

Peracetylation as a Means of Enhancing *In vitro* Bioactivity and Bioavailability of Epigallocatechin-3-gallate

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Abbreviations: AcEGCG, peracetylated (-)-epigallocatechin-3-gallate; $AUC_{0 \rightarrow \infty}$, area under the curve from 0 to infinity; C_{max} , maximum concentration; EGCG, (-)-epigallocatechin-3-gallate; MTT, 3,[4,5-dimethylthiazol-2-y]-2,5-diphenyltetrazolium bromide; $t_{1/2}$, half-life; V_{max} , maximum velocity

Abstract

(-)-Epigallocatechin-3-gallate (EGCG) is the widely studied catechin in green tea (*Camellia sinensis*). Previously, we have reported the low bioavailability of EGCG in rats and mice. As a means of improving the bioavailability of EGCG, we have prepared a peracetylated EGCG derivative (AcEGCG), and herein, report its growth inhibitory activity and cellular uptake *in vitro*, as well as, bioavailability in mice. AcEGCG exhibited enhanced growth inhibitory activity relative to EGCG in both KYSE150 human esophageal (IC₅₀ = 10 vs. 20 μM) and HCT116 human colon cancer cells (IC₅₀ = 32 vs. 45 μM). AcEGCG was rapidly converted to EGCG by HCT116 cells, and treatment of cells with AcEGCG resulted in a 2.8 - 30-fold greater intracellular concentration of EGCG as compared to treatment with EGCG. AcEGCG was also more potent than EGCG at inhibiting nitric oxide-production (4.4-fold) and arachidonic acid-release (2.0-fold) from lipopolysaccharide-stimulated RAW264.7 murine macrophages. Intra-gastric administration of AcEGCG to CF-1 mice resulted in higher bioavailability compared to administration of equimolar doses of EGCG. The plasma AUC_{0→∞} of total EGCG was 465.0 and 194.6 ((μg/mL)·min) from the administration of AcEGCG and EGCG, respectively. The t_{1/2} of EGCG was also increased following administration of AcEGCG compared to EGCG (441.0 vs. 200.3 min). The AUC_{0→∞} and t_{1/2} were also increased in small intestinal (2.8- and 4.3-fold, respectively) and colonic tissues (2.4 and 6.0-fold, respectively). These data suggest that acetylation represents a means of increasing the biological potency *in vitro*, increasing the bioavailability of EGCG *in vivo*, and may improve cancer preventive activity.

Introduction

Green tea (*Camellia sinensis*, Theaceae) and its catechin components, especially epigallocatechin-3-gallate (EGCG, mol. formula: C₂₂H₁₈O₁₁, Fig. 1), have shown cancer preventive activity in a number of animal models (Lambert and Yang, 2003b; Lambert et al., 2005). Numerous mechanisms have been proposed for this activity based on studies of human cancer cell lines, including inhibition of growth factor signaling, inhibition of kinases, inhibition of DNA methyltransferase, and others (Higdon and Frei, 2003; Hou et al., 2004). Due to the low bioavailability of EGCG, it is unclear which of these mechanisms play primary roles in cancer prevention.

We have previously shown that the bioavailability of EGCG in mice and rats is 26.5% and 1.6%, respectively (Chen et al., 1997; Lambert et al., 2003). EGCG has a large number of hydrogen bond donors (*i.e.* hydroxyl groups), has a large polar molecular surface area, and is predicted by “Lipinski’s Rule of 5” to be poorly absorbed (Clark, 1999; Lipinski et al., 2001). EGCG is subjected to extensive glucuronidation, methylation, and sulfation, as well, as to microbial degradation in the colon (Kida et al., 2000; Lu et al., 2003a; Lu et al., 2003b). The small intestine appears to be a major barrier to the bioavailability of EGCG. Cai *et al.* found that although the oral bioavailability of EGCG is low in the rat, only a small percentage of EGCG, administered by the portal vein, is metabolized by the liver (Cai et al., 2002). This suggests that poor absorption from the small intestine may play an important role in limiting EGCG bioavailability. Similarly, our laboratory has reported that the intestine-specific human UDP-glucuronosyltransferase 1A8 and that mouse small intestine microsomes were the more active at glucuronidation of EGCG than the liver (Lu et al., 2003a). Cell line

studies have also suggested that EGCG and other catechins are subject to active efflux by multidrug resistance related proteins (Hong et al., 2003; Vaidyanathan and Walle, 2003). Chemical modifications which can improve the physio-chemical properties of EGCG or reduce its biotransformation may be useful in improving its bioavailability.

