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Suppressive effect of (-)-epigallocatechin gallate on 7,12-dimethylbenz[*a*]anthracene-induced chromosome aberrations in rat bone marrow cells

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Summary

The suppressive effect of (-)-epigallocatechin gallate (EGCG), the major polyphenolic constituent present in green tea, on 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced chromosome aberrations (CA) in rat bone marrow cells was studied. Rats given EGCG before the DMBA injection displayed a considerably suppressed frequency of DMBA-induced CA in their bone marrow cells. The suppressive effect of EGCG (60 mg/kg body weight) given 24 h before was observed 24, 30, 48 and 72 h after the DMBA injection, but not at the early period (6, 12 and 18 h) after the DMBA treatment. On the other hand, EGCG (60 mg/kg body weight) given 0.5 h before DMBA suppressed DMBA-induced CA at all periods after the DMBA injection. The suppression of EGCG given 24 h or 0.5 h before was observed for all doses of DMBA (25, 50, 75 and 100 mg/kg) investigated. EGCG given at 60 mg/kg body weight 0.5 h before the DMBA injection showed greater suppressive effect than the same dose given 24 h before. The suppressive effect of EGCG given 0.5 h before was dose-dependent in the range of 20−60 mg/kg body weight. Methyl methanesulfonate (MMS: direct-acting carcinogen)-induced CA were not suppressed by EGCG.

The administration of dehydroepiandrosterone (DHEA), a typical substrate for hydroxysteroid sulfotransferases, 0.5 h before DMBA injection also significantly suppressed DMBA-induced CA but DHEA given 24 h before did not.

These results suggest that EGCG has two different suppression mechanisms for DMBA-induced CA depending on the administration time. The suppression of DMBA-induced CA by EGCG given 24 h or 0.5 h before may result from the modification of microsomal enzyme system or the inhibition of sulfotransferase activity by EGCG, respectively.

Keywords: (-)-epigallocatechin gallate, 7,12-dimethylbenz[*a*]anthracene, methyl methanesulfonate, chromosome aberration, rat bone marrow cells

Introduction

Green tea is a popular beverage in Asian countries, mainly in Japan, China and India. The relationship between tea consumption and human cancer incidence is an important concern. Several epidemiological studies (Gao et al., 1994; Katiyar and Mukhtar, 1996; Kato et al., 1990; Kono et al., 1988; Oguni et al., 1992; Ohno et al., 1995; Yang et al., 1993) showed a lower risk of certain types of cancers among people who consume a large amount of green tea.

The hot water extract from green tea (GTE) is known to possess various beneficial pharmacological and physiological effects, such as antibacterial (Fukai et al., 1991; Toda et al., 1991; 1992), antiviral (Green, 1949; Nakayama et al., 1990; 1993), antifungal (Okubo et al., 1991), antioxidative (Matsuzaki and Hara, 1985; Osawa et al., 1988), antihemolysin (Ikigai et al., 1990; Okubo et al., 1989), antimutagenic (Apotolides et al., 1996; Jain et al., 1989; Wang et al., 1989) and antitumor (Katiyar et al., 1993a; 1993b; Wang et al., 1992; 1994) activities. These effects of GTE are

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Table 1 Specific details of rat bone marrow cell CA at various times after DMBA injection and EGCG pretreatments[a]

