

Pharmacokinetics of Tea Catechins after Ingestion of Green Tea and (–)-Epigallocatechin-3-gallate by Humans: Formation of Different Metabolites and Individual Variability¹

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Abstract

Green tea and tea polyphenols have been studied extensively as cancer chemopreventive agents in recent years. The bioavailability and metabolic fate of tea polyphenols in humans, however, are not clearly understood. In this report, the pharmacokinetic parameters of (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), and (–)-epicatechin (EC) were analyzed after administration of a single oral dose of green tea or decaffeinated green tea (20 mg tea solids/kg) or EGCG (2 mg/kg) to eight subjects. The plasma and urine levels of total EGCG, EGC, and EC (free plus conjugated forms) were quantified by HPLC coupled to an electrochemical detector. The plasma concentration time curves of the catechins were fitted in a one-compartment model. The maximum plasma concentrations of EGCG, EGC, and EC in the three repeated experiments with green tea were 77.9 ± 22.2 , 223.4 ± 35.2 , and 124.03 ± 7.86 ng/ml, respectively, and the corresponding AUC values were 508.2 ± 227 , 945.4 ± 438.4 , and 529.5 ± 244.4 ng·h·ml⁻¹, respectively. The time needed to reach the peak concentrations was in the range of 1.3–1.6 h. The elimination half-lives were 3.4 ± 0.3 , 1.7 ± 0.4 , and 2.0 ± 0.4 h, respectively. Considerable interindividual differences and variations between repeated experiments in the pharmacokinetic parameters were noted. Significant differences in these pharmacokinetic parameters were not observed when EGCG was given in decaffeinated green tea or in pure

form. In the plasma, EGCG was mostly present in the free form, whereas EGC and EC were mostly in the conjugated form. Over 90% of the total urinary EGC and EC, almost all in the conjugated forms, were excreted between 0 and 8 h. Substantial amounts of 4'-O-methyl EGC, at levels higher than EGC, were detected in the urine and plasma. The plasma level of 4'-O-methyl EGC peaked at 1.7 ± 0.5 h with a half life of 4.4 ± 1.1 h. Two ring-fission metabolites, (–)-5-(3',4',5'-trihydroxyphenyl)- γ -valerolactone (M4) and (–)-5-(3',4'-dihydroxyphenyl)-valerolactone (M6), appeared in significant amounts after 3 h and peaked at 8–15 h in the urine as well as in the plasma. These results may be useful for designing the dose and dose frequency in intervention studies with tea and for development of biomarkers of tea consumption.

Introduction

Tea, a water infusion prepared from the dried leaves of *Camellia sinensis*, is one of the most popular beverages in the world. In addition to its pleasant flavor and aroma, green tea has been reported to possess beneficial health activities, such as the prevention of cancer and cardiovascular diseases (1). The active constituents of green tea are believed to be the polyphenols, commonly known as tea catechins. The major tea catechins are EGCG, EGC, ECG, and EC. In brewed green tea, the water-extractable material, which usually accounts for one-third of the tea leaves in dry weight, contains about 30% catechins, 3% flavonols, 3–6% caffeine, and other constituents (2). Studies in many animal models and cell lines have demonstrated that tea and tea polyphenols possess anticarcinogenic activity (1, 3–5). Many ecological, case-control, and cohort studies have been conducted to investigate the effects of tea consumption on human cancer incidence, but no clear-cut conclusion can be drawn (3, 6–8). Whereas some epidemiological studies have suggested that tea consumption may reduce the risk for certain cancers, such a protective effect has not been observed in other studies (3, 6–8). Similarly, there are conflicting results concerning the possible preventive effect of tea consumption against cardiovascular diseases (1, 9).

The interpretation of epidemiological data and the understanding of biological effects of tea consumption in humans are hampered by inadequate information on the bioavailability and biotransformation of tea catechins. Human studies with tea, especially on the pharmacokinetics of tea catechins, have been limited in scope (10–19). In preparation for intervention trials, we have previously conducted studies to examine the acceptability of tea beverage preparations by human volunteers and some pharmacokinetic parameters of tea polyphenols (10, 11).

