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Effects of Tea, Decaffeinated Tea, and Caffeine on UVB Light-induced Complete Carcinogenesis in SKH-1 Mice: Demonstration of Caffeine as a Biologically Important Constituent of Tea[1]

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ABSTRACT

Oral administration of green or black tea inhibited UVB light-induced complete carcinogenesis in the skin of SKH-1 mice. Green tea was a more effective inhibitor than black tea. Oral administration of decaffeinated green or black tea resulted in substantially less inhibitory activity than did administration of the regular teas, and in one experiment, administration of a high-dose level of the decaffeinated teas enhanced the tumorigenic effect of UVB. Oral administration of caffeine alone had a substantial inhibitory effect on UVB-induced carcinogenesis, and adding caffeine to the decaffeinated teas restored the inhibitory effects of these teas on UVB-induced carcinogenesis. In additional studies, topical application of a green tea polyphenol fraction after each UVB application inhibited UVB-induced tumorigenesis. The results indicate that caffeine contributes in an important way to the inhibitory effects of green and black tea on UVB-induced complete carcinogenesis.

INTRODUCTION

Sunlight-induced nonmelanoma skin cancer is a major cancer in the United States and in other temperate parts of the world (1, 2). Although most skin cancers observed by dermatologists are squamous cell carcinomas and basal cell carcinomas that are easily cured if detected early, people still die from these cancers, as well as from the more dangerous sunlight-induced melanomas. In earlier studies, Wang *et al.* (3) reported an inhibitory effect of a p.o. administered green tea polyphenol fraction on UVB light-induced complete tumorigenesis in the skin of SKH-1 mice, but the histology of the tumors was not investigated. In additional studies, our laboratory reported inhibitory effects of p.o. administered green tea, black tea, decaffeinated green tea, and decaffeinated black tea on UVB-induced formation of papillomas, keratoacanthomas, and squamous cell carcinomas in mice previously initiated with DMBA[4] (4, 5). It was observed that the decaffeinated teas were somewhat less effective than the regular teas (5). In the present study, we evaluated the effect of p.o. administered green tea, black tea, decaffeinated green tea, decaffeinated black tea, and caffeine on UVB-induced complete carcinogenesis in the skin of SKH-1 mice. The results of this study indicate an inhibitory effect of p.o. administered green and black tea on UVB-induced complete carcinogenesis, but the decaffeinated teas were either inactive (at moderate dose levels) or they enhanced the tumorigenic effect of UVB (at a high dose level). Oral administration of caffeine had an inhibitory effect on UVB-induced complete carcinogenesis.

MATERIALS AND METHODS

Chemicals. Purified water was prepared by reverse osmosis and was used for the preparation of all tea infusions. Acetone and 10% buffered formalin phosphate were obtained from Fisher Scientific (Springfield, NJ). Methanol and ethyl acetate were obtained from Fisher Scientific. Caffeine (>99% purity) was obtained from the Sigma Chemical Co. (St. Louis, MO).

Animals. Female SKH-1 hairless mice (6–7 weeks old) were purchased from Charles River Breeding Laboratories (Kingston, NY). The animals were kept in our animal facility for at least 1 week before use. Mice were given water and Purina Laboratory Chow 5001 diet from the Ralston-Purina Co. (St. Louis, MO) *ad libitum*, and they were kept on a 12 h light/12 h dark cycle.

Preparation and Composition of Teas. Commercial-grade tea leaves and lyophilized hot water extracts of commercial-grade tea leaves (tea extract solids; lyophilized tea solids) were provided by the United States Tea Association (New York, NY). Decaffeinated tea leaves, prepared by extracting the leaves with supercritical CO_2 (CO_2 under high pressure at ~65°C), were used for the preparation of decaffeinated teas. This process removed almost all caffeine from the tea leaves without removing the polyphenolic catechins (5). It should also be noted that the decaffeination process removes small amounts of tea components in addition to caffeine,[5] but the identification of these components requires further investigation.

For experiments 1 and 2, we prepared solutions of caffeine (0.24 or 0.72 mg/ml water; 0.024 or 0.072% solutions) or solutions of lyophilized tea solids (3 or 9 mg/ml water; 0.3 or 0.9% solutions). For experiment 3, we prepared tea infusions from tea leaves with a commercial Bunn automatic basket tea brewer as described earlier (5). Briefly, tea leaves (50 g) were placed in a filter paper-lined brewing basket, and 4 liters of hot deionized water were passed in the brewing machine through the tea leaves to obtain a 1.25% tea infusion (1.25 g of tea leaf/100 ml of water). The resulting tea brews in experiment 3 were similar to those consumed by humans, and they contained about 4 mg of tea solids per ml. The compositions of the tea leaf infusions and the reconstituted lyophilized teas were similar to what was reported earlier (5). Notably, the concentrations of polyphenolic catechins in the decaffeinated tea brews did not differ from the concentrations of these substances in the regular tea brews (5).

Preparation and Composition of Green Tea Polyphenol Fraction. Commercial-grade green tea leaves (100 g) were extracted three times with 300 ml of methanol at 50°C for 3 h, and the samples were filtered after each extraction. Solvent was removed from the combined extract with a vacuum rotary evaporator. The residue was dissolved in 500 ml of water (50°C) and extracted three times with 200 ml of hexane to remove pigments and three times with 200 ml of chloroform to remove caffeine. The aqueous phase was extracted three times with 180 ml of ethyl acetate, and the ethyl acetate was evaporated to dryness. The residue was redissolved in 300 ml of water, and this solution was lyophilized to obtain 8–9 g of green tea polyphenol fraction. The green tea polyphenol fraction used in the present study was prepared and analyzed as described earlier (6). The composition of the green tea polyphenol fraction was: 49.5% (−)-epigallocatechin gallate, 11.5% (−)-epigallocatechin, 11.4% (−)-epicatechin gallate, 7.6% caffeine, 6.1% (−)-epicatechin, 0.5% (+)-catechin, and 0.4% gallic acid.