Time (h)	Treatment	Percentage of cells with[b]				No. of aberration per cell	Percentage of aberrant cells[c]
		Gap	Break	Ex.	Multi.		
0	Non-DMBA	1.8 ± 0.5	0.8 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.01 ± 0.01	0.8 ± 1.0
6	DMBA only	3.8 ± 3.0	2.6 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	0.03 ± 0.01	2.6 ± 1.3
	EGCG(−24 h) + DMBA	2.5 ± 1.0	2.5 ± 1.9	0.0 ± 0.0	0.0 ± 0.0	0.03 ± 0.03	2.5 ± 1.9(4)
	EGCG(−0.5 h) + DMBA	2.8 ± 1.0	1.8 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.02 ± 0.01	1.8 ± 1.2(31)
12	DMBA only	7.6 ± 3.3	9.4 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	0.13 ± 0.03	9.4 ± 1.3
	EGCG(−24 h) + DMBA	5.7 ± 1.9	9.2 ± 3.0	0.2 ± 0.4	0.0 ± 0.0	0.12 ± 0.06	9.3 ± 3.3(1)
	EGCG(−0.5 h) + DMBA	7.0 ± 2.7	7.8 ± 2.6	0.0 ± 0.0	0.0 ± 0.0	0.11 ± 0.05	7.8 ± 2.6(17)
18	DMBA only	11.0 ± 3.6	19.3 ± 3.9	0.0 ± 0.0	0.0 ± 0.0	0.24 ± 0.09	19.3 ± 3.9
	EGCG(−24 h) + DMBA	8.7 ± 1.9	18.4 ± 3.1	0.6 ± 0.8	0.3 ± 0.5	0.30 ± 0.08	19.3 ± 4.1(0)
	EGCG(−0.5 h) + DMBA	5.7 ± 2.2	10.3 ± 3.9	0.2 ± 0.4	0.0 ± 0.0	0.18 ± 0.09	10.5 ± 4.0(46)**
24	DMBA only	11.1 ± 3.4	22.7 ± 3.6	0.5 ± 0.7	0.5 ± 0.7	0.39 ± 0.12	23.6 ± 4.0
	EGCG(−24 h) + DMBA	9.5 ± 2.1	17.2 ± 4.2	0.8 ± 1.0	0.2 ± 0.4	0.27 ± 0.11	18.2 ± 5.1(23)*
	EGCG(−0.5 h) + DMBA	7.6 ± 1.3	15.3 ± 4.5	0.4 ± 0.5	0.0 ± 0.0	0.23 ± 0.09	15.8 ± 4.3(33)**
30	DMBA only	7.4 ± 2.7	16.4 ± 3.8	0.2 ± 0.4	1.0 ± 1.2	0.34 ± 0.17	19.6 ± 3.7
	EGCG(−24 h) + DMBA	7.6 ± 1.7	14.0 ± 4.3	0.6 ± 0.5	0.4 ± 0.5	0.26 ± 0.13	15.0 ± 3.0(23)*
	EGCG(−0.5 h) + DMBA	6.3 ± 2.7	12.3 ± 5.5	0.3 ± 0.5	0.5 ± 0.5	0.28 ± 0.14	13.2 ± 4.0(33)*
48	DMBA only	8.2 ± 2.7	16.2 ± 3.4	0.6 ± 0.5	0.4 ± 0.5	0.29 ± 0.16	17.2 ± 3.7
	EGCG(−24 h) + DMBA	6.9 ± 2.5	12.9 ± 4.5	0.2 ± 0.4	0.0 ± 0.0	0.19 ± 0.09	13.1 ± 3.4(24)*
	EGCG(−0.5 h) + DMBA	7.2 ± 3.6	9.8 ± 3.3	0.2 ± 0.4	0.0 ± 0.0	0.16 ± 0.09	10.0 ± 3.4(42)*
72	DMBA only	4.3 ± 2.5	5.7 ± 2.2	0.0 ± 0.0	0.0 ± 0.0	0.07 ± 0.03	5.7 ± 2.2
	EGCG(−24 h) + DMBA	4.8 ± 0.8	4.2 ± 1.6	0.4 ± 0.9	0.0 ± 0.0	0.05 ± 0.03	4.6 ± 1.8(19)
	EGCG(−0.5 h) + DMBA	2.6 ± 1.9	3.0 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	0.03 ± 0.01	3.0 ± 0.8(47)*

[a] Chromosome specimens were prepared at various times after 100 mg DMBA/kg body weight was injected. A dose of 60 mg EGCG/kg body weight was orally given 24 h or 0.5 h before the DMBA injection. Values are means ± SD.

[b] Ex., exchange; Multi., multiple CA: cells having more than 10 aberrations.

[c] Cells with gaps are not included in the percentage of aberrant cells. Figures in the parenthesis indicate the suppression percent.

* Statistical significance ($p < 0.05$) of the difference from the rat group given only DMBA was determined by Student's *t*-test.

** Statistical significance ($p < 0.01$) of the difference from the rat group given only DMBA was determined by Student's *t*-test.

thought to be due to polyphenolic constituents contained in green tea. (-)-Epigallocatechin gallate (EGCG) is one of the main green tea polyphenols (GTP).

We have previously reported (Ito et al., 1989) that the administration of GTE or GTP mixture before aflatoxin B_1 (AFB_1) injection in rats significantly suppressed AFB_1-induced chromosome aberrations (CA) in bone marrow cells. Furthermore, we have reported (Ito and Ito, 2001) the suppressive effect of EGCG on AFB_1-induced CA in rat bone marrow cells. EGCG given 24 h before the AFB_1 injection suppressed AFB_1-induced CA but not when given 2 h before; the same was true for GTE or GTP mixture. This suppression seems to be due to modification of the microsomal enzyme system by EGCG.

