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Inhibition of human catechol-*O*-methyltransferase (COMT)-mediated *O*-methylation of catechol estrogens by major polyphenolic components present in coffee

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ABSTRACT

In the present study, we investigated the inhibitory effect of three catechol-containing coffee polyphenols, chlorogenic acid, caffeic acid and caffeic acid phenethyl ester (CAPE), on the *O*-methylation of 2- and 4-hydroxyestradiol (2-OH-E_2 and 4-OH-E_2, respectively) catalyzed by the cytosolic catechol-*O*-methyltransferase (COMT) isolated from human liver and placenta. When human liver COMT was used as the enzyme, chlorogenic acid and caffeic acid each inhibited the *O*-methylation of 2-OH-E_2 in a concentration-dependent manner, with IC_{50} values of 1.3–1.4 and 6.3–12.5 μM, respectively, and they also inhibited the *O*-methylation of 4-OH-E_2, with IC_{50} values of 0.7–0.8 and 1.3–3.1 μM, respectively. Similar inhibition pattern was seen with human placental COMT preparation. CAPE had a comparable effect as caffeic acid for inhibiting the *O*-methylation of 2-OH-E_2, but it exerted a weaker inhibition of the *O*-methylation of 4-OH-E_2. Enzyme kinetic analyses showed that chlorogenic acid and caffeic acid inhibited the human liver and placental COMT-mediated *O*-methylation of catechol estrogens with a mixed mechanism of inhibition (competitive plus noncompetitive). Computational molecular modeling analysis showed that chlorogenic acid and caffeic acid can bind to human soluble COMT at the active site in a similar manner as the catechol estrogen substrates. Moreover, the binding energy values of these two coffee polyphenols are lower than that of catechol estrogens, which means that coffee polyphenols have higher binding affinity for the enzyme than the natural substrates. This computational finding agreed perfectly with our biochemical data.

1. Introduction

In humans, catechol estrogens such as 2- and 4-hydroxyestradiol (2-OH-E_2 and 4-OH-E_2)[3] are rapidly *O*-methylated to form monomethyl ethers (structures shown in Fig. 1) catalyzed by catechol-*O*-methyltransferase (COMT) using *S*-adenosyl-L-methionine (AdoMet) as the methyl donor (reviewed in Ref. [1]). Many earlier studies on the potential genotoxicity of catechol estrogens have led to the suggestion that this metabolic *O*-methylation may provide a rapid inactivation/detoxification for the chemically reactive catechol estrogen intermediates, and this suggestion was, in part, based on the well-known functions of the COMT-mediated *O*-methylation of endogenous catecholamines in the detoxification of these reactive catechols. Additional studies have also shown that 2-methoxyestradiol (the major *O*-methylation product of 2-OH-E_2) has strong apoptotic, antiangiogenic, and anticancer activities [1,2]. These observations have led to the suggestion that the metabolic *O*-methylation of catechol estrogens may not only inactivate the chemically reactive catechol estrogen intermediates, but it may also simultaneously produce estrogen derivatives with potential anticancer activities.

The human COMT has a low substrate specificity, and it can catalyze the *O*-methylation of various catechol-containing endobiotics and xenobiotics [3–10]. Therefore, the rate for *O*-methylation of catechol estrogen intermediates *in vivo* may subject to modulation by other endogenous or exogenous catechol substrates that are present in the body in significant quantities. Notably, earlier studies by Zhu et al. [11,12] has shown that chronic treatment of Syrian hamsters with dietary quercetin, a substrate and potent inhibitor of hamster renal COMT, significantly enhanced 17β-estradiol-induced tumorigenesis in the kidney. This interesting observation has led to the suggestion that strong inhibition of COMT-mediated *O*-methylation of catechol estrogens by xenobiotics may facilitate the

Abbreviations: 2-OH-E_2, 2-hydroxyestradiol; 4-OH-E_2, 4-hydroxyestradiol; COMT, catechol-*O*-methyltransferase; AdoMet, *S*-adenosyl-L-methionine; AdoHcy, *S*-adenosyl-L-homocysteine; CAPE, caffeic acid phenethyl ester.

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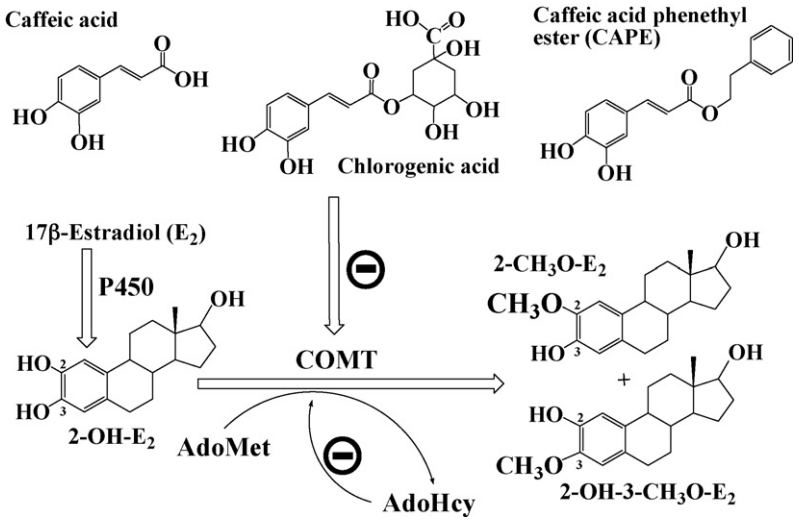

Fig. 1. Metabolic formation of catechol estrogens (2-OH-E_2 and 4-OH-E_2) and the COMT-mediated further *O*-methylation of these catechol metabolites. Note that the metabolic *O*-methylation is subject to regulation by various endogenous factors (such as catecholamines, *S*-adenosyl-L-homocysteine, and homocysteine) as well as exogenous factors (such as the catechol-containing dietary polyphenols).

development of estrogen-induced tumors as a result of decreased formation of 2-methoxyestradiol and increased accumulation of the reactive catechol estrogen intermediates in target cells.

In our recent study that was aimed to evaluate the modulating effect of the catechol-containing dietary polyphenols on human COMT-mediated *O*-methylation of endogenous catechols, we found that (-)-epigallocatechin-3-*O*-gallate (EGCG), a common tea polyphenol, is an exceptionally strong inhibitor of human COMT-mediated *O*-methylation of 2-OH-E_2 and 4-OH-E_2, with IC_{50} values at approximately 70 nM [13–16]. We describe here our study that evaluated the potential modulating effect of chlorogenic acid, caffeic acid, and caffeic acid phenethyl ester (CAPE) (three catechol-containing polyphenols richly present in coffee) on human liver and placental COMT-mediated *O*-methylation of 2-OH-E_2 and 4-OH-E_2. Our data show that these coffee polyphenols are highly effective inhibitors of the human liver and placental COMT-mediated *O*-methylation of catechol estrogens *in vitro*. In addition, computational molecular modeling analysis was also conducted to shed light on the mechanism of COMT inhibition by these coffee polyphenols.

