Pharmacokinetics and Safety of Green Tea Polyphenols after Multiple-Dose Administration of Epigallocatechin Gallate and Polyphenon E in Healthy Individuals[1]

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ABSTRACT

Purpose: **Green tea and green tea polyphenols have been shown to possess cancer preventive activities in preclinical model systems. In preparation for future green tea intervention trials, we have conducted a clinical study to determine the safety and pharmacokinetics of green tea polyphenols after 4 weeks of daily p.o. administration of epigallocatechin gallate (EGCG) or Polyphenon E (a defined, decaffeinated green tea polyphenol mixture). In an exploratory fashion, we have also determined the effect of chronic green tea polyphenol administration on UV-induced erythema response.**

Experimental Design: **Healthy participants with Fitzpatric skin type II or III underwent a 2-week run-in period and were randomly assigned to receive one of the five treatments for 4 weeks: 800 mg EGCG once/day, 400 mg EGCG twice/day, 800 mg EGCG as Polyphenon E once/day, 400 mg EGCG as Polyphenon E twice/day, or a placebo once/day (8 subjects/group). Samples were collected and measurements performed before and after the 4-week treatment period for determination of safety, pharmacokinetics, and biological activity of green tea polyphenol treatment.**

Results: **Adverse events reported during the 4-week treatment period include excess gas, upset stomach, nausea, heartburn, stomach ache, abdominal pain, dizziness, headache, and muscle pain. All of the reported events were rated as mild events. For most events, the incidence reported in the polyphenol-treated groups was not more than that reported in the placebo group. No significant changes were observed in blood counts and blood chemistry profiles after repeated administration of green tea polyphenol products. There was a >60% increase in the area under the plasma EGCG concentration-time curve after 4 weeks of green tea polyphenol treatment at a dosing schedule of 800 mg once daily. No significant changes were observed in the pharmacokinetics of EGCG after repeated green tea polyphenol treatment at a regimen of 400 mg twice daily. The pharmacokinetics of the conjugated metabolites of epigallocatechin and epicatechin were not affected by repeated green tea polyphenol treatment. Four weeks of green tea polyphenol treatment at the selected dose and dosing schedule did not provide protection against UV-induced erythema.**

Conclusions: **We conclude that it is safe for healthy individuals to take green tea polyphenol products in amounts equivalent to the EGCG content in 8–16 cups of green tea once a day or in divided doses twice a day for 4 weeks. There is a >60% increase in the systemic availability of free EGCG after chronic green tea polyphenol administration at a high daily bolus dose (800 mg EGCG or Polyphenon E once daily).**

INTRODUCTION

Tea (*Camellia sinensis*) is one of the most consumed beverages in the world, especially in Asian countries. Tea consumption may be linked to low incidences of various pathological conditions, including cardiovascular disease, diabetes, obesity, and cancer. Green tea, GTEs,[3] and EGCG have been shown to inhibit carcinogenesis induced by a wide variety of carcinogens in rodent cancer models. Cancer chemopreventive activity of green tea has been demonstrated in the following target organs: colon, duodenum, esophagus, forestomach, large intestine, liver, lung, mammary glands, and skin (reviewed in Refs. 1, 2). The principal active polyphenols in green tea include EGCG, EGC, EC, and epicatechin gallate, with EGCG being the most abundant and possessing the most potent antioxidative activity. The cancer chemopreventive activities of green tea have been attributed, in part, to the antioxidative and free radical scavenging activities of green tea polyphenols (3, 4). Studies have also suggested that the cancer preventive properties of green tea are related to inhibition of tumor promotion and cell

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[3] The abbreviations used are: GTE, green tea extract; EGCG, epigallocatechin gallate; CL/F, oral clearance; EGC, epigallocatechin; EC, epicatechin; AUC, area under the plasma concentration-time curve; MED, minimum erythema dose; HPLC, high-performance liquid chromatography; C_{max}, maximum plasma concentration; T_{max}, time to reach the maximum plasma concentration; $t_{1/2}$, half-time; Vβ/F, oral apparent volume of distribution.

proliferation (5), and induction of Phase II detoxification enzymes (6, 7).

At present, epidemiological evidence of the protective effect of tea consumption against the development of human cancers is not conclusive. This may be attributed to variables related to individual differences in tea preparation and consumption patterns, and seasonal and geographic differences in tea production. Controlled prospective human intervention trials to evaluate the chemopreventive activity of ingestion of tea or tea components are clearly necessary. Because it is not easy to change the dietary habits of an individual, ingesting green tea products in oral formulations may be more acceptable for chronic use in healthy populations. We have reported recently the pharmacokinetics and safety of two oral green tea polyphenol formulations (EGCG and Polyphenon E, a defined mixture of green tea polyphenols) after single-dose administration (8). Peak plasma EGCG levels of 200–400 ng/ml (0.4–0.8 μM) can be achieved after the administration of these formulations at doses equivalent to the EGCG content in 8–16 cups of green tea (depending on the cup size). Here, we report results from a follow-up study designed to determine the safety and pharmacokinetics of oral tea polyphenol products after 4 weeks of daily administration. In an exploratory fashion, the study has also determined the effect of chronic green tea polyphenol administration on UV-induced erythema response. This study provides the fundamental knowledge needed to conduct future intervention trials using oral green tea polyphenol products.