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[4] The abbreviations used are: DMBA, 7,12-dimethylbenz[a]anthracene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

[5] D. Balentine, personal communication.

UVB Light. Treatment of SKH-1 mice with UVB was done as described earlier (5). We used UV lamps (model no. FS72T12-UVB-HO) that emit UVB (280–320 nm; 75–80% of total energy) and UVA (320–375 nm; 20–25% of total energy). The UV lamps were obtained from the Voltare Co. (Fairfield, CT). Although all data are expressed as exposure to UVB, some additional exposure to UVA also occurred, as indicated above. Exposure to UV (UVA plus UVB) was performed as follows. Mice were housed in 25.4 × 45.7 cm plastic boxes, with 10 mice per box. Six boxes (without tops) were placed under eight UV lamps (50.8 × 182.9 cm), and the boxes were systematically rotated during the course of the study to compensate for possible small differences in flux at various positions under the lamps. The distance between the UV lamps and the backs of the mice or the UVB detector was 43.2 cm. The amount of exposure time for a 30 mJ/cm^2 dose of UVB was 25–30 s.

UVB-induced Tumorigenesis Protocol. In experiments 1–3, SKH-1 female mice (30 per group) were given either water or tea preparations as their sole source of drinking fluid. Two weeks before twice-weekly UVB treatment (30 mJ/cm^2) began, the mice were given tea solutions that were 25, 50, and 75% of full-strength (2 days at each concentration), and then, beginning 1 week before the initiation of UVB treatment, mice were given full-strength tea preparations, and these preparations were given until the end of the experiment. For the study on the effect of topical application of a green tea polyphenol fraction on UVB-induced tumorigenesis, we treated mice topically with 200 μl of acetone or a caffeine-containing green tea polyphenol fraction (2.4 or 7.2 mg) in 200 μl of acetone immediately after each application of UVB (30 mJ/cm^2) twice a week.

For all animal studies, body weights and skin tumors greater than 1 mm in diameter were recorded every 2 weeks. Tumor volumes were determined in live animals by measuring the three-dimensional size (height, length, and width) of each tumor and by using the average of the three measurements as the diameter. The radius (diameter/2) was determined, and the volume was calculated by:

$$\text{Volume} = \frac{4\pi r^3}{3}$$

For histopathology studies, the animals were killed, and the dorsal skins were removed and stapled flat to a plastic sheet before they were placed in 10% buffered formalin phosphate for histological examination. For histology, thin skin samples were taken to include each of the grossly counted tumors. In specimens lacking macroscopic tumors, a single representative sample of skin was processed. Four- to 5-μm sections were stained with H&E and evaluated for tumor classification by light microscopy. In experiment 1, histopathology examination was done only for large masses (>5 mm in diameter) from mice given water, 0.3% green tea, or 0.9% green tea as their drinking fluid. In experiments 2 and 3, histopathology examination was done with all masses.

Statistical analysis was by the Fisher's exact test and the Student's *t* test.

RESULTS

Effect of Oral Administration of Green Tea, Black Tea, Decaffeinated Green Tea, Decaffeinated Black Tea, and Caffeine on UVB-induced Complete Carcinogenesis. In experiment 1, SKH-1 mice were treated with UVB (30 mJ/cm^2) twice a week for 35 weeks, and the animals were killed 4 weeks later. The animals had an average of 8.9 tumors per mouse, and the tumor volume per mouse was 351 mm^3 just before the animals were killed, at 39 weeks after the start of UVB treatment (Table 1, experiment 1). The oral administration of 0.3% lyophilized green tea (3 mg of tea solids/ml) and 0.9% lyophilized green tea (9 mg of tea solids/ml) as the drinking fluid to the UVB-treated mice decreased the number of tumors per mouse by 35 and 94%, respectively, and the tumor volume per mouse was decreased by 49 and 97%, respectively. Administration of 0.3–0.9% lyophilized decaffeinated green tea had little or no effect on the number of tumors per mouse and had a modest but significant inhibitory effect on tumor volume per mouse (Table 1, experiment 1). Administration of 0.24 and 0.72 mg of caffeine per ml of drinking water (about the same concentrations that are present in 0.3 and 0.9%

Table 1 *Effect of oral administration of green tea, black tea, decaffeinated green tea, decaffeinated black tea, and caffeine on UVB-induced skin tumorigenesis: observations on live animals*