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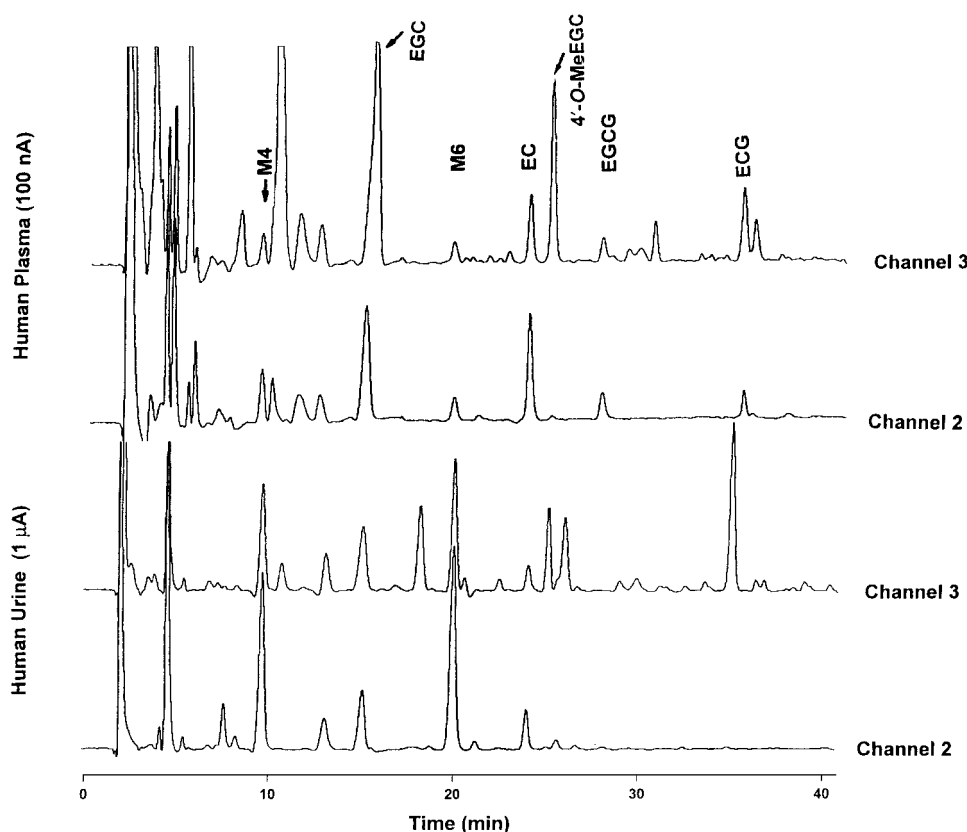


Fig. 1. HPLC chromatograms of tea catechins and their metabolites in human plasma and urine after a single oral dose of GT (20 mg/kg body weight). The plasma and urine samples were digested with β -glucuronidase and sulfatase, extracted, and analyzed as described in "Materials and Methods." The column was eluted with buffer A (30 mM NaH_2PO_4 containing 1.75% acetonitrile and 0.12% tetrahydrofuran, pH 3.45) and buffer B (15 mM NaH_2PO_4 buffer containing 58.5% acetonitrile and 12.5% tetrahydrofuran, pH 3.45). The gradient was set initially as 96% buffer A and 4% buffer B until 7 min; then it was changed by increasing buffer B to 17% at 25 min, 28% at 31 min, 33% at 37 min, 98% at 38 min, and maintaining at 98% up to 43 min, and finally switching buffer B back to 4% at 44 min. The retention times of EGCG, EGC, EC, 4'-O-MeEGC, M4, and M6 were 28.1, 15.3, 24.1, 25.3, 10.0, and 20.1 min, respectively.

The time-dependent blood levels of EGCG,⁴ EGC, and EC as well as the urine levels of EGC and EC in individuals after taking different amounts of DGT have been determined. The peak plasma concentrations of EGCG, EGC, and EC, reached between 1.5 and 2.5 h after consumption of 1.5 g of DGT, were 119, 148, and 55 ng/ml, respectively. The half-lives of EGCG was \sim 5 h and of EGC and EC were \sim 3 h. The levels of their metabolites were not measured. The reproducibility of the results in specific individuals and the interindividual variability of plasma catechin levels, however, are not known.

The objective of the present study is to gain an understanding about the pharmacokinetic properties of tea catechins as well as the interindividual differences and reproducibility of the repeated experiments in humans. The tea catechins were given to human subjects in the form of GT, DGT, or as pure EGCG. In addition to the major catechins, methylated and ring-fission metabolites were also analyzed.

Materials and Methods

Chemicals and Reagents. EGCG, EGC, EC, β -D-glucuronidase (G-7896, EC 3.2.1.31, from *Escherichia coli* with 9,000,000 units/g solid), and sulfatase (S-9754, EC 3.1.6.1, from Abalone entrails with 23,000 units/g solid) were purchased from Sigma Chemical Co. (St. Louis, MO). The GT and

DGT solids and EGCG were provided by Thomas J. Lipton Co. (Englewood Cliffs, NJ). The GT solids were prepared by freeze-drying the water extracts of green tea leaves (1 g of powder was derived from 6 g of dry leaves). For preparing DGT, green tea leaves were decaffeinated using supercritical carbon dioxide. The residual caffeine was $<$ 0.1%. The contents of EGCG, EGC, and EC were 13.9, 11.0 and 3.2%, respectively, in GT solids and 5.2, 4.3, and 1.3%, respectively, in DGT solids.

Other reagents and HPLC grade solvents were obtained from EM Sciences (Gibbstown, NJ) and were of the highest grade available commercially. A standard stock solution containing EGCG, EGC, and EC (10 $\mu\text{g}/\text{ml}$ each) was made in 0.2% ascorbic acid-0.005% EDTA solution (pH 3.8) and stored in small aliquots at -80°C until use. The stock solutions were stable for at least 6 months.