Ester-based prodrugs are classical means to improve the bioavailability and reduce the toxicity of a compound (Liederer and Borchardt, 2006). For example, acetylation of salicylic acid to form aspirin accomplishes this, as does acetylation of morphine to produce heroin (Sollman, 1957; Klaassen, 1996). This improvement is accomplished by occlusion of polar side chains, increasing hydrophobicity, and making hydroxyl groups unavailable for Phase II biotransformation or oxidative degradation. Previously Lam *et al.* have reported that peracetylated EGCG (AcEGCG, mol. formula $C_{38}H_{34}O_{19}$, Fig. 1) represents a prodrug for inhibition of proteasome activity (Lam et al., 2004). The authors found that this compound had increased activity against proteasome activity in intact cells, but not against pure enzymes.

In the present study, we have examined the *in vitro* growth inhibitory and anti-inflammatory activity of AcEGCG, the kinetics of AcEGCG de-esterification, and the oral bioavailability of this compound in mice.

Methods

Chemicals EGCG (100% pure) was isolated from a crude green tea polyphenol extract and provided by Dr. Chi-Tang Ho (Dept. of Food Science, Rutgers, University). AcEGCG was synthesized using a pyridine catalyzed reaction of EGCG with acetic anhydride. β -D-glucuronidase (G-7896, EC 3.2.1.31, from *Escherichia coli* with 9×10^6 U/g solid), and sulfatase (S-9754, EC 3.1.6.1, from Abalone entrails with 2.3×10^5 U/g solid) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest grade commercially available. Dosing solutions of EGCG or AcEGCG were prepared in 0.9% NaCl or polyethylene glycol 300: ethanol:polysorbate 80: water (2:1:0.98:0.02, v:v:v:v), respectively. For analytical purposes, a standard stock solution of EGCG, epigallocatechin, epicatechin, and epicatechin-3-gallate (10 mg/L each) was prepared in 11.4 mmol/L ascorbic acid-0.13 mmol/L EDTA (pH 3.8) and stored at -80°C .

Cell Culture KYSE150 human esophageal squamous cell carcinoma cells were maintained in log-phase growth in Ham's F-12:RPMI 1640 (1:1) supplemented 5% fetal bovine serum. HCT116 human colon adenocarcinoma cells and RAW264.7 murine macrophages were maintained in log-phase growth in Dulbecco's modified Eagle's Medium supplemented with 10% fetal bovine serum. All medium was supplemented with 100 U/mL penicillin and 0.1 mg/mL streptomycin.

Animals Male CF-1 mice (6 – 8 wks) were purchased from Charles River Laboratories (Wilmington, MA) and allowed to acclimate for at least one week prior to

the start of the experiment. The mice were housed 10 per cage, and maintained in air-conditioned quarters with a room temperature of $20 \pm 2^{\circ}\text{C}$, relative humidity of $50 \pm 10\%$, and an alternating 12 h light/dark cycle. Mice were given Purina Rodent Chow 5001 (Research Diets, New Brunswick, NJ) and water *ad libitum*. For bioavailability studies, animals were deprived of food for 12 h prior to administration of test compounds.

Growth Inhibition and Intracellular Concentration To compare the growth inhibitory activity of EGCG and AcEGCG, KYSE150 human esophageal cancer cells and HCT116 human colon adenocarcinoma cells were plated in 96-well plates ($2 - 5 \times 10^3$ cells per well) and allowed to attach for 24 h. The medium was replaced with fresh, serum free medium containing 0 – 40 μM of EGCG or AcEGCG. Cells were incubated for 24 h at 37°C . The medium was then replaced with fresh serum-complete medium and the cells were incubated for an additional 24 h at 37°C . Growth inhibition was determined using the 3,[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay (Bold et al., 2001).

Cellular uptake and cytosolic levels of EGCG and AcEGCG following treatment of HCT116 cells with either AcEGCG or EGCG were determined as previously reported (Hong et al., 2003). In brief, cells were allowed to grow to 70 – 90% confluency in 12 – well plates. The medium was replaced with fresh medium containing 20 μM EGCG or AcEGCG and 5 U/mL superoxide dismutase (to stabilize the EGCG). The cells were incubated for 0.25 – 24h at 37°C , after which the medium was removed, the cells were washed 2 times with cold PBS, 2% ascorbic acid was added to each well, and the cells were scraped and sonicated. The resulting solution was centrifuged at 10,000g for 20

min. The supernatant was combined with an equal volume of ice-cold methanol and centrifuged for 20 min at 10,000g to precipitate the protein. The resulting supernatant was analyzed by HPLC. Cytosolic EGCG was normalized to cytosolic protein concentration.

Inhibition of Arachidonic Acid release and NO production The ability of EGCG and AcEGCG to inhibit the release of arachidonic acid and production of NO by lipopolysaccharide (LPS)-stimulated murine macrophages was determined using previously described methods (Sang et al., 2004). In brief, to determine arachidonic acid release, RAW264.7 cells were incubated overnight with 0.1 $\mu\text{Ci/mL}$ [5,6,8,9,11,12,14,15- ^3H](N) arachidonic acid to allow membrane incorporation. Cells were then washed with PBS containing 0.1% BSA. Cells were then stimulated with 2 $\mu\text{g/mL}$ LPS for 1 h, the cells were washed, and fresh medium containing AcEGCG or EGCG (0 – 40 μM) was added. Following 18 h incubation, radioactivity in the medium was determined by scintillation counting. To determine inhibition of NO formation, cells were stimulated and treated with test compounds as above. NO levels were determined by measuring nitrite production spectrophotometrically using the Greiss reagent (Ryu et al., 2003).