Experiment	Treatment[a]	Tea solids or caffeine (mg/ml)	No. of mice	Body weight (g)	% of mice with tumors	Tumors per mouse	Tumor volume per mouse (mm^3)
1[b]	Water	0	28	30.5 ± 0.5	97	8.9 ± 0.9	351 ± 84
	0.3% LGT	3.0	28	31.9 ± 0.4[c]	79[c]	5.8 ± 1.0[c]	178 ± 68[d]
	0.9% LGT	9.0	29	30.4 ± 0.4	31[c]	0.5 ± 0.2[c]	9 ± 7[c]
	0.3% LDGT	3.0	29	32.2 ± 0.5[c]	100	8.4 ± 1.0	157 ± 34[c]
	0.9% LDGT	9.0	29	30.3 ± 0.4	100	8.8 ± 0.7	149 ± 48[c]
	0.24% caffeine	0.24	30	32.3 ± 0.6[c]	96	6.2 ± 0.6[c]	159 ± 35[c]
	0.072% caffeine	0.72	28	30.7 ± 0.4	93	6.1 ± 0.8[c]	66 ± 21[c]
2[e]	Water	0	27	32.4 ± 0.6	100	15.6 ± 1.7	130 ± 35
	0.9% LGT	9.0	28	30.9 ± 0.6[c]	79[c]	5.5 ± 0.9[c]	21 ± 14[c]
	0.9% LBT	9.0	29	31.0 ± 0.5[c]	100	7.3 ± 0.8[c]	41 ± 26[c]
	0.9% LDBT	9.0	27	31.1 ± 0.6[d]	100	14.9 ± 1.5	230 ± 67[d]
	0.9% LDGT	9.0	28	33.7 ± 0.5[c]	100	15.9 ± 1.4	136 ± 36
3[f]	Water	0	24	32.2 ± 0.7	100	13.5 ± 1.8	173 ± 96
	1.25% GT	4.0	29	33.1 ± 0.5	93	6.6 ± 1.0[c]	37 ± 17[d]
	1.25% BT	4.4	29	33.4 ± 0.5[d]	100	7.9 ± 0.7[c]	52 ± 17[d]
	1.25% DGT	3.6	26	31.9 ± 0.6	100	10.2 ± 1.4[d]	70 ± 21
	1.25% DBT	3.9	28	33.0 ± 0.6	100	13.5 ± 1.0	192 ± 77
	0.036% caffeine	0.36	27	32.3 ± 0.7	93	6.3 ± 1.1[c]	82 ± 54
	DGT + caffeine	4.0	30	33.3 ± 0.5[d]	100	7.1 ± 0.8[c]	15 ± 5[d]
	DBT + caffeine	4.3	29	34.4 ± 0.4[c]	100	10.3 ± 0.8[d]	41 ± 11[d]

[a] LGT, lyophilized green tea solids; LBT, lyophilized black tea solids; LDGT, lyophilized decaffeinated green tea solids; LDBT, lyophilized decaffeinated black tea solids; GT, green tea leaf; BT, black tea leaf; DGT, decaffeinated green tea leaf; DBT, decaffeinated black tea leaf.

[b] Female SKH-1 mice (30 per group) were treated with UVB (30 mJ/cm^2) twice a week for 35 weeks, and the mice were killed 4 weeks later. LGT, LDGT, or caffeine was administered as the sole source of drinking fluid from 2 weeks before the start of UVB treatment until the animals were killed. Gradually increasing doses of the teas and caffeine were administered during the first week and full-strength solutions were used thereafter.

[c] The Student's *t* test was used for all statistical analyses except for the evaluation of the data on percent of mice with tumors where the Fisher's exact test was used. Value is significantly different from water control group ($P < 0.05$).

[d] The Student's *t* test was used for all statistical analyses except for the evaluation of the data on percent of mice with tumors where the Fisher's exact test was used. Value is significantly different from water control group ($P < 0.10$).

[e] Female SKH-1 mice (30 per group) were treated with UVB (30 mJ/cm^2) twice a week for 45 weeks, and the animals were killed. Two weeks before the first dose of UVB, the mice were given gradually increasing doses of lyophilized tea solids for 1 week and full-strength teas until completion of the study.

[f] Female SKH-1 mice (30 per group) were treated with UVB (30 mJ/cm^2) twice weekly for 40 weeks, and the animals were killed 4 weeks later. Two weeks before the first dose of UVB, the mice were given gradually increasing doses of tea leaf extracts (1.25 g of tea leaf/100 ml of hot water) or caffeine for 1 week and full-strength teas or caffeine solutions until the completion of the study. All tumor data described here for experiments 1–3 represent grossly measured masses in living animals prior to histological examination.

lyophilized green tea, respectively) inhibited UVB-induced tumorigenesis, but a dose-dependent effect was not observed (Table 1 and Fig. 1, experiment 1). The oral administration of 0.24 and 0.72 mg of caffeine per ml decreased the number of UVB-induced tumors per mouse by 53 and 61%, respectively, at 35 weeks after the start of UVB (Fig. 1, experiment 1) and by 30–31% at 39 weeks after the start of UVB (Table 1 and Fig. 1, experiment 1). Oral administration of 0.24 or 0.72 mg of caffeine per ml decreased tumor volume per mouse by 55 and 82%, respectively, at 39 weeks after the start of UVB (Table 1 and Fig. 1, experiment 1). Histopathological examination of all large masses (>5 mm in diameter) from the UVB plus water positive control group and from the mice drinking 0.3 or 0.9% solutions of lyophilized green tea during the administration of UVB revealed that almost all of the large masses examined were squamous cell carcinomas. The number of large squamous cell carcinomas per mouse was 1.50 in the UVB positive control group, 0.93 in the 0.3% lyophilized green tea plus UVB group, and 0.10 in the 0.9% lyophilized green tea plus UVB group. Histopathology studies were not done with the remaining masses observed in experiment 1.

