ABSTRACT:
Tea polyphenols—including (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC)—are believed to be responsible for the beneficial effects of tea. This study was conducted to investigate the absorption, distribution, and elimination of EGCG, EGC, and EC in rats after administration of decaffeinated green tea (DGT). For comparison, pure EGCG was also studied. The plasma and tissue levels of EGCG, EGC, and EC were quantified by HPLC, and the results were analyzed by the PCNONLIN program. Following intravenous injection of DGT (25 mg/kg), the plasma concentration-time curves of EGCG, EGC, and EC were fitted in a two-compartment model. The β-elimination half-lives (t1/2b) were 212, 45, and 41 min for EGCG, EGC, and EC, respectively; the clearances were 2.0, 7.0, and 13.9 ml·min/kg, and the apparent distribution volumes (Vd) were 1.5, 2.1, and 3.6 dl/kg. When pure EGCG (10 mg/kg) was given, however, a shorter t1/2b (135 min), a larger clearance (72.5 ml·min/kg), and a larger Vd (22.5 dl/kg) for EGCG were observed, suggesting that other components in DGT could affect the plasma concentration and elimination of EGCG. After intragastric administration of DGT (200 mg/kg), −13.7% of EGC and 31.2% of EC were shown in the plasma, but only 0.1% of EGCG was bioavailable as judged by the ratio of AUC0–t1/2/AUC0–t. After intravenous administration of DGT (25 mg/kg), the level of EGCG was found to be the highest in the intestine samples, and the intestinal EGCG level declined with a t1/2 of 173 min. The highest levels of EGC and EC were observed in the kidney, and the levels declined rapidly with a t1/2 of 29 and 28 min, respectively. The AUC of EGCG in the intestine was 4-fold higher than that in the kidney, but the AUCs of EGC and EC in the intestine were similar to those in the kidney. The liver and lung levels of EGC, EGC, and EC were generally lower than those in the intestine and the kidney. The distribution results suggest that EGCG is mainly excreted through bile, and that EGC and EC are excreted through both the bile and urine.

Tea is a widely consumed beverage in many countries. An estimated 2.5 million metric tons of dried teas are manufactured annually. Of that amount, ~20% is green tea, which is mainly consumed in China and Japan, and ~78% is black tea, which is consumed in many Western countries. Many laboratory studies have demonstrated the inhibition of tumorigenesis by tea and tea polyphenols in different animal models, including mouse skin, mouse lung, rat and mouse esophagi, mouse forestomach, mouse duodenum, rat small intestine, rat colon, and rat and mouse livers (1). In contrast to the consistently observed inhibition of tumorigenesis by tea in many animal models, studies concerning the effects of tea on the incidence of human cancers have been inconclusive. Some epidemiological studies on the effect of tea ingestion on cancer risk have suggested an inhibitory effect (2–5), others an enhancing effect, and still others a lack of an effect (6–8). However, none of these human studies are conclusive, and more epidemiological research is needed.

A major problem in investigating the association of tea consumption with cancer incidence is the lack of quantitative data. It is not known whether the inconclusive results in the human studies were due to a presumed lower amount of tea consumption by humans than by experimental animals, or were due to species differences in the bioavailability and actions of the active components involved. Even in studies with animals, mechanistic understanding of the inhibitory effect of tea on tumorigenesis is hampered by insufficient information regarding the absorption, distribution, metabolism, and elimination of the effective components of tea. It is believed that most of the cancer-inhibitory activity of tea is due to the tea polyphenols present in the tea. The major polyphenols in green tea, commonly known as tea catechins, are EGCG, EGC, ECG, and EC (1, 9, 10) (fig. 1). However, the pharmacokinetic properties of these tea polyphenols are largely unknown.

The objective of the present study is to gain an understanding about the pharmacokinetic properties and bioavailabilities of EGCG, EGC, and EC in rats. These tea polyphenols were given to rats either in the form of DGT or as pure EGCG. EGCG was selected as a prototype tea polyphenol for study because it is the most abundant in green tea with demonstrated biological activities, and it is available in high purity. Both administration routes (i.v. and i.g.) were used. The compounds were analyzed with an HPLC method newly developed in our laboratory (11).

