Carcinogenesis vol.19 no.10 pp.1771–1776, 1998

Wide distribution of [^{3}H](–)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue

Masami Suganuma, Sachiko Okabe, Masumi Oniyama, Yukiko Tada, Hideyuki Ito[1] and Hirota Fujiki[2]

Saitama Cancer Center Research Institute, Ina, Kitaadachi-gun, Saitama 362-0806 and [1]Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700-0082, Japan

[2]To whom correspondence should be addressed
Email: masami@saitama-cc.go.jp

The increasing recognition of green tea and tea polyphenols as cancer preventives has created a need for a study of their bioavailability. For this purpose, we synthesized [^{3}H](–)-epigallocatechin gallate ([^{3}H]EGCG) with a specific activity of 48.1 GBq/mmol and directly administered the solution into the stomachs of CD-1 female or male mice. Radioactivity in the digestive tract, various organs, blood, urine and feces was measured with an oxidizer at various times after administration and significant radioactivity was found in the previously reported target organs of EGCG and green tea extract (digestive tract, liver, lung, pancreas, mammary gland and skin), as well as other organs (brain, kidney, uterus and ovary and testes) in both sexes. Incorporation of radioactivity in the cells was confirmed by microautoradiography. Within 24 h, 6.6 (females) and 6.4% (males) of total administered radioactivity was excreted in the urine and 37.7 and 33.1% in feces. HPLC analysis of urine from both sexes revealed that 0.03–0.59% of administered [^{3}H]EGCG, along with at least five metabolites, was excreted. In addition, we found that a second, equal administration to female mice after a 6 h interval enhanced tissue levels of radioactivity in blood, brain, liver, pancreas, bladder and bone 4–6 times above those after a single administration. These results suggest that frequent consumption of green tea enables the body to maintain a high level of tea polyphenols and this paper is the first pharmacological evidence of a wide distribution of [^{3}H]EGCG in mouse organs, indicating a similar wide range of target organs for cancer prevention in humans.

Introduction

In 1987, our results showing inhibition of tumor promotion by (–)-epigallocatechin gallate (EGCG), a tea polyphenol with a molecular weight of 458, opened up the study of the cancer preventive properties of EGCG and other tea polyphenols (1,2). The inhibitory effects of EGCG and green tea extract on carcinogenesis in various organs in rodents have now been demonstrated over the past decade. Specifically, EGCG and green tea extract in drinking water effectively inhibit carcinogenesis in the esophagus, glandular stomach, duodenum, colon, liver, pancreas, lung, breast and skin (3–13) and EGCG in drinking water also inhibits metastasis of B16 melanoma cells into the lungs (14). At present, both EGCG and green tea extract are acknowledged practical cancer preventive agents (15), but to fully appreciate their significance, it was necessary to study how EGCG and green tea extract in drinking water are incorporated into various organs, along with their metabolism.

Tea (*Camellia sinensis*) is widely consumed throughout the world as black tea, oolong tea and green tea. Approximately 26% of the solid weight of green tea extract is tea polyphenols and EGCG constitutes 11% of these (16). The composition and content of tea polyphenols undergo changes during the fermentation process: The content of EGCG in black tea, the most fermented tea, is less than in green tea, whereas the content of theaflavins is higher in black tea. All this was known and, moreover, a prospective cohort study in Saitama Prefecture revealed the cancer preventive effects of drinking green tea in humans (17). However, the bioavailability and metabolic fate of tea polyphenols have not been fully understood. A few papers had reported on HPLC analysis of tea polyphenols in plasma and urinary samples of humans or rats after ingestion of green tea (18–21). These papers demonstrated that tea polyphenols, including EGCG, (–)-epigallocatechin (EGC) and (–)-epicatechin (EC), were incorporated into the blood only a few hours after oral administration and excreted into urine as conjugated forms, e.g. glucuronide and sulfate. However, a more detailed study of their bioavailability, using radiolabeled tea polyphenols, was needed.

Accordingly, we recently obtained [^{3}H]EGCG, labeled with ^{3}H with a specific activity of 48.1 GBq/mmol, as a tool for a bioavailability study (Figure 1). The ^{3}H label in this EGCG was quite stable: it did not, for example, exchange with ^3H_2O after incubation for 24 h (Amersham, UK, personal communication). First, we studied the distribution of [^{3}H]EGCG in mouse organs by oral administration: Radioactivity was found in various organs, including many where inhibition of carcinogenesis by EGCG or green tea extract has already been shown. HPLC analyses of urine samples showed that small amounts of [^{3}H]EGCG were excreted in urine. A second, equal administration of [^{3}H]EGCG after a 6 h interval increased the radioactivity in blood and various organs. This paper provides the first evidence that the radioactivity of [^{3}H]EGCG is distributed in various organs, a finding supported by microautoradiography.

Materials and methods

Chemicals

4(*n*)-[^{3}H](–)-EGCG was labeled with tritium gas (Amersham, Aylesbury, UK). The radiochemical purity of [^{3}H]EGCG was 92.9% by analysis by HPLC and ^{3}H NMR. The positions labeled with ^{3}H were in one of the aromatic rings estimated from the ^{3}H NMR; it was not possible to specifically identify the sites. The ^{3}H label in EGCG was quite stable, confirming that it did not exchange with water over a period of 24 h. The specific activity of [^{3}H]EGCG was 48.1 GBq/mmol and the EGCG used for all the experiments was 99.7% pure.

