

Short Communication

Blood and Urine Levels of Tea Catechins after Ingestion of Different Amounts of Green Tea by Human Volunteers¹

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Abstract

The inhibitory activity of tea against tumorigenesis has been demonstrated in many animal models and has been suggested by some epidemiological studies. Such activity has generally been attributed to tea catechins. To understand the bioavailability of tea catechins in humans, we gave 18 individuals different amounts of green tea and measured the time-dependent plasma concentrations and urinary excretion of tea catechins. After taking 1.5, 3.0, and 4.5 g of decaffeinated green tea solids (dissolved in 500 ml of water), the maximum plasma concentration (C_{\max}) of (–)-epigallocatechin-3-gallate (EGCG) was 326 ng/ml, the C_{\max} of (–)-epigallocatechin (EGC) was 550 ng/ml, and the C_{\max} of (–)-epicatechin (EC) was 190 ng/ml. These C_{\max} values were observed at 1.4–2.4 h after ingestion of the tea preparation. When the dosage was increased from 1.5 to 3.0 g, the C_{\max} values increased 2.7–3.4-fold, but increasing the dose to 4.5 g did not increase the C_{\max} values significantly, which suggested a saturation phenomenon. The half-life of EGCG (5.0–5.5 h) seemed to be higher than the half-life of EGC or EC (2.5–3.4 h). EGC and EC, but not EGCG, were excreted in the urine. Over 90% of the total urinary EGC and EC was excreted within 8 h. When the tea dosage was increased, the amount of EGC and EC excretion seemed to increase, but a clear dose-response relationship was not observed. The present study provides basic pharmacokinetic parameters of green tea catechins in humans; these parameters may be used to estimate the levels of these compounds after drinking tea.

Introduction

Tea (*Camellia sinensis*) is consumed by a very large population worldwide, and its health effects are an important topic for

scientific investigation. Much attention has been paid recently to the anticancer activities of tea (1–3). The inhibitory actions of tea and various tea polyphenol preparations have been demonstrated in a variety of rodent models in organ sites such as the skin, lung, liver, esophagus, forestomach, stomach, small intestine, and colon (1, 3). The relationship between tea consumption and human cancer, however, is not clear. Whereas some recent 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 (1, 4). One of the difficulties in studying the effects of tea consumption on cancer incidence as well as on other health parameters is the lack of pharmacokinetic data on the possible effective components of tea. It is not clear how much of these compounds are absorbed and distributed in tissues in humans and animals.

It has been hypothesized that most of the cancer-inhibitory activity of tea found in animal models is due to the polyphenolic constituents in tea, also known as tea catechins (2, 3). In green tea, the major polyphenols are EGCG,³ EGC, ECG, and EC (5, 6). The anticarcinogenic activities of EGCG and green tea polyphenol preparations have been demonstrated in several animal models (7–10). These polyphenolic compounds have also been demonstrated to inhibit the growth of a variety of cancer cell lines or cancer cells in athymic mice (11–14), but the effective concentrations are usually rather high. The precise mechanisms for these activities are not known. These polyphenols are known for their antioxidant activities, and these activities may play a key role in the anticancer activities (1). Other mechanisms have also been suggested (13, 15), but the precise mechanisms of the inhibitory activity against carcinogenesis remain to be elucidated. Whether these beneficial effects occur in humans might be learned through more detailed epidemiological studies. Well-controlled intervention trials are especially needed.

In preparation for future intervention trials, we have conducted a Phase I study to examine the acceptability of tea beverage preparations by human volunteers. We have also studied the pharmacokinetics of tea catechins to understand their bioavailability and in an effort to develop tea catechins as biomarkers for tea consumption. In this article, we report the time-dependent blood levels of EGCG, EGC, and EC in individuals after taking different amounts of green tea as well as the urinary excretion of EGC and EC by these individuals.

Received 8/7/97; revised 12/19/97; accepted 1/8/98.