In this study, we investigated the suppressive effect of EGCG on CA induced by 7,12-dimethylbenz[*a*]anthracene (DMBA: indirect-acting carcinogen, as is AFB_1) and by methyl methanesulfonate (MMS: direct-acting carcinogen, needs no metabolic activation).

Materials and Methods

1. Chemicals

EGCG was purchased from Kurita Kogyo Co. (Tokyo, Japan). DMBA, dehydroepiandrosterone (DHEA) and colchicine were obtained from Wako Pure Chemicals Co. (Tokyo, Japan), MMS was from Aldrich (Milwaukee, WI), and dimethyl fulfoxide (DMSO: spectrophotometric grade) was from E. Merck A.G., (Darmstadt, F.R.G.).

2. Animal experiments

Male rats of the Wistar strain (Charles River Japan, Inc., Kanagawa, Japan), aged 28−35 days and weighing 80−110 g, were used. Each experimental group consisted of at least 6 rats. They were kept in an air-conditioned room and fed diet (Oriental MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum.

DMBA and MMS were dissolved in DMSO or in water, respectively. They were administered by i.p. injection. EGCG and DHEA were dissolved in water or sesame oil, respectively. These were administered by gastric instillation to lightly ether-anesthetized rats at various times before the carcinogen injection. Colchicine (0.3 mg/rat) was injected i.p. 1 h before sacrifice. Chromosome specimens were prepared from the femoral bone marrow by the conventional method (Sugiyama, 1971) at various times after the carcinogen injection, stained in 2% Giemsa solution (pH 6.8) for 15 min, and then analyzed microscopically.

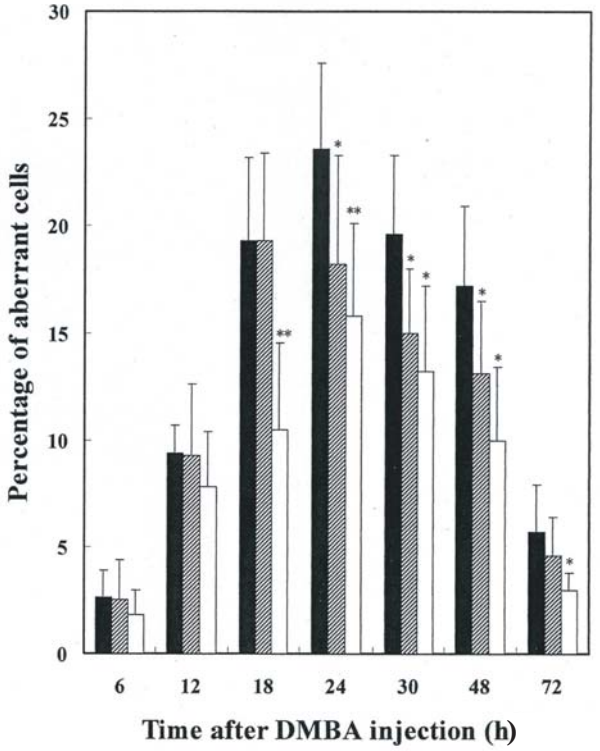

Fig. 1 Variation of the frequency of aberrant cells in bone marrow of rats (■) receiving only the DMBA injection, rats (▨) given EGCG 24 h before and rats (□) given EGCG 0.5 h before the DMBA injection. Chromosome specimens were prepared at various times after 100 mg DMBA/kg body weight was injected i.p. A dose of 60 mg EGCG/kg body weight was orally given 24 h or 0.5 h before the DMBA injection. Values represent the mean ± SD. Significant difference from corresponding control group ($^{**}p<0.01$, $^{*}p<0.05$).