In experiment 2, SKH-1 mice treated with UVB (30 mJ/cm^2) twice a week for 45 weeks had an average of 15.6 tumors per mouse, and the average tumor volume was 130 mm^3 per mouse just before the animals were killed. Oral administration of 0.9% lyophilized green or black tea decreased the number of UVB-induced tumors per mouse by 53–65%, and the tumor volume per mouse was decreased by 68–84% (Table 1, experiment 2). Administration of 0.9% lyophilized decaffeinated green or black tea did not inhibit UVB-induced tumorigenesis (Table 1, experiment 2). Administration of green or black tea substantially decreased the number of large tumors per mouse, but this was not observed for mice treated with decaffeinated green or black tea. The numbers of large tumors (>5 mm in diameter) per mouse in animals treated with UVB plus water, UVB plus green tea, UVB plus black tea, UVB plus decaffeinated green tea, and UVB plus decaffeinated black tea in experiment 2 were 0.56, 0.07, 0.10, 0.59, and 0.36, respectively (data not presented). Examination of all tumors histologically revealed that oral administration of 0.9% lyophilized green or black tea lowered the percent of mice with UVB-induced keratoacanthomas and carcinomas (Table 2, experiment 2). In contrast to these observations, oral administration of 0.9% lyophilized decaffeinated green or black tea increased the percent of mice with UVB-induced squamous cell papillomas, keratoacanthomas, and carcinomas, although some of these changes were not statistically significant (Table 2, experiment 2). The results of the histology studies revealed that 0.9% lyophilized green or black tea decreased the average number of UVB-induced tumors per mouse (combined papillomas, keratoacanthomas, and carcinomas) by 75 and 60%, respectively, decreased the average number of keratoacanthomas per mouse by 67 and 76%, respectively, and decreased the average number of carcinomas (squamous cell carcinomas and carcinomas *in situ*) by 80 and 41%, respectively (Table 3, experiment 2). In contrast to these results, the oral administration of 0.9% lyophilized decaffeinated green or black tea increased the average number of histologically identified tumors per mouse by 123 and 102%, respectively, increased the average number of keratoacanthomas per mouse by 166 and 109%, respectively,

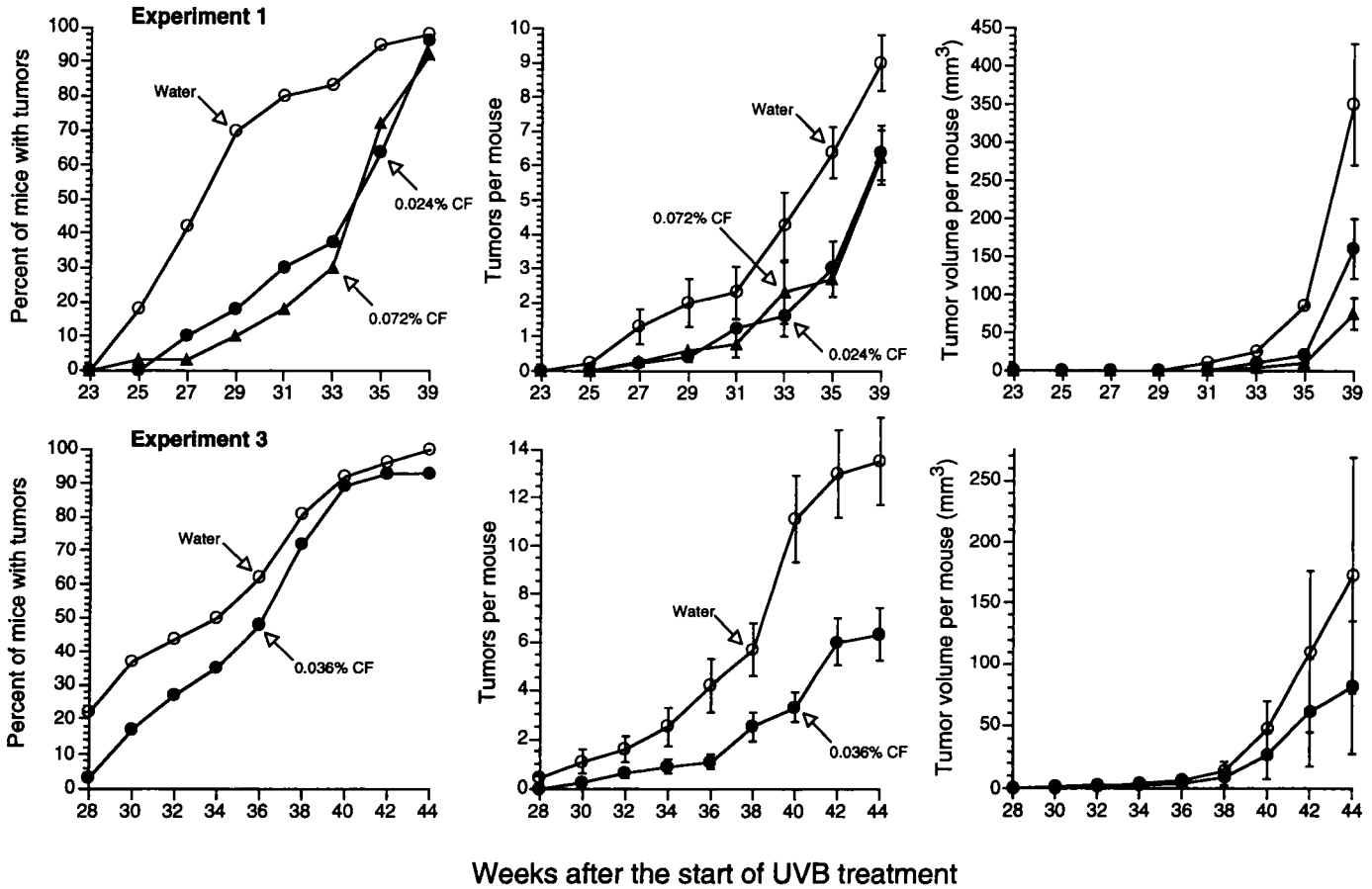

Fig. 1. Effect of oral administration of caffeine on UVB-induced complete carcinogenesis in SKH-1 mice. Female SKH-1 mice (30 per group) were treated p.o. with gradually increasing doses of caffeine (*CF*) during the first week and full-strength caffeine thereafter, as described for experiments 1 and 3 in the legend of Table 1. UVB (30 mJ/cm^2) was administered twice a week starting 1 week after full-strength caffeine administration. Caffeine in the drinking water was administered for 35 weeks in experiment 1 and for 40 weeks in experiment 3.

