molecular pharmaceutics

reviews

# Biotransformation of Green Tea Polyphenols and the Biological Activities of Those Metabolites

**Joshua D. Lambert,* Shengmin Sang, and Chung S. Yang**

*Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854*

Received June 5, 2007; Revised Manuscript Received September 4, 2007; Accepted September 13, 2007

**Abstract:** Green tea (*Camellia sinensis*, Theaceae) and its major polyphenol constituents, the catechins, have been reported to have many health benefits including the prevention of cancer and heart disease. Many mechanisms of action have been proposed based on *in vitro* models; however, the importance of most of these mechanisms remains to be determined *in vivo*. The bioavailability and biotransformation of tea catechins play a key role in determining the importance of various mechanisms *in vivo*. Likewise, the biological activity and bioavailability of tea catechin metabolites, an understudied area, are important in understanding the potential beneficial effects of tea. In this article, we review the data available on the biotransformation of the tea catechins and the limited data set available on the biological activities of the catechin metabolites. Careful interpretation of available data, carefully designed animal experiments, and integration of bioavailability and biological activity data are needed if the disease preventive activity of tea is to be understood. We hope this article will spark research efforts on some of the important questions regarding tea polyphenol bioavailability, biotransformation, and the biological activities of tea catechin metabolites.

**Keywords:** Green tea; catechins; epigallocatechin-3-gallate; biotransformation

## Introduction

Green tea (*Camellia sinensis*, Theaceae) and its major polyphenolic compounds, the catechins, have been extensively studied for potential health beneficial effects including prevention of cancer, heart disease, diabetes, and neurodegenerative disease.[1–4] There are three major commercial varieties of tea: green (20% of consumption), oolong (2% of consumption), and black tea (78% of consumption).[5] Green tea is most commonly consumed in Asia whereas black tea is more popular in the United States and Europe. Green tea is chemically characterized by the presence of large amounts of polyphenolic compounds known as catechins. The major catechins are shown in Figure 1.

A typical cup of brewed green tea contains 30–40% catechins, by dry weight, including epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG). EGCG is the most abundant and widely studied green tea catechin, representing ~16.5 wt % of the water extractable fraction of tea.[5]

Tea and tea polyphenols have been the subject of a great deal of research in both *in vitro* and *in vivo* models of carcinogenesis.[1,6] Whereas these compounds have been

* To whom correspondence should be addressed: Fax (732)445-0687; e-mail joshua_lambert@hotmail.com.

(1) Lambert, J. D.; Hong, J.; Yang, G. Y.; Liao, J.; Yang, C. S. Inhibition of carcinogenesis by polyphenols: evidence from laboratory investigations. *Am. J. Clin. Nutr.* **2005**, *81* (1), 284S–291S.

(2) Yang, C. S.; Lambert, J. D.; Hou, Z.; Ju, J.; Lu, G.; Hao, X. Molecular targets for the cancer preventive activity of tea polyphenols. *Mol. Carcinog.* **2006**, *45* (6), 431–435.

(3) Higdon, J. V.; Frei, B. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Crit. Rev. Food Sci. Nutr.* **2003**, *43* (1), 89–143.

(4) Weisburger, J. H. Tea and health: the underlying mechanisms. *Proc. Soc. Exp. Biol. Med.* **1999**, *220* (4), 271–275.

(5) Balentine, D. A.; Wiseman, S. A.; Bouwens, L. C. The chemistry of tea flavonoids. *Crit. Rev. Food Sci. Nutr.* **1997**, *37* (8), 693–704.

(6) Yang, C. S.; Maliakal, P.; Meng, X. Inhibition of carcinogenesis by tea. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 25–54.

10.1021/mp700075m CCC: $37.00 
Published on Web 10/27/2007

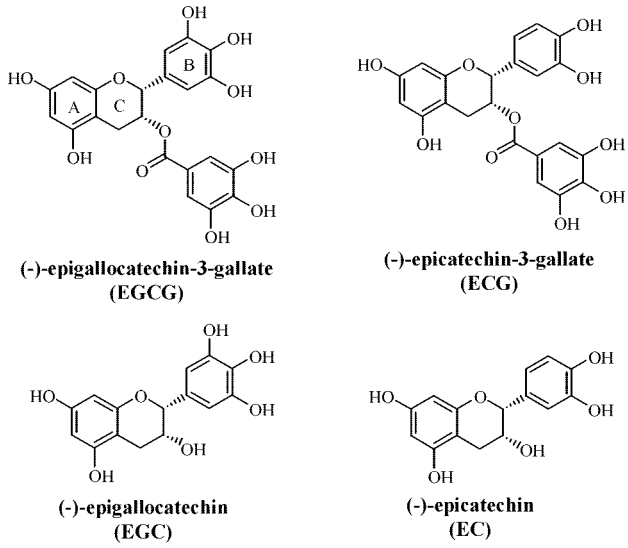


***Figure 1.*** Major catechins in green tea.

shown to be efficacious in a number of models of carcinogenesis, the epidemiological data of cancer prevention remain mixed. There are several possible explanations for the inconsistency of epidemiological data, including inaccuracies in assessing tea exposure, differences in tea preparation used, lifestyle factors such as smoking status, and differences in the bioavailability of tea polyphenols among different study populations.

Although a number of studies have examined the biotransformation and bioavailability of EGCG and the other catechin, considerably fewer studies have examined the biological activities of catechin metabolites. Biotransformative pathways are typically regarded as a means of inactivating biologically active compounds and facilitating their excretion.[7] Many examples of biologically active metabolites of drugs and dietary compounds have been reported.[8–10] Indeed, in some cases, biotransformative pathways result in metabolites that are more active than the parent compounds.[8] It is necessary to determine plasma and tissue levels and the biological activities of individual catechin metabolites. This will allow determination of the relative contribution of the pool of catechin metabolites to the observed biological activity of green tea *in vivo*.