Materials and Methods

Chemicals and Reagents. DGT and purified EGCG, EGC, and EC were obtained from Thomas J. Lipton, Inc. (Englewood Cliffs, NJ). The DGT solids were dehydrate preparations of water extracts of DGT leaves and contained 73, 68, and 27 mg/g of EGCG, EGC, and EC, respectively (11). All other chemicals and solvents were the highest grades of commercially available materials.

1 Abbreviations used are: EGCG, (−)-epigallocatechin-3-gallate; EGC, (−)-epigallocatechin; EC, (−)-epicatechin; DGT, decaffeinated green tea; i.v., intravenously; i.g., intragastrically; CL, clearance; t1/2, time to maximum concentration; Cmax, maximum concentration; AUC, area under the plasma concentration vs. time curve.
Treatment of Animals and Blood Sample Collection. Male Sprague-Dawley rats with body weights of $\sim$310 g were obtained from Taconic Farms (Germantown, NY) and maintained in air-conditioned quarters with 12-hr light/dark cycles. They were given a commercial rat chow (Ralston Purina Co., St. Louis, MO) and water ad libitum. The experiments started after acclimation for at least 1 week. EGCG and DGT solutions were made fresh in saline and were administered to rats i.v. (in $\sim$1 ml) and i.g. (in $\sim$2 ml). In the first experiment, EGCG was administered i.v. at a dose of 10 mg/kg. Blood samples were administered to rats i.v. (in $\sim$1 ml) and i.g. (in $\sim$2 ml). In the first experiment, EGCG was administered i.v. at a dose of 10 mg/kg. Blood samples were collected into heparinized tubes from the orbital sinus at 5, 10, 20, 40, 60, 90, 120, 180, and 300 min after the injection. The blood samples were centrifuged at 2,000 g for 10 min. The resultant plasma was mixed with one-tenth the volume of a preservative solution (20% ascorbic acid and 0.05% Na2EDTA dissolved in a 0.4 M sodium phosphate buffer, with a final pH of 6.5). After centrifugation at 16,000 rpm for 5 min, 200 µl of the liver, lung, and kidney were homogenized with 1 ml of 0.4 M sodium phosphate buffer containing 6 mg of ascorbic acid and 0.05% Na2EDTA (final pH of 7.2), and the mixture was stored at $\sim$80°C until use. In a second experiment, rats were given EGCG i.g. at a dose of 75 mg/kg. The blood samples were taken at 10, 20, 40, 60, 90, 120, 180, 240, and 360 min after the administration. Similar experiments were conducted using DGT at a dose of 25 mg/kg given i.v. and a dose of 200 mg/kg through i.g. administration.

Tissue Sample Collection. Rats were divided randomly into six groups (four rats per group), and given DGT i.v. at a dose of 25 mg/kg. The animals were killed at 5, 15, 30, 60, 120, and 240 min after the injection. The liver, kidneys, lungs, and intestine (30 cm from the stomach) were removed. About 0.5 g of the liver, lung, and kidney were homogenized with 1 ml of 0.4 M sodium phosphate buffer containing 6 mg of ascorbic acid and 0.05% Na2EDTA (final pH of 6.5). After centrifugation at 16,000 g for 5 min, 200 µl of the supernatant of tissue homogenates was used. The plasma sample was digested with $\beta$-glucuronidase and sulfatase, ex- extracted, and injected onto the HPLC as described in Materials and Methods. Numbers 1–4 denote different channels of a coulochem electrode array detector.

