STRONG INHIBITORY EFFECTS OF COMMON TEA CATECHINS AND BIOFLAVONOIDs ON THE O-METHYLATION OF CATECOL ESTROGENS CATALYzed BY HUMAN LIVER CYTOSOLIC CATECHOL-O-METHYLTRANSFERASE

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Received November 24, 2003; accepted January 29, 2004

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

In the present investigation, we studied the inhibitory effects of three tea catechins [catechin, epicatechin, and (−)-epigallocatechin-3-O-gallate] and two bioflavonoids (quercetin and fisetin) on the O-methylation of 2- and 4-hydroxyestradiol (2-OH-E2 and 4-OH-E2, respectively) by human liver cytosolic catechol-O-methyltransferase (COMT). We found that catechin and epicatechin each inhibited the O-methylation of 2-OH-E2 and 4-OH-E2 in a concentration-dependent manner. The IC50 values for inhibition of 2-OH-E2 methylation by catechin and epicatechin were 14 to 17 µM and 44 to 65 µM, respectively, and their IC50 values for inhibition of 4-OH-E2 methylation were 5 to 7 µM and 10 to 18 µM, respectively. Our data showed that these two catechins had 2- to 6-fold higher inhibition potency for the O-methylation of 4-OH-E2 than for the O-methylation of 2-OH-E2. (−)-Epigallocatechin-3-O-gallate was found to have a distinctly high inhibition potency for the O-methylation of 2- and 4-OH-E2 (IC50 values of 0.04–0.07 µM and 0.2–0.5 µM, respectively). The crude extracts from green tea and black tea also showed very strong activity in inhibiting human liver COMT-mediated O-methylation of catechol estrogens. We also determined, for comparison, two common bioflavonoids (quercetin and fisetin) for their inhibitory effects on human liver COMT-mediated O-methylation of catechol estrogens. The IC50 values for quercetin and fisetin were 0.9 to 1.5 µM and 3.3 to 4.5 µM, respectively, for inhibiting the O-methylation of 2-OH-E2 and 0.5 to 1.2 µM and 2.6 to 4.2 µM, respectively, for inhibiting the O-methylation of 4-OH-E2. Enzyme kinetic analyses showed that both tea catechins and bioflavonoids inhibited human liver COMT-mediated O-methylation of 4-OH-E2 (a representative substrate) with a mixed mechanism of inhibition (competitive plus noncompetitive). In summary, the catechol-containing tea catechins and bioflavonoids are strong inhibitors of human liver COMT-mediated O-methylation of catechol estrogens. More studies are warranted to determine the extent of such inhibition in human subjects and the potential biological consequences.

In humans, catechol estrogens such as 2- and 4-hydroxyestradiol (2-OH-E2 and 4-OH-E2) are rapidly O-methylated to form monomethyl ethers (structures shown in Fig. 1) catalyzed by catechol-O-methyltransferase (COMT) using S-adenosyl-L-methionine (SAM) as the methyl donor (reviewed by Zhu and Conney, 1998). Metabolic O-methylation of catechol estrogens has been recognized since the 1950s, but it was unclear why the human body would produce large amounts of more lipophilic estrogen metabolites that have longer half-lives (t1/2) than the parent hormone but essentially are devoid of estrogenic activity. Earlier studies on the potential genotoxicity of catechol estrogens have led to the suggestion that this metabolic O-methylation may provide an additional or alternative mechanism of detoxification for the chemically reactive catechol estrogen intermediates, as was commonly accepted for catecholamines. However, studies in the past decade have also shown that 2-methoxyestradiol (the major O-methylation product of 2-OH-E2) has strong apoptotic, antiangiogenic, and anticancer activities (Zhu and Conney, 1998; Pribulda et al., 2000). Therefore, the metabolic O-methylation of catechol estrogens may not only inactivate the chemically reactive catechol estrogen intermediates, but it may also simultaneously generate estrogen derivatives with potential anticancer activities. These two concurrent processes are thought to be beneficial for protection against estrogen-induced tumorigenesis (Zhu and Conney, 1998).

