Note

**Absorption and Distribution of Tea Catechin,
(–)-Epigallocatechin-3-Gallate, in the Rat**

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Summary To investigate the absorption and metabolism of an anti-carcinogenic tea catechin, (–)-epigallocatechin-3-gallate (EGCg), in rats, a newly developed chemiluminescence-detection high-performance liquid chromatography (CL-HPLC) method was employed and the EGCg concentrations in blood plasma, liver, brain, small intestinal mucosa and colon mucosa were determined before and after EGCg administration. The recovery of EGCg, extracted consecutively with ethyl acetate and methanol, was 86.1% from plasma and 64.5–74.2% from the tissue samples. The EGCg concentrations of plasma and tissue samples from the control rat (before EGCg administration) were all below the detection limit (<0.002 nmol/mL, 0.002 nmol/g), but 60 min after a single oral administration of EGCg (500 mg/kg body weight), the levels increased, reaching 12.3 nmol/mL in plasma, 48.4 nmol/g in liver, 0.5 nmol/g in brain, 565 nmol/g in small intestinal mucosa and 68.6 nmol/g in colon mucosa. The EGCg levels found in the tissues corresponded to 0.0003–0.45% of ingested EGCg. The results indicate that tea catechin, EGCg, is absorbed from the digestive tract, with the intestinal mucosa the most enriched of the organs. This may explain the potent antioxidant function of EGCg in inhibiting colon mucosal phospholipid hydroperoxidation in the prevention of rat colonic carcinogenesis.

Key Words epigallocatechin gallate, tea catechin, metabolism, antioxidant, rats

(–)-Epigallocatechin-3-gallate (EGCg) is a tea catechin and is consumed as a popular beverage in Japan and other Asian countries. In recent years, several epidemiologic studies have suggested a lower risk of gastric cancer for green tea drinkers (1).

Several tea catechins have been reported to act as water-soluble antioxidants *in vitro* by scavenging oxygen radicals, chelating metal ions, and inhibiting lipoxy-

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genase (2). If EGCg in free form could be efficiently incorporated into the tissue organelles, its antioxidant activity may provide a beneficial anti-carcinogenic effect in cases involving membrane phospholipid peroxidation, such as the development of colon cancer (3).

The incorporation of EGCg into the tissue organelles of mammals except for body fluids such as plasma and urine has never been confirmed. It is therefore of interest to examine whether EGCg orally ingested is absorbed and incorporated directly in free form into intestinal mucosa, liver and other tissues.

In this study, we employed a chemiluminescence-detection high-performance liquid chromatography (CL-HPLC) method (4) which was recently established to determine EGCg in biological fluids, and confirmed the absorption and distribution of EGCg in free form into rat tissues.

Materials and methods

EGCg. EGCg (above 95% purity) extracted from green tea leaf was obtained from Taiyo Kagaku (Yokkaichi, Japan) and was used without further purification.

CL-HPLC. The CL-HPLC system used in the EGCg assay was the same as that reported in a previous paper (4). The standard EGCg solution was newly made by dissolving EGCg in a Vc-EDTA solution which consisted of 2% ascorbic acid and 0.1% ethylenediamine tetraacetic acid disodium salt (EDTA) in a 0.4 M NaH_2PO_4 buffer at pH 3.9.

Experimental animals. Male Sprague-Dawley rats were obtained at 9 wk of age from Funabashi Farm (Chiba, Japan; 290–300 g body wt, $n=12$), and housed at 25°C with a 12-h light:dark cycle with free access to commercial chow (F-2 pellet rations from Funabashi Farm) and distilled water for one week. After that, rats were starved for 24 h and weighed. Body weights after food deprivation were 304 ± 15 g (mean $\pm$ SD). Six of the rats (EGCg rats) received, by stomach tube, one dose of EGCg (500 mg/kg body weight) dissolved in 2.5 mL distilled water. The other six rats (control rats) were not treated with the EGCg aqueous solution. After 60 min of EGCg administration, all rats were anesthetized with ether, and blood was collected from the abdominal artery with a heparinized syringe. Then, the liver and brain were perfused *in situ* with ice-cold 0.15 M saline, and the liver, brain and intestines were excised and washed thoroughly with 0.15 M saline. The mucosa of the small intestine and colon were carefully abraded with a razor, and the mucosa collected were further washed with 0.15 M saline to remove the hampering EGCg adhered to the surface of the mucosa. The stomach walls and intestinal mucosa were not affected at all by the oral EGCg intake.

