Phase I Pharmacokinetic Study of Tea Polyphenols following Single-dose Administration of Epigallocatechin Gallate and Polyphenon E[1]

H-H. Sherry Chow,[2] Yan Cai, David S. Alberts, Iman Hakim, Robert Dorr, Farah Shahi, James A. Crowell, Chung S. Yang, and Yukihiko Hara

Arizona Cancer Center, The University of Arizona, Tucson, Arizona 85724 [H-H. S. C., Y. C., D. S. A., I. H., R. D., F. S.]; Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland 20892 [J. A. C.]; College of Pharmacy, Rutgers University, Piscataway, New Jersey 08854 [C. S. Y.]; and Mitsui Norin Co., Ltd., Shizuoka 426-01, Japan [Y. H.]

Abstract

Green tea has been shown to exhibit cancer-preventive activities in preclinical studies. Its principal active components include epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), and epicatechin gallate, of which EGCG is the most abundant and possesses the most potent antioxidative activity. We performed a Phase I pharmacokinetic study to determine the systemic availability of green tea catechins after single oral dose administration of EGCG and Polyphenon E (decaffeinated green tea catechin mixture). Twenty healthy subjects (five subjects/dose level) were randomly assigned to one of the dose levels (200, 400, 600, and 800 mg based on EGCG content). All subjects were randomly crossed-over to receive the two catechin formulations at the same dose level. Blood and urine samples were collected for up to 24 h after oral administration of the study medication. Tea catechin concentrations in plasma and urine samples were determined using high-performance liquid chromatography with the coulometric electrode array detection system. After EGCG *versus* Polyphenon E administration, the mean area under the plasma concentration-time curves (AUC) of unchanged EGCG were 22.5 *versus* 21.9, 35.4 *versus* 52.2, 101.9 *versus* 79.7, and 167.1 *versus* 161.4 min·μg/ml at the 200-, 400-, 600-, and 800-mg dose levels, respectively. EGC and EC were not detected in plasma after EGCG administration and were present at low/undetectable levels after Polyphenon E administration. High concentrations of EGC and EC glucuronide/sulfate conjugates were found in plasma and urine samples after Polyphenon E administration. There were no significant differences in the pharmacokinetic characteristics of EGCG between the two study medications. The AUC and maximum plasma concentration (C_{max}) of EGCG after the 800-mg dose of EGCG were found to be significantly higher than those after the 200- and 400-mg dose. The AUC and C_{max} of EGCG after the 800-mg dose of Polyphenon E were significantly higher than those after the three lower doses. We conclude that the two catechin formulations resulted in similar plasma EGCG levels. EGC and EC were present in the body after the Polyphenon E administration; however, they were present predominantly in conjugated forms. The systemic availability of EGCG increased at higher doses, possibly due to saturable presystemic elimination of orally administered green tea polyphenols.

Introduction

During the last decade, the relationship between tea consumption and cancer has been a subject of research interest for many investigators. Several recent studies have thoroughly reviewed and summarized epidemiological and experimental studies on tea and cancer prevention (1–6). Green tea, green tea extract, and one of the major green tea polyphenols, EGCG,[3] have been shown to inhibit carcinogenesis induced by a wide variety of carcinogens in rodent cancer models. Cancer chemopreventive activity has been demonstrated in the following target organs: colon; duodenum; esophagus; forestomach; large intestine; liver; lung; mammary glands; and skin (1, 2, 4). The preventive activity is believed to be due to the antioxidative and antiproliferative effects of tea polyphenols. The polyphenols may also inhibit carcinogenesis by suppressing the activation of carcinogens and trapping genotoxic agents. The principal polyphenols in green tea include EGCG, EGC, EC, and epicatechin gallate; of these, EGCG is the most abundant and possesses the most potent antioxidative activity (2). 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 an individual's dietary habits, ingesting purified green tea polyphenol products in oral formulations may be more acceptable for chronic use in individuals who do not customarily consume green tea. We have

Received 7/19/00; revised 11/2/00; accepted 11/7/00.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by Contract N01-CN-85179 from the National Cancer Institute.
[2] To whom requests for reprints should be addressed, at Arizona Cancer Center, The University of Arizona, Tucson, AZ 85724. Phone: (520) 626-3358; Fax: (520) 626-5348; E-mail: schow@azcc.arizona.edu.

[3] The abbreviations used are: EGCG, epigallocatechin gallate; EGC, epigallocatechin; EC, epicatechin; HPLC, high-performance liquid chromatography; AUC, area under the plasma concentration-time curves.

performed a Phase I pharmacokinetic study to determine the systemic availability of tea polyphenols after a single oral dose administration of purified tea polyphenol products and the effects of dose and polyphenol formulation on the pharmacokinetics of EGCG and other tea catechins.

