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Cytochrome P450 isoforms catalyze formation of catechol estrogen quinones that react with DNA

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Abstract

Accumulating evidence suggests that specific metabolites of estrogens, namely, catechol estrogen quinones, react with DNA to form adducts and generate apurinic sites, which can lead to the mutations that induce breast cancer. Oxidation of estradiol (E_2) produces 2 catechol estrogens, 4-hydroxyestradiol (4-OHE_2) and 2-OHE_2 among the major metabolites. These, in turn, are oxidized to the quinones, E_2-3, 4-quinone (E_2-3,4-Q) and E_2-2,3-Q, which can react with DNA. Oxidation of E_2 to 2-OHE_2 is mainly catalyzed by cytochrome P450 (CYP) 1A1, and CYP3A4, whereas oxidation of E_2 to 4-OHE_2 in extrahepatic tissues is mainly catalyzed by CYP1B1 as well as some CYP3As. The potential involvement of CYP isoforms in the further oxidation of catechols to semiquinones and quinones has, however, not been investigated in detail. In this project, to identify the potential function of various CYPs in oxidizing catechol estrogens to quinones, we used different recombinant human CYP isoforms, namely, CYP1A1, CYP1B1, and CYP3A4, with the scope of oxidizing the catechol estrogens 2-OHE_2 and 4-OHE_2 to their respective estrogen quinones, which then reacted with DNA. The depurinating adducts 2-OHE_2-6-N3Ade, 4-OHE_2-1-N3Ade, and 4-OHE_2-1-N7Gua were observed in the respective reaction systems by ultraperformance liquid chromatography/tandem mass spectrometry. Furthermore, more than 100-fold higher levels of estrogen-glutathione (GSH) conjugates were detected in the reactions. Glutathione conjugates were observed, in much smaller amounts, when control microsomes were used. Depurinating adducts, as well as GSH conjugates, were obtained when E_2-3,4-Q was incubated with CYP1B1 or control microsomes in a 30-minute reaction, further demonstrating that GSH is present in these recombinant enzyme preparations. These experiments demonstrated that CYP1A1, CYP1B1, and CYP3A4 are able to oxidize catechol estrogens to their respective quinones, which can further react with GSH, protein, and DNA, the last resulting in depurinating adducts that can lead to mutagenesis.

1. Introduction

The estrogens estrone (E_1) and estradiol (E_2) have been implicated in the development of breast cancer by simultaneously causing DNA damage via their genotoxic catechol estrogen quinones (CE-Qs) and stimulating cell proliferation and gene expression via the estrogen receptor [1-4]. Catechol estrogens (CEs), namely, 2-CE [2-$OHE_1(E_2)$] and 4-CE [4-$OHE_1(E_2)$], are produced in a series of linked oxidations of E_2 or E_1, catalyzed by phase I cytochrome P450 (CYP) enzymes [5,6]. These enzymes, along with other oxidation enzymes such as lactoperoxidase, tyrosinase, and prostaglandin H synthase, are postulated to further oxidize the CEs to reactive semiquinones and quinones. The electrophilic CE-Qs react directly with DNA to form predominantly depurinating adducts that generate apurinic sites [4]. It has been shown that a large induction of apurinic sites in DNA by CE-Q is highly mutagenic in both mouse skin and rat mammary gland [3,7].

The main evidence for the genotoxicity of the oxidative estrogen metabolism pathway comes from animal models in which the administration of E_2 or 4-OHE_2 induces renal cancer in male Syrian hamsters [8,9]; and E_2, 2-OHE_2, and 4-OHE_2 induce endometrial cancer in CD-1 mice [10]. Intramammillary treatment of ACI rat mammary glands with E_2-3,4-Q induced A → G mutations in the Harvey-*ras* reporter gene within 6 hours [7]; similar mutations were observed in SENCAR mouse skin within 6 hours of topical

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Table 1
UPLC/MS/MS parameters for estrogen-DNA adducts and estrogen GSH conjugates