Since COMT also catalyzes the O-methylation of endogenous catecholamines as well as many other catechol-containing xenobiotics (Axelrod and Tomchick, 1958; Axelrod, 1966; Zhu et al., 1994, 2000, 2001; Zhu, 2002), the rate for O-methylation of catechol estrogen intermediates in vivo may be subject to modulation by other endogenous or exogenous catechol substrates that are present in significant quantities. An earlier study by Zhu and Liehr (1993) showed that very high concentrations of endogenous catecholamines supported in part by grants from the National Institutes of Health (RO1 CA 97109), the American Parkinson Disease Association, and the California Table Grape Commission.

Abbreviations used are: 2-OH-E2, 2-hydroxyestradiol; 4-OH-E2, 4-hydroxyestradiol; COMT, catechol-O-methyltransferase; SAM, S-adenosyl-L-methionine; EGCG, (−)-epigallocatechin-3-O-gallate; BTP, black tea polyphenol(s); GTP, green tea polyphenol(s); SAH, S-adenosylhomocysteine.

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Printed in U.S.A.
Fig. 1. Metabolic formation of catechol estrogens and the COMT-mediated further O-methylation of the catechol metabolites.

Note that only the structures of 2-OH-E₂ and its methylated products are shown as examples. The metabolic formation of 4-OH-E₂ and its methylation follows the same metabolic pathways. As depicted, the metabolic 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).

(substrates and inhibitors of COMT) appeared to be selectively present in the target organs of estrogen-induced tumorigenesis in several well known animal models. Additional studies by Zhu and Liehr (1994, 1996) also showed that chronic treatment of Syrian hamsters with dietary quercetin, a substrate and potent inhibitor of hamster kidney COMT, significantly enhanced 17β-estradiol-induced tumorigenesis in the kidney. Taken together, these data suggested that strong inhibition of the COMT-mediated O-methylation of catechol estrogens by xenobiotics or by the endogenous catecholamines may facilitate the 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 the present study, we evaluated the effects of several common tea catechins (catechin, epicatechin, and (-)-epigallocatechin-3-O-gallate (EGCG)) and bioflavonoids (quercetin and fisetin) on the O-methylation metabolism of 2-OH-E₂ and 4-OH-E₂ catalyzed by the cytosolic COMT prepared from human liver samples. Our data showed that these catechol-containing common dietary polyphenols are all strong inhibitors for the human liver COMT-mediated O-methylation of catechol estrogens, and EGCG was the most potent inhibitor for the O-methylation of catechol estrogens, with IC₅₀ values ranging from ~0.04 μM to 0.5 μM.

Materials and Methods

Chemicals. 2-OH-E₂, 4-OH-E₂, SAM, dithiothreitol, catechin, epicatechin, EGCG, quercetin, and fisetin were purchased from Sigma-Aldrich (St. Louis, MO). A black tea polyphenol (BTP) extract and a green tea polyphenol (GTP) extract were gifts from the Thomas J. Lipton Company (Englewood Cliffs, NJ). The compositions of the BTP and GTP mixtures were described earlier (Huang et al., 1992; Zhu et al., 1998). [methyl-³H]SAM (specific activity 11.2–13.5 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). All solvents used in this study were of high-performance liquid chromatography grade or better and were obtained from Fisher Scientific Co. (Pittsburgh, PA).

Preparation of Human Liver Cytosolic Fraction. Human liver samples were obtained from Caucasians undergoing liver tumor removal surgery at the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School (New Brunswick, NJ). The procedure for procurement of human liver samples was approved by the institutional review boards of the University of South Carolina (Columbia, SC), Rutgers University (Piscataway, NJ), and the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School (New Brunswick, NJ). Within 30 min after the liver tumor(s) was removed, a portion of the surrounding normal tissue was collected and snap-frozen in liquid nitrogen for transport to a laboratory at Rutgers University (~2 miles away from the surgery room) for temporary storage in a ~80°C freezer. The frozen human liver 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 subcellular fractions and the enzymatic assays were carried out.

On the day of preparation of cytosolic fractions, the liver 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 to 3 min followed by a Teflon homogenizer (DuPont, Wilmington, DE) for another 2 to 3 min. Tissue homogenates were centrifuged at 9000 rpm 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 rpm (4°C) for 90 min. The resulting pellets are the microsomal fraction and the supernatants are the cytosolic fraction. Aliquots of each cytosolic preparation were stored separately in small vials at ~80°C until used. The protein concentration was determined by using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) with bovine serum albumin as standard.

