O-Methylation of Catechol Estrogens by Human Placental Catechol-O-Methyltransferase: Interindividual Differences in Sensitivity to Heat Inactivation and to Inhibition by Dietary Polyphenols

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ABSTRACT:

The human catechol-O-methyltransferase (COMT) is a polymorphic enzyme that catalyzes the O-methylation of catechol estrogens. Recent animal studies showed that placental COMT is involved in the development of placentas and embryos, probably via the formation of 2-methoxyestradiol. In this study, we analyzed a total of 36 human term placentas to determine their cytosolic COMT activity for the O-methylation of catechol estrogens as well as their sensitivity to inhibition by heat and dietary compounds. Large variations (up to 4-fold) in the COMT activity for the formation of methoxyestrogens were noted with different human placental samples. The cytosolic COMTs in different human placentas also displayed considerable differences in their sensitivity to heat

inactivation. This differential sensitivity was not associated with the overall catalytic activity for the O-methylation of catechol estrogen substrates. It was observed that there was a positive correlation ($r = 0.760$) between the sensitivity of the human placental COMT to heat inactivation and its sensitivity to inhibition by (–)-epigallocatechin-3-gallate (a well known tea polyphenol with COMT-inhibiting activity) but an inverse correlation ($r = 0.544$) between heat inactivation and inhibition by quercetin (another dietary COMT inhibitor). The differences in inhibition by these two dietary compounds are due to different mechanisms of COMT inhibition involved.

Introduction

Endogenous estrogens, such as 17 β -estradiol (E₂) and estrone, undergo rapid oxidative metabolism in humans, mostly catalyzed by cytochrome P450 enzymes, resulting in the formation of hydroxylated or keto metabolites (Zhu and Conney, 1998a; Zhu, 2002). Catechol estrogens, such as 2-hydroxyestradiol (2-OH-E₂) and 4-hydroxyestradiol (4-OH-E₂), are among the major oxidative estrogen metabolites formed in the liver. These estrogen metabolites are further O-methylated to form corresponding monomethyl ethers, catalyzed by catechol-O-methyltransferase (COMT) using S-adenosyl-L-methionine (AdoMet) as the methyl donor (depicted in Fig. 1).

During human pregnancy, there is a dramatic increase in the daily production of endogenous estrogens. The placenta contributes significantly to the biosynthesis of endogenous estrogens. This organ also

contains rather high levels of estrogen 2-hydroxylase (Osawa et al., 1993; Zhu, 2002) and COMT (Boadi et al., 1992; Zhu et al., 2000, 2009), and the concerted actions of these two estrogen-metabolizing enzymes lead to increased formation of 2-methoxyestradiol (2-MeO-E₂). Earlier studies have shown that the concentration of 2-MeO-E₂ increased considerably during human pregnancy, and its circulating level at the third trimester was approximately 10 nM (Berg et al., 1983). However, the levels of 2-MeO-E₂ and COMT were found to be significantly lower in women with preeclampsia (Rosing and Carlström, 1984; Barnea et al., 1988). A recent study using *Comt*(–/–) mice provided definitive evidence showing that COMT is important for the normal development of the placentas and embryos (Kanasaki et al., 2008). Furthermore, administration of 2-MeO-E₂ could effectively rescue the pregnant *Comt*(–/–) phenotype in these mice, thus suggesting that COMT plays an important role during pregnancy via the formation of 2-MeO-E₂. In that same study, it was shown that COMT and 2-MeO-E₂ also play a role in regulating the uteroplacental vascular homeostasis, blood pressure, kidney glomerular structure, and hypoxia response during pregnancy. In addition to these physiological actions, many earlier studies have shown that 2-MeO-E₂ has strong anticancer activity both in vitro and in vivo (Zhu and Conney, 1998b; Zhu, 2002), and these observations have led to the speculation

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ABBREVIATIONS: E₂, 17 β -estradiol; 2-OH-E₂, 2-hydroxyestradiol; 4-OH-E₂, 4-hydroxyestradiol; COMT, catechol-O-methyltransferase; AdoMet, S-adenosyl-L-methionine; 2-MeO-E₂, 2-methoxyestradiol; EGCG, (–)-epigallocatechin-3-gallate; AdoHcy, S-adenosyl-L-homocysteine.