2. Materials and methods

2.1. Chemicals

2-OH-E_2, 4-OH-E_2, AdoMet, dithiothreitol, caffeic acid, chlorogenic acid, and CAPE were purchased from the Sigma Chemical Co. (St. Louis, MO). [Methyl-^{3}H]AdoMet (specific activity of 11.2–13.5 Ci/mmol) was purchased from New England Nuclear Research Products (Boston, MA). The biodegradable scintillation cocktail (SintiVerse BD) and all other organic solvents used in this study (HPLC grade or better) were obtained from Thermo Fisher Scientific Inc. (Waltham, MA).

2.2. Preparation of cytosolic fractions from human liver and placenta samples

Human liver samples were obtained from Caucasians undergoing liver tumor removal surgery at the University of Medicine and Dentistry of New Jersey (UMDNJ)—Robert Wood Johnson Medical School (New Brunswick, NJ). Human term placental samples from Caucasian nonsmokers (mean age ± S.D., 29.5 ± 7.8 years) were obtained from women (at 36–40 weeks of gestation) after normal pregnancy and vaginal delivery at the St. Peter's University Hospital (New Brunswick, NJ). The procedures for the procurement of human liver and placental samples were approved beforehand by the Institutional Review Boards (IRBs) of the University of South Carolina (Columbia, SC), Rutgers University (Piscataway, NJ), and the UMDNJ-Robert Wood Johnson Medical School (New Brunswick, NJ). Within 30 min after the liver tumor(s) was removed, a portion of the surrounding normal liver tissue was collected and snap-frozen in liquid nitrogen. Similarly, a portion (50–100 g) of the term placenta sample was collected and snap-frozen in liquid nitrogen within 30 min the term placenta was expelled. The samples were then immediately transported to a laboratory at Rutgers University (~2 miles away from the surgery room) for storage in a −80 °C freezer. The frozen human liver and placental samples in the presence of adequate amounts of dry-ice were later air-shipped overnight to the University of South Carolina where the preparation of human liver and placental subcellular fractions and the enzymatic assays were carried out.

On the day of preparation of cytosolic fractions, the liver or placental samples were first thawed at room temperature and then rinsed with ice-cold normal saline. Connective tissues were removed with a pair of sharp eye-surgery scissors. The tissues were then minced in 3 volumes of an ice-cold solution (pH 7.4) containing 0.05 M Tris–HCl and 1.15% KCl and were then homogenized with a Tri-R homogenizer (Model K41) for 2–3 min followed by a Teflon homogenizer for another 2–3 min. Tissue homogenates were centrifuged at 9000 × *g* for 10 min, and supernatants were pooled and filtered through two layers of cheesecloth to remove lipid clots. The filtrates were then recentrifuged at 105,000 × *g* (4 °C) for 90 min. The resulting pellets are microsomal fraction and the supernatants are cytosolic fraction. Aliquots of each cytosolic preparation were stored separately in small vials at −80 °C until use. The protein concentration was determined by using the BioRad protein assay kit with bovine serum albumin (BSA) as a standard.

2.3. O-Methylation of 2-OH-E_2 and 4-OH-E_2 by human liver and placental cytosolic COMTs

The COMT-mediated *O*-methylation of catechol estrogens was carried out as described earlier [12,13]. The reaction mixture consisted of 0.5 mg human liver or placental cytosolic protein, 1.2 mM

$MgCl_2$, 250 μM AdoMet (containing 0.5 μCi [methyl-^{3}H]AdoMet), 1 mM dithiothreitol, and varying concentrations of 2-OH-E_2 or 4-OH-E_2 in a final volume of 0.5 mL Tris–HCl buffer (50 mM, pH 7.4). The reaction was initiated by addition of liver or placental cytosolic protein and carried out at 37 °C for 10 min. The reaction was arrested by immediately cooling to ice-cold temperatures, addition of 250 μL ice-cold 0.9% NaCl, and extraction with 5 mL of ice-cold toluene. After centrifugation at 1000 × *g* for 10 min, portions of the organic extracts were measured for radioactivity content with a liquid scintillation analyzer (Packard Tri-CARB 2900TR; Downers Grove, IL).

To measure the inhibitory effect of chlorogenic acid or caffeic acid, the above organic extraction method was used because the methylated products formed from these two water-soluble polyphenols could not be extracted with toluene. However, when CAPE (which is more hydrophobic than chlorogenic acid and caffeic acid) was used as the inhibitor, the above organic extraction method could not be used because the methylated products of CAPE could also be extracted. Therefore, an HPLC method was used when we tested the effect of CAPE. The methylated products of catechol-E_2 and CAPE were first extracted with ethyl acetate, and the products were separated on the HPLC for quantification individually. The HPLC system consisted of a Waters 2690 separation module (Milford, MA), a radioactivity detector (β-RAM, INUS System, Inc., Tampa, FL), a Waters UV detector (Model 484), and an Ultracarb 5 μm ODS column (150 mm × 4.60 mm, Phenomenex, Torrance, CA). The solvent system for the separation of methoxy-E_2 products consisted of water and methanol, at the flow rate of 1.0 mL/min. The composition of the mobile phase included 80% methanol and 20% water for the separation of 4-OH-E_2 and its methylated products, and 70% methanol and 30% water for the separation of 2-OH-E_2 and its methylated products.

The baseline values at the zero time point were determined in each experiment and were subtracted. The rate of methylation was expressed as "pmol of methylated product formed/mg of liver cytosolic protein/min" (abbreviated as "pmol/mg protein/min").

2.4. Computational modeling analysis of human COMT

Energy minimizations and molecular dynamics simulation were performed with Discovery Studio modeling program (Version 1.7, Accelrys Inc., San Diego, CA) installed in Red Hat Enterprise Linux WS4.0 operation system (Red Hat Inc., Raleigh, NC) on a Dell Precision 690 workstation. Homology modeling was performed with InsightII modeling program (Version 2005 Accelrys Inc., San Diego, CA). CHARMm force field was used for energy minimization and dynamics simulation.