Analyte	Parent ion (m/z)	Daughter ion (m/z)	Capillary (kV)	Cone (V)	Collision (eV)	RT (min)	LOD (pmol/mL)	LOQ (pmol/mL)
	[M + H]							
2-OHE_2-1-SG	594	318.9	3.0	30	21	1.72	0.5	1.5
2-OHE_2-4-SG	594	318.9	3.0	30	21	2.14	0.3	1.0
2-OHE_2-1+4-NAcCys	450.2	162	3.0	30	14	4.10	0.5	1.7
2-OHE_2-1+4-Cys	408	319	3.0	30	16	1.72	4.4	13.5
4-OHE_2-2-SG	594	318.9	5.0	35	20	2.23	1.1	3.4
4-OHE_2-2-NAcCys	450.2	162	3.5	35	17	5.61	2.7	8.6
4-OHE_2-2-Cys	408	319	3.5	30	14	2.04	0.2	0.3
2-OHE_2-6-N3Ade	422.2	136	3.0	25	11	1.22	0.5	1.4
2-OHE_1-6-N3Ade	420	135.9	3.0	25	10	1.40	0.2	0.7
4-OHE_2-1-N3Ade	422.3	135.9	3.0	55	44	1.54	5.5	17.0
4-OHE_2-1-N7Gua	438.1	152.2	3.0	60	45	1.68	2.7	8.3
2-OH-3-OCH_3E_1	301.1	136.9	3.0	30	18	8.86	12.7	38.6
	[M − H]							
4-OHE_2-d_5	292	163	4.0	65	46	5.94	34.1	NA

RT indicates retention time; LOD, limit of detection; LOQ, limit of quantification; NA, not available.

treatment with E_2-3,4-Q [3]. In addition, 4-OHE_2, but not 2-OHE_2, was found to be mutagenic in Big Blue rat 2 embryonic cells, with significantly higher fractions of A → G mutations observed in mutants than in controls [11].

Direct experimental evidence for each step of the pathway and the underlying mechanism of enzyme kinetics is limited to studies of the CYP-mediated conversion of E_2 to 2-OHE_2 and 4-OHE_2 [5,6,12] and the subsequent conversion to the respective glutathione (GSH)–estrogen conjugates, estrogen-DNA adducts [4,13-17], or the methoxy estrogens by catechol-*O*-methyltransferase [18]. Several studies have shown the formation of CE-DNA adducts when CEs are activated by liver microsomes, as well as lactoperoxidase, tyrosinase, horseradish peroxidase, or prostaglandin H synthase [17,19,20].

Rat microsomal CYP1A family isoforms induced by β-naphthoflavone were reported to catalyze most efficiently the oxidation of estrogenic hydroquinones to the corres2-ponding quinones [21]. In these studies, formation of the quinones was detected by increases in the UV absorbance of the reaction mixtures. Thus, more direct evidence for the involvement of CYPs in oxidizing CE to CE-Q and subsequent formation of DNA adducts is warranted.

Several human CYPs have been characterized [22,23]. The highest concentrations of most CYPs occur in the liver, with the exception of CYP1B1, whereas lower concentrations of many CYPs are expressed in extrahepatic tissues. CYP1A1 and CYP1B1 are expressed or inducible in human breast epithelial cells [24]. Breast tissue from women with breast cancer has higher levels of 4-CE and CE-3,4-Q than breast tissue from women without breast cancer [25]. The metabolic oxidation of CE, the precursor to the ultimate carcinogenic CE-Q, has not been fully characterized. As mentioned above, CYP1A1, CYP1B1, and CYP3As are primarily responsible for the formation of CE. High levels of CYP1A1, CYP1B1, and CYP3As have been found in breast and prostate tissues [26-28], especially more CYP1B1 in adjacent nontumor tissue than in tumor tissue [27]. It is also important to know what role CYPs play in the conversion of CE to CE-Q.

To help dissect the role(s) of CYPs in estrogen-initiated cancer from a metabolic perspective, CYP1A1, CYP1B1, and CYP3A4 were examined for their ability to oxidize CE to CE-Q, which reacted with DNA to form depurinating adducts in vitro. The reaction products were analyzed by ultraperformance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS).