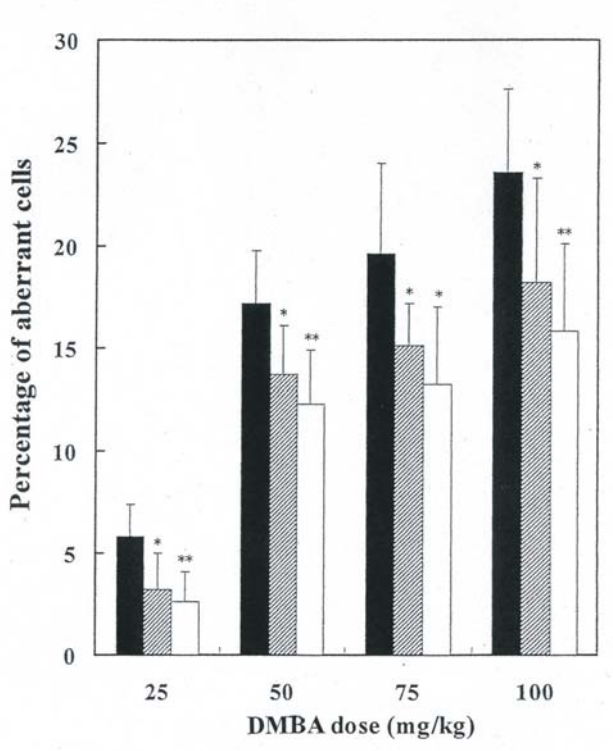

Fig. 2 Relationship between the DMBA dose and the frequency of aberrant cells in the bone marrow of rats (■) receiving only the DMBA injection, rats (▨) given EGCG 24 h before and rats (□) given EGCG 0.5 h before the DMBA injection. Chromosome specimens were prepared 24 h after various doses of DMBA were injected i.p. A dose of 60 mg EGCG/kg body weight was orally given 24 h or 0.5 h before the DMBA injection. Values represent the mean ± SD. Significant difference from corresponding control group ($^{**}p<0.01$, $^{*}p<0.05$).

3. Chromosome analysis

Metaphase cells with one or more CA were scored from 50 well-spread metaphases per rat (therefore 300 metaphases per experimental group). Gaps were defined as achromatic lesions in one or both chromatids not exceeding the width of a chromatid, and breaks as a discontinuity greater than the width of a chromatid, irrespective of whether or not the distal fragment was dislocated. Cells with multiple CA were defined as cells in which the number of CA was too great to count (numerous, above 10). Cells were classified into 1 of 4 categories according to the degree of CA: cells with gaps only, cells with breaks, cells with exchanges, and cells with multiple CA. In the tabulated data, the column headed "percentage of aberrant cells" gives the percentage of damaged cells in the total population of cells analyzed. Damaged cells include the cells with breaks, exchanges and multiple CA, but not the cells with gaps. The severity of damage within a cell is also given as the number of aberrations per cell; cells with multiple CA were counted as 10 aberrations. The suppression rate was calculated from the frequency of aberrant cells.

Results

1. DMBA-induced CA and suppression by EGCG

DMBA-induced CA consisted mainly of gaps and breaks. Cells with multiple CA or exchanges were observed infrequently. On the other hand, the rats which had received DMSO without DMBA showed only a few gaps and breaks in their bone marrow cells. Cells with exchange or multiple CA were not observed. The frequency of aberrant cells in the bone marrow of rats injected with DMBA increased with the lapse of time from exposure, as did the number of aberrations per cell. Maximum levels were seen 24 h after the DMBA injection, after which they declined (Table 1, Fig. 1). The frequency of aberrant cells induced by DMBA increased in proportion to the dose of DMBA (Fig. 2).

Rats given EGCG 24 h before the DMBA injection displayed a considerably suppressed frequency of CA in their bone marrow cells. The suppression was observed 24, 30, 48 and 72 h after the DMBA injection but no suppressive