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¹ Supported by NIH Grant CA56673 and facilities from National Institute of Environmental Health Sciences Center Grant ES05022 and Cancer Center Support Grant CA72720.

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³ The abbreviations used are: EGCG, (–)-epigallocatechin-3-gallate; EGC, (–)-epigallocatechin; ECG, (–)-epicatechin-3-gallate; EC, (–)-epicatechin; DGT, decaffeinated green tea extract solid; HPLC, high-performance liquid chromatography; AUC, area under the plasma concentration-time curve; $t_{1/2}$, elimination half-life; C_{\max} , maximum plasma concentration; T_{\max} , the time to reach C_{\max} ; MRT, mean residence time; k , terminal elimination rate constant.

Materials and Methods

Chemicals and Reagents. Green tea leaves were decaffeinated using supercritical carbon dioxide. The residual caffeine in the dried tea was less than 0.1%. DGTs were prepared from the tea leaves by extraction with boiling water and lyophilization. The yield was 300 g of DGT from 1 kg of tea leaves. One g of this DGT powder contained 73 mg of EGCG, 68 mg of EGC, 22 mg of ECG, and 25 mg of EC. Standard EGC, EC, EGCG, and ECG were isolated from green tea and purified as described previously (16); the purity of each compound was >98%. β -Glucuronidase (G-7896) and sulfatase (S-9754) were obtained from Sigma Chemical Co. (St. Louis, MO). Other reagents and HPLC grade solvents were from EM Sciences (Gibbstown, NJ).

Human Subjects. The study had the participation of 18 volunteers who were healthy adults and employees of the Memorial Sloan-Kettering Cancer Center. This study was approved by the Institutional Review Board of the Memorial Sloan-Kettering Cancer Center. The subjects refrained from drinking tea for 2 days before the experiment. They were given a beverage preparation formulated by combining DGT (1.5, 3.0, or 4.5 g) with 45 g of sucrose, 7.5 g of coffee whitener (Borden 1-22007), and vanilla flavor (Tastmaker Lot SOS 5019). These beverage preparations were reconstituted in 500 ml of hot water and drunk by the volunteers in the morning after an overnight fast. Blood samples were then collected in heparin-containing tubes at 0, 0.5, 2, 4, 8, and 24 h. After centrifugation, 1 ml of each plasma sample was mixed with 20 μ l of the ascorbate-EDTA solution [0.4 M NaH_2PO_4 buffer containing 20% ascorbic acid and 0.1% EDTA (pH 3.6)], and the mixture was stored at -80°C until analysis. Urine samples were collected before the dose and during periods of 0-2, 2-6, 6-8, 8-24, and 24-48 h after the dose. The 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 adjusted to pH 6.8 with 10% NaOH and stored at -80°C until analysis.

Quantitation of Tea Polyphenols. The plasma levels of EGCG, EGC, and EC, as well as the urinary levels of EGC and EC, were analyzed by HPLC with a coulochem electrode array detector (ESA, Inc., Bedford, MA) as described in the work of Lee *et al.* (16). In brief, each plasma sample (100 μ l) was thawed and mixed with 10 μ l of a mixture of β -glucuronidase (250 units) and sulfatase (20 units). The incubation mixture, containing 0.1 M sodium phosphate and 0.27 μM EDTA, with a final pH of 3.65, was incubated at 37°C for 45 min. The reaction mixture was extracted twice with ethyl acetate. The combined ethyl acetate extracts were added to 10 μ l of a 20% ascorbic acid solution and then evaporated to dryness in a vacuum centrifuge concentrator. The residues were redissolved in 100 μ l of a 10% acetonitrile aqueous solution. The resultant solution was centrifuged, and 50 μ l of the supernatant were injected onto the HPLC system. The eluent was monitored by the coulochem electrode array system with potential settings at -90 , -10 , 70, and 150 mV, and four chromatograms were obtained simultaneously. The peak height was used to calculate the plasma and urine concentrations of EGCG, EGC, and EC. This method provided the total amounts, including the free and conjugated forms, of each of EGCG, EGC, and EC. ECG was eluted after 32 min in this HPLC system and was not analyzed. The urine sample was incubated, extracted, and analyzed similarly (16).