Sample preparation. Liver, brain, small intestinal mucosa and colon mucosa (500 mg of each) were minced and homogenized in 2.5 mL of Vc-EDTA solution with a Teflon-glass homogenizer under ice-cold conditions. The plasma was obtained by centrifuging the heparinized blood at $1,000 \times g$ for 15 min. Tissue homogenates (20% w/w) and plasma were employed in the recovery test and the determination of EGCg as described below.

Recovery test of EGCg. To study the recovery of EGCg throughout the sample preparation procedures in the CL-HPLC assay, 218 pmol of EGCg dissolved in 20 μ L of Vc-EDTA solution was added to 480 μ L of 20% tissue homogenates (liver, brain, small intestinal mucosa and colon mucosa) prepared from control rats. On the other hand, 218 pmol EGCg dissolved in 250 μ L of Vc-EDTA solution was added to 250 μ L plasma of control rats.

Extraction and determination of EGCg. For EGCg determination, plasma (250 μ L, diluted with the same volume of Vc-EDTA solution) and tissue homogenates (500 μ L, 20% w/w) were used. To each sample of plasma and tissue homogenates, 500 μ L of acetonitrile was added, and the mixture was vortexed for 5 min, after which 3 mL of ethyl acetate was added. This mixture was vortexed again vigorously for 4 min and centrifuged ($1,000 \times g$) at 4°C for 15 min. The supernatant ethyl acetate layer was collected. This ethyl acetate extraction was repeated three times. The combined ethyl acetate layer was evaporated to dryness with a rotary evaporator. The dried extract was redissolved in 900 μ L of methanol/water (8:1 v/v) and passed through a HPLC chromatodisc (GL chromatodisc 13A, pore size 0.45 μ m; GLC Science, Tokyo, Japan) with 4 mL of methanol as the eluant to exclude contaminated cell debris. The methanol filtrate was evaporated to dryness and dissolved in an appropriate amount of 10% acetonitrile aqueous solution. An aliquot of this acetonitrile aqueous solution was injected into the CL-HPLC system to determine the EGCg concentration. The EGCg peak on the chemiluminescence chromatogram was identified by comparing its retention time with that of standard EGCg.

Results

The recoveries of EGCg throughout the sample preparation procedures in the CL-HPLC assay are given in Table 1. The EGCg recoveries by successive extraction with ethyl acetate and methanol were in the range from 64.5 to 86.1%.

In the CL-HPLC chromatograms of extracts from blood plasma, liver, brain, small intestinal mucosa and colon mucosa of the rat before and after 60 min of EGCg administration (500 mg/kg body weight), an EGCg peak was not found in these tissue samples before administration, but a substantial amount of EGCg (10.7 min of retention time) appeared in plasma and tissue organelles after EGCg supplementation.

Table 2 shows the EGCg concentrations in blood plasma and tissues after 60 min of EGCg administration (500 mg/kg body weight). Although the tissue EGCg levels before administration were below the detection limit (<0.002 nmol/mL plasma and <0.002 nmol/g tissues), substantial amounts of EGCg in free form were detected in all tissue samples of the EGCg-treated rats. The total amount of EGCg in the rat blood mass was calculated to be 37 μ g/rat, accounting for 0.024% of the ingested EGCg (500 mg/kg body weight); the whole blood mass was estimated provisionally to be 15 mL (corresponding to 6.5 mL plasma) per rat. Therefore, 0.0003–0.45% of the ingested EGCg was recognized to be incorporated into the tissues, among which the small intestinal mucosa was the most enriched.

Table 1. Recovery of EGCg from blood plasma and tissue organelles of rats.

	EGCg added (pmol)	EGCg found with CL-HPLC (pmol)	Recovery (%)
Control plasma 250 μ L	0	n.d. ^a	—
EGCg-added plasma 250 μ L ^b	218	188 $\pm$ 12	86.1 $\pm$ 5.4
Control liver ^c	0	n.d.	—
EGCg-added liver ^d	218	153 $\pm$ 6	70.2 $\pm$ 2.6
Control brain ^c	0	n.d.	—
EGCg-added brain ^d	218	141 $\pm$ 15	64.5 $\pm$ 6.7
Control small intestinal mucosa ^c	0	n.d.	—
EGCg-added small intestinal mucosa ^d	218	153 $\pm$ 13	70.0 $\pm$ 6.1
Control colon mucosa ^c	0	n.d.	—
EGCg-added colon mucosa ^d	218	162 $\pm$ 11	74.2 $\pm$ 5.2

The data are expressed as the mean $\pm$ SD of four determinations. EGCg extraction was described in the Methods section; CL-HPLC was performed under the conditions described in a previous paper (4).

^aNot detected. ^b218 pmol EGCg was added to 250 μ L plasma. ^cControl tissues (480 μ L, 20% homogenate) untreated with EGCg. ^d218 pmol EGCg was added to 20% tissue homogenates (480 μ L).