Materials and Methods

Study Drugs. Bulk EGCG and Polyphenon E were supplied to the Chemoprevention Agent Development Research Group, National Cancer Institute (Bethesda, MD) from the Food Research Laboratories, Mitsui Norin Co., Ltd., (Fujieda City, Japan). EGCG and Polyphenon E formulated in capsules were supplied by the Chemoprevention Agent Development Research Group, National Cancer Institute. On average, each EGCG capsule contained 200 mg of EGCG, and each Polyphenon E capsule contained 200 mg of EGCG, 37 mg of EGC, 31 mg of EC, and other tea polyphenols. Caffeine was not present in either formulation. The study medications were stored at room temperature, protected from environmental extremes.

Participants. Twenty healthy male and female subjects $\geq$30 years of age were recruited to participate in the study. To be eligible, the participants were required to be able to give informed consent, to have a performance status of 0–1 (as determined by the Southwest Oncology Group Performance Status Criteria), and to have normal liver and renal function. Individuals with performance status of 0–1 are fully active or restricted only in physically strenuous activity. Participants were excluded if they were pregnant, had had cancers of any types within the past 5 years, had severe metabolic disorders or other life-threatening acute or chronic diseases, had weight loss of >10% in the 6 months preceding study entry, and had a prior history of gastric ulcer. The study was approved by the University of Arizona Human Subjects Committee. Written informed consent was obtained from all participants.

Study Design. All study participants were required to refrain from the ingestion of tea or tea products for 7 days before the first pharmacokinetic study and until the end of the second pharmacokinetic study. Study participants (five subjects/dose level) were randomly assigned to a dose level (200, 400, 600, or 800 mg based on EGCG content). All subjects were randomly crossed-over to receive the two polyphenol formulations at the same dose level, with a 2-week wash-out period. The diet of the subjects was not standardized or controlled before the study.

The day before the pharmacokinetic study, study participants were instructed to fast after midnight except for drinking water. On the pharmacokinetic study day, study subjects skipped breakfast and took no over the counter medications, vitamins, or health food products. Study participants came to the clinic in the early morning (6–8 a.m.) and were provided with one to two bagels for breakfast. Immediately after or during breakfast, study subjects swallowed one of the tea polyphenol formulations at the assigned dose level with a glass of water. Study subjects were allowed unlimited water intake throughout the study day. Other drinks were not allowed. Blood samples (5–7 ml each) were collected before the administration of the study medication and at time points 0.5, 1, 2, 4, 6, 8, and 24 h after drug administration. Study subjects self-collected urine before dosing and at three intervals during the 24-h period after dosing (0–4, 4–8, and 8–24 h). After the 4 h blood collection, a vegetarian or turkey bagel sandwich was provided to the study subjects.

Sample Collection and Processing. Once collected, blood samples were kept in the refrigerator and centrifuged at 4°C within 2 h of collection. After centrifugation, 1 ml of plasma was aliquoted into cryotubes containing 20 μl of ascorbate-EDTA solution [0.4 M NaH_2PO_4 buffer containing 20% ascorbic acid and 0.1% EDTA (pH 3.6)]. The samples were stored at −80°C until analysis. Before each urine collection period, 5 ml of ascorbate-EDTA solution were added to the urine collection containers, which were kept in a cooler containing frozen ice-packs. At the end of the urine collection, urine volume was recorded, and an aliquot of 15 ml was transferred into a storage tube that contained 20 mg of ascorbic acid and 0.5 mg of EDTA. The pH of the urine samples was adjusted to 6.8 with 10% NaOH, and an aliquot was stored at −80°C until analysis.

Tea Polyphenol Concentration Measurements. EGCG, EC, and EGC concentrations in plasma and urine samples were determined within 1 month of collection using a previously published HPLC procedure (7). Briefly, for determination of the unchanged tea polyphenols in plasma, lipid soluble components in plasma were removed with a methylene chloride extraction. The remaining aqueous phase was extracted with ethyl acetate. The ethyl acetate fraction was collected, mixed with a small volume of 0.1% ascorbic acid, and dried by vacuum centrifugation. The dried sample was redissolved in an aliquot of 15% acetonitrile aqueous solution. The reconstituted sample was centrifuged at 16,000 $\times$ g for 10 min, and an aliquot of the sample was injected onto HPLC. For determination of the glucuronic acid/sulfate conjugates of tea polyphenols in plasma, the samples were mixed with an aliquot of ascorbate-EDTA solution and a mixture of β-glucuronidase and sulfatase (to convert the conjugates to the unchanged form). The resulting mixture was incubated at 37°C for 45 min. Blank plasma spiked with polyphenol standards was incubated with a similar mixture at 37°C for 23 min (half of the incubation time of the sample). The reaction was stopped by adding methylene chloride and water and extracted as described for the unchanged polyphenols. Differences between the polyphenol concentrations determined before and after enzyme hydrolysis allowed the estimation of the concentrations of the conjugated polyphenols. The extraction procedures for the urine samples were similar to those for the plasma samples, except that the urine samples were not subjected to the clean-up procedures with methylene chloride. For each analytical run, a standard curve was prepared in the appropriate matrix and used to determine the tea polyphenol concentration. Quality control samples were prepared and analyzed along with the authentic samples. The whole batch of samples was reprocessed and reanalyzed if the variation of the quality control samples was greater than 15% of the expected values.

