Plasma and Tissue Levels of Tea Catechins in Rats and Mice During Chronic Consumption of Green Tea Polyphenols

Sungbin Kim, Mao-Jung Lee, Jungil Hong, Chuan Li, Theresa J. Smith, Guang-Yu Yang, Darren N. Seril, and Chung S. Yang

Abstract: To understand the relationship between tea consumption and its biological effects, plasma and tissue levels of (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC) were measured after rats and mice were given a 0.6% green tea polyphenol preparation as the drinking fluid for different periods of time. EGC and EC levels in rat plasma increased over time and reached peak values (3 times the Day 1 values) on Day 14. Then the plasma levels of tea catechins decreased, to Day 1 values on Day 28. The plasma concentrations of EGCG were much lower than those of EGC or EC. High levels of EGC and EC were found in urine, whereas high levels of EGCG were found in feces. The changes in the urinary and fecal excretions of tea catechins could not account for the above-described changes in the plasma levels. The amounts of catechins in different tissues reflected the ingestion, absorption, and excretion pattern. When the green tea polyphenol preparation was given to mice, the “increase-and-then-decrease” pattern of catechin levels was also observed in the plasma, lung, and liver; the EGCG levels were much higher than in the rats. The results suggest that consumption of tea by rodents could induce adaptive responses affecting blood and tissue levels of tea catechins with time and that investigation of a similar phenomenon in humans is warranted.

Introduction

Tea, made from the leaves of the plant *Camellia sinensis*, is a popular beverage worldwide. Commercial green tea contains polyphenols, which include flavanols, flavandiols, flavonols, and phenolic acids; these compounds account for up to 30% of the dry weight. The majority of polyphenols are catechins (Figure 1), characterized by di- or trihydroxyl group substitution of the B ring and meta-5,7-dihydroxy substitution of the A ring; such structures contribute to the bitterness and astringency of green tea (1). (−)-Epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC), (+)-gallocatechin, and (+)-catechin are the major catechins in tea (2,3).

In recent years, many studies have demonstrated that green tea and green tea polyphenol (GTPP) preparations possess anticarcinogenic effects in different animal tumor bioassay systems, and tea catechins are believed to be key active constituents. For example, feeding of GTPP in drinking water resulted in significant protection against chemically induced colon cancers in rats (4,5) as well as lung and forestomach tumors in mice (6). Topical application of GTPP to mouse skin protected against skin carcinogenesis induced by various agents (7,8). Significant protection against lung tumor formation occurred when decaffeinated green or black tea or 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) was given to mice during or after NNK treatment (9,10). EGCG, the major epicatechin in green tea, was also demonstrated to inhibit NNK-induced lung tumorigenicity in *A/J* mice (11). Collectively, these data suggest that GTPP, mainly catechins, possess significant anticarcinogenic effects. Antioxidant activities, inhibition of carcinogen activation, moderate enhancement of detoxifying enzymes, suppression of activator protein-1 activity and cell proliferation, and induction of apoptosis have been proposed as the mechanisms for the inhibitory action of tea against tumorigenesis (12–16).

Despite clear demonstration of anticarcinogenic activity of tea in animals, the effects of tea consumption on cancer incidence in humans are inconclusive (12,17–19). It is possible that tea consumption by humans indeed has protective effects against carcinogenesis, but the effect is masked by confounding factors. Another possible explanation is that the amounts of tea consumed by humans are much lower than the amounts given to animals in the anticarcinogenesis experiments and, thus, may not produce a significant effect. Therefore, it is important to know the blood and tissue levels of catechins in animals in which an anticarcinogenic activity can be demonstrated and to compare this information with blood levels of these compounds found in humans after tea consumption.
consumption. Previously, we studied the blood, saliva, tissue, and urine levels as well as the pharmacokinetic parameters of the catechins in rats and humans after the administration of a single dose of tea or purified catechins (19–23). The tissue distribution of EGCG in rats after an oral dose of EGCG was also studied by Nakagawa and Miyazawa (24). Suganuma and co-workers (25) followed the tissue distribution of radioactivity after a single dose of [3H]EGCG. Judging from the fact that the observed blood radioactivity at 24 hours was >10 times the level at 1 hour (25) and the previously observed half-lives of EGCG in rats (165 min) and humans (5 h) (21,23), it is likely that most of the radioactivity measured was not due to EGCG or its conjugates. The blood and tissue levels of catechins after repeated tea consumption are not known. In this study, we determined the plasma and tissue levels of tea catechins in rats and mice after they had consumed GTTP through drinking fluid for different time periods and investigated different factors that might affect these levels. To achieve rather high blood and tissue levels of catechins, a 0.6% GTTP solution (comparable to 2% of brewed green tea) was used for most of the experiments. Rats and mice were used to study possible species differences.