Human Studies. The study had the participation of eight healthy adult volunteers (5 males and 3 females) between 25 and 35 years of age and weighing between 45 and 85 kg. The protocol (No. 92-034) was approved by the Institutional Review Board for the Protection of Human Subjects in Research at Rutgers University (Piscataway, NJ). The subjects did not use tobacco products and only occasionally drank alcoholic beverages. They did not ingest tea or tea-related beverages for at least 2 days before the experiment and during the urine sample collection period. The subjects were fasted overnight and given a single oral dose of GT solids (20 mg/kg body weight) dissolved in 200 ml of warm water between 9 and 10 a.m. Blood samples (5 ml each) were collected in heparinized tubes at 0, 0.25, 0.5, 1, 2, 3, 5, 8, 12, and 24 h after ingesting the GT. After centrifugation, 1 ml of each plasma

⁴ The abbreviations used are: EGCG, (–)-epigallocatechin gallate; EGC, (–)-epigallocatechin; ECG, (–)-epicatechin gallate; EC, (–)-epicatechin; GT, green tea; DGT, decaffeinated GT; AUC, area under the plasma concentration time curve; 4-O-MeEGC, 4'-O-methyl EGC; M4, (–)-5-(3',4',5'-trihydroxyphenyl)- γ -valerolactone; M6, (–)-5-(3',4'-dihydroxyphenyl)- γ -valerolactone.

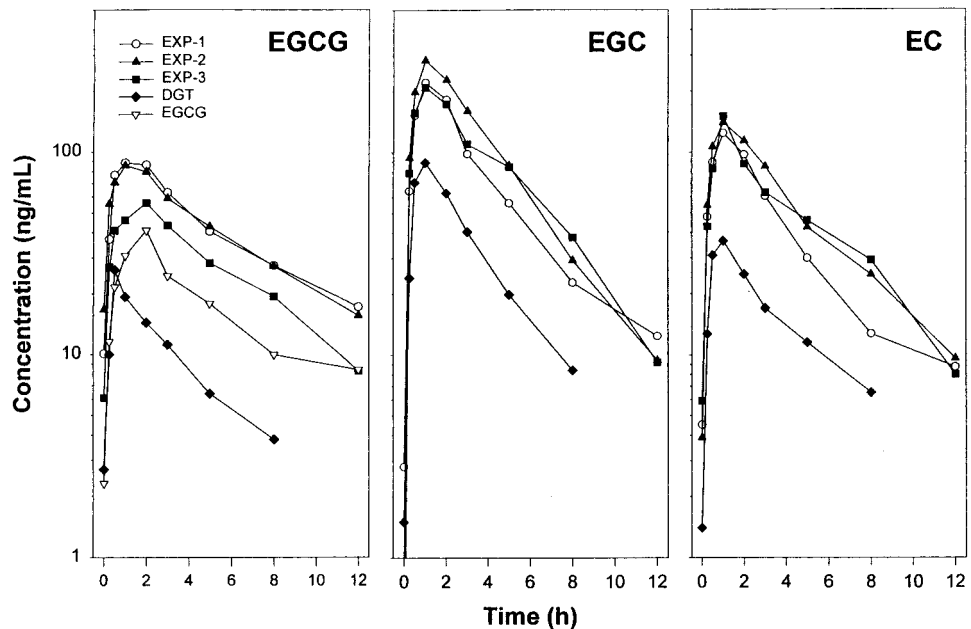


Fig. 2. Plasma concentration versus time plot of EGCG, EGC and EC after ingestion of GT, DGT, or pure EGCG. The subjects were given a single oral dose of GT (20 mg/kg; experiment was repeated three times) or DGT (20 mg/kg) or EGCG (2 mg/kg). Blood samples were collected and analyzed as described in Fig. 1. The quantitation of EGC was complicated by interfering peaks in some samples, and therefore, EGC was not included in the pharmacokinetic analysis. Data are the mean of eight subjects for GT and DGT and seven subjects for EGCG. For clarity of the figure, the SDs are not shown.

Table 1 Pharmacokinetic parameters for EGCG, EGC, and EC after a single oral administration of GT, DGT, or EGCG^a

Catechin and treatment	C_{max} ng/ml	T_{max} h	AUC ng · h/ml	$t_{1/2}$ h
EGCG				
GT1	91.69 ± 36.86	1.38 ± 0.37	587.9 ± 200.6	3.76 ± 2.22
GT2	89.80 ± 45.29	1.60 ± 1.05	564.8 ± 333.4	3.08 ± 1.45
GT3	52.30 ± 15.64	1.71 ± 0.93	372.8 ± 147.7	3.37 ± 0.71
DGT	24.37 ± 15.76	1.20 ± 0.67	90.5 ± 46.97	2.32 ± 2.55
EGCG	34.71 ± 22.87	1.61 ± 0.39	213.7 ± 86.4	3.70 ± 2.22
EGC				
GT1	208.1 ± 87.0	1.25 ± 0.61	797.1 ± 355.3	1.37 ± 0.54
GT2	263.6 ± 139.3	1.39 ± 0.39	1081.9 ± 456.9	1.54 ± 0.48
GT3	198.4 ± 72.2	1.34 ± 0.47	957.1 ± 503.1	2.15 ± 1.80
DGT	80.34 ± 22.26	1.09 ± 0.26	294.9 ± 92.38	1.45 ± 0.65
EC				
GT1	120.8 ± 67.6	1.26 ± 0.41	436.5 ± 157.5	1.53 ± 0.73
GT2	133.0 ± 75.8	1.35 ± 0.34	593.8 ± 286.9	2.06 ± 1.01
GT3	118.3 ± 73.7	1.18 ± 0.56	558.2 ± 288.8	2.52 ± 1.95
DGT	32.53 ± 12.5	1.02 ± 0.19	154.7 ± 72.3	2.51 ± 1.47

