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

Mao-Jung Lee, Pius Maliakal, Laishun Chen, Xiaofeng Meng, Florideliza Y. Bondoc, Saileta Prabhu, George Lambert, Sandra Mohr, and Chung S. Yang


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 EGC, 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 EGC, 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-valorolactone (M4) and (−)-5-(3′,4′-dihydroxyphenyl)-valorolactone (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 ecologic, 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).
The time-dependent blood levels of EGCG, 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 5 h and of EGC and EC were 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 dried 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 g/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...
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 Fig. 1. The quantitation 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.

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

<table>
<thead>
<tr>
<th>Catechin and treatment</th>
<th>$C_{max}$ (ng/ml)</th>
<th>$T_{max}$ (h)</th>
<th>AUC (ng · h/ml)</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT1</td>
<td>91.69 ± 36.86</td>
<td>1.38 ± 0.37</td>
<td>587.9 ± 200.6</td>
<td>3.76 ± 2.22</td>
</tr>
<tr>
<td>GT2</td>
<td>89.80 ± 45.29</td>
<td>1.60 ± 0.10</td>
<td>564.8 ± 333.4</td>
<td>3.08 ± 1.45</td>
</tr>
<tr>
<td>GT3</td>
<td>52.30 ± 15.64</td>
<td>1.71 ± 0.93</td>
<td>372.8 ± 147.7</td>
<td>3.37 ± 0.71</td>
</tr>
<tr>
<td>DGT</td>
<td>24.37 ± 15.76</td>
<td>1.20 ± 0.67</td>
<td>90.5 ± 46.97</td>
<td>2.32 ± 2.55</td>
</tr>
<tr>
<td>EGCG</td>
<td>34.71 ± 22.87</td>
<td>1.61 ± 0.39</td>
<td>213.7 ± 86.4</td>
<td>3.70 ± 2.22</td>
</tr>
<tr>
<td>EGC</td>
<td>208.1 ± 87.0</td>
<td>1.25 ± 0.61</td>
<td>797.1 ± 355.3</td>
<td>1.37 ± 0.54</td>
</tr>
<tr>
<td>GT2</td>
<td>263.6 ± 139.3</td>
<td>1.39 ± 0.39</td>
<td>1081.9 ± 456.9</td>
<td>1.54 ± 0.48</td>
</tr>
<tr>
<td>GT3</td>
<td>198.4 ± 72.2</td>
<td>1.34 ± 0.47</td>
<td>957.1 ± 503.1</td>
<td>2.15 ± 1.80</td>
</tr>
<tr>
<td>DGT</td>
<td>80.34 ± 22.26</td>
<td>1.09 ± 0.26</td>
<td>294.9 ± 92.38</td>
<td>1.45 ± 0.65</td>
</tr>
<tr>
<td>EC</td>
<td>120.8 ± 67.6</td>
<td>1.26 ± 0.41</td>
<td>436.5 ± 157.5</td>
<td>1.53 ± 0.73</td>
</tr>
<tr>
<td>GT2</td>
<td>133.0 ± 75.8</td>
<td>1.35 ± 0.34</td>
<td>593.8 ± 286.9</td>
<td>2.06 ± 1.01</td>
</tr>
<tr>
<td>GT3</td>
<td>118.3 ± 73.7</td>
<td>1.18 ± 0.56</td>
<td>558.2 ± 288.8</td>
<td>2.52 ± 1.95</td>
</tr>
<tr>
<td>DGT</td>
<td>32.53 ± 12.5</td>
<td>1.02 ± 0.19</td>
<td>154.7 ± 72.3</td>
<td>2.51 ± 1.47</td>
</tr>
</tbody>
</table>

$a$ 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.
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).
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:

\[ C = F \times D \times (V_d \times (K_e - K_{10})) \times (e^{-K_{10}t} \times e^{-K_{at}}) \]

where \( C \) is the concentration, \( F \) is the fraction absorbed, \( D \) is dose, \( V_d \) is the volume of distribution, \( K_e \) 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 \( 77.9 \pm 22.2 \) 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 the EGCG has a longer half-life. The variations among the repeated experiments were rather large in some subjects. For example, in S3 the coefficient of variation for the \( C_{\text{max}} \) 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 \( C_{\text{max}} \) 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 form) 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
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-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 µg/ml (1.7 ± 0.4 µg/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 M6 and M4, 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. M6 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
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 \( T_{\text{max}} \) values of 1.6 ± 0.2 h are comparable with the first study and lower than the second study, in which the \( T_{\text{max}} \) appeared to increase with the dose of EGCG administered (15). The \( C_{\text{max}} \) 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 \( \mu \)g/ml. 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 \( \mu \)g/ml 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.

Acknowledgments

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References


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

<table>
<thead>
<tr>
<th>Dose of EGCG (ng)</th>
<th>Present study (a)</th>
<th>Yang et al. (b) (1999)</th>
<th>Chow et al. (c) (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>195</td>
<td>110</td>
<td>220</td>
<td>200</td>
</tr>
<tr>
<td>110</td>
<td>220</td>
<td>330</td>
<td>400</td>
</tr>
<tr>
<td>200</td>
<td>400</td>
<td>600</td>
<td>800</td>
</tr>
</tbody>
</table>

\(a\) 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.


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