compounds 15α-OH-E2, 2-OH-E1, and 15α-OH-E1 were obtained from Steraloids, Inc. (Newport, RI).

Expression, purification, reconstitution, and analysis of recombinant enzymes. The modified CYP1A1 cDNAs for expression of the CYP1A1 variants were constructed according to Chernogolov et al. (29). Enzymes were expressed in *Spodoptera frugiperda* (Sf9) insect cells as COOH-terminal His-tag proteins and purified by Ni-affinity chromatography to a specific content of about 11 nmol CYP per milligram of protein as previously described (29). Reduced CO-difference spectroscopy proved the expressed variants to be free of any inactive cytochrome P420; homogeneity was proven by SDS-PAGE. Human P450 reductase was expressed in Sf9 cells and purified to a specific catalytic activity of 18.2 units/mg protein according to Tamura et al. (30); homogeneity was confirmed by SDS-PAGE. Recombinant baculovirus for expression of P450 reductase was kindly provided by Dr. F.J. Gonzalez.

Reconstitution of enzymatic activity of CYP1A1 and P450 reductase was achieved by incubation with insect cell control microsomes as described recently (31). Control microsomes were prepared from HighFive insect cells (BTI-TN-5B1-4 cells from Invitrogen, Groningen, the Netherlands) which were free from baculovirus infection. They contained no CYP and no P450 reductase and exhibited no activity towards several substrates such as ethoxyresorufin, benzo(*a*)pyrene, arachidonic acid, and the estrogens studied here.

CYP content was measured by reduced CO-difference spectroscopy (32). Protein concentration was determined using the Coomassie Plus protein assay (Pierce, Rockford, IL). P450 reductase concentration was determined spectrally using an extinction coefficient of 21 $mmol/L^{-1}$ cm^{-1} (at 450 nm). P450 reductase activity was determined as NADPH-cytochrome *c* reductase activity (33).

Estrogen hydroxylation assays. For NADPH-mediated reactions, 20 pmol CYP1A1 were reconstituted with a 5-fold molar excess of recombinant human P450 reductase in 80 μg of control microsomes and added to 50 mmol/L Tris-HCl buffer (pH 7.5) containing 100 mmol/L NaCl and 2 mmol/L ascorbate. DMSO (2.5 μL) containing varying concentrations of E1 (or E2) were added and reactions were started after short preincubation (1 minute at 37°C) by addition of NADPH (final concentration, 1 mmol/L) to a final volume of 250 μL. Reactions proceeded at 37°C under gentle shaking in a water bath and were stopped by addition of 50 μL 1 N HCl after 20 minutes. Metabolites and substrate were extracted twice in 1 mL ethyl acetate. Extracts were combined and, after evaporation, reconstituted in 100 μL of methanol/water (80:20, v/v).

Substrate stock solutions were prepared from estrogen stocks (2.5 mmol/L in ethanol) as follows: "cold" substrate was mixed with [4-^{14}C]E2 (or [4-^{14}C]E1) corresponding to about 200,000 cpm in ethanol. The solvent was evaporated and estrogens dissolved in 2.5 μL of DMSO.

HPLC analysis. A HPLC system (LC-10Avp, Shimadzu, Japan) equipped with a radioactivity detector (LB509, Berthold, Germany) and a reversed-phase column Nucleosil 100-5C18HD (250 × 4 mm; Macherey-Nagel, Dueren, Germany) was used for separation and analysis of the metabolites. HPLC was done at a flow rate of 1 ml/min beginning with a 10-minute isocratic elution with 30% A (acetonitrile/0.5% acetic acid)/70% B (water/0.5% acetic acid). This was followed by a 10-minute linear gradient from 30% A/70% B to 60% A/40% B and a 15-minute gradient from 60% A/40% B to 100% B. Elution was complete after another 15 minutes of 100% B. Metabolites were identified by comparing their retention times with authentic standards.