Esterase-mediated Deacetylation To determine the capability of plasma esterases to convert AcEGCG to EGCG, freshly isolated plasma from CF-1 mice was combined with AcEGCG (final concentration 5 μM) and incubated at 37°C for 0 – 60 min. The reaction was stopped by addition of CH_2Cl_2 , and EGCG was extracted and analyzed as described below. Time-dependent de-acetylation by plasma or hepatic microsomal esterases was

determined by incubating 10 μ M AcEGCG with mouse plasma or hepatic microsomes (20 μ g protein) in Tris-HCl (20 mM, pH 6.8 containing 0.02% ascorbic acid and 1 mM CaCl_2). Samples were pre-incubated for 3 min at 37°C and the reaction was started by addition of AcEGCG. Reactions were carried out for 0 – 30 min at 37°C and terminated by addition of an equal volume of acetonitrile. Following centrifugation at 10,000g for 10 min, 100 μ L aliquots of supernatant were analyzed by HPLC-UV to determine the disappearance of AcEGCG. To determine the K_m and V_{max} for plasma-mediated de-esterification of AcEGCG, the reactions were prepared as above with the modification that the concentration of AcEGCG was changed from 2 – 200 μ M. The reactions were pre-incubated and started as above. The reaction time was held constant at 15 min. Samples were then prepared and analyzed as above.

Oral Bioavailability Male, CF-1 mice (20 – 25g, 6 per group) were given a single dose of EGCG or AcEGCG (163.8 μ mol/kg, *i.g.*) and sacrificed at 20, 50, 90, 180, and 300 min. Plasma, small intestine, and colon were collected and processed by previously reported methods (Lee et al., 2002; Lambert et al., 2003). In brief, 100 μ L of plasma was hydrolyzed with 1 U sulfatase, and 250 U β -glucuronidase at 37°C for 45 min, and extracted with methylene chloride and ethyl acetate. The ethyl acetate fractions were pooled and dried under vacuum. Samples were reconstituted in 10% aqueous acetonitrile and analyzed by HPLC.

Tissue samples were homogenized in 2 volumes of ice-cold 2% ascorbic acid using a mechanical dounce homogenizer and 200 μ L aliquots were hydrolyzed, extracted, and analyzed in a manner identical to plasma. EGCG and its methylated metabolites

were identified by comparing samples with the retention times of authentic standards. Quantification was based on comparison of peak heights with standard plasma containing a known amount of compound.

HPLC Analysis of AcEGCG and EGCG HPLC analysis of EGCG in cell culture medium and biological samples was performed using our previously reported methods (Lee et al., 2002; Hong et al., 2003). AcEGCG concentrations in enzyme reactions were analyzed by HPLC-UV with $\lambda_{\max} = 280$ nm. The mobile phase was a binary gradient of solvent A (4% sodium monophosphate buffer containing 5% acetonitrile, pH 3.2) and solvent B (1% sodium monophosphate buffer containing 70% acetonitrile, pH 3.2). The initial phase was 12 min gradient from 57 to 86% B, followed by a 6 min isocratic period at 86% B. The mobile phase was then re-equilibrated at 57% B for 7 min. The flow rate was 1.5 mL/min. The limit of detection for the method was determined as the minimum quantifiable peak at a signal-to-noise ratio of 3 to 1.

Statistical Analyses Pharmacokinetic parameters were calculated using Microsoft Excel functions developed by Usansky and colleagues (<http://www.boomer.org/pkin/soft.html>). The Area Under the Curve ($AUC_{0 \rightarrow \infty}$) was calculated by the linear trapezoidal rule, elimination rate constant (k_{elim}) was calculated from least squares curve fit of the plot of \ln concentration as a function of time, and the half-life ($t_{1/2}$) was determined as $\ln(2) / k_{\text{elim}}$. K_m and V_{\max} values for the de-esterification of AcEGCG were determined using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA). Differences in

pharmacokinetic parameters, inhibition values, and cytosolic levels were assessed using the paired Students *t* test. $p < 0.05$ was regarded as significant.

