Inhibitory Effect of Green and Black Tea on Tumor Growth (44371)

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Abstract. **The administration of green tea, black tea, or (–)-epigallocatechin gallate inhibited the growth of established nonmalignant and malignant tumors in tumor-bearing mice. In experiments with black tea, we found that its oral administration inhibited DNA synthesis and enhanced apoptosis in both nonmalignant and malignant tumors in tumor-bearing mice.** [P.S.E.B.M. 1999, Vol 220]

During the past several years, our laboratory and many others have carried out studies on factors that modulate carcinogenesis in mouse skin. We believe that such studies with mouse skin serve as a useful model not only for cancer formation in the skin but also for carcinogenesis in other organs with epithelial cells such as the lung, colon, breast, esophagus, trachea, larynx, and bladder. In the present manuscript, we describe some studies on the inhibitory effects of green and black tea on carcinogenesis and on tumor growth.

Inhibitory Effect of Green and Black Tea on Tumorigenesis in Cancer Chemoprevention Studies

In earlier studies, we found that oral administration of green tea or black tea to DMBA-pretreated mice inhibited ultraviolet B (UVB)-induced carcinogenesis (1). In this cancer chemoprevention study, tea administration inhibited the percentage of mice with tumors and the number of tumors per mouse. It was of considerable interest that tea administration not only decreased the number of UVB-induced tumors per mouse, but the tumors from mice treated with UVB + tea were smaller in size than the tumors from animals treated only with UVB (Table I).

In an additional cancer chemoprevention study, we treated SKH-1 mice with 30 mJ/cm^2 twice a week until one mouse developed a tumor (at 22 weeks). This mouse was discarded, and UVB-administration was stopped. The remaining tumor-free mice had a high risk of developing tumors during the next several months in the absence of further UVB administration. Oral administration of green or black tea to these high-risk mice for 23 weeks inhibited the formation of tumors, and the tumors that were observed were smaller in size than those observed in mice that were not treated with tea (Table II). The data described in Tables I and II indicate that tea slowed the formation of skin tumors and/or inhibited the growth of these tumors once they were formed.

Inhibitory Effect of Green and Black Tea on the Growth of Established Skin Tumors in Mice

To test the possibility that tea inhibits the growth of well-established tumors, we generated papilloma-bearing animals by treatment of CD-1 mice with 7,12-dimethylbenz[a]anthracene (DMBA) or UVB followed by twice a week topical administration of 12-O-tetradecanoylphorbol-13-acetate (TPA). After obtaining papilloma-bearing mice, TPA was stopped, and 2 weeks later we treated the tumor-bearing mice with green tea as their sole source of drinking fluid or we injected a green tea polyphenol fraction or (–)-epigallocatechin gallate (EGCG) i.p. 3 times a week (Figs. 1, 2). The results of this study indicated that continuous oral administration of green tea, i.p. injections of a green tea polyphenol fraction three times a week, or i.p. injections of EGCG three times a week for 4–10 weeks inhibited the growth of established skin papillomas (Figs. 1, 2) (2). In some experiments, administration of green tea or tea constituents caused complete inhibition of tumor growth or tumor regression. Examination of the data from all ten experiments revealed that none of the 220 papilloma-bearing control mice given only water as their drinking fluid exhibited complete tumor regression, but complete tumor regres-

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0037-9727/99/2204-0229$10.50/0

Table I. Effect of Oral Administration of Green Tea on the Number and Size of Skin Tumors Induced by Ultraviolet B Light (UVB) in SKH-1 Mice

Treatment	Number of mice	Percentage of mice with tumors	Number of tumors	Total volume of tumors (mm^3)	Volume per tumor (mm^3)
60 mJ/cm^2 twice weekly					
Water	30	100	363	22,506	62 ± 24
Green tea (2 mg/ml)	29	86	165	1,485	9 ± 2
Green tea (4 mg/ml)	30	77	116	1,624	14 ± 4
30 mJ/cm^2 twice weekly					
Water	30	83	117	3,627	31 ± 13
Green tea (2 mg/ml)	28	70	67	938	14 ± 7
Green tea (4 mg/ml)	29	66	55	275	5 ± 2

Note. Female SKH-1 mice (6–8 weeks old) were treated topically with DMBA (200 nmol). Two weeks later the mice were treated with UVB twice a week together with water or green tea (2–4 mg tea solids/ml) as their sole source of drinking fluid continuously for 30 weeks. Each value represents the mean or the mean ± SE. (Taken from Ref. 1.)