MATERIALS AND METHODS

Study Drugs. EGCG, Polyphenon E, and placebo capsules were supplied by the Chemoprevention Agent Development Research Group, National Cancer Institute (Bethesda, MD). On average, each EGCG capsule contained 200 mg EGCG and pharmaceutical excipients consisting of pregelatinized starch, colloidal silicon dioxide, and magnesium stearate. Each Polyphenon E capsule contained 200 mg EGCG, 37 mg EGC, 31 mg EC, other green tea polyphenols, and pharmaceutical excipients consisting of pregelatinized starch, colloidal silicon dioxide, and magnesium stearate. Placebo capsules contained only pharmaceutical excipients consisting of microcrystalline cellulose, pregelatinized starch, colloidal silicon dioxide, and magnesium stearate. Caffeine was not present in any of the formulations. The study medications were stored at room temperature and protected from environmental extremes. On the basis of the content analysis performed every 6 months, green tea polyphenols were found to be stable under the above storage condition.

Participants. Forty healthy men and women ≥18 years of age with Fitzpatric skin type II or III participated in the study. Individuals with skin type II have skin that burns and peels easily after short initial sun exposure and tends to develop a light tan. Individuals with skin type III typically develop a slight tender burn after short initial sun exposure and a moderate tan. These skin types allow evaluation of UV-induced erythema response without resulting painful burn. The participants were in performance status 0–1 (determined by Southwest Oncology Group Performance Status Criteria) and have normal liver and renal function. Participants were excluded if they were pregnant, had cancers of any type within the past 5 years, had severe metabolic disorders or other life-threatening acute or chronic diseases, had weight loss >10%, or had gastric ulcer within the last 6 months. The study was approved by the University of Arizona Human Subjects Committee. Written informed consent was obtained from all of the participants.

Study Design. During the initial clinic visit, study participants completed a medical history form and underwent a brief physical examination. A fasting blood was collected and subjected to a complete blood count with differential leukocyte count and the following blood chemistry analyses: glucose, urea nitrogen, creatinine, uric acid, sodium, potassium, chloride, total protein, albumin, globulin, cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, calcium, phosphorus, alkaline phosphatase, γ glutamyl transpeptidase, alanine amino transferase, aspartate amino transferase, lactate dehydrogenase, total bilirubin, and iron. Eligible subjects were required to refrain from the ingestion of tea, tea products, dietary supplements, and herbal products 2 weeks before the placebo run-in period and throughout the entire green tea polyphenol treatment period. Study subjects were randomly assigned to receive one of the five treatments (8 subjects/group): 800 mg EGCG formulation once daily, 400 mg EGCG formulation twice daily, 800 mg EGCG as Polyphenon E once daily, 400 mg EGCG as Polyphenon E twice daily, or placebo once daily.

All of the subjects underwent a 2-week placebo run-in period in which they were instructed to ingest the placebo capsules once a day or twice a day depending on their assigned dosing schedule. Subjects with ≥80% compliant during placebo run-in based on pill count were entered into the green tea polyphenol treatment period. The baseline UV light-induced MED was determined before the initiation of the polyphenol/placebo treatment phase. On the first treatment day, a fasting blood was collected before ingestion of the study medication. Subsequently, subjects took 800 or 400 mg dose of the assigned study capsules with a standardized light breakfast. Blood samples (5–7 ml each) were collected at 0.5, 1, 2, 3.5, 5, 6.5, 8, and 24 h after drug administration. After the first study day, study subjects were provided with a 4-week supply of the assigned study agent and instructions on how to take the study drug. Study subjects were also provided with a medication intake calendar to write down the time and quantity of any medication usage (including the study medication). A daily diary form was provided to record side effects experienced during this period with documentation of time of onset and resolution, severity, and remedial measures taken. At the end of treatment period, study subjects underwent a post-treatment MED evaluation. On the last treatment day, most subjects underwent procedures similar to that described for the first treatment day for determination of plasma pharmacokinetics of green tea catechins. For subjects assigned to receive the placebo capsules, only a fasting blood sample was collected on the last treatment day. The fasting blood sample collected on the last treatment day was subjected to post-treatment clinical laboratory evaluation. All of the study participants were followed for 4 weeks for any potential adverse events related to the study procedure or the study agent.