Enzyme kinetics. Reaction kinetics were determined in duplicate with six different concentrations of E2 and E1, respectively. Data were fitted with nonlinear regression to determine the kinetic parameters (K_m and V_{max}) using Sigma Plot 2001 with the Enzyme Kinetics module (SPSS Science Software, Erkrath, Germany). Kinetic data represent mean ± SE as determined by nonlinear regression fitting.

Results

Wild-type and variant CYP1A1 catalyzed E2 and E1 hydroxylations at C-2, C-4, C-6, and C-15. Enzyme kinetics were analyzed and the resulting K_m and V_{max} values and catalytic efficiencies (V_{max}/K_m) for E2 and E1 hydroxylations are presented in Tables 1 and 2, respectively. Note that the rate of 15α-hydroxylation also possibly includes the rate of 7α-hydroxylation, because 15α- and 7α-products co-elute under our HPLC conditions. Small amounts of 7α-hydroxylated metabolites were also detected as minor products in previous studies with CYP1A1 (e.g., ref. 1). Comparison of total catalytic efficiencies of hydroxylation showed the CYP1A1.2 variant to be the most active catalyst. Its overall E2 hydroxylation efficiency (V_{max}/K_m) was more than 3-fold higher, and its E1 hydroxylation efficiency was more than 9-fold higher than those of wild-type CYP1A1 and CYP1A1.4. The differences in catalytic efficiencies are even more pronounced when certain regiospecific reactions are compared. 2-OH-E2 (E1) formation by CYP1A1.2 was nearly 6-fold (12-fold) greater than those by wild-type CYP1A1 and nearly 12-fold (17-fold) greater than those by CYP1A1.4 (Tables 1 and 2; Fig. 1). Interestingly, differences between variants were greatest for 2-hydroxlation, whereas efficiencies in 6α-hydroxylation are almost the same (Fig. 1).

Regioselectivities of the variants differed (Fig. 1). The main products of wild-type CYP1A1 and of CYP1A1.2 were 2-hydroxylated products, 2-OH-E2 and 2-OH-E1, followed by 15α-hydroxylated products. The major product of CYP1A1.4-catalyzed E2 hydroxylation was 6α-OH-E2, followed by 15α-OH-E2. However, E1 was transformed by this variant mainly to 2-OH-E1.

Differences in catalytic efficiencies in E2 hydroxylation in positions 2 and 4 were mainly caused by V_{max} values, whereas the K_m values of the different variants were almost the same (Table 1). This is most clearly shown by the Lineweaver-Burk plots; as an example, we show the formation of the major metabolite 2-OH-E2 (Fig. 2). For 6α- and 15α-hydroxylation, in contrast, both V_{max} and K_m were different, indicating a complex influence of the mutations.

E1 was most efficiently hydroxylated by the CYP1A1.2 variant (Table 2). Its higher V_{max} values and lower K_m values for hydroxylations in positions 2 and 4 indicate a higher affinity of CYP1A1.2 for E1 binding. Note, that this property of CYP1A1.2 could be important for the human mammary epithelium because E1 is likely to be the more abundant primary estrogen available for 2- and 4-hydroxylation in breast parenchyma (34). For 6α- and 15α-hydroxylation, K_m values of CYP1A1.2 were lower, whereas V_{max} values were similar for all three variants. Interestingly, for both estrogenic substrates, all the K_m values for 2- and 4-hydroxylation were considerably smaller than those for 6α- and 15α-hydroxylation (2- to 10-fold).