2.4.1. Construction of homology model of human S-COMT

The primary sequence of the human S-COMT (GI 6466450) and the rat S-COMT (GI 1633081) were obtained from the NCBI database. Sequence alignment was done with the Homology Modeling module of InsightII, which showed >80% sequence similarity. Using the known X-ray structure of the rat S-COMT (PDB code: 1VID) as template, we have recently constructed the homology model of human S-COMT with the Modeler in the Homology Modeling module of InsightII [6]. The substrate 3,5-dinitrocatechol (DNC), methyl donor AdoMet, Mg^{2+} ion, and the crystallographic water that coordinates with Mg^{2+} were included in the model. The simulation was carried out with the Standard Dynamics Cascade Protocol in Discovery Studio. For energy minimizations, the steepest descent method was employed first to a 10 kcal/(mol Å) root mean square (RMS) energy gradient and followed by the Polak and Ribiere conjugate gradient method until the final convergence criterion reached 0.01 kcal/(mol Å) RMS gradient. Then the whole system was heated from 100 K to 300 K in 2 ps and equilibrated in 300 K for 100 ps. One hundred conformations were collected in 20 ps production phase at 300 K. The conformation with the lowest potential energy was further minimized and used for binding energy analysis. DNC, Mg^{2+}, H_2O, AdoMet and key residues in the catalytical site (namely, D141, K144, D169, N170, and E199) were constrained during the whole simulation process. The structure of the human COMT was verified with Prostat function in the Homology Modeling module.

2.4.2. Binding energy calculation

The structure of 2-OH-E_2, 4-OH-E_2, caffeic acid and chlorogenic acid were built with the Builder module in InsightII and minimized with CHARMm. The catechol ring of the substrates was superimposed onto the catechol ring of DNC in the constructed human S-COMT homology model. Similar energy simulation was carried out with the Standard Dynamics Cascade Protocol in Discovery Studio as described above. The binding energy was calculated by the Binding Energy Calculation protocol in Discovery Studio.

3. Results

3.1. Optimization of the conditions for the in vitro enzymatic *O*-methylation of catechol estrogens

A total of four human liver cytosol preparations (HL1C, HL2C, HL6C and HL8C) and four human placenta cytosol preparations (HP8C, HP17C, HP18C and HP20C) were used in the present study. Before we tested the inhibitory effects of coffee polyphenols on the *O*-methylation of 2-OH-E_2 and 4-OH-E_2, we optimized the assay conditions by determining the effects of incubation time, cytosolic protein concentrations, AdoMet concentrations and reaction pH on the formation of *O*-methylated estrogen metabolites by human liver and placental cytosolic COMTs (data not shown). An optimized reaction condition suitable for the *in vitro O*-methylation of both 2-OH-E_2 and 4-OH-E_2 by human liver or placenta cytosolic COMT was used, which included an incubation time of 10 min, a cytosolic protein concentration of 0.5 mg/mL, a AdoMet concentration of 250 μM and a reaction pH at 7.4.

3.2. Inhibition of human liver and placental COMT-mediated *O*-methylation of catechol estrogens by coffee polyphenols

When different concentrations (from 0.313 to 20 μM) of chlorogenic acid were added to the incubation mixture, the rate of *O*-methylation of 2-OH-E_2 or 4-OH-E_2 by human liver and placental COMT was similarly inhibited in a concentration-dependent manner (Fig. 2). The average IC_{50} values of chlorogenic acid for inhibiting the *O*-methylation of 2-OH-E_2 by human liver or placental cytosolic COMTs were 1.2–1.4 and 0.8–0.9 μM, respectively, and the average IC_{50} values for inhibiting the *O*-methylation of 4-OH-E_2 were 0.7–0.8 and 0.2–0.3 μM, respectively (Fig. 2 and Table 1). Notably, our data consistently showed that chlorogenic acid had 1.5–7-fold higher inhibition potency for the *O*-methylation of 4-OH-E_2 than for the *O*-methylation of 2-OH-E_2 by human liver or placental cytosolic COMT (Fig. 2, Table 1).

Similarly, when different concentrations (from 1.6 to 100 μM) of caffeic acid were added to the incubation mixture, the rate of *O*-methylation of 2-OH-E_2 or 4-OH-E_2 by human liver or placental COMT was also inhibited in a concentration-dependent manner (Fig. 3). The average IC_{50} values of caffeic acid for inhibiting the *O*-methylation of 2-OH-E_2 by human liver and placental COMTs were 6.3–12.5 and 13–14 μM, respectively, and the average IC_{50} values for inhibiting the *O*-methylation of 4-OH-E_2 were 1.3–3.1 and 3.1 μM, respectively (Fig. 3, Table 1). Caffeic acid also had a higher inhibition potency (4–6 fold) for the *O*-methylation of 4-OH-E_2 than for