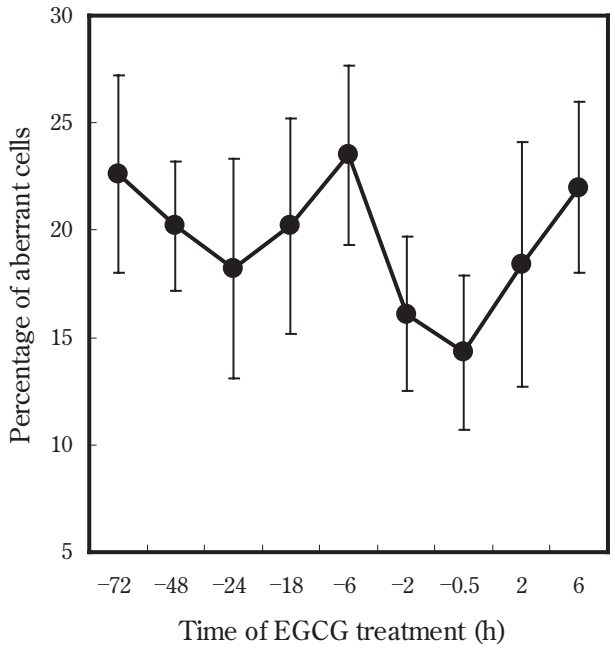

Fig. 3 Effect of EGCG treatment time on the frequency of aberrant cells induced by DMBA. Chromosome specimens were prepared 24 h after 100 mg DMBA/kg body weight was injected i.p. A dose of 60 mg EGCG/kg body weight was orally given at various times before or after the DMBA injection. Each point represents the mean ± SD.

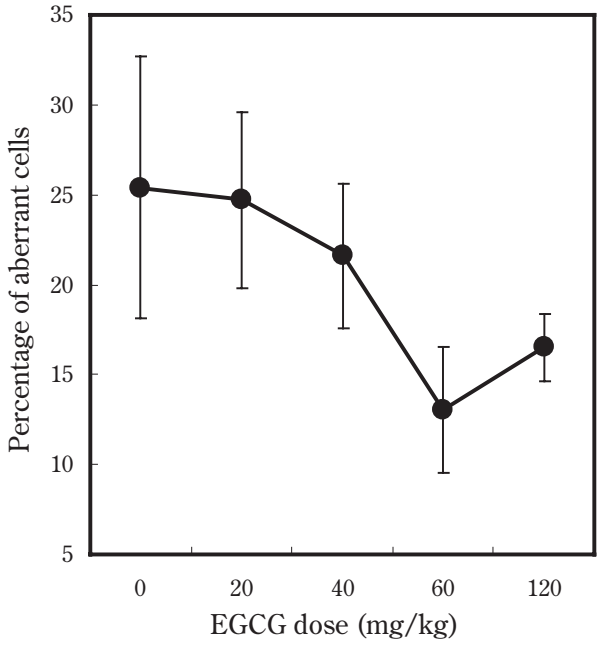

Fig. 4 Relationship between the EGCG dose and the frequency of aberrant cells induced by DMBA. Chromosome specimens were prepared 24 h after 100 mg DMBA/kg body weight was injected i.p. EGCG was orally administered at various doses 0.5 h before the DMBA injection. Each point represents the mean ± SD.

Table 2 Specific details of rat bone marrow cell CA at various times after MMS injection and EGCG pretreatments [a]

Time (h)	Treatment	Percentage of cells with [b]				No. of aberration per cell	Percentage of aberrant cells [c]
		Gap	Break	Ex.	Multi.		
0	Non-MMS	1.8 ± 0.5	0.8 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.01 ± 0.01	0.8 ± 1.0
6	MMS only	6.7 ± 2.7	11.0 ± 4.0	0.3 ± 0.5	0.3 ± 0.5	0.23 ± 0.11	11.7 ± 4.1
	EGCG(−0.5 h) + MMS	5.7 ± 0.5	11.8 ± 4.6	0.2 ± 0.4	0.0 ± 0.0	0.20 ± 0.08	12.0 ± 4.7
12	MMS only	9.1 ± 2.0	18.9 ± 5.2	3.8 ± 1.4	18.0 ± 1.9	2.58 ± 0.33	40.6 ± 5.6
	EGCG(−0.5 h) + MMS	8.6 ± 1.5	17.4 ± 2.5	2.3 ± 1.8	18.9 ± 2.5	2.51 ± 0.22	38.6 ± 2.8
18	MMS only	9.0 ± 1.2	12.6 ± 3.1	2.4 ± 1.1	17.8 ± 6.0	2.39 ± 0.69	32.8 ± 6.1
	EGCG(−24 h) + MMS	9.7 ± 2.1	12.6 ± 5.3	2.6 ± 1.0	18.3 ± 7.1	2.34 ± 0.71	33.4 ± 5.6
	EGCG(−0.5 h) + MMS	9.1 ± 2.1	13.5 ± 5.4	3.0 ± 1.6	16.2 ± 3.3	2.24 ± 0.39	32.7 ± 6.2
24	MMS only	9.5 ± 1.8	11.0 ± 2.4	0.3 ± 0.8	4.5 ± 1.8	0.76 ± 0.21	15.8 ± 3.1
	EGCG(−0.5 h) + MMS	6.5 ± 1.6	10.0 ± 2.4	1.3 ± 1.0	2.8 ± 3.6	0.52 ± 0.27	14.0 ± 3.7
48	MMS only	1.8 ± 0.4	3.0 ± 1.8	0.2 ± 0.4	0.0 ± 0.0	0.04 ± 0.03	3.2 ± 1.8
	EGCG(−0.5 h) + MMS	2.7 ± 1.8	2.4 ± 1.5	0.2 ± 0.4	0.0 ± 0.0	0.03 ± 0.03	2.6 ± 1.8
72	MMS only	1.5 ± 0.8	1.0 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.01 ± 0.01	1.0 ± 0.6