2. Materials and methods

2.1. Chemicals

4-OHE_2, 4-OHE_2-d_5, 2-OHE_2, $E_1(E_2)$-3,4-Q, 4-OHE_1 (E_2)-1-N7Gua, 4-$OHE_1(E_2)$-1-N3Ade, 2-$OHE_1(E_2)$-6-Ade, and estrogen conjugates were synthesized and purified in our laboratory [17,19,29,30]. Calf thymus DNA was purchased from USB (Cleveland, OH). Baculovirus-insect cells expressing CYP1A1, CYP1B1, CYP3A4, or control enzymes were purchased from BD Gentest (Bedford, MA). Other chemicals were purchased from Sigma-Aldrich (St Louis, MO).

2.2. Cytochrome P450–catalyzed oxidation of CE in the presence or absence of DNA

2-OHE_2 or 4-OHE_2 (0.87 mmol/L, delivered in 50 μL of dimethyl sulfoxide) was incubated with CYP1A1, CYP1B1, or CYP3A4 in the presence of 0.6 mmol/L reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) with or without calf thymus DNA (3 mmol/L DNA-P) in 0.067 mol/L Na-K phosphate (pH 7.0) in a total volume of 2.5 mL at 37°C for 30 minutes or 10 hours as indicated in the respective tables. The reaction was terminated with

2 volumes of ethanol and the precipitated DNA was removed by centrifugation. The supernatant from the mixture was condensed and reconstituted in 50% methanol in water. Samples were filtered through 10 000 molecular weight cutoff filters before analysis on UPLC/MS/MS. For the study of CE-3,4-Q, NADPH was eliminated and the other conditions were the same as above. Control reactions were conducted under multiple conditions, including without enzyme, without DNA, without cofactor, or in the presence of control enzyme. 2-OH-3-OCH_3E_1 was added right after termination of reactions to monitor compound recovery in sample processing. The results were obtained by averaging the data from 3 replicate experiments.

2.3. UPLC/MS/MS analysis

Ultraperformance liquid chromatography was performed by using a Waters (Manchester, UK) Acquity UPLC system, equipped with an autosampler and a UV detector. The chromatography was performed on a Waters Acquity C_{18} 1.7-μm (1.0 × 100 mm) column. Ten microliters of sample was injected into the system. The analytical gradient started with 80% A (water with 0.1% formic acid) and 20% B (acetonitrile with 0.1% formic acid) at a flow rate of 150 μL/min. Then B was linearly increased to 21% in 4 minutes. Buffer B was further linearly increased to 55% in the next 6 minutes. The gradient was returned to the starting proportion at 11 minutes and equilibrated for another 4 minutes before injection of the next sample.

The Acquity UPLC system was connected to a Micromass QuattroMicro triple quadrupole mass spectrometer (Waters). The UPLC/MS/MS system was controlled by MassLynx software V4.0 SP4 (QuanLynx, Waters). Adducts, conjugates, and metabolites were analyzed online by electrospray ionization in positive mode and the standard 4-OHE_2-d_5 in negative mode. The desolvation temperature was set at 200°C with nitrogen flow of 400 L/h, source temperature set at 100°C, and capillary voltage set at 3000 V. Cone gas flow was set at 60 L/h with argon used as collision gas. Tandem mass spectrometry conditions were optimized for each compound by using pure standard, and multiple reaction monitoring was used in quantification, whereas both parent and daughter ions were used to detect compounds. For quantification, the responses of sample ion peaks with the standards (standard curve) were compared using QuanLynx. The precision and accuracy of the UPLC/MS/MS method were determined and will be reported together with other parameters in a separate article. The calculated limit of detection (LOD = 3 × SD/slope) and limit of quantification (LOQ = 10 × SD/slope) are listed in Table 1, along with collision energy and other parameters for each compound. To ensure the quality of analysis, a single concentration of 4-OHE_2-d_5, prepared in our laboratory with substitution of [1,2,16,16,17α-d_5]-4-OHE_2, was added to each sample before UPLC/MS/MS analysis. Reported data were adjusted for the deviation of detected 4-OHE_2-d_5 in each sample from standard that was dissolved in the sample buffer. Data were further adjusted for extraction efficiency by calculating recoveries of 2-OH-3-OCH_3E_1 against its standard curve. Data were the average of triplicate experiments and reported as average ± SD.