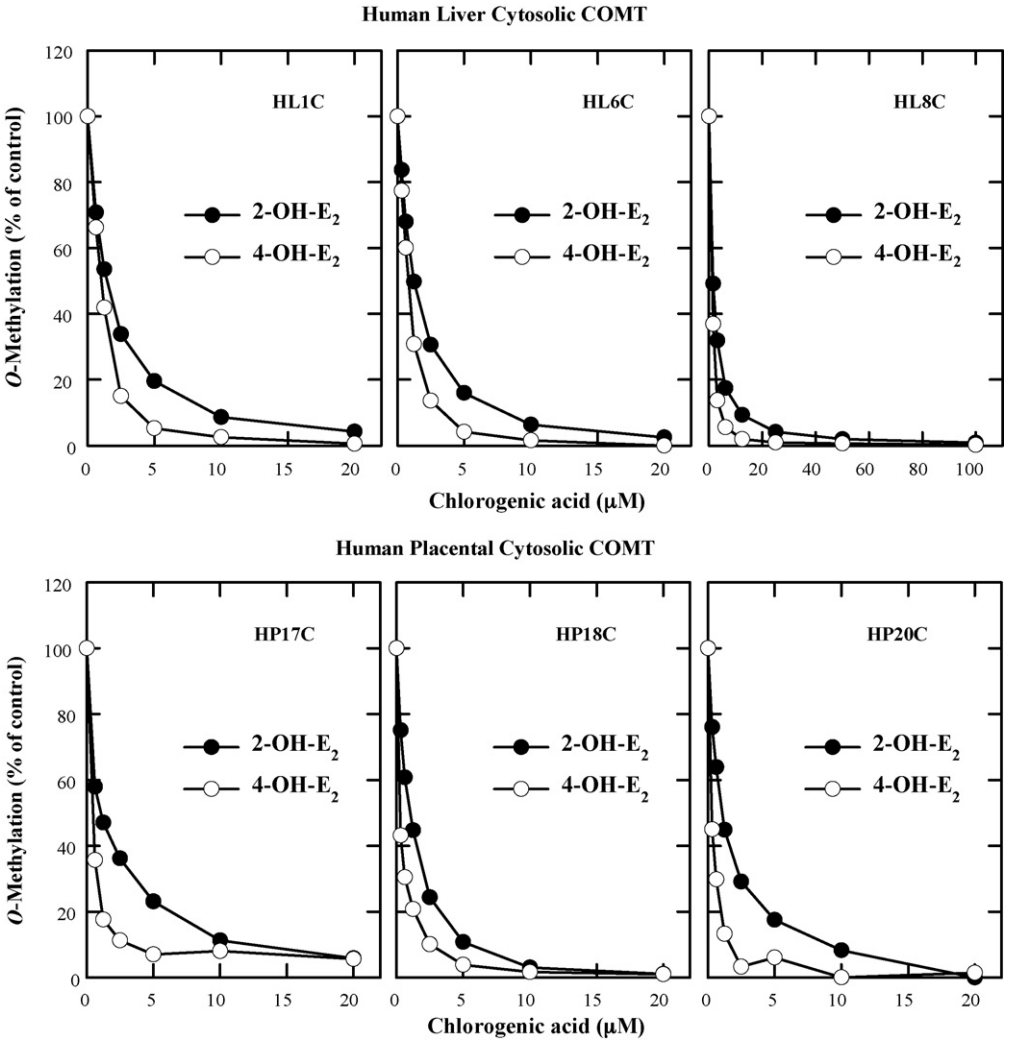

Fig. 2. Inhibition of human liver and placental cytosolic COMT-mediated *O*-methylation of 2-OH-E_2 and 4-OH-E_2 by different concentrations of chlorogenic acid. The incubation mixture consisted of 10 μM of 2-OH-E_2 or 4-OH-E_2, chlorogenic acid (at the indicated concentrations), 250 μM [methyl-^{3}H]AdoMet (containing 0.2 μCi), 0.5 mg/mL of human liver cytosolic protein, 1 mM dithiothreitol, and 1.2 mM $MgCl_2$ in a final volume of 0.25 mL Tris–HCl buffer (10 mM, pH 7.4). Incubations were carried at 37 °C for 10 min. Each point is the mean of duplicate determinations (with average variation usually <5%).

Table 1
The IC_{50} values for chlorogenic acid, caffeic acid, and CAPE in inhibiting the *O*-methylation of 2-OH-E_2 and 4-OH-E_2 catalyzed by representative human liver and placental cytosolic COMTs.

Inhibitor	Cytosol sample	IC_{50} value (μM)	
		2-OH-E_2	4-OH-E_2
Chlorogenic acid	Human liver HL1C	1.4	0.8
	Human liver HL6C	1.3	0.7
	Human liver HL8C	1.3	0.8
	Mean ± S.D. (for liver)	1.3 ± 0.1	0.8 ± 0.1
	Human placenta HP17C	1.0	0.3
	Human placenta HP18C	0.8	0.2
	Human placenta HP20C	0.9	0.2
	Mean ± S.D. (for placenta)	0.9 ± 0.1	0.2 ± 0.1
Caffeic acid	Human liver HL1C	12.5	2.0
	Human liver HL2C	12.5	3.1
	Human liver HL6C	6.3	1.3
	Human liver HL8C	6.3	3.1
	Mean ± S.D. (for liver)	8.4 ± 3.6	2.5 ± 1.0
	Human placenta HP18C	13.0	3.1
	Human placenta HP20C	14.0	3.1
	Mean (for placenta)	13.5	3.1
CAPE	Human liver HL2C	10.2	12.0
	Human liver HL6C	5.6	8.4
	Mean (for liver)	7.9	10.2

the *O*-methylation of 2-OH-E_2 by human liver or placental cytosolic COMT.

When CAPE was used as the inhibitor, human liver cytosolic COMT-mediated *O*-methylation of 2-OH-E_2 and 4-OH-E_2 was inhibited to a comparable extent. Its IC_{50} values were 5.6–10.2 and 8.4–12.0 μM, respectively, for inhibiting the *O*-methylation of 2- and 4-OH-E_2 (Fig. 4, Table 1).

In summary, of the three coffee polyphenols tested, chlorogenic acid has the highest inhibition potency, with IC_{50} values of 0.2–0.8 μM for inhibiting the methylation of 4-OH-E_2 and 0.8–1.4 μM for inhibiting the methylation of 2-OH-E_2. In comparison, caffeic acid and CAPE are significantly less potent. Notably, while chlorogenic acid and caffeic acid each have a preferential activity for inhibiting the methylation of 2-OH-E_2 than the methylation of 4-OH-E_2, CAPE inhibits the methylation of both catechol estrogens comparably.

3.3. Mechanism of inhibition of human COMT-mediated *O*-methylation of catechol estrogens by coffee polyphenols

3.3.1. Human placental cytosolic COMT

As we have described in our earlier study [13], the *O*-methylation of both 2-OH-E_2 and 4-OH-E_2 by human placental cytosolic COMT