FIG. 1. Metabolic formation of catechol estrogens and COMT-mediated further *O*-methylation of the catechol metabolites. Note that only the structures for 2-OH-E₂ and its methylated products are shown as examples. The formation of 4-OH-E₂ and its *O*-methylation of endogenous catechol estrogens is subject to modulation by various endogenous factors (such as catecholamines, *S*-adenosyl-L-homocysteine, and homocysteine) as well as exogenous factors (such as various catechol-containing dietary polyphenolic compounds). CYP, cytochrome P450 enzymes; 2-OH-3-MeO-E₂, 2-hydroxyestradiol 3-methyl ether.

that a marked increase in the production of 2-MeO-E₂ during human pregnancy may provide an important endogenous protective mechanism against the development of estrogen-inducible tumors in various target organs.

It has been known for years that human COMT is a polymorphic enzyme. Genetic analysis showed that the V108M variant of soluble COMT (*S*-COMT) (or V158M variant of membrane-bound COMT) is a common variation in the *COMT* gene resulting from a single nucleotide mutation (Lachman et al., 1996; Shield et al., 2004). This mutated COMT had significantly lower catalytic activity than the wild-type enzyme. An earlier study also showed that the maternal *COMT* polymorphism with a low enzyme activity was associated with an increased occurrence of fetal growth restriction, in addition to maternal preeclampsia (Sata et al., 2006).

In addition to catalyzing the *O*-methylation of catechol estrogens, COMT also catalyzes the *O*-methylation of endogenous catecholamines

as well as that of many other catechol-containing xenobiotics, owing to its broad substrate specificity (Axelrod and Tomchick, 1958; Zhu, 2002). Therefore, the rate of catechol estrogen *O*-methylation *in vivo* is subject to modulation by other endogenous substrates and in particular exogenous dietary catechol substrates that are usually present in much larger quantities. However, it is not known whether the polymorphic COMT enzyme in human placenta has differential sensitivity to inhibition by various dietary catechols. In the present study, we compared the *O*-methylation of 2-OH-E₂ and 4-OH-E₂ *in vitro* catalyzed by the cytosolic COMT from a total of 36 human term placentas. In addition, we also analyzed their differential sensitivity to heat inactivation as well as to inhibition by representative polyphenolic components present in our diet.

Materials and Methods

Chemicals. 2-OH-E₂, 4-OH-E₂, AdoMet, dithiothreitol, (–)-epigallocatechin-3-gallate (EGCG), and quercetin were purchased from Sigma-Aldrich (St. Louis, MO). [Methyl-³H]AdoMet (specific activity 11.2–13.5 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). All solvents used in this study were of high-performance liquid chromatography-grade or better and were obtained from Thermo Fisher Scientific (Waltham, MA).

Preparation of Human Placental Cytosolic Fraction. Human term placental samples were obtained from 36 whites (at 36–40 weeks of gestation) after normal vaginal delivery at the St. Peter's University Hospital (New Brunswick, NJ). The mean age of the 36 participants was 32.4 ± 9.5 years (mean ± S.D.). The procedure for procurement of human term placenta samples was approved by the institutional review boards of the University of South Carolina (Columbia, SC), Rutgers University (Piscataway, NJ), University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School (New Brunswick, NJ), and the St. Peter's University Hospital (New Brunswick, NJ). Within 30 min after the placenta was expelled, a portion (50–100 g) was collected and snap-frozen in liquid nitrogen for transport to the laboratory for storage in a –80°C freezer until it was used for the preparation of placental cytosol.

On the day of preparation of cytosolic fraction, the placental samples were first thawed at room temperature and then rinsed with ice-cold normal saline to remove blood clots. The chorionic membrane tissues were removed with a pair of sharp eye surgery scissors. The tissues were then minced in 3 volumes of ice-cold 0.1 M Tris-HCl and 0.1% KCl solution and were then homogenized with a Tri-R homogenizer (model K41) for 2 to 3 min followed by a Teflon homogenizer for another 2 to 3 min. Tissue homogenates were centrifuged at 9000g (4°C) for 10 min, and supernatants were pooled and filtered through two layers of cheesecloth to remove lipid clots. The filtrates were then recentrifuged at 105,000g (4°C) for 90 min. The supernatants (the cytosolic fractions from each placenta) were combined, and aliquots of the cytosol preparations were stored at –80°C until use. The protein concentration was determined by using a protein assay kit (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as standard.