**Materials and Methods**

**Chemicals and Reagents**

GTTP was a generous gift from Thomas J. Lipton (Englewood Cliffs, NJ). It contained 590, 76, and 86 mg of EGCG, EGC, and EC per gram of solid, respectively. β-Glucuronidase (G-7896) and sulfatase (S-9754) were purchased from Sigma Chemical (St. Louis, MO). High-performance liquid chromatography (HPLC)-grade solvents were obtained from EM Sciences (Gibbstown, NJ). Methoxyflurane (Metofane) was purchased from Mallinckrodt Veterinary (Mundelein, IL). All other chemicals and solvents were the highest grades of commercially available materials.

**Treatment of Animals**

Male Sprague-Dawley rats with average weights of about 300 g (10 wk old) were purchased from Taconic Farm (Germantown, NY). Female A/J mice with average body weights of about 18 g (6 wk old) were obtained from Jackson Laboratory (Bar Harbor, ME). Animals were given an AIN-76A diet (Research Diet, New Brunswick, NJ) and water ad libitum during acclimation. The experiments started after acclimation for at least one week. Rats were housed one per cage or metabolism cage, and mice were housed five per cage. GTTP solutions (0.1% or 0.6%, wt/vol) were made fresh with Millipore-purified water every other day and were given to rats ad libitum as the sole source of drinking fluid. During this two-day period, tea catechin concentrations in the drinking fluid, at room temperature, did not show any significant change. Fluid consumption was measured at the time of fluid change.

**Blood Sample Collection**

Rats were anesthetized by inhalation of methoxyflurane, and then blood was withdrawn from the retroorbital plexus in the eye with use of heparinized capillary tubes. Blood was collected into tubes containing heparin. Blood samples were collected from mice into heparinized tubes on sacrifice by decapitation, and the samples were mixed thoroughly to prevent blood clotting. The blood samples were centrifuged at 2,000 g for 10 minutes. The resultant plasma was mixed with one-tenth the volume of an ascorbate-EDTA preservation solution (20% ascorbic acid and 0.01% Na2-EDTA dissolved in a 0.4 M sodium phosphate buffer, final pH 3.6), and the mixture was stored at −80°C until use.

**Tissue Sample Collection**

The tissues were removed from animals on sacrifice, washed twice in ice-cold saline, and blot dried with tissue paper. For bladder and heart, tissues were cut to open the cavity and washed thoroughly. For large intestine and esophagus, the luminal sides were flushed by injection of ice-cold saline. Then they were cut longitudinally and washed extensively. The tissues were weighed and immediately snap frozen in liquid nitrogen. Tissues were stored at −80°C until use. For the analysis of the samples, tissues were thawed and homogenized with a Polytron with two volumes (wt/vol) of the ascorbate-EDTA preservation solution. In Protocol I, which was employed with most of the tissue sample preparations, the homogenates were centrifuged at 16,000 g for five minutes, and the supernatant was used for the analysis. In Protocol II, which was used only for the determination of tissue catechin levels in heart and thyroid, the homogenates were used directly for HPLC analysis. The recoveries of the spiked catechins were 60% and 75% for Protocols I and II, respectively. The catechins in the homog-

![Figure 1. Structures of major tea catechins in green tea: (−)-epigallocatechin (EGC), (−)-epicatechin (EC), and (−)-epigallocatechin-3-gallate (EGCG).](image-url)
enated or supernatant were not stable on long-term storage. All homogenization and sample preparations were carried out on the same day, and the HPLC analysis was carried out on the same day or the following day. The levels of EGCG, EGC, and EC in the tissue were expressed as nanograms per gram of wet weight.

