Epigallocatechin Gallate Modulates CYP450 Isoforms in the Female Swiss-Webster Mouse

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This study was designed to determine the effect of the in vivo administration of epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) on enzymes involved in the synthesis and metabolism of estradiol. EGCG (12.5, 25, or 50 mg/kg/day, ip) or ECG (12.5 or 25 mg/kg/day, ip) was administered to female Swiss-Webster mice for 7 days. The chemicals were well tolerated by the mice with the exception of EGCG given at 50 mg/kg, which resulted in severe hepatic necrosis and a 67% mortality rate. Following the administration of nontoxic doses of EGCG and ECG, aromatase (CYP19), CYP3A, CYP1A, and catechol O-methyltransferase (COMT) were measured. Additionally, the activity of CYP2E1 was determined, since this CYP450 isoform is important in the bioactivation of numerous carcinogens. The results demonstrated that ovarian aromatase activity was inhibited 56% by EGCG (25 and 12.5 mg/kg), but not ECG, while hepatic CYP3A catalytic activity and polypeptide levels were increased 31 ± 4 and 47 ± 2%, respectively, by 25 mg/kg of EGCG. However, ECG (but not EGCG) inhibited CYP1A catalytic activity and polypeptide levels (31 ± 5 and 47 ± 5%, respectively). Hepatic and renal COMT, as well as renal CYP3A remained unchanged following catechin dosing. Hepatic CYP2E1 catalytic activity and polypeptide levels were significantly increased (37 ± 3 and 22 ± 3%) following administration of EGCG (25 mg/kg). These results indicate that EGCG modulates enzymes responsible for both the synthesis and metabolism of estradiol, which may provide a potential mechanism for the reported action of EGCG, reported action as an inhibitor of breast tumor growth.

Key Words: epigallocatechin gallate, CYP19, CYP3A, CYP1A, catechol O-methyltransferase.

The catechins are a group of polyphenolic chemicals that belong to the flavanol family, which is a subclass of flavonoids. Flavonoids are plant-derived chemicals that are contained in a variety of foods and beverages (Arts et al., 2000). In these sources, the predominant catechins are epigallocatechin gallate (EGCG), epicatechin gallate (ECG), and epicatechin (EC). There has been scientific interest in this chemical family due to their various pharmacological actions. For example, the catechins inhibit the proliferation of human breast cancer cells (Kavanagh et al., 2001; Morre et al., 2000; Valcic et al., 1996) and epidemiological studies have suggested that green tea consumption is linked to a decrease in both the rate of development and recurrence of breast cancer (Nakachi et al., 1998). Additionally, in the rat DMBA mammary carcinogenesis model, the administration of green-tea extract has decreased both tumor number (Tanaka et al., 1997) and weight (Kavanagh et al., 2001). However, the effect of purified catechins on tumor growth has only been examined in mice. The results demonstrated that EGCG caused regression of MCF-7 cell implanted tumors and reduced the growth of implanted prostate tumors (Liao et al., 1995). The effect on prostate tumors was catechin-specific as EC, EGC, and ECG all failed to decrease tumor growth. However, EGCG was the only catechin examined in the mammary tumor model. Overall, the results demonstrate that EGCG may have beneficial characteristics that could be exploited in the treatment of breast cancer.

Numerous studies with either green-tea extract or specific members of the catechin family have postulated a wide variety of potential mechanisms of action, such as estrogen receptor (ER) antagonism (Komori et al., 1993), proapoptotic (Morre et al., 2000), antiangiogenic (Kondo et al., 2002) and anti-oxidative (Guo et al., 1999). None of these mechanisms have been conclusively proven in either breast cancer cells or breast tumor models. However, ER antagonism is not likely to be involved, since EGCG, EGC, and ECG all failed to antagonize estradiol-induced responses in both uterotrophic and ERα reporter gene assays (Goodin et al., 2002). Alternatively, alterations in the synthesis and/or metabolism of estradiol may be involved in the antitumor actions of the catechins. Therefore, aromatase (CYP19) would be a key target to examine, as it is responsible for the synthesis of estrogens from androgens and aromatase expression in breast tumor epithelial cells positively correlates with cellular proliferation (Lu et al., 1996). CYP3A and CYP1A are also important targets, as these CYP450 isoforms are responsible for the formation of the antiestrogenic metabolite, 2-hydroxyestradiol (Kerlan et al., 1992; Shou et al., 1997). This metabolite is then rapidly converted by cate-
O-methyltransferase (COMT) to 2-methoxyestradiol, which displays antiangiogenic properties (Fotis et al., 1994; Klauber et al., 1997). Therefore, the overall balance of the activity of these enzymes is important in the regulation of estradiol-mediated breast tumor growth.