Animals

Female and male CD-1 mice, 7 weeks old, were obtained from Charles River Japan (Kanagawa, Japan). Mice were given no nourishment for ~15 h before gastric intubation.

Abbreviations: EC, (–)-epicatechin; ECG, (–)-epicatechin gallate; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin gallate.

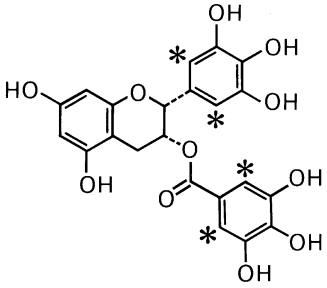

Fig. 1. Structure of [^{3}H]EGCG. *Potential ^{3}H labeled positions estimated by ^{3}H NMR.

Distribution of radioactivity in mice after a single administration of [^{3}H]EGCG

Each mouse was given 200 μl 0.05% EGCG solution containing 3.7 MBq [^{3}H]EGCG by gastric tube and then kept in a metabolic cage with controlled temperature, humidity and light. At various intervals after administration, samples of digestive tract, blood and various organs were taken for measurements of radioactivity.

Digestive tract. The digestive tract was divided into three parts, stomach, small intestine and colon. Total radioactivity of each part, including dietary content, was measured with an oxidizer (Packard Japan, Tokyo, Japan) (22). The oxidizer converts ^{3}H radioactivity in tissue samples to ^3H_2O in a process of perfect combustion. The ^3H_2O was measured with a scintillation counter (LS 6500; Beckman Instrument, Fullerton, CA).

Blood. Radioactivity in blood was measured with an oxidizer using filter paper blotted with 50 μl blood.

Various organs. Radioactivity of various organs was measured with an oxidizer using 100–150 mg tissue from each organ. All radioactivity in the organs was expressed as d.p.m./100 mg tissue and total radioactivity incorporated was obtained from radioactivity per 100 mg tissue multiplied by the weight of the organ. More than 98% of total radioactivity was recovered by the oxidizer. Data represent the means of two mice of each sex.

Cumulative excretion of radioactivity in feces and urine

Feces and urine of female and male mice were collected for 0–3, 0–6 or 0–24 h in a metabolic cage after a single administration of [^{3}H]EGCG. Radioactivity of all feces samples collected in each period was measured with an oxidizer. The volume of urine samples was measured and 50 μl of each urine sample dissolved in 10 ml Aquazol was counted in a scintillation counter.

HPLC analysis of radioactive metabolites in urine

The urine of female and male mice was mixed with an equal volume of 0.1 N HCl before analysis by HPLC. The urine mixture plus 10 μg EGCG was applied to a C18 Bond Elut cartridge (Varian, Harbor, CA) and eluted with water followed by methanol. More than 95% of EGCG was recovered in the methanol eluent from C18 Bond Elut. The radioactivity of urine samples in the methanol eluent was 57.3–77.7% of applied radioactivity. The methanol eluent from C18 Bond Elut was analyzed by HPLC as follows: column, YMC Pack ODS-A (YMC, Kyoto, Japan); solvent, water/methanol/acetic acid (80:16:4); flow rate, 1 ml/min; detection, UV 280 nm. Fractions of 1 ml were collected and radioactivity was measured in a scintillation counter.

Tissue distribution of radioactivity in female mice after a duplicate administration of [^{3}H]EGCG

Six hours after the first administration of [^{3}H]EGCG with 3.7 MBq/200 μl 0.05% EGCG solution, a second administration of [^{3}H]EGCG with the same radioactivity as the first was given in the female mouse stomach. Six hours after a second administration, the radioactivity of blood, digestive tract, various organs, urine and feces was measured as mentioned above. Total radioactivity after two administrations was compared with that after a single administration.

Microautoradiography of mouse tissue

Twenty-four hours after administration of [^{3}H]EGCG, various organs of female mice were immediately frozen in O.C.T. Compound (Miles, Elkhart, IN) in dry ice/ethanol. Tissue sections (3 μm) were coated with Hypercoat emulsion (Amersham) (23). After being kept for 3 weeks in a darkroom at 4°C, slides were developed with D-19 developer (Eastman Kodak, Rochester, NY). The tissues were stained with DiffQuick (International Reagents, Kobe, Japan). Silver grains were observed by microscopic examination. Tissues of non-treated female mice were used as controls.

Results

Distribution of radioactivity in mice after a single administration of [^{3}H]EGCG

Digestive tract. Average percent of total administered radioactivity in the digestive tract, including dietary content, of female mice was 30.7, 40.6 and 3.9% for the stomach, small intestine and colon, respectively, 1 h after administration (Table I). The results with male mice were almost identical to those for female mice (Table I). Twenty-four hours after administration, radioactivity in the digestive tract (stomach + small intestine + colon) had decreased, but 14.5% of total administered radioactivity for female mice and 18.2% for male mice remained (Table I). Specifically, after 24 h radioactivity per 100 mg tissue in female mice was measured at 328.0×10^3, 213.7×10^3 and 269.0×10^3 d.p.m. for the stomach, small intestine and colon respectively. In male mice, the figures were 145.5×10^3, 109.9×10^3 and 440.3×10^3 d.p.m. respectively. These are the highest counts among various organs measured in both sexes. It is clear that some of the EGCG given in drinking water remained in the digestive tract, resulting in direct interaction with the cells.