Urine and Fecal Sample Collection

Rats were placed in metabolism cages, and urine was collected daily into the reservoir containing 200 µl of the preservation solution. Once urine volume was measured, more preservation solution was added to make up to one-tenth of total urine volume. The urine samples were then stored at −80°C until use. Fecal samples were collected at the time of blood collection. During anesthesia, rats usually push out two to three pellets of fresh feces. The feces were weighed, snap frozen, and stored at −80°C until use. For analysis, six volumes (wt/vol) of preservation solutions were added to the feces, and the samples were homogenized extensively with a Polytron. The homogenates were centrifuged, and the supernatants were diluted 100-fold with the preservation solution before injection onto the HPLC. The recovery of spiked catechins in the analysis was 95% and 90% for urine and fecal samples, respectively.

Quantitation of Tea Polyphenols

The levels of EGCG, EGC, and EC in rat or mouse samples were determined by HPLC with a Coulochem electrode array detector, as previously described (20) with slight modifications. ECG had a retention time of >32 minutes in this HPLC system and was not analyzed. For the determination of total amount, including free and conjugated forms, of each of EGCG, EGC, and EC, the sample (200 µl of plasma and 50 µl of urine) was incubated with a mixture of β-glucuronidase (250 U) and sulfatase (10 U) at 37°C for 45 minutes. The reaction was stopped by addition of methylene chloride. For the analysis of free catechins, the mixture of β-glucuronidase and sulfatase was omitted from the incubation. The reaction mixture was extracted twice with ethyl acetate. The combined ethyl acetate solutions were added with 10 µl of a 0.2% ascorbate-0.0005% EDTA solution and then evaporated to dryness in a vacuum centrifuge concentrator. The residues were redisolved in 100 µl of a 10% acetonitrile aqueous solution, the resultant solution was centrifuged at 16,000 g for 5 minutes, and 50 µl of the sample were injected onto the HPLC. A reference plasma containing EGC, EC, and EGCG (441.4, 212.8, and 112.8 ng/ml, respectively) was also subjected to the same extraction and incubation steps. The catechins were separated by an NBS C18 column (5 µm, 150 × 4.6 mm; ESA, Bedford, MA), and the eluant was monitored by a Coulochem electrode array system (ESA) with potential settings at −90, −10, 70, and 150 mV. The peak height was used to calculate the concentrations of EGCG, EGC, and EC by comparison with the standard plasma. The level of ECG was not analyzed because of interfering peaks in some samples. The standard plasma samples were stored at −80°C, and freshly thawed aliquots were used. Under these conditions, the samples were stable for at least six months, comparable to that of pure catechin standards.

Results

Sampling Time and Plasma Levels of Tea Catechins in Rats

Five rats were given 0.6% GTPP as the sole source of drinking fluid for eight days, and then blood samples were drawn from the orbital sinus at 6 AM, 9 AM, 12 noon, and 6 PM. GTPP solution was given to the rats ad libitum during the sampling period. The result showed substantial differences in the tea catechin levels from the plasma samples collected at the selected time points in one day (Table 1). The highest levels of all three tea catechins were observed at 6 AM; the lowest concentrations were found at noon, possibly because the rats drank very little fluid during this time period. Over a six-hour period, the plasma concentrations for EGC and EC decreased by factors of 5 and 7, respectively. The EGCG levels were lower than the other two catechins, and the decrease was not pronounced. The plasma levels were much higher at 6 PM than at noon. Apparently, the rats started to drink GTPP solutions again in the afternoon.