^a The subjects were given an oral dose of GT or DGT (20 mg/kg) or EGCG (2 mg/kg). Blood samples were collected and treated as described in "Materials and Methods." The data were analyzed, and the pharmacokinetic parameters were calculated using PCNONLIN. Data are the mean ± SD of eight subjects for GT and DGT and seven subjects for EGCG.

sample was mixed with 20 μ l of an ascorbate-EDTA solution (0.4 M NaH₂PO₄ buffer containing 20% ascorbic acid-0.1% EDTA, pH 3.6) as a preservative, and the mixture was stored at -80°C until analysis. Urine samples were collected before the dose and for the time periods of 0-3, 3-8, and 8-24 h after the dose. The total volume of each urine sample was recorded. Aliquots of 20 ml of each urine sample were transferred into plastic tubes that contained 20 mg of ascorbic acid and 0.5 mg of EDTA. The urine samples were stored at -80°C until analysis. The same experiment was repeated two more times on the same eight subjects. In the fourth experiment, the same subjects ingested a dose of pure EGCG (2 mg/kg body weight) dissolved in 200 ml of water. One subject was unable to complete the study, and the data presented are the mean of seven subjects for EGCG. In the fifth experiment, the eight subjects received a single oral dose of DGT (20 mg/kg body

weight) dissolved in 200 ml of water. Each succeeding experiment was conducted after at least a 1-week washout period. Aliquots of the GT, DGT, and EGCG solutions were stored in the presence of ascorbate-EDTA solution at -80°C for determination of the concentrations of the tea polyphenols.

Quantitation of Tea Polyphenols. The plasma and urinary levels of catechins and their metabolites were analyzed by HPLC with a Coulochem electrode array detector (ESA, Inc., Bedford, MA) as described previously (10) but with some modifications. For determination of the total amounts of EGCG, EGC, and EC, including free and conjugated forms as well as their metabolites, the urine and plasma samples were incubated with β -glucuronidase and sulfatase before extraction. Reference standards of M4 and M6 were isolated from human urine (14), and 4'-O-MeEGC was synthesized chemically (20).