Blood. Radioactivity in blood of female mice was found to be 24.0×10^3 d.p.m./ml 1 h after administration of [^{3}H]EGCG into the stomach and reached 235.0×10^3 d.p.m./ml at 6 h after. There was a steady accumulation, which reached 288×10^3 d.p.m./ml after 24 h (Table IIa). In male mice, radioactivity in blood was 22.0×10^3, 189.0×10^3 and 269.0×10^3 d.p.m./ml at 1, 6 and 24 h after respectively (Table IIb), i.e. ~2% of total administered radioactivity had been incorporated into total blood in female and male mice 24 h after intubation. This radioactivity was not separated into free EGCG, its metabolites or the protein-bound forms of EGCG; it was calculated as 1.2 μg/ml EGCG under the assumption that all radioactivity was [^{3}H]EGCG.

Various organs. Radioactivity was found in various organs in female and male, such as the brain, lung, heart, liver, kidney, spleen, pancreas, uterus and ovary, mammary gland, testes, bladder, bone and skin. The radioactivity per 100 mg tissue of these organs at different intervals after administration is shown in Table II. Although there are individual variations, it is clear that radioactivity was found in the organs in which inhibition of carcinogenesis by EGCG or green tea extract in drinking water has previously been demonstrated, as well as in other organs. Radioactivity in almost all organs gradually increased up to 24 h and was found to be ~$1.7–22.5\times10^3$ d.p.m./100 mg tissue in female brain and $1.3–17.3\times10^3$ d.p.m./100 mg tissue in male brain. Radioactivity was equally distributed among almost all organs in mouse of both sexes 24 h after administration. Total radioactivity incorporated was 0.89% for liver, 0.32% for brain, 0.28% for kidney and 0.16% for lung in female and 0.67, 0.33, 0.43 and 0.16% for the same organs respectively in male. The percentages are those of total administered radioactivity and differences obviously reflect the sizes of the organs.

Cumulative excretion of radioactivity in feces and urine

Radioactivity excreted in feces and urine of both sexes for different intervals (0–3, 0–6 and 0–24 h) was determined. In female, 0.9, 23.4 and 37.7% of total administered radioactivity was excreted in feces and 1.7, 1.4 and 6.6% in urine within 3, 6 and 24 h respectively (Figure 2A). In male, 2.0, 20.9 and 33.0% was excreted in feces and 1.9, 4.0 and 6.4% in urine

Table I. Distribution of radioactivity into the digestive tract of female and male mice after a single administration of [^{3}H]EGCG

Time after administration (h)	Radioactivity ($\times 10^4$ d.p.m.) (% of total administered radioactivity)[a]		
	Stomach	Small intestine	Colon
Female			
1	766.6 ± 182.0[b] (30.7)	1014.5 ± 82.9 (40.6)	98.4 ± 19.1 (3.9)
6	569.8 ± 15.7 (22.8)	346.6 ± 33.3 (13.9)	413.6 ± 120.2 (16.7)
24	98.4 ± 50.5 (3.9)	151.7 ± 38.6 (6.1)	113.0 ± 77.1 (4.5)
Male			
1	1423.0 ± 27.5 (56.9)	646.2 ± 186.9 (25.8)	22.7 ± 10.2 (1.0)
6	620.9 ± 86.2 (24.8)	524.9 ± 86.6 (21.1)	515.0 ± 21.0 (20.6)
24	58.2 ± 20.3 (2.3)	126.4 ± 50.0 (5.0)	273.0 ± 101.7 (10.9)

Each mouse was given 200 µl 0.05% EGCG solution containing 3.7 MBq [^{3}H]EGCG. At various intervals after administration, samples of digestive tract were taken for measurements of radioactivity.
[a]Total radioactivity in stomach, small intestine and colon, including dietary content, was measured with an oxidizer.
[b]Total radioactivity is expressed as the mean ± variation of two mice.

within 3, 6 and 24 h respectively (Figure 2B). To test what percentages of radioactivity were original [^{3}H]EGCG and its metabolites, a urine methanol fraction was analyzed by HPLC. Figure 3 shows the HPLC profile of a methanol eluent of a C18 Bond Elut cartridge column of urine collected at 0–6 h from a female mouse. One of the radioactive peaks coincided with a peak of unlabeled EGCG eluted at 10 min. HPLC profiles of urine samples of male mice were similar to those of female (data not shown). The results allowed us to calculate that original [^{3}H]EGCG excreted in urine collected for 6 h was ~6% of total radioactivity in urine of female mouse and ~5% in male. Urine samples collected after 3 and 24 h from females contained ~2 and 9% [^{3}H]EGCG; the figures were ~3 and 9% in male (data not shown). These results indicate that ~0.03–0.59% of total administered [^{3}H]EGCG was excreted in the urine of both sexes. The other five peaks in the HPLC profile may represent EGCG metabolites, based on available literature (24).