Human Placental COMT-Mediated *O*-Methylation of 2-OH-E₂ and 4-OH-E₂. The COMT-catalyzed *O*-methylation of catechol estrogens was performed as described previously (Zhu et al., 2000, 2009). The reaction mixture consisted of 0.25 mg of human placenta cytosolic protein, 1.2 mM MgCl₂, 250 μM AdoMet iodide (containing ~0.2 μCi of [methyl-³H]AdoMet), 1 mM dithiothreitol, and various concentrations of 2-OH-E₂

FIG. 2. Substrate concentration dependence for the *O*-methylation of 2-OH-E₂ and 4-OH-E₂ catalyzed by four human placental cytosolic COMT preparations. The incubation mixture consisted of 0 to 36 μM 2-OH-E₂ or 4-OH-E₂, 250 μM AdoMet (containing 0.2 μCi of [methyl-³H]AdoMet), 0.5 mg/ml placental cytosolic protein, 1 mM dithiothreitol, and 1.2 mM MgCl₂ in a final volume of 0.5 ml of Tris-HCl buffer (10 mM, pH 7.4). The incubations were performed at 37°C for 10 min. Each point is the mean of duplicate determinations (average variation <5%).

FIG. 3. Human placental cytosolic COMT-mediated *O*-methylation of 2-OH-E₂ and 4-OH-E₂ (top panel) and their sensitivity to heat inactivation at 42°C (middle panel) and 46°C (bottom panel). The incubation mixture consisted of 10 μ M 2-OH-E₂ or 4-OH-E₂, 250 μ M [methyl-³H]AdoMet (containing 0.2 μ Ci), 0.5 mg/ml human placental cytosolic protein, 1 mM dithiothreitol, and 1.2 mM MgCl₂ in a final volume of 0.25 ml of Tris-HCl buffer (10 mM, pH 7.4). Incubations were performed at 37°C for 10 min. For heat inactivation, the cytosolic proteins were first incubated at 42 or 46°C for 15 min before analysis of their catalytic activity. Each point is the mean of duplicate determinations (with variations <5%). H/C refers to the ratio of the catalytic activity (H) of COMT that was preincubated at 42°C or 46°C for 15 min over the control catalytic activity that was preincubated at 30°C for 15 min.

or 4-OH-E₂ in 0.5 ml of Tris-HCl buffer (10 mM, pH 7.4). For the inhibitor experiments, the incubation mixture also contained the inhibitor at the indicated concentrations. The reaction was initiated by addition of placental cytosolic protein and performed at 37°C for 10 to 45 min. Each reaction was run in duplicate. The reaction was arrested by immediate cooling to ice-cold temperatures, addition of 50 μ l of NaOH (0.5 M), and extraction with 3 ml of ice-cold toluene. After centrifugation at 1000g for 10 min, portions of the organic extracts (2 ml) were analyzed for radioactivity content by liquid scintillation counting (model LS 5000TD; Beckman Coulter, Fullerton, CA).

The rate for the enzymatic *O*-methylation of 2-OH-E₂ and 4-OH-E₂ *in vitro* was expressed as picomoles of the methyl ethers formed by 1 mg of total cytosolic protein in 1 min (abbreviated as pmol/mg protein/min). The enzyme kinetics (K_m and V_{max}) for the *O*-methylation of these catechol estrogens were calculated using the curve regression program of SigmaPlot software (version 9; Systat Software, Inc., San Jose, CA).

Temperature Inhibition Studies. To determine the temperature stability of placental COMT, the cytosolic enzyme fraction was preincubated in a water bath set at a given temperature for 15 min, and then the enzyme was immediately assayed for its catalytic activity for the *O*-methylation of catechol estrogen substrates *in vitro*. The corresponding control enzyme was preincubated at 30°C for 15 min. The remaining catalytic activity of the enzyme after preincubation was expressed as a percentage of the control enzyme activity (without preincubation).