Table 2 *Effect of oral administration of green tea, black tea, decaffeinated green tea, decaffeinated black tea, and caffeine on UVB-induced carcinogenesis: Histopathology evaluation of tumor incidence*

Treatment[a]	No. of mice per group	% of mice with tumors	% of mice with squamous cell papillomas	% of mice with keratoacanthomas	% of mice with carcinomas *in situ*	% of mice with squamous cell carcinomas	% of mice with carcinomas (total)
Experiment 2[b]							
Water	27	70	4	48	11	44	44
0.9% LGT	27	37[c]	0	30[e]	0[e]	15[d]	15[d]
0.9% LBT	29	34[c]	0	10[c]	3	28	31
0.9% LDGT	27	85	37[c]	74[d]	22	67[e]	67[e]
0.9% LDBT	28	96[c]	29[c]	71[e]	11	68[e]	71[d]
Experiment 3[f]							
Water	24	83	21	83	38	63	79
1.25% GT	29	72	3[e]	62[e]	24	31[d]	41[c]
1.25% BT	29	100[e]	0[d]	83	21	72	76
1.25% DGT	26	96	15	88	31	65	77
1.25% DBT	28	96	4[e]	86	39	75	86
0.036% Caffeine	27	93	0[d]	74	30	52	66
1.25% DGT + caffeine	30	100[e]	7	90	10[d]	40[e]	50[d]
1.25% DBT + caffeine	29	90	3[e]	79	3[c]	69	69

[a] LGT, lyophilized green tea solids; LBT, lyophilized black tea solids; LDGT, lyophilized decaffeinated green tea solids; LDBT, lyophilized decaffeinated black tea solids; GT, green tea leaf; BT, black tea leaf; DGT, decaffeinated green tea leaf; DBT, decaffeinated black tea leaf.

[b] Female SKH-1 mice (30 per group) were treated with UVB (30 mJ/cm^2) twice a week for 45 weeks, and the animals were killed. Two weeks before the first dose of UVB, the mice were given gradually increasing doses of lyophilized tea solids for 1 week, which was followed by full-strength tea until completion of the study.

[c] Value is statistically different from the corresponding water control group, as determined by the Fisher's exact test. ($P < 0.01$).

[d] Value is statistically different from the corresponding water control group, as determined by the Fisher's exact test. ($P < 0.05$).

[e] Value is statistically different from the corresponding water control group, as determined by the Fisher's exact test. ($P < 0.10$).

[f] Female SKH-1 mice (30 per group) were treated with UVB (30 mJ/cm^2) twice weekly for 40 weeks, and the animals were killed 4 weeks later. Two weeks before the first dose of UVB, the mice were given gradually increasing doses of tea leaf extracts or caffeine for 1 week, which was followed by full-strength tea or caffeine solutions until the completion of the study. Histopathology examination was done for all tumors.

increased the average number of squamous cell papillomas per mouse 5–7-fold, and increased the average number of carcinomas per mouse by 54–55% (Table 3, experiment 2). Only some of these changes were statistically significant.

In experiment 3, SKH-1 mice were treated with UVB (30 mJ/cm^2) twice weekly for 40 weeks, and the animals were killed 4 weeks later. The animals had an average of 13.5 tumors per mouse, and the tumor volume per mouse was 173 mm^3 just before the animals were killed at 44 weeks after the start of UVB administration (Table 1, experiment 3). Oral administration of 1.25% green or black tea leaf extract (1.25 g of tea leaf/100 ml of water) containing 4.0 or 4.4 mg of tea solids per ml, respectively, as the drinking water to the UVB-treated mice decreased the number of tumors per mouse by 51 and 41%, respectively, and tumor volume per mouse was decreased 79 and 70%, respectively, just before the animals were killed at 44 weeks after the start of UVB administration (Table 1, experiment 3). Decaffeinated green or black tea leaf extract (1.25%) containing 3.6 or 3.9 mg of tea solids per ml, respectively, was less effective than regular green or black tea, and decaffeinated black tea was somewhat less effective than decaffeinated green tea at inhibiting the formation of skin tumors (Table 1, experiment 3). Adding 0.36 mg of caffeine per ml to the decaffeinated teas either fully or partially restored the inhibitory effects of these teas on UVB-induced tumorigenesis (Table 1, experiment 3). This concentration of caffeine was approximately equivalent to that which was present in the regular teas used in experiment 3. Administration of only caffeine (0.36 mg/ml) in the drinking water

Table 3 *Effect of oral administration of green tea, black tea, decaffeinated green tea, decaffeinated black tea, and caffeine on UVB-induced carcinogenesis: Histopathology evaluation of tumor multiplicity*