Table II. Effect of Oral Administration of Green or Black Tea on Tumor Formation in Mice Previously Treated with UVB for 22 Weeks (High Risk Mice)

Treatment	Keratoacanthomas			Squamous cell carcinomas		
	Percentage of mice with tumors	Tumors per mouse	Volume per tumor (mm^3)	Percentage of mice with tumors	Tumors per mouse	Volume per tumor (mm^3)
Water	79	4.00 ± 0.65	2.8 ± 0.5	61	1.82 ± 0.41	40 ± 20
Green tea	46	1.25 ± 0.34	0.5 ± 0.1	39	0.68 ± 0.22	13 ± 4
Black tea	56	1.26 ± 0.38	0.4 ± 0.1	44	0.70 ± 0.19	13 ± 7

Note. Female SKH-1 mice were treated with UVB (30 mJ/cm^2) twice a week for 22 weeks. One week later, mice (30/group) with no observable tumors were given green or black tea (6 mg tea solids/ml) as their sole source of drinking fluid for 23 weeks. Each value is the mean ± SE.

sion was observed in 14 of 346 papilloma-bearing mice (4%) that were treated with green tea in the drinking water or with i.p. injections of green tea constituents. These observations indicated that oral administration of green tea, i.p. administration of a green tea polyphenol fraction, or i.p. administration of EGCG inhibited the growth and/or caused the regression of established experimentally induced skin papillomas.

In another experiment, skin tumors were generated by treating SKH-1 mice with UVB (30 mJ/cm^2) twice weekly for 22 weeks. UVB administration was stopped, and nonmalignant and malignant tumors were allowed to develop during the following 13 weeks. These tumor-bearing mice were then treated with black tea (6 mg tea solids/ml) as their sole source of drinking fluid for 11 weeks. In this experiment, tumor growth as measured by an increase in tumor volume per mouse, was inhibited by 70% (Fig. 3) (3). Histologic examination revealed that tea-treated mice had a 58% decrease in the number of nonmalignant tumors (primarily keratoacanthomas) per mouse and a 54% decrease in the number of squamous cell carcinomas per mouse (Table III). In addition, administration of black tea decreased the volume per tumor by 60% for nonmalignant tumors and by 84% for squamous cell carcinomas (Table IV). Mechanistic studies with tumors from these mice revealed that administration of black tea decreased the bromodeoxyuridine labeling index in squamous cell papillomas, keratoacanthomas, and squamous cell carcinomas by 56%, 45%, and 35%, respectively (Table V), and the apoptosis index (TUNEL assay) was increased by 44%, 100%, and 95%, respectively (Table VI). Similar results were observed when apoptosis was measured by counting morphologically distinct apoptotic cells. The results of this study indicate that oral administration of black tea to mice with well-established nonmalignant and malignant skin tumors inhibited proliferation and enhanced apoptosis in these tumors (3). Recent studies indicate that oral administration of a green tea polyphenol fraction inhibits the progression of papillomas to carcinomas (4) and that oral administration of EGCG inhibits the metastasis of B16 mouse melanoma cells in mice (5).

Effect of Tea or Tea Components on the Growth of Transplanted Tumors

In one study, oral administration of a concentrated green tea infusion twice a day starting 1 week before i.p. injection of Ehrlich ascites tumor cells and continuing for an additional 8 days resulted in a 41% inhibition of tumor growth (6). In another study, oral administration of 400–800 mg/kg of a decaffeinated instant green tea powder once a day for 4 days starting 24 hr after s.c. inoculation of mice with sarcoma 180 cells resulted in a 50%–59% inhibition of