Results

Human Placental COMT-Mediated *O*-Methylation of 2-OH-E₂ and 4-OH-E₂. We first determined the effect of different incubation time, protein concentration, AdoMet concentration, and reaction pH on the rate of *O*-methylation of 2-OH-E₂ and 4-OH-E₂ for several representative placenta COMT preparations (data not shown). The optimized conditions for the *in vitro* enzymatic *O*-methylation included an incubation time of 10 min, a final protein concentration at 0.5 mg/ml for the placental cytosolic protein preparation, an AdoMet concentration at 250 μ M, and the reaction pH at 7.4.

Next, we determined the K_m and V_{max} values for the cytosolic COMT from several representative human placentas (Fig. 2). Based on the curve patterns, it was apparent that there was autoinhibition of the enzyme activity when high concentrations of the substrates were present. The apparent K_m values for placentas 1, 4, and 7 were approximately 2 μ M, and plateaus were reached when substrate concentrations reached approximately 10 μ M. The apparent K_m value for placenta 8 was substantially lower, at approximately 1 μ M, and a plateau was reached at approximately 5 μ M. The same assay for this placenta was repeated twice, and similar results were obtained.

With use of 10 μ M 2-OH-E₂ and 4-OH-E₂ as substrates, the

FIG. 4. The sensitivity of eight representative human placental cytosolic preparations (placentas 3, 5, 7, 13, 18, 20, 24, and 33) to a 10-min heat inactivation at 37 to 50°C. The incubation mixture consisted of 10 μ M 2-OH-E₂ or 4-OH-E₂, 250 μ M AdoMet (containing 0.2 μ Ci of [methyl-³H] AdoMet), 0.5 mg/ml human placental cytosolic protein, 1 mM dithiothreitol, and 1.2 mM MgCl₂ in a final volume of 0.25 ml of Tris-HCl buffer (10 mM, pH 7.4). Incubations were performed at 37°C for 15 min. Each point is the mean of duplicate determinations (with variations <5%).

catalytic activity for the cytosolic COMT prepared from a total of 36 human term placentas was determined (data are summarized in Fig. 3, top panel). Considerable variations in the COMT activity were noted with different placenta samples, with activity for the *O*-methylation of 2-OH-E₂ and 4-OH-E₂ ranging from 48 to 197 and 35 to 125 pmol/mg protein/min, respectively.

Differential Sensitivity to Heat Inactivation. Human cytosolic COMT is a polymorphic enzyme. Earlier studies with human liver and red blood cells have shown that the low-activity form of the COMT is slightly more sensitive to heat inactivation than its high-activity form (Scanlon et al., 1979; Weinshilboum and Dunnette, 1981). Using eight representative human placenta samples, we determined in the present study their sensitivity to inhibition by gradually increasing the preincubation temperatures from 30 to 50°C (Fig. 4). Our data showed that when the cytosolic fraction of the human placentas was preincubated for 10 min at 35 to 50°C, the COMT activities for the *O*-methylation of 2-OH-E₂ and 4-OH-E₂ were each inhibited in a temperature-dependent manner. However, the sensitivity of different placental cytosols to a given temperature showed substantial differences. Although some of the samples were more sensitive to heat inactivation, with IT₅₀ values (the preincubation temperature that deactivates 50% of the catalytic activity of COMT) at approximately 40°C, other samples were less sensitive (with the IT₅₀ values of as high as 47°C). For the same placenta sample, the pattern of heat inhibition of the *O*-methylation of 2-OH-E₂ and 4-OH-E₂ remained almost the same, although the pattern for different placenta samples varied considerably.

Based on the above data, next we chose to determine the sensitivity of all 36 human placenta cytosolic COMT preparations to heat inactivation at two selected preincubation temperature points (42 and 46°C) (data shown in Fig. 3, middle and bottom panels). Our data showed that the COMT present in different human placenta samples had markedly different sensitivity to heat inactivation. There was no apparent correlation between the apparent COMT activity present in the cytosolic fraction of different human placentas and the sensitivity to heat inactivation either at 42 or 46°C (Fig. 5, top and middle

panels). However, there was a high degree of correlation between the sensitivity to heat inactivation at 42 and 46°C (Fig. 5, bottom panel).

We also determined the effect of heat treatment on the kinetic parameters for the *O*-methylation of 2-OH-E₂ and 4-OH-E₂ by two representative placenta samples (placentas 13 and 15). Heat treatment had nearly the same effect on the *O*-methylation of 2-OH-E₂ and 4-OH-E₂. For instance, heat treatment at 42°C not only decreased the apparent $V_{\max}$ values of the enzyme for the *O*-methylation of 2-OH-E₂ and 4-OH-E₂, but it also increased its apparent K_m values (data not shown).