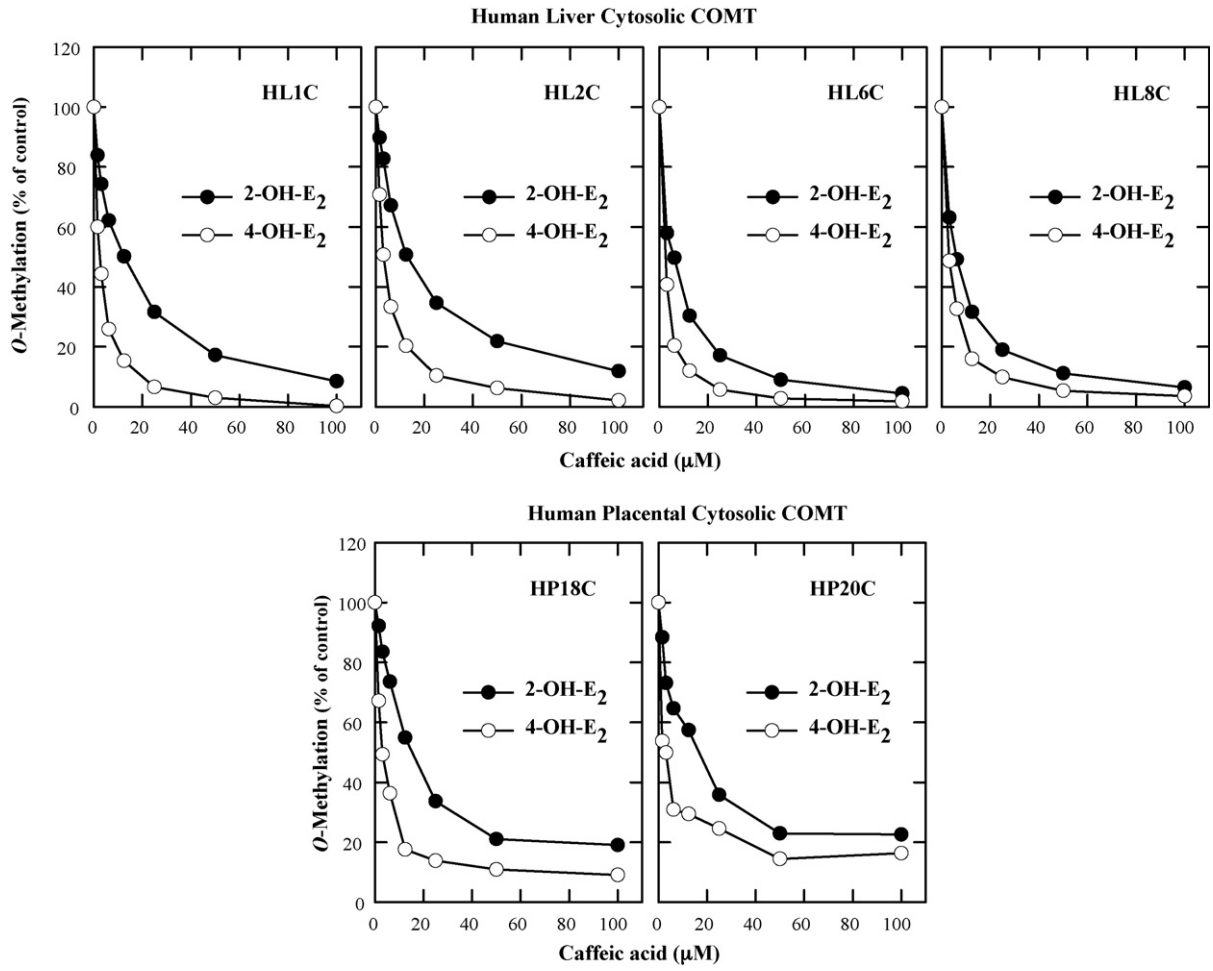

Fig. 3. Inhibition of human liver and placental cytosolic COMT-mediated *O*-methylation of 2-OH-E_2 and 4-OH-E_2 by different concentrations of caffeic acid. The incubation mixture consisted of 10 μM of 2-OH-E_2 or 4-OH-E_2, caffeic acid (at the indicated concentrations), 250 μM [methyl-^{3}H]AdoMet (containing 0.2 μCi), 0.5 mg/mL of human cytosolic protein, 1 mM dithiothreitol, and 1.2 mM $MgCl_2$ in a final volume of 0.25 mL Tris–HCl buffer (10 mM, pH 7.4). Incubations were carried at 37 °C for 10 min. Each point is the mean of duplicate determinations (with average variation usually <5%).

in the absence of inhibitors followed typical Michaelis–Menten curve patterns. When chlorogenic acid was present at 0.25 and 0.5 μM with 2-OH-E_2 as a substrate, the K_M value was increased by approximately 2- and 4-fold, respectively, whereas the V_{MAX} value was decreased by only approximately 20% (Fig. 5A, Table 1). When chlorogenic acid was present at 0.25 and 0.5 μM with 4-OH-E_2 as a substrate, the K_M value was increased by approximately 1.2- and 2-fold, respectively, whereas the V_{MAX} value was decreased

Table 2
Kinetic parameters (K_M and V_{MAX} values) for the *O*-methylation of 2-OH-E_2 and 4-OH-E_2 catalyzed by cytosolic COMT prepared from representative human placenta and liver samples in the absence or presence of an inhibitor (chlorogenic acid or caffeic acid). These parameters were calculated from Fig. 5 for placental cytosolic COMT and Fig. 6 for liver cytosolic COMT, respectively, according the Edie–Hofstee plots.

Human cytosol samples	2-OH-E_2 as substrate		4-OH-E_2 as substrate	
	K_M (μM)	V_{MAX} (pmol/mg protein/min)	K_M (μM)	V_{MAX} (pmol/mg protein/min)
Placenta (Fig. 5)				
HP18C (no inhibitor)	1.0	4815	4.2	1918
+0.25 μM chlorogenic acid	1.6	3960	5.1	1480
+0.5 μM chlorogenic acid	3.0	3648	8.3	1146
HP20C (no inhibitor)	0.7	1650	3.5	759
+0.5 μM chlorogenic acid	2.5	1311	6.4	312
HP17C (no inhibitor)	1.3	2274	3.2	1827
+0.5 μM caffeic acid	2.8	2078	8.1	1572
+2 μM caffeic acid	5.3	1893	5.4	531
Liver (Fig. 6)				
HL1C (no inhibitor)	–	–	4.5	6408
+1 μM chlorogenic acid	–	–	5.9	2825
HL6C (no inhibitor)	–	–	7.1	6310
+2 μM chlorogenic acid	–	–	6.6	3292
HL1C (no inhibitor)	–	–	6.5	6401
+2 μM caffeic acid	–	–	12.0	4063

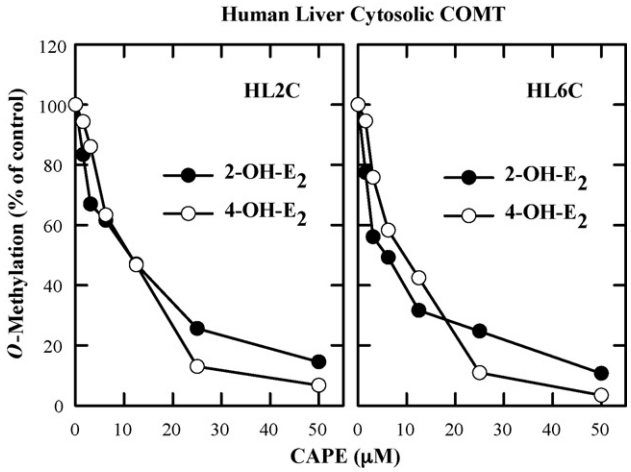

Fig. 4. Inhibition of human liver cytosolic COMT-mediated *O*-methylation of 2-OH-E_2 and 4-OH-E_2 by different concentrations of CAPE. The incubation mixture consisted of 10 μM of 2-OH-E_2 or 4-OH-E_2, CAPE (at the indicated concentrations), 250 μM [methyl-^{3}H]AdoMet (containing 0.2 μCi), 0.5 mg/mL of liver cytosolic protein, 1 mM dithiothreitol, and 1.2 mM $MgCl_2$ in a final volume of 0.25 mL Tris–HCl buffer (10 mM, pH 7.4). Incubations were carried at 37 °C for 10 min. Each point is the mean of duplicate determinations (with average variation <5%).

approximately 50% (Fig. 5B, Table 1). Those results suggested that chlorogenic acid inhibited the human placenta COMT-mediated *O*-methylation of 2-OH-E_2 and 4-OH-E_2 with a mixed (competitive plus noncompetitive) mechanism of enzyme inhibition. Similar kinetic alterations were also observed with caffeic acid (Fig. 5C, Table 2).

3.3.2. Human liver cytosolic COMT

When 1 μM chlorogenic acid were used as the inhibitor, the apparent V_{MAX} value was decreased by approximately 50%, whereas the apparent K_M value was slightly increased, suggesting that chlorogenic acid inhibited human liver COMT-mediated *O*-methylation of 4-OH-E_2 with primarily a noncompetitive mechanism of enzyme inhibition (Fig. 6A, Table 2). Similar kinetic changes were also observed with caffeic acid (Fig. 6B, Table 2).