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Table 1. NADPH-supported E2 hydroxylation activities of human CYP1A1 variants

	2-OH-estradiol			4-OH-estradiol			6α-OH-estradiol			15α-OH-estradiol		
	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m
CYP1A1.1	0.6 ± 0.1	9.5 ± 4.6	0.063	0.02 ± 0.002	11.8 ± 4.8	0.002	3.6 ± 0.8	79 ± 26	0.046	0.9 ± 0.1	19.6 ± 7.3	0.046
CYP1A1.2	3.3 ± 0.3	9.2 ± 2.3	0.359	0.08 ± 0.02	12.9 ± 6.5	0.006	1.3 ± 0.2	21.7 ± 6.2	0.060	9.3 ± 0.6	86 ± 8.0	0.108
CYP1A1.4	0.3 ± 0.03	9.8 ± 3.2	0.031	0.02 ± 0.002	12.3 ± 3.6	0.002	0.9 ± 0.1	18.6 ± 2.9	0.048	3.7 ± 0.6	89 ± 20	0.042

NOTE: NADPH-supported reactions were done in a reconstituted system consisting of purified human CYP1A1 variant, purified human P450 reductase and insect control microsomes. The final concentrations for determination of activities were 80 nmol/L, 400 nmol/L, and 320 μg/mL for CYP1A1, P450 reductase, and control microsomes, respectively. Formation of hydroxylated products was determined using HPLC and kinetic analyses were done by nonlinear regression as described under Materials and Methods.
V_{max} data are given in picomoles of product per minute/picomoles of CYP1A1, K_m data are in micromoles per liter, and catalytic efficiencies V_{max}/K_m are expressed in liter/micromoles per minute. Data represent mean ± SE as determined by nonlinear regression using software described under Materials and Methods.

To illustrate the marked differences in regiospecificity of the different hydroxylations, the relative ratios 2-OH/X-OH (X: 4, 15α, and 6α) were calculated (Table 3). As can be seen from the data, ratios differ by a factor of 2 to 10 between the variants. Clearly, CYP1A1.2 is the most efficient 2-hydroxylase with 2-OH/4-OH ratios of about 60 and 10 for E2 and E1 hydroxylation, respectively.

Discussion

In the present *in vitro* study, we characterized the metabolite profiles which resulted from NADPH-dependent oxidation of E2 and E1 by three of the most common allelic variants of human CYP1A1. Our results show that all variants are actively involved in the oxidative metabolism of E2 and E1, yet with a varying degree of catalytic efficiency and distinct regiospecificity. Most remarkably, catalytic efficiencies of CYP1A1.2 (Ile^{462}Val) for the formation of the major metabolites, 2-OH-E2 and 2-OH-E1, were 5.7- and 12-fold, respectively, higher than those of the wild-type enzyme, clearly demonstrating that this polymorphism is functionally significant.

Comparison with earlier wild-type data. With regard to wild-type CYP1A1, our findings with reconstituted purified CYP1A1 are in good agreement with several earlier investigations using natural microsomal as well as selectively expressed CYP1A1 (see ref. 1 and citations therein). These studies revealed that CYP1A1 catalyzed 2-, 4-, 6α-, and 15α-hydroxylations of estrogens, and that CYP1A1 exhibited highest catalytic activity for 2-hydroxylation. However, small amounts of other metabolites were also identified, e.g., 6β-OH-, 7α-OH-, and 16α-OH-E2 and 7α-OH- and 16α-OH-E1. Lee et al. (1) reported that ratios of 2- to 4-hydroxylation of E2 were higher than those for E1, a finding which is also in good agreement with our results. For wild-type CYP1A1, in our study and in the studies of others, E2 turned out to be the better substrate. Smaller differences in activity and the metabolic profile reported by the different groups certainly are due to the use of different expression systems. In this relation, one should mention that the use of selectively expressed or reconstituted purified enzymes certainly makes the experiments much easier and unambiguous; however, one should be aware that catalytic properties can vary depending on the expression system and may differ from those under *in vivo* conditions.