Time-Dependent Changes in Plasma Levels of Tea Catechins in Rats

The HPLC analysis of blood samples from the control group (drinking water) showed that tea catechins were not present in the plasma of these animals (limit of detection 5 ng/ml). Substantial amounts, ranging from 300 to 1,000 ng/ml of EGC and EC, were detected in the 9 AM blood from rats after they were fed 0.6% GTPP for different time periods (Figure 2). Interestingly, during Days 1–4, there was a gradual increase in plasma concentrations of EGC, EC, and EGCG. The EGC and EC levels on Day 14 were about three times higher than those on Day 1. After Day 14, however, the tea catechin levels decreased, and by Day 28 the EGC and EC concentrations returned to levels comparable to those of Day 1. The plasma levels of EGCG were significantly lower (50–200 ng/ml), and the changes were not as

Table 1. Plasma Levels of Tea Catechins in Samples Taken at Different Times of the Day in Ratsa,b

<table>
<thead>
<tr>
<th>Time</th>
<th>Plasma Tea Catechins, ng/ml</th>
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<tbody>
<tr>
<td></td>
<td>EGC</td>
</tr>
<tr>
<td>6 AM</td>
<td>983.9 ± 114.2</td>
</tr>
<tr>
<td>9 AM</td>
<td>372.89 ± 56.7</td>
</tr>
<tr>
<td>Noon</td>
<td>186.89 ± 34.5</td>
</tr>
<tr>
<td>6 PM</td>
<td>548.1 ± 221.6</td>
</tr>
</tbody>
</table>

a: Values are means ± SE of 5 Sprague-Dawley male rats.
b: Rats were given 0.6% green tea polyphenol preparation for 8 days, and blood samples were collected at 9 AM. Total (free + conjugated form) amount of each catechin was determined. EGC, (−)epigallocatechin; EC, (−)epicatechin; EGCG, (−)epigallocatechin-3-gallate.
apparent as with EGC or EC. After this 28-day cycle, water was given to animals as the drinking fluid in a 10-day wash-out period. Then 0.6% GTPP solution was reintroduced; the plasma levels of EGC and EC fluctuated for the next 28 days around values one-half of the maximal levels observed in the first cycle (Figure 2).

The plasma samples taken on Days 4, 14, and 28 in the first cycle and Day 4 in the second cycle were analyzed for unconjugated (free) and total amount of each catechin. These time points were selected because the specific samples collected at each time point represented the most significant changes in plasma levels of total tea catechins over time. The results showed that most of the EC and EGC were in the conjugated form in the plasma, whereas a much larger portion of EGCG was unconjugated (Figure 3). Interestingly, the concentrations of free catechins in the plasma were not changed significantly over time, whereas those of conjugated catechins underwent the time-dependent changes.

**Time-Dependent Changes in the Excretion of Tea Catechins in Urine and Feces in Rats**

The daily fluid consumption was monitored during the entire experimental period (Figure 4). The intake of the GTPP solution was low on Day 1, increased sharply on Day 4, and increased only slightly thereafter. The daily average intake of the 0.6% GTPP was significantly lower than the water consumption of the control rats. Urine volumes of rats in the GTPP group were lower than those of the controls (Figure 4), reflecting the lower fluid consumption in the tea group. EGC was the major catechin that was excreted through urine, and EC was present at much lower levels (Figure 5, top). EGCG was undetectable or present in very low concentrations in the urine. The urinary excretion of EGC also changed significantly over time. The urinary concentrations of EGC on Days 1 and 4 were the same (275 ng/ml) and increased on Days 8 and 14 (approx 500 ng/ml). On Day 21, however, the urinary EGC level was lowered to 100 ng/ml, one-fifth of the peak value. The changes in EC were not as apparent. It was noteworthy that urinary concentrations of catechins were much lower on Day 21 than at other time points. Total daily excretion of EGC peaked on Day 14 and was the lowest on Day 21 (Figure 5, bottom), and this pattern resembled the increase-and-then-decrease trend in the plasma EGC levels.