The HPLC system consisted of an ESA Model 540 refrigerated autosampler, an ESA Model 582 two-pump solvent delivery system, an ESA 5600 coulochem electrode array system, and a Supelcosil C18 reversed-phase column (150 $\times$ 4.6 mm; particle size, 5 μm; Supelco Inc., Bellefonte, PA). The autosampler and column temperatures were maintained at 6°C and 35°C, respectively. Buffer A consisted of 30 mM NaH_2PO_4 buffer, acetonitrile, and tetrahydrofuran in a 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 a volume ratio of 41.5:58.5:12.5 (pH 3.45). The flow rate was maintained at 1 ml/min. The column was eluted at 96% buffer A and 4% buffer B from 0–7 min, and 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

Table 1 Subject demographic data by dose levels

	200 mg	400 mg	600 mg	800 mg
No. of subjects	5	5	5	5
No. of males	1	2	1	0
Age (yr)	44.6 ± 9.0[a]	49.2 ± 11.6	48.8 ± 11.5	48.0 ± 19.3
Height (inches)	63.0 ± 0.7	65.8 ± 4.0	64.0 ± 3.7	63.0 ± 1.4
Weight (lbs)	146.8 ± 25.3	166.6 ± 9.3	160.0 ± 26.2	145.2 ± 14.2

[a] Mean ± 1 SD.

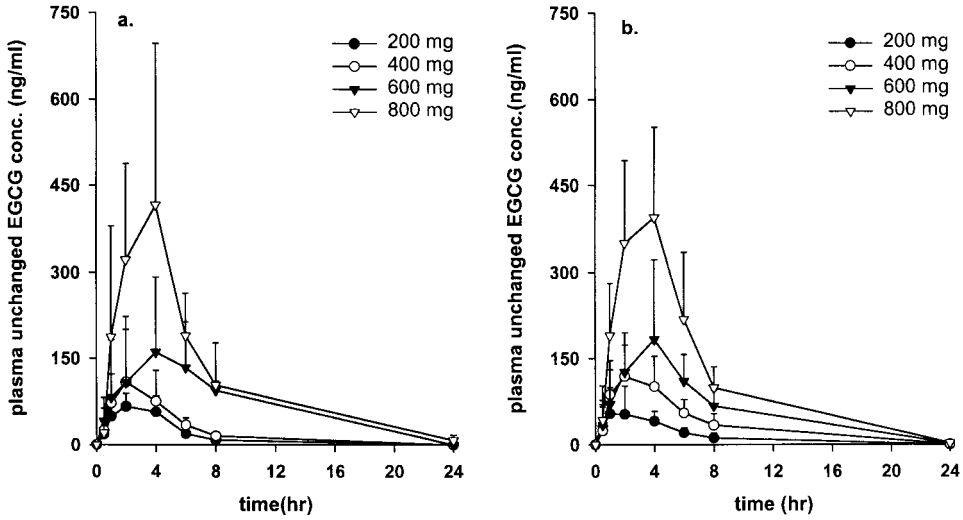

Fig. 1. Average plasma EGCG concentration *versus* time profiles after oral administration of EGCG or Polyphenon E at different dose levels. *a,* EGCG formulation; *b,* Polyphenon E formulation. Each point represents the average of five subjects, and the *cross-vertical bars* represent 1 SD of the mean. ●, 200 mg; ○, 400 mg; ▼, 600 mg; ▽, 800 mg.

min. It was maintained at 98% buffer B from 38–43 min and finally changed back to 4% buffer B at 44 min for the analysis of the next sample. The eluent was monitored by the coulochem electrode array system with potential settings at −90, −10, 70, and 150 mV, and four chromatograms were obtained simultaneously.

