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Effects of Oral Administration of Tea, Decaffeinated Tea, and Caffeine on the Formation and Growth of Tumors in High-Risk SKH-1 Mice Previously Treated With Ultraviolet B Light

You-Rong Lou, Yao-Ping Lu, Jian-Guo Xie, Mou-Tuan Huang, and Allan H. Conney

Abstract: *Treatment of SKH-1 mice with ultraviolet B light (UV-B, 30 mJ/cm^2) twice a week for 22–23 weeks resulted in tumor-free animals with a high risk of developing malignant and nonmalignant tumors during the next several months in the absence of further UV-B treatment (high-risk mice). In three separate experiments, oral administration of green tea or black tea (4–6 mg tea solids/ml) as the sole source of drinking fluid for 18–23 weeks to these high-risk mice inhibited the formation and decreased the size of nonmalignant squamous cell papillomas and keratoacanthomas as well as the formation and size of malignant squamous cell carcinomas. In one experiment all these inhibitory effects of tea were statistically significant, whereas in the two other experiments many but not all of the inhibitory effects of tea were statistically significant. The decaffeinated teas were inactive or less effective inhibitors of tumor formation than the regular teas, and adding caffeine back to the decaffeinated teas restored biological activity. Oral administration of caffeine alone (0.44 mg/ml) as the sole source of drinking fluid for 18–23 weeks inhibited the formation of nonmalignant and malignant tumors, and this treatment also decreased tumor size in these high-risk mice.*

Introduction

Sunlight-induced skin cancer is a major cancer in the United States and in other temperate parts of the world (1–3). Ultraviolet B light (UV-B) and, to a much lesser extent, ultraviolet A light (UV-A) are responsible for sunlight-induced cancers (2,4,5), and animal studies indicate that the UV-B part of the solar spectrum is responsible for most of the carcinogenic effects of sunlight (5). Molecular studies on mutations observed in human cancers have also implicated UV as a major cause of human skin cancer (6,7).

The incidence of skin cancer appears to be increasing and is predicted to increase even further because of an increase in recreational exposure to sunlight and also because of the depletion of the stratospheric ozone layer (2,7). Skin cancer is the most common type of human cancer (8). Although most skin cancers seen by dermatologists are squamous cell carcinomas and basal cell carcinomas that are easily cured if detected early, many people still die from these cancers, as well as from the more dangerous sunlight-induced melanomas. The development of strategies to prevent UV-induced cancers would have a major impact in decreasing the total load of human cancer.

In earlier studies, Wang and co-workers (9) reported an inhibitory effect of an orally administered green tea polyphenol fraction on UV-B-induced complete tumorigenesis in the skin of SKH-1 mice, but the histology of the tumors was not investigated. The green tea polyphenol fraction used in this study also contained caffeine (Z. Y. Wang, personal communication). In additional studies, our laboratory reported inhibitory effects of orally administered green tea, black tea, decaffeinated green tea, and decaffeinated black tea on UV-B-induced formation of papillomas, keratoacanthomas, and squamous cell carcinomas in mice previously initiated with 7,12-dimethylbenz[*a*]anthracene (DMBA) (10,11). In these experiments we observed that the decaffeinated teas were somewhat less effective than the regular teas (11). In a recent study we evaluated the effect of orally administered green tea, black tea, decaffeinated green tea, decaffeinated black tea, and caffeine on UV-B-induced complete carcinogenesis in the skin of SKH-1 mice. The results of this study indicated an inhibitory effect of orally administered green and black tea on UV-B-induced complete carcinogenesis, but the decaffeinated teas were either inactive (at moderate dose levels) or they enhanced the tumorigenic effect of UV-B (at a high dose level) (12). Oral administration of caffeine had a strong inhibitory effect on UV-B-induced complete carcinogenesis (12).