**Results**

Pharmacokinetics of EGCG after i.v. Administration. After giving EGCG i.v. to rats at a dose of 10 mg/kg, blood samples were collected at different time points. The plasma samples were analyzed by an HPLC method that can monitor EGCG, EGC, and EC simultaneously (fig 2). EGCG and EC were not observed in any of the plasma samples, suggesting that there was no conversion of EGCG to EGC or EC. The plasma EGCG concentration-time curve is shown in fig 3. The concentration-time data were analyzed by the PCNONLIN program. The best fit was achieved with a two-compartment i.v. input model, which was described by the mathematical equation of

$$C = D \cdot \left( K_{21} - \alpha \right) \cdot \left( V_d \cdot \left( \beta - \alpha \right) \right) \cdot e^{-\alpha t} + D \cdot \left( K_{21} - \beta \right) \cdot \left( V_d \cdot \left( \beta - \alpha \right) \right) \cdot e^{-\beta t},$$

where $\alpha + \beta = K_{12} + K_{21} + K_{10} \cdot \alpha \cdot \beta = K_{21} \cdot K_{10} \cdot C = \text{concentration}, D = \text{dose}, V_d = \text{apparent distribution volume}, K_{21} = \text{distribution rate constant from the peripheral compartment to the central compartment}, K_{12} = \text{distribution rate constant from the central compartment to the peripheral compartment}, K_{10} = \text{rate constant associated with the elimination from the central compartment}, \alpha =$...
rate constant associated with the distribution phase of the concentra-
tion–time curve, $\beta = \text{rate constant associated with the terminal phase}
$ of the concentration–time curve, and $t = \text{time (14). As shown in table 1,}$
EGCG had a distribution half-life ($t_{1/2,d}$) of 11.8 min, an elimination
half-life from the central compartment ($t_{1/2,K_{a}}$) of 21.3 min, and a
$\beta$-elimination half-life ($t_{1/2,b}$) of 135.1 min. The CL of EGCG was
72.5 ml × min/kg. The $V_d$ was 22.0 dl/kg.

**Absorption and Elimination of EGCG after i.g. Administration of
EGCG.** To study EGCG absorption properties and to determine
whether conversion of EGCG to EGC or EC occurs in the gastrodu-
odenal tract, EGCG was given to rats i.g. at a dose of 75 mg/kg. No
peak of EGC or EC was shown in the HPLC profiles, suggesting again
conversion from EGCG to EGC or EC. A concentration vs. time
curve is shown in fig. 3. The best fit of the data sets was achieved with
a one-compartment oral input model, which is described by the
mathematical equation of

$$C = F \cdot D \cdot V_d \cdot (K_{a} - K_{10}) \cdot (e^{-K_{a}t} - e^{-K_{10}t}),$$

(2)

where $C = \text{concentration, } F = \text{fraction of absorption, } D = \text{dose, } V_d = \text{volume of distribution, } K_a = \text{absorption rate constant, } K_{10} = \text{elimination rate constant, and } t = \text{time (14).}$ $t_{\text{max}}$ and $C_{\text{max}}$ were 85.5
min and 19.8 ng/ml, respectively. The absorption rate constant ($K_a$) was
$1.4 \times 10^{-3}$ min⁻¹. The fraction of absorption calculated by the
equation of $\text{AUC}_{i.g.} \cdot \text{Dose}_{i.g.} / \text{AUC}_{i.v.} \cdot \text{Dose}_{i.v.}$ was 1.6%. Other
EGCG pharmacokinetic parameters, such as $t_{1/2,K_{a}}, V_d,$ and CL, were
similar to the results obtained from i.v. administration of EGCG
(table 2).