Tissue distribution of radioactivity in female mice after a duplicate administration of [^{3}H]EGCG

Based on the fact that the Japanese drink green tea throughout the day, we performed an experiment to determine whether a duplicate administration of [^{3}H]EGCG would enhance radioactivity in blood and organs. As Table III shows, a second, equal administration of [^{3}H]EGCG increased radioactivity in blood by as much as 5.9 times over a single administration at 6 h after the second administration. Increases >4-fold were found in the brain, lung, liver, pancreas, bladder and bone. However, the radioactivity of the digestive tract, including dietary content, showed a less dramatic increase, 1.3- to 2.0-fold. Interestingly, radioactivity of urine increased 6.1-fold after the second dose. In addition, increased incorporation was also observed at 24 h after the second administration, i.e. radioactivity in blood, brain, lung, liver, pancreas and bone had increased >3-fold compared with that at 24 h after a single administration (data not shown). These results indicate that drinking green tea many times a day increases the level of tea polyphenols in blood and various organs, suggesting that a high concentration of tea polyphenols can be maintained with the usual Japanese lifestyle.

Microautoradiography of mouse tissue

To confirm whether administered [^{3}H]EGCG is incorporated into cells of various organs, tissue sections of female mice administered [^{3}H]EGCG were subjected to microautoradiography 24 h later. Silver grains indicating radioactive compounds were found in cells of the tested organs, such as colon mucosa and lung, but not in non-treated organs (Figure 4). It is important to note that silver grains were found in some cells of the tissues, but not in all cells homogeneously, suggesting the presence of unique specific mechanisms of incorporation of EGCG.

Table II. Distribution of radioactivity in organs other than digestive tract of mice after a single administration of [^{3}H]EGCG

	Radioactivity ($\times 10^3$ d.p.m./100 mg tissue)		
	1 h	6 h	24 h
(a) Female			
Blood (/ml)	24.0 ± 2.0[a]	235.0 ± 37.0	288.0 ± 2.0
Brain	1.7 ± 1.0	9.0 ± 6.7	22.5 ± 10.4
Lung	43.6 ± 34.1	15.3 ± 1.5	22.0 ± 5.8
Heart	13.8 ± 10.9	15.1 ± 1.3	24.4 ± 8.1
Liver	2.1 ± 1.7	14.7 ± 6.0	27.3 ± 3.7
Kidney	3.7 ± 2.7	12.5 ± 1.6	24.7 ± 6.5
Spleen	6.3 ± 0.7	15.9 ± 0.7	20.0 ± 7.1
Pancreas	5.9 ± 3.5	25.6 ± 16.4	21.4 ± 12.3
Uterus and ovary	0.8 ± 0.5	10.8 ± 1.7	21.8 ± 8.0
Mammary gland	2.3 ± 1.8	6.8 ± 2.4	18.4 ± 4.3
Bladder	5.5 ± 2.0	6.1 ± 2.0	6.6 ± 3.6
Bone	3.8 ± 3.0	11.3 ± 0.8	18.5 ± 6.5
Skin	3.1 ± 2.0	26.2 ± 13.3	19.3 ± 0.4
(b) Male			
Blood (/ml)	22.0 ± 10.0[a]	189.0 ± 78.0	269.0 ± 55.0
Brain	1.3 ± 0.1	8.6 ± 0.0	17.3 ± 7.6
Lung	4.3 ± 2.1	13.5 ± 2.9	16.1 ± 2.6
Heart	1.3 ± 0.3	7.6 ± 1.0	14.6 ± 2.8
Liver	34.8 ± 12.3	15.8 ± 1.9	13.9 ± 4.0
Kidney	4.1 ± 2.1	7.8 ± 1.4	19.8 ± 1.7
Spleen	9.6 ± 7.9	11.7 ± 7.3	33.8 ± 19.4
Pancreas	4.6 ± 2.7	43.3 ± 31.9	11.7 ± 8.1
Testes	1.1 ± 0.2	7.8 ± 1.8	9.1 ± 6.7
Bladder	1.2 ± 0.5	10.0 ± 3.2	12.2 ± 8.0
Bone	1.1 ± 0.0	5.7 ± 2.1	6.7 ± 1.0
Skin	1.5 ± 0.3	7.6 ± 0.1	9.8 ± 4.3

Each mouse was given 200 µl 0.05% EGCG solution containing 3.7 MBq [^{3}H]EGCG. At various intervals after administration, samples of each organ were taken for measurements of radioactivity.
[a]Each value represents the mean ± variation of two mice.