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**Figure 2.** Time-dependent changes in plasma levels of catechins in rats given green tea polyphenol (GTPP) solution as drinking fluid. Animals were given 0.6% GTPP as sole source of drinking fluid for 2 consecutive cycles of 28 days with 10 days of washout between cycles. Each data point represents total amount of each catechin and is mean ± SEM of 5 rats.

**Figure 3.** Plasma levels of free and total tea catechins in rats given GTPP solution. Plasma samples were selected from Days 4, 14, and 28 of 1st cycle and Day 4 of 2nd cycle of experiment described in Figure 2. Samples were incubated with or without β-glucuronidase and sulfatase before analysis by high-performance liquid chromatography to obtain amount of “total” or “free” catechin, respectively. Values are means ± SEM of 5 samples (from 5 rats). Number above bar is level of “free” catechin expressed as a percentage of “total” catechin.
Fecal excretion of tea catechins was also measured in the study (Figure 6). Free EGCG (unconjugated) was the major tea catechin excreted in feces; on Day 8, it was about 15 times higher than EGC. The highest concentration of fecal EGCG was observed on Days 4 and 8, and then the levels decreased. By Day 28, fecal EGCG decreased to a level comparable to that of Day 1. EGC and EC were also increased during Day 1 to Day 8, but the levels of these catechins did not significantly decrease thereafter.

**Tissue Distribution of Tea Catechins in Rats**

Five rats were given 0.6% GTPP for eight days, the rats were sacrificed at 9 AM the next day, and catechin concentrations in different tissues were measured (Table 2). The highest concentration of EGC was found in the bladder, whereas the highest concentration of EGCG was found in the large intestine. In the kidney, prostate, and lung, substantial concentrations of EGC and EGCG were found; the concentration of EGCG was much lower than that of EGC, similar to the situation in plasma. In the liver, spleen, heart, and thyroid, tea catechins were present in low levels. Large intestine and esophagus are the tissues in direct contact with ingested tea solutions. In these tissues, all three catechins were present in rather high concentrations.

**Time-Dependent Changes in Plasma and Tissue Concentrations of Tea Catechins in Mice**

On the basis of the results from rats, the changes in plasma and tissue concentrations over time were investigated using female A/J mice. Female A/J mice were given 0.1% and 0.6% GTPP for 1, 4, 9, and 14 days. In contrast to the results with rats, the level of EGCG was much higher than levels of EGC and EC, suggesting a high bioavailability.

**Figure 4.** Daily fluid consumption and urine volume of rats given 0.6% GTPP solution. Animals were the same as described for Figure 2. Values are means ± SEM of 5 rats.

**Figure 5.** Urinary concentrations and total daily excretion of tea catechins by rats. Animals were the same as described for Figure 2. Urine was collected for 24 h at selected time points in metabolism cages. Values are means ± SEM of 5 rats; error bar is not shown when SEM is smaller than symbol.

**Figure 6.** Fecal levels of catechins during chronic consumption of GTPP. Animals were the same as described for Figure 2, and samples were analyzed without digestion with β-glucuronidase and sulfatase. Values are means ± SEM of 5 rats. Inset: levels of EGC and EC in an expanded scale.
of EGCG in mice. With 0.1% GTPP, the plasma catechin concentrations were highest on Day 1, and the levels remained steady (approx 80 ng/ml) between Days 4 and 14 (Figure 7). With 0.6% GTPP, the plasma concentrations of EGCG and EGC peaked at Day 4 and decreased thereafter. The concentration of EC was the highest on Day 1 and then decreased. The time-dependent changes in lung and liver