Results

Synthesis and HPLC Analysis of AcEGCG AcEGCG was obtained in 95% yield following peracetylation by reaction with acetic anhydride in a pyridine-catalyzed reaction. The structure was confirmed by 1D (^1H and ^{13}C) and 2D (HMBC and HMQC) NMR. ^1H NMR ($(\text{CD}_3)_2\text{CO}$, 600 MHz): δ 5.53 (1H, s, H-2), 5.77 (1H, m, H-3), 3.22 (1H, dd, $J=4.8, 18.0$ Hz, H-4a), 3.11 (1H, dd, $J=1.8, 18.0$ Hz, H-4b), 6.61 (1H, d, $J=2.4$ Hz, H-6), 6.74 (1H, d, $J=2.4$ Hz, H-8), 7.42 (2H, s, H-2' and H-6'), 7.63 (2H, s, H-2'' and H-6''), 2.29 (3H, CH_3CO), 2.28 (6H, CH_3CO), 2.28 (3H, CH_3CO), 2.252 (3H, CH_3CO), 2.251 (3H, CH_3CO), 2.23 (6H, CH_3CO); ^{13}C NMR ($(\text{CD}_3)_2\text{CO}$, 150 MHz): δ 79.0 (d, C-2), 71.1 (d, C-3), 28.1 (t, C-4), 152.7 (s, C-5), 111.8 (d, C-6), 152.6 (s, C-7), 110.3 (d, C-8), 157.5 (s, C-9), 112.6 (s, C-10), 130.1 (s, C-1'), 121.6 (d, C-2' and C-6'), 146.4 (s, C-3' and C-5'), 137.3 (s, C-4'), 141.9 (s, C-1''), 124.5 (d, C-2'' and C-6''), 146.3 (s, C-3'' and C-5''), 138.4 (s, C-4''), 166.0 (s, C-7''), 22.6 (q, CH_3CO), 22.3 (q, CH_3CO), 22.2 (q, $2 \times \text{CH}_3\text{CO}$), 22.1 (q, $2 \times \text{CH}_3\text{CO}$), 21.7 (q, CH_3CO), 21.6 (q, CH_3CO), 171.1 (s, CH_3CO), 170.6 (s, CH_3CO), 170.0 (s, $2 \times \text{CH}_3\text{CO}$), 169.9 (s, $2 \times \text{CH}_3\text{CO}$), 169.1 (s, CH_3CO), 168.8 (s, CH_3CO).

AcEGCG was not detectable using our standard electrochemical method for EGCG (Lee et al., 2002; Hong et al., 2003). We therefore developed an HPLC-UV method to analyze AcEGCG in cell culture medium and enzyme incubations. Analysis of standard solutions of AcEGCG revealed a single peak with a retention time of 12 min with a $\lambda_{\text{max}} = 280$ nm. The apparent limit of detection was 400 ng/mL (Fig. 1). Although this limit of detection was not sensitive enough to detect AcEGCG levels in plasma and

other biological samples, it was sufficient to monitor enzyme assays and cell uptake studies.

Inhibition of Cell Growth, NO Production, and Arachidonic acid Release The growth inhibitory activity of AcEGCG and EGCG were compared in KYSE150 human esophageal squamous cell carcinoma and HCT116 human colon adenocarcinoma cells (Fig 2a). The IC₅₀ values of AcEGCG were approximately 10 and 32 μ M against KYSE150 and HCT116 cells, respectively. These values were lower than those of EGCG (approximately 20 and 45 μ M against KYSE150 and HCT116 cells) under similar treatment conditions.

The ability of AcEGCG and EGCG to inhibit NO production and arachidonic acid release by LPS-stimulated RAW 264.7 murine macrophages were compared. At 40 μ M, AcEGCG inhibited NO production by 81%, whereas EGCG treatment reduced NO by only 15% (Fig 2b). Similarly, AcEGCG reduced LPS-induced arachidonic acid release dose-dependently and was approximately 2-fold more potent than EGCG (Fig. 2c).

In order to determine if the increased biological potency of AcEGCG was due to increased cytosolic levels of EGCG in treated cells, we determined the cytosolic levels of EGCG in AcEGCG-treated HCT116 cells. Cells treated with AcEGCG had a maximal cytosolic concentration of 2088.0 ng/mg EGCG at 2 h after treatment, whereas those treated with EGCG had a maximal cytosolic level of 65.0 ng/mg at 6 h after treatment (Fig 2d). After 24 h, the cytosolic levels of EGCG were 2.3-fold higher in cells treated with AcEGCG than in those treated with EGCG.

Enzyme-mediated De-acetylation of AcEGCG EGCG could be detected within 5 min following incubation of AcEGCG in mouse plasma at 37°C (Fig. 3a). Time-dependent incubation with lower concentrations of mouse plasma and with hepatic microsomes resulted in decreases in the levels of AcEGCG and the formation of at least two putative metabolites (Fig. 3b and 3c). We are currently trying to identify the structures of these compounds. Incubations in the absence of plasma or hepatic microsomes did not result in a significant decrease in the concentration of AcEGCG, nor the formation of M4.8 and M7.01 (data not shown). The kinetics of de-esterification of AcEGCG were determined in mouse plasma (Fig. 3d). Following a 10 min incubation, the V_{\max} and K_m of de-esterification were determined as 10615 pmol/min/mg and 16.7 μ M, respectively.