The only previous reports of catechin-mediated alterations of CYP450 isoforms are restricted to either in vitro investigations or to in vivo studies in which animals were treated with EC, (+)-catechin, or herbal supplements containing a mixture of various plant extracts (Lhoste et al., 2003; Muto et al., 2001; Ryu and Chung, 2003; Siegers et al., 1982; Wang et al., 1988). Therefore, the aim of the present study was to determine if either EGCG or ECG would modulate the effect of enzymes involved in the synthesis and metabolism of estradiol, namely, aromatase, CYP1A, CYP3A, or COMT, at doses that were previously reported to cause tumor regression in athymic mice (Liao et al., 1995). Since COMT inhibition has led to an increase in the renal carcinogenic potential of estradiol (Zhu and Liehr, 1994), the effect of these two catechins on renal enzymes was also determined. Additionally, catechin-mediated changes in hepatic CYP2E1 were characterized, as this enzyme is expressed in breast tumors (Iscan et al., 2001) and also activates numerous carcinogens (Constan et al., 1999; Sohn et al., 2001). EGCG and ECG were used in this investigation, as catechins that contain a gallate group in the 3’ position demonstrate potent inhibition of CYP450 in vitro (Muto et al., 2001; Wang et al., 1988).

**MATERIALS AND METHODS**

**Chemicals.** EGCG, ECG, β-naphthoflavone, dexamethasone, acetone, NADPH, alanine aminotransferase (ALT) kit, blood urea nitrogen (BUN) kit, S-adenosyl-L-methionine (SAM), antirabbit IgG, erythromycin, aniline, and ethoxyresorufin were purchased from Sigma Chemical Co. (St. Louis, MO). [1H]-androstenedione (25.9 Ci/mmol) was purchased from New England Nuclear. NADPH, alanine aminotransferase (ALT) kit, and blood urea nitrogen (BUN) kit, 5-bromo-4-chloro-3-indoyl phosphate-p-toluidine salt (BCIP), avidin-alkaline phosphatase conjugate, biotinylated sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) broad-range standards, and nitrocellulose were purchased from Bio Rad (Hercules, CA). Primary antibodies antirat CYP1A, CYP3A, and CYP1E1 were purchased from Amersham (Auckland, N.Z.). All other chemicals were of the highest purity commercially available.

**Animals.** Female Swiss-Webster mice (5–6 weeks old) were purchased from Hercus Taieri Resource Unit (Dunedin). All procedures were approved by the University of Otago Animal Ethics Committee (96/01). The animals were housed in micro-isolator cages on shredded paper bedding and had continuous access to Reliance rodent diet (Dunedin, NZ) and water. They were maintained at 21–24°C with a 12-h light/dark cycle and allowed to acclimatize for one week prior to experimentation. Mice were randomly assigned to the various treatment groups, which consisted of 9 mice in each group. Mice were dosed with EGCG (12.5, 25, or 50 mg/kg/day, ip), ECG (12.5 or 25 mg/kg, ip), or saline control (8 ml/kg) for 7 days. To control for potential catechin-mediated weight loss, saline control mice were pair-fed. Mice were sacrificed by CO2 inhalation 24 h following the final catechin dose. The doses and route of administration used were based on previous work, which demonstrated that EGCG (50 mg/kg/day, 14 days, ip) caused tumor regression in a murine MCF-7 cell implant model (Liao et al., 1995). Mice used as positive controls were treated with β-naphthoflavone (80 mg/kg, ip, 3 consecutive days), dexamethasone (75 mg/kg, ip, 4 consecutive days), or acetone (4.8 g/kg, po, 16 h prior to necropsy).