Differential formation of catechol estrogens by CYP1A1 variants. Enzyme kinetics showed that the superior activity of CYP1A1.2 is mainly caused by a higher V_{max}, whereas K_m values of all variants were similar—at least for 2- and 4-hydroxylations. This leads to the conclusion that variant-specific interactions with P450 reductase including differential electron transfer play a decisive role in NADPH-dependent oxidations of estrogens. Homology modeling of the location of the mutated residues within the three-dimensional structure of CYP1A1 supports such a hypothesis. A homology model of human CYP1A1, based on the CYP2C5 crystallographic template, locates the mutated residues Val^{462} and Asn^{461} on the proximal face of the heme group (adjacent to the thiolate ligand; ref. 35), a region where the interaction with P450 reductase is generally assumed to take place (36). The interpretation is further supported by preliminary studies of hydroperoxide-mediated CYP1A1 hydroxylations of estrogens which showed that differences in catalytic efficiencies between the variants disappear or at least are much less pronounced when cumene hydroperoxide was used as cofactor (and P450 reductase was absent).[4] Providing a further argument for this explanation, no comparable striking differences between CYP1A1 variants were found in an earlier study of cumene hydroperoxide-supported hydroxylation of progesterone and testosterone using microsomal CYP1A1 systems devoid of any P450 reductase (37). However, final evidence should be obtained by direct comparison of NADPH- and CuOOH-dependent hydroxylation reactions catalyzed by the different CYP1A1 variants which are currently under investigation.

Two active site-binding modes for estrogens in CYP1A1 variants. Lee et al. (1) studied 15 human CYP isoforms and found that CYP1A1 was the only one with a distinctly high catalytic activity for the 15α-hydroxylation of estrogens. Our study did not only confirm this result for the wild-type enzyme but showed that this also holds for the variant CYP1A1 enzymes. This remarkable hydroxylation activity at C-15 towards both estrogenic substrates and at C-6 at least towards E2, suggests two different binding positions at the active site of CYP1A1. Both estrogenic substrates can probably enter the enzyme and position either ring A or, alternatively, ring D proximal to the heme; this results in 2 and 4 hydroxylation and in hydroxylation at C-15 and C-6, respectively.

[4] D. Schwarz, unpublished results.

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Table 2. NADPH-supported E1 hydroxylation activities of human CYP1A1 variants

	2-OH-estrone			4-OH-estrone			6α-OH-estrone			15α-OH-estrone		
	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m
CYP1A1.1	0.4 ± 0.03	12.2 ± 1.2	0.033	0.1 ± 0.03	22.6 ± 11.7	0.004	0.2 ± 0.1	86 ± 40	0.002	1.2 ± 0.1	85 ± 12	0.014
CYP1A1.2	2.5 ± 0.3	6.3 ± 2.0	0.397	0.5 ± 0.1	12.7 ± 1.9	0.039	0.2 ± 0.02	27.3 ± 4.6	0.008	1.0 ± 0.1	20.5 ± 5.8	0.049
CYP1A1.4	0.3 ± 0.03	13.1 ± 3.0	0.023	0.1 ± 0.03	24.4 ± 9.8	0.004	0.1 ± 0.04	45 ± 29	0.002	1.0 ± 0.2	46 ± 21	0.022

NOTE: For conditions of experiments and analysis see legend of Table 1. V_{max} data are given in picomoles of product per minute/picomoles of CYP1A1, K_m data are in micromoles per liter, and catalytic efficiencies V_{max}/K_m are expressed in liter/micromoles per minute. Data represent mean ± SE as determined by nonlinear regression using software described under Materials and Methods.