3.4. Computational molecular modeling analysis of the binding interaction of human S-COMT with chlorogenic acid and caffeic acid

Using the homology model of human S-COMT that we have recently constructed [6], we studied the binding interactions of chlorogenic acid and caffeic acid with human S-COMT (Fig. 7). Both of 2-OH-E_2 (Fig. 7A) and chlorogenic acid (Fig. 7B) fit quite well inside the binding pocket in a similar manner. Moreover, hydrogen bonds were formed between the catecholic hydroxyl groups and the side chain of D170 for both chemicals, revealing that both chemicals can serve as substrates for the *O*-methylation catalyzed by this enzyme, and each of them would also serve as a competitive inhibitor of the other. This would explain the competitive component of enzyme inhibition.

The binding energy values ($\Delta E_{binding}$) for the interaction of coffee polyphenols (chlorogenic acid and caffeic acid) and catechol estrogens (2-OH-E_2 and 4-OH-E_2) with the enzyme were also calculated (Table 3). The $\Delta E_{binding}$ values for 2-OH-E_2 and 4-OH-E_2 were very close to each other, which was consistent with the close K_M values of 2-OH-E_2 and 4-OH-E_2 determined experimentally for human placental cytosolic COMT. The $\Delta E_{binding}$ values of chlorogenic acid and caffeic acid were lower than the values for 2-OH-E_2 and 4-OH-E_2, which means that these inhibitors (particularly chlorogenic acid) can bind to human S-COMT more tightly than catechol estrogens. This can be explained by the more rigid ring structure of 2-OH-E_2 and 4-OH-E_2, which limits their contact surface with the enzyme and thus accounts for their lower binding affinity compared to chlorogenic acid.

Table 3
The binding energy values ($\Delta E_{binding}$, kcal/mol) of 2-OH-E_2, 4-OH-E_2, caffeic acid and chlorogenic acid with human S-COMT.

Substrate	$\Delta E_{binding}$ (kcal/mol)
2-OH-E_2	−124.5
4-OH-E_2	−121.9
Caffeic acid	−155.5
Chlorogenic acid	−236.4

$\Delta E_{binding}$ was calculated using the following equation: $\Delta E_{binding} = E_{complex} - (E_{ligand} + E_{COMT})$, where $E_{complex}$ is the potential energy for the complex of human S-COMT with the ligand, E_{ligand} is the potential energy of the ligand itself and E_{COMT} is the potential energy of the enzyme itself.

In addition, the computational values showed that the binding energy of chlorogenic acid was much lower than caffeic acid, which suggests that the binding affinity of the former was much higher than the latter and the competitive component of COMT inhibition would be stronger by chlorogenic acid than caffeic acid. The computational data agreed perfectly with our experimental data which also showed that chlorogenic acid had a much lower IC_{50} value for inhibiting the *O*-methylation of 2-OH-E_2 and 4-OH-E_2 compared to caffeic acid. Mechanistically, the higher binding affinity of chlorogenic acid for the S-COMT was, in part, due to its more flexible and hydroxylated tail structure that can be readily bent to form more molecular contacts with amino acid residues inside the catalytic pocket of the enzyme.

4. Discussion

In the present study, we demonstrated that chlorogenic acid, caffeic acid and CAPE (three common coffee polyphenols) are effective inhibitors of the *O*-methylation of 2-OH-E_2 and 4-OH-E_2 catalyzed by human liver and placental cytosolic COMT. The inhibition potencies (IC_{50} values) of chlorogenic acid are markedly higher than those of caffeic acid and CAPE. Chlorogenic acid and caffeic acid have 1.5–7-fold higher inhibition potency for the *O*-methylation of 4-OH-E_2 than for the *O*-methylation of 2-OH-E_2. Notably, these two coffee polyphenols are significantly less potent than EGCG for inhibition of the human COMT-mediated *O*-methylation of catechol estrogens *in vitro*, but they are more potent when compared to catechin and epicatechin [13].

Our enzyme kinetic analyses showed that chlorogenic acid and caffeic acid inhibited human liver and placental COMT-mediated *O*-methylation of 2-OH-E_2 and 4-OH-E_2 with a mixed (competitive plus noncompetitive) mechanism of inhibition. We believe this mixed mechanism of COMT inhibition can be explained on the basis of the available data. Since the catechol-containing chlorogenic acid and caffeic acid (like tea catechins) are also substrates for human COMT, they will serve as competitive inhibitors for the COMT-mediated *O*-methylation of the catechol estrogen substrates. This suggestion was supported by the molecular computational analysis which showed that chlorogenic acid and caffeic acid can bind to the active site of COMT in a perfect substrate-binding conformation. The estimated binding affinity (based on the calculated binding energy values) of chlorogenic acid and caffeic acid for COMT is significantly higher than that of catechol estrogen substrates. The higher binding affinity of chlorogenic acid for COMT is partly attributable to the presence of the additional hydroxylated cyclohexane moiety, which increases the binding interactions with the amino acid residues inside the active site of the enzyme.

In addition, during the metabolic *O*-methylation of these catechol-containing coffee polyphenols by COMT, *S*-adenosyl-L-homocysteine (AdoHcy, the demethylated product of AdoMet)