In previous studies, tea or tea components were given together with UV-B so that it was not possible to evaluate the effects of tea or the tea components on postinitiation

The authors are affiliated with the Laboratory for Cancer Research, Department of Chemical Biology, College of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854-8020.

events that occur after UV-B administration was stopped. In the present report we describe the results of new experiments designed to test the hypothesis that oral administration of green tea, black tea, and caffeine inhibits the formation of malignant and nonmalignant skin tumors in tumor-free SKH-1 mice previously treated with UV-B (high-risk mice).

Materials and Methods

Chemicals

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

Animals

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

Preparation 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 US Tea Association (New York, NY). Decaffeinated tea leaves, prepared by extracting the leaves with supercritical CO_2 (CO_2 under high pressure at approx 65°C), were used for the preparation of lyophilized decaffeinated tea solids. The supercritical CO_2 process removed almost all caffeine from the tea leaves without removing the polyphenolic catechins (11).

For Experiment 1 we prepared tea infusions from tea leaves with a commercial Bunn automatic basket tea brewer, as described earlier (11). Briefly, tea leaves (50 g) were placed in a filter paper-lined brewing basket, and 4 liters of hot deionized water were passed into the brewing machine through the tea leaves to obtain a 1.25% tea infusion (1.25 g tea leaf/100 ml water). The resulting tea brews in Experiment 1 were similar to those consumed by humans, and they contained about 4 mg of hot water-extracted tea solids per milliliter. The composition of the tea leaf infusions (Experiment 1) and the reconstituted lyophilized teas (Experiments 2 and 3) were similar to what was reported earlier (11). Notably, the concentrations of (−)-epigallocatechin gallate, epicatechin gallate, epigallocatechin, and epicatechin (polyphenolic catechins) in the decaffeinated tea brews did not differ substantially from the concentration of these substances in the regular tea brews (11).

In Experiments 2 and 3 we prepared solutions of caffeine (0.44 mg/ml, 0.044% solutions) or solutions of lyophilized tea solids (6 mg/ml water, 0.6% solutions). In these studies the concentrations of tea solids and caffeine were about 50% higher than normally consumed by humans. The concentration of caffeine (0.44 mg/ml) used in Experiments 2 and 3 was the same as that in the 0.6% green tea and black tea infusions.

Treatment of Mice With UV-B and Teas

Female SKH-1 mice (6-7 wk old) were treated with UV-B (30 mJ/cm^2) twice a week for 22–23 weeks, as described earlier (11,12). For these studies, UV lamps (FS72T12-UVB-HO) that emit UV-B (280–320 nm, 75–80% of total energy) and UV-A (320–375 nm, 20–25% of total energy) were obtained from Voltare (Fairfield, CT). In each experiment, when one animal developed a small skin nodule, the UV-B treatment was stopped and the mouse was discarded. Histological examination of the epidermis of 40 of these treated mice revealed a lack of histologically identified tumors. Additional treated mice without tumors and with a high risk for the development of tumors in the absence of continued UV-B exposure were then given various teas or caffeine solutions as their sole source of drinking fluid in the absence of further UV-B treatment. In these studies the mice were given tea solutions or caffeine that were 25%, 50%, and 75% of full strength (2 days at each concentration), and then full-strength solutions were given until the end of each experiment. None of the treatments had an effect on food intake or the body weights of the mice. There were no differences in caffeine ingestion (fluid consumption) for the green tea, black tea, caffeine, decaffeinated green tea plus caffeine, and decaffeinated black tea plus caffeine groups, except the decaffeinated green tea plus caffeine and the decaffeinated black tea plus caffeine groups consumed 6–10% less caffeine than the caffeine-alone group. The mean caffeine intake between the above groups varied by <5.5% from the mean of the groups during a five-week interval when the mice received the full-strength teas or caffeine and fluid consumption was measured. The mean daily caffeine intake by the mice for the above five groups was 2.75 mg of caffeine per mouse per day.