**Plasma markers of hepatic and renal injury.** Plasma ALT activity and BUN were used as indicators of hepatic and renal damage, respectively. Immediately following euthanasia, blood was collected from the inferior vena cava and stored on ice. Plasma ALT activity was determined kinetically, using a Sigma diagnostic kit, and the results are expressed as IU/l. BUN was determined using a Sigma diagnostic kit, and the results are expressed as mg/dl.

**Liver histology.** Liver slices were obtained from the distal portion of the left lateral lobe and the tissue was fixed for at least 48 h in 10% neutral buffered formalin. The samples were then embedded in paraffin, cut into 5 μm sections, and stained with hematoxylin and eosin for examination by light microscopy. The examiner of the slides was blind to the various treatment groups.

**Tissue preparation.** Hepatic and renal cytosolic extracts and microsomes were prepared from individual mice by differential centrifugation (Guengerich, 1989). Ovarian tissue was pooled from 3 mice and microsomes were prepared as described (Kellis and Vickery, 1984). Protein concentration of the resulting microsomes or cytosolic extract was immediately determined by the bicinchoninic acid method (Smith et al., 1985). The microsomes and cytosolic extracts were stored at −80°C until experimentation.

**Aromatase catalytic activity.** The release of tritiated water from [1H]-androstenedione was used as an indicator of aromatase activity, as this assay has been validated as an appropriate indicator of murine ovarian aromatase activity (Toda et al., 2001). The assay was conducted as described (Vinggaard et al., 2000) with the following modifications. The incubation mixture contained 100 mM [1H]-androstenedione, 25 μg protein, 250 μM NADPH, and 50 mM KH2PO4 buffer in a total volume of 500 μl. Samples were preincubated for 2 min at 37°C, and the reaction was initiated by the addition of NADPH. After 10 min, the reaction was terminated by the addition of chloroform and 0.9% NaCl. The samples were vortexed for 30 s and then centrifuged at 1700 × g for 15 min. One hundred μl of the aqueous phase was transferred to scintillation vials and the radioactivity was counted on a Beckman LS8301 scintillation counter. Results are expressed as pmol/mg/h.

**CYP1A catalytic activity.** Ethoxyresorufin O-deethylation was used to determine changes in the catalytic activity of CYP1A (Ryan and Levin, 1990) and was performed as described previously (Bray et al., 2002). Results are expressed as nmol/mg/min.

**CYP3A catalytic activity.** Erythromycin N-demethylation was used as a selective probe for changes in the catalytic activity of CYP3A (Wrighton et al., 1985) and was performed as described previously (Bray et al., 2002). Results are expressed as nmol/mg/min.

**CYP2E1 catalytic activity.** Aniline hydroxylation was used as a selective probe for changes in the catalytic activity of CYP2E1 and was performed as described previously (Inder et al., 1999). Results are expressed as nmol/mg/min.

**COMT catalytic activity.** Methylation of 4-nitrocatechol was used as a selective probe for changes in COMT activity (Herblin, 1973) with the following modifications. Hepatic or renal cytosolic extract (2 mg) was incubated with 25 μM 4-nitrocatechol, 0.2 mM SAM, 0.01 M MgCl2, and 1 mM Tris-HCl buffer (pH 7.0) in a total volume of 2 ml. Samples were preincubated for 2 min at 37°C and a 60-min incubation period was initiated by the addition of SAM. The reaction was terminated by addition of 12 N NaOH and the samples were centrifuged at 1000 × g for 10 min. Absorbance was determined at 520 nm and the results are expressed as nmol/mg/min.