[a] Chromosome specimens were prepared at various times after 75 mg MMS/kg body weight was injected. A dose of 60 mg EGCG/kg body weight was orally given 24 h or 0.5 h before the MMS injection. Values are means ± SD.

[b] Ex., exchange; Multi., multiple CA: cells having more than 10 aberrations.

[c] Cells with gaps are not included in the percentage of aberrant cells.

effect was observed at early periods (6, 12 and 18 h) after the DMBA injection (Fig. 1). On the other hand, rats which received EGCG 0.5 h before the DMBA injection showed a considerable suppression of CA at all periods after the DMBA injection (Fig. 1). The number of aberrations per cell showed the same tendency as the frequency of aberrant cells (Table 1). The significant suppression by EGCG was observed for all doses (25, 50, 75 or 100 mg/kg) of DMBA investigated (Fig. 2). In studying the effect of EGCG pretreatment time on DMBA-induced CA, there was a clear cycling function with 2 points of maximal effect at 24 h and 0.5 h (Fig. 3). The suppressive effect of EGCG given 0.5 h before the DMBA injection was greater than that of EGCG given 24 h before the DMBA

Table 3 Specific details of rat bone marrow cell CA after various doses of MMS with or without EGCG pretreatment[a]

Dose (mg/kg)	Treatment	Percentage of cells with[b]				No. of aberration per cell	Percentage of aberrant cells[c]
		Gap	Break	Ex.	Multi.		
25	MMS only	3.5 ± 1.2	3.3 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.08 ± 0.06	3.3 ± 1.0
	EGCG(−0.5 h) + MMS	2.8 ± 1.3	2.8 ± 1.8	0.3 ± 0.5	0.5 ± 0.5	0.12 ± 0.11	3.7 ± 2.4
50	MMS only	6.3 ± 1.0	8.0 ± 1.5	1.6 ± 1.0	2.4 ± 1.5	0.57 ± 0.18	12.0 ± 1.8
	EGCG(−0.5 h) + MMS	5.4 ± 1.5	6.2 ± 1.1	0.4 ± 0.5	3.4 ± 1.9	0.52 ± 0.18	10.0 ± 1.9
75	MMS only	9.3 ± 1.4	13.5 ± 3.6	2.7 ± 1.2	18.8 ± 5.9	2.48 ± 0.66	35.0 ± 7.7
	EGCG(−24 h) + MMS	9.7 ± 2.1	12.6 ± 5.3	2.6 ± 1.0	18.3 ± 7.1	2.34 ± 0.71	33.4 ± 5.6
	EGCG(−0.5 h) + MMS	9.1 ± 2.1	13.5 ± 5.4	3.0 ± 1.6	16.2 ± 3.3	2.24 ± 0.39	32.7 ± 6.2
100	MMS only	8.8 ± 2.6	18.4 ± 4.6	3.4 ± 2.4	20.4 ± 3.8	2.82 ± 0.50	42.2 ± 7.6

[a] Chromosome specimens were prepared at 18 h after various doses of MMS were injected. A dose of 60 mg EGCG/kg was orally given 24 h or 0.5 h before the MMS injection. Values are means ± SD.
[b] Ex., exchange; Multi., multiple CA: cells having more than 10 aberrations.
[c] Cells with gaps are not included in the percentage of aberrant cells.