For tumor size and 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. Tumor volume was determined for each mass by measuring the three-dimensional size (height, length, and width) of each mass and by using the average of the three measurements as the diameter. The radius (r) was determined, and the volume was calculated as follows

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

For histology, the skin samples were taken to include each of the grossly observed masses. Sections (4–5 μm) were stained with hematoxylin and eosin and evaluated for tumor classification by light microscopy.

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

Results and Discussion

In the experiments reported here, we treated SKH-1 mice with UV-B for several months until one mouse developed a small skin nodule. This animal was discarded, thereby providing a population of tumor-free mice with a high risk of developing papillomas, keratoacanthomas, and squamous cell carcinomas during the following several months in the absence of further treatment with UV-B (high-risk mice). In three separate experiments, treatment of these high-risk mice with green tea or black tea (4–6 mg tea solids/ml) as their sole source of drinking fluid for several months in the absence of further UV-B exposure inhibited the formation of papillomas, keratoacanthomas, and squamous cell carcinomas (nonmalignant and malignant skin tumors) (Table 1, Experiments 1–3). In one experiment all these inhibitory effects of tea were statistically significant (Table 1, Experiment 3). In the two additional experiments many but not all of the inhibitory effects of tea were statistically significant (Table 1, Experiments 1 and 2). The concentrations of tea solids in hot water extracts of tea leaves used in our studies can be compared with a concentration of about 4 mg of tea solids per milliliter, which is commonly found in teas that are ingested as a beverage by humans.

It was of interest that administration of decaffeinated green tea or black tea was inactive or considerably less effective at inhibiting the formation of malignant and nonmalignant tumors than the regular teas, and adding caffeine (equivalent to the concentration present in the regular teas, 0.44 mg/ml) back to the decaffeinated teas restored their inhibitory activities (Table 1, Experiments 2 and 3). In addition, we found that oral administration of caffeine alone (0.44 mg/ml) inhibited the formation of malignant and nonmalignant tumors in high-risk mice (Table 1, Experiments 2 and 3). Earlier studies indicated that the effects of caffeine on carcinogenesis are complex. Some studies demonstrated an inhibitory effect of caffeine administration on carcinogenesis in animals (12–21), whereas other studies indicated a stimulatory effect of caffeine on carcinogenesis (22–26). Whether caffeine inhibits or stimulates carcinogenesis depends on the experimental animal model used.

Administration of green or black tea to high-risk mice not only inhibited the formation of malignant and nonmalignant tumors, but the size of the tumors was also decreased (Table 2). In three experiments where mice received green or black tea, the volume per squamous cell carcinoma was decreased by 54–80% and the volume per nonmalignant tumor (papillomas + keratoacanthomas) was decreased by 32–92%. Administration of green or black tea decreased the volume of squamous cell carcinomas per mouse by 75–88%, and the volume of nonmalignant tumors per mouse was decreased by 58–97%. In one of the three experiments all the inhibitory effects of green and black tea on tumor size were statistically significant (Table 2, Experiment 3). In the two other experiments many but not all of the inhibitory effects of green and black tea were statistically significant (Table 2, Experiments 1 and 2). Administration of decaffeinated green tea, decaffeinated black tea, or caffeine alone also decreased tumor size (Table 2, Experiments 2 and 3). Administration of only caffeine to high-risk mice decreased the size of malignant and nonmalignant tumors per mouse by 90–96%. The results of our studies indicated that although decaffeinated tea was not effective at inhibiting the formation (number) of malignant and nonmalignant tumors (Table 1), these tea preparations did decrease tumor size (Table 2). It is possible that the inhibitory effects of decaffeinated teas on tumor size may be related to an inhibitory effect of (−)-epigallocatechin gallate or other polyphenolic substances in these teas on tumor growth. Earlier studies have shown an inhibitory effect of intraperitoneal injections of (−)-epigallocatechin gallate on tumor growth (27,28).