Pharmacokinetic Analysis. The plasma concentration-time data for the tea polyphenols were analyzed by the PCNONLIN

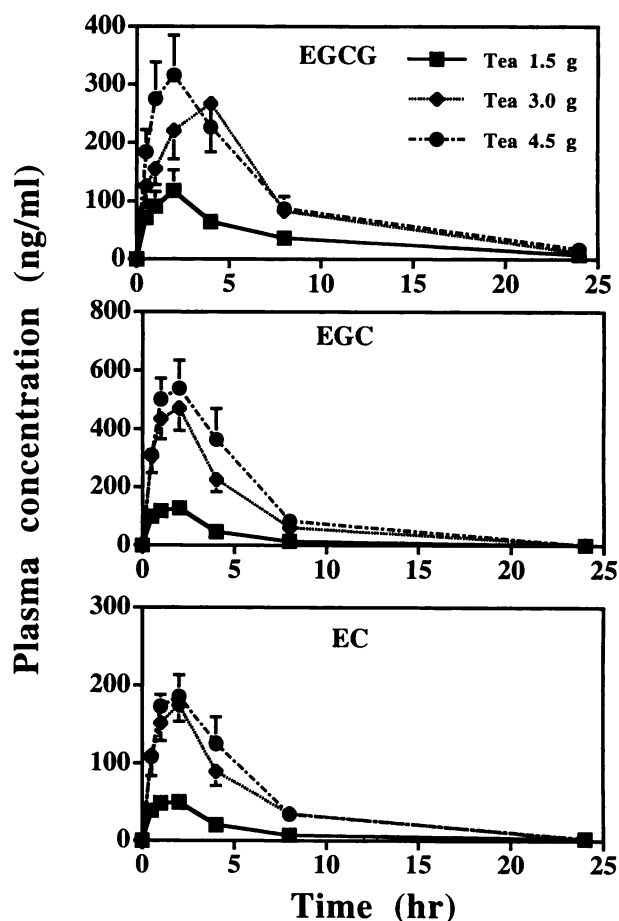


Fig. 1. Plasma level of tea catechins after ingestion of DGT. Each data point and error bar represent the mean \pm SE for six subjects. The pharmacokinetic parameters are shown in Table 1.

software package (Version 4.2; Clin Trials, Lexington, KY) with a noncompartment model. The AUCs for EGCG, EGC, and EC were determined trapezoidally and extrapolated to infinity by using the k for each compound. The $t_{1/2}$ was obtained from the ratio of $0.693/k$.

Results and Discussion

After ingestion of DGT, the plasma concentrations of EGCG, EGC, and EC reached peak levels between 1.5 and 2.5 h in almost all of the subjects and declined to undetectable levels after 24 h (Fig. 1). The pharmacokinetic parameters calculated by the PCNONLIN program with a noncompartment model are shown in Table 1. When the dose of DGT was increased from 1.5 to 3.0 g, the C_{\max} for EGCG increased 2.7-fold (from 120 to 326 ng/ml), the C_{\max} for EGC increased 3.4-fold (from 148 to 508 ng/ml), and the C_{\max} for EC increased 3.4-fold (from 55 to 189 ng/ml). The AUC, which reflects the extent of absorption, increased 2.5-fold (from 897 to 2223 ng/h/ml) for EGCG, 4.0-fold (from 617 to 2493 ng/h/ml) for EGC, and 3.8-fold (from 279 to 1059 ng/h/ml) for EC. However, increasing the dose of DGT from 3.0 to 4.5 g did not further increase the C_{\max} or the AUC for any of the three catechins. The T_{\max} for EGCG (1.6-2.7 h) seemed to be longer than that for EGC and EC (1.3-1.8 h) and was not affected by the dose increase for these