Sample Collection and Processing. Blood samples were collected into Vacutainer tubes containing sodium heparin. Once collected, blood samples were kept in the refrigerator and centrifuged at 4°C within 2 h of collection. Plasma was aliquoted into cryotubes containing a small aliquot of ascorbate-EDTA solution [0.4 M NaH_2PO_4 buffer containing 20% ascorbic acid and 0.1% EDTA (pH 3.6)] and stored at −80°C until analysis.

Tea Polyphenol Concentration Measurements. EGCG, EC, and EGC concentrations in plasma samples were determined within 1 month of collection using a published HPLC procedure (9). In brief, for determination of free green tea polyphenols, plasma samples or spiked plasma standards were extracted with methylene chloride to remove lipid components. The remaining aqueous phase was extracted with ethyl acetate. The ethyl acetate layer was mixed with a small aliquot of 0.1% ascorbic acid before drying by vacuum centrifugation. The dried residue was redissolved in 15% acetonitrile and injected onto HPLC. For determination of the total of free and glucuronic acid/sulfate conjugates of tea polyphenols, plasma samples were mixed with an aliquot of β-glucuronidase and sulfatase in the presence of ascorbate-EDTA solution. After pretreatment, the samples were extracted as described above for the free polyphenols.

The HPLC system consisted of an ESA Model 540 refrigerated autosampler, an ESA Model 580 two-pump solvent delivery system, an ESA 5600 coulochem electrode array system, and a Supelcosil C_{18} reversed-phase column (150 × 4.6 mm; particle size, 5 μm; Supelco Inc.). The autosampler and column temperatures were maintained at 6°C and 35°C, respectively. This assay used a gradient of two mobile phases. Buffer A consisted of 30 mM NaH_2PO_4 buffer, acetonitrile, and tetrahydrofuran in the volume ratio of 98.13:1.75:0.12 (pH 3.35). Buffer B consisted of 15 mM NaH_2PO_4 buffer, acetonitrile, and tetrahydrofuran in the volume ratio of 41.5:58.5:12.5 (pH 3.45). The column was eluted with 96% buffer A and 4% buffer B from 0 to 7 min. Then the linear gradient was changed progressively to 17% buffer B at 25 min, 28% buffer B at 31 min, 33% buffer B at 37 min, and 98% buffer B at 38 min. It was maintained at 98% buffer B from 38 to 43 min and finally changed back to 4% buffer B at 44 min for the analysis of the next sample. The flow rate was maintained at 1 ml/min. The eluent was monitored by the coulochem electrode array system with potential settings at −90, −10, 70, and 150 mV.

Determination of MED. Erythema was induced by applying UV radiation at six doses ranging from 10 to 42 mj/cm^2 to six sites of 1-cm in diameter in a horizontal row on midbuttock skin. A multiport solar UV simulator (Model 600; Solar Light Co., Philadelphia, PA) was used for the UV irradiation. The simulator was equipped with a 150W Xenon lamp emitting a continuous spectrum of radiation beginning at 240 nm through the infrared spectrum and maximally peaking at 360 nm. UVC and visible wavelengths were reduced with a liquid filter and 1-mm Schott WG 320 filter. Spectroradiometric assessment of the lamp indicated that relative emission in the UVA (320–400 nm), UVB (290–320 nm), and UVC (200–290 nm) wavebands was 32%, 61%, and 7%, respectively. The lamp was housed in a black plastic tube with six apertures, 1-cm in diameter. The apparatus was calibrated before each use. The UV irradiation time lasted for 1 min. The MED was determined visually 22–24 h after irradiation and defined as the lowest UV dose causing uniform redness filling the irradiated site. The evaluator was blinded to the treatment assignment of the subject.

Data Analysis. Plasma EGCG concentration-time data were analyzed with the model-independent approach (10). Data in the terminal, log-linear phase were analyzed by linear regression to estimate terminal elimination rate constant (λn) and $t_{1/2} = 0.693/\lambda n$. In general, there were four data points in the terminal log-linear phase. The $AUC_{0-\infty}$ after the first dose was determined by trapezoidal rule up to the last measured concentration-time value to which was added the terminal area. The terminal area was calculated by dividing the concentration at the last time point by λn. To correct for drug accumulation because of repeated dosing and to allow for comparison with the AUC obtained after the first dose, the AUC after the last catechin dose ($AUC_{last\ dose}$) was calculated by trapezoidal rule up to 24 h and 12 h after dosing for the once daily and twice daily dosing schedules, respectively. Concentrations at 12 h after the last catechin dose for the twice daily treatment group were interpolated from the regression line. C_{max} and T_{max} were obtained by visual inspection of the plasma concentration *versus* time profile. CL/F (systemic clearance/oral bioavailability) was estimated from the quotient of dose and $AUC_{0-\infty}$ or $AUC_{last\ dose}$ after the first or last tea catechin dose, respectively. Vβ estimated from the oral data are also influenced by the F value [$V\beta/F = (CL/F)/\lambda n$]. $AUC_{0-\infty}$ and $AUC_{last\ dose}$ of total (free and glucuronic acid/sulfate conjugates) EGC and EC were also estimated with the model-independent approach.