<table>
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<tr>
<th>Type of Tissue</th>
<th>Protocol I</th>
<th>Protocol II</th>
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<tbody>
<tr>
<td>Large intestine</td>
<td>303.2 ± 7.0</td>
<td>30.1 ± 6.6</td>
</tr>
<tr>
<td>Esophagus</td>
<td>185.9 ± 8.1</td>
<td>36.9 ± 8.9</td>
</tr>
<tr>
<td>Prostate</td>
<td>250.6 ± 66.1</td>
<td>20.7 ± 5.9</td>
</tr>
<tr>
<td>Spleen</td>
<td>93.0 ± 22.5</td>
<td>36.9 ± 8.9</td>
</tr>
<tr>
<td>Bladder</td>
<td>810.4 ± 299.4</td>
<td>20.7 ± 5.9</td>
</tr>
<tr>
<td>Lung</td>
<td>187.3 ± 66.9</td>
<td>20.7 ± 5.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>508.3 ± 70.8</td>
<td>20.7 ± 5.9</td>
</tr>
<tr>
<td>Liver</td>
<td>50.2 ± 17.4</td>
<td>20.7 ± 5.9</td>
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</tbody>
</table>

Table 2. Tissue Distribution of Tea Catechins in Rats

<table>
<thead>
<tr>
<th>Type of Tissue</th>
<th>Tea Catechins, ng/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large intestine</td>
<td>EGC 303.2 ± 7.0, EC 250.6 ± 66.1, EGCG 93.0 ± 22.5</td>
</tr>
<tr>
<td>Esophagus</td>
<td>EGC 185.9 ± 8.1, EC 93.0 ± 22.5, EGCG 810.4 ± 299.4</td>
</tr>
<tr>
<td>Prostate</td>
<td>EGC 250.6 ± 66.1, EC 93.0 ± 22.5, EGCG 810.4 ± 299.4</td>
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<td>Liver</td>
<td>EGC 50.2 ± 17.4, EC 93.0 ± 22.5, EGCG 810.4 ± 299.4</td>
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</table>

Figure 7. Time-dependent changes of plasma levels of tea catechins in mice given GTPP solution. Female A/J mice were given 0.1% or 0.6% GTPP solutions. Plasma samples were obtained from 10 mice at each data point. Values (means ± SEM of 10 mice) are total amounts of each catechin.

Figure 8. Time-dependent changes in tissue levels of tea catechins in mice. Female A/J mice were given 0.6% GTPP solution. Lung and liver tissues from 3 mice (same as those in Figure 7) were isolated and pooled for high-performance liquid chromatography analysis of catechins at different time points. Values (means ± SEM of 3 pooled samples) are total amounts of different catechins.

Discussion

In recent years, there have been extensive studies concerning the effects of tea on carcinogenesis. Yet quantitative data on the blood and tissue levels of tea catechins are scarce. This hinders our understanding of the biological activities of these compounds. Previously, we determined the plasma and urinary levels of tea catechins and their pharmacokinetic properties in humans and rats after administration of a dose of tea or purified catechins (20–23). These results helped us design the present studies to obtain information on the effects of multiple doses of tea on plasma, urine, and tissue levels of tea catechins. Because rats eat and drink mostly at night, as expected, the plasma levels of EGC and EC were highest at 6 AM and were lowest at noon (Table 1). EGCG levels, however, did not change significantly over the same period of time, consistent with our previous result that
EGCG has a longer β-elimination half-life (212 min) than EGC and EC (45 and 41 min, respectively) (21). These results demonstrated that, in studying the plasma and tissue levels of catechins, the sampling time is of great importance. In our subsequent studies, we chose 9 AM as the sampling time for all experiments.

The most interesting result of the present study is that in rats given 0.6% GTPP as the drinking fluid, the plasma concentrations of tea catechins increased in the first two weeks and then decreased. The increased fluid (GTPP solution) consumption during the first four days (Figure 4) and the accumulation of catechins may contribute to the increased plasma levels of catechins observed. The reason for the decline of plasma catechin level after Day 14 is not known. Chronic oral administration of green tea has been shown to induce the activity of UDP-glucuronosyltransferase in the liver (26). Initially, it was suspected that this was due to the increase in the elimination of tea catechins. However, urinary and fecal concentrations did not increase, and furthermore they were lowered as plasma concentrations decreased. The decrease in plasma and tissue catechins may be due to the conversion of tea catechins to unidentified metabolites. Recently, EC was reported to be present mainly as 3′-O-methyl-(-)-epicatechin conjugates in the urine (27). Methylated catechins could be present in the sample from Days 21 to 28 in high concentrations and were not detected in our assay system. Microbial breakdown products of catechins have been observed previously (28,29) and could significantly reduce the urinary and fecal levels of catechins.