In the present review, we will discuss the current knowledge on the biotransformation and bioavailability of green tea polyphenols. We also discuss the relatively small body of literature concerning the biological activities for catechin metabolites. It is our intent to stimulate discussion and research on this area which is understudied and is likely to be important in understanding the health beneficial effects of green tea.

**Biotransformation and Bioavailability of Tea Polyphenols. Phase II Biotransformation of Tea Polyphenols.** We and others have reported that the green tea polyphenols undergo methylation, glucuronidation, sulfation, and ring-fission metabolism.[11–14] Figure 2 shows the major biotransformative pathways for the tea catechins.

Studies on the enzymology of catechol-*O*-methyltransferase (COMT) have shown that EGC and EGCG are readily methylated to form 4′-*O*-methyl-(–)-EGC and 4″-*O*-methyl-(–)-EGCG or 4′,4″-*O*-dimethyl-(–)-EGCG, respectively.[15] Rat liver cytosol shows higher COMT activity toward EGCG and EGC than did human or mouse liver cytosol. At low concentrations of EGCG, the dimethylated compound is the major product, where as at high EGCG concentrations, monomethylated EGCG metabolites predominate.

EGCG-4″-*O*-glucuronide is the major metabolite formed by human, mouse, and rat microsomes.[16] Mouse small intestinal microsomes have the greatest catalytic efficiency ($V_{max}/K_m$) for glucuronidation followed by mouse liver, human liver, rat liver, and rat small intestine. Human UGT1A1, 1A8, and 1A9 had the greatest glucuronidation activity toward EGCG. UGT1A8, an intestinal-specific isoform, had the highest catalytic efficiency. EGC-3′-*O*-glucuronide is the major product formed by microsomes from mice, rats, and humans with the liver microsomes having a higher efficiency than intestinal microsomes.

(7) Klaassen, C. D. *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 5th ed.; McGraw-Hill: New York, 1996; p 1111.
(8) Monks, T. J.; Jones, D. C.; Bai, F.; Lau, S. S. The role of metabolism in 3,4-(+)-methylenedioxyamphetamine and 3,4-(+)-methylenedioxymethamphetamine (ecstasy) toxicity. *Ther. Drug Monit.* **2004**, *26* (2), 132–136.
(9) Bai, F.; Lau, S. S.; Monks, T. J. Glutathione and N-acetylcysteine conjugates of alpha-methyldopamine produce serotonergic neurotoxicity: possible role in methylenedioxyamphetamine-mediated neurotoxicity. *Chem. Res. Toxicol.* **1999**, *12* (12), 1150–1157.
(10) Harris, R. M.; Wood, D. M.; Bottomley, L.; Blagg, S.; Owen, K.; Hughes, P. J.; Waring, R. H.; Kirk, C. J. Phytoestrogens are potent inhibitors of estrogen sulfation: implications for breast cancer risk and treatment. *J. Clin. Endocrinol. Metab.* **2004**, *89* (4), 1779–1787.
(11) Lambert, J. D.; Yang, C. S. Cancer chemopreventive activity and bioavailability of tea and tea polyphenols. *Mutat. Res.* **2003**, *523–524*, 201–208.
(12) Li, C.; Meng, X.; Winnik, B.; Lee, M. J.; Lu, H.; Sheng, S.; Buckley, B.; Yang, C. S. Analysis of urinary metabolites of tea catechins by liquid chromatography/electrospray ionization mass spectrometry. *Chem. Res. Toxicol.* **2001**, *14* (6), 702–707.
(13) Kohri, T.; Nanjo, F.; Suzuki, M.; Seto, R.; Matsumoto, N.; Yamakawa, M.; Hojo, H.; Hara, Y.; Desai, D.; Amin, S.; Conaway, C. C.; Chung, F. L. Synthesis of (-)-[4-3H]epigallocatechin gallate and its metabolic fate in rats after intravenous administration. *J. Agric. Food Chem.* **2001**, *49* (2), 1042–1048.
(14) Hu, M.; Chen, J.; Lin, H. Metabolism of flavonoids via enteric recycling: mechanistic studies of disposition of apigenin in the Caco-2 cell culture model. *J. Pharmacol. Exp. Ther.* **2003**, *307* (1), 314–321.
(15) Lu, H.; Meng, X.; Yang, C. S. Enzymology of methylation of tea catechins and inhibition of catechol-O-methyltransferase by (-)-epigallocatechin gallate. *Drug Metab. Dispos.* **2003**, *31* (5), 572–579.
(16) Lu, H.; Meng, X.; Li, C.; Sang, S.; Patten, C.; Sheng, S.; Hong, J.; Bai, N.; Winnik, B.; Ho, C. T.; Yang, C. S. Glucuronides of tea catechins: enzymology of biosynthesis and biological activities. *Drug Metab. Dispos.* **2003**, *31* (4), 452–461.