Data Analysis. The following pharmacokinetic parameters of unchanged EGCG were estimated using the WINNONLIN program with the model-independent approach (8): time to reach maximum plasma concentration (T_{max}); maximum plasma concentration (C_{max}); AUC; clearance/bioavailability (CL/F); volume of distribution/bioavailability (V_d/F); terminal elimination half-life ($t_{1/2}$); and terminal elimination rate constant (λ_n). The AUC, T_{max}, and C_{max} of total (unchanged and glucuronic acid/sulfate conjugates) EGC, EC, and EGCG were also estimated using the WINNONLIN program with the mode-independent approach. The amount of tea polyphenol excreted into the urine was estimated by the product of the polyphenol concentration in the urine and urine volume.

Pharmacokinetic parameters were compared among different dose levels for the same catechin formulation using ANOVA. Bonferroni's *t* test was used for the pairwise multiple comparisons. Pharmacokinetic measurements of EGCG between the two catechin formulations were compared by a paired *t* test.

Results

Table 1 presents the demographic data of the study participants. A total of 20 subjects (5 subjects/dose level) completed the study. Because of the small number of male participants, we did not have a completely balanced male:female subject ratio among the dose levels. There were no significant differences in other demographic characteristics of the participants among the four dose groups.

Fig. 1 illustrates the average plasma unchanged EGCG concentration-time profiles after EGCG or Polyphenon E administration. After oral administration, plasma EGCG levels increased toward a peak and declined rapidly, with very low/undetectable levels at 24 h after dosing. The average EGCG concentrations increased as the dose was increased. Similar plasma EGCG concentration-time profiles were observed after EGCG and Polyphenon E administration for each dose level.

Fig. 2*a* illustrates the average plasma catechin concentration *versus* time profile after a 600-mg dose of EGCG. After EGCG administration, EGCG but not EGC or EC was detected in the plasma samples. Treating the plasma samples with sulfatase/glucuronidase did not significantly increase the EGCG levels (Fig. 2*b*), suggesting that small amounts of EGCG were present as the glucuronic acid/sulfate conjugates. Fig. 3 shows the average plasma catechin concentration *versus* time profiles after a 600-mg dose of Polyphenon E. Unchanged plasma EGCG levels observed after Polyphenon E administration were similar to those after EGCG administration (see Fig. 2). Similarly, EGCG was present predominantly as the unchanged form. Unchanged plasma EGC and EC levels were low or undetectable after Polyphenon E administration. After sulfatase/glucuronidase treatment, EGC and EC concentrations increased dramatically, suggesting that EGC and EC were present predominantly as the glucuronic acid/sulfate conjugates.

The average pharmacokinetic parameters of EGCG after EGCG or Polyphenon E administration are summarized in Table 2. The C_{max} values were 73.7 ± 25.3, 111.8 ± 98.6,

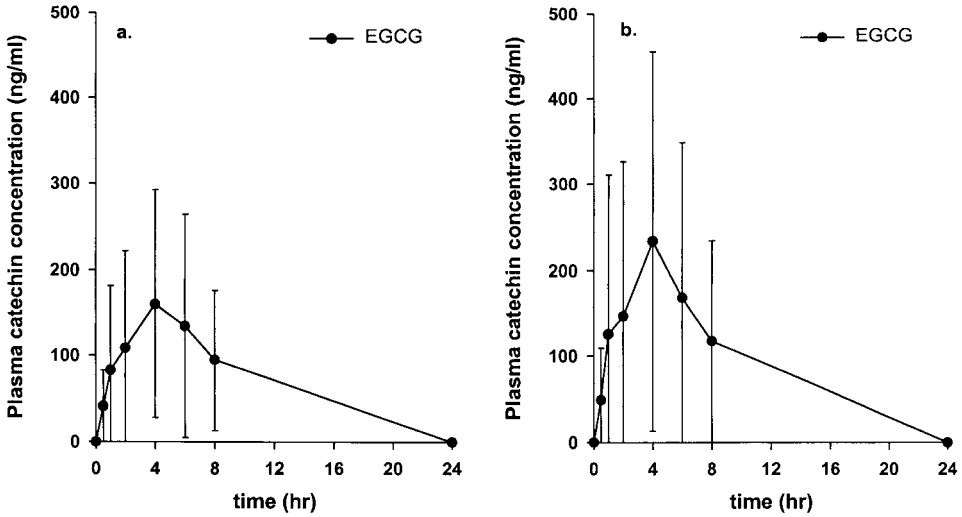

Fig. 2. Average plasma tea polyphenol concentration *versus* time profiles after a 600-mg dose of EGCG. *a*, data obtained from plasma samples without glucuronidase/sulfatase treatment. *b*, data obtained from plasma samples treated with glucuronidase/sulfatase. Each point represents the average of five subjects, and the *cross-vertical bars* represent 1 SD of the mean. ●, EGCG.