O-Methylation of 2-OH-E₂ and 4-OH-E₂ by Human Liver Cytosolic COMT. The COMT-mediated O-methylation of catechol estrogens was carried out as described earlier (Zhu and Liehr, 1994, 1996). The reaction mixture consisted of 0.5 mg of human liver cytosolic protein, 1.2 mM MgCl₂, 250 μM SAM (containing 0.5–1 mCi [methyl-³H]SAM), 1 mM dithiothreitol, and 10 μM 2-OH-E₂ or 4-OH-E₂ in 0.5 ml of Tris-HCl buffer (50 mM, pH 7.4). The reaction was initiated by addition of liver cytosolic protein and carried out at 37°C for 10 to 60 min. After the incubation, the reaction was arrested by immediately cooling to ice-cold temperatures, addition of 250 μl of ice-cold 0.9% NaCl, and extraction with 5 ml of ice-cold n-heptane. After centrifugation at 1000g for 10 min, portions of the organic extracts were measured for radioactivity content with a liquid scintillation analyzer (Packard Tri-CARB 2900TR; PerkinElmer Life and Analytical Sciences). The rate of methylation was expressed as picomoles of methylated product formed per milligram of liver cytosolic protein per minute (abbreviated as pmol/mg protein/min).
The rates for the 2-OH-E2 and 4-OH-E2 were first optimized the assay conditions by determining the effects of incubation time, cytosolic protein concentrations, SAM concentrations, and reaction pH on the formation of O-methylated estrogen metabolites by human liver cytosolic COMT. Two representative liver samples were assayed for this purpose, and a representative data set is shown in Fig. 2. Based on our data, an optimal reaction condition was devised for most of the in vitro enzymatic O-methylation of 2-OH-E2 and 4-OH-E2, which included an incubation time of 10 min, a cytosolic protein concentration of 0.5 mg/ml, a SAM concentration of 250 μM, and a reaction pH of 7.4. The rates for the O-methylation of 10 μM 2-OH-E2 and 4-OH-E2 by each of the eight human liver cytosolic COMT preparations determined under the optimized in vitro metabolic conditions are summarized in Table 1.

Here it should be noted that because the average Km values for the O-methylation of 4-OH-E2 by several human liver samples were ~10 μM (described later in Fig. 7), a 10 μM concentration of 2-OH-E2 or 4-OH-E2 was chosen in the present study for testing the inhibitory effects of various dietary chemicals. Also, in some of the experiments designed to determine the kinetic parameters (Km and Vmax), several relatively low substrate concentrations (<10 μM) were also used. To increase the detection accuracy, the incubation time was increased from 10 to 20 min so that more products could be formed at the low substrate concentrations. Since we confirmed that the 20-min incubation time was still within the linear range (Fig. 2), this increase would not affect the kinetic parameters.

**Inhibition of Human Liver COMT-Mediated O-Methylation of Catechol Estrogens by Tea Polyphenols. Catechin and epicatechin.** When different concentrations (from 1.6 to 100 μM) of catechin and epicatechin were introduced into the incubation mixture, the rate of O-methylation of 2-OH-E2 and 4-OH-E2 (at 10 μM) by human liver COMT was inhibited in a concentration-dependent manner (Fig. 3). The IC50 values for inhibiting the O-methylation of 10 μM 2-OH-E2 by catechin and epicatechin were 14 to 17 and 44 to 65 μM, respectively, and the IC50 values for inhibiting the O-methylation of 10 μM 4-OH-E2 were 5 to 7 and 10 to 18 μM, respectively (Fig. 3; Table 2). Notably, our data consistently showed that catechin and epicatechin have 2- to 6-fold higher inhibition potency for the O-methylation of 4-OH-E2 than for the O-methylation of 2-OH-E2 by human liver cytosolic COMT (Fig. 3). At the highest concentration (100 μM) tested, catechin had a slightly higher inhibition efficacy for the methylation of 4-OH-E2 than for the methylation of 2-OH-E2, and this difference was more evident with epicatechin (Fig. 3).

**Epigallocatechin gallate (EGCG).** We also tested the effects of EGCG on the O-methylation of 2-OH-E2 and 4-OH-E2 by two human liver cytosolic preparations (HL9C and HL8C). We were surprised to find that EGCG had an exceptionally strong inhibitory effect (Fig. 4, upper left and middle panels) compared with catechin and epicatechin. The assays were repeated more than twice with each of the human liver cytosolic samples tested, and consistent results were obtained. Representative data for the repeat of the assay using human liver cytosol HL8C were shown in Fig. 4 (upper right panel). The estimated IC50 values for inhibition of the O-methylation of 2-OH-E2 by EGCG was only 40 to 70 nM (Fig. 4; Table 2), and EGCG was ~300 times more potent than catechin and ~1000 times more potent than epicatechin. In comparison, the IC50 values for inhibition of the O-methylation of 4-OH-E2 by EGCG were relatively lower, 0.23 to 0.46 μM (Fig. 4; Table 2), but it is still 20 to 50 times more potent than catechin and 50 to 100 times more potent than epicatechin. It is also of note that EGCG had a much higher inhibitory potency for the O-methylation of 2-OH-E2 than for the O-methylation of 4-OH-E2, which is exactly opposite to the differential inhibitory effect observed with catechin and epicatechin (refer to Fig. 3).

We found that the inhibition pattern and potency of EGCG with human placenta cytosolic COMT were similar to those with human liver cytosolic COMT (data not shown). When rat liver cytosolic COMT was tested, EGCG displayed only moderate inhibitory potency.

**TABLE 1**

<table>
<thead>
<tr>
<th>Human Liver Cytosolic Sample</th>
<th>Rate of O-Methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-OH-E2 as substrate</td>
</tr>
<tr>
<td>HL1C</td>
<td>822 ± 12</td>
</tr>
<tr>
<td>HL2C</td>
<td>930 ± 18</td>
</tr>
<tr>
<td>HL3C</td>
<td>1207 ± 13</td>
</tr>
<tr>
<td>HL4C</td>
<td>1014 ± 22</td>
</tr>
<tr>
<td>HL5C</td>
<td>618 ± 7</td>
</tr>
<tr>
<td>HL6C</td>
<td>294 ± 8</td>
</tr>
<tr>
<td>HL7C</td>
<td>857 ± 21</td>
</tr>
<tr>
<td>HL8C</td>
<td>641 ± 14</td>
</tr>
<tr>
<td>HL9C</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2.** Dependence of the in vitro O-methylation of 2-OH-E2 and 4-OH-E2 on incubation time (upper left), cytosolic protein concentration (upper right), SAM concentration (lower left), and incubation pH (lower right).
(IC$_{50}$ value of ~1 μM) for the O-methylation of both 2-OH-E$_2$ and 4-OH-E$_2$, but the distinctly high potency of EGCG for inhibiting the methylation of 2-OH-E$_2$ as observed with human liver cytosolic COMT was not observed with rat liver COMT (data not shown).

**Crude extracts from green and black tea.** We also tested the crude extracts from green or black tea for their activity in inhibiting the O-methylation of 2-OH-E$_2$ and 4-OH-E$_2$ by human liver cytosolic COMT. The extracts containing either GTP or BTP each strongly inhibited the O-methylation of 2-OH-E$_2$ and 4-OH-E$_2$ in a concentration-dependent manner (Fig. 5, lower panels). Notably, GTP and BTP appeared to have an inhibition pattern similar to that of EGCG. To make a more detailed comparison of the inhibition pattern, GTP and BTP (at low concentrations, from 0.0125–0.4 μg/ml) were reanalyzed by using human liver cytosol sample HL8C as the enzyme source, and the data are shown in Fig. 5 (upper panel). Our data confirmed that GTP and BTP had distinctly higher inhibition potency for the methylation of 2-OH-E$_2$ as compared with the methylation of 4-OH-E$_2$. The estimated IC$_{50}$ values for GTP were ~0.02 and 0.3 μg/ml for inhibiting the O-methylation of 2-OH-E$_2$ and 4-OH-E$_2$, respectively, and the IC$_{50}$ values for BTP were 0.3 and 0.6 μg/ml, respectively.

**Inhibition of Human Liver COMT-Mediated O-Methylation of Catechol Estrogens by Bioflavonoids.** Zhu et al. (1994) and Zhu and Liehr (1996) have previously shown that quercetin and fisetin (two common bioflavonoids) are substrates and also strong inhibitors for 

**TABLE 2**

The IC$_{50}$ values for tea catechins and bioflavonoids in inhibiting the O-methylation of 2-OH-E$_2$ and 4-OH-E$_2$ catalyzed by human liver cytosolic COMT

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Cytosol Sample</th>
<th>IC$_{50}$ value/μM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-OH-E$_2$</td>
<td>4-OH-E$_2$</td>
</tr>
<tr>
<td>Catechin</td>
<td>Liver HL7C</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Liver HL8C</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Liver HL9C</td>
<td>14</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>Liver HL1C</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Liver HL2C</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Liver HL3C</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Liver HL4C</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Liver HL6C</td>
<td>44</td>
</tr>
<tr>
<td>Epigallocatechin gallate (EGCG)</td>
<td>Liver HL8C</td>
<td>0.07</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Liver HL8C</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Liver HL9C</td>
<td>1.5</td>
</tr>
<tr>
<td>Fisetin</td>
<td>Liver HL8C</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Liver HL9C</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Liver HL8C</td>
<td>3.3</td>
</tr>
</tbody>
</table>

**FIG. 3.** Inhibition of human liver COMT-mediated O-methylation of 2-OH-E$_2$ and 4-OH-E$_2$ by increasing concentrations of catechin and epicatechin.

The incubation mixture consisted of 10 μM 2-OH-E$_2$ or 4-OH-E$_2$, 250 μM [methyl-$^3$H]SAM (containing 0.2 μCi), 0.5 mg/ml human liver cytosolic protein, catechin or epicatechin (at indicated concentrations), 1 mM dithiothreitol, and 1.2 mM MgCl$_2$ in a final volume of 0.25 ml of Tris-HCl buffer (10 mM, pH 7.4). Incubations were carried out at 37°C for 10 min. Each point is the mean of duplicate determinations (with variations <5%).

**FIG. 4.** Inhibition of COMT-mediated O-methylation of 2-OH-E$_2$ and 4-OH-E$_2$ by increasing concentrations of (−)-epigallocatechin-3-O-gallate (EGCG).

The incubation mixture consisted of 10 μM 2-OH-E$_2$ or 4-OH-E$_2$, 250 μM [methyl-$^3$H]SAM (containing 0.2 μCi), 0.5 mg/ml human cytosolic protein, EGCG, 1 mM dithiothreitol, and 1.2 mM MgCl$_2$ in a final volume of 0.25 ml of Tris-HCl buffer (10 mM, pH 7.4). Incubations were carried out at 37°C for 10 min. Each point is the mean of duplicate determinations (with variations <5%).
The incubation mixture consisted of 10 μM of 2-OH-E2, or 4-OH-E2, 250 μM [methyl-3H]SAM (containing 0.2 μCi), 0.5 mg/ml liver cytosolic protein, a GTP or BTP extract (at indicated concentrations), 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 carried out at 37°C for 10 min. Each point is the mean of duplicate determinations (average variation <5%).

**Mechanism of Inhibition of Human COMT-Mediated O-Methylation of Catechol Estrogens by Dietary Polyphenols.** We first determined the enzyme kinetics for the O-methylation of 2-OH-E2 and 4-OH-E2 by human liver cytosolic COMT. Our data showed that both quercetin and fisetin had a high inhibitory potency (Fig. 6). Their IC50 values are 0.9 to 1.5 μM, respectively, for inhibiting the O-methylation of 2-OH-E2, and 0.5 to 1.2 μM, respectively, for inhibiting the O-methylation of 4-OH-E2 (Table 2). Although the O-methylation of 4-OH-E2 is somewhat more prone to inhibition by quercetin and fisetin than the O-methylation of 2-OH-E2, the difference is much smaller than the difference observed with catechin and epicatechin. Notably, similar inhibitory effects of quercetin and fisetin on the O-methylation of 2-OH-E2 and 4-OH-E2 at different substrate concentrations by several human liver cytosol preparations (representative data are shown in Fig. 7). Our data showed that the O-methylation of 4-OH-E2 by human liver cytosolic COMT followed the typical Michaelis-Menten curve patterns, and the K_M and V_max values for the O-methylation could be readily and reliably determined according to the corresponding Eadie-Hofstee plots (plots not shown). The estimated K_M and V_max values are summarized in Fig. 7. In comparison, the O-methylation of 2-OH-E2 by the same cytosolic preparations showed significant deviation from the typical Michaelis-Menten curve patterns in which the substrate at higher concentrations had a marked concentration-dependent self-inhibition. This irregular curve pattern also prevented us from meaningfully estimating the apparent K_M and V_max values for each of these samples.

To evaluate the kinetic mechanism of enzyme inhibition by dietary polyphenols, we only focused on analyzing the enzyme inhibition with 4-OH-E2 as substrate, and only purified dietary polyphenols (catechin, epicatechin, and quercetin) were studied. In the presence of a relatively low concentration of catechin (5 μM) or epicatechin (7.5 μM), the K_M values were increased while the V_max values were decreased, suggesting that these two tea catechins inhibited human liver COMT-mediated O-methylation of 4-OH-E2 with a mixed (competitive plus noncompetitive) mechanism of enzyme inhibition (Fig. 8). However, when the concentrations of catechin and epicatechin were further increased by ~4-fold (to 20 and 30 μM, respectively), the V_max was further decreased, whereas the apparent K_M values were basically not further increased, suggesting that at high concentrations the noncompetitive inhibition became more predominant.

Similarly, kinetic analyses also showed that quercetin at 1 and 2 μM concentrations inhibited human liver COMT-mediated O-methylation of 4-OH-E2 with a clear mixed mechanism of inhibition (competitive plus noncompetitive) (Fig. 9). The kinetic analysis was repeated with two human liver cytosolic COMT preparations, and almost exactly the same results were obtained.

**Discussion**

In the present study, we demonstrated that catechin and epicatechin are strong inhibitors of the human liver cytosolic COMT-mediated O-methylation of 2-OH-E2 and 4-OH-E2, and these two tea catechins have 2- to 6-fold higher inhibition potency for the O-methylation of...
FIG. 7. Substrate concentration dependence for the O-methylation of 2-OH-E2 and 4-OH-E2 catalyzed by representative human liver cytosolic preparations.

The incubation mixture consisted of 0 to 100 μM 2-OH-E2 or 4-OH-E2, 250 μM [methyl-3H]SAM (containing 0.2 μCi), 0.5 mg/ml liver 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 carried out at 37°C for 20 min. Each point is the mean of duplicate determinations (with average variation <5%).

4-OH-E2, than for the O-methylation of 2-OH-E2. Surprisingly, EGCG had a much higher inhibiting activity toward the O-methylation of 2-OH-E2 (IC50 of 0.04–0.07 μM) and 4-OH-E2 (IC50 of 0.2–0.5 μM). The crude extracts from green tea and black tea also showed very strong activity in inhibiting human liver COMT-mediated O-methylation of catechol estrogens, and the strong inhibitory effects likely are attributable to EGCG that was contained in these tea extracts.

Notably, inhibition of the enzymatic O-methylation of 2-OH-E2 by EGCG was clearly biphasic (Fig. 4). The first-phase inhibition occurred at the EGCG concentrations <0.1 μM, with the maximal inhibition between 50 and 70%. In comparison, EGCG at the same concentrations (≤0.1 μM) did not show any appreciable inhibition of the enzymatic O-methylation of 4-OH-E2. The mechanism underlying the biphasic inhibition of the O-methylation of 2-OH-E2, but not 4-OH-E2, is not known at present. Since earlier molecular cloning studies showed that human COMT only has one gene coding for the cytosolic COMT, it is not impossible that EGCG at very low concentrations may bind to the cytosolic COMT with a high affinity, preferentially affecting the lodging of 2-OH-E2 to the catalytic site, but not 4-OH-E2.

Since quercetin and fisetin (two common bioflavonoids) were previously shown to be substrates and also strong inhibitors for the O-methylation of catechol estrogens catalyzed by hamster and porcine COMT (Zhu et al., 1994; Zhu and Liehr, 1994, 1996), we also tested, for comparison, the inhibitory effects of these two bioflavonoids on the O-methylation of 2-OH-E2 and 4-OH-E2 by human liver cytosolic COMT. We found that quercetin and fisetin are two strong inhibitors of human COMT-mediated O-methylation of catechol estrogens. The inhibitory potencies of quercetin and fisetin are markedly stronger than those of catechin and epicatechin. Notably, the IC50 values for quercetin and fisetin determined in the present study for human hepatic COMT are very similar to the values obtained earlier with hamster and porcine COMT (Zhu et al., 1994; Zhu and Liehr, 1994, 1996).

Our enzyme kinetic analyses showed that tea catechins and bioflavonoids inhibited human liver COMT-mediated O-methylation of 4-OH-E2 (a representative substrate) with a mixed (competitive plus noncompetitive) mechanism of inhibition. We believe that this mixed mechanism of COMT inhibition can be readily explained on the basis of the available knowledge. Our earlier studies showed that tea catechins and bioflavonoids are excellent substrates for human or rodent COMT (Zhu et al., 1994, 2000, 2001), and thus it is reasonable to believe that these polyphenols, when concomitantly present, will serve as competitive inhibitors for the COMT-mediated O-methylation of the catechol estrogen substrates. Moreover, during the metabolic O-methylation of the catechol-containing tea catechins and bioflavonoids, S-adenosyl-L-homocysteine (SAH, the demethylated product of SAM) is formed in equimolar quantities with the methylated dietary polyphenols. It is known that SAH is a strong noncompetitive inhibitor for the COMT-mediated O-methylation of catechol estrogens as well as other catechol substrates (Ueland, 1982; Zhu et al., 1994, 2000, 2001; Zhu and Liehr, 1996). As a result, we believe that the competitive component of enzyme inhibition is due to the copresence of multiple substrates of COMT (i.e., 4-OH-E2 and the catechol-containing dietary polyphenol) that would compete for the same enzyme for methylation, and the noncompetitive component of enzyme inhibition is due to an increased formation of SAM when two substrates are copresent. In addition, the decreased availability of SAM may also partially add to the reduction of the catalytic activity when a dietary polyphenolic inhibitor is present together with a catechol estrogen substrate.

Several recent studies showed that dietary bioflavonoids and common tea catechins such as (−)-epicatechin and (−)-epigallocatechin could be quite readily absorbed in human subjects after drinking tea or fruit juice, and some of them may reach submicromolar concentrations (0.1–0.4 μM) in the plasma (Lee et al., 1995; Unno et al., 1996; Li et al., 2000; Chow et al., 2001, 2003). A representative earlier study using 3H-labeled (−)-EGCG demonstrated that this tea polyphenol can be readily absorbed and is widely distributed in various mouse tissues (Suganuma et al., 1998). Notably, inhibition of the COMT-mediated O-methylation of endogenous 2- and 4-hydroxylated estrogens by dietary polyphenols is expected to result in an increase in the tissue levels of the procarcinogenic 4-OH-E2 plus a decrease in the tissue levels of the anticarcinogenic 2-methoxyestradiol. These effects...
The upper and lower left insets illustrate the substrate concentration dependence for the O-methylation of 4-OH-E2 in the presence of a tea catechin. The incubation mixture consisted of 0 to 20 μM 4-OH-E2, 250 μM [methyl-3H]SAM (containing 0.2 μCi), 0.5 mg/ml liver cytosolic protein, catechin or epicatechin, 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 carried out at 37°C for 20 min. Each point is the mean of duplicate determinations (average variation <5%).

may facilitate the development of estrogen-induced tumors [discussed by Zhu and Conney, 1998; Zhu, 2002]. In partial support of this notion, our earlier studies in animal models have shown that chronic administration of dietary quercetin enhanced 17β-estradiol-induced carcinogenesis. In the Syrian hamsters (Zhu and Liehr, 1994; B. T. Zhu, unpublished data) and azoxymethanol-induced colonic neoplasms in mice (Deschner et al., 1991). These results suggest that the selective enhancing effect of quercetin on 17β-estradiol-induced carcinogenesis was largely due to its inhibitory effect on the COMT-mediated O-methylation metabolism of catechol estrogens.

In addition to the inhibition of catechol estrogen O-methylation, it is also known that dietary catechol-containing tea catechins and bioflavonoids have multiple beneficial health effects, including their strong antioxidant and antimutagenic activity in certain chemically induced animal tumor models (Verma et al., 1988; Deschner et al., 1991; Lu et al., 2002; Yang et al., 2002; Conney, 2003; Lambert and Yang, 2003). Therefore, more studies are needed to carefully evaluate not only the extent of in vivo inhibition of the metabolic O-methylation of catechol estrogens in animal models and humans, but also the net effects on estrogen-induced tumorigenesis which may be associated with the long-term inhibition of the metabolic O-methylation of catechol estrogens.

References