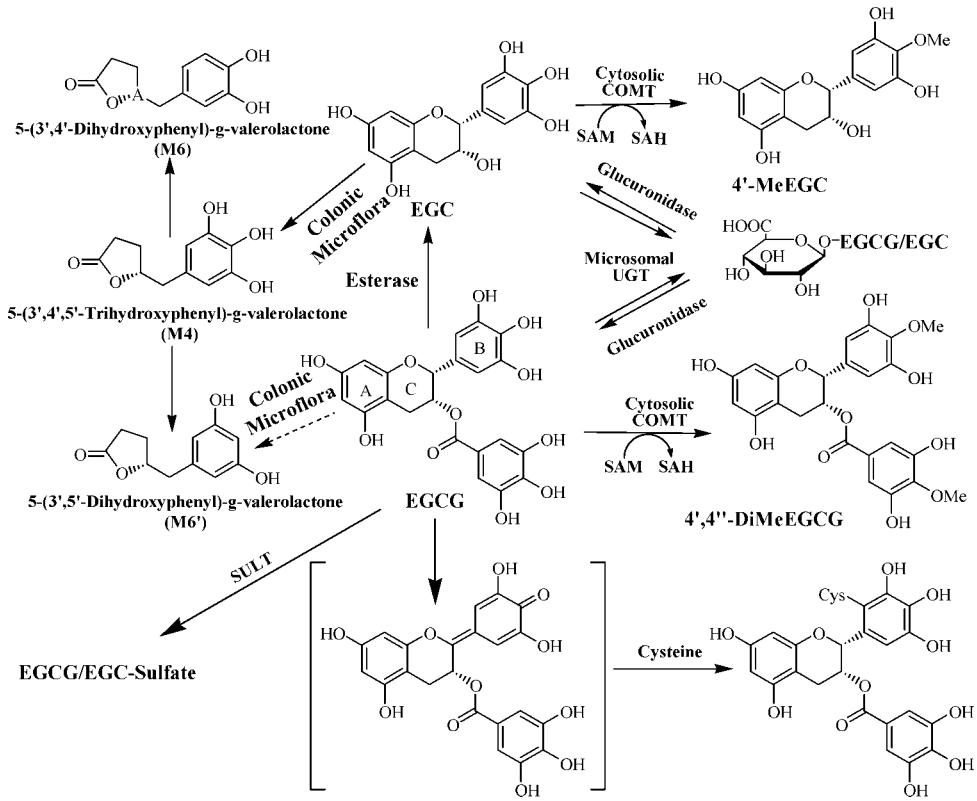


***Figure 2.*** Biotransformation of the green tea catechins. Abbreviations: 4′-MeEGC, 4′-*O*-methyl-(–)-epigallocatechin; 4′,4″-di-*O*-methylEGCG, 4′,4″-di-*O*-methyl-(–)-epigallocatechin-3-gallate; COMT, catechol-*O*-methyltransferase; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin-3-gallate; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SULT, sulfotransferase; UGT, UDP-glucuronosyltransferase.

Vaidyanathan et al. have shown that EC undergoes sulfation catalyzed by human and rat intestinal and liver enzymes in cytosol, with the human liver being the most efficient.[17] Sulfotransferase (SULT)1A1 is largely responsible for this activity in the liver, whereas both SULT1A1 and SULT1A3 are active in the human intestine. The catalytic efficiency for SULT1A1- and SULT1A3-mediated sulfation of EC are 5834 and 55 $\mu$L/(min mg). EGCG is also time- and concentration-dependently sulfated by human, mouse, and rat liver cytosol.[18] The rat has the greatest activity followed by the mouse and the human.

On the basis of these *in vitro* studies, it appears that mice are more similar to humans in terms of biotransformative ability toward tea catechins than are rats. While these similarities must still be confirmed *in vivo*, this information will aid in choosing the most appropriate animal model to study the potential health benefits of tea constituents.

Our recent results from data-dependent tandem mass spectrometric analysis of mouse urine samples after EGCG treatment have shown that methylated EGCG (or glucuronidated or sulfated EGCG) can be further glucuronided and/or sulfated (or methylated) to form related mixed EGCG metabolites (Sang, unpublished data).

At toxic doses, EGCG is metabolized to EGCG-2″-cysteine and EGCG-2′-cysteine *in vivo*.[19] These metabolites can be detected in the urine following administration of 200–400 mg/kg, i.p., or 1500 mg/kg, i.g., EGCG to mice. We hypothesize that these metabolites form as the result of oxidation of EGCG to a quinone or semiquinone which then reacts with the sulfhydryl group of cysteine. Similar metabolites formed by reaction with glutathione and *N*-acetylcysteine have not yet been discovered.

**Active Efflux of Tea Polyphenols.** Active efflux, also referred to as phase III metabolism, has been shown to limit the bioavailability and cellular accumulation of many compounds. The multidrug resistance-associated proteins (MRP) are ATP-dependent efflux transporters that are expressed in many tissues and overexpressed in many human cancer. MRP1 is located on the basolateral side of cells and is present in nearly all tissues and transports compounds from the interior of the cells into the interstitial space.[20] MRP2 is

(17) Vaidyanathan, J. B.; Walle, T. Glucuronidation and sulfation of the tea flavonoid (-)-epicatechin by the human and rat enzymes. *Drug Metab. Dispos.* **2002**, *30* (8), 897–903.

(18) Lu, H. Mechanistic studies on the Phase II metabolism and absorption of tea catechins. Doctoral, Rutgers, The State University of New Jersey, New Brunswick, 2002.

(19) Sang, S.; Lambert, J. D.; Hong, J.; Tian, S.; Lee, M. J.; Stark, R. E.; Ho, C. T.; Yang, C. S. Synthesis and structure identification of thiol conjugates of (-)-epigallocatechin gallate and their urinary levels in mice. *Chem. Res. Toxicol.* **2005**, *18* (11), 1762–1769.

(20) Leslie, E. M.; Deeley, R. G.; Cole, S. P. Toxicological relevance of the multidrug resistance protein 1, MRP1 (ABCC1) and related transporters. *Toxicology* **2001**, *167* (1), 3–23.