Oral Bioavailability of AcEGCG and EGCG The oral bioavailability of EGCG in CF-1 mice was compared following administration of equimolar doses of AcEGCG and EGCG. The peak concentration (C_{\max}) of total EGCG in the plasma, small intestine, and colon were not significantly different between the two treatment groups (Fig 4). By contrast, the $AUC_{0 \rightarrow \infty}$ of total EGCG increased by 2.4-, 2.8-, and 2.4-fold in the plasma, small intestine, and colon, respectively of mice treated with AcEGCG compared to those treated with EGCG (Table 1). Likewise, the $t_{1/2}$ of total EGCG was increased by 2.2-, 2.4-, and 6.0-fold in the plasma, small intestine, and colon, respectively, in mice treated with AcEGCG compared to those treated with EGCG (Table 1). There was no

significant difference in the lung levels of EGCG of the two treatment groups (data not shown).

Discussion

EGCG is the most abundant catechin in green tea (*Camellia sinensis*, Theaceae). This compound has been reported to have cancer preventive activity *in vivo* and associated activities *in vitro*, however it is extensively biotransformed and has relatively poor bioavailability (Lambert and Yang, 2003b; Lambert and Yang, 2003a). In an effort to improve the bioavailability of EGCG, we prepared AcEGCG and investigated the effect of peracetylation on the *in vitro* biological activities of EGCG related to cancer prevention and the bioavailability of AcEGCG *in vivo*.

Acetylation improved the cell uptake and growth inhibitory activity of EGCG against both human esophageal squamous cell carcinoma cells (KYSE150) and human colon adenocarcinoma cells (HCT-116). Incubation of AcEGCG with KYSE150 cells resulted in rapid conversion to EGCG and in the formation of at least two unknown compounds. These unknown compounds may represent partially deacetylated AcEGCG products. We are attempting to identify these putative metabolites. It appears that AcEGCG can increase the intracellular concentrations due to increased cell uptake over the short-term (1 h), but once the compound is converted back to EGCG, it is then subject to biotransformation and efflux at a rate similar to EGCG over the long-term (24 h). This may explain why the increase in growth inhibitory activity, which is measured after 24 h treatment, was only a 2-fold. Based on these data, it would appear that AcEGCG in combination with inhibitors of phase II metabolism and active efflux would result in an even greater increase in biological potency.

AcEGCG increased the oral bioavailability of EGCG, as measured by $AUC_{0 \rightarrow \infty}$. Increases in the $AUC_{0 \rightarrow \infty}$ and $t_{1/2}$ of plasma (240% and 220%), small intestine (280% and

240%), and colon (240% and 600%) were observed following oral gavage with AcEGCG compared to treatment with equimolar doses of EGCG. Interestingly, there was little difference in the C_{\max} of EGCG between the AcEGCG and EGCG-treated groups. This could be due to incomplete conversion of AcEGCG to EGCG following oral administration to mice. It is also possible that although acetylation is expected to increase absorption of the test compound, it is not expected to affect biotransformation and elimination of the deacetylated product, EGCG.

Due to limitations in the sensitivity of our current analytical method, we were unable to measure AcEGCG or partially-deacetylated AcEGCG in the tissues of treated mice. Although we demonstrated that AcEGCG is readily deacetylated by mouse plasma and hepatic microsomes, it is possible that a pool of partially-deacetylated products is present in the tissue and plasma, and that these products may have biological activity. We are currently developing a more sensitive method to study these partially deacetylated AcEGCG products.

In summary, we have demonstrated that AcEGCG has improved biological activity *in vitro* and improved bioavailability *in vivo*. This compound is apparently readily converted back to EGCG both *in vitro* and *in vivo*, and the improved biological activity *in vitro* appears to be due to increased intracellular levels of EGCG. These data suggest that AcEGCG may have improved cancer preventive activity *in vivo*. Future studies in animal models of carcinogenesis are needed to test this hypothesis.

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Footnotes

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Figure Legends

Figure 1. HPLC analysis of EGCG and peracetylated EGCG.

Figure 2. Biological activities and cellular uptake of AcEGCG. (A) Growth inhibition of KYSE150 human esophageal cancer cells and HCT116 human colon adenocarcinoma cells following treatment with either EGCG or AcEGCG for 24 h. Inhibition of NO production (B) or arachidonic acid release (C) by LPS-stimulated RAW264.7 murine macrophages by AcEGCG or EGCG (0 – 40 μ M, 18 h treatment). (D) Cytosolic levels of EGCG in HCT116 human colon cancer cells treated with EGCG or AcEGCG (10 μ M) for 0.25 – 24 h. N = 4 \pm SD. * = $p < 0.05$, ** = $p < 0.01$.