In earlier studies we showed that oral administration of green or black tea inhibited the growth of papillomas (27,29). A recent study also showed that administration of black tea inhibited the growth of squamous cell carcinomas and keratoacanthomas (29). The inhibitory effect of green tea, black tea, and caffeine on the formation and size of malignant and nonmalignant tumors in high-risk mice previously treated with UV-B described here may be related in part to the inhibitory effect of tea administration on tumor growth (27,29). Recent studies showed that administration of black tea to mice with preformed malignant and nonmalignant skin tumors inhibited the growth of these tumors by inhibiting DNA synthesis (bromodeoxyuridine incorporation into DNA) and stimulating apoptosis in the tumors (29). Further studies are needed to elucidate the mechanisms of the inhibitory effects of tea and caffeine on skin carcinogenesis in tumor-free mice with a high risk for skin cancer and to evaluate whether tea administration inhibits UV-B-induced carcinogenesis in humans.

Table 1. Effect of Oral Administration of Green Tea, Black Tea, Decaffeinated Green Tea, Decaffeinated Black Tea, and Caffeine on Incidence and Multiplicity of Tumors in SKH-1 Mice Previously Treated with UV-B (High-Risk Mice)[a–c]

			Squamous Cell Papillomas		Keratoacanthomas		Total Nonmalignant Tumors		Squamous Cell Carcinomas		Total Tumors	
Treatment	*n*	Body Wt, g	% of mice with tumors	Tumors/ mouse	% of mice with tumors	Tumors/ mouse	% of mice with tumors	Tumors/ mouse	% of mice with tumors	Tumors/ mouse	% of mice with tumors	Tumors/ mouse
						Experiment 1						
Water	35	33.5 ± 0.7	31	0.46 ± 0.13	60	2.06 ± 0.42	66	2.51 ± 0.48	51	1.11 ± 0.27	74	3.63 ± 0.67
GT	37	31.5 ± 0.9	14[‡]	0.16 ± 0.07[†]	49	1.43 ± 0.38	54	1.59 ± 0.39[‡]	41	0.70 ± 0.16[‡]	62	2.30 ± 0.50[‡]
			(55)	(65)	(18)	(31)	(18)	(37)	(20)	(37)	(16)	(37)
BT	34	33.2 ± 0.7	12[†]	0.21 ± 0.10[‡]	59	1.53 ± 0.31	62	1.74 ± 0.35[‡]	56	0.91 ± 0.22	74	2.65 ± 0.52
			(61)	(54)	(2)	(26)	(6)	(31)	(0)	(18)	(0)	(27)
						Experiment 2						
Water	35	30.9 ± 0.4	17	0.20 ± 0.08	57	1.17 ± 0.25	60	1.37 ± 0.28	23	0.43 ± 0.15	60	1.80 ± 0.38
0.6% GT	34	30.9 ± 0.3	9	0.09 ± 0.05	38[‡]	0.85 ± 0.27	38[‡]	0.94 ± 0.29	21	0.24 ± 0.09	47	1.18 ± 0.32
			(47)	(55)	(33)	(27)	(37)	(31)	(9)	(44)	(22)	(34)
0.6% BT	35	30.3 ± 0.5	11	0.11 ± 0.05	37[‡]	0.80 ± 0.27	40[‡]	0.91 ± 0.28	20	0.26 ± 0.09	46	1.17 ± 0.31[‡]
			(35)	(45)	(35)	(32)	(33)	(34)	(13)	(40)	(23)	(35)
0.6% DGT	33	30.4 ± 0.6	18	0.27 ± 0.12	49	1.39 ± 0.39	52	1.70 ± 0.43	24	0.48 ± 0.17	55	2.18 ± 0.56
			(0)	(0)	(14)	(0)	(13)	(0)	(0)	(0)	(8)	(0)
0.6% DBT	34	30.6 ± 0.5	21	0.26 ± 0.10	53	1.62 ± 0.34	62	1.88 ± 0.39	38	0.56 ± 0.14	68	2.44 ± 0.45
			(0)	(0)	(7)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
0.044% CF	36	30.9 ± 0.3	6	0.11 ± 0.09	44	0.81 ± 0.17	47	0.92 ± 0.18[‡]	14	0.25 ± 0.13	50	1.17 ± 0.27[‡]
			(65)	(45)	(23)	(31)	(22)	(33)	(39)	(42)	(17)	(35)
DGT + CF	34	30.3 ± 0.3	15	0.15 ± 0.06	24*,[‖]	0.56 ± 0.23[†,#]	32[†,#]	0.71 ± 0.26[†,‖]	15	0.32 ± 0.17	38[‡]	1.03 ± 0.41[‡,#]
			(12)	(25)	(58)	(52)	(47)	(48)	(35)	(26)	(37)	(43)
DBT + CF	36	30.7 ± 0.5	3[†,‖]	0.03 ± 0.03[†,‖]	36[‡]	0.56 ± 0.16[†,§]	36[†,‖]	0.58 ± 0.16*,[§]	25	0.33 ± 0.11	44[‖]	0.92 ± 0.25[†,§]
			(82)	(85)	(37)	(52)	(40)	(58)	(0)	(23)	(27)	(49)