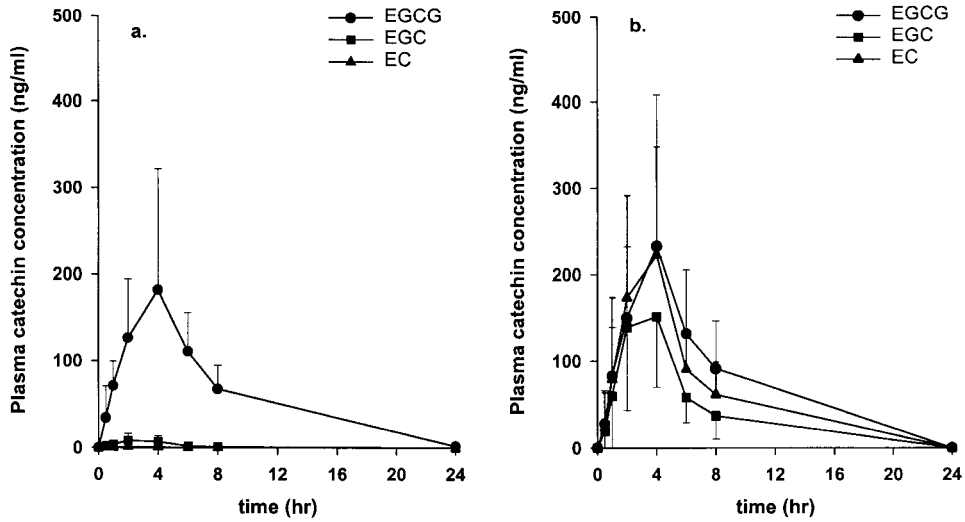

Fig. 3. Average plasma tea polyphenol concentration *versus* time profiles after a 600-mg dose of Polyphenon E. *a*, data obtained from plasma samples without glucuronidase/sulfatase treatment. *b*, data obtained from plasma samples treated with glucuronidase/sulfatase. Each point represents the average of five subjects, and the *cross-vertical bars* represent 1 SD of the mean. ●, EGCG; ■, EGC; ▲, EC.

Table 2 Pharmacokinetic parameters of EGCG after oral administration of EGCG or Polyphenon E

Parameter	200 mg		400 mg		600 mg		800 mg	
	EGCG	Polyphenon E	EGCG	Polyphenon E	EGCG	Polyphenon E	EGCG	Polyphenon E
AUC (min·μg/ml)	22.5 ± 7.3[a,b]	21.9 ± 12.0[c]	35.4 ± 21.5[b]	52.2 ± 22.8[c]	101.9 ± 99.7	79.7 ± 39.2[c]	167.1 ± 57.0	161.4 ± 57.0
C_{max} (ng/ml)	73.7 ± 25.3[b]	72.7 ± 66.4[c]	111.8 ± 98.6[b]	125.3 ± 50.4[c]	169.1 ± 139.6	165.7 ± 126.9[c]	438.5 ± 284.4	377.6 ± 149.8
T_{max} (min)	127.1 ± 76.6	144.7 ± 90.7	108.7 ± 26.4	170.9 ± 64.7	180.0 ± 84.8	216.0 ± 53.7	240.6 ± 84.6	249.0 ± 85.4
CL/F (liters/min)	11.4 ± 4.3	14.6 ± 10.1	18.0 ± 10.2	10.7 ± 5.6	12.8 ± 12.5	10.3 ± 4.8	6.0 ± 1.7	6.5 ± 1.8
V_β/F (liters)	2009 ± 1514	3068 ± 2229	4774 ± 3582	2760 ± 2211	4368 ± 5883	2544 ± 1414	1044 ± 543	1091 ± 438
$t_{1/2}$ (min)	118.0 ± 77.0	156.9 ± 96.2	162.3 ± 84.3	180.5 ± 112.3	183.7 ± 67.6	163.1 ± 31.8	114.0 ± 33.3	113.5 ± 20.6
λ_n (min^{-1})	0.0085 ± 0.0058	0.0059 ± 0.0031	0.0054 ± 0.0030	0.0051 ± 0.0028	0.0041 ± 0.0012	0.0044 ± 0.0011	0.0065 ± 0.0019	0.0063 ± 0.0010

[a] Mean ± 1 SD.
[b] Significantly different from that of 800 mg of EGCG, $P < 0.05$.
[c] Significantly different from that of 800 mg of Polyphenon E, $P < 0.05$.