Figure 3. Enzyme-mediated de-acetylation of AcEGCG. Conversion of AcEGCG (5 μ M) to EGCG by mouse plasma (A). Time-dependent deacetylation (10 μ M) by mouse plasma (B) and hepatic microsomes (C). Concentration-dependent deacetylation by mouse plasma (D). Time and concentration-dependent kinetics were based on the disappearance of AcEGCG. Each point represents the average of three experiments. Error bars represent the standard deviation.

Figure 4. Plasma and tissue levels of EGCG in male CF-1 mice treated with equimolar doses of EGCG (75 mg/kg, *i.g.*) and AcEGCG (130 mg/kg, *i.g.*). N = 6, error bars represent the standard error of the mean.

Table 1. Plasma and tissue pharmacokinetic parameters of EGCG following administration of 75 mg/kg, *i.g.* EGCG or 130 mg/kg, *i.g.* AcEGCG to male CF-1 mice.^a

Tissue	EGCG (75 mg/kg, <i>i.g.</i>)				AcEGCG (130 mg/kg, <i>i.g.</i>)			
	C _{max}	t _{1/2}	AUC _{0→∞}	k _{elim}	C _{max}	t _{1/2}	AUC _{0→∞}	k _{elim}
Plasma	0.73 ± 0.16	200.3 ± 3.6	194.6 ± 54.9	0.003 ±	0.76 ± 0.05	441.0 ± 17.6 ^b	465.0 ±	0.002 ±
				0.0001				65.7 ^b
S. Int.	20.9 ± 5.8	128.2 ± 8.8	3.43x10 ³ ± 456.5	0.005 ±	13.0 ± 3.0	545.0 ± 125.8 ^b	9.57x10 ³ ±	0.001 ±
				0.0004				324.0 ^b
Colon	19.7 ± 13.1	58.0 ± 5.0	1.78x10 ³ ± 594.7	0.012 ±	25.9 ± 8.33	348.7 ± 104.3 ^b	4.28x10 ³ ±	0.002 ±
				0.0001				90.6 ^b

^a Values represent the mean ± SEM. Units: C_{max} = µg/g or µg/mL; t_{1/2} = min; AUC_{0→∞} = (µg/mL) · min or (µg/g) · min; k_{elim} = min⁻¹

^b Value is significantly different from EGCG-treated group by paired Students' *t*-test, *P* < 0.01