Substantial levels of catechins were observed in different tissues of rats. The tissues that have direct contact or are involved in the excretion of tea catechins, such as the esophagus, large intestine, bladder, and kidney, appeared to have higher levels of catechins than the heart or liver. Rat prostate and lung also had high catechin levels (500–2,000 ng/g combined). When individual catechins were examined, the highest concentration of EGC was detected in bladder and kidney (calculated to be 2.6 and 1.7 µM, respectively, with assumption that 1 g tissue equates to 1 ml), whereas the highest EGCG level was found in large intestine (1.1 µM calculated as shown above). This observation is consistent with our previous results showing that intravenously administered EGC and EC were excreted through the urine, whereas most of EGCG was found in the intestine of rats (21). The unabsorbed EGCG, of course, will go through the intestine. The poor absorption of EGCG by rats is suggested by 1) the much higher levels of fecal EGCG than EGC and EC and 2) the much lower levels of EGCG than EGC in the plasma and tissues, even though the GTPP solution contained a much higher level of EGCG. In addition, our unpublished results indicate that when green tea was given to rats by intraperitoneal injection, the plasma catechin level of EGCG was much higher than that obtained by gastric intubation, and the EGCG-to-ECG ratio resembled the composition of the tea (unpublished results). The lower blood level of EGCG in comparison to EGC was also observed in our previous single-dose experiment with rats in which conversion of EGCG to EGC was not observed (22).

In comparison to EGCG and EGC, the concentrations of EC in the plasma, intestine, and bladder were rather high, but the urinary and fecal levels of EC were unexpectedly low. The reasons for this discrepancy are not known. One possibility is the intestinal microbial conversion of EC to 5-(3′,4′-dihydroxyphenyl)valerolactone (29). This product was found in substantial quantities in human urine and feces (30).

The decrease of plasma catechin levels during repeated consumption of GTPP was also observed in mice that consumed 0.6% GTPP (Figure 7), although the decrease started much earlier than in the rats. The reason for the difference between mice and rats is not known. The decrease in catechin levels in mouse lung and liver levels after Day 4 suggests decreased absorption or increased elimination of catechins on prolonged tea administration. The peak levels of EGCG in the plasma and lung are calculated to be 2.4 and 2.9 µM, respectively. The ratio of concentrations of EGCG to EGC in the mouse plasma roughly resembles that in the GTPP solution. In this study, female mice and male rats were used because of the carcinogenesis models in our laboratory (19). The presently observed difference in EGCG levels between mice and rats is likely due to species differences rather than gender differences. In humans, a gender difference in plasma EGCG levels has not been observed (22; unpublished results). In human plasma, the EGCG levels were only slightly lower than EGC levels after tea preparations containing a slightly higher amount of EGCG than EGC were taken (22). Thus the mouse appears to be closer than the rat to humans in terms of the bioavailability of EGCG.

To our knowledge, this is the first detailed study of blood, urine, and tissue levels of catechins after repeated treatments of rats and mice with tea polyphenols for different periods of times. The increase in blood levels in the first phase of the experiment may be contributed by several factors, including the accumulation of catechin due to continuous consumption of GTPP from the drinking fluid and increased fluid consumption. The decrease in catechin levels in blood and tissues in the second phase of the experiment is unexpected. It may be due to an adaptation by the animals and their intestinal microflora with an increased conversion of catechins to metabolites that were not measured in our study. It is not known whether the presently observed phenomenon is dose dependent or occurs in humans. This may have important implications in understanding the cancer-preventive or other health benefit effects of tea consumption. The difference between rats and mice in handling EGCG and EGC, as well as the tissue distribution of catechins, is also important in understanding the cancer-preventive activities of tea in these animals. Further studies in this area, especially in humans, are needed for better understanding of the bioavailability, tissue distribution, and biological activities of tea.

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