**Pharmacokinetics of EGCG, EGC, and EC after i.v. Adminis-
tration of DGT.** The plasma concentrations of EGCG, EGC, and EC
for the rats treated with DGT (25 mg/kg i.v.) were plotted against time
(fig. 3). The plasma EGC and EC levels were declined to <1% in 300
min. However, the plasma EGCG level was decreased to 12% in the
same time period, suggesting a slower rate of elimination of EGCG
than that of EGC and EC. The pharmacokinetic parameters for EGCG,
EGC, and EC were analyzed by the PCNONLIN program based on
the assumptions: 1) EGCG, EGC, and EC are not interconvertible; and
2) the conjugation with glucurone or sulfate is not a rate-limiting
step. The best fit of the concentration–time data of the three tea
polyphenols was achieved with a two-compartment i.v. input model.
The pharmacokinetic parameters are shown in table 1. The parameters
related to elimination of EGC and EC were similar [$t_{1/2,K_{a}}, 20.2$ and
18.1 min; $t_{1/2,b}, 44.9$ and 41.2 min, respectively]. The CL of EGC was
~50% of that of EC, indicating that EC was more quickly eliminated
from the rats. As reflected by the $t_{1/2,K_{a}}$ of 51 min, $t_{1/2,b}$ of 212 min,
and CL of 2.0 ml · min/kg, the EGCG elimination was slower than
EGC and EC. The distribution half-lives ($t_{1/2,K_{a}}$) of the three tea
polyphenols, however, were not significantly different (~8–10 min). The
$V_d$ of EGCG, EGC, and EC were 1.5, 2.1, and 3.6 dl/kg,
respectively. Rats receiving EGCG in DGT displayed a 2.8-fold
higher plasma concentration at 5 min than those receiving pure
EGCG, even though the dose of EGCG in DGT was one-fifth the dose
of pure EGCG (1.8 vs. 10 mg/kg). A slower elimination was shown when
EGCG was given in DGT than given as the pure compound (fig.
4). The difference was also indicated by other pharmacokinetic
parameters; for example, EGCG given in DGT showed a smaller $V_d,$
slower $t_{1/2,b}$, and smaller CL than EGCG given in the pure
form (table 1).

**Absorption and Elimination of EGCG, EGC, and EC after i.g.
Administration of DGT.** After an i.g. dose of DGT (200 mg/kg),
the plasma concentration–time data of EGCG, EGC, and EC were fitted in
a one-compartment oral input model. The $t_{\text{max}}$’s and $C_{\text{max}}$’s were 74.4, 64.2, and
54.6 min, and the $C_{\text{max}}$’s were 16.3, 1,432.8, and 685.4 ng/ml
for EGCG, EGC, and EC, respectively. The fractions of absorption
for these three polyphenols were 0.1, 13.7, and 31.2%, respectively. The
absorption rate constants ($K_a$) of EGCG and EC were ~2.4-fold
higher than that of EGCG. The CL of the three polyphenols were similar to
the results obtained from i.v. injection.

**Tissue Distribution of Tea Polyphenols after i.v. Administration
of DGT.** Rats were given DGT i.v. at a dose of 25 mg/kg. The tissue
levels of tea polyphenols against time are shown in fig. 5. The highest
EGCG level was found in the intestine samples, and the level declined
slowly with an estimated $t_{1/2}$ of 173.3 min. The AUC of EGCG were
1.7, 0.3, and 0.4 mg · min/g, respectively, in the intestine, kidney, and
lung. The highest EGC and EC levels were observed in the kidney, but
the levels declined rapidly ($t_{1/2} = 28.9$ and 27.7 min, respectively). The
AUC of EGC were 1.4, 1.2, and 0.9 mg · min/g in the kidney,
lung, and intestine, respectively, and the AUC of EC was 0.3, 0.1, and
0.4 mg · min/g, respectively. Low levels of EGC, EC, and EC were
also detected in the liver. Consistent with the observations in the
plasma, the $t_{1/2}$ for EGCG was longest among the three tea poly-
phenols in the tissues examined (table 3).

**Discussion**

The possible cancer-preventive activity of tea is receiving a great
dead of attention. Information on the bioavailability and disposition of
tea polyphenols such as EGCG, EGC, and EC is important for
understanding the biological effects of tea. To our knowledge, this is
the first report on the absorption, distribution, and elimination of
EGCG, EGC, and EC in rodents that have been used extensively in the
studies of cancer chemoprevention. Although there are similarities in
their chemical structures (fig. 1), EGCG, EGC, and EC displayed
different pharmacokinetics. When DGT was used as the source of tea
polyphenols by i.v. injection, the $K_{12}$ and $K_{21}$ (the distribution rate
constants between the central compartment and the peripheral com-
partment) were similar for EGC and EC. But for EGCG, the $K_{12}$ was
3-fold larger than $K_{21},$ suggesting that EGCG tends to distribute into
the peripheral compartment (table 1). The longer $t_{1/2,b}$ and smaller CL
of EGCG also indicate that EGCG could stay in the body for a longer
period of time than EGC and EC (table 1).