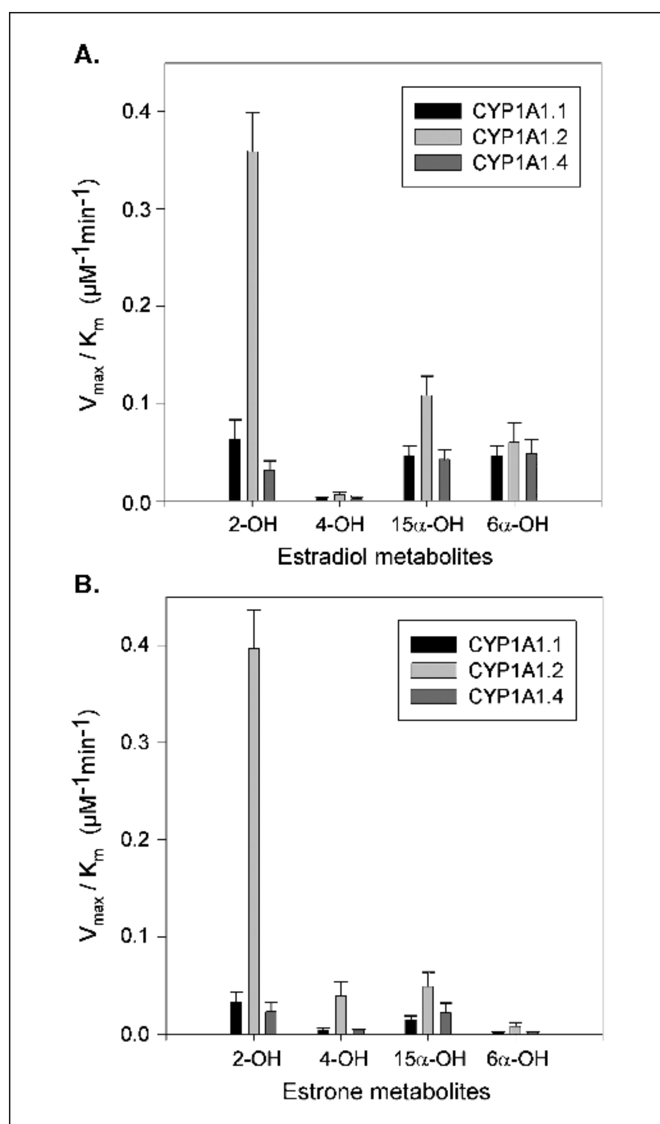

Figure 1. Comparison of catalytic efficiencies of the different CYP1A1 variants in E2 (*A, top*) and E1 (*B, bottom*) hydroxylation supported by NADPH and NADPH-cytochrome P450 reductase. 2-OH, 4-OH, 15α-OH, and 6α-OH (*columns*) designate the catalytic efficiencies V_{max}/K_m (in units of L/µmol min^{-1}) of 2-, 4-, 15α-, and 6α-hydroxylation, respectively. Catalytic efficiencies are means based on duplicate experiments; *bars,* SD.

Consequently, this hypothesis suggests that both E2 and E1 bind to two locations with antiparallel orientation of the molecules' long axes. Support for this hypothesis comes from the measured K_m values. For both estrogenic substrates and all variants, the K_m values for 2- and 4-hydroxylation were considerably smaller than those for 6α- and 15α-hydroxylation, indicating a higher binding affinity of both substrates in the position optimal for 2- and 4-hydroxylation. It is conceivable that in both positions, OH-groups (3-OH and 17-OH, respectively) support the substrate's docking into the active site. The rates for 15α- and 6α-hydroxylation of E2 were several-fold higher than those of E1, possibly because of the differential effects that the 17-OH-group (for E2) and 17-keto-group (for E1) have on these hydroxylation reactions. The hypothesis of two (or multiple) binding orientations for the two estrogens is consistent with recent evidence for multiple substrate binding modes from X-ray analysis of CYP2C5-substrate complexes (38) and with the observation that CYPs can hydroxylate a substrate at multiple distal positions (37, 39).