169.1 ± 139.6, and 438.5 ± 284.4 ng/ml after a 200-, 400-, 600-, and 800-mg dose of EGCG, respectively. The AUC and C_{max} of EGCG after EGCG administration among the three lower doses were not significantly different. The AUC and C_{max} of EGCG after an 800-mg dose of EGCG were significantly higher than the corresponding parameters after the 200- and 400-mg doses. There were no significant differences in other pharmacokinetic parameters among the four EGCG doses. Plasma EGCG levels observed after Polyphenon E administration were similar to those seen after EGCG administration, with an average C_{max} of 72.7 ± 66.4, 125.3 ± 50.4, 165.7 ± 126.9, and 377.6 ± 149.8 ng/ml at the four dose levels.

Table 3 AUC of total catechins (sum of unchanged and conjugated forms) after oral administration of EGCG or Polyphenon E

	Dose levels			
	200 mg[a]	400 mg[b]	600 mg[c]	800 mg[d]
EGCG formulation				
EGCG AUC (min·μg/ml)	22.0 ± 8.8[e]	37.3 ± 25.4	132.6 ± 149.9	166.4 ± 70.7
Polyphenon E formulation				
EGCG AUC (min·μg/ml)	23.0 ± 7.8[f]	64.9 ± 32.8[f]	111.1 ± 66.3[f]	258.2 ± 111.0
EGC AUC (min·μg/ml)	34.7 ± 10.0[f]	47.1 ± 23.3[f]	61.3 ± 22.0[f]	94.3 ± 8.2
EC AUC (min·μg/ml)	39.0 ± 10.5[f]	52.4 ± 31.2[f]	90.8 ± 35.7[f]	167.8 ± 43.9

[a] 200 mg of EGCG in the EGCG formulation; 200 mg of EGCG, 37 mg of EGC, and 31 mg of EC in the Polyphenon E formulation.
[b] Twice the tea polyphenol contents compared with the 200-mg dose.
[c] Three times the tea polyphenol contents compared with the 200-mg dose.
[d] Four times the tea polyphenol contents compared with the 200-mg dose.
[e] Mean ± 1 SD.
[f] Significantly different from the 800-mg dose level ($P < 0.05$).

Similar to the EGCG formulation, the AUC and C_{max} values of EGCG among the three lower doses of Polyphenon E were not significantly different. The AUC and C_{max} of EGCG after an 800-mg dose of Polyphenon E were significantly higher than those after the three lower doses. There were no significant differences in other pharmacokinetic parameters among the four Polyphenon E doses. The pharmacokinetic parameters of EGCG after EGCG and Polyphenon E administration were not significantly different.

The AUCs of total (unchanged and glucuronic acid/sulfate conjugates) catechins are summarized in Table 3. The AUCs of total EGCG after Polyphenon E administration appeared to be greater than that after the EGCG administration in three of the doses studied; however, the differences were not statistically significant. Although the content of EGC or EC in the Polyphenon E formulation is less than 20% of that of EGCG, the AUC of total EGC or EC was 36–170% of total EGCG.

Neither EGCG nor its glucuronic acid/sulfate conjugates were detectable in urine after EGCG or Polyphenon E administration. EGC and EC were detected in urine after Polyphenon E administration, primarily as glucuronic acid/sulfate conjugates. The average amount of total EGC (unchanged and glucuronic acid/sulfate conjugates) recovered in the 0–24 h urine was 418 ± 194, 1395 ± 1390, 3513 ± 2356, and 3710 ± 1786 μg after a single oral dose administration of Polyphenon E containing 37, 74, 111, and 148 mg of EGC, respectively. The average amount of total EC (unchanged and glucuronic acid/sulfate conjugates) recovered in the 0–24 h urine was 620 ± 326, 1194 ± 1718, 2305 ± 1466, and 5041 ± 3077 μg after a single dose administration of Polyphenon E containing 31, 62, 93, and 124 mg of EC, respectively.

Discussion

In our study, the EGCG and Polyphenon E formulations containing the same amount of EGCG resulted in similar plasma EGCG levels. This is likely to be attributable to the fact that EGCG was the major component in the Polyphenon E formulation (EGCG:EGC:EC ratio of 20:3.7:3.1). EGC and EC were detected in plasma and urine samples after Polyphenon E administration, predominantly as glucuronic acid/sulfate conjugates. It is not known whether the tea catechin conjugates possess any biological activities. In a recent study (9), the glucuronic acid/sulfate conjugates of catechins were shown to have the same electrochemical behavior as the parent drug across different electrode potentials. Considering that the oxidation potential of chemicals may represent their antioxidant capacity, the electrochemical behavior of the conjugates suggests that they are effective antioxidants. Nevertheless, because the glucuronic acid/sulfate conjugates are generally more hydrophilic than the parent compound, the tissue distributions of these metabolites are likely to be more limited than those of the parent catechins. Therefore, it is not known whether the glucuronic acid/sulfate conjugates of tea catechins will be pharmacologically relevant in target tissues. Based on the plasma polyphenol levels, the EGCG and Polyphenon E formulations should have similar pharmacological responses if the conjugates do not contribute to the activities. Otherwise, the Polyphenon E formulation should be more potent because of the presence of high plasma levels of EGC and EC conjugates.