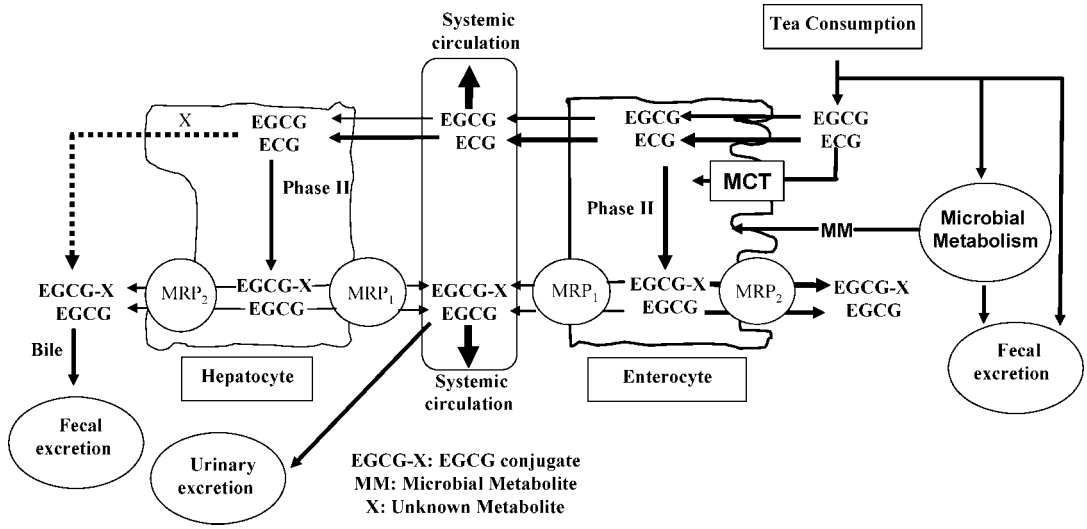


***Figure 3.*** Proposed model summarizing the effects of phase II and phase III (active efflux) metabolism on the bioavailability of the green tea catechins. Arrows between tissues which do not pass through an indicated protein represent passive diffusion. Abbreviations: ECG, (–)-epicatechin-3-gallate; EGCG, (–)-epigallocatechin-3-gallate; EGCG-X, unknown EGCG metabolite; MCT, monocarboxylate transporter; MM, microbial metabolite; MRP, multidrug resistance related protein; X, unknown metabolite.

located on the apical surface of the intestine, kidney, and liver, where it transports compounds from the bloodstream into the lumen, urine, and bile, respectively.[20] Recent studies on EGCG uptake in Madin-Darby canine kidney (MDCKII) cells have shown that indomethacin (MRP inhibitor) increased the intracellular accumulation of EGCG, EGCG 4″-*O*-methyl-EGCG, or 4′,4″-di-*O*-methyl-EGCG by 10-, 11-, or 3-fold overexpressing MRP1.[21] Similarly, treatment of MRP2 overexpressing MDCKII cells with MK-571 (an MRP2 inhibitor) resulted in 10-, 15-, or 12-fold increase in the intracellular levels of EGCG, 4″-*O*-methyl-EGCG, and 4′,4″-di-*O*-methyl-EGCG, respectively. Treatment of PGP-overexpressing MDCKII cells with PGP inhibitors, however, resulted in no significant effect on the intracellular levels of EGCG or its metabolites. Treatment of HT-29 human colon cancer cells with indomethacin resulted in increase intracellular accumulation of EGCG and its methylated and glucuronidated metabolites.[22] These data suggest a role for MRPs, but not PGP, in affecting the bioavailability of EGCG.

Vaidyanathan and Walle have shown that treatment of Caco-2 cells with MK-571 enhances apical to basolateral movement of EC and ECG.[23,24] MK-571 reduced the efflux of EC-sulfates from the cytosol to the apical well, suggesting the EC-sulfates are also substrates for MRP2.[23] Although we have previously suggested that the uptake of EGCG into HT-29 cells is mainly through passive diffusion, others have demonstrated that ECG is a substrate for the monocarboxylate transporter (MCT). Inhibition MCT-1 by benzoic acid or phloretin significantly reduced ECG uptake by Caco-2 cells.[24]

The combined effects of MRP1, MRP2, and MCT on the bioavailability of the tea polyphenols remain to be determined *in vivo*. As shown in Figure 3, the apical location of MRP2 suggests that it acts to limit EGCG bioavailability by actively exporting EGCG back into the intestinal lumen.

The remaining fraction of EGCG would then be absorbed into the portal circulation, enter the liver, and could subsequently be effluxed by MRP2 located on the canalicular membrane of the hepatocytes. In contrast, MRP1 is located on the basolateral side of enterocytes, hepatocytes, and other tissues. Substrates of this pump are effluxed from the interior of the cells into the intestinal space. The role of MRP1 would be expected to increase the bioavailability of EGCG *in vivo*. The influence of MRP1 and 2 *in vivo*, however, is likely to depend on their relative tissue distribution. It was reported that the MRP2 mRNA level was over 10-fold higher than that of MRP1 in the human jejunum; therefore, efflux of EGCG by MRP2 may be predominant in the intestine, resulting in a decrease of EGCG bioavailability.[25]

**Microbial Metabolism of Tea Polyphenols.** Our laboratory has identified several ring fission products of tea catechins in human urine and plasma after ingestion of 20 mg/kg decaffeinated green tea.[26] The compounds, 5-(3′,4′,5′-

(21) Hong, J.; Lambert, J. D.; Lee, S. H.; Sinko, P. J.; Yang, C. S. Involvement of multidrug resistance-associated proteins in regulating cellular levels of (-)-epigallocatechin-3-gallate and its methyl metabolites. *Biochem. Biophys. Res. Commun.* **2003**, *310* (1), 222–227.