3. Results and discussion

Catechol estrogen quinones have been postulated to play a key role in the oxidative estrogen metabolism pathway. Reactive oxygen species formed during redox cycling of estrogen semiquinones/quinones are capable of causing oxidative DNA damage [31], whereas the electrophilic CE-Qs themselves readily react with a variety of physiologic compounds, ranging from amino acids, such as lysine and cysteine, to proteins and DNA [17,20,29,30]. Because CE-Qs are too reactive to be reliably quantified, stable conjugates, such as estrogen-GSH conjugates, have been used as surrogate markers for the presence of CE-Q [25,32]. Because of the important role of estrogen-DNA adducts in carcinogenesis, we directly analyzed formation of depurinating DNA adducts in vitro in the presence of calf thymus DNA to determine whether specific CYP isoforms can oxidize CEs to CE-Qs. 4-OHE_2-1-N3Ade and 4-OHE_2-1-N7Gua were analyzed by UPLC/MS/MS (Fig. 1A and B).

The 4-OHE_2-1-N7Gua adduct depurinates with a half-life of approximately 3 hours and reaches a plateau at 10 hours, whereas the 4-OHE_2-1-N3Ade depurinates instantaneously [20]. Therefore, depurinating DNA adducts were analyzed after incubation of CE with DNA and CYP for 10 hours (Table 2). Serial concentrations of 4-OHE_2 from 0 to 0.87 mmol/L, including 0.087 mmol/L, were previously reacted with DNA in the presence of tyrosinase [20]. The 0.87 mmol/L concentration of 4-OHE_2 clearly saturated the enzyme and was chosen for this study. Data were normalized based on total protein and on micromoles of CYP. Blanks that contained no enzyme protein are not shown in the table. As expected, with 4-OHE_2 as substrate, both 4-OHE_2-1-N3Ade and 4-OHE_2-1-N7Gua were formed (Table 2). Comparing CYP1B1 to CYP3A4, we found little difference in the amounts of the two 4-OHE_2 adducts formed. The amounts of these adducts were much higher, however, in the presence of either CYP1B1 or CYP3A4 than in the presence of CYP1A1. With 2-OHE_2 as the substrate, 2-OHE_2-6-N3Ade was formed and a minor amount of 2-OHE_1-6-N3Ade was also detected (Table 2). Formation of 2-OHE_2-6-N3Ade was slightly higher in the presence of CYP3A4 than CYP1A1 or CYP1B1. Formation of all 3 adducts was at least 10 times higher than in the presence of control microsomes, which have no detectable CYP activity.

In addition to the depurinating adducts, we observed formation of GSH and *N*-acetylcysteine (NAcCys) conjugates (Fig. 1C and D). NAcCys conjugates are breakdown products from GSH conjugates, formed by the mercapturic