Fig. 4 shows the relationship between the AUC of unchanged EGCG and the tea polyphenol dose ingested. The AUC of EGCG showed less than proportional increases at the three lower doses and more than proportional increases as the dose was increased to 800 mg. This phenomenon is consistent with that observed for chemicals undergoing extensive presystemic elimination. Because the presystemic elimination is saturated at higher doses, the extents of the unchanged form available in the systemic circulation are increased. In a previous clinical study using reconstituted green tea beverages (10), the C_{max} of EGCG increased approximately 2–3-fold when the dose of green tea solids was increased from 1.5 to 3.0 grams, but increasing the dose to 4.5 grams did not increase the C_{max} values significantly. These findings differed from our current observation. In the previous study, EGCG is not the major component in the green tea solids, with EGC content similar to that of EGCG. The presence of other tea polyphenols in significant quantities might affect the absorption and disposition of EGCG (10). Furthermore, the tea polyphenol concentrations were reported as the sum of unchanged and conjugated forms in the previous study (10), although the kinetic disposition of the conjugates can be very different from that of the parent catechins.

Large variations in the systemic availability of tea polyphenols after oral consumption were observed in our study, as in other studies (10, 11). With the limited number of study subjects, we did not find significant correlations between the age, weight, and height of the subject and the systemic tea polyphenol levels. The oral bioavailability of tea polyphenols has been found to be very low in rodents (12) but has not been reported in humans because of the lack of pharmacokinetic studies after i.v. administration. The large CL/F and V_d/F values observed in our study suggest that the oral bioavailability of tea polyphenols in humans is also low. Therefore, a significant fraction of the orally administered tea polyphenols is likely to

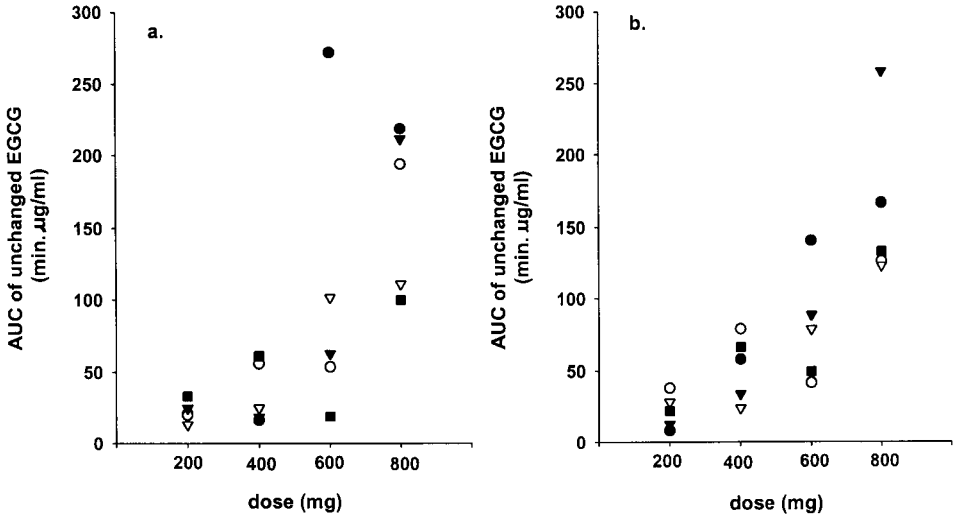

Fig. 4. The relationship between the AUC of the unchanged EGCG and the tea polyphenol dose ingested. *a,* EGCG formulation. *b,* Polyphenon E formulation. Within each dose level, five different symbols were used to identify data derived from different subjects, and the same symbol was used to represent data obtained from the same subject receiving EGCG and Polyphenon E.

be eliminated presystemically. Small changes in the presystemic elimination of green tea catechins could have a significant impact on the systemic availability of these compounds. Large intersubject and intrasubject variability in the systemic exposure of tea polyphenols could be an additional contributing factor to the inconsistent epidemiological findings on the relationship between tea and cancer.