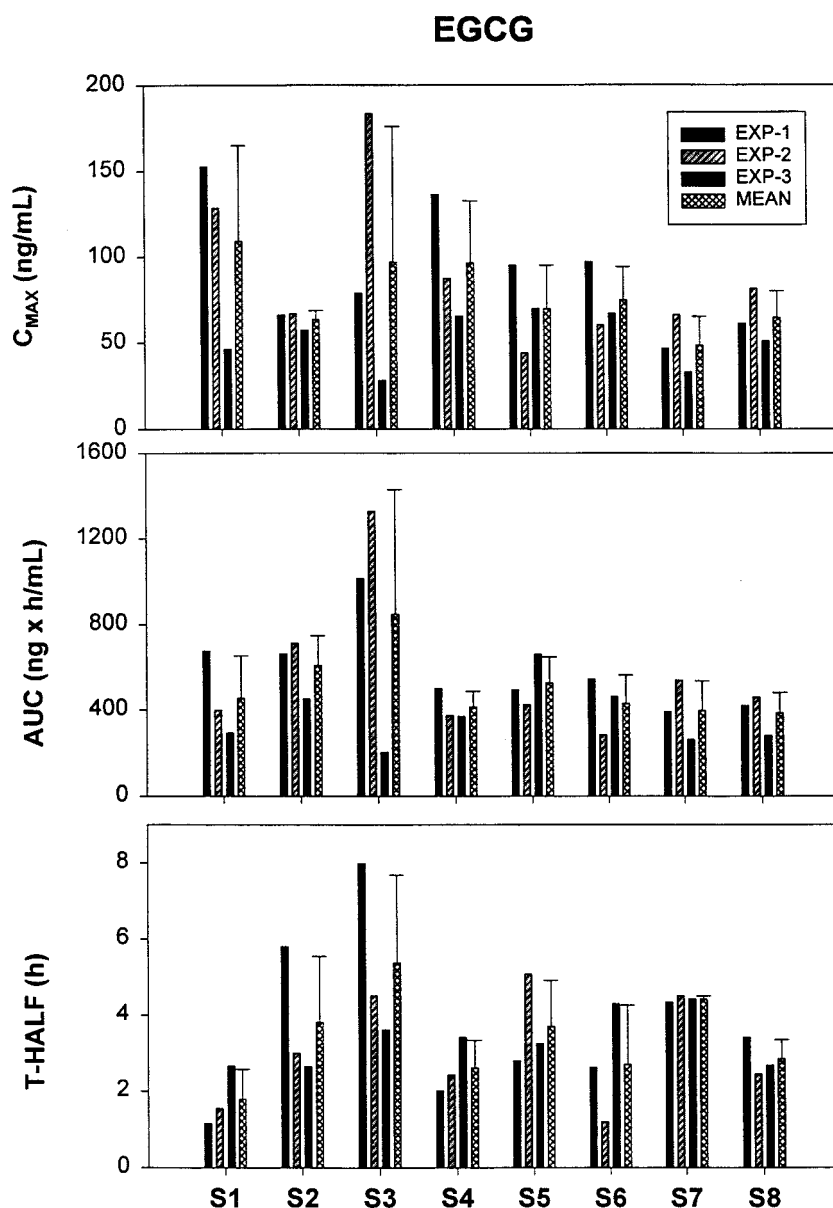


Fig. 3. Variability in pharmacokinetic parameters among subjects and repeated experiments. The data were from the same three experiments as described in Fig. 2 for the eight subjects (S1–S8) receiving GT. Bars, SD.

For determination of the free (nonconjugated) form of each catechin, the samples were analyzed without the enzymatic digestion. The potential settings of channels 1, 2, 3, and 4 were at -100 , 100 , 300 , and 500 mV. The peak height was used to calculate the plasma and urine concentrations of EGCG, EGC, and EC.

Pharmacokinetic Analysis. The plasma concentration time data for the tea polyphenols were analyzed by the PCNONLIN software package (version 4.2; Clin Trials, Lexington, KY) with a one-compartment model. Data for model-fitting were reweighted iteratively by modulating the reciprocal of the predicted concentrations. An appropriate fitting was assessed by correlation coefficient (r^2), the Akaike Information Criterion, and the Schwartz Criterion (21, 22). The AUCs for EGCG, EGC, and EC were determined trapezoidally and extrapolated to infinity by using the terminal elimination rate constant (k) for

each compound. The elimination half-life ($t_{1/2}$) was obtained from the ratio of $0.693/k$.

Results

Pharmacokinetic Profile of Tea Catechins. After ingesting GT, different tea catechins and their metabolites were detected and quantified by HPLC (Fig. 1). The major catechins detected in the plasma were EGC, EC, EGCG, and ECG as well as their metabolites 4'-*O*-MeEGC, $(-)$ -5-(3',4',5'-trihydroxyphenyl)- γ -valerolactone (M4) and $(-)$ -5-(3',4'-dihydroxyphenyl)- γ -valerolactone (M6). These compounds were also found at higher concentrations in the urine samples, except for EGCG and ECG, which were only present in trace amounts. In plasma and urine samples collected before tea administration, these compounds were not detected. The plasma levels of EGCG, EGC, and EC were used for pharmacokinetic analysis (Fig. 2).

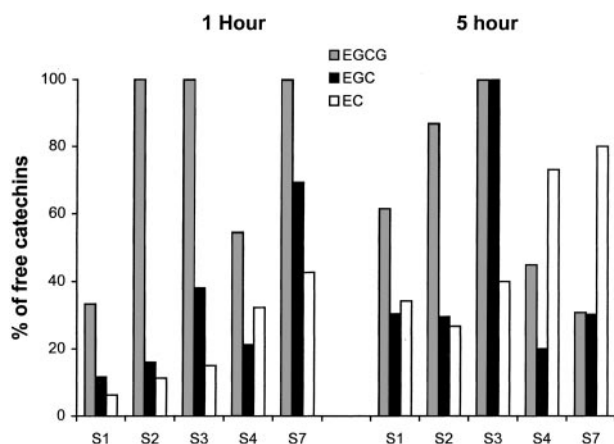


Fig. 4. Plasma levels of free EGCG, EGC, and EC as a percentage of the total amount (free + conjugated) of each catechin in human plasma after ingestion of a single dose of GT (20 mg/kg body weight). The samples were selected from those analyzed in Fig. 2 and analyzed before and after digestion with β -glucuronidase and sulfatase.

The peak plasma concentrations of these catechins were reached in 1–2 h in all of the subjects and gradually reduced to undetectable levels in 24 h. The pharmacokinetic data of catechins were analyzed using the PCNONLIN program. The best data fit was achieved with a one-compartment oral input model with probable lowest values of Akaike Information Criterion. This model is described by the equation:

$$C = F \times D / [V_d \times (K_a - K_{10})] \times (e^{-K_{10}t} \times e^{-K_a t})$$

where C is the concentration, F is the fraction absorbed, D is dose, V_d is the volume of distribution, K_a is the absorption rate constant, K_{10} is the elimination rate constant, and t is time.

The calculated pharmacokinetics parameters are summarized in Table 1. The analysis was performed with the total amount of each catechin, and these data represent a composite of pharmacokinetic parameters of free and conjugated catechins. In the three repeated experiments, the peak plasma concentration of EGCG was 77.9 ± 22.2 ng/ml. This value was lower than the values of EGC and EC, although the GT contained a higher level of EGCG than EGC and EC. However, the AUC values of EGCG were similar to those of EC, but they were still lower than those of EGC. The T_{max} values for EGCG, EGC, and EC were in the range of 1.3–1.6 h. The elimination half-life of EGCG at 3.4 ± 0.3 h appears to be higher than those of EGC and EC. When EGCG was given as a constituent of DGT or in pure form, the dose administered was lower than that given in GT, and the resulting C_{max} (maximum plasma concentration) and AUC were lower, but no significant difference was found in the $t_{1/2}$ values.