(22) Hong, J.; Lu, H.; Meng, X.; Ryu, J. H.; Hara, Y.; Yang, C. S. Stability, cellular uptake, biotransformation, and efflux of tea polyphenol (-)-epigallocatechin-3-gallate in HT-29 human colon adenocarcinoma cells. *Cancer Res.* **2002**, *62* (24), 7241–7246.

(23) Vaidyanathan, J. B.; Walle, T. Transport and metabolism of the tea flavonoid (-)-epicatechin by the human intestinal cell line Caco-2. *Pharm. Res.* **2001**, *18* (10), 1420–1425.

(24) Vaidyanathan, J. B.; Walle, T. Cellular uptake and efflux of the tea flavonoid (-)epicatechin-3-gallate in the human intestinal cell line Caco-2. *J. Pharmacol. Exp. Ther.* **2003**, *307* (2), 745–752.

trihydroxyphenyl)-$\gamma$-valerolactone (M4), 5-(3′,4′-dihydroxyphenyl)-$\gamma$-valerolactone (M6), and 5-(3′,5′-dihydroxyphenyl)-$\gamma$-valerolactone (M6′), shown in Figure 2, have a $T_{max}$ of 7.5–13.5 h and reach peak plasma concentrations of 100–200 nM. Maximal urine concentrations of 8, 4, and 8 $\mu$M have been demonstrated for M4, M6, and M6′, respectively, following ingestion of 200 mg EGCG. These metabolites are believed to be result from microbial metabolism in the colon. Anaerobic fermentation of EGC, EC, and ECG with human fecal microflora has been shown to result in the production of M4, M6, and M6′.[27]

**Biological Activities of Tea Polyphenol Metabolites.** We and others have examined the biological activities of the metabolites of tea polyphenols. The small number of studies have focused on antioxidant/prooxidant activity, inhibition of cancer cell growth, anti-inflammatory activity, and inhibition of specific enzymes.

**Antioxidant/Prooxidant Activities.** Our laboratory has examined the antioxidant activities of the glucuronide metabolites of EGCG and EGC. Both EGC-3′-glucuronide and EGC-7-glucuronide had reduced radical scavenging activity compared to EGC as measured by 1,1-diphenyl-2-picrylhydryl (DPPH) radical assay.[16] The $EC_{50}$'s, in terms of molar ratio of test compound/DPPH, were 0.08, 0.11, and 0.19 for EGC, EGC-7-glucuronide, and EGC-3′-glucuronide, respectively. Overall, the glucuronide metabolites of EGCG were more active than EGC and its glucuronides. The effect of glucuronidation on the DPPH scavenging ability of EGCG showed significant variation depending on the site of glucuronidation. EGCG-7-glucuronide and EGCG-4″-glucuronide were less active than EGCG ($EC_{50}$ = 0.081 and 0.084 vs 0.039), whereas EGCG-3′-glucuronide ($EC_{50}$ = 0.037) and EGCG-3″-glucuronide ($EC_{50}$ = 0.035) had equivalent radical scavenging ability to EGCG.

We and others have previously shown that tea catechins undergo auto-oxidation under cell culture conditions and generate $H_2O_2$.[28–30] This $H_2O_2$ has been implicated in some of the biological activities of the catechins, especially EGCG. For example, we have reported that treatment of human esophageal cancer cells with EGCG results in dose-dependent decrease in the levels of phosphorylated and nonphosphorylated epidermal growth factor receptor.[31] These effects are diminished by inclusion of superoxide dismutase and catalase which stablizes EGCG and apparently prevents oxidative damage of EGFR. Methylation of EGCG at the 4′-hydroxyl or 4″-hydroxyl group has been shown to decreases its ability to reduce Fe(III) and, in the case of 4′-*O*-methylEGCG, to reduce it is ability to produce $H_2O_2$ (85% reduction compared to EGCG).[32] By contrast, EGCG-2″-cysteine was more redox active as measured by production of $H_2O_2$ (Lambert, unpublished results). Under cell culture conditions, EGCG and EGCG-2″-cysteine produced similar total amounts of $H_2O_2$ over 24 h, but the time to half-maximal $H_2O_2$ was 15 min for EGCG-2″-cysteine compared to 60 min for EGCG.

**Inhibition of Cancer Cell Growth.** A limited number of studies have examined the growth inhibitory activity of EGCG metabolites against cancer cell lines. Overall, these studies indicate that the metabolites are less effective at inhibiting cell growth than EGCG. Treatment of HT-29 human colon and KYSE150 human esophageal cancer cells with 50$\mu$M M4 in serum-free medium resulted in ~40% growth inhibition after 48 h.[33,34] The structure of M4 is shown in Figure 2. By contrast, treatment of KYSE150 cells with EGCG for 48 h resulted in 50% growth inhibition at 20 $\mu$M.[34,35] HCT-116 human colon cancer cells, INT-407 human, and IEC-6 rat immortalized intestinal cell lines were not sensitive to the growth inhibitory effects of M4. The $IC_{50}$ of EGCG against HCT-116 cells 45 $\mu$M after 48 h.[34]

(25) Taipalensuu, J.; Tornblom, H.; Lindberg, G.; Einarsson, C.; Sjoqvist, F.; Melhus, H.; Garberg, P.; Sjostrom, B.; Lundgren, B.; Artursson, P. Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers. *J. Pharmacol. Exp. Ther.* **2001**, *299* (1), 164–170.

(26) Li, C.; Lee, M. J.; Sheng, S.; Meng, X.; Prabhu, S.; Winnik, B.; Huang, B.; Chung, J. Y.; Yan, S.; Ho, C. T.; Yang, C. S. Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion. *Chem. Res. Toxicol.* **2000**, *13* (3), 177–184.