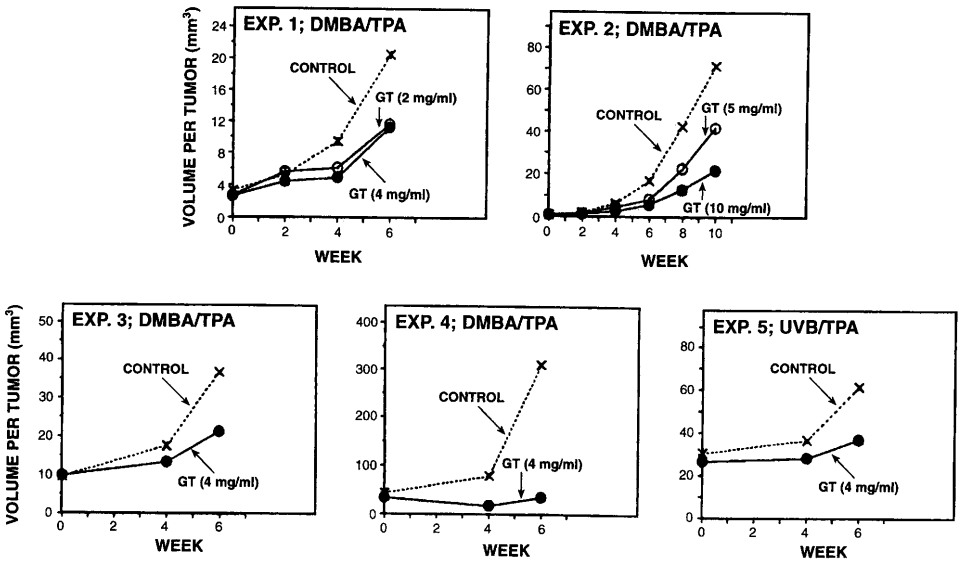

Figure 1. Effect of oral administration of green tea on the growth and regression of skin papillomas in mice. Female CD-1 mice (6 weeks old) were initiated with DMBA or UVB and promoted with TPA as described elsewhere (2). One week after stopping TPA administration, tumor-bearing mice were treated with green tea as their sole source of drinking fluid for 6–10 weeks. The concentration of tea (expressed as mg of tea solids per ml) is indicated (taken from Ref. 2).

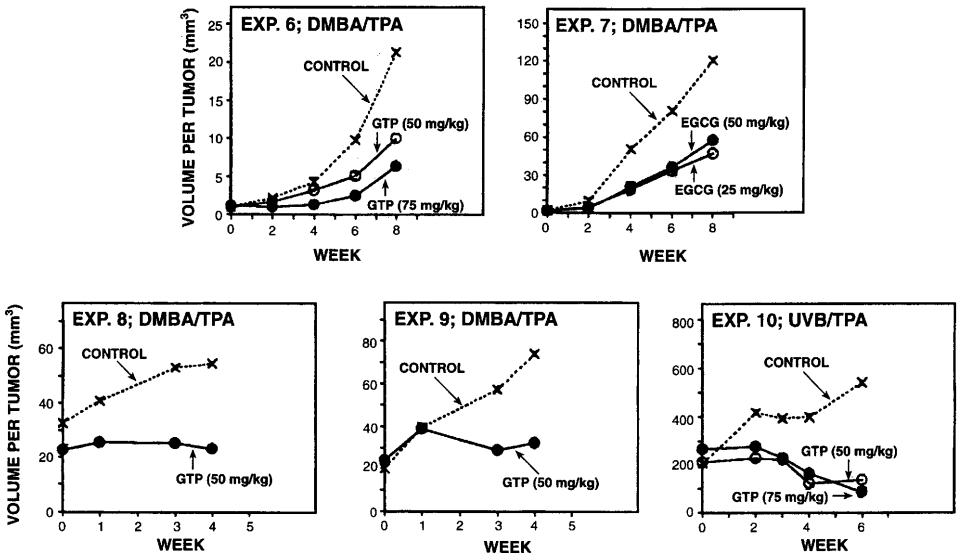

Figure 2. Effect of i.p. administration of a green tea polyphenol fraction or (−)-epigallocatechin gallate (EGCG) on the growth and regression of skin papillomas in mice. Female CD-1 mice (6 weeks old) were initiated with DMBA or UVB and promoted with TPA as described elsewhere (2). One week after stopping TPA administration, tumor-bearing mice were treated with i.p. injections of a green tea polyphenol fraction or EGCG 3 times a week for 4–8 weeks. The amount of tea constituent administered for each injection is given in the figure (taken from Ref. 2).

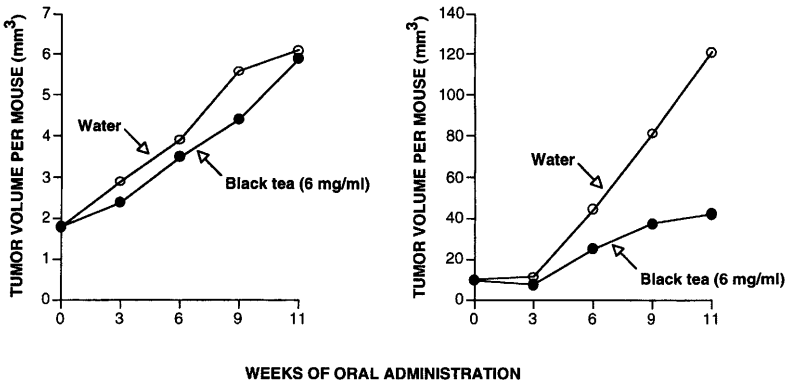

Figure 3. Effect of oral administration of black tea on the development and growth of skin tumors in tumor-bearing SKH-1 mice. Female SKH-1 mice (7–8 weeks old) were irradiated with UVB (30 mJ/cm^2) twice weekly for 22 weeks, and UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) were then treated with lyophilized black tea (6 mg tea solids/ml) or water as the drinking fluid for 11 weeks (taken from Ref. 3).

tumor growth during a 21-day interval (7). In another study, i.p. or s.c. injections of a green tea polyphenol fraction or (−)-epigallocatechin gallate (EGCG) into mice that were inoculated s.c. with cells from a solid Ehrlich tumor or with cells from a sarcoma 180 tumor inhibited tumor growth by 20%–56% (8). Feeding a green tea polyphenol fraction in the diet was also reported to inhibit the growth of sarcoma 180 cells that were inoculated into mice (8).