Table 1 Comparison in humans of pharmacokinetic parameters of EGCG, EGC, and EC with various doses of green tea powder^a

Tea (g)	T_{max} (h)	C_{max} (ng/ml)	k (h^{-1})	$t_{1/2}$ (h)	AUC (ng/h/ml)	MRT (h)
A. EGCG						
1.5	1.6 ± 0.7 ^b	119 ± 84 ^b	0.13 ± 0.04 ^b	5.5 ± 1.3 ^b	897 ± 732 ^b	5.9 ± 0.8 ^b
3.0	2.4 ± 1.4 ^b	326 ± 187 ^c	0.15 ± 0.04 ^b	5.0 ± 1.4 ^b	2223 ± 1106 ^c	5.8 ± 0.9 ^b
4.5	2.7 ± 1.0 ^b	321 ± 165 ^c	0.15 ± 0.06 ^b	4.9 ± 1.4 ^b	2462 ± 1217 ^c	5.5 ± 0.8 ^b
B. EGC						
1.5	1.4 ± 0.7 ^b	148 ± 54 ^b	0.32 ± 0.15 ^b	2.7 ± 1.4 ^b	617 ± 363 ^b	3.5 ± 1.3 ^b
3.0	1.8 ± 0.6 ^b	508 ± 182 ^c	0.30 ± 0.11 ^b	2.8 ± 1.4 ^b	2493 ± 677 ^c	4.0 ± 0.7 ^b
4.5	1.3 ± 0.5 ^b	550 ± 240 ^c	0.28 ± 0.05 ^b	2.5 ± 0.5 ^b	3281 ± 1923 ^c	3.9 ± 0.6 ^b
C. EC						
1.5	1.4 ± 0.6 ^b	55 ± 24 ^b	0.14 ± 0.05 ^b	5.7 ± 2.0 ^b	279 ± 148 ^b	5.1 ± 1.4 ^b
3.0	1.8 ± 0.6 ^b	189 ± 51 ^c	0.23 ± 0.08 ^c	3.4 ± 1.1 ^c	1059 ± 468 ^c	4.5 ± 0.7 ^b
4.5	1.8 ± 1.1 ^b	190 ± 62 ^c	0.26 ± 0.12 ^b	3.2 ± 1.2 ^c	1199 ± 577 ^c	4.3 ± 0.8 ^b

^a Data are expressed as mean ± SE for six subjects.

^{b, c} The means are significantly different ($P < 0.05$) between different doses of each catechin by the ANOVA test followed by the Fisher protected least significant difference test.

three catechins. The $t_{1/2}$ for EGCG was approximately 5 h, and the $t_{1/2}$ for EGC was approximately 2.7 h; the $t_{1/2}$ values were not affected by the increase in dose. We think that this is also true for EC, although the $t_{1/2}$ for EC decreased at higher doses. The MRT was also unaffected by the dose, and the values for EGCG seemed to be higher than for EGC.

The similar T_{max} observed for the three doses suggests that the dose may not affect the absorption and access of these tea catechins to the bloodstream. The higher C_{max} value for EGC than EGCG (although the amount of EGCG is slightly higher than that of EGC in DGT), suggests that EGCG has a lower absorbability or a larger volume of distribution than EGC. Conversion of EGCG to EGC or EC was not observed in a recent study with eight subjects.⁴ In addition, because the $t_{1/2}$ and MRT for the three catechins were not prolonged by the increase in dose (Table 1), these tea catechins seem to have linear pharmacokinetic properties. The dose-response relationship is more complex. It was unexpected that the C_{max} and AUC of EGCG, EGC, and EC would increase more than 2-fold when the dose was increased from 1.5 to 3.0 g, but no significant additional increase was observed when the DGT dose reached 4.5 g. The reason for this is not known. It may be that the capacity of plasma to take up or bind catechins is saturated at 3.0 g of DGT, but there may be other factors involved.