(27) Meselhy, M. R.; Nakamura, N.; Hattori, M. Biotransformation of (-)-epicatechin 3-O-gallate by human intestinal bacteria. *Chem. Pharm. Bull. (Tokyo)* **1997**, *45* (5), 888–893.

(28) Yang, G. Y.; Liao, J.; Li, C.; Chung, J.; Yurkow, E. J.; Ho, C. T.; Yang, C. S. Effect of black and green tea polyphenols on c-jun phosphorylation and H(2)O(2) production in transformed and non-transformed human bronchial cell lines: possible mechanisms of cell growth inhibition and apoptosis induction. *Carcinogenesis* **2000**, *21* (11), 2035–2039.

(29) Shen, J. Z.; Zheng, X. F.; Wei, E. Q.; Kwan, C. Y. Green tea catechins evoke a phasic contraction in rat aorta via H2O2-mediated multiple-signalling pathways. *Clin. Exp. Pharmacol. Physiol.* **2003**, *30* (1–2), 88–95.

(30) Elbling, L.; Weiss, R. M.; Teufelhofer, O.; Uhl, M.; Knasmueller, S.; Schulte-Hermann, R.; Berger, W.; Micksche, M. Green tea extract and (-)-epigallocatechin-3-gallate, the major tea catechin, exert oxidant but lack antioxidant activities. *FASEB J.* **2005**, *19* (7), 807–809.

(31) Hou, Z.; Sang, S.; You, H.; Lee, M. J.; Hong, J.; Chin, K. V.; Yang, C. S. Mechanism of Action of (-)-Epigallocatechin-3-Gallate: Auto-oxidation-Dependent Inactivation of Epidermal Growth Factor Receptor and Direct Effects on Growth Inhibition in Human Esophageal Cancer KYSE 150 Cells. *Cancer Res.* **2005**, *65* (17), 8049–8056.

(32) Nakagawa, H.; Hasumi, K.; Takami, M.; Aida-Hyugaji, S.; Woo, J. T.; Nagai, K.; Ishikawa, T.; Wachi, M. Identification of two biologically crucial hydroxyl groups of (-)-epigallocatechin gallate in osteoclast culture. *Biochem. Pharmacol.* **2007**, *73* (1), 34–43.

(33) Lambert, J. D.; Rice, J. E.; Hong, J.; Hou, Z.; Yang, C. S. Synthesis and biological activity of the tea catechin metabolites, M4 and M6 and their methoxy-derivatives. *Bioorg. Med. Chem. Lett.* **2005**, *15* (4), 873–876.

(34) Lambert, J. D.; Sang, S.; Hong, J.; Kwon, S. J.; Lee, M. J.; Ho, C. T.; Yang, C. S. Peracetylation as a Means of Enhancing In vitro Bioactivity and Bioavailability of Epigallocatechin-3-gallate. *Drug Metab. Dispos.* **2006**, in press.

(35) Hou, Z.; Xiao, H.; Lambert, J.; You, H.; Yang, C. S. Green tea polyphenol, (-)-epigallocatechin-3-gallate, induces oxidative stress and DNA damage in cancer cell lines, xenograft tumors, and mouse liver. *Proc. Am. Assoc. Cancer Res.* **2006**, in press.

The cytotoxic effects of EGCG, 4″-*O*-MeEGCG, and 4′,4″-di-*O*-methylEGCG were compared in Jurkat T cells using the Trypan Blue exclusion assay.[36] Treatment for 24 h with 25 $\mu$M resulted in 10–20% growth inhibition by all compounds: methylation appeared to have no significant effect on cytotoxic activity.

Nakagawa et al. have reported that methylated metabolites of EGCG had decreased growth inhibitory and pro-apoptotic activity compared to EGCG against murine osteoclasts.[32] The $IC_{50}$'s of EGCG, 4′-*O*-MeEGCG, and 4″-*O*-methylEGCG for growth inhibition were 58, >100, and >100 $\mu$M, respectively. The authors speculate that this decrease in growth inhibitory activity is related to changes in pro-oxidant activity of the compounds. Both 4′-*O*-MeEGCG and 4″-*O*-methylEGCG had reduced Fe(III)-reducing power, and 4′-*O*-MeEGCG had decreased $H_2O_2$-producing ability.

We have examined the growth inhibitory effects of EGCG-2′-cys and EGCG-2″-cys after 48 h treatment against HCT-116 ($IC_{50}$'s $\sim$ 100 $\mu$M) cells in the presence of SOD and catalase (Lambert et al., unpublished data). The growth inhibitory activity of EGCG and EGCG-2′-cys were compared in CL13 mouse lung adenocarcinoma cells. Both compounds were only weakly active after 24 h in the presence of SOD and catalase (inhibition less than 20% at 100 $\mu$M), but in the absence of SOD and catalase the growth inhibitory activity of the two compounds was much greater ($IC_{50} \sim 60$ $\mu$M) (Lambert, unpublished results).