(Continued)

Table 1. *(Continued)*

Treatment	*n*	Body Wt, g	Squamous Cell Papillomas		Keratoacanthomas		Total Nonmalignant Tumors		Squamous Cell Carcinomas		Total Tumors	
			% of mice with tumors	Tumors/ mouse	% of mice with tumors	Tumors/ mouse	% of mice with tumors	Tumors/ mouse	% of mice with tumors	Tumors/ mouse	% of mice with tumors	Tumors/ mouse
						Experiment 3						
Water	28	32.0 ± 0.7	36	0.36 ± 0.09	79	4.00 ± 0.65	82	4.36 ± 0.69	61	1.82 ± 0.41	89	6.18 ± 0.96
0.6% GT	28	32.1 ± 0.6	7*	0.07 ± 0.05*	46†	1.25 ± 0.34*	46*	1.32 ± 0.38*	39‡	0.68 ± 0.22*	61†	2.00 ± 0.50*
			(80)	(81)	(41)	(69)	(43)	(70)	(35)	(63)	(32)	(68)
0.6% BT	27	32.5 ± 0.6	15‡	0.15 ± 0.07†	56‡	1.26 ± 0.38*	59‡	1.41 ± 0.42*	44	0.70 ± 0.19*	74	2.11 ± 0.55*
			(59)	(58)	(29)	(69)	(28)	(68)	(27)	(62)	(17)	(66)
0.6% DGT	27	32.9 ± 0.4	15‡	0.19 ± 0.09‡	59‡	2.93 ± 0.69	59‡	3.11 ± 0.72	67	1.85 ± 0.48	78	4.96 ± 1.14
			(59)	(47)	(25)	(27)	(28)	(29)	(0)	(0)	(13)	(20)
0.6% DBT	26	32.1 ± 0.4	12†	0.15 ± 0.09‡	77	2.88 ± 0.57‡	81	3.04 ± 0.56‡	54	1.58 ± 0.39	81	4.62 ± 0.86
			(68)	(58)	(2)	(28)	(2)	(30)	(11)	(13)	(10)	(25)
0.044% CF	27	32.0 ± 0.4	7†	0.07 ± 0.05*	63	1.70 ± 0.50*	63‡	1.78 ± 0.50*	37‡	0.63 ± 0.20*	63†	2.41 ± 0.63*
			(79)	(81)	(20)	(58)	(23)	(59)	(39)	(65)	(29)	(61)
DGT + CF	25	32.3 ± 0.5	4*	0.04 ± 0.04*,$^{\#}$	56‡	1.04 ± 0.25*,$^{\|\|}$	56†	1.08 ± 0.26*,§	44$^{\#}$	0.92 ± 0.27$^{\dagger,\#}$	64†	2.00 ± 0.48*,$^{\|\|}$
			(89)	(89)	(29)	(74)	(32)	(75)	(28)	(49)	(28)	(68)
DBT + CF	29	31.5 ± 0.4	10†	0.10 ± 0.06*	38*,§	0.69 ± 0.20*,§	41*,§	0.79 ± 0.22*,§	28$^{\dagger,\|\|}$	0.38 ± 0.14*,§	52*,$^{\|\|}$	1.17 ± 0.26*,§
			(71)	(72)	(52)	(83)	(50)	(82)	(55)	(79)	(42)	(81)