In summary, the cytosolic COMT activity in different human placentas had substantial differences in sensitivity to heat inactivation. This differential sensitivity appears to be dissociated from the overall catalytic activity for the *O*-methylation of catechol estrogen substrates.

Differential Sensitivity to Inhibition by Dietary Polyphenols.

We showed earlier that many catechol-containing dietary polyphenols are excellent substrates for placental COMT that *O*-methylates catechol estrogens (Zhu et al., 2000). In the present study, we compared the sensitivity of all 36 placental cytosolic COMT preparations to inhibition by two representative dietary polyphenols, EGCG and quercetin, using 4-OH-E₂ as a substrate (data are summarized in Figs. 6 and 7). We found that there was a positive correlation ($r = 0.760$) between the sensitivity of the human placental COMT to heat inactivation and the sensitivity to inhibition by EGCG (a common tea polyphenol) (Fig. 6). There was an inverse relationship ($r = 0.544$) between these two when quercetin (a dietary bioflavonoid) was tested as a COMT inhibitor, i.e., the higher sensitivity of the placental COMT to heat inactivation was associated with decreased sensitivity to inhibition by quercetin.

Our earlier studies showed that EGCG and quercetin inhibit human COMT-mediated *O*-methylation of catechol estrogens via different mechanisms (Zhu and Liehr, 1994, 1996; Zhu et al., 1994). Whereas EGCG inhibits COMT mainly through direct binding to the enzyme, quercetin (a catechol-containing bioflavonoid) inhibits COMT through a combination of two mechanisms: one was through the

FIG. 5. Lack of correlation between total COMT catalytic activity and the heat sensitivity (top and middle panels) from 36 human placental cytosolic preparations. The bottom panel shows the correlation between the sensitivity to heat inactivation at 46 or 42°C. The original data were obtained from Fig. 3. H/C refers to the ratio of the catalytic activity (H) of COMT that was preincubated at 42°C or 46°C for 15 min over the control catalytic activity that was preincubated at 30°C for 15 min.

formation of *S*-adenosyl-L-homocysteine (AdoHcy) as a result of its own rapid *O*-methylation catalyzed by COMT (a major mechanism), and the other one was its direct competitive inhibition of the enzyme by serving as a substrate. Therefore, we also determined the sensitivity of these human placental COMT preparations to inhibition by AdoHcy (data are shown in Fig. 8). We found that the sensitivity to AdoHcy inhibition was also inversely correlated with their sensitivity to heat inactivation, which was similar to the pattern seen with the differential sensitivity to inhibition by quercetin.

Discussion

A recent study showed that COMT, probably via the formation of 2-MeO-E₂, plays an important role in the development of placenta and embryos as well as in the regulation of uteroplacental vascular homeostasis, blood pressure, kidney glomerular structure, and hypoxia response during pregnancy (Kanasaki et al., 2008). In the present study, we analyzed the cytosolic COMT activity contained in 36 human term placenta samples to evaluate their differential sensitivity to inhibition by heat and dietary polyphenolic compounds. We found that the cytosolic COMT activity in different human placentas dis-

played a large interindividual variation, with up to 4-fold differences noted. In addition, substantial differences (up to 3-fold) were also noted in their sensitivity to heat inactivation. These observations are mostly in agreement with an earlier study showing that considerable interindividual variability existed in human placental COMT activity (Barnea et al., 1988).

Earlier studies of the human COMT activity in red blood cells (Scanlon et al., 1979) and also other tissues (Sladek-Chelgren and Weinshilboum, 1981) had led to the discovery of a common polymorphism in the two codominant alleles of the *COMT* gene. Grossman et al. (1992) and Goodman et al. (2002) later found that the Val108 and Met108 variations of the human COMT correlated with the phenotypes of high and low levels of COMT catalytic activity, respectively. However, a more detailed analysis of the catalytic activity of these two forms of COMT revealed a similar catalytic property, although the Met108 variant was more thermolabile (37°C), which could be stabilized by AdoMet binding (Weinshilboum and Dunnette, 1981). Studies have also shown that there was a significant correlation between the COMT heated/control ratios and the levels of enzyme activity in the lysates prepared from hepatic tissue and red blood cells. However, the results of our present study with human term placental samples did not show a significant correlation between the COMT heated/control ratios and the enzyme activity (Fig. 5). This probably suggests that factors other than genetic polymorphism, such as differences in the levels of *COMT* gene expression in the placentas of different individuals and/or in the post-translational modifications of the enzyme proteins, may contribute to the difference in the overall cytosolic COMT activity and/or thermolability of the enzyme in human placentas.