Impact on susceptibility to cancer and cardiovascular diseases. CYP1A1 is a versatile monooxygenase that is involved in the metabolism of environmental pollutants such as polycyclic aromatic hydrocarbons and endogenous substances such as estrogens. Since the conversion of both types of substrates has the potential to yield products with mutagenic and carcinogenic properties, polymorphisms in the human *CYP1A1* gene have been frequently discussed as risk factors for the development of cancer. However, one of the main difficulties in understanding the clinical relevance of the CYP1A1 polymorphism has been the fact that it remained largely unclear whether or not the mutations in the *CYP1A1* gene are indeed functionally significant. Earlier studies indicated different inducibilities among the allelic variants (40, 41), however, the generally observed huge interindividual variation in CYP1A1 expression and inducibility is probably more associated with regulatory factors than polymorphisms in the *CYP1A1* gene itself (42, 43). The present study clearly shows that the $Ile^{462}Val$ substitution is functionally significant and that its effect is dependent on the substrate. Taking into account previous results from different laboratories including ours (26–28), which showed no significantly changed ethoxyresorufin-*O*-dealkylase and benzo(*a*)pyrene oxidation activities of all CYP1A1 variants, the superior catalytic efficiency of the CYP1A1.2 ($Ile^{462}Val$) variant in the formation of 2-hydroxylated metabolites from the most important physiologic estrogens, E2 and E1, was probably our study's main result.

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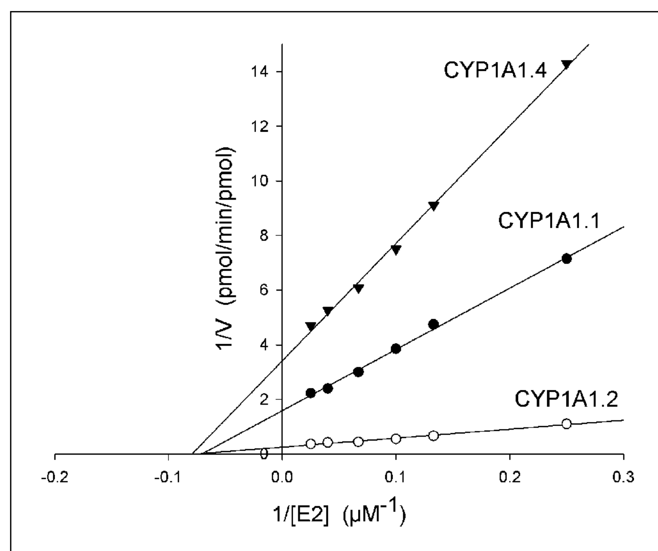

Figure 2. Lineweaver-Burk plots of 2-hydroxylation of E2 by CYP1A1 allelic variants. Activities V were measured at 4, 7.5, 10, 25, and 40 μmol/L substrate (E2) concentration. Data represent means of duplicate assays.

This new finding may help to identify the specific conditions under which the *CYP1A1*2* (Ile^{462}Val) allele actually contributes to an increased cancer risk. Almost all genes involved in estrogen metabolism are known to be polymorphic and only certain combinations of different polymorphic genes can be expected to have a significant effect on cancer susceptibility (reviewed in ref. 44). Considering the metabolic fate of the 2-hydroxyestrogens produced by CYP1A1, a particular risk may arise if *CYP1A1*2* (Ile^{462}Val) occurs simultaneously with both (*a*) a "low-activity" *COMT* allele (*COMT-L,* 108/158Met substitution), and/or (*b*) a defect in one of the glutathione-S-transferase (*GST*) genes required to detoxify the quinones by conjugation with glutathione (e.g., in carriers of the *GSTM1* and/or *GSTT1* null genotypes). In theory, this combination would cause an accumulation of 2-hydroxylated estrogens and eventually of the corresponding genotoxic semiquinones and quinones. Simultaneously, the production of methoxyestrogens may be reduced, which was recently shown to exert feedback inhibition on CYP1A1-catalyzed estrogen metabolism (45) and to have tumor-growth inhibiting properties (see discussion below). The methoxyestrogens also act as inhibitors of CYP1B1-catalyzed estrogen-4-hydroxylation (45) leading to 4-OH-derived semiquinones and quinones with their known higher potential of carcinogenicity (compare the discussion under Introduction).