Table 2. EGCg detected in blood plasma and tissue organelles of rats as determined by CL-HPLC.

Tissue	Before administration	60 min after administration ^a	EGCg detected ^d (%)
Plasma (nmol/mL)	<0.002 ^b	12.3 $\pm$ 6.2	(0.024 $\pm$ 0.012) ^c
Liver (nmol/g)	<0.002	48.4 $\pm$ 19.7	(0.19 $\pm$ 0.09) ^c
Brain (nmol/g)	<0.002	0.5 $\pm$ 0.3	(0.0003 $\pm$ 0.0002) ^c
Small intestinal mucosa (nmol/g)	<0.002	565 $\pm$ 153	(0.45 $\pm$ 0.14) ^c
Colon mucosa (nmol/g)	<0.002	68.6 $\pm$ 40.4	(0.013 $\pm$ 0.008) ^c

The data are expressed as the mean $\pm$ SD of six rats.

^aRats received a single oral administration of EGCg (500 mg/kg body weight) after 24 h of food deprivation. ^bBelow the detection limit (<0.002 nmol). ^cPercentage against ingested EGCg. ^dCalculated from the blood mass estimated to be 15 mL/rat and from the tissue weights (liver, 13.0 $\pm$ 1.7 g/rat; brain, 2.6 $\pm$ 0.5 g/rat; small intestinal mucosa, 1.9 $\pm$ 0.2 g/rat; colon mucosa, 0.6 $\pm$ 0.2 g/rat for mean $\pm$ SD of six rats).

Discussion

The occurrence of tea catechins, especially of EGCg, in body fluids has been reported (5–7), but not in the tissue organelles. This study demonstrated the incorporation of EGCg in free form into the liver, brain, small intestinal mucosa and colon mucosa of the rat after EGCg ingestion. Relatively high levels of EGCg were detected in alimentary tract tissues such as the small intestinal mucosa and colon mucosa (Table 2). This may mean that the intestines are the major absorption site of EGCg in mammals. This data also indicates that EGCg is transportable in small amounts to the most conservative organ, the brain.

Liver and intestinal walls contain enzymes which would be responsible for the conjugation and methylation of EGCg (i.e., UDP-glucuronosyl transferase and catechol-O-methyl transferase). Intestinal microorganisms contain certain enzymes involved in the ring-fission of EGCg (8). In this study, two unknown minor chemiluminescence peaks, speculated to be the metabolites of EGCg, were observed in the liver of rats at 60 min after EGCg intake. On the other hand, only EGCg in free form was found as the major chemiluminescence peak in both small intestinal mucosa and colon mucosa. The EGCg incorporated into the blood stream could be transported to the tissue organelles as discussed by Hackett (9). The EGCg could also be excreted into bile from the liver. On the other hand, as a result of biliary excretion, EGCg could be excreted into the duodenum. It is probable that some EGCg would then be further reabsorbed from the intestines to the liver thus creating an enterohepatic circulation system as has been suggested to occur in the rat (9).

As metabolites of EGCg, Lee et al (5) reported that the glucuronide and sulfate of EGCg appear in human plasma after the ingestion of green tea extract. Hackett (9) has found (+)-catechin in the form of methylate, glucuronide and sulfate in plasma, urine and bile after its administration to rats. Oshima and Watanabe (10) reported that the oral administration of (+)-catechin to rabbit results in the excretion of its ring-fission product in urine. Also for 3-O-methyl-(+)-catechin and flavonoid (7-mono-O-(β -hydroxyethyl) rutin), the glucuronide conjugates have been found in the urine and plasma of mammals (9). Thus some degree of conjugation, methylation and ring-fission reaction seem to be involved in EGCg metabolism.

In terms of the possible therapeutic use of EGCg against digestive cancer, we recently reported that green tea extract, mainly consisting of EGCg, clearly prevents 1,2-dimethylhydrazine-induced colonic carcinogenesis in rats, in which the levels of phospholipid hydroperoxide as a peroxidized membrane lipid marker were significantly and dose-dependently lowered in colon mucosal cells as compared to the control rats (3). This directly indicated at first that the potent antioxidant function of EGCg is involved essentially in the prevention of colonic carcinogenesis. As the antioxidant mechanisms, metal chelation, hydrogen donation, lipoxxygenase inhibition and reactive oxygen elimination would be responsible. As shown here, since relatively high concentrations of EGCg were found in the intestinal mucosa in the EGCg-supplemented rats, the ingestion of EGCg as an antioxidative nutrient to prevent intestinal carcinogenesis may be recommendable. The findings presented

here help to explain the anticarcinogenic properties of green tea as revealed in several epidemiological studies (1).

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