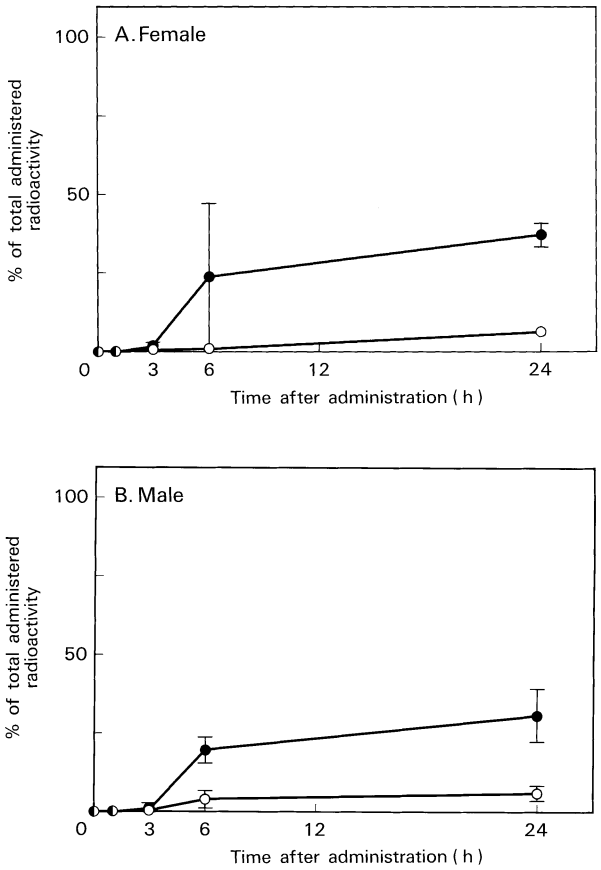

Fig. 2. Cumulative excretion of radioactivity in feces and urine after a single administration of [^{3}H]EGCG. (**A**) Female and (**B**) male radioactivity of feces (●) and urine (○) collected at 3, 6 and 24 h was measured as described in Materials and methods.

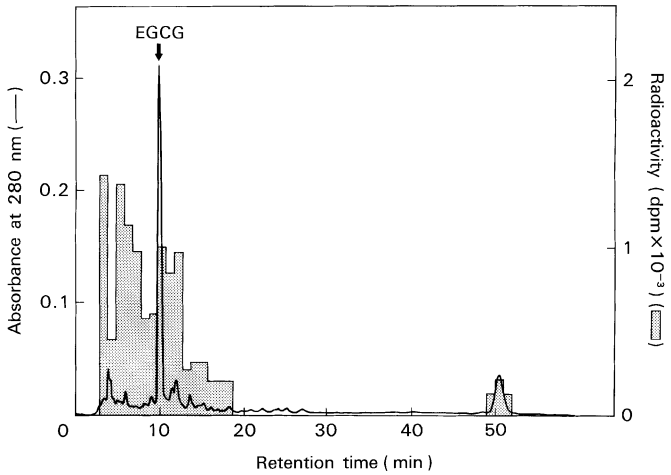

Fig. 3. HPLC profile of a methanol fraction of a urine sample from a female mouse collected at 6 h. Fractions were collected at 1 min intervals. The line and shaded areas indicate absorbance at 280 nm and radioactivity of fractions respectively.

Discussion

This paper provides significant evidence that EGCG, the main constituent of green tea, is easily absorbed from the digestive tract and widely distributed into various organs of mouse. Although ^{3}H label is thought to be easily exchanged with water, the actual exchange rate of [^{3}H]EGCG with water was <5% for 24 h incubation (unpublished data). Therefore, we think this radioactivity indicates mostly [^{3}H]EGCG itself, its metabolites and their protein-bound forms. Thus, these experiments with [^{3}H]EGCG revealed that the target organs of EGCG and green tea extract, such as the digestive tract, lung, liver, pancreas and skin, showed significant incorporation of radioactivity after oral administration of [^{3}H]EGCG. In addition, similar amounts of radioactivity were found in other organs, such as kidney, brain, uterus and ovary and testes, all of which are assumed to be additional target organs for cancer prevention. A small amount of the original [^{3}H]EGCG was excreted in urine, so the possibility exists that EGCG and green tea extract can prevent bladder cancer. The wide distribution of [^{3}H]EGCG in mouse organs suggests that EGCG and green tea extract have a wide range of target organs. Since it was previously reported that flavonoids, such as 3-*O*-methyl-[^{14}C](+)-catechin and [^{14}C]cianidanol-(+)-catechin, were distributed in various organs, including the liver, kidney and skin, in rat and marmoset (24,25), we think that wide distribution is an important and specific feature of EGCG, a tea polyphenol.

HPLC analyses with a methanol fraction of urine indicated that tea polyphenols have various metabolites, based on evidence that intestinal microorganisms metabolize flavonoids through ring fission, hydrolysis, methylation, oxidation and conjugation with glucuronide and sulfate (24,26). It has been reported that one conjugate form of EGCG, EGCG 4′-monosulfate, is metabolized by arylsulfotransferase of human intestinal bacteria (27). Sulfate forms and glucuronide forms of catechins (EGCG, EGC and EC) were also found in plasma and urine after oral administration of green tea extract to healthy volunteers (18). Since the conjugated forms of [^{3}H]EGCG might be contained in the water fraction of urine, further analysis of metabolites is needed.

Table III. Increased incorporation of radioactivity in organs after a duplicate administration of [^{3}H]EGCG compared with that after a single administration

	Total radioactivity ($\times 10^4$ d.p.m.)		Fold increase
	Single[a]	Duplicate[b]	
Blood (/ml)	25.13 ± 3.96[c]	149.40 ± 9.60	×5.9
Brain	3.00 ± 2.04	20.29 ± 1.47	×6.8
Lung	3.47 ± 0.56	16.14 ± 4.05	×4.7
Heart	2.08 ± 0.11	5.13 ± 1.03	×2.5
Liver	18.94 ± 8.18	87.06 ± 30.17	×4.6
Kidney	4.68 ± 0.39	14.68 ± 4.75	×3.1
Spleen	1.33 ± 0.34	2.03 ± 0.14	×1.5
Pancreas	0.77 ± 0.49	3.29 ± 0.16	×4.3
Uterus and ovary	2.04 ± 0.62	7.23 ± 2.44	×3.5
Bladder	0.14 ± 0.07	0.74 ± 0.31	×5.3
Mammary gland (100 mg)	0.29 ± 0.07	0.72 ± 0.01	×2.5
Bone (100 mg)	0.96 ± 0.01	4.50 ± 0.44	×4.7
Skin (100 mg)	3.41 ± 1.73	4.91 ± 2.09	×1.4
Urine	35.63 ± 11.18	215.77 ± 35.67	×6.1
Feces	585.67 ± 388.30	217.19 ± 5.94	×0.4