Table 4 Suppression of DMBA-induced CA by DHEA[a]

Treatment	Percentage of cells with[b]				No. of aberration per cell	Percentage of aberrant cells[c]
	Gap	Break	Ex.	Multi.		
DMBA only	9.8 ± 2.9	23.0 ± 5.1	0.4 ± 0.9	0.4 ± 0.9	0.39 ± 0.15	23.8 ± 4.9
DHEA(−0.5 h, 30 mg/kg) + DMBA	8.2 ± 2.9	16.7 ± 3.8	0.7 ± 0.8	0.3 ± 0.5	0.32 ± 0.06	17.7 ± 3.3(26) *
DHEA(−0.5 h, 60 mg/kg) + DMBA	8.8 ± 1.7	14.8 ± 3.2	0.0 ± 0.0	0.2 ± 0.4	0.26 ± 0.09	15.0 ± 3.3(37) **
DHEA(−24 h, 60 mg/kg) + DMBA	9.1 ± 1.4	22.9 ± 4.9	0.4 ± 0.5	0.5 ± 0.7	0.42 ± 0.14	23.8 ± 5.4

[a] Chromosome specimens were prepared 24 h after 100 mg DMBA/kg body weight was injected. DHEA dissolved in sesame oil was orally given 24 h or 0.5 h before the DMBA injection. Values are means ± SD.
[b] Ex., exchange; Multi., multiple CA: cells having more than 10 aberrations.
[c] Cells with gaps are not included in the percentage of aberrant cells. Figures in the parenthesis indicate the suppression percent.
* Significantly different from the rat group given only DMBA ($p < 0.05$).
** Significantly different from the rat group given only DMBA ($p < 0.01$).

injection. The suppressive effect of EGCG was dose-dependent in the range of 20−60 mg/kg body weight; higher EGCG doses (120 mg/kg) produced no additional suppression (Fig. 4). Rats given only EGCG (180 mg/kg) without carcinogen displayed no induction of CA in their bone marrow cells (data not shown).

2. MMS-induced CA and effect of EGCG

The frequency of MMS-induced CA in rat bone marrow cells after MMS injection also increased with time and was at the maximum level 12 h post-injection, decreasing thereafter (Table 2). Cells with multiple CA or exchanges induced by MMS were at a higher frequency compared with DMBA. The frequency of aberrant cells induced by MMS increased in proportion to the dose of MMS (Table 3). MMS-induced CA were not suppressed significantly by EGCG given 24 h or 0.5 h before the MMS injection (Table 2).

3. Suppression of DMBA-induced CA by DHEA

DHEA, a typical substrate for hydroxysteroid sulfotransferases, significantly suppressed DMBA-induced CA when given at 0.5 h before DMBA injection but not at 24 h (Table 4).

Discussion

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants. DMBA is one of the most potent carcinogenic PAH and can induce in experimental animals not only skin tumors (Boyland et al., 1965; Kinoshita and Gelboin, 1972; Slaga et al., 1979) but also leukemia (Huggins and Sugiyama, 1966) and mammary cancers (Huggins et al., 1961; Russo and Russo, 1991). DMBA, in common with other PAH or AFB_1, requires metabolic activation by hepatic microsomal enzyme systems to exert its mutagenic or carcinogenic activity. Two ultimate carcinogenic metabolites of DMBA, a bay region diol-epoxide (DMBA-trans-3,4-diol-1,2-epoxide) (Huberman et al., 1979; Moschel et al., 1977) and 7-hydroxymethyl-12-methylbenz[*a*]anthracene (7-HMBA) sulfate ester (Watabe et al., 1982; 1985), are well known. DMBA is metabolized by cytochrome P450 (CYP) enzymes and microsomal epoxide hydrolase into both inert metabolites and electrophilic metabolites capable of producing DNA adducts. CYP1B1 oxidizes DMBA to DMBA-3,4-epoxide, which is hydrolysed by microsomal epoxide hydrolase to DMBA-3,4-diol. This metabolite is further oxidized by either CYP1A1 or CYP1B1 to the ultimate carcinogenic

metabolite, DMBA-3,4-diol-1,2-epoxide (Miyata et al., 1999). The first step of another pathway of DMBA metabolic activation is the hydration of the position 7 methyl group. DMBA is metabolized by hepatic microsomal cytochrome P450 CYP1A1 to 7-HMBA (Christou et al., 1984; Wilson et al., 1984), which is transformed to reactive 7-HMBA sulfate ester by hepatic sulfotransferase. 7-HMBA sulfate ester is nonenzymatically converted into DMBA-7-methylene carbonium ion which rapidly reacts with endogenous substrates such as DNA and protein (Watabe et al., 1983; 1985).