The pharmacokinetic parameters obtained after the first dose were compared among treatment groups using one-way ANOVA followed by a Bonferroni adjusted *t* test for the pairwise multiple comparisons. The pharmacokinetic parameters obtained after repeated treatment were compared with those obtained after the first dose using a paired *t* test. The baseline MEDs were compared among treatment groups using one-way ANOVA. Ratios of the post-treatment MED measurements to those obtained at baseline were calculated and used to compare the treatment effect using one-way ANOVA. A $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 summarizes the demographic data of the study participants. A total of 40 subjects (8/group) completed the study. There were no significant differences in the average age, weight, and height among treatment groups. There were between 2 and 4 male participants in each treatment group.

Adverse events reported during the 4-week treatment period are summarized in Table 2. Data are presented as the number of events reported during the 4-week green tea polyphenol/placebo treatment period, and values in parentheses represent the number of individuals experienced the event. The reported events include excess gas, upset stomach, nausea, heartburn, stomachache, abdominal pain, dizziness, headache, and muscle pain. All of the reported events have been rated as mild events (grade 1). For most events, the incidence reported in the treatment groups was not significantly more than that in the placebo group. Mild nausea was more frequent after the 800-mg

Table 1 Subject demographic data by treatment group

	Placebo	800 mg EGCG once daily	800 mg EGCG as Polyphenon E once daily	400 mg EGCG twice daily	400 mg EGCG as Polyphenon E twice daily
Number of subjects	8	8	8	8	8
Fraction of male participants	3/8	2/8	3/8	4/8	2/8
Age (yr)	34.5 ± 10.6[a]	39.5 ± 10.4	32.4 ± 10.1	29.4 ± 10.7	34.1 ± 11.9
Height (inches)	67.1 ± 2.7	64.9 ± 4.6	67.9 ± 3.2	66.1 ± 5.3	65.1 ± 3.1
Weight (lbs)	169 ± 36	165 ± 52	167 ± 30	160 ± 40	150 ± 19

[a] Mean ± 1 SD.

Table 2 Adverse events reported during the 4-week green tea polyphenol/placebo treatment period

Data are presented as the number of events reported and values in parenthesis represent the number of individuals experienced the event.

	Placebo ($n = 8$)	800 mg EGCG once daily ($n = 8$)	800 mg EGCG as Polyphenon E once daily ($n = 8$)	400 mg EGCG twice daily ($n = 8$)	400 mg EGCG as Polyphenon E twice daily ($n = 8$)
Headache	3 (1)	2 (2)	0	1 (1)	1 (1)
Stomach ache	0	1 (1)	1 (1)	1 (1)	1 (1)
Upset stomach	0	0	0	1 (1)	0
Heartburn	2 (1)	0	0	0	0
Abdominal pain	2 (1)	2 (2)	1 (1)	0	0
Excess gas	1 (1)	0	0	0	1 (1)
Nausea	1 (1)	5 (2)	3 (1)	0	1 (1)
Dizziness	0	1 (1)	0	0	0
Muscle pain	0	1 (1)	0	0	0

once daily treatment than that in the placebo group (5, 3, and 1 occurrence for 800 mg EGCG once daily, 800 mg EGCG as Polyphenon E once daily, and placebo, respectively). Complete blood count and a panel of blood chemistry profiles were obtained before and after 4 weeks of daily administration of the study agent. No significant changes were observed in these clinical laboratory measurements (data not shown).