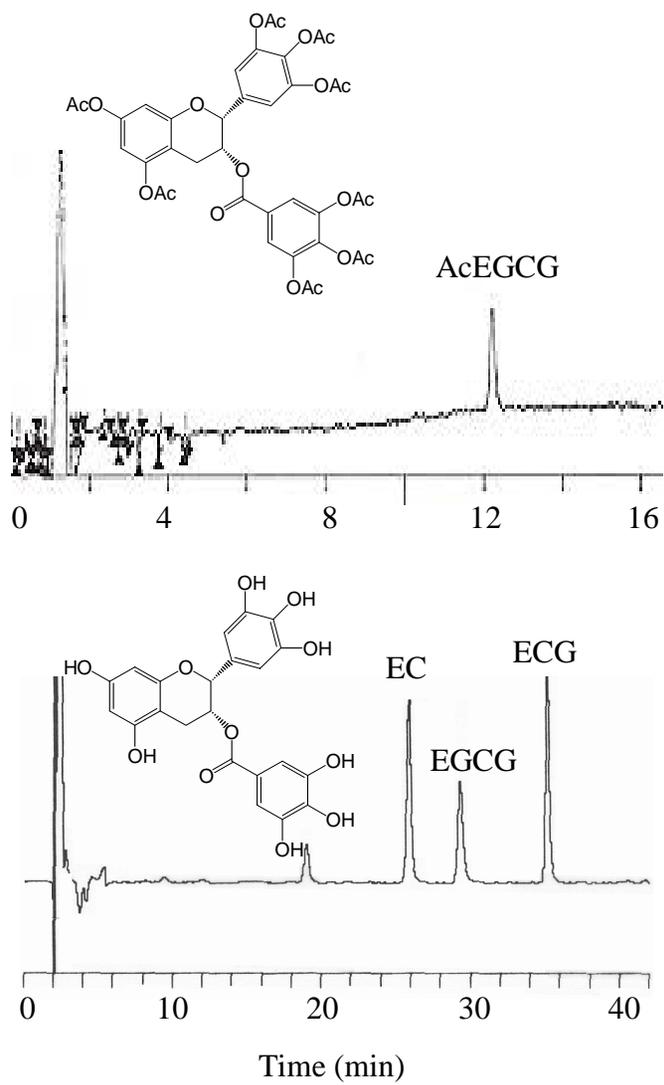


Figure 1

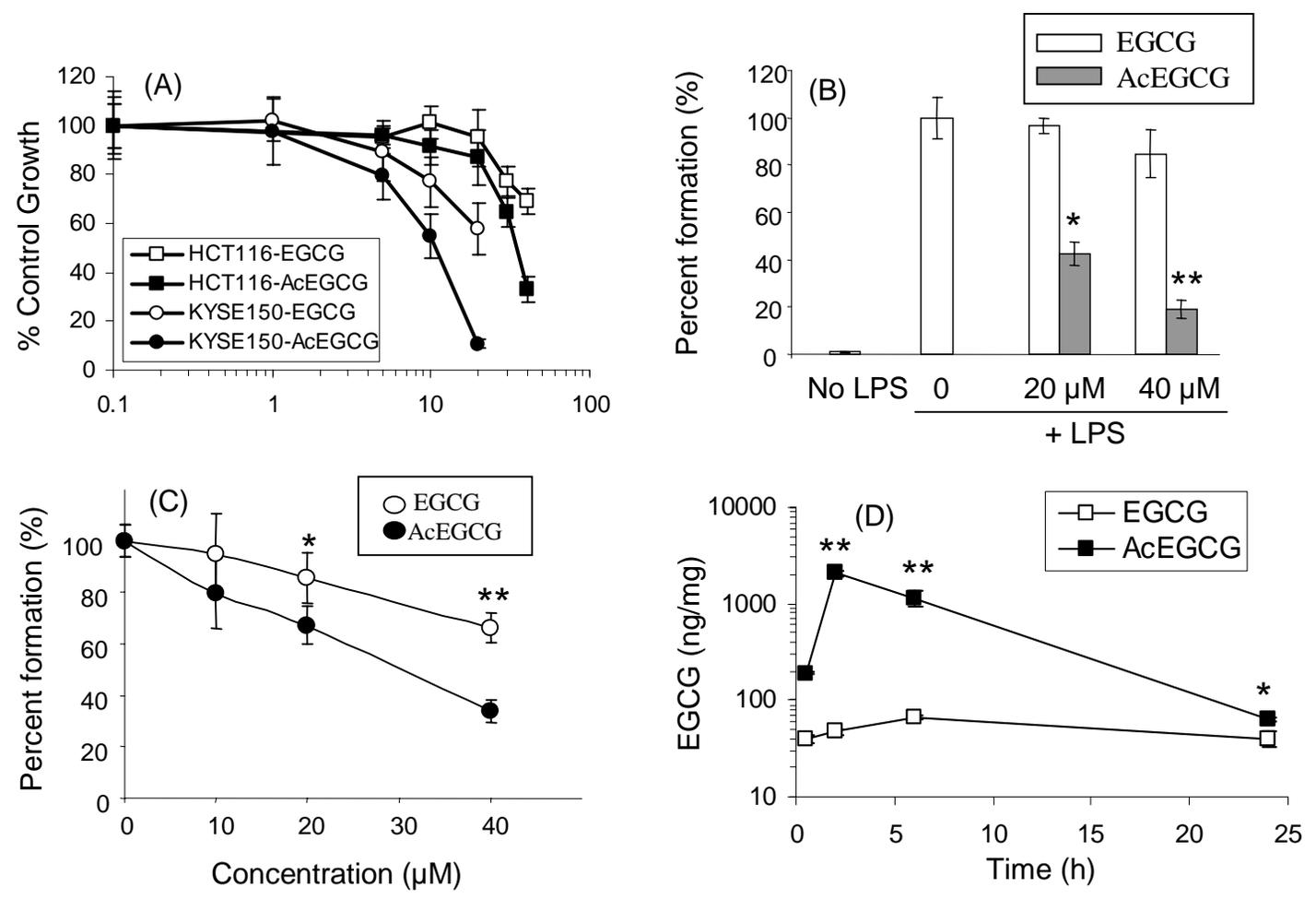


Figure 2