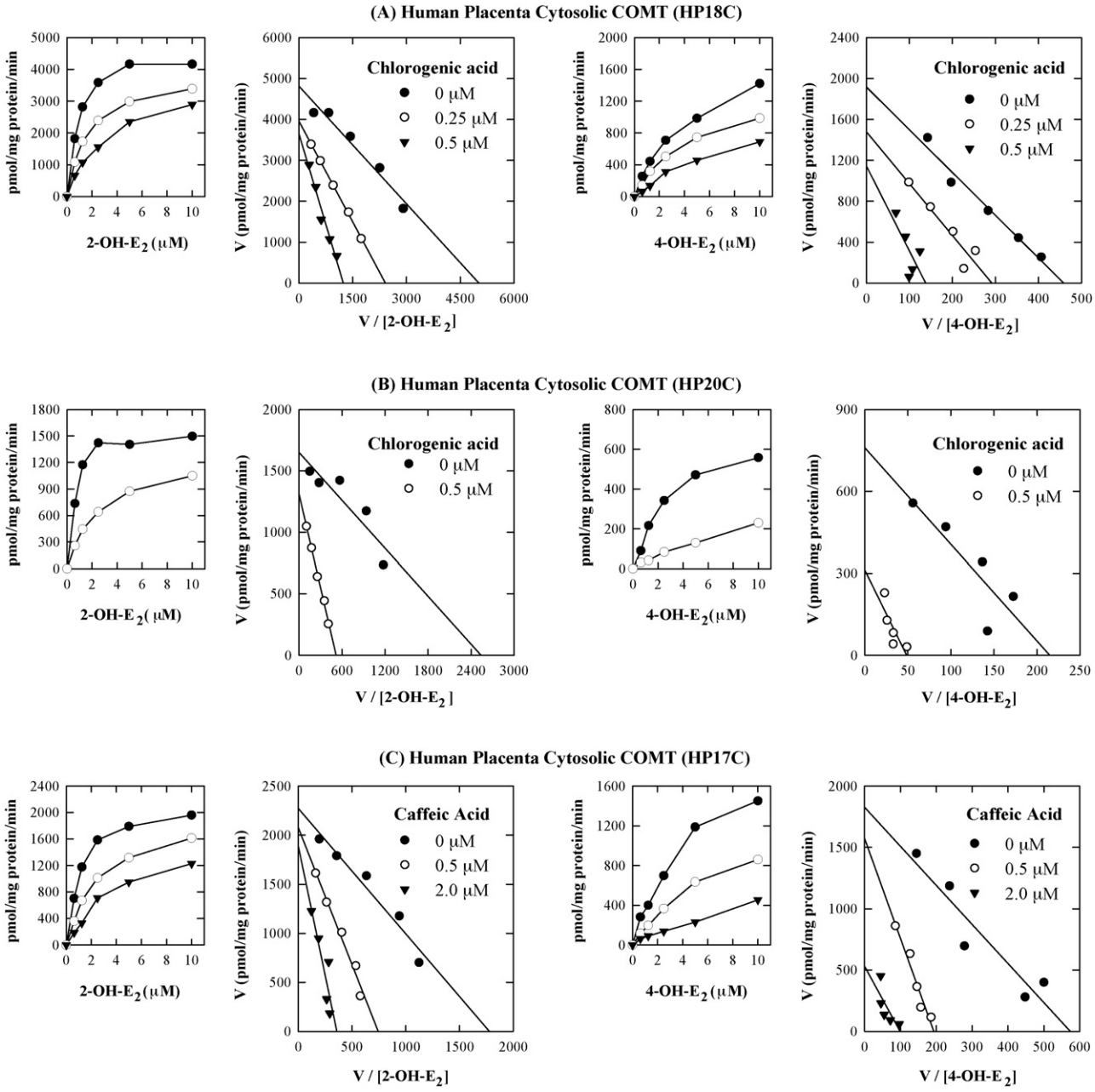

Fig. 5. Eadie–Hofstee plots for the inhibition of human placental cytosolic COMT-mediated *O*-methylation of 2-OH-E_2 and 4-OH-E_2 by chlorogenic acid (panels A and B) and caffeic acid (panel C). The left insets illustrate the substrate concentration dependence for the *O*-methylation of 2-OH-E_2 or 4-OH-E_2 in the presence of chlorogenic acid or caffeic acid. The incubation mixture consisted of 0–10 μM 2-OH-E_2 or 4-OH-E_2, chlorogenic acid or caffeic acid (at the indicated concentrations), 250 μM [methyl-^{3}H]AdoMet (containing 0.2 μCi), 0.5 mg/mL of placental cytosolic protein, 1 mM dithiothreitol, and 1.2 mM $MgCl_2$ in a final volume of 0.5 mL Tris–HCl buffer (10 mM, pH 7.4). The incubations were carried out at 37 °C for 20 min. Each point is the mean of duplicate determinations (with average variation <5%).

is formed in equimolar quantities with the methylated dietary polyphenols. AdoHcy is a strong noncompetitive inhibitor of the COMT-mediated *O*-methylation of catechol estrogens as well as other catechol substrates [8–10,12,17]. Therefore, we believe the noncompetitive component of enzyme inhibition is, in a significant part, due to increased formation of AdoHcy when two substrates are co-present, although the component of a direct noncompetitive inhibition by coffee polyphenols might also co-exist. In addition, the decreased availability of AdoMet may also partially add to the reduction of the catalytic activity when a dietary polyphenolic inhibitor is present along with a catechol estrogen substrate.

A number of earlier studies showed that several coffee polyphenols, e.g., chlorogenic acid and caffeic acid, could be readily absorbed in humans after drinking coffee, and they could reach rather high concentrations under certain conditions [18–21]. Since coffee polyphenols are often ingested in rather large quantities by those who regularly consume large amounts of coffee, it is expected that collectively these coffee polyphenols may exert a biologically meaningful inhibition of the *O*-methylation of various endogenous catechols *in vivo*. It will be of interest to determine in human subjects the extent of inhibition of the metabolic methylation of endogenously formed catechol estrogens and possibly other endogenous catechol substrates (such as catecholamines), and also to determine what are the biological consequences that are associated with the long-term inhibition of these metabolic pathways due to chronic consumption of large amounts of coffee.