At the time point T_{max} (the time to reach C_{max}), 0.16, 0.58, and 1.1% of the ingested doses of EGCG, EGC, and EC from GT administration were present in the circulating plasma (calculated as a mean value and considered the average blood volume as 4 liters in a typical subject). In the case of DGT administration, the values in the blood were 0.13, 0.53, and 0.71%, respectively. When pure EGCG was administered, only 0.1% of the ingested dose appeared in the blood at T_{max} . The results indicate that a lower fraction of EGCG than EGC and EC appears in the blood, but the EGCG has a longer half-life.

Differences in Pharmacokinetic Parameters among Subjects and Repeated Experiments. Considerable differences in the pharmacokinetic parameters in the repeated experiments

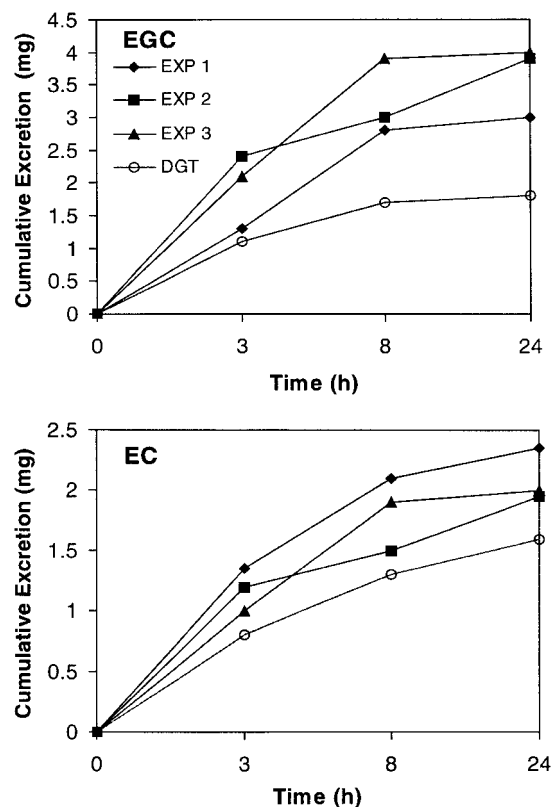


Fig. 5. Cumulative urinary excretion of tea catechins after ingestion of GT or DGT. The subjects were given a single dose of GT or DGT as described in Fig. 2. Urine samples were collected for the period of 0–3, 3–8, and 8–24 after tea consumption. The urine samples were digested with β -glucuronidase and sulfatase and analyzed as described in "Materials and Methods." The values are the means of eight subjects.

and among the individual subjects (S1–S8) were observed. In Fig. 3, only the data for EGCG are shown to illustrate this point. The variations among the repeated experiments were rather large in some subjects. For example, in S3 the coefficient of variation for the C_{max} and AUC values of EGCG in the repeated experiments were 81 and 68%, respectively. The EGC and EC levels appeared to be slightly less variable than EGCG among the three repeated experiments. Taking the EGCG, EGC, and EC levels as a whole, S1 and S4 appeared to have higher C_{max} values than other subjects, whereas S7 had the lowest. S7, however, together with S3, might have had higher $t_{1/2}$ values than others.

Free versus Conjugated Catechins in Plasma. A recent report by Chow *et al.* (15) indicated that after ingestion of EGCG and Polyphenon E (a tea polyphenol preparation) by human volunteers, plasma EGCG was mainly in the free (unconjugated) form, but EGC and EC were mainly present in the conjugated form. In the present study, the free *versus* total (free plus conjugated) levels of each catechin were determined at two time points (1 and 5 h) in five subjects (Fig. 4). Although large individual differences were observed, the results generally agree with the report by Chow *et al.* (15) that EGCG was mostly in the free form in the plasma, whereas EGC and EC were mostly in the conjugated form. In the samples collected at 1 h, in which the catechin contents were higher and the analysis tended to be more accurate, 77% of the EGCG was present in