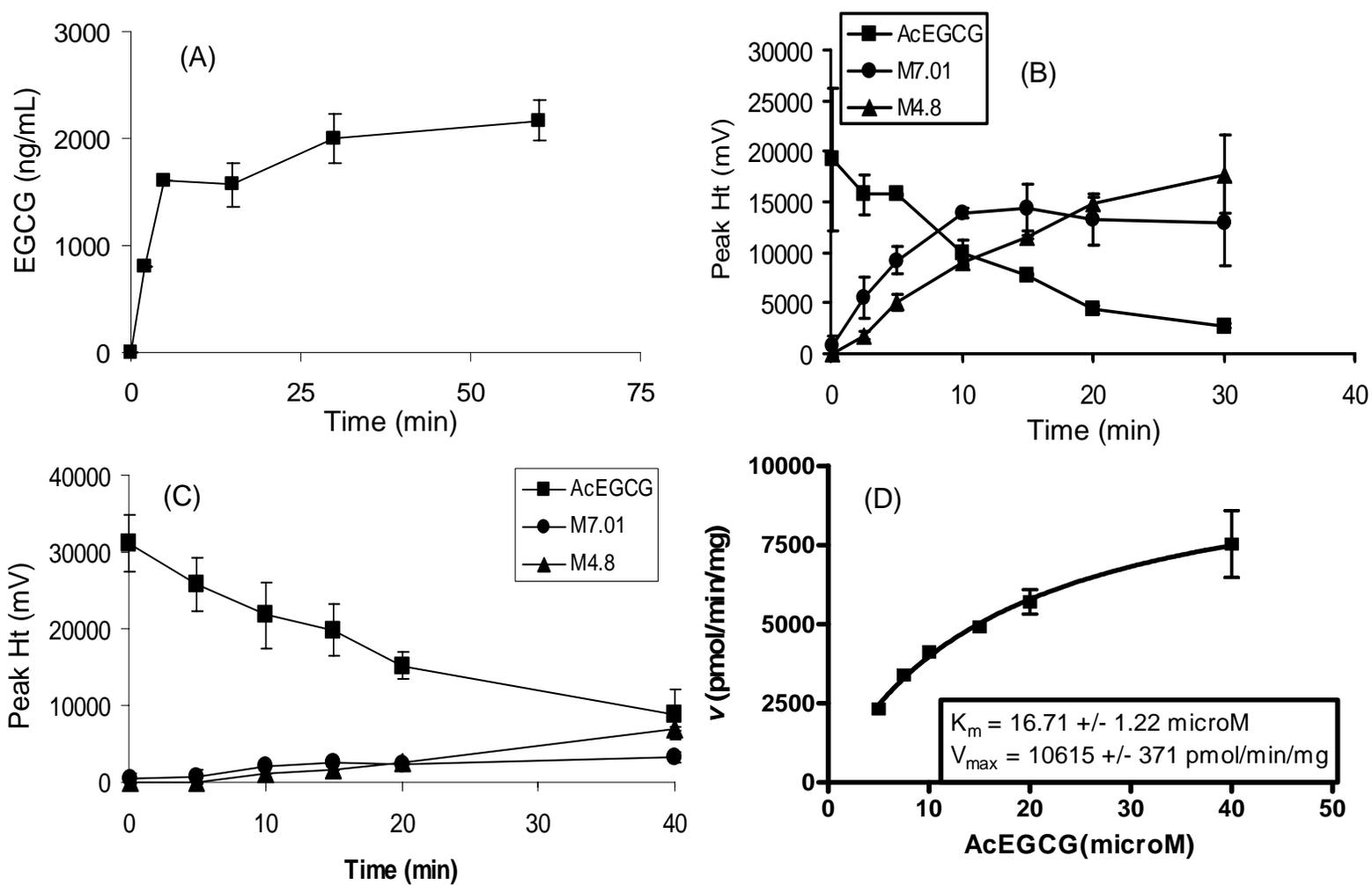


Figure 3

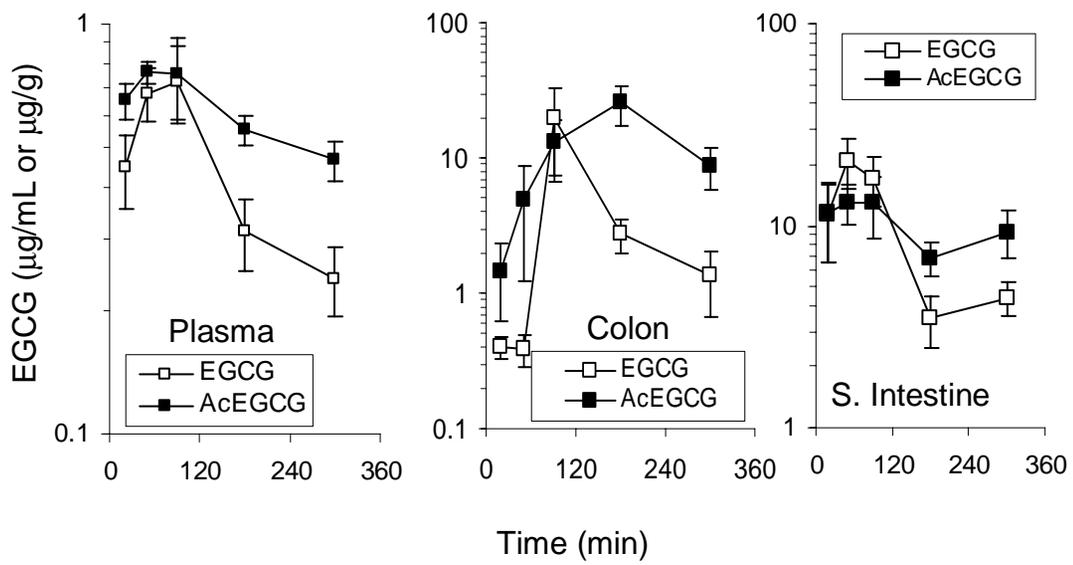


Figure 4