The observed plasma catechin levels with consumption of 1.5 g of DGT were similar to previously reported levels with consumption of 1.2 g of DGT, and most of the catechins were in the sulfate- and glucuronide-conjugated forms according to our previous observations (16). Consistent with previous observations (16), the level of EGCG in urine was undetectable. As in rats (17), most of the EGCG ingested by humans is probably excreted in the bile. The total amount of EGC excreted in urine seemed to increase with the increase in dose, but the difference was not statistically significant (Fig. 2). Although urinary excretion of EC increased when the dose of DGT was increased from 1.5 to 3.0 g, no additional increase was observed when the dose was increased to 4.5 g. Over 90% of the total urinary EGC and EC was excreted within 8 h. After 24 h, the levels of EGC and EC in the urine were below the limit of detection. No correlation was observed between urinary excretion of EGC and the AUC for EGC or between urinary excretion of EC and the AUC for EC. Previous studies indicated that

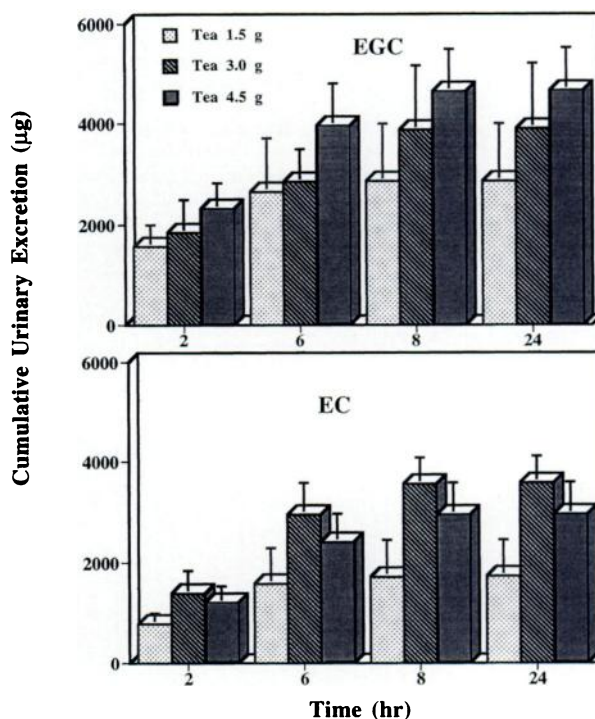


Fig. 2. Cumulative urinary excretion of tea catechins after ingestion of DGT. The values are the mean ± SE for six subjects.

both EGC and EC were excreted predominantly as glucuronide and sulfate conjugates at a ratio of approximately 2:1 (16). Possible catechin urinary metabolites that were eluted at 18.5 and 20.5 min had been reported previously (16) and were also observed in the current study.

The current study demonstrates that the tea preparation is acceptable to volunteers and provides pharmacokinetic parameters of green tea catechins. To our knowledge, this is the first report on the pharmacokinetic properties of EGCG, EGC, and EC after ingestion of green tea by humans. The results are useful for the designing of future experiments in the development of tea as a chemopreventive agent and for the use of tea catechins as biomarkers for tea consumption. For example, 3 g

⁴ C. S. Yang, M.-J. Lee, and L. Chen, unpublished results.

of DGT may be an optimal dose to be administered, and higher doses of tea may not give a significantly higher blood level of tea catechins. Moreover, using the C_{\max} , T_{\max} , and $t_{1/2}$ values, we can estimate the tea catechin levels at different times after drinking tea, for example, at breakfast daily or three times a day (at breakfast, lunch, and dinner). In this plan, on the basis of our results, we predict that most tea catechins would be cleared from the body in 10 to 12 h and would not accumulate in the body, because tea would not be ingested at night. All these predictions remain to be confirmed in future experiments. Additional studies are also needed to better understand the dose-response relationships in the plasma levels of catechins and in the urinary excretion of EGC and EC after ingestion of tea.

Acknowledgments

We thank Drs. Harold Newmark and Allan Conney for helpful discussions.

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Cancer Epidemiol Biomarkers Prev 1998;7:351-354.

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