**Electrophoresis and Western blotting.** SDS-PAGE was performed as previously described (Bray et al., 2002). Briefly, 10 μg of microsomal protein was loaded onto a 10% gel with a 4% stacking gel. Polypeptide levels of CYP1A, CYP3A, and CYP2E1 were quantified by Western immunoblotting as previously described (Bray et al., 2002). Upon transfer to a nitrocellulose membrane, proteins were incubated with CYP1A, CYP3A, or CYP2E1 antirat

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primary antibody. Each antibody was proven by the manufacturer to cross-react with the specific mouse CYP450 isoform examined. After washing, membranes were incubated with antirabbit IgG (an alkaline phosphate conjugate) secondary antibody. The bands were visualized using the NBT/BCIP system and quantified by scanning densitometry.

**Statistical analysis.** Individual groups were analyzed using a two-way ANOVA coupled with the Student-Newman-Keuls post hoc test with \( p < 0.05 \) as the minimum requirement for a statistically significant difference.

**RESULTS**

EGCG (50 mg/kg/d) administration resulted in a mortality rate of 67%, as 5 mice died after either 4 or 5 days of EGCG administration and one mouse appeared sick and was euthanized on day 5. EGCG-induced significant weight loss and extensive hepatotoxicity as indicated by a mean body weight (bw) loss of 2.4 g, elevated plasma ALT activity (2123 ± 1040 IU/l) (Table 1) and extensive hepatic necrosis throughout the entire liver (Fig. 1), while pair-fed vehicle control mice remained normal. Spleen, kidney, and liver weights were also increased (82, 23, and 19%, respectively) following EGCG (50 mg/kg) treatment, while the gross weight of the uterus and ovaries remained unchanged (Table 2). However, there was no major renal impairment as the levels of BUN (20–28 mg/dl) remained in the normal range (Table 2). The toxicity elicited by EGCG was dose-dependent, as lower doses of EGCG (25 and 12.5 mg/kg) produced normal ALT activities, normal liver histology, and no change in bw or organ weight.

Since 50 mg/kg of EGCG produced extensive mortality, enzymatic parameters were only examined following lower doses of both EGCG and ECG (25 and 12.5 mg/kg). The results showed that both doses of EGCG decreased ovarian aromatase activity approximately 56% from control, while there was no change in aromatase following ECG treatment (Fig. 2). EGCG (25 mg/kg) also increased hepatic CYP3A catalytic activity 31% ± 4% above control (Fig. 3). This was supported by a 47% ± 2% increase in CYP3A polypeptide levels (Fig. 4). This response was dose-dependent, as the catalytic activity and polypeptide levels for CYP3A remained unchanged following 12.5 mg/kg of EGCG. The ability to modulate CYP3A was both catechin- and organ-specific, as ECG did not alter hepatic CYP3A (Figs 3 and 4), and renal CYP3A remained unchanged following the administration of both catechins (data not shown). Interestingly, ECG (25 mg/kg) decreased hepatic CYP1A catalytic activity by 31% ± 5% of control (Fig. 5) and polypeptide levels by 47% ± 5% (Fig. 6), while EGCG failed to alter the levels of CYP1A. Examination of renal CYP1A failed to determine a catechin-specific effect, as renal CYP1A remained below levels that could be detected by either catalytic or Western immunoblotting techniques. However, renal CYP1A catalytic activity was increased to 0.53 ± 0.10 mmol/min and visualized by Western immunoblotting following administration of the potent inducer β-naphthoflavone. Another enzyme examined with relevance to estradiol metabolism was COMT. The results demonstrated

### TABLE 1

<table>
<thead>
<tr>
<th>Treatment (dose)</th>
<th>Mortality (%)</th>
<th>ALT (IU/l)</th>
<th>BUN (mg/dl)</th>
<th>Body-weight change (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (8 ml/kg)</td>
<td>0</td>
<td>25 ± 3</td>
<td>24 ± 2</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>EGCG (12.5 mg/kg)</td>
<td>0</td>
<td>23 ± 3</td>
<td>27 ± 3</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>EGCG (25 mg/kg)</td>
<td>0</td>
<td>32 ± 5</td>
<td>25 ± 3</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>EGCG (50 mg/kg)</td>
<td>67</td>
<td>2123 ± 1040</td>
<td>22 ± 4</td>
<td>-2.4 ± 1.1**</td>
</tr>
<tr>
<td>ECG (12.5 mg/kg)</td>
<td>0</td>
<td>24 ± 4</td>
<td>28 ± 3</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>ECG (25 mg/kg)</td>
<td>0</td>
<td>25 ± 5</td>
<td>24 ± 3</td>
<td>1.5 ± 0.4</td>
</tr>
</tbody>
</table>