**Antiinflammatory Activity.** The anti-inflammatory activity of tea polyphenol metabolites have been examined by measuring NO production by LPS-stimulated macrophages and aberrant arachidonic acid metabolism in [$^{13}$C]-arachidonic acid-labeled human colon cancer cells. M4 inhibited the NO production by RAW264.7 murine macrophages by $\sim$50% at 20 $\mu$M compared to vehicle treated controls.[33]

The ability of EGCG to inhibit aberrant arachidonic acid metabolism in HT29 was unaffected by glucuronidation.[16] EGCG and its 3′-, 3″-, 4″-, and 7-glucuronide metabolites had $IC_{50}$'s of 2 $\mu$M. By contrast, glucuronidation of EGC at the 3′-OH decreased the inhibitory potency of this molecule by 20% compared to EGC. EGC and EGC-7-glucuronide were equipotent and inhibited aberrant arachidonic acid metabolism by 60% at 10 $\mu$M. M4 showed no inhibitory activity against aberrant arachidonic acid metabolism when tested in the RAW 264.7 murine macrophage model.[33]

**Inhibition of Specific Enzymes.** COMT is an essential detoxification pathway for both endogenous and exogenous substrates.[7] We and others have examined the inhibitory effects of EGCG, EGC, and their methylated and glucuronidated metabolites.[37,38] EGCG potently inhibited the methylation of hydroxyestradiol metabolites ($IC_{50}$ = 0.07–0.08 $\mu$M). The inhibition was mixed type. Molecular modeling studies have shown that the strong binding of EGCG to COMT was due to the formation of a hexacoordination complex with the active site $Mg^{2+}$ of COMT and interaction between the 4″-OH of EGCG and Lys144-$NH_2$.[38] Binding of EGCG to COMT was stabilized by hydrophobic interactions between the D-ring of EGCG and Trp38, Leu198, Pro174, and Trp143 of COMT.[38]

Both 4″-*O*-methylEGCG and 4′,4″-di-*O*-methylEGCG were less potent than EGCG ($IC_{50}$ = 0.10 and 0.15 $\mu$M, respectively).[38] Kinetic analysis of 4′,4″-di-*O*-methylEGCG revealed a noncompetitive inhibitory mechanism. Methylation of the 4′- and 4″-hydroxyl groups interferes with the interaction with Lys144 and eliminates the competitive inhibitory activity of 4′,4″-di-*O*-methylEGCG. The binding site of this molecule to COMT remains to be determined.

The glucuronide metabolites of EGCG had differential inhibitory activity against COMT-mediated methylation of hydroxyestradiol depending on the location of glucuronidation.[38] EGCG-7-glucuronide had submicromolar potency (0.6 $\mu$M) whereas EGCG-3′-glucuronide, EGCG-3″-glucuronide, and EGCG-4″-glucuronide were significantly less potent (1.8–2.5 $\mu$M). These findings indicate the importance of 4′- and 4″-hydroxyl groups and suggest that addition of a bulky, charged substituent reduces access to the COMT active site.

EGC was significantly less active than EGCG ($IC_{50}$ = 44–50 $\mu$M) at COMT-mediated methylation of hydroxyestradiol. Methylation of the 4′-hydroxyl group did not significantly affect the inhibitory activity of EGC ($IC_{50}$ = 32.0 $\mu$M).[38] This suggests that 4′-MeEGC acts as a noncompetitive inhibitor. Further studies on the kinetics of EGC and 4′-MeEGC are necessary to clarify the mechanism of inhibition of these compounds.

The 20S proteasome is an essential protease enzyme which catalyzes the targeted degradation of intracellular proteins, and inhibitors of this enzyme have been identified as potential new anticancer agents.[39] EGCG has been reported to potently inhibit ($IC_{50}$ = 86–194 nM) the chymotryptic activity of the 20S proteasome.[40] Landis-Piwowar et al. studied the effect of *O*-methylation on the potency EGCG as a proteasome inhibitor. Both of the major *in vivo* methyl metabolites, 4″-*O*-MeEGCG, and 4′,4″-di-*O*-methylEGCG, had significantly reduced activity compared to EGCG.[36] The reported $IC_{50}$'s of EGCG, 4″-*O*-MeEGCG, and 4′,4″-di-*O*-methylEGCG

(36) Landis-Piwowar, K. R.; Wan, S. B.; Wiegand, R. A.; Kuhn, D. J.; Chan, T. H.; Dou, Q. P. Methylation suppresses the proteasome-inhibitory function of green tea polyphenols. *J. Cell Physiol.* **2007**, in press.

(37) Nagai, M.; Conney, A. H.; Zhu, B. T. Strong inhibitory effects of common tea catechins and bioflavonoids on the O-methylation of catechol estrogens catalyzed by human liver cytosolic catechol-O-methyltransferase. *Drug Metab. Dispos.* **2004**, *32* (5), 497–504.

(38) Chen, D.; Wang, C. Y.; Lambert, J. D.; Ai, N.; Welsh, W. J.; Yang, C. S. Inhibition of human liver catechol-O-methyltransferase by tea catechins and their metabolites: structure-activity relationship and molecular-modeling studies. *Biochem. Pharmacol.* **2005**, *69* (10), 1523–1531.

(39) Bold, R. J.; Virudachalam, S.; McConkey, D. J. Chemosensitization of pancreatic cancer by inhibition of the 26S proteasome. *J. Surg. Res.* **2001**, *100* (1), 11–17.

(40) Nam, S.; Smith, D. M.; Dou, Q. P. Ester bond-containing tea polyphenols potently inhibit proteasome activity in vitro and in vivo. *J. Biol. Chem.* **2001**, *276* (16), 13322–13330.

were 0.2, 6.9, and 8.2, respectively. Previous studies by the same laboratory used molecular modeling techniques to gain insight into the mechanism for the loss of potency. The *in silico* study suggested that methylation of the 4″-hydroxyl group shifts the EGCG molecule relative to the $\beta$5 subunit of the 20S proteasome and prevents the ester carbon of EGCG from interacting with Thr1 on the $\beta$5 subunit.[41] This interaction between the ester carbon of EGCG and Thr1 is believed to be key to the inhibitory activity of EGCG.