On the other hand, it is tempting to speculate that the *CYP1A1*2* (Ile^{462}Val) allele could even play a protective role, if combined with a "high-activity" *COMT* allele (*COMT-H,* 108/158Val substitution). Under these conditions, an enhanced production of methoxyestrogens may be predicted in those extrahepatic organs and tissues where CYP1A1 is known to be expressed and/or highly inducible including breast, lung, and the vasculature. As shown for 2-MeO-E2, methoxyestrogens are powerful inhibitors of the proliferation of several cell types and have strong antiangiogenic and anticancerogenic properties (4). Therefore, 2-MeO-E2 is considered as a promising anticancer agent and its potential is tested in first clinical trials (46–48). Moreover, it is interesting to note that the CYP1A1-COMT pathway may be also relevant for cardiovascular diseases. 2-MeO-E2 shows protective activities in the cardiovascular and renal systems by inhibiting the proliferation of vascular smooth muscle cells and of glomerular mesangial cells (5–7). Thus, CYP1A1-COMT-dependent production of 2-MeO-E2 in these cell types may counteract vascular and glomerular remodeling processes involved in the pathophysiology of hypertension and renal damage.

Taken together, from a biochemical point of view, the extraordinary high estrogen-2-hydroxylase activity of the CYP1A1.2 (Ile^{562}Val) variant may either increase or reduce the susceptibility to cancer depending on its combination with other genetic and environmental risk factors. The results of epidemiologic studies are indeed controversial and obviously dependent on the ethnicity, gender, and lifestyle of the study group (reviewed in refs. 44, 49, 50). For example, an increased breast cancer risk associated with the *CYP1A1*2* (Ile^{462}Val) allele and smoking was observed in Caucasian women (51, 52), whereas Chinese and Japanese women carrying the *CYP1A1*2* (Ile^{462}Val) allele have no or a significantly reduced risk of breast cancer as compared with carriers of the wild-type allele (53, 54). Interestingly, ethnic differences in the frequency of *CYP1A1* alleles are considerable: the *CYP1A1*2* (Ile^{462}Val) allele occurs with a much higher frequency in Asians (18-33%) than in Caucasians (2-10%) and the *COMT-L* allele is less frequent in Asians (24-33%) compared with Caucasians (49-63%; e.g., ref. 49). Of course, besides *CYP1A1*, *COMT*, and *GST*, other polymorphic genes may contribute to either activation or inactivation of the catechol estrogens or their metabolites, e.g., CYP1B1, another member of the CYP1 family. Several CYP1B1 variants have about 2- to 3-fold higher catalytic efficiency than the wild-type allele with respect to the 4-hydroxylation of E2, whereas no data were reported for E1 (see ref. 13; and discussion in Introduction). Recent epidemiologic studies increasingly consider the whole range of polymorphic genes involved in estrogen biosynthesis and metabolism and it remains to be seen whether the combinations discussed before or additional gene-gene interactions are actually decisive in determining

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Table 3. Ratios of formation of metabolites in estrogen hydroxylation catalyzed by human CYP1A1 variants

	2-OH/4-OH		2-OH/15α-OH		2-OH/6α-OH	
	E2	E1	E2	E1	E2	E1
CYP1A1.1	31.5	8.3	1.4	2.4	1.4	16.5
CYP1A1.2	59.8	10.2	3.3	8.1	6.0	50.0
CYP1A1.4	15.5	5.8	0.7	1.2	0.6	11.5

NOTE: Ratios were calculated based on catalytic efficiencies V_{max}/K_m. For conditions of experiments and determination, see legend of Table 1.

whether carriers of the *CYP1A1*2* (Ile[462]Val) allele are at increased or reduced risk of cancer.

Conclusions. The detailed analysis of CYP1A1-dependent metabolism of physiologic estrogens, E2 and E1, showed considerable differences in the metabolite profiles and in the catalytic efficiencies of the CYP1A1 variants tested. This might enhance our understanding of factors that influence the metabolism of estrogens and its metabolites, particularly the function of CYP1A1, in different target cells. Our data suggest that CYP1A1 genotype-dependent variation in estrogen metabolism, alone or in combination, might explain a portion of an individual's susceptibility towards estrogen-induced cancers and cardiovascular diseases.

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