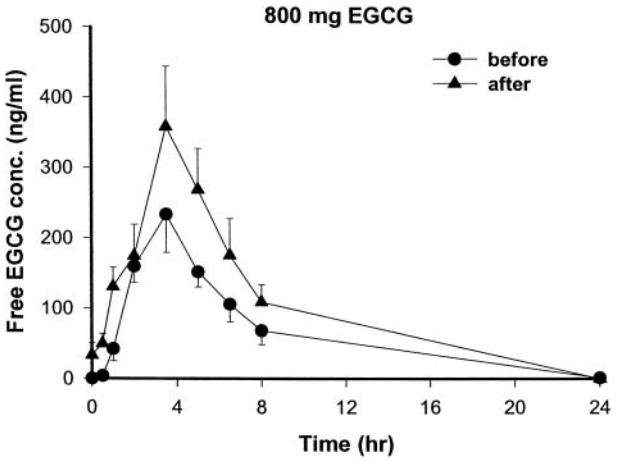
Because EGCG was present mostly in the free form in the systemic circulation [>92% as the free form, based on the AUC ratio of free *versus* total (free and conjugated) EGCG], Figs. 1 and 2 illustrate the average plasma concentration-time profiles of free EGCG after EGCG or Polyphenon E administration. The average pharmacokinetic parameters of free EGCG after p.o. administration of tea polyphenols are summarized in Table 3. Before repeated tea polyphenol treatment, pharmacokinetic parameters of EGCG were similar among the different treatment groups. The AUC and C_{max} of EGCG after the administration of 800-mg dose of EGCG or Polyphenon E were higher than those obtained after 400-mg dose of the respective formulation, but the differences did not reach statistical significance. After repeated tea polyphenol treatment, the AUC of EGCG obtained after the 800-mg dose of Polyphenon E was significantly higher than that obtained from the 400-mg dose of either product. The AUC of EGCG obtained after the 800-mg dose of EGCG was significantly higher than that after the 400-mg dose of EGCG. Four weeks of repeated tea polyphenol administration at 800 mg once daily resulted in significant changes in the AUC of free EGCG, whereas repeated administration at 400 mg twice daily did not result in significant changes in the pharmacokinetics of free EGCG. The AUC of free EGCG increased from 95.6 ± 46.8 to 145.6 ± 85.1 min μg/ml ($P < 0.05$) and from 98.1 ± 46.5 to 158.4 ± 89.8 min μg/ml ($P < 0.05$) for the 800 mg once daily EGCG and Polyphenon E treatment, respectively. A decreasing trend was observed in the CL/F and Vβ/F of EGCG after repeated treatment at 800 mg once daily; however, the changes did not reach statistical significance. Repeated administration of green tea polyphenols did not result in significant changes in C_{max}, T_{max}, and $t_{1/2}$ of EGCG.

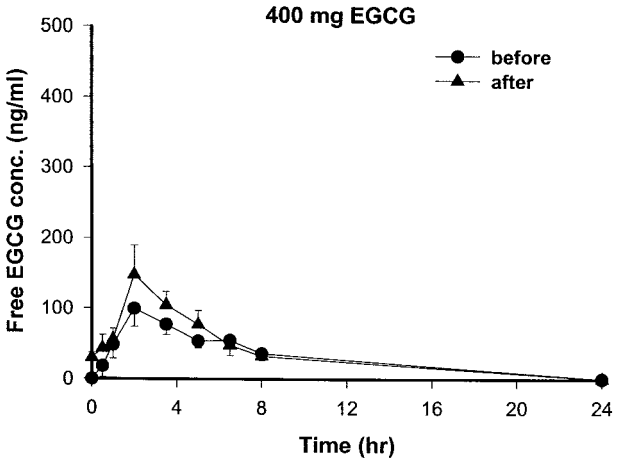
The AUCs of total EGC and EC after p.o. administration of Polyphenon E before and after 4 weeks of treatment are summarized in Table 4. Because the concentrations of free EGC and EC were below the limit of quantification in most plasma samples [0–4% present as the free form, based on the AUC ratio of free *versus* total (free and conjugated) catechins], AUCs presented represent mostly the levels of conjugated metabolites of EGC and EC. Four weeks of tea polyphenol treatment with either dosing schedule did not result in significant changes in the levels of conjugated tea catechins.

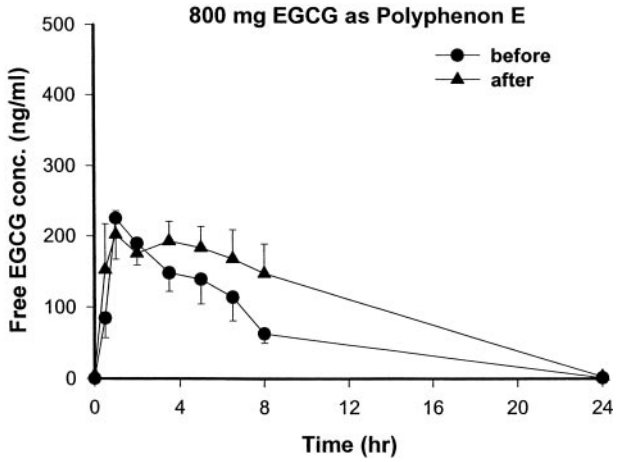
Table 5 shows the changes in MED after 4 weeks of green tea polyphenol/placebo treatment. The data are presented as the ratios of MED determined after repeated treatment over that obtained before treatment. As shown in the data, the ratios of MED approximated unity for all of the study groups, suggesting that the intervention did not change the UV-induced erythema response.

