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Inhibitory Effect of Green Tea in the Drinking Water on Tumorigenesis by Ultraviolet Light and 12-*O*-Tetradecanoylphorbol-13-acetate in the Skin of SKH-1 Mice[1]

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ABSTRACT

Green tea was prepared by extracting 12.5 g of green tea leaves twice with 500 ml of boiling water, and the extracts were combined. This 1.25% green tea extract (1.25 g of tea leaves/100 ml of water) contained 4.69 mg of green tea extract solids per ml and was similar in composition to some green tea beverages consumed by humans. A 2.5% green tea extract (2.5 g of tea leaves/100 ml of water) was prepared similarly. Treatment of female SKH-1 mice with 180 mJ/cm² of ultraviolet B light (UVB) once daily for 7 days resulted in red sunburn lesions of the skin. The intensity of red color and area of these lesions were inhibited in a dose-dependent fashion by the administration of 1.25 or 2.5% green tea extract as the sole source of drinking water before and during UVB treatment. Treatment of female SKH-1 mice with 180 mJ/cm² of UVB once daily for 10 days followed 1 wk later by twice weekly application of 12-*O*-tetradecanoylphorbol-13-acetate for 25 wk resulted in the development of skin tumors. The formation of skin tumors was inhibited by administration of 1.25% green tea extract as the sole source of drinking water prior to and during the 10 days of UVB treatment and for 1 wk after UVB treatment. In additional experiments, female SKH-1 mice were treated with 200 nmol of 7,12-dimethylbenz(*a*)anthracene followed 3 wk later by irradiation with 180, 60, or 30 mJ/cm² of UVB twice weekly for 30 wk. UVB-induced formation of skin tumors and increased spleen size were inhibited by administration of 1.25% green tea extract as the sole source of drinking water prior to and during the 30 wk of UVB treatment. In these experiments, treatment of the animals with the green tea extract not only decreased the number of skin tumors but also decreased substantially the size of the tumors. In additional studies, SKH-1 mice were initiated by topical application of 200 nmol of 7,12-dimethylbenz(*a*)anthracene followed by twice weekly application of 12-*O*-tetradecanoylphorbol-13-acetate for 25 wk. Administration of 1.25% green tea extract as the sole source of drinking water during promotion with 12-*O*-tetradecanoylphorbol-13-acetate reduced the number and incidence of skin tumors.

INTRODUCTION

Green tea is widely used as a beverage in Japan, China, and other Far Eastern countries. Although green tea is also used in North America and Europe, black tea is a more popular beverage in the Western countries. Studies on the effects of tea on the incidence of cancer in humans have been inconclusive. Some epidemiology studies have suggested an inhibitory effect, others an enhancing effect, and still others a lack of effect of tea ingestion on cancer risk (1). However, none of these studies was definitive, and more epidemiological research is needed.

In 1987, Yoshizawa *et al.* (2) reported that topical application of (−)-epigallocatechin gallate, a major polyphenolic catechin in green tea, inhibited teleocidin-induced tumor promotion in the skin of mice previously initiated with 7,12-dimethylbenz(*a*)anthracene. In subsequent studies, it was found that p.o. administration of (−)-epigallocatechin gallate inhibited the formation of tumors in the duodenum of mice previously initiated with ENNG[3] (3), and p.o. administration of a polyphenol fraction of green tea inhibited 7,12-dimethylbenz(*a*)anthracene-induced tumorigenesis in mouse skin (4). In recent studies, we found that topical application of a green tea polyphenol fraction to CD-1 mice inhibited the tumor-initiating activities of benzo(*a*)pyrene and 7,12-dimethylbenz(*a*)anthracene and the tumor-promoting activity of TPA[4] (Ref. 5). We also found that topical application of the green tea polyphenol fraction inhibited TPA-induced inflammation, ornithine decarboxylase activity, hyperplasia, and hydrogen peroxide production in the epidermis of mice (Footnote 4; Ref. 5). Preliminary reports have recently appeared that describe an inhibitory effect of p.o. administered green tea (6) or a green tea polyphenol fraction (7) on UVB-induced tumorigenesis. In this paper we describe the inhibitory effects of green tea in the drinking water on UVB-induced sunburn lesions and UVB-induced tumorigenesis in SKH-1 mouse skin. We also report an inhibitory effect of p.o. administration of green tea on TPA-induced tumor promotion in mouse skin.

MATERIALS AND METHODS

Chemicals and Green Tea. Purified water was prepared by reverse osmosis and was used for the preparation of green tea and for all of the animal studies. (−)-Epigallocatechin gallate, (−)-epicatechin gallate, (−)-epigallocatechin, and (−)-epicatechin were purchased from the Kurita Industrial Co., Ltd., Chromato Division (Tokyo, Japan). TPA was obtained from the LC Services Corporation (Woburn, MA). Acetone, methanol, chloroform, ethyl acetate, and 10% buffered formalin phosphate were obtained from Fisher Scientific (Springfield, NJ). DMBA was obtained from Calbiochem-Behringer (San Diego, CA). Green tea (special gunpowder) was exported by the China National Native Produce and Animal By-Products Import and Export Corporation, Zhejiang Tea Branch (Zhejiang, China), and was purchased from the Kam Kuo Co. (New York, NY).

Animals. Female SKH-1 hairless mice (6 to 8 wk old) were purchased from Charles River Laboratories (Kingston, NY). The animals were kept in our animal facility for at least 1 wk before use. Mice were given water and Purina Laboratory Chow 5001 diet *ad libitum* (Ralston-Purina Co., St. Louis, MO) and kept on a 12-h-light, 12-h-dark cycle.

Preparation and Composition of Green Tea. Green tea leaves (12.5 g)

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[3] The abbreviations used are: ENNG, *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; UVB, ultraviolet B light; UVA, ultraviolet A light; DMBA, 7,12-dimethylbenz(*a*)anthracene; HPLC, high-pressure liquid chromatography.

[4] M-T. Huang, C-T. Ho, T. Ferraro, T. Finnegan-Olive, Y-R. Lou, Z. Y. Wang, H. Newmark, C. S. Yang, J. M. Mitchell, J. D. Laskin, and A. H. Conney. Inhibitory effect of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin, submitted for publication.

were added to 500 ml of boiling water and were steeped for 15 min. The infusion was cooled to room temperature in an ice bath and then filtered. The tea leaves were extracted a second time with 500 ml of boiling water and filtered, and the two filtrates were combined to obtain a 1.25% green tea water extract (1.25% g of tea leaves/100 ml of water). The resulting clear solution is similar to tea brews consumed by humans. In some experiments, 25 g of tea leaves were extracted as described above to obtain a 2.5% green tea water extract (2.5 g of tea leaves/100 ml of water). In other experiments, the 1.25% green tea water extract was diluted 1:1 with water to make a 0.63% green tea extract. The amount of solids present in the 1.25% green tea extract was determined to be 4.69 mg per ml by drying samples in an air convection oven (18 h at 65°C) and weighing the dry