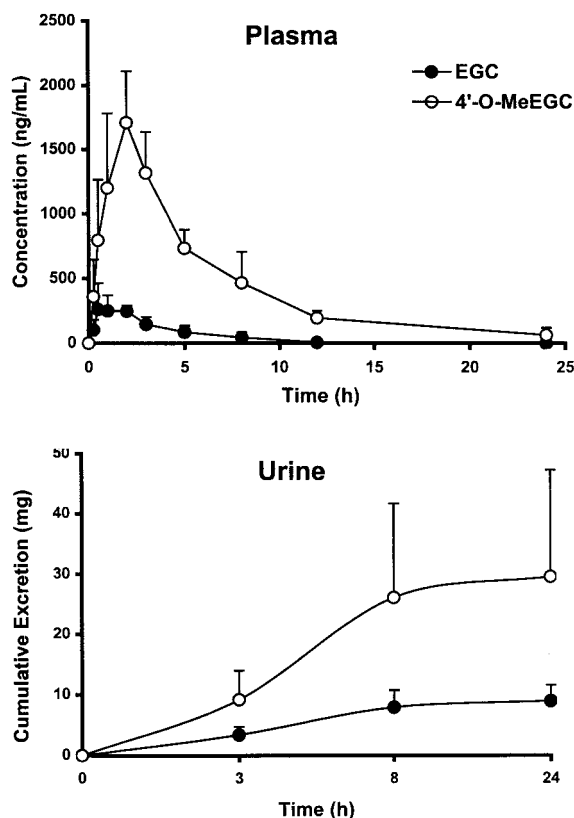


Fig. 6. The plasma concentration *versus* time curves and urinary excretion profiles of 4'-*O*-MeEGC after ingestion of a single dose of GT (20 mg/kg). The plasma and urine samples were digested with β -glucuronidase and sulfatase and analyzed as described in "Materials and Methods." The results are the means of samples from four subjects; bars, SD.

the free form whereas 31% of EGC and 21% of EC were in the free form. In the samples collected at 5 h, the content of free EGCG was 64%, and those of free EGC and EC were 40–50%. Most of the conjugated catechins were in the glucuronide form.

Urinary Excretion of Catechins. Urine samples were collected just before ingesting the tea or EGCG and for the time intervals of 0–3, 3–8, and 8–24 h after taking the tea or EGCG. Tea catechins were mostly (>95%) in the conjugated forms and were analyzed after hydrolysis with β -glucuronidase and sulfatase. The cumulative urinary excretion of EGC and EC are shown in Fig. 5. The amounts of excreted catechins were rather high in the first 3 to 6 hours, and >90% of the urinary catechins were excreted in the first 8 h. With DGT, lower amounts of catechins were ingested than with GT, and lower levels of catechins were excreted in the urine. However, a higher percentage of ingested EGC and EC appeared in the urine with DGT (3.3 and 8.9%, respectively, *versus* 2.3 and 4.6%, with GT). When pure EGCG was administered, urinary EGC or EC was not detected in a significant amount. In all these experiments, EGCG was present in trace or nondetectable amounts in the urine.

Formation and Pharmacokinetic Profile of Methylated and Ring-Fission Metabolites. Formation of the methylated and ring-fission metabolites was monitored and quantified in some of the plasma and urine samples. The plasma concentration *versus* time curves and urinary excretion profiles of 4'-*O*-

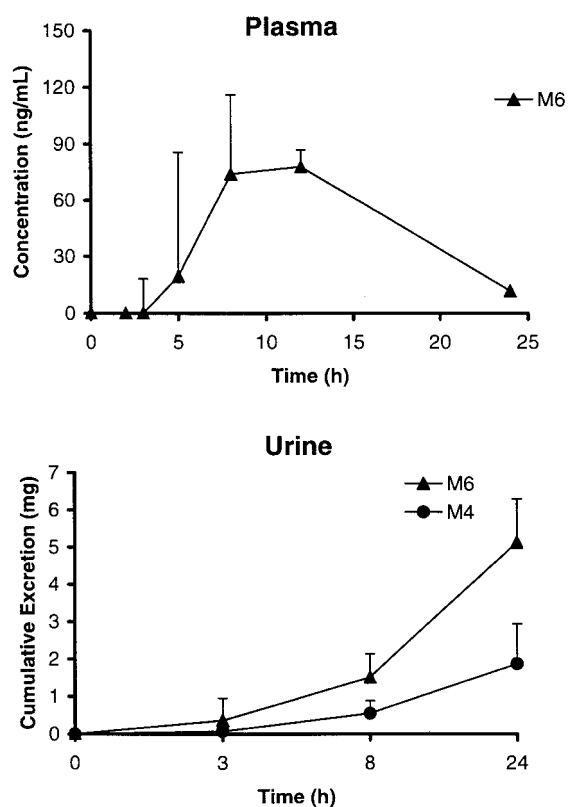


Fig. 7. The plasma concentration *versus* time and the urinary excretion profiles of M6 and the urinary excretion profile of M4 after ingestion of a single dose of GT (20 mg/kg). The plasma and urine samples were digested with β -glucuronidase and sulfatase, extracted, and injected onto the HPLC as described in "Materials and Methods." The results are the means of samples from four subjects; bars, SD.

MeEGC from GT are shown in Fig. 6. The plasma 4'-*O*-MeEGC reached peak levels at 1.7 ± 0.5 h and were eliminated with a half-life of 4.4 ± 1.1 h. The peak concentrations ranged from 1.2 to 2.2 μ g/ml (1.7 ± 0.4 μ g/ml), much higher than those for EGC. Most of the urinary 4'-*O*-MeEGC was excreted in the urine within 8 h after the ingestion of GT, and the amount of 4'-*O*-MeEGC recovered in the urine accounted for 8–31% (19 ± 10 mg) of the ingested EGC.