Many catechol-containing dietary polyphenols (including those tested in the present study) can serve as both substrates and inhibitors of the human and rodent COMTs with different mechanisms (Zhu and Liehr, 1994, 1996; Zhu et al., 1994). An earlier study showed that EGCG inhibited the COMT activity mainly through tight binding interaction with the catalytic site of the enzyme, whereas the inhibitor itself was essentially not methylated by the enzyme (Zhu et al., 2008). In comparison, quercetin is a good substrate of COMT (Zhu and Liehr, 1994), and our earlier study showed that it inhibits the COMT activity largely via the formation of AdoHcy (a noncompetitive feedback inhibitor of the COMT). In this study, we also tested, for comparison, the ability of these dietary compounds to inhibit the *O*-methylation of 4-OH-E₂ catalyzed by representative human placental cytosolic COMTs that exhibited differential sensitivity to heat inactivation. We found that there was a marked difference in the sensitivity of different human placental samples to inhibition by these two dietary compounds. A positive correlation was noted between the sensitivity of the cytosolic COMT in different human placentas to heat inactivation and its sensitivity to EGCG inhibition. However, an inverse correlation was seen between the two when quercetin or AdoHcy was used as the inhibitor. The similarity between the inhibition patterns of quercetin and AdoHcy supports the mechanistic explanation that COMT inhibition by quercetin is mostly mediated by the formation of AdoHcy. Accordingly, it was suggested that whereas EGCG might have a relatively higher binding affinity for the thermo-unstable COMT, AdoHcy might have a relatively higher binding affinity for the thermo-stable COMT. This explanation was supported by our recent study (Bai et al., 2008) showing that a novel mutant form of the human COMT protein that also exhibits an increased sensitivity to heat inactivation has a decreased sensitivity to AdoHcy inhibition compared with the wild-type COMT.

It should be noted that in ancient Chinese folklore medicine, pregnant women, particularly at late stages of gestation, were strongly

FIG. 6. Comparison of the sensitivity of 36 placental cytosolic COMT preparations to inhibition by EGCG. The incubation mixture consisted of 10 μ M 4-OH-E₂, 250 μ M AdoMet (containing 0.2 μ Ci of [methyl-³H]AdoMet), 0.1 μ g/ml EGCG, 0.5 mg/ml placental cytosolic protein, 1 mM dithiothreitol, and 1.2 mM MgCl₂ in a final volume of 0.5 ml of Tris-HCl buffer (10 mM, pH 7.4). The incubations were performed at 37°C for 15 min. Each point is the mean of duplicate determinations (average variation <5%). H/C refers to the ratio of the catalytic activity (H) of COMT that was preincubated at 42°C or 46°C for 15 min over the control catalytic activity that was preincubated at 30°C for 15 min.

FIG. 7. Comparison of the sensitivity of 36 placental cytosolic COMT preparations to inhibition by quercetin. The incubation mixture consisted of 10 μ M 4-OH-E₂, 250 μ M AdoMet (containing 0.2 μ Ci [³H-methyl] AdoMet), 2 μ M quercetin, 0.5 mg/ml placental cytosolic protein, 1 mM dithiothreitol, and 1.2 mM MgCl₂ in a final volume of 0.5 ml of Tris-HCl buffer (10 mM, pH 7.4). The incubations were performed at 37°C for 15 min. Each point is the mean of duplicate determinations (average variation <5%). H/C refers to the ratio of the catalytic activity (H) of COMT that was preincubated at 42°C or 46°C for 15 min over the control catalytic activity that was preincubated at 30°C for 15 min.