a: Values are means ± SE; values in parentheses represent percent inhibition (comparison with water positive control group); *n*, number of mice at time of sacrifice.

b: SKH-1 mice were treated with ultraviolet B light (UV-B) for 22–23 wk, and UV-B administration was stopped. These tumor-free mice had a high risk of developing skin tumors during the next several months. In Experiment 1, tumor-free mice previously treated with UV-B (40 mice/group) were treated with a 1.25% green tea leaf or black tea leaf extract (GT or BT, 1.25 g tea leaf/100 ml water, approx 4 mg tea solids/ml) as the sole source of drinking fluid for 23 wk. In Experiment 2, tumor-free mice previously treated with UV-B (40 mice/group) were treated with 0.6% lyophilized green tea (GT, 6 mg tea solids/ml), black tea (BT, 6 mg tea solids/ml), or decaffeinated green and black teas (DGT and DBT, 6 mg tea solids/ml) as their sole source of drinking fluid for 18 wk. Other mice received 0.044% caffeine (CF, 0.44 mg caffeine/ml) or 0.6% lyophilized decaffeinated teas plus 0.044% caffeine. In Experiment 3, tumor-free mice previously treated with UV-B (30 mice/group) were treated as described for Experiment 2, but duration of treatment was 23 wk. In each experiment, masses were characterized by histological evaluation.

c: Statistical evaluation of differences from water positive control group (*, $p < 0.01$; †, $p < 0.05$; ‡, $p < 0.10$) or between DGT and DGT + CF or DBT and DBT + CF (§, $p < 0.01$; ||, $p < 0.05$; #, $p < 0.10$) was done by Student's *t*-test or Fisher's exact test.

Table 2. Effect of Oral Administration of Green Tea, Black Tea, Decaffeinated Green Tea, Decaffeinated Black Tea, and Caffeine on Size of Tumors in SKH-1 Mice Previously Treated With UV-B (High-Risk Mice)[a–c]