Table III. Effect of Oral Administration of Black Tea on the Incidence and Multiplicity of Squamous Cell Papillomas, Keratoacanthomas and Squamous Cell Carcinomas in Tumor-Bearing SKH-1 Mice

Treatment	Number of mice	Squamous cell papillomas		Keratoacanthomas		Total nonmalignant tumors		Squamous cell carcinomas		Total tumors	
		Percentage of mice with tumors	Tumors per mouse	Percentage of mice with tumors	Tumors per mouse	Percentage of mice with tumors	Tumors per mouse	Percentage of mice with tumors	Tumors per mouse	Percentage of mice with tumors	Tumors per mouse
Water	15	33.3	0.53 ± 0.22	73.3	3.33 ± 0.80	80.0	3.87 ± 0.94	53.3	1.40 ± 0.43	86.7	5.27 ± 1.22
Black tea	14	14.3 (57)	0.21 ± 0.15 (60)	57.1 (22)	1.43 ± 0.47 (57)	64.3 (20)	1.64 ± 0.49 (58)	42.9 (20)	0.64 ± 0.25 (54)	71.4 (18)	2.29 ± 0.65 (57)

Note. Female SKH-1 mice (7–8 weeks old) were irradiated with UVB (30 mJ/cm^2) twice weekly for 22 weeks, and UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) with the same number and size of tumors per group were then treated with lyophilized black tea (6 mg tea solids/ml) or water as the drinking fluid for 11 weeks (see Fig. 3). The animals were sacrificed, and the tumors were characterized by histopathology studies. Each value is the mean ± SE and the numbers in parentheses represent percentage inhibition. (Taken from Ref. 3.)

Table IV. Effect of Oral Administration of Black Tea on the Size of Established Papillomas, Keratoacanthomas and Squamous Cell Carcinomas in SKH-1 Mice

Treatment	Number of mice	Squamous cell papillomas		Keratoacanthomas		Total nonmalignant tumors		Squamous cell carcinomas		Total tumors	
		Volume per tumor (mm^3)	Tumor volume per mouse (mm^3)	Volume per tumor (mm^3)	Tumor volume per mouse (mm^3)	Volume per tumor (mm^3)	Tumor volume per mouse (mm^3)	Volume per tumor (mm^3)	Tumor volume per mouse (mm^3)	Volume per tumor (mm^3)	Tumor volume per mouse (mm^3)
Water	15	1.8 ± 0.6	0.9 ± 0.5	1.5 ± 0.2	4.9 ± 1.4	1.5 ± 0.2	5.8 ± 1.8	233.5 ± 135.2	326.9 ± 196.7	63.2 ± 37.2	332.7 ± 196.6
Black tea	14	1.7 ± 1.2 (6)	0.4 ± 0.3 (56)	0.4 ± 0.1 (73)	0.6 ± 0.2 (88)	0.6 ± 0.2 (60)	1.0 ± 0.3 (83)	37.7 ± 25.9 (84)	24.3 ± 17.0 (93)	11.0 ± 7.6 (83)	25.2 ± 17.3 (92)

Note. Female SKH-1 mice (7–8 weeks old) were irradiated with UVB (30 mJ/cm^2) twice weekly for 22 weeks, and UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) with the same number and size of tumors per group were then treated with lyophilized black tea (6 mg tea solids/ml) or water as the drinking fluid for 11 weeks (see Fig. 3). The animals were sacrificed, the size of each tumor was determined, and the tumors were characterized by histopathology studies. Each value is the mean ± SE, and the numbers in parentheses represent percentage inhibition. (Taken from Ref. 3.)