FIG. 8. Comparison of the sensitivity of 36 placental cytosolic COMT preparations to inhibition by AdoHcy. The incubation mixture consisted of 10 μ M 4-OH- E_2 , 250 μ M AdoMet (containing 0.2 μ Ci of [methyl- 3 H]AdoMet), 5 μ M AdoHcy, 0.5 mg/ml placental cytosolic protein, 1 mM dithiothreitol, and 1.2 mM $MgCl_2$ in a final volume of 0.5 ml of Tris-HCl buffer (10 mM, pH 7.4). The incubations were performed at 37°C for 15 min. Each point is the mean of duplicate determinations (average variation <5%). H/C refers to the ratio of the catalytic activity (H) of COMT that was preincubated at 42°C or 46°C for 15 min over the control catalytic activity that was preincubated at 30°C for 15 min.

advised against drinking concentrated tea, for it was believed that drinking tea would cause various abnormalities, ranging from pregnancy complications and miscarriages to stillbirth. Recent studies have made a similar observation that pregnant women in Western countries who consumed four to seven cups of coffee a day have a markedly increased risk for stillbirth and fetal abnormalities (Bech et al., 2005). Although the mechanisms for the adverse effects of drinking tea and coffee on human pregnancy are still not understood, it has been suggested that these adverse effects might be partially associated with the intake of caffeine from these beverages. Because the polyphenolic components abundantly present in tea and coffee can modulate DNA methylation and epigenetic control of gene expression (Fang et al., 2003; Lee et al., 2005; Lee and Zhu, 2006; Yang et al., 2008), it has also been suggested that alterations of these vital processes during pregnancy and fetal development may contribute to the occurrence of the adverse effects. The ability of the dietary phenolic compounds to inhibit the placental COMT activity as well as the formation of methoxyestrogens (such as 2-MeO- E_2), as observed in the present study, is probably another factor that may contribute to the development of adverse effects associated with tea and coffee beverages during pregnancy. In this context, it is of note that because placentas from different women have rather wide differences in their sensitivity to inhibition by dietary polyphenolic compounds, these differences may partially contribute to the differential sensitivity of pregnant women to the adverse actions of drinking tea or coffee beverages.

Last, many earlier studies have shown that 2-MeO- E_2 has strong apoptotic, antiangiogenic, and antitumorigenic activities (Zhu and Conney, 1998; Pribluda et al., 2000; Fukui and Zhu, 2009). Because the circulating 2-MeO- E_2 levels are dramatically increased during

human pregnancy, it has been speculated that the large amounts of 2-MeO- E_2 formed during pregnancy may be an important endogenous protective factor against the development of estrogen-inducible tumors in various target organs (discussed by Zhu and Conney, 1998; Zhu, 2002). In partial support of this notion, an earlier study in an animal model showed that chronic administration of dietary quercetin enhanced 17 β -estradiol-induced, but not diethylstilbesterol-induced, kidney tumor formation in male Syrian hamsters (Zhu and Liehr, 1994; B.T. 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 (Verma et al., 1988) and azoxymethanol-induced colonic neoplasms in mice (Deschner et al., 1991). These results suggest that the selective enhancing effect of quercetin on 17 β -estradiol-induced carcinogenesis may be due to its inhibitory effect on the COMT-mediated *O*-methylation metabolism of catechol estrogens. Likewise, human epidemiological studies have also shown that the occurrence of the low-activity COMT polymorphism, under certain conditions, is associated with an increased risk for development of human hormone-associated cancers (Lavigne et al., 1997; Thompson et al., 1998).

In summary, we found that there are large person-to-person differences in the cytosolic COMT activity and sensitivity to heat inactivation with different human placenta samples. This differential sensitivity was not associated with the overall catalytic activity for the *O*-methylation of the catechol estrogen substrates. Overall, there was a positive correlation between the sensitivity of the human placental COMT to heat inactivation and its sensitivity to inhibition by EGCG, but an inverse relationship between heat inactivation and inactivation by quercetin. The difference was due to the different mechanisms of COMT inhibition exerted by EGCG and quercetin. Our data suggest

that the placental cytosolic COMT activities from different individuals have different sensitivities to heat inactivation as well as to inhibition by dietary compounds. The observed interindividual differences may contribute to the person-to-person differences in the sensitivity of pregnant women to the possible adverse effects associated with tea or coffee drinking during pregnancy.

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