## Discussion

Green tea and tea polyphenols have been extensively studied for their potential health beneficial effects, including prevention of cancer, diabetes, heart disease, and neurodegenerative diseases.[1–4] Our own laboratory has published many studies related to the lung and colon cancer preventive activities of green tea and its major catechin component, EGCG.[42–45] Although numerous mechanisms of action have been proposed for the disease preventive activities of EGCG and the other catechins, the majority of these are based on *in vitro* studies with purified compounds. Few of the proposed mechanisms have been validated in animal models of disease. Most of the *in vitro* studies have used concentrations of test compounds 10–100-fold greater than those observed *in vivo* following administration of dietary or pharmacological doses of tea catechins.[11,46] Possible explanations for the discrepancy in effective concentrations between *in vitro* and *in vivo* studies may be differences in treatment times (*in vitro* studies are short-term, *in vivo* studies are long-term), lack of epithelial–stroma interactions *in vitro*, or inherent genetic and physiological differences between cell lines and animal models. Another possibility is that the metabolites of EGCG and the other catechins are biologically active and contribute to the overall activity of green tea *in vivo*.

We and others have extensively studied the biotransformation and bioavailability of green tea polyphenols, especially EGCG, both in animal models and in human volunteers.[11,46–51] Tea polyphenols undergo extensive phase II and microbial metabolism and may be subject to active efflux. Further studies are needed to fully elucidate the relative of importance of various pathways in different species, to carefully establish the plasma and tissue levels of individual metabolites, and to establish the role of active efflux *in vivo*. A complete understanding of the biotransformation and bioavailability of EGCG will aid in designing future intervention studies and in interpreting the results of epidemiological studies and laboratory studies of green tea. Relatively few studies have been conducted on the biological activities of the metabolites of green tea polyphenols. In most cases, these studies have shown that metabolism of EGCG to glucuronide, methylated, or ring fission products reduces its biological activity. Some metabolites, however, retain equivalent activity in some systems (e.g., EGCG-2″-cys has equivalent or greater pro-oxidant activity than EGCG). Further systematic studies are needed to characterize the biological activities of the major catechin metabolites in different experimental systems. Precise measurements of the plasma and tissue levels of these compounds are also needed. Taken together, these data could provide insight into the relative contribution of catechin metabolites to the disease preventive effects of green tea.

**Acknowledgment.** This manuscript was supported by AICR grant 05A047 and NCI grant CA125780 (to J.D.L.) and NIH grant CA88961 (to C.S.Y.).

MP700075M

(41) Daniel, K. G.; Landis-Piwowar, K. R.; Chen, D.; Wan, S. B.; Chan, T. H.; Dou, Q. P. Methylation of green tea polyphenols affects their binding to and inhibitory poses of the proteasome beta5 subunit. *Int. J. Mol. Med.* **2006**, *18* (4), 625–632.

(42) Landau, J. M.; Wang, Z. Y.; Yang, G. Y.; Ding, W.; Yang, C. S. Inhibition of spontaneous formation of lung tumors and rhabdomyosarcomas in A/J mice by black and green tea. *Carcinogenesis* **1998**, *19* (3), 501–507.

(43) Liao, J.; Yang, G. Y.; Park, E. S.; Meng, X.; Sun, Y.; Jia, D.; Seril, D. N.; Yang, C. S. Inhibition of lung carcinogenesis and effects on angiogenesis and apoptosis in A/J mice by oral administration of green tea. *Nutr. Cancer* **2004**, *48* (1), 44–53.

(44) Lu, G.; Liao, J.; Yang, G.; Reuhl, K. R.; Hao, X.; Yang, C. S. Inhibition of adenoma progression to adenocarcinoma in a 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis model in A/J mice by tea polyphenols and caffeine. *Cancer Res.* **2006**, *66* (23), 11494–11501.

(45) Ju, J.; Hong, J.; Zhou, J. N.; Pan, Z.; Bose, M.; Liao, J.; Yang, G. Y.; Liu, Y. Y.; Hou, Z.; Lin, Y.; Ma, J.; Shih, W. J.; Carothers, A. M.; Yang, C. S. Inhibition of intestinal tumorigenesis in Apcmin/+ mice by (-)-epigallocatechin-3-gallate, the major catechin in green tea. *Cancer Res.* **2005**, *65* (22), 10623–10631.

(46) Lambert, J. D.; Yang, C. S. Mechanisms of cancer prevention by tea constituents. *J. Nutr.* **2003**, *133* (10), 3262S–3267S.

(47) Chow, H. H.; Cai, Y.; Alberts, D. S.; Hakim, I.; Dorr, R.; Shahi, F.; Crowell, J. A.; Yang, C. S.; Hara, Y. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. *Cancer Epidemiol., Biomarkers Prev.* **2001**, *10* (1), 53–58.

(48) Chow, H. H.; Cai, Y.; Hakim, I. A.; Crowell, J. A.; Shahi, F.; Brooks, C. A.; Dorr, R. T.; Hara, Y.; Alberts, D. S. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clin. Cancer Res.* **2003**, *9* (9), 3312–3319.

(49) Lee, M. J.; Maliakal, P.; Chen, L.; Meng, X.; Bondoc, F. Y.; Prabhu, S.; Lambert, G.; Mohr, S.; Yang, C. S. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. *Cancer Epidemiol., Biomarkers Prev.* **2002**, *11* (10 Pt 1), 1025–1032.

(50) Lambert, J. D.; Lee, M. J.; Lu, H.; Meng, X.; Hong, J. J.; Seril, D. N.; Sturgill, M. G.; Yang, C. S. Epigallocatechin-3-gallate is absorbed but extensively glucuronidated following oral administration to mice. *J. Nutr.* **2003**, *133* (12), 4172–4177.

(51) Chen, L.; Lee, M. J.; Li, H.; Yang, C. S. Absorption, distribution, elimination of tea polyphenols in rats. *Drug Metab. Dispos.* **1997**, *25* (9), 1045–1050.