In the present study, we have investigated the suppressive effect of EGCG, the major polyphenolic constituent of green tea, on DMBA-induced CA in rat bone marrow cells. EGCG given before the DMBA injection significantly suppressed DMBA-induced CA. The suppressive effect of EGCG given 24 h before was observed 24, 30, 48 and 72 h after the DMBA injection, but no suppressive effect at the early period (6, 12 and 18 h) after the DMBA treatment. On the other hand, EGCG given 0.5 h before the DMBA injection suppressed DMBA-induced CA at all periods after the DMBA injection (Fig. 1). Rats given EGCG 0.5 h before DMBA showed greater suppressive effects than rats given EGCG 24 h before DMBA. We have been studying the suppressive effect of antimutagens on carcinogen-induced CA for more than 20 years, and EGCG is the first antimutagen that displays two temporal points of maximal action. This result suggests that EGCG has two different suppression mechanisms for DMBA-induced CA depending on the administration time. However, the suppression mechanism of EGCG has not yet been elucidated sufficiently.

We have previously reported (Ito et al., 1982; 1984; Sugiyama et al., 2002) that the administration of Sudan III, an inducer of drug-metabolizing enzymes, 24 h before the DMBA injection potently suppressed DMBA-induced CA, although its administration 2 h before did not. The suppression of DMBA-induced CA by Sudan III was observed at all periods after the DMBA treatment. It was suggested that the suppressive effect of Sudan III is due to the induction of cytochrome P450 and GST by Sudan III in rat liver. However, the administration of EGCG 24 h before sacrifice did not result in a significant increase in cytochrome P450 content or GST activity (Ito and Ito, 2001). Sohn et al. (1994) and Bu-Abbas et al. (1994) have reported that rats administered GTE for 4 or 6 weeks displayed no significant increase in total cytochrome P450 content in the liver, but did display a significant increase in *O*-dealkylase activity of ethoxyresorufin (CYP1A1), methoxyresorufin (CYP1A2) and pentoxyresorufin (CYP2B1). Of the phase II enzymes, UDP-glucuronyltransferase was increased, but GST was not.

The peak in the percentage of aberrant cells induced by DMBA seems to shift from 24 h to 18 h by EGCG given 24 h before the DMBA injection (Fig. 1), that is to say, EGCG given 24 h before seems to accelerate the rate of DMBA metabolism in rat liver. Furthermore, CA induced by MMS or *n*-butyl-*N*-nitrosourea (data not shown), a direct-acting carcinogen which does not require metabolic activation by microsomal enzyme systems, were not suppressed by EGCG. Therefore, the suppression of DMBA-induced CA by EGCG given 24 h before may result from the modification of microsomal enzyme systems.

We have previously reported (Ito and Ito, 2001) that EGCG given 24 h before an AFB_1 injection significantly suppressed AFB_1-induced CA but EGCG given 2 h before did not. The suppression of AFB_1-induced CA by EGCG was also observed only at the late period (18, 24 and 48 h) after the AFB_1 injection and was explained by the modification of microsomal enzyme system, that is, the administration of EGCG decreased the active metabolites of AFB_1 reaching target cells by the modulation of AFB_1 metabolism in rat liver and consequently suppressed AFB_1-induced CA. The major ultimate metabolite of AFB_1 is considered to be AFB_1-2,3-epoxide (Schoenhard et al., 1976; Schoental, 1970; Swenson et al., 1977). Recent evidence indicates that cytochromes P450 CYP3A2 and CYP2C11 are responsible for AFB_1-epoxidation. Inactivation of AFB_1 to AFM_1 is mediated via CYP1A1 and CYP1A2; these cytochrome P450 species are not responsible for AFB_1-epoxidation. Similarly, AFQ_1 formation is mediated via CYP3A2 and CYP2B (Eaton and Gallagher, 1994; Buetler et al., 1996). Furthermore, Qin et al. (1997) have reported that the pretreatment of rats with 0.5% green tea in their drinking water for 2 or 4 weeks did not produce a significant increase in cytochrome P450 content but enhanced microsome-mediated formation of non-toxic hydroxylated metabolites of AFB_1 by 2−3 fold. These reports support our explanation.

Surh et al. (1991) reported that the sulfotransferase activity for 7-HMBA was strongly inhibited by DHEA, a typical substrate for hydroxysteroid sulfotransferases. DHEA given 0.5 h before the DMBA injection significantly suppressed DMBA-induced CA but DHEA given 24 h before did not (Table 4). Therefore, the suppression of DMBA-induced CA by EGCG given 0.5 h before may be due to the inhibition of sulfotransferase activity by EGCG. Further studies are needed for clarification.

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