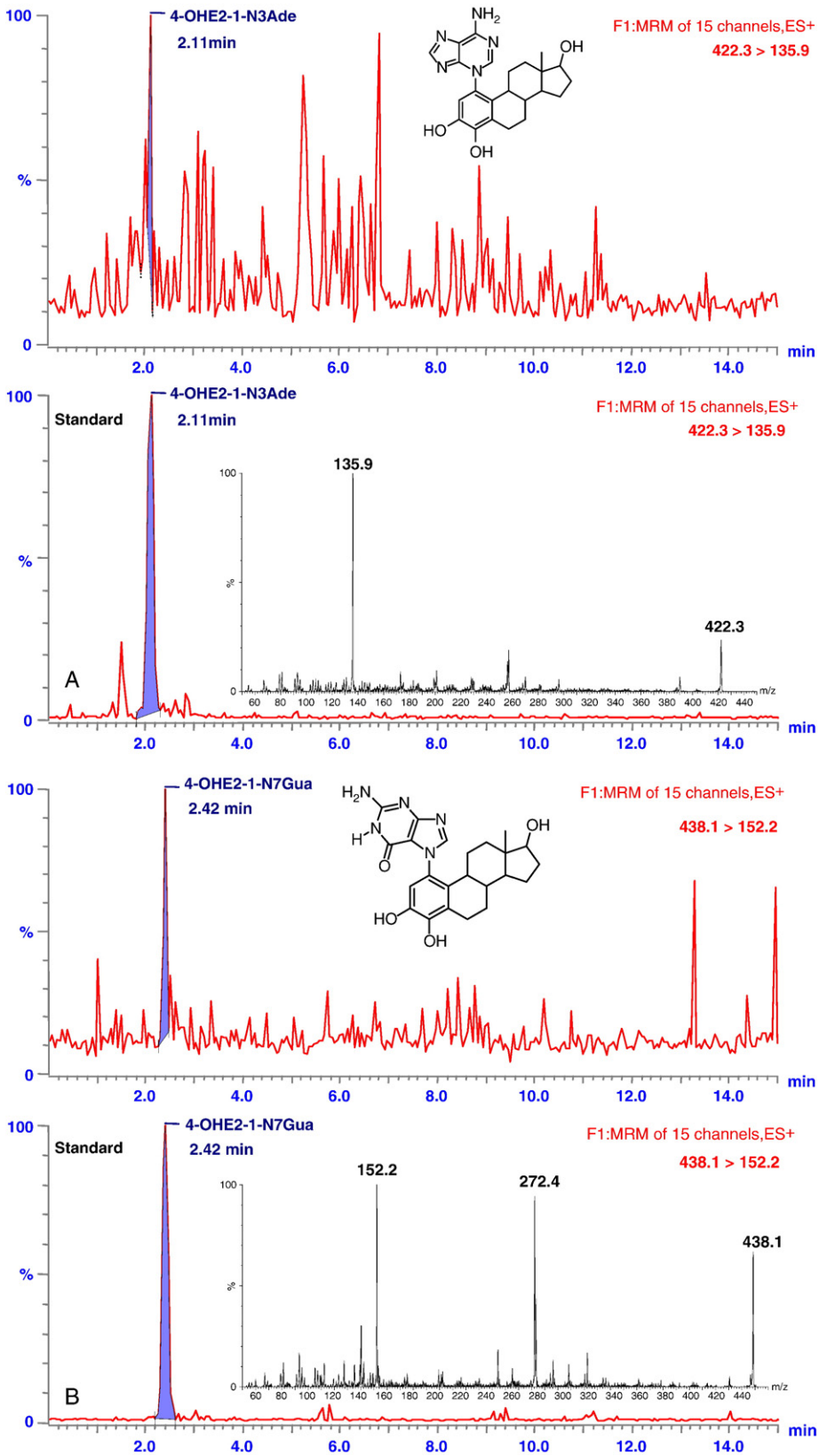

Fig. 1. Representative UPLC/MS/MS chromatograms of depurinating DNA adducts and thiol conjugates formed by incubation of catechol estrogens with recombinant human CYP and DNA. Chromatography and fragmentation of (A) 4-OHE_2-1-N3Ade, (B) 4-OHE_2-1-N7Gua, (C) 4-OHE_2-2-SG, and (D) 4-OHE_2-2-NAcCys.