Table V. Effect of Oral Administration of Black Tea on Bromodeoxyuridine Incorporation into the DNA of Established Papillomas, Keratoacanthomas, and Squamous Cell Carcinomas in SKH-1 Mice

Treatment	Number of mice	Bromodeoxyuridine labeling index					
		Focal epidermal hyperplasia	Squamous cell papillomas	Keratoacanthomas	Nonmalignant tumors	Squamous cell carcinomas	Total tumors
Water	15	14.1 ± 1.5	33.9 ± 6.5	35.2 ± 2.9	35.0 ± 2.6	54.5 ± 4.3	40.2 ± 2.4
Black tea	14	14.3 ± 1.9 (–1)	15.0 ± 4.4 (56)	19.5 ± 2.4 (45)	19.0 ± 2.1 (46)	35.2 ± 4.2 (35)	23.5 ± 2.3 (42)

Note. Female SKH-1 mice (7–8 weeks old) were irradiated with UVB (30 mJ/cm^2) twice weekly for 22 weeks, and UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) were then treated with lyophilized black tea (6 mg tea solids/ml) or water as the drinking fluid for 11 weeks (see Fig. 3). Bromodeoxyuridine (BrdU; 50 mg/kg) was injected i.p., and the animals were sacrificed 1 hr later. Bromodeoxyuridine positive cells in each area of focal epidermal hyperplasia and in each tumor were counted and expressed as the number of positive cells per 100 cells counted. Each value is the mean ± SE, and the numbers in parentheses represent percentage inhibition. (Taken from Ref. 3.)

The possibility that EGCG could inhibit the growth of human breast or prostate tumors was recently evaluated in athymic immunodeficient mice by S. Liao at the University of Chicago (9). In these studies it was found that treatment of athymic mice with daily i.p. injections of EGCG (≈50 mg/kg) inhibited the growth of transplanted MCF-7 human breast cancer cells and LNCaP 104R human prostate cancer cells (Fig. 4).

Summary and Concluding Remarks

In conclusion, the administration of green tea, black tea, or EGCG inhibits the growth of established nonmalignant and malignant skin tumors in tumor-bearing mice. In experiments with black tea, we found that its oral administration inhibited DNA synthesis and enhanced apoptosis in both nonmalignant and malignant tumors in tumor-bearing

Table VI. Effect of Oral Administration of Black Tea on Apoptosis in Established Papillomas, Keratoacanthomas, and Squamous Cell Carcinomas in SKH-1 Mice

Treatment	Number of mice	Apoptosis index (mean ± SE)					
		Focal epidermal hyperplasia	Squamous cell papillomas	Keratoacanthomas	Total nonmalignant tumors	Squamous cell carcinomas	Total tumors
Water	15	1.37 ± 0.22	1.16 ± 0.32	0.79 ± 0.10	0.84 ± 0.10	0.77 ± 0.19	0.82 ± 0.09
Black tea	14	2.15 ± 0.24 (57%)	1.67 ± 0.44 (44%)	1.58 ± 0.22 (100%)	1.59 ± 0.19 (89%)	1.50 ± 0.33 (95%)	1.56 ± 0.17 (95%)

Note. Female SKH-1 mice (7–8 weeks old) were irradiated with UVB (30 mJ/cm^2) twice weekly for 22 weeks, and UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) were then treated with lyophilized black tea (6 mg tea solids/ml) or water as the drinking fluid for 11 weeks (see Fig. 3). The apoptosis index was determined by evaluating the number of cells stained and the intensity of staining. Each value is the mean ± SE, and the numbers in parentheses represent percentage increase. (Taken from Ref. 3.)

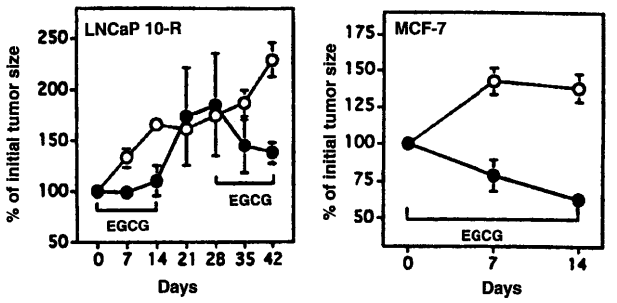

Figure 4. Growth inhibition and regression of human prostate and breast tumors in athymic mice by daily i.p. injections of EGCG (1 mg per mouse). The open circles are from vehicle-treated mice, and the closed circles are from EGCG-treated mice (taken from Ref. 9).

mice. In some cancer chemoprevention studies, caffeine was found to be a major inhibitory substance in both green and black tea (10, 11), and further work is needed to determine its effect on tumor growth. Our studies and those by others suggest the initiation of carefully controlled clinical trials to determine whether administration of tea, EGCG, caffeine or other tea constituents alone or together with other agents can prevent the formation of tumors or inhibit tumor growth in humans. Patients with leukoplakia or head neck cancer may be particularly good patient populations for these studies.

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