		Squamous Cell Papillomas		Keratoacanthomas		Total Nonmalignant Tumors		Squamous Cell Carcinomas		Total Tumors	
Treatment	*n*	Tumor volume/ tumor	Tumor volume/ mouse	Tumor volume/ tumor	Tumor volume/ mouse	Tumor volume/ tumor	Tumor volume/ mouse	Tumor volume/ tumor	Tumor volume/ mouse	Tumor volume/ tumor	Tumor volume/ mouse
						Experiment 1					
Water	35	3.4 ± 0.9	1.6 ± 0.5	2.7 ± 0.5	5.5 ± 1.3	2.8 ± 0.5	7.1 ± 1.4	418 ± 361	466 ± 403	131 ± 111	475 ± 403
GT	37	1.9 ± 1.0 (44)	0.3 ± 0.2* (81)	1.9 ± 0.4 (30)	2.7 ± 0.8† (51)	1.9 ± 0.4‡ (32)	3.0 ± 0.9* (58)	143 ± 94 (66)	101 ± 67 (78)	45 ± 29 (66)	104 ± 67 (78)
BT	34	0.3 ± 0.1* (91)	0.1 ± 0* (94)	1.3 ± 0.3* (52)	2.0 ± 0.9† (64)	1.2 ± 0.3* (57)	2.1 ± 0.9* (70)	83 ± 33 (80)	76 ± 30 (84)	29 ± 12 (78)	78 ± 30 (84)
						Experiment 2					
Water	35	5.2 ± 2.4	1.0 ± 0.6	2.1 ± 0.4	2.4 ± 0.8	2.5 ± 0.5	3.5 ± 1.0	225 ± 178	96 ± 81	55 ± 43	100 ± 81
0.6% GT	34	0.4 ± 0.2† (92)	0 ± 0‡ (100)	1.2 ± 0.3† (43)	1.1 ± 0.5* (54)	1.2 ± 0.3† (52)	1.1 ± 0.5† (69)	103 ± 62 (54)	24 ± 16 (75)	22 ± 14 (60)	25 ± 16 (75)
0.6% BT	35	0.2 ± 0.1† (96)	0 ± 0‡ (100)	0.6 ± 0.2* (71)	0.5 ± 0.2† (79)	0.6 ± 0.2* (76)	0.5 ± 0.2* (86)	69 ± 25 (69)	18 ± 9 (81)	16 ± 7 (71)	18 ± 9 (82)
0.6% DGT	33	3.5 ± 2.0 (33)	1.0 ± 0.6 (0)	0.6 ± 0.2* (71)	0.8 ± 0.2† (67)	1.1 ± 0.4† (56)	1.8 ± 0.7‡ (49)	28 ± 21 (88)	14 ± 11 (85)	7 ± 5 (87)	15 ± 11 (85)
0.6% DBT	34	2.0 ± 0.7 (62)	0.5 ± 0.2 (50)	2.6 ± 1.1 (0)	4.3 ± 2.0 (0)	2.6 ± 0.9 (0)	4.8 ± 0.2 (0)	81 ± 41 (64)	45 ± 25 (53)	21 ± 10 (62)	50 ± 25 (50)
0.044% CF	36	0.4 ± 0.1† (92)	0 ± 0‡ (100)	0.5 ± 0.1* (76)	0.4 ± 0.1* (83)	0.5 ± 0.1* (80)	0.4 ± 0.1* (89)	14 ± 9 (94)	4 ± 3 (96)	3 ± 2 (95)	4 ± 3 (96)
DGT + CF	34	0.2 ± 0.1†,# (96)	0 ± 0‡,# (100)	1.2 ± 0.5‡ (43)	0.7 ± 0.4† (71)	1.0 ± 0.4† (60)	0.7 ± 0.4* (80)	34 ± 31 (85)	11 ± 10 (89)	11 ± 10 (80)	12 ± 10 (88)
DBT + CF	36	1.8[d] (65)	0 ± 0‡,‖ (100)	0.5 ± 0.2*,# (76)	0.3 ± 0.1*,‖ (88)	0.5 ± 0.2*,‖ (80)	0.3 ± 0.1*,§ (91)	73 ± 43 (68)	24 ± 15 (75)	27 ± 16 (51)	25 ± 15 (75)

(Continued)