EGC and EC seemed to be absorbed faster (larger $K_a$) than EGCG,
and EGCG had much lower bioavailability in terms of fraction of
absorption (table 2). The low bioavailability of EGCG was found
was much larger than our previously observed plasma levels of EGCG when given either in DGT or as pure EGCG. The difference in absorption was also indicated by the much higher $C_{\text{max}}$ values for EGC and EC than that for EGCG (table 2). This difference in the $C_{\text{max}}$ was much larger than our previously observed plasma levels of EGC and EC vs. that of EGCG in the rats receiving 0.9% DGT as the sole source of drinking fluid for 3 weeks (11). It seems that EGCG is better absorbed when given through drinking fluid than i.g. administration. In addition, the relatively higher AUC value of EGCG in the intestine samples after i.v. injection suggests that EGCG is excreted mainly through the bile. EGC and EC are likely excreted through both the urine and bile, because similar AUC values of EGC and EC were obtained in both the kidney and intestine. These results are in agreement with the previous observation that EGC and EC, but not EGCG, were recovered from human urine samples (11).

It is worth noting that EGCG displayed different pharmacokinetic behavior when EGCG was given to rats in the DGT, in comparison to when it was given as pure EGCG. When administered i.g., EGCG in DGT showed a 3.6-fold higher absorption rate constant ($K_{\alpha}$) than pure EGCG. Based on the AUC and $C_{\text{max}}$ produced by per unit of EGCG, DGT seems to deliver EGCG into the bloodstream more effectively than when EGCG is given as a pure compound (table 2). The molecular basis for this absorption difference is not known. It is possible that complex formation between EGCG and other components in the source of drinking fluid for 3 weeks (11). It seems that EGCG is better absorbed when given through drinking fluid than i.g. administration.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>EGCG</th>
<th>DGT</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_d$</td>
<td>dl/kg</td>
<td>22.0 ± 4.2</td>
<td>1.5 ± 0.3</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td>$K_{10}$</td>
<td>min$^{-1} \times 10^{-3}$</td>
<td>33.0 ± 4.7</td>
<td>14.1 ± 3.5</td>
<td>35.5 ± 7.0</td>
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<tr>
<td>$K_{12}$</td>
<td>min$^{-1} \times 10^{-3}$</td>
<td>22.5 ± 4.7</td>
<td>75.9 ± 55.2</td>
<td>24.0 ± 19.3</td>
</tr>
<tr>
<td>$K_{21}$</td>
<td>min$^{-1} \times 10^{-3}$</td>
<td>9.3 ± 1.2</td>
<td>26.0 ± 14.3</td>
<td>34.5 ± 16.0</td>
</tr>
<tr>
<td>AUC</td>
<td>µg × min/ml</td>
<td>143.2 ± 32.1</td>
<td>982.6 ± 328.5</td>
<td>252.4 ± 55.2</td>
</tr>
<tr>
<td>$t_{1/2A}$</td>
<td>min</td>
<td>21.3 ± 3.0</td>
<td>51.3 ± 11.2</td>
<td>20.2 ± 4.1</td>
</tr>
<tr>
<td>$t_{1/2K_{10}}$</td>
<td>min</td>
<td>59.7 ± 9.6</td>
<td>112.7 ± 72.3</td>
<td>78.4 ± 39.0</td>
</tr>
<tr>
<td>$t_{1/2K_{21}}$</td>
<td>min</td>
<td>5.2 ± 0.5</td>
<td>3.3 ± 0.5</td>
<td>15.7 ± 2.3</td>
</tr>
<tr>
<td>$V_d$</td>
<td>ml × min/kg</td>
<td>72.5 ± 15.6</td>
<td>2.0 ± 0.7</td>
<td>7.0 ± 1.5</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD of four rats. The equivalent dose of EGCG in DGT was 1.8 mg/kg.