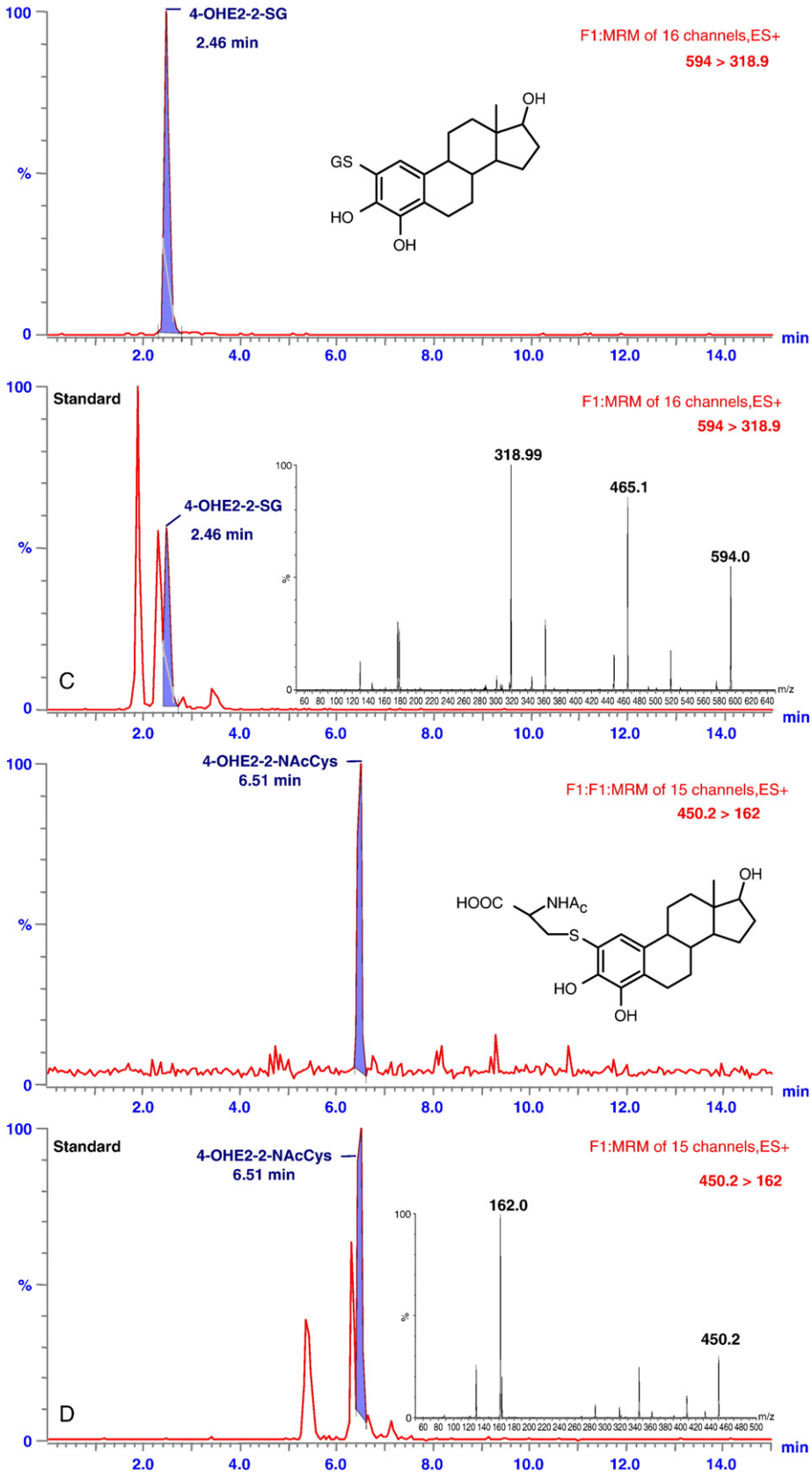

Fig. 1 (continued).

Table 2
Oxidation of 2-OHE_2 and 4-OHE_2 by CYP isoforms as measured by formation of conjugates and depurinating DNA adducts during 10 hours of incubation

	2-OHE_2			
Enzyme	Picomoles per milligram of protein (picomoles per micromole of CYP)			
	2-OHE_2-6-N3Ade	2-OHE_1-6-N3Ade	2-OHE_2-1-SG	2-OHE_2-1 + 4-NAcCys
CYP1A1	4.1 ± 1.9 (39 ± 18)	0.2 ± 0.1 (1.9 ± 1.0)	1083 ± 145 (10310 ± 1380)	22.5 ± 6.5 (214 ± 62)
CYP3A4	12.9 ± 5.6 (75 ± 33)	0.1 ± 0.0 (0.6 ± 0.1)	1725 ± 236 (10074 ± 1378)	93.7 ± 22.7 (547 ± 133)
CYP1B1	2.5 ± 0.4 (10.8 ± 1.7)	0.2 ± 0.1 (0.9 ± 0.4)	387 ± 193 (1672 ± 834)	10.4 ± 5.1 (45 ± 23)
Control enzyme[a]	0.3 ± 0.2	<0.05	262 ± 57	9.3 ± 4.0
	4-OHE_2			
Enzyme	4-OHE_2-1-N3Ade	4-OHE_2-1-N7Gua	4-OHE_2-2-SG	4-OHE_2-2-NAcCys
CYP1A1	0.6 ± 0.1 (5.7 ± 1.0)	1.2 ± 0.1 (11.4 ± 1.0)	441 ± 77 (4198 ± 733)	1.5 ± 0.9 (14.3 ± 8.6)
CYP3A4	4.3 ± 1.3 (25 ± 8)	5.7 ± 0.9 (33 ± 5)	3430 ± 846 (20031 ± 4941)	26.5 ± 4.3 (155 ± 25)
CYP1B1	6.2 ± 2.6 (27 ± 11)	5.2 ± 1.1 (22 ± 5)	1184 ± 112 (5115 ± 484)	41.2 ± 8.1 (178 ± 35)
Control enzyme[a]	0.3 ± 0.2	0.3 ± 0.2	252 ± 71	10.8 ± 1.9