[a]Single administration. Each mouse was given 0.05% EGCG solution containing 3.7 MBq [^{3}H]EGCG. At 6 h after administration, samples of each organ were taken for measurements of radioactivity.
[b]Duplicate administration. Each mouse was given 0.05% EGCG solution containing 3.7 MBq [^{3}H]EGCG. At 6 h after the first administration, a second aliquot of 0.05% EGCG solution containing 3.7 MBq [^{3}H]EGCG was given. At 6 h after the second administration, samples of each organ were taken for measurements of radioactivity.
[c]Each value represents the mean ± variation of two female mice.

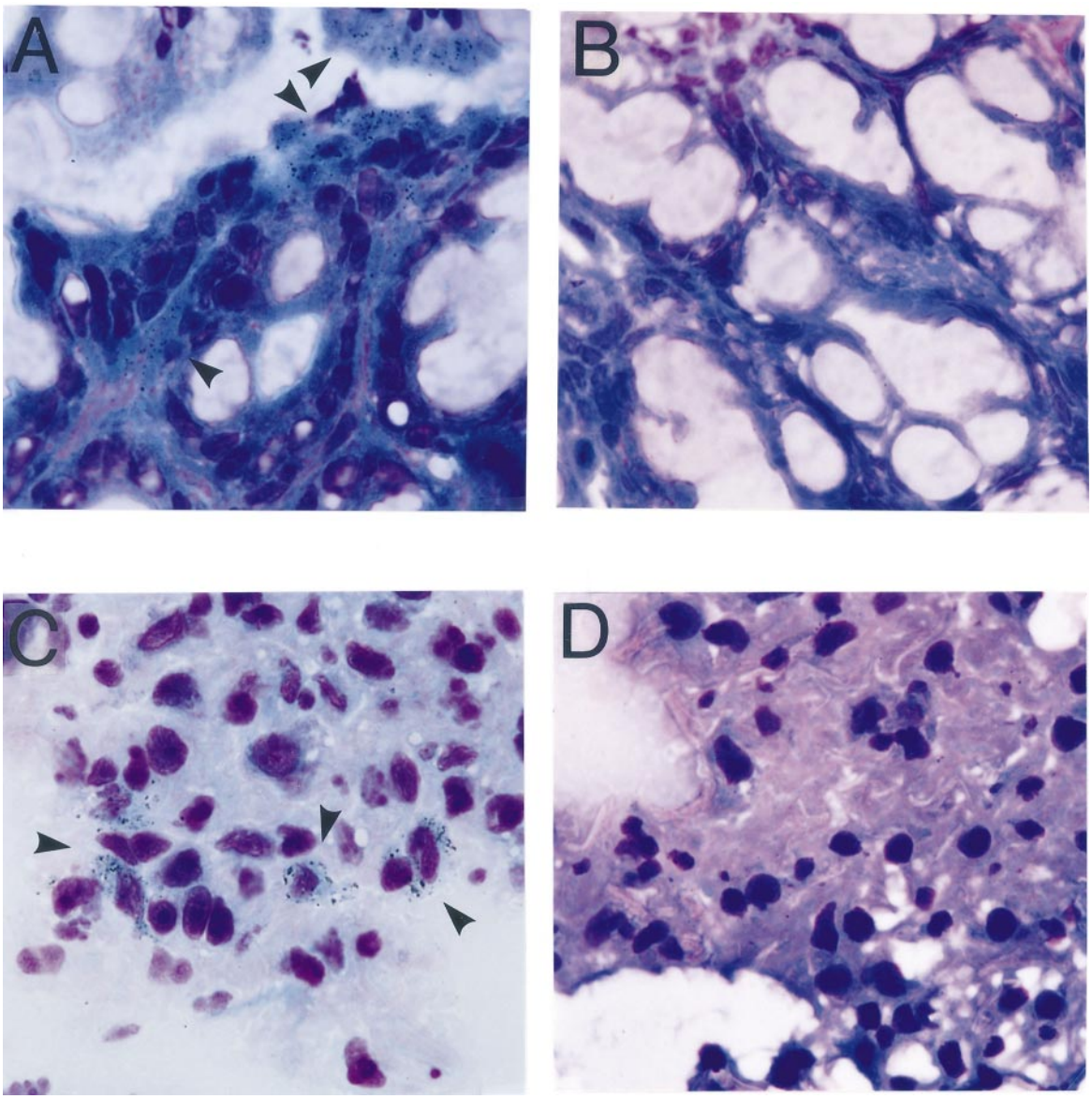

Fig. 4. Microautoradiography in colon mucosa and lung after [^{3}H]EGCG administration. Silver grains (arrow heads) indicate radioactivity in colon mucosa (**A**) and lung (**C**). No silver grains were observed in colon mucosa (**B**) and lung (**D**) of non-treated mouse.