DISCUSSION

There have been no reports of clinical toxicity when green tea is consumed as a beverage throughout the day. Consumption of up to 20 cups of green tea per day is not uncommon in certain populations. Oral pills of GTE or green tea polyphenol products







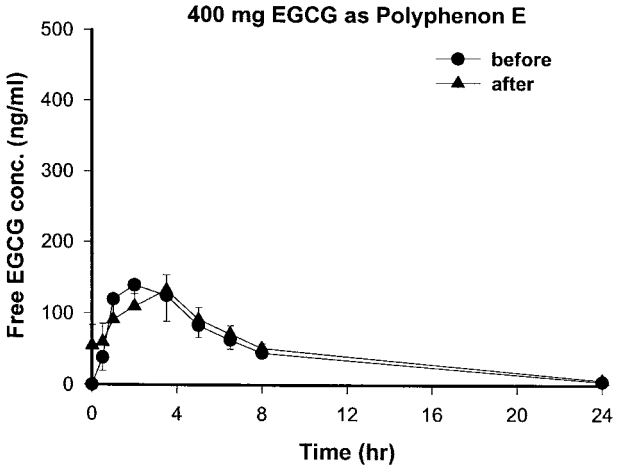


Fig. 1 Average plasma concentration-time profiles of free EGCG after an 800-mg dose of EGCG as EGCG or Polyphenon E formulation. The profiles were obtained before and after 4 weeks of green tea polyphenol treatment with a dosing schedule of 800 mg once daily. Each *point* represents the mean of data from 7 or 8 subject, *bars*, ±SE.

Fig. 2 Average plasma concentration-time profiles of free EGCG after a 400-mg dose of EGCG as EGCG or Polyphenon E formulation. The profiles were obtained before and after 4 weeks of green tea polyphenol treatment with a dosing schedule of 400 mg twice daily. Each *point* represents the mean of data from 8 subjects; *bars*, ±SE.

are available commercially as dietary supplements. Use of standardized oral products can facilitate the conduct of controlled human intervention trials to evaluate the biological activity of green tea or green tea components. However, safety data based on human consumption cannot be extrapolated to chronic consumption of large amounts of an isolated component (*e.g.*, EGCG) or an enriched extract (*e.g.*, Polyphenon E) at regimens that would gain better compliance (*e.g.*, once or twice daily dosing) in intervention trials. A recent study has determined the toxicity of oral GTE administered once daily or three times daily in adult patients with solid tumors (11). Dose levels of 0.5 to 5.05 g/m^2 once daily and 1.0 to 2.2 g/m^2 three times daily were explored. The study found dose-limiting side effects of gastrointestinal complaints (abdominal bloating, dyspepsia, flatulence, nausea, and vomiting) and central nervous system stimulation (agitation, dizziness, insomnia, tremors, and restlessness). These side effects are likely to be related to the 7% caffeine content in the study formulation. The study reported that a dosing regimen of 1.0 g/m^2 three times daily is well tolerated for at least 6 months. To achieve this dose, study participants have been required to swallow 7–10 capsules each time and three times a day. This dose level is roughly equivalent to drinking 7–8 Japanese-style cups (one cup = 120 ml) of green tea three times daily (a total of 21–24 cups of tea/day). In the current study, standardized, defined, and decaffeinated green tea polyphenol oral products in amounts similar to the EGCG content in 16 Japanese-style cups of green tea were consumed once daily or in divided doses twice daily (4 capsules/day) for 4 weeks. On the basis of the reported adverse events and clinical laboratory data, the study agents and dosing schedules have been found to be safe and well tolerated by the study participants for at least 1 month. The reported adverse events were rated as mild events. The more common events include headache, stomach ache, abdominal pain, and nausea, which have been reported in sub-

Table 3 Pharmacokinetic parameters of free EGCG obtained after p.o. administration of EGCG or Polyphenon E before and after 4 weeks of green tea polyphenol treatment