Treatment[a]	No. of mice per group	Total tumors per mouse	Squamous cell papillomas per mouse	Keroacathomas per mouse	Carcinomas *in situ* per mouse	Squamous cell carcinomas per mouse	Total carcinomas per mouse
Experiment 2[b]							
Water	27	1.89 ± 0.40	0.07 ± 0.07	0.89 ± 0.24	0.19 ± 0.12	0.74 ± 0.23	0.93 ± 0.28
0.9% LGT	27	0.48 ± 0.14[c]	0	0.29 ± 0.09[c]	0	0.19 ± 0.09[d]	0.19 ± 0.09[c]
0.9% LBT	29	0.76 ± 0.27[c]	0	0.21 ± 0.13[c]	0.03 ± 0.03[e]	0.52 ± 0.19	0.55 ± 0.19[e]
0.9% LDGT	27	4.22 ± 0.65[c]	0.41 ± 0.11[c]	2.37 ± 0.46[c]	0.25 ± 0.10	1.19 ± 0.21[e]	1.44 ± 0.24[e]
0.9% LDBT	28	3.82 ± 0.50[c]	0.53 ± 0.22[d]	1.86 ± 0.36[d]	0.11 ± 0.06	1.32 ± 0.22[d]	1.43 ± 0.25[e]
Experiment 3[f]							
Water	24	7.54 ± 1.32	0.21 ± 0.08	5.75 ± 1.04	0.46 ± 0.13	1.17 ± 0.27	1.58 ± 0.32
1.25% GT	29	3.10 ± 0.61[c]	0.03 ± 0.03[d]	2.21 ± 0.46[c]	0.34 ± 0.13	0.52 ± 0.18[d]	0.86 ± 0.23[d]
1.25% BT	29	5.30 ± 0.52[e]	0[d]	3.72 ± 0.50[d]	0.24 ± 0.09[e]	1.34 ± 0.23	1.58 ± 0.23
1.25% DGT	26	6.39 ± 0.75	0.15 ± 0.07	4.58 ± 0.64	0.31 ± 0.09	1.35 ± 0.29	1.66 ± 0.30
1.25% DBT	28	7.36 ± 0.87	0.04 ± 0.04[d]	5.29 ± 0.79	0.64 ± 0.21	1.39 ± 0.18	2.03 ± 0.24[e]
0.036% Caffeine	27	2.81 ± 0.50[c]	0[d]	1.81 ± 0.44[c]	0.37 ± 0.12	0.63 ± 0.14[d]	1.00 ± 0.20[e]
1.25% DGT + caffeine	30	3.17 ± 0.49[c]	0.07 ± 0.05[e]	2.53 ± 0.43[c]	0.10 ± 0.06[c]	0.47 ± 0.11[c]	0.57 ± 0.12[c]
1.25% DBT + caffeine	29	3.72 ± 0.58[c]	0.03 ± 0.03[d]	2.66 ± 0.49[c]	0.03 ± 0.03[c]	1.00 ± 0.18	1.03 ± 0.19[e]

[a] LGT, lyophilized green tea solids; LBT, lyophilized black tea solids; LDGT, lyophilized decaffeinated green tea solids; LDBT, lyophilized black tea solids; GT, green tea leaf; BT, black tea leaf; DGT, decaffeinated green tea leaf; DBT, decaffeinated black tea leaf.

[b] Female SKH-1 mice (30 per group) were treated with UVB (30 mJ/cm^2) twice a week for 45 weeks, and the animals were killed. Two weeks before the first dose of UVB, the mice were given gradually increasing doses of lyophilized tea solids for one week which was followed by full strength tea until completion of the study. Histopathology examination was done for all tumors.

[c] Value is statistically different from the corresponding water group as determined by the Student's t test ($P < 0.01$).

[d] Value is statistically different from the corresponding water group as determined by the Student's t test ($P < 0.05$).

[e] Value is statistically different from the corresponding water group as determined by the Student's t test ($P < 0.10$).

[f] Female SKH-1 mice (30/group) were treated with UVB (30 mJ/cm^2) doses of tea leaf extracts or caffeine for one week which was followed by full strength tea or caffeine solutions until the completion of the study. Histopathology examination was done for all tumors.

Table 4 *Effect of topical administration of a green tea polyphenol fraction on UVB-induced skin tumorigenesis*

Female SKH-1 mice (6–8 weeks old; 30 per group) were treated with UVB (30 mJ/cm^2) twice a week for 35 weeks. Two hundred μl of acetone or green tea polyphenol fraction in 200 μl of acetone was applied topically immediately after each UVB treatment for 35 weeks. Topical application of vehicle or green tea polyphenol fraction twice a week was continued until the animals were killed 4 weeks later. Each value represents the mean ± SE from 28–30 mice.

Treatment	% of mice with tumors		Tumors per mouse		Tumor volume per mouse (mm^3)	
	35 wk	39 wk	35 wk	39 wk	35 wk	39 wk
Acetone (solvent control)	97	100	9.8 ± 1.1	11.2 ± 0.8	172 ± 48	712 ± 303
Green tea polyphenols (2.4 mg)	79[a]	100	4.4 ± 0.8[c]	6.4 ± 0.6[c]	71 ± 19[d]	366 ± 167
Green tea polyphenols (7.2 mg)	80[a]	83[b]	3.0 ± 0.5[c]	5.7 ± 0.9[c]	23 ± 11[c]	123 ± 36[d]

[a] Value is significantly different from the solvent control group ($P < 0.05$), as determined by the Fisher's exact test.
[b] Value is significantly different from the solvent control group ($P < 0.10$), as determined by the Fisher's exact test.
[c] Value is significantly different from the solvent control group ($P < 0.01$) as determined by the Student's t test.
[d] Value is significantly different from the solvent control group ($P < 0.05$) as determined by the Student's t test.

decreased the number of UVB-induced skin tumors per mouse by 53%, and UVB-induced tumor volume per mouse was also decreased by 53% (Table 1, Fig. 1, experiment 3). The number of large tumors (>5 mm diameter) per mouse in animals treated with UVB plus water, UVB plus green tea, UVB plus black tea, UVB plus decaffeinated green tea, UVB plus decaffeinated black tea, UVB plus caffeine, UVB plus decaffeinated green tea plus caffeine, or UVB plus decaffeinated black tea plus caffeine in experiment 3 was 1.25, 0.24, 0.76, 0.81, 0.86, 0.44, 0.10, or 0.45, respectively (data not shown). Histological examination of all skin masses revealed moderate effects of administering the various 1.25% tea leaf infusions or caffeine on the incidence of histologically identified tumors (Table 2, experiment 3). Oral administration of green tea or decaffeinated green tea plus caffeine was the most effective regimens for decreasing the percent of mice with squamous cell carcinomas (Table 2). Histological examination of all tumors revealed that oral administration of 1.25% green or black tea leaf extract decreased the average number of UVB-induced tumors per mouse (combined histologically identified papillomas, keratoacanthomas and carcinomas) by 59 and 30%, respectively, lowered the number of keratoacanthomas per mouse by 62 and 35%, respectively, and lowered the number of carcinomas per mouse (squamous cell carcinomas and carcinomas *in situ*) by 46 and 0%, respectively (Table 3, experiment 3). The decaffeinated green and black teas were markedly less effective than the regular teas at inhibiting UVB-induced carcinogenicity (Table 3, experiment 3). The average number of carcinomas per mouse in animals treated with UVB and decaffeinated black tea as the sole source of drinking fluid was 28% higher than in animals treated with UVB and water alone as the drinking fluid (Table 3, experiment 3). Adding caffeine back to the decaffeinated teas resulted in restoration of their inhibitory effects on UVB-induced papilloma, keratoacanthoma, and carcinoma formation (Table 3, experiment 3). Administration of only caffeine in the drinking water decreased the total number of histologically identified UVB-induced tumors by 63%, the number of keratoacanthomas per mouse by 69%, and the number of carcinomas per mouse by 37% (Table 3, experiment 3).