DNA adduct data were adjusted against blanks that contained the catechol and DNA, but no enzyme. In all cases, the blank values were less than 0.1 pmol. No conjugates were observed in the blanks. Reaction mixtures contained either 2-OHE_2 or 4-OHE_2 (0.87 mmol/L), 125 pmol CYP, and 3 mmol/L calf thymus DNA with or without 0.6 mmol/L NADPH. Reactions were adjusted with 0.067 mol/L Na-K phosphate buffer (pH 7.0) to a total volume of 2.5 mL and incubated at 37°C for 10 hours. The results are presented as the average ± SD for 3 replicate experiments.

[a] Control enzyme, baculovirus-insect cell–expressed microsomes (BD Gentest, cat no. 456200).

acid biosynthesis pathway [33]. More than 100-fold higher levels of GSH conjugates were formed compared to the DNA adducts with both recombinant CYP microsomes, as well as control microsomes (Table 2). The oxidized catechol metabolite E_2-3,4-Q was added to CYP1B1 or control microsomes and incubated for 30 minutes at 37°C (Table 3) to verify that GSH and NAcCys were present in these microsomes. As expected, GSH conjugates were found in the reaction mixtures that contained either CYP1B1 or control microsomes. This indicated that, indeed, GSH was present in both the recombinant and control microsomes. Similar amounts of GSH conjugates were observed in the presence of either CYP1B1 or control microsomes plus E_2-3,4-Q (Table 3), indicating that CYPs do not contribute to the conjugation with GSH but only to the oxidation of 4-OHE_2 to E_2-3,4-Q.

Catechol estrogen quinones are reactive compounds and have very short half-lives, as indicated above. This is why the reaction time with E_2-3,4-Q as substrate was set at 30 minutes. For comparison, reactions with 4-OHE_2 as substrate were also analyzed at 30 minutes (Table 3). When 4-OHE_2 was incubated with recombinant CYP1B1 microsomes, the 4-OHE_2-2-SG and 4-OHE_2-2-NAcCys conjugates were detected at the highest levels (Table 3), indicating that GSH is present in the microsomes, as well as an oxidizing cofactor. In the absence of DNA, 10-fold higher levels of 4-OHE_2-2-SG (1460 vs 141 pmol/mg protein) were observed in the CYP1B1 reaction mixtures

Table 3
Formation of depurinating DNA adducts and GSH conjugates after incubation of 4-OHE_2 or E_2-3,4-Q with CYP1B1 for 30 minutes

Reaction components	Picomoles per milligram of protein (picomoles per micromoles of CYP)			
	4-OHE_2-1-N3Ade	4-OHE_2-1-N7Gua	4-OHE_2-2-SG	4-OHE_2-2-NAcCys
4-OHE_2 + CYP1B1			2015 ± 184 (8075 ± 795)	2.0 ± 0.4 (8.6 ± 1.7)
4-OHE_2 + CYP1B1 + NADPH			1460 ± 235 (6307 ± 1015)	3.5 ± 1.7 (15.1 ± 7.3)
4-OHE_2 + CYP1B1 + NADPH + DNA	0.7 ± 0.4 (3.0 ± 1.7)	<0.05	293 ± 1 (1266 ± 4)	3.3 ± 0.7 (14.3 ± 3.0)
4-OHE_2 + control enzyme + NADPH			141 ± 19	0.5 ± 0.2
4-OHE_2 + control enzyme + NADPH + DNA	0.2 ± 0.1	<0.05	40 ± 3	0.2 ± 0
E_2-3,4-Q + CYP1B1			144 ± 33 (622 ± 142)	1.9 ± 0.6 (8.2 ± 2.6)
E_2-3,4-Q + CYP1B1 + DNA	1.1 ± 0.6 (4.8 ± 2.6)	<0.05	33 ± 7 (143 ± 30)	1.9 ± 0.4 (8.2 ± 1.7)
E_2-3,4-Q + control enzyme			102 ± 24	2.1 ± 0.2
E_2-3,4-Q + control enzyme + DNA	1.3 ± 0.2	<0.05	29 ± 5	1.3 ± 0.2
E_2-3,4-Q + control enzyme + GSH			554 ± 95	2.2 ± 0.32
E_2-3,4-Q + control enzyme + GSH + DNA	1.1 ± 0.2	<0.05	340 ± 57	1.6 ± 0.3