Table 2. *(Continued)*

Treatment	*n*	Squamous Cell Papillomas		Keratoacanthomas		Total Nonmalignant Tumors		Squamous Cell Carcinomas		Total Tumors	
		Tumor volume/ tumor	Tumor volume/ mouse	Tumor volume/ tumor	Tumor volume/ mouse	Tumor volume/ tumor	Tumor volume/ mouse	Tumor volume/ tumor	Tumor volume/ mouse	Tumor volume/ tumor	Tumor volume/ mouse
						Experiment 3					
Water	28	27.5 ± 11.4	9.8 ± 4.7	2.8 ± 0.5	11.0 ± 2.8	4.8 ± 1.2	20.9 ± 5.6	40.1 ± 19.6	73.1 ± 35.7	15.2 ± 5.9	94.0 ± 36.0
0.6% GT	28	0.3 ± 0.2† (99)	0† (100)	0.5 ± 0.1* (82)	0.6 ± 0.3* (95)	0.5 ± 0.1* (90)	0.7 ± 0.3* (97)	13.3 ± 4.3‡ (67)	9.0 ± 3.5† (88)	4.8 ± 1.6† (68)	9.7 ± 3.6† (90)
0.6% BT	27	0.7 ± 0.4† (97)	0.1 ± 0.1† (99)	0.4 ± 0.1* (86)	0.5 ± 0.2* (95)	0.4 ± 0.1* (92)	0.6 ± 0.3* (97)	12.7 ± 7.0‡ (68)	8.9 ± 5.0† (88)	4.5 ± 2.4† (70)	9.6 ± 5.0† (90)
0.6% DGT	27	2.2 ± 0.8† (92)	0.4 ± 0.3† (96)	1.2 ± 0.2* (57)	3.4 ± 0.8* (69)	1.2 ± 0.2* (75)	3.8 ± 1.0* (82)	12.4 ± 5.1‡ (69)	22.9 ± 12.7‡ (69)	5.4 ± 1.9‡ (64)	26.6 ± 13.0† (72)
0.6% DBT	26	66.7 ± 57.8 (0)	10.3 ± 10.2 (0)	0.8 ± 0.2* (71)	2.2 ± 0.8* (80)	4.1 ± 3.0 (15)	12.5 ± 10.2 (40)	13.7 ± 4.6‡ (66)	21.6 ± 9.1‡ (70)	7.4 ± 2.6 (51)	34.0 ± 13.2‡ (64)
0.044% CF	27	2.4 ± 1.8† (91)	0.2 ± 0.2† (98)	0.5 ± 0.1* (82)	0.8 ± 0.2* (93)	0.5 ± 0.1* (90)	1.0 ± 0.3* (95)	12.9 ± 10.5 (68)	8.1 ± 6.6† (89)	3.8 ± 2.8† (75)	9.1 ± 6.6† (90)
DGT + CF	25	0.5[d] (98)	0 ± 0$^{\dagger,\#}$ (100)	0.3 ± 0.2*,§ (89)	0.4 ± 0.2*,§ (96)	0.3 ± 0.2*,§ (94)	0.4 ± 0.2*,§ (98)	17.7 ± 9.3 (56)	16.3 ± 9.4‡ (78)	8.3 ± 4.4 (45)	16.7 ± 9.5† (82)
DBT + CF	29	1.7 ± 1.2† (94)	0.2 ± 0.1† (98)	0.6 ± 0.2* (79)	0.4 ± 0.2*,‖ (96)	0.8 ± 0.2* (83)	0.6 ± 0.2* (97)	26.9 ± 18.2 (33)	10.2 ± 8.5† (86)	9.1 ± 6.1 (40)	10.7 ± 8.5$^{\dagger,\#}$ (89)

a: Values are means ± SE in mm^3; values in parentheses represent percent inhibition (comparison with water positive control group); *n*, number of mice at time of sacrifice.

b: SKH-1 mice were treated with UV-B for 22–23 wk, and UV-B administration was stopped. These tumor-free mice with a high risk of developing skin tumors during the next several months were treated orally with green tea (GT) or black tea (BT) leaf extract (1.25 g tea leaf/100 ml water; approximately 4 mg tea solids/ml; Experiment 1) or with GT (6 mg tea solids/ml), BT (6 mg tea solids/ml), decaffeinated green tea (DGT), decaffeinated black tea (DBT), or caffeine (CF) for Experiments 2 and 3 as described in Table 1 footnote. Other mice received DGT or DBT plus CF as described in Table 1 footnote. Size of each histologically characterized tumor described in Table 1 was determined.

c: Statistical evaluation of differences from water positive control group (*, $p < 0.01$; †, $p < 0.05$; ‡, $p < 0.10$) or between DGT and DGT + CF or DBT and DBT + CF (§, $p < 0.01$; ‖, $p < 0.05$; #, $p < 0.10$) was done by Student's *t*-test.

d: Only 1 tumor was observed.

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