Effect of Topical Application of a Green Tea Polyphenol Fraction on UVB-induced Complete Carcinogenesis. SKH-1 mice were treated with UVB (30 mJ/cm^2) twice a week for 35 weeks. Either 200 μl of acetone or a caffeine-containing green tea polyphenol fraction in 200 μl of acetone was applied to the backs of the mice immediately after each treatment with UVB. UVB treatment was stopped after 35 weeks, but twice-weekly topical application of acetone or green tea polyphenol fraction in acetone was continued for another 4 weeks. Mice treated with acetone vehicle and UVB for 35 weeks had 9.8 tumors per mouse, and tumor volume per mouse was 172 mm^3 (Table 4). When UVB treatment was stopped but acetone vehicle treatment continued for an additional 4 weeks, there were 11.2 tumors per mouse, and the tumor volume was 712 mm^3 (Table 4). The results described in Table 4 indicate that topical application of 2.4 or 7.2 mg of a green tea polyphenol fraction after each administration of UVB for 35 weeks decreased the number of UVB-induced tumors per mouse by 55 or 69%, respectively, and the tumor volume per mouse was decreased by 59 or 87%, respectively. The continued topical application of 2.4 or 7.2 mg of green tea polyphenol fraction twice a week for an additional 4 weeks after discontinuation of UVB administration decreased the number of tumors per mouse by 43 and 49%, respectively, and the tumor volume per mouse was lowered by 49 and 83%, respectively (Table 4).

DISCUSSION

In an earlier study, we demonstrated a potent inhibitory effect of p.o. administered green tea, black tea, decaffeinated green tea, or decaffeinated black tea (~2–4 mg of tea solids/ml) as the sole source of drinking fluid on UVB-induced carcinogenesis in mice previously initiated with DMBA (5). Although each of the tea preparations studied in our earlier investigation had a strong inhibitory effect in this DMBA/UVB carcinogenesis model, the regular teas were somewhat more effective than the decaffeinated teas (5). The results presented here using a different model of UVB-induced carcinogenesis indicate that oral administration of green or black tea (~4–9 mg of tea solids/ml) inhibited UVB-induced complete carcinogenesis and that green tea was somewhat more effective than black tea (Tables 1–3). These tea preparations can be compared with tea brews containing ~4 mg of tea solids/ml that are commonly ingested by humans. The lack of a substantial inhibitory effect of the decaffeinated teas in the complete carcinogenesis model described here differs from the marked inhibitory effects of the decaffeinated teas given to DMBA-initiated mice treated with UVB. The reason(s) for the different effects of the decaffeinated teas in the two animal models are not known. The mechanism of the inhibitory effects of green and black tea on UVB-induced complete carcinogenesis is unknown but may not be closely related to the antioxidant and free radical-scavenging activity of the teas. Although the decaffeinated teas retained high polyphenol concentrations and had strong ability to scavenge superoxide anion radicals (5), they lost much of their ability to inhibit UVB-induced complete carcinogenesis (Tables 1–3). The results of the present study, indicating that oral administration of decaffeinated green tea or decaffeinated black tea had little or no inhibitory effect on UVB-induced complete carcinogenesis and that, in one experiment with a high dose level, they enhanced the tumorigenic effect of UVB, were unexpected (Tables 1–3). Adding caffeine back to the decaffeinated teas restored their inhibitory effects (Tables 1–3, experiment 3), and administration of aqueous solutions of caffeine alone as the sole source of drinking fluid also inhibited UVB-induced complete carcinogenesis (Tables 1–3 and Fig. 1, experiments 1 and 3). These results indicate that caffeine is a biologically important component of green and black tea that is responsible for a substantial portion of the inhibitory effects of the teas on UVB-induced complete carcinogen-

esis. An earlier report indicated that oral administration of a green tea polyphenol fraction also inhibited UVB-induced tumor formation in a complete tumorigenesis model (3), but it is likely that the green tea polyphenol fraction used in this earlier study also contained caffeine. It should be pointed out that semipurified polyphenol fractions isolated from tea may contain varying amounts of caffeine, which is difficult to remove from the tea polyphenols, and residual caffeine may influence the results obtained with the polyphenol fraction studied. Unfortunately, many of the earlier reports on the effects of green tea polyphenol fractions on carcinogenesis did not describe the concentration of caffeine in the material used. In the present study, we found that topical application of a caffeine-containing green tea polyphenol fraction inhibited UVB-induced complete carcinogenesis on mouse skin, but the relative roles of caffeine and the polyphenolic compounds for this inhibitory effect are not known. The tea polyphenol fraction used in this study contained about 80% known polyphenolic compounds and 7.6% caffeine (see "Materials and Methods" section for composition).