Microautoradiography indicated that radioactivity was incorporated into cells of organs, but that this incorporation was not equal in all cells. Therefore, we think that some cells may readily incorporate EGCG by specific mechanisms. When [^{3}H]EGCG was incubated with the human lung cancer cell line PC-9, radioactivity was incorporated into cytosol and nuclei *in vitro* (28). Thus, radioactivity may also be incorporated into the nuclei of organs. Recently, we discovered that incorporation of [^{3}H]EGCG into PC-9 cells is stimulated by EC, suggesting that whole green tea is more effective than pure EGCG (M.Suganuma *et al.*, manuscript in preparation). Whether EGCG in green tea distributes more effectively than EGCG alone should be examined.

Imai *et al.*, in their prospective cohort study of 8552 individuals in Saitama Prefecture, Japan, found delayed cancer onset among patients who drink >10 cups of green tea per day, compared with that of those consuming <3 cups per day. Specifically, cancer onset of patients was delayed by 3.0 years (from 65.3 ± 1.5 to 68.3 ± 1.2) among males and 8.7 years (65.7 ± 1.7 to 74.4 ± 2.5) among females (17). These epidemiological results strongly support evidence that frequent consumption of green tea results in high levels of tea polyphenols in the blood and organs. The study also revealed that relative risk of cancers of the lung, colon and liver decreased from 1.0 to 0.36–0.55 (29). Interestingly, these organs coincide with those organs having high incorporation of radioactivity in mouse after oral administration of [^{3}H]EGCG. Thus, we conclude that a wide range of target organs for green tea as a cancer preventive in humans correlates well with a wide distribution of [^{3}H]EGCG in organs of mice.

Acknowledgements

We thank Dr Hidehiko Koya and Mr Seiji Kuwahara for their assistance with the microautoradiography. This work was supported by Grants-in-Aid from for Scientific Research on Priority Areas for Cancer Research from the Ministry of Education, Science, Sports and Culture, Japan, and for a Second-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare, Japan. Grants were also received from the Uehara Memorial Life Science Foundation, the Foundation for Promotion of Cancer Research, the Smoking Research Fund, the MOA Health Science Foundation,

Suzuken Memorial Foundation, the Asahi Glass Foundation and the Plant Science Research Foundation of the Faculty of Agriculture, Kyoto University.