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>EGCG</th>
<th>DGT</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{10}$</td>
<td>min$^{-1} \times 10^{-3}$</td>
<td>1.4 ± 0.6</td>
<td>5.0 ± 2.6</td>
<td>11.6 ± 3.7</td>
</tr>
<tr>
<td>$K_{12}$</td>
<td>min$^{-1} \times 10^{-3}$</td>
<td>57.5 ± 44.3</td>
<td>38.1 ± 24.1</td>
<td>21.8 ± 8.9</td>
</tr>
<tr>
<td>AUC</td>
<td>µg × min/ml</td>
<td>17.4 ± 7.0</td>
<td>5.3 ± 1.7</td>
<td>277.1 ± 60.1</td>
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<tr>
<td>$t_{1/2A}$</td>
<td>min</td>
<td>537.7 ± 189.4</td>
<td>165.2 ± 72.7</td>
<td>66.3 ± 27.7</td>
</tr>
<tr>
<td>$t_{1/2K_{10}}$</td>
<td>min</td>
<td>16.8 ± 9.3</td>
<td>32.1 ± 32.9</td>
<td>35.3 ± 11.7</td>
</tr>
<tr>
<td>$t_{1/2K_{21}}$</td>
<td>min</td>
<td>85.5 ± 42.0</td>
<td>74.4 ± 30.1</td>
<td>64.2 ± 4.0</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>ng/ml</td>
<td>19.8 ± 3.5</td>
<td>16.3 ± 6.5</td>
<td>1,432.8 ± 245.5</td>
</tr>
<tr>
<td>$F$</td>
<td>%</td>
<td>1.6 ± 0.6</td>
<td>0.1 ± 0.0</td>
<td>13.7 ± 3.0</td>
</tr>
<tr>
<td>$V_d$</td>
<td>dl/kg</td>
<td>16.9 ± 0.9</td>
<td>0.9 ± 0.9</td>
<td>3.4 ± 1.1</td>
</tr>
<tr>
<td>CL</td>
<td>ml × min/kg</td>
<td>69.8 ± 0.0</td>
<td>1.9 ± 0.0</td>
<td>6.8 ± 0.0</td>
</tr>
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</table>

Data are expressed as the mean ± SD of 3 or 4 rats. The equivalent dose of EGCG in DGT was 15 mg/kg.

![Fig. 4. Comparison of plasma concentration-time profile of EGCG between the rats given pure EGCG and the rats given DGT as the source of EGCG.](image-url)

The dose of pure EGCG was 10 mg/kg and the equivalent dose of EGCG in DGT was 1.8 mg/kg.

In addition to absorption, there were also differences in the distribution between these two dosage forms. The plasma level and AUC of EGCG due to i.v. administration of pure EGCG were lower and smaller than those due to i.v. administration of a lower dose of EGCG in DGT. Correspondingly, a larger $V_d$ was observed in the rats receiving pure EGCG i.v. (table 1). It is possible that upon i.v.
administration, EGCG is rapidly distributed in the body (before the first measurement at 5 min) or is rapidly converted to metabolites (which are not measured in our assays). Other components in DGT, such as EGC and EC, may hinder the processes by competing for the binding sites or competitively inhibiting the metabolic conversion and thus increase the initial concentration of EGCG (decreased $V_d$). This analysis is also applicable to experiments in which DGT and EGCG were administered i.g.

Pure EGCG also seems to be eliminated more readily from the body when compared with EGCG from DGT (table 1, fig. 4). Such differences in these parameters of EGCG were also shown when pure EGCG or DGT was administered through intragastrical intubation (table 2). Because catechins are known to bind with proteins tightly (15), it is possible that other tea components in DGT compete with EGCG for binding to plasma and tissue proteins, thus changing the EGCG pharmacokinetic behavior. This concept is similar to the previous results with warfarin, a drug with high levels of plasma and tissue protein binding (16). When warfarin was used together with other protein binding displacers, its $V_d$ was decreased and clearance delayed (16). Another possibility is that, because the glucuronidation and sulfation of tea polyphenols are the major elimination pathways (11), the competition among tea polyphenols for glucuronosyltransferase and sulfotransferase may also result in inhibition of EGCG elimination.

The present results on pharmacokinetic properties and tissue distribution of EGCG, EGC, and EC provide a base for understanding the biological effects of tea in rats. To understand the cancer prevention and other health effects of tea in humans, we are studying the pharmacokinetics of tea polyphenols in human volunteers.

References