**Note.** Mice were treated as indicated for 7 days. Each value represents the mean ± SE of 9 animals. Significance was determined with a 2-way ANOVA coupled with the Students-Newman-Keuls post hoc test in which \( p < 0.05 \) was required for a statistically significant difference.

*Significantly increased from vehicle control, \( p < 0.05 \).

**Significantly decreased from vehicle control, \( p < 0.01 \).
that the catalytic activity of both hepatic and renal COMT remained unchanged following 7 days of catechin treatment, as the values ranged between 16 ± 1 and 19 ± 3 nmol/mg/min. Lastly, CYP2E1 was also profiled following catechin treatment. While ECG had no effect on CYP2E1, EGCG (25 mg/kg/d, 7d) increased CYP2E1 catalytic activity by 37 ± 3% (Fig. 7) and polypeptide levels by 22 ± 3% (Fig. 8).

**DISCUSSION**

The ability of EGCG to modulate the enzymes responsible for the synthesis and metabolism of estradiol was initially intended to be conducted at doses relevant to the reported tumor inhibitory properties of EGCG. However, administration of EGCG (50 mg/kg, ip) resulted in extensive hepatic necrosis, significant weight loss, and mortality in 67% of the mice. Other groups have reported a decrease in bw following the administration of (-)-epicatechin (EC) (30 –35 mg/rat) (Lhoste et al., 2003) or 5% green tea extract in male rats (Satoh et al., 2002). Following 2 weeks of EC, the rats lost 14% of their body weight (Lhoste et al., 2003), while administration of green-tea extract for 2–8 weeks caused a 10–15% loss in bw (Satoh et al., 2002). Only one other study has examined the more potent

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment (dose)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Uterus</th>
<th>Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (8 ml/kg)</td>
<td>5.2 ± 0.1</td>
<td>1.3 ± 0.03</td>
<td>0.41 ± 0.02</td>
<td>0.36 ± 0.03</td>
<td>0.041 ± 0.004</td>
</tr>
<tr>
<td>EGCG (12.5 mg/kg)</td>
<td>5.2 ± 0.1</td>
<td>1.4 ± 0.03</td>
<td>0.45 ± 0.02</td>
<td>0.45 ± 0.03</td>
<td>0.047 ± 0.004</td>
</tr>
<tr>
<td>EGCG (25 mg/kg)</td>
<td>5.5 ± 0.2</td>
<td>1.4 ± 0.04</td>
<td>0.50 ± 0.03</td>
<td>0.40 ± 0.03</td>
<td>0.046 ± 0.003</td>
</tr>
<tr>
<td>EGCG (50 mg/kg)</td>
<td>6.2 ± 0.3*</td>
<td>1.6 ± 0.05*</td>
<td>0.75 ± 0.10*</td>
<td>0.38 ± 0.04</td>
<td>0.048 ± 0.005</td>
</tr>
<tr>
<td>ECG (12.5 mg/kg)</td>
<td>4.8 ± 0.1</td>
<td>1.3 ± 0.04</td>
<td>0.36 ± 0.01</td>
<td>0.40 ± 0.04</td>
<td>0.045 ± 0.003</td>
</tr>
<tr>
<td>ECG (25 mg/kg)</td>
<td>5.3 ± 0.1</td>
<td>1.3 ± 0.03</td>
<td>0.38 ± 0.02</td>
<td>0.39 ± 0.09</td>
<td>0.035 ± 0.003</td>
</tr>
</tbody>
</table>