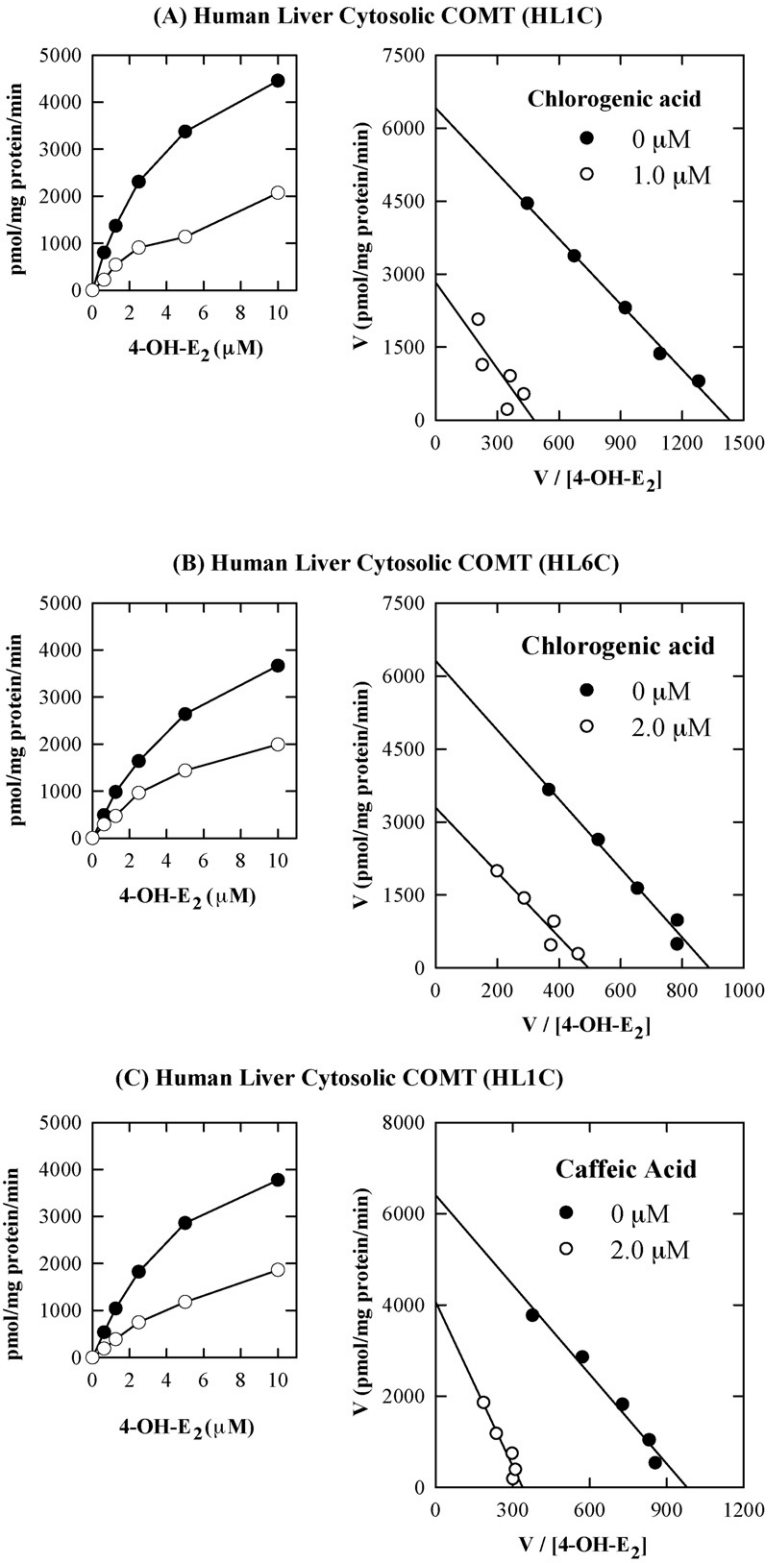

Fig. 6. Eadie–Hofstee plots for the inhibition of human liver cytosolic COMT-mediated *O*-methylation of 4-OH-E_2 by chlorogenic acid (panels A and B) and caffeic acid (panel C). The left insets illustrate the substrate concentration dependence for the *O*-methylation of 4-OH-E_2 in the presence of chlorogenic acid or caffeic acid. The incubation mixture consisted of 0–10 μM 4-OH-E_2, chlorogenic acid or caffeic acid (at the indicated concentration), 250 μM [methyl-^{3}H]AdoMet (containing 0.2 μCi), 0.5 mg/mL of liver cytosolic protein, 1 mM dithiothreitol, and 1.2 mM $MgCl_2$ in a final volume of 0.5 mL Tris–HCl buffer (10 mM, pH 7.4). The incubations were carried out at 37 °C for 20 min. Each point is the mean of duplicate determinations.

Earlier studies have suggested that a common COMT polymorphism (Val108 → Met108) that encodes an enzyme with a lower catalytic activity may be associated with increased risk of breast cancer in humans [22–28]. These observations are in line with the hypothesis that decreased methylation of endogenous 2- and 4-hydroxylated estrogens by dietary polyphenols would result in a decrease in the tissue levels of the anticarcinogenic 2-methoxyestradiol [1,7] plus an increase in the tissue levels of

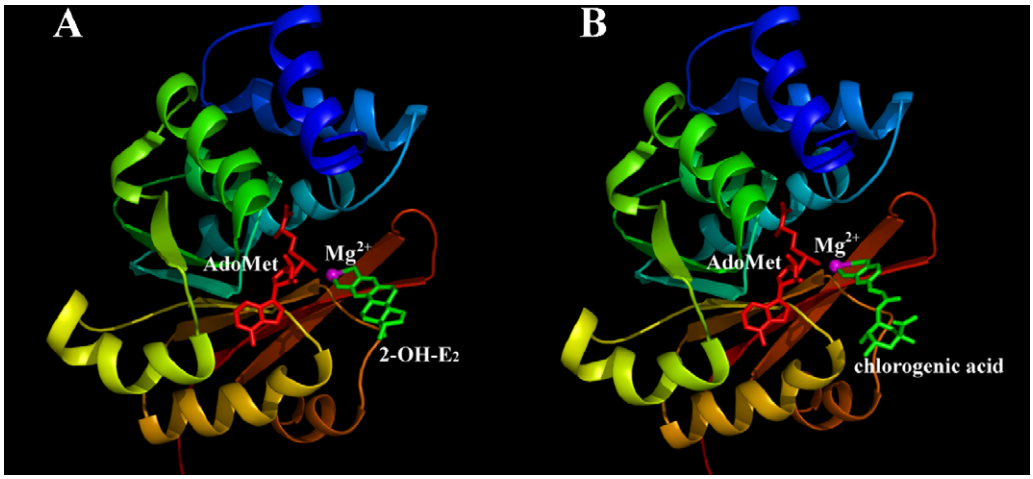

Fig. 7. Computational models for human S-COMT bound with 2-OH-E_2 (A) and chlorogenic acid (B). The red molecule shows AdoMet and the green molecule shows the ligand (2-OH-E_2 or chlorogenic acid). Hydrogens and water molecules were omitted from both structures. The atom with magenta color is Mg^{2+}. The secondary structure of human S-COMT is colored from blue (N-terminus) to red (C-terminus). This figure is drawn using the PyMOL software.

the potentially procarcinogenic 4-OH-E_2 [29–32]. These effects may increase the risk for developing estrogen-induced tumors in humans (discussed in Refs. [1,7]). Similarly, inhibition of COMT-mediated *O*-methylation of endogenous catechol estrogens by dietary polyphenols, if it should occur *in vivo*, is expected to have a similar effect. In partial support of this possibility, our earlier studies in an animal model have shown that chronic administration of dietary quercetin enhanced 17β-estradiol-induced, but not diethylstilbesterol (DES)-induced, kidney tumor formation in male Syrian hamsters (Ref. [15]; Zhu, unpublished data). It is of note that quercetin did not increase, but instead inhibited 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors in rats [29] and azoxymethanol-induced colonic neoplasms in mice [33]. Taken together, these results suggest that the selective potentiation of 17β-estradiol-induced carcinogenesis by quercetin may be, in part, due to its inhibition of the COMT-mediated *O*-methylation of catechol estrogens.

In summary, the results of our present study showed that chlorogenic acid and caffeic acid are rather strong inhibitors of the *O*-methylation of 2-OH-E_2 and 4-OH-E_2 catalyzed by human liver and placental cytosolic COMT. Enzyme kinetic studies revealed a mixed (competitive plus noncompetitive) mechanism of enzyme inhibition by these dietary polyphenols. Molecular computational modeling analysis showed that chlorogenic acid and caffeic acid can bind to human S-COMT at the active site in a similar manner as the catechol estrogen substrates. Moreover, these two coffee polyphenols have higher binding affinity for the enzyme than the endogenous catechol estrogen substrates, which agreed perfectly with our biochemical data. More studies are needed to determine the extent of such inhibition in human subjects as well as its potential long-term health consequences.

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