DNA adduct data were adjusted against blanks that contained 4-OHE_2 or E_2-3,4-Q and DNA. In all cases, the blank values were less than 0.1 pmol. No conjugates were observed in blanks. Reaction mixtures contained either E_2-3,4-Q or 4-OHE_2 (0.87 mmol/L), 125 pmol CYP1B1, and 3 mmol/L calf thymus DNA with or without 0.6 mmol/L NADPH. Reaction mixtures were adjusted with 0.067 mol/L Na-K phosphate buffer (pH 7.0) to a total volume of 2.5 mL and incubated at 37°C for 30 minutes. For the reaction containing control enzyme, the same volume as used in CYP1B1 reactions was added. Data were normalized with total protein. The results are presented as the average ± SD for 3 replicate experiments. Control enzyme, baculovirus-insect cell–expressed microsomes (BD Gentest, cat no. 456200). In reactions that contained added GSH, 100 μmol/L GSH was added to the reaction mixture.

compared with that in the control microsome mixtures. The observation of much more conjugates and DNA adducts in reactions containing CYP1B1 (rather than control) microsomes in 30 minutes, as well as at 10 hours, indicated that CYP1B1 is able to oxidize 4-OHE_2 to E_2-3,4-Q. The 10-hour data (Table 2) indicated that CYP1A1 and CYP3A4 are able to oxidize CEs to their respective CE-Qs too. In addition, CYP1A1 oxidized 4-OHE_2 and CYP1B1 oxidized 2-OHE_2 to much smaller extents, based on both GSH conjugate and DNA adduct formation, which is in line with the observation that in estrogen metabolism, CYP1A1 did not have significant catalytic activity in forming 4-OHE_2, or CYP1B1 in forming 2-OHE_2 [5].

In the presence of DNA, low amounts of 4-OHE_2-1-N3Ade, but not 4-OHE_2-1-N7Gua, were observed (Table 3) in the 30-minute reactions. This is expected because the N3Ade adduct is lost from DNA by rapid depurination, whereas the N7Gua adduct is lost from DNA with a half-life of approximately 3 hours and is maximal at 10 hours [20]. As expected, in the presence of DNA, a smaller amount of 4-OHE_2-2-SG (293 vs 1460 pmol/mg protein) was observed. This could be due to competition between DNA and GSH for the reactive E_2-3,4-Q.

NADPH is an important cofactor for CYP oxidation reactions, but it also can reduce E_2-3,4-Q back to 4-OHE_2 [34]. For this reason, no NADPH was added to the reaction mixture when E_2-3,4-Q was used as the substrate. For the same reason, a smaller amount of 4-OHE_2-2-SG was observed in reaction in the presence of NADPH compared with reactions without NADPH when 4-OHE_2 was used as the substrate (Table 3).

In summary, the results of our study clearly show that CYP1A1 and CYP3A4 can oxidize 2-OHE_2 to E_2-2,3-Q, and CYP1B1 and CYP3A4 can oxidize 4-OHE_2 to E_2-3,4-Q. CYP1A1 can also oxidize 4-OHE_2 to E_2-3,4-Q and CYP1B1 can oxidize 2-OHE_2 to E_2-2,3-Q, but to much smaller extents. These quinones react with GSH and other thiol-containing compounds such as NAcCys, or DNA, the latter resulting predominantly in depurinating adducts that can generate mutations. The formation of the 4-OHE_2-2-SG conjugate demonstrates that significant amounts of GSH were present in the recombinant human CYP preparations. This study definitively demonstrates that the oxidation of CE to CE-Q is catalyzed by the CYPs that also catalyze the oxidation of estrogens to CE. The CYPs show catalytic specificity not only for the formation of 2-CE and 4-CE, but also for the oxidation of the CE to CE-Q. Thus, induction of CYP1B1 will not only increase formation of 4-CE, but also CE-3,4-Q.

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