It is worth noting that although the EGC or EC content in the Polyphenon E formulation was less than 20% of that of EGCG, the AUCs of total (unchanged and conjugated) EGC or EC were 36–170% of total EGCG. If a nonspecific assay were used, we might have concluded that orally administered EGC and EC were more bioavailable than EGCG. However, total EGC or EC consisted mostly of conjugated forms, whereas total EGCG was constituted mostly of the unchanged form. Having specifically determined the unchanged tea polyphenol concentrations and compared the dose-adjusted AUC of the unchanged forms, we found EGCG to be more bioavailable than EGC and EC.

Throughout the study, we recorded all side effects experienced by our study subjects. Both tea polyphenol formulations administered as a single oral dose over the dose range studied were well tolerated by the study participants. The highest dose used was equivalent to consuming up to 8 cups of green tea at once. Some subjects experienced mild headache and fatigue, possibly related to the study products. These adverse events could also have been consequences of the procedures and restrictions that the subjects encountered on the pharmacokinetic study days (such as refraining from beverages containing caffeine).

We conclude that both tea polyphenol formulations administered as a single oral dose over the dose range studied were well tolerated by the study participants. Oral administration of EGCG and Polyphenon E at the same dose level (based on EGCG content) resulted in similar plasma EGCG levels. From the economical standpoint in chemoprevention, these results are encouraging because it would be less expensive to produce the Polyphenon E formulation than the pure EGCG formulation. Orally ingested tea polyphenols undergo extensive and saturable presystemic elimination and have large intersubject variations in systemic availability. Additional studies are needed to address factors affecting the systemic availability of tea polyphenols. Future clinical studies are planned to determine the safety, pharmacokinetics, and pharmacological activity of tea polyphenols after chronic EGCG/Polyphenon E treatment.

References

1. Katiyar, S. K., and Mukhtar, H. Tea in chemoprevention of cancer: epidemiologic and experimental studies. Int. J. Oncol., *8:* 221–238, 1996.

2. National Cancer Institute, Division of Cancer Prevention and Control, Chemoprevention Branch, and Agent Development Committee. Clinical development plan: tea extracts green tea polyphenols epigallocatechin gallate. J. Cell. Biochem. *26S:* 236–257, 1996.

3. Kohlmeier, L., Weterings, K. G. C., Steck, S., and Kok, F. J. Tea and cancer prevention: an evaluation of the epidemiologic literature. Nutr. Cancer, *27:* 1–13, 1997.

4. Dreosti, I. E., Wargovich, M. J., and Yang, C. S. Inhibition of carcinogenesis by tea: the evidence from experimental studies. Crit. Rev. Food Sci. Nutr., *37:* 761–770, 1997.

5. Blot, W. J., Chow, W-H., and McLaughlin, J. K. Tea and cancer: a review of the epidemiological evidence. Eur. J. Cancer Prev., *5:* 425–438, 1996.

6. Yang, C. S., and Wang, Z. Y. Tea and cancer. J. Natl. Cancer Inst. (Bethesda), *85:* 1038–1049, 1993.

7. Lee, M-J., Wang, Z-Y., Li, H., Chen, L., Sun, Y., Gobbo, S., Balentine, D. A., and Yang, C. S. Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol. Biomark. Prev., *4:* 393–399, 1995.

8. Gibaldi, M., and Perrier, D. Pharmacokinetics, 2nd ed. New York: Dekker, 1982.

9. Manach, C., Texier, O., Morand, C., Crespy, V., Régérat, F., Demigné, C., and Rémésy, C. Comparison of the bioavailability of quercetin and catechin in rats. Free Radic. Biol. Med., *27:* 1259–1266, 1999.

10. Yang, C. S., Chen, L., Lee, M-J., Balentine, D. A., Kuo, M. C., and Schantz, S. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer Epidemiol. Biomark. Prev., *7:* 351–354, 1998.

11. Bell, J. R. C., Donovan, J. L., Wong, R., Waterhouse, A., German, J. B., Walzem, R. L., and Kasim-Karakas, S. E. (+)-Catechin in human plasma after ingestion of a single serving of reconstituted red wine. Am. J. Clin. Nutr., *71:* 103–108, 2000.

12. Chen, L., Lee, M-J., and Yang, C. S. Absorption, distribution, and elimination of tea polyphenols in rats. Drug Metab. Dispos., *25:* 1045–1050, 1997.

Phase I Pharmacokinetic Study of Tea Polyphenols following Single-dose Administration of Epigallocatechin Gallate and Polyphenon E

H-H. Sherry Chow, Yan Cai, David S. Alberts, et al.

Cancer Epidemiol Biomarkers Prev 2001;10:53-58.