	800 mg EGCG ($n = 7$)[a]		800 mg EGCG as Polyphenon E ($n = 8$)		400 mg EGCG ($n = 8$)		400 mg EGCG as Polyphenon E ($n = 8$)	
	First dose	On the last treatment day	First dose	On the last treatment day	First dose	On the last treatment day	First dose	On the last treatment day
AUC[b] (min·μg/ml)	95.6 ± 46.8[c]	145.6 ± 85.1[d]	98.1 ± 46.5	158.4 ± 89.8[d]	46.9 ± 21.4	43.9 ± 25.6[e,f]	71.2 ± 36.9	54.8 ± 23.7[e]
C_{max} (ng/ml)	234.9 ± 140.9	390.3 ± 231.4	263.8 ± 135.7	287.6 ± 124.2	137.6 ± 66.5	161.4 ± 100.5[f]	179.9 ± 114.3	155.4 ± 61.9[f]
T_{max} (min)	224.4 ± 33.4	210.0 ± 73.5	112.5 ± 65.6	248.5 ± 184.9	183.9 ± 117.1	135.6 ± 48.4	150.8 ± 109.7	146.6 ± 102.2
CL/F (liter/min)	10.5 ± 4.4	7.3 ± 3.4	9.6 ± 3.4	8.0 ± 7.1	11.2 ± 5.2	13.1 ± 8.6	7.0 ± 3.4	9.0 ± 4.2
V_β/F (liter)	1910 ± 866	1686 ± 1241	2760 ± 1901	1551 ± 795	2516 ± 750	3456 ± 3200	2139 ± 716	3759 ± 2089
$t_{1/2}$ (min)	136.7 ± 54.4	158.9 ± 78.7	200.4 ± 94.7	163.0 ± 56.2	183.0 ± 75.3	170.5 ± 50.2	241.2 ± 115.6	296.6 ± 152.9

[a] Data from only 7 subjects were used in the analysis because of incomplete sample collection in 1 study participant.
[b] $AUC_{0-\infty}$ was calculated after the first catechin dose. After repeated dosing, AUC_{0-24h} was calculated after administration of 800-mg dose of the study agent on the last treatment day, and AUC_{0-12h} was calculated after administration of 400-mg dose of the study agent on the last treatment day.
[c] Mean ± SD.
[d] Significantly different from that of the first dose, $P < 0.05$.
[e] Significantly different from that after 800-mg dose of EGCG as Polyphenon E on the last treatment day, $P < 0.05$.
[f] Significantly different from that after 800-mg dose of EGCG on the last treatment day, $P < 0.05$.

Table 4 AUC[a] (μg·min/ml) of total (free and conjugated) EGC and EC obtained after p.o. administration of Polyphenon E before and after 4 weeks of Polyphenon E treatment

	800 mg EGCG as Polyphenon E ($n = 8$)		400 mg EGCG as Polyphenon E ($n = 8$)	
	First dose	After repeated dosing	First dose	After repeated dosing
Total EGC	103.4 ± 41.4[b]	95.9 ± 16.2	50.4 ± 19.1[c]	63.6 ± 47.2
Total EC	130.4 ± 72.2	154.5 ± 37.6	55.4 ± 16.6[c]	77.8 ± 81.5

[a] $AUC_{0-\infty}$ was calculated after the first catechin dose. After repeated dosing, AUC_{0-24h} was calculated after administration of 800-mg dose of Polyphenon E on the last treatment day, and AUC_{0-12h} was calculated after administration of 400-mg dose of Polyphenon E on the last treatment day.
[b] Mean ± SD.
[c] Significantly different from that at the 800-mg dose level, $P < 0.05$.

Table 5 Changes in MED after 4 weeks of green tea polyphenol/placebo treatment

Data are expressed as ratios of the post-treatment values to those obtained at baseline.

	Placebo ($n = 8$)	800 mg EGCG once daily ($n = 8$)	800 mg EGCG as Polyphenon E once daily ($n = 8$)	400 mg EGCG twice daily ($n = 8$)	400 mg EGCG as Polyphenon E twice daily ($n = 8$)
MED (after/before)	1.09 ± 0.08[a]	1.15 ± 0.11	1.11 ± 0.14	1.07 ± 0.08	1.08 ± 0.09

[a] Mean ± SD.

jects receiving green tea polyphenol treatment as well as in subjects receiving placebo. There were no significant changes in blood counts and blood chemistry profiles after 4 weeks of green tea polyphenol treatment.

On the basis of the observed plasma half-lives of EGCG, we do not expect EGCG to accumulate in the body after repeated dosing at a once daily schedule. The accumulation ratio was calculated based on the half-life and dosing interval (12), and was found to be <1.05. Consistently, EGCG was not detected or was detected at low levels in the predose sample collected on the last treatment day for the once daily schedule. Nevertheless, small amounts of EGCG are expected to accumulate after repeated dosing at a twice daily schedule with an average accumulation ratio of 1.07–1.24. This is reflected by the presence of measurable concentrations of EGCG in most of the predose samples collected on the last treatment day. Average predose EGCG levels of 29.9 and 54.8 ng/ml were observed after repeated dosing of EGCG and Polyphenon E, respectively, at the twice daily schedule. Some of the subjects had a short elapsed time from the time the predose sample was collected to the time the previous dose was taken, which could additionally contribute to the predose EGCG levels.