Although the present study appears to be the first to indicate an inhibitory effect of p.o. administered caffeine on UVB-induced carcinogenesis, an earlier study indicated an inhibitory effect of topically applied caffeine on UVB-induced carcinogenesis (7, 8). The effects of caffeine on carcinogenesis in animals are complex. Several earlier studies have indicated inhibitory effects of caffeine administration on carcinogenesis in animals (7–15), but other studies have indicated a stimulatory effect of caffeine administration on carcinogenesis (16–20). It was found that topical application of caffeine inhibited cigarette smoke condensate-induced skin carcinogenesis (9), UV-induced skin carcinogenesis (7, 8), and TPA-induced tumor promotion in mouse skin (10). In addition, s.c. injections of caffeine immediately after urethane or 4-nitroquinoline-1-oxide administration inhibited urethane- or 4-nitroquinoline-1-oxide-induced lung tumors in mice (11–13), and the i.p. injection of caffeine 3 times a week inhibited the formation of spontaneous or urethane-induced pulmonary adenomas in strain A mice (14). In another study, treatment of GR mice with caffeine in the drinking water (0.5 mg/ml) inhibited ovarian hormone-induced breast tumorigenesis (15). In a recent study, we found an inhibitory effect of topically applied caffeine on TPA-induced tumor promotion on mouse skin (data not shown) that was similar to that reported earlier (10). In contrast to the inhibitory effects of caffeine on carcinogenesis described above, treatment of mice with caffeine in the drinking water (0.25–0.50 mg/ml) increased spontaneous or DMBA-induced breast tumorigenesis (16, 20), treatment of rats with caffeine in the drinking water (0.25–0.50 mg/ml) enhanced DMBA-induced breast tumorigenesis (18, 19), and multiple topical applications of caffeine together with 4-nitroquinoline-1-oxide increased the tumorigenic effects of 4-nitroquinoline-1-oxide administration after a single exposure of mouse skin to β-radiation (17). The results of these studies indicate that the effects of caffeine on carcinogenesis are complex, and whether caffeine inhibits or stimulates carcinogenesis depends on the experimental model used.

Although the mechanisms of the effects of caffeine on chemically induced, UVB-induced, and spontaneous carcinogenesis are unknown, some biochemical effects of caffeine that may be relevant include: an inhibitory effect of caffeine on phosphodiesterase activity and the accumulation of cAMP (21); an inhibitory effect of caffeine on the repair of UV-damaged DNA (22); effects of caffeine to enhance or diminish the mutagenic effect of UV (23); an enhancing effect of caffeine on the cytotoxic and lethal/apoptosis-inducing effects of certain alkylating agents (24); strong antioxidant effects of caffeine (25); and effects of caffeine to uncouple mitosis from the completion of DNA replication (26). In contrast to the antioxidant activity of caffeine described above, other studies have been unable to demonstrate appreciable antioxidant activity for caffeine.[5]

The nature of the substance(s) in decaffeinated green or black tea that enhance UVB-induced complete carcinogenesis at high-dose levels of the decaffeinated teas is unknown. In some animal models, the administration of high doses of certain tea components or related substances has been reported to have tumor-enhancing effects. Repeated s.c. injections of a chloroform-isolated polyphenolic fraction of black tea (8 mg once a week for 45–77 weeks) resulted in fibrous histiocytomas in rats (27). Repeated topical applications of a black tea extract was reported to increase the incidence of skin tumors in mice previously treated with benzo(*a*)pyrene (28). In contrast, using a similar model, the incidence of skin tumors was unchanged but the latent period for tumor appearance was decreased (29). Studies in our laboratory demonstrated an inhibitory effect of a topically applied green or black tea polyphenol fraction on TPA-induced tumor promotion on the skin of DMBA-initiated mice (6).[6]

An early study indicated that topical application of (−)-epigallocatechin gallate, a major green tea constituent, inhibits the tumor-promoting effect of teleocidin (30). During the past several years, tea and some of its constituents have been shown to have a very broad spectrum of cancer chemopreventive activity (reviewed in Refs. 31 and 32). Oral administration of green tea, black tea, (−)-epigallocatechin gallate, or a green tea polyphenol fraction has been reported to inhibit chemically-induced carcinogenesis in many organs, including esophagus (33, 34), forestomach (35–37), stomach (38), duodenum/small intestine (39, 40), colon (41, 42), lung (35–37, 43–45), skin (6, 30), liver (46), and pancreas (47). p.o.-administered decaffeinated green or black tea has been reported to effectively inhibit chemically induced esophageal, lung, and forestomach carcinogenesis (34, 36), indicating that caffeine may not play a major role in the inhibitory effects of tea in these chemically-induced carcinogenesis models. In addition to these studies, oral administration of (−)-epigallocatechin gallate (the major polyphenol in green tea) or a green tea polyphenol fraction inhibits *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine-induced duodenal tumors (39), azoxymethane- or 1,2-dimethylhydrazine-induced colon tumors (41, 42), and the formation of spontaneous liver tumors (48).

It is important to point out that both green and black tea are very complex mixtures (see Ref. 5 for partial composition), and more research is needed to identify the active constituents in these teas and to determine the possibility of synergistic or antagonistic effects of the multiple constituents in tea. The results of the present study indicate that caffeine, which is present in black and green tea, is an important inhibitor of UVB-induced complete carcinogenesis. The broad profile of inhibition of carcinogenesis by green and black tea in several animal models has attracted considerable attention with regard to the possible inhibitory effects of tea on human cancer. The results of epidemiology studies on the effects of tea on human cancer have been reviewed (31, 32, 49–52), but clear-cut conclusions cannot be made. More definitive epidemiology studies on the effects of tea on human cancer are needed.

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[6] Unpublished observations.

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