References

1. Yoshizawa,S., Horiuchi,T., Fujiki,H., Yoshida,T., Okuda,T. and Sugimura,T. (1987) Antitumor promoting activity of (–)-epigallocatechin gallate, the main constituent of 'tannin' in green tea. *Phytother. Res*., **1**, 44–47.
2. Fujiki,H. and Okuda,T. (1992) (–)-Epigallocatechin gallate. *Drugs Future*, **17**, 462–464.
3. Wang,Z.Y., Wang,L.-D., Lee,M.J., Ho,C.-T., Huang,M.-T., Conney,A.H. and Yang,C.S. (1995) Inhibition of *N*-nitrosomethylbenzylamine-induced esophageal tumorigenesis in rats by green and black tea. *Carcinogenesis*, **16**, 2143–2148.
4. Yamane,T., Takahashi,T., Kuwata,K., Oya,K., Inagake,M., Kitao,Y., Suganuma,M. and Fujiki,H. (1995) Inhibition of *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine-induced carcinogenesis by (–)-epigallocatechin gallate in the rat glandular stomach. *Cancer Res*., **55**, 2081–2084.
5. Fujita,Y., Yamane,T., Tanaka,M., Kuwata,K., Okuzumi,J., Takahashi,T., Fujiki,H. and Okuda,T. (1989) Inhibitory effect of (–)-epigallocatechin gallate on carcinogenesis with *N*-ethyl-*N′*-nitro-*N*-nitrosoguanidine in mouse duodenum. *Jpn. J. Cancer Res*., **80**, 503–505.
6. Yamane,T., Hagiwara,N., Tateishi,M., Akachi,S., Kim,M., Okuzumi,J., Kitao,Y., Inagake,M., Kuwata,K. and Takahashi,T. (1991) Inhibition of azoxymethane-induced colon carcinogenesis in rat by green tea polyphenol fraction. *Jpn. J. Cancer Res*., **82**, 1336–1339.
7. Narisawa,T. and Fukaura,Y. (1993) A very low dose of green tea polyphenols in drinking water prevents *N*-methyl-*N*-nitrosourea-induced colon carcinogenesis in F344 rats. *Jpn. J. Cancer Res*., **84**, 1007–1009.
8. Nishida,H., Omori,M., Fukutomi,Y., Ninomiya,M., Nishiwaki,S., Suganuma,M., Moriwaki,H. and Muto,Y. (1994) Inhibitory effects of (–)-epigallocatechin gallate on spontaneous hepatoma in C3H/HeNCrj mice and human hepatoma-derived PLC/PRF/5 cells. *Jpn. J. Cancer Res*., **85**, 221–225.
9. Fujiki,H., Suganuma,M., Okabe,S., Komori,A., Sueoka,E., Sueoka,N., Kozu,T. and Sakai,Y. (1996) Japanese green tea as a cancer preventive in humans. *Nutr. Rev*., **54**, S67–S70.
10. Wang,Z.Y., Hong,J.-Y., Huang,M.-T., Reuhl,K.R., Conney,A.H. and Yang,C.S. (1992) Inhibition of *N*-nitrosodiethylamine- and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced tumorigenesis in A/J mice by green tea and black tea. *Cancer Res*., **52**, 1943–1947.
11. Xu,Y., Ho,C.-T., Amin,S.G., Han,C. and Chung,F.-L. (1992) Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. *Cancer Res*., **52**, 3875–3879.
12. Yang,C.S. and Wang,Z.Y. (1993) Tea and cancer. *J. Natl Cancer Inst*., **85**, 1038–1049.
13. Wang,Z.-Y., Huang,M.-T., Ferraro,T., Wong,C.-Q., Lou,Y.-R., Reuhl,K., Iatropoulos,M., Yang,C.S. and Conney,A.H. (1992) Inhibitory effect of green tea in the drinking water on tumorigenesis by ultraviolet light and 12-*O*-tetradecanoylphorbol-13-acetate in the skin of SKH-1 mice. *Cancer Res*., **52**, 1162–1170.
14. Taniguchi,S.-i., Fujiki,H., Kobayashi,H., Go,H., Miyado,K., Sadano,H. and Shimokawa,R. (1992) Effect of (–)-epigallocatechin gallate, the main constituent of green tea, on lung metastasis with mouse B16 melanoma cell lines. *Cancer Lett*., **65**, 51–54.
15. Fujiki,H., Komori,A. and Suganuma,M. (1996) Chemoprevention of cancer. In Bowden,G.T. and Fischer,S.M. (eds) *Comprehensive Toxicology.* Pergamon Elsevier Science, Oxford, UK, pp. 453–471.
16. Balentine,A.D. (1992) Manufacturing and chemistry of tea. In Chi-Tang,H., Chang,Y.L. and Mou-Tuan,H. (eds) *Phenolic Compounds in Food and Their Effects on Health I*. ACS Washington, DC, pp. 103–117.
17. Imai,K., Suga,K. and Nakachi,K. (1997) Cancer-preventive effects of drinking green tea in a Japanese population. *Preventive Med*., **26**, 769–775.
18. Lee,M.-J., Wang. Z.-Y., Li,H., Chen,L., Sun,Y., Gobbo,S., Balentine,D.A. and Yang,C.S. (1995) Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol*., **4**, 393–399.
19. Unno,T. and Takeo,T. (1995) Absorption of (–)-epigallocatechin gallate into the circulation system of rats. *Biosci. Biotechnol. Biochem*., **59**, 1558–1559.
20. Unno,T., Kondo,K., Itakura,H. and Takeo,T. (1996) Analysis of (–)-epigallocatechin gallate in human serum obtained after ingesting green tea. *Biosci. Biotechnol. Biochem*., **60**, 2066–2068.
21. Yang,C.S., Yang,G.-Y., Lee,M.-J. and Chen,L. (1997) Mechanistic consideration of the inhibition of carcinogenesis by tea. In Ohigashi,H., Osawa,T., Terao,J., Watanabe,S. and Yoshikawa,T. (eds) *Food Factors for Cancer Prevention*. Springer-Verlag, Tokyo, Japan, pp. 113–117.
22. Nishiwaki,R., Ohta,T., Sueoka,E., Suganuma,M., Harada,K.-i., Watanabe, M.F. and Fujiki,H. (1994) Two significant aspects of microcystin-LR: specific binding and liver specificity. *Cancer Lett*., **83**, 283–289.
23. Stanulis,B.M., Sheldon,S., Grove,G.L. and Cristofalo,V.J. (1979) Scintillation fluid shortens exposure times in autoradiography. *J. Histochem. Cytochem*., **27**, 1303–1307.
24. Hackett,A.M. (1986) The metabolism of flavonoid compounds in mammals. In Cody,V., Middleton,E.Jr and Harborne,J.B. (eds) *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure–Activity Relationships*. Alan R.Liss, New York, NY, pp. 177–194.
25. Awata,N., Miura,S., Hamada,T., Satomi,O. and Midorikawa,T. (1983) Metabolic fate of cianidanol (KB-53) (1) absorption, distribution and excretion in rats. *Pharmacometrics*, **25**, 993–1006.
26. Winter,J., Moore,L.H., Dowell,V.R.Jr and Bokkenheuser,V.D. (1989) C-Ring cleavage of flavonoids by human intestinal bacteria. *Appl. Environ. Microbiol*., **55**, 1203–1208.
27. Koizumi,M., Akao,T., Imamura,L., Dohi,K., Yoshida,T., Okuda,T. and Kobashi,K. (1992) Enzymatic sulfation of isoamyl gallate and (–)-epigallocatechin gallate by bacterial arylsulfotransferase. *Chem. Pharm. Bull*., **40**, 1864–1867.
28. Okabe,S., Suganuma,M., Hayashi,M., Sueoka,E., Komori,A. and Fujiki,H. (1997) Mechanisms of growth inhibition by tea polyphenols on human lung cancer cell line, PC-9. *Jpn. J. Cancer Res*., **88**, 639–643.
29. Nakachi,K., Imai,K. and Suga,K. (1997) Cancer-preventive effects of drinking green tea in a Japanese population. *Proc. Am. Assoc. Cancer Res*., **38**, 261.

Received on October 28, 1997; revised on May 12, 1998; accepted on June 2, 1998