On average, there was a >60% increase in the AUC of free EGCG after 4 weeks of tea polyphenol treatment at a dosing schedule of 800 mg once daily. The observed increase in the systemic exposure of free EGCG is not related to drug accumulation after repeated dosing, because the AUC calculation has corrected for this factor (see "Data Analysis" for details). A dosing schedule of 400 mg twice daily did not result in significant changes in the AUC of free EGCG. Because the AUC

calculation has corrected for the accumulation factor, comparisons of the EGCG AUC after the first dose *versus* that on the last treatment day do not reveal the expected small accumulation effect. The relative proportions of free *versus* total (free and conjugated) EGCG did not change consistently after 1 month of treatment. Neither dosing schedule resulted in significant changes in the AUC of EGC and EC. EGC and EC are present in plasma mostly as the conjugated form, and the relative proportions of free *versus* total EGC or EC did not change consistently after 1 month of treatment. These data suggest that the conjugation process is not affected by repeated treatment of EGCG/Polyphenon E. Because EGCG is the only component in the EGCG formulation and the major component in the Polyphenon E formulation, it is likely that the 800-mg dose of EGCG/Polyphenon E resulted in significantly elevated EGCG levels in the gastrointestinal tract and subsequently inhibited presystemic elimination of EGCG but not EGC or EC. The 400-mg twice daily regimen apparently did not result in tea catechin concentrations that would exert a significant inhibitory effect. The mechanism(s) responsible for the observed increase in the AUC of free EGCG after chronic treatment of EGCG/Polyphenon E at a high daily bolus dose remain(s) to be studied. Inhibitions in nonenzymatic degradation, intestinal flora metabolism, methylation, and/or intestinal efflux of EGCG are plausible contributing factors. It is not known whether ingestion of green tea polyphenols at a high daily bolus dose for >4 weeks will result in additional enhancement in the systemic exposure of EGCG.

In animal model systems, topical treatment or p.o. administration of green tea or green tea polyphenols has been shown to inhibit UV radiation-induced skin tumorigenesis, formation of cutaneous edema, and depletion of antioxidant-defense system (13–15). Katiyar *et al.* (16) have shown that topical application of green tea polyphenols to human skin before UV irradiation significantly reduced the UV-induced erythema response and pyrimidine dimer formation. In a follow-up study, topical application of EGCG to human skin before UV irradiation markedly decreased UV-induced changes in markers of oxidative stress and antioxidant enzymes (17). In these studies, EGCG/green tea polyphenols prepared in acetone were applied topically 20 min before UV irradiation. This route of administration with acetone as the vehicle is likely to give rise to high levels of green tea polyphenol in epidermis and/or dermis during UV irradiation. In the current study, no significant changes in MED were observed after 4 weeks of p.o. EGCG/Polyphenon E administration at a daily dose of 800 mg of EGCG. Interestingly, some of the study participants indicated that they have experienced less-intensive sunburn reactions when receiving the green tea polyphenol treatment. One of the disparities between our study and that reported by Katiyar *et al.* (16) is the route of tea polyphenol administration. Topical application of EGCG/tea polyphenols in acetone is likely to have resulted in high local concentrations of green tea polyphenols, whereas p.o. administration of green tea polyphenols may not result in accumulation of high levels of green tea polyphenols in the skin during UV irradiation. This would be an important factor of consideration if protection against UV-induced erythema response requires the presence of high concentrations of green tea polyphenols at the target site. Insufficient treatment duration could also potentially contribute to our observations, because UV-induced erythema on dorsal skin has been shown not to be affected after 4 weeks of p.o. administration of carotenoids and vitamin E in healthy human subjects, but diminished significantly 8–12 weeks after treatment (18). Incorporation of other sensitive markers of photodamage should also be considered in future trials. In addition, the green tea polyphenol products used in the current study are decaffeinated products. Studies have compared the inhibitory effects of green tea and decaffeinated green tea in UV-induced skin carcinogenesis models and found that the decaffeinated products were either effective but less active or not effective (13, 14, 19), suggesting that caffeine contributes to the biological activity of green tea. However, the decreased effectiveness of decaffeinated green tea may be because the decaffeinated process also reduces the levels of green tea polyphenols.

We conclude that p.o. administration of EGCG or Polyphenon E at a daily dose of 800 mg (based on the EGCG content) for 4 weeks is safe and well tolerated in healthy human subjects. Repeated green tea polyphenol administration at a high daily bolus dose (800 mg once daily) results in a >60% increase in the systemic exposure of EGCG, possibly because of inhibition of presystemic elimination of this catechin. Repeated administration of EGCG and Polyphenon E at a daily dose equivalent to the EGCG content in 16 Japanese-style cups of green tea for 4 weeks did not provide protection against UV-induced erythema.

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