The two ring-fission metabolites, eluting at 10 and 20 min, observed in the urine have been identified as M4 and M6, respectively (14). M6 appeared in the plasma 3–5 h after the ingestion of GT and reached peak levels in 8–12 h (Fig. 7). The level of M6 varied considerably among the subjects, and in some subjects, it was much higher than that of EC, the direct precursor of M6. The majority of it was eliminated from the plasma in 24 h. The levels of urinary M6 (5.0 ± 2 mg) accounted for 11.2% of the ingested EC. M4 was present at lower levels in the plasma, and the levels were not accurately determined. M4 was determined in the urine, and high amounts were observed in the 8–24 h sample (Fig. 7). The amount of M4 excreted corresponded to 1.4% of the ingested EGC, the direct precursor of M4 (14).

Discussion

A comparison of the pharmacokinetic parameters of EGCG from the present and two previous studies are shown in Table

Table 2 Comparison of the EGCG pharmacokinetics of the present and previous studies

	Present study ^a	Yang <i>et al.</i> (11) 1998 ^b			Chow <i>et al.</i> (15) 2001 ^c		
Dose of EGCG (ng)	195	110	220	330	200	400	800
T_{\max} (h)	1.6	1.6	2.4	2.7	2.1	1.8	4.0
$t_{1/2}$ (h)	3.4	5.5	5.0	4.9	2.0	2.7	1.9
C_{\max} (ng/ml) (μM)	78	119	326	321	74	112	438
AUC (ng·h/ml)	508	897	2223	2462	367	623	2779

^a GT was used; the data are for EGCG and its conjugates (analyzed together).

^b DGT was used; the data are for EGCG and its conjugates (analyzed together).

^c EGCG tea was used; the data are for EGCG.

2. The dose of GT used in the present study (20 mg tea solids/kg) corresponds to 195 mg of EGCG, 154 mg of EGC, and 45 mg of EC for an individual with a body weight of 70 kg. This dose is within the range of our first study on the pharmacokinetics of tea polyphenols in humans in which DGT solids were given to volunteers at doses of 1.5, 3.0, and 4.5 g in 500 ml of water; 1 g of DGT solids contained 73 mg of EGCG, 68 mg of EGC, and 25 mg of EC (11). The presently used EGCG dose is close to the dose of 3.0 g of DGT (220 mg of EGCG) used previously. Our current EGCG dose is also close to the lowest dose used in a second study (15) in which 200, 400, 600, and 800 mg of EGCG were given to human subjects. Because more time points were used in the present study, we believe the presently reported pharmacokinetic data are more accurate than our previous results (11). The presently observed $t_{1/2}$ value of EGCG (3.4 ± 0.3 h) appears to be slightly lower than that observed in our first study (11) and slightly higher than the second study (15). The T_{\max} values of 1.6 ± 0.2 h are comparable with the first study and lower than the second study, in which the T_{\max} appeared to increase with the dose of EGCG administered (15). The C_{\max} and AUC levels of EGCG were lower than those observed with 219 mg of EGCG in the first study but are close to the values obtained with 200 mg of EGCG in the second study. These pharmacokinetic parameters are useful for selecting the dose and dose frequency for intervention studies with tea.

The present study, consistent with the two previous studies, demonstrated that only a small percentage of the p.o.-ingested catechins appears in the blood. In the present study, after drinking the equivalent of ~ 2 cups of tea, the mean peak plasma EGCG level was $0.17 \mu\text{M}$. These values could serve as a reference for designing *in vitro* experiments for elucidating the mechanisms of action of EGCG. Most of the published studies in cell culture systems used 10–100 μM of EGCG (5). The rather poor bioavailability of tea catechins needs to be considered when we extrapolate results obtained *in vitro* to situations *in vivo*. Most of the ingested EGCG apparently does not get into the blood, and the absorbed EGCG is preferentially excreted through the bile to the colon (23). EGC and EC appear to be more bioavailable, but the fractions of these compounds that appeared in the plasma are also low, and only 3.3 and 8.9% of the ingested EGC and EC were excreted in the urine. The presence of substantial amounts of 4'-O-MeEGC, M4, and M6 in the plasma and urine account for some of the ingested catechins. On the basis of the structures, these compounds could possess biological activities, and this topic needs to be investigated further.

The present results will help us develop biomarkers for tea consumption. Because of the rather high concentrations of 4'-O-MeEGC in plasma and urine, it may be used together with

tea catechins as biomarkers of tea consumption. It remains to be determined whether the level of 4'-O-MeEGC is influenced by the polymorphism of catechol-O-methyl transferase (24), an enzyme that catalyzes the methylation of EGC (25). In theory, M4 and M6 could also serve as useful markers of tea consumption and intestinal microflora metabolism of catechins. Because the appearance of M4 and M6 lags a 6 to 9 hours behind the catechins and they have long half-lives, they may be detectable in plasma and urine after most of the catechins have been eliminated. A possible complication is that with the same dose of tea, M4 and M6 levels in the plasma and urine varied among individuals (14), possibly because of the difference in intestinal microorganism population. More research is needed to improve the analytical method to measure accurately catechins and their metabolites with good reproducibility, to understand the factors which affect the bioavailability and biotransformation of catechins, and to understand the biological activities of these compounds.

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