Note. Mice were treated as indicated for 7 days. Each value is the indicated organ weight as a percentage of body weight and represents the mean ± SE from 9 animals. Significance was determined with a 2-way ANOVA coupled with the Students-Newman-Keuls post hoc test in which \( p < 0.05 \) was required for a statistically significant difference. *Significantly increased from vehicle control, \( p < 0.01 \).
catechins. Specifically, 85 mg/kg/day of EGCG (but not EC, EGC or ECG) for 7 days caused a 15–21% loss of initial body weight in male Sprague-Dawley rats, while 92 mg/kg/d for 7 days caused a 10% loss of body weight in female rats (Kao et al., 2000). This study supports our results, which demonstrate that only EGCG causes a loss of body weight in female C57Bl/6 (Goodin et al., 2002) and Swiss-Webster mice. While the studies in rats provide conflicting results regarding catechin-mediated weight loss, none of them have reported hepatotoxicity. Therefore, we are the only group to have previously reported mild hepatotoxicity following EGCG treatment (Goodin et al., 2002). Since Liao et al. (1995) administered EGCG (50 mg/kg, ip) to athymic nude female mice for 14 to 28 days and did not report mortality or hepatotoxicity, we had previously postulated that the mild hepatotoxicity elicited by EGCG (50 mg/kg/d, 3d) in immature C57Bl/6 female mice was an age-specific response (Goodin et al., 2002). However, this clearly is not the case, as four or more days of 50 mg/kg of EGCG induced hepatotoxicity and mortality in adult female Swiss-Webster mice. Therefore, a species- and strain-specific response occurs following high doses of EGCG since hepatotoxicity was observed in female Swiss-Webster and C57Bl/6 mice, but not in rats or athymic mice. However, in the athymic mouse study, markers of hepatotoxicity were not included as part of the findings. Therefore, it is recommended that further investigations with this compound, especially in mice, include indicators of hepatotoxicity. This will help elucidate the exact species- and strain-specific response of EGCG.

Due to the hepatotoxicity and mortality that occurred, enzymatic activities were only performed following the administration of lower doses of the catechins. Aromatase was initially examined, as it is responsible for the synthesis of estradiol from testosterone, and a catechin-specific inhibition of aromatase in vitro has previously been reported. Specifically, Satoh et al. (2002), reported IC_{50} values of 60 µM and 100 µM for EGCG and EGC, respectively, while 100 µM of ECG only produced 20% inhibition. However, other groups have reported that EGC (Kao et al., 1998) and (-)-catechin fail to inhibit aromatase (Campbell and Kurze, 1993). Our in vivo results support a catechin-specific inhibition of aromatase, since EGCG (12.5 or 25 mg/kg) inhibited ovarian aromatase activity by 56%, while ECG did not alter aromatase activity. A decrease in aromatase in vivo would reduce the production of estradiol and thus decrease estradiol-mediated events. There-

![FIG. 4. Hepatic CYP3A immunoreactive polypeptide levels following treatment with EGCG and ECG: Mice were dosed with EGCG, ECG (25 or 12.5 mg/kg, ip), or vehicle for 7 days. Dexamethasone (Dex, 75 mg/kg, ip) was administered for 4 days. Necropsies were performed 24 h after the final dose. (A) Representative Western immunoblot for CYP3A. The positions of the molecular weight markers are indicated on the right (kDa). The lanes are, from left to right, (1) dexamethasone; (2) saline; (3) EGCG, 12.5 mg/kg; (4 and 5) EGCG, 25 mg/kg; (6) ECG, 12.5 mg/kg; (7 and 8) ECG, 25 mg/kg. (B) Results from scanning densitometry of Western immunoblots: the bars represent the mean ± SE of the optical density from 9 animals. Significance was analyzed with a 2-way ANOVA coupled with the Student-Newman-Keuls post hoc test in which p < 0.05 was required for a statistical difference; *significantly increased from vehicle treatment at a level of p < 0.05; **significantly increased from all other treatments at a level of p < 0.01.]

![FIG. 5. Hepatic CYP1A catalytic activity following treatment with EGCG and ECG: Mice were dosed with EGCG, ECG (25 or 12.5 mg/kg, ip), or vehicle for 7 days. ß-Naphthoflavone (ß-NF, 80 mg/kg, ip) was administered for 3 days. Necropsies were performed 24 h after the final dose. The bars represent the mean ± SE of the optical density from 9 animals. Significance was analyzed with a 2-way ANOVA coupled with the Student-Newman-Keuls post hoc test in which p < 0.05 was required for a statistical difference; *significantly decreased from vehicle treatment at a level of p < 0.05; **significantly increased from all other treatments at a level of p < 0.001.]
fore, aromatase inhibition may be partly responsible for the antitumor actions of EGCG in rodent models of mammary cancer. However, other mechanisms are likely to be involved in the antitumor effect produced by EGCG, as other aromatase inhibitors have been reported to cause a decrease in tumor growth but not tumor regression (Yue and Brodie, 1993). Further studies examining the effect of EGCG on breast tumor aromatase activity in a murine model will be conducted to confirm this hypothesis.

The effect of EGCG on breast tumor growth could also be due to changes in estradiol metabolism via the modulation of either CYP1A, CYP3A, or COMT. Our results demonstrated that EGCG increased the catalytic activity of hepatic but not renal CYP3A, and ECG decreased the catalytic activity of hepatic CYP1A, while renal CYP1A remained below detectable levels. The renal enzymatic results are not unexpected: we have previously reported that renal CYP1A is undetectable (Bray et al., 2001), while rodent renal CYP3A remains at low but detectable levels following administration of the classic inducer, dexamethasone (Zerilli et al., 1998). While there are no other in vivo studies with EGCG or ECG, two studies have measured CYP450 activity in male rats following treatment with either (+)-catechin or EC. Specifically, Siegers et al. (1982) demonstrated that (+)-catechin (200 mg/kg/day, 7 or 28 days, po) did not alter total hepatic CYP450 or CYP2E1 activity in Sprague-Dawley rats. However, Lhoste et al. (2003) reported that both (+)-catechin and EC (30–35 mg/rat/day, 14 days, po) decreased CYP2E1 (37 and 25%, respectively) in male F344 rats. The discrepancy in the response following (+)-catechin in rats may be due to the fact that Lhoste et al. (2003) administered the catechins in 10% ethanol in order to simulate catechin exposure from drinking wine. However, it appears as though the effect of catechins on CYP2E1 activity is both catechin- and species-specific, as we demonstrated a modest increase in hepatic CYP2E1 following EGCG. It is worth noting that the majority of the in vivo data is not supported by in vitro inhibition studies. Specifically, studies, using bacterial cells transfected with plasmids for specific human CYP450 isoforms, demonstrated that both ECG and EGCG acted as either mixed or noncompetitive inhibitors of CYP1A1, CYP1A2, CYP2A6, CYP2C9, CYP2E1, and CYP3A4 (Muto et al., 2001). ECG exhibited the greatest potency against CYP1A2 as 10 μM caused 50% inhibition. However, the effect of ECG on CYP1A in vivo is exhibited via

FIG. 6. Hepatic CYP1A immunoreactive polypeptide levels following treatment with EGCG and ECG: Mice were dosed with EGCG, ECG (25 or 12.5 mg/kg, ip), or vehicle for 7 days. β-Naphthoflavone (β-NF, 80 mg/kg, ip) was administered for 3 days. Necropsies were performed 24 h after the final dose. (A) Representative Western immunoblot for CYP1A: the positions of molecular weight markers are indicated on the right (kDa). The lanes from left to right: (1) β-naphthoflavone; (2) saline; (3) EGCG, 12.5 mg/kg; (4 and 5) EGCG, 25 mg/kg; (6) ECG, 12.5 mg/kg; (7 and 8) ECG, 25 mg/kg. (B) Results from scanning densitometry of Western immunoblots: the bars represent the mean ± SE of the optical density from 9 animals. Significance was analyzed with a 2-way ANOVA coupled with the Student-Newman-Keuls post hoc test in which p < 0.05 was required for a statistical difference; *significantly decreased from vehicle treatment at a level of p < 0.05; **significantly increased from all other treatments at a level of p < 0.001.

FIG. 7. Hepatic CYP2E1 catalytic activity following treatment with EGCG and ECG: Mice were dosed with EGCG, ECG (25 or 12.5 mg/kg, ip) or vehicle for 7 days. Necropsies were performed 24 h after the final dose with the exception of acetone (Ace, 4.8 mg/kg, po), which was administered 16 h prior to necropsy. The bars represent the mean ± SE from 9 animals. Significance was analyzed with a 2-way ANOVA coupled with the Student-Newman-Keuls post hoc test in which p < 0.05 was required for a statistical difference; *significantly increased from vehicle treatment at a level of p < 0.05; **significantly increased from all other treatments at a level of p < 0.01.
increased from all other treatments at a level of significance both from each other and in comparison to vehicle treatment. This catechin-specific modulation of CYP450 isoforms may be responsible for the formation of approximately 85% of 2-hydroxyestradiol, which is then rapidly converted to 2-methoxyestradiol by COMT (Kerlan et al., 1992; Shou et al., 1997). Importantly, EGCG did not inhibit COMT activity as has been demonstrated with the in vivo administration of other gallate-containing compounds (Mannisto and Kaakkola, 1999). Therefore, the rapid conversion to 2-methoxyestradiol should not be altered by EGCG, thus allowing the induction of CYP3A to shift the metabolism toward the production of 2-methoxyestradiol. Since nM and μM concentrations of 2-methoxyestradiol disrupt microtubule formation (D’Amato et al., 1994), inhibit angiogenesis and induce apoptosis (Lakhani et al., 2003), small increases in 2-methoxyestradiol may have significant inhibitory effects on breast tumor growth.

Overall, the modest increase in CYP3A activity, paired with the 56% inhibition of aromatase, may work together to both decrease estradiol production and shift the metabolism of estradiol toward the production of antiestrogenic and antiangiogenic metabolites. However, CYP3A is also responsible for the formation of 15–20% of 4-hydroxyestradiol, a metabolite with estrogenic properties (Weisz et al., 1992). Therefore, to determine the exact effect on estradiol metabolism, future studies will measure the production of estradiol and its metabolites following EGCG treatment in tumor-bearing mice. Additionally, future studies will also determine the specific isoform of CYP3A (i.e., 3A11, 3A41, and/or 3A44) that is increased by EGCG.

In addition to examining the enzymes responsible for the synthesis and metabolism of estradiol, the modulatory effect on CYP2E1 by EGCG and ECG was also determined, as this enzyme plays an important role in activation of numerous carcinogens (Constan et al., 1999; Sohn et al., 2001) and is also expressed in breast tumors (Iscan et al., 2001). Therefore, this examination would demonstrate if catechin administration had the potential to produce drug/chemical interactions. While ECG failed to alter CYP2E1, EGCG (25 mg/kg/day, 7 days) increased CYP2E1 catalytic activity by 37% and polypeptide levels by 22%. Unfortunately this increase in CYP2E1 occurred following the same dose of EGCG that modulated aromatase and CYP3A. This small increase in CYP2E1 is unlikely to significantly increase the activation of carcinogens. However, the combined increase in CYP2E1 and CYP3A may cause drug-interactions with substances, such as acetaminophen, which utilize both of these enzymes in their metabolism (Kostrubsky et al., 1997; Raucy et al., 1989). This effect may be limited to the mouse, as, in the male rat, CYP2E1 is either unchanged (Siegers et al., 1982) or decreased by (+)-catechin and EC (Lhoste et al., 2003). However, further work needs to be performed to determine the extent of this potential species-specific response.

In summary, this is the first study to examine both hepatic and renal enzymes responsible for the synthesis and metabolism of estradiol following in vivo administration of specific catechins. The resulting catechin-specific alteration in ovarian aromatase and hepatic CYP3A by EGCG may prove to be an important component of the mechanism of EGCG-mediated breast tumor suppression. However, this result is tempered by...
the finding that high doses of EGCG produce significant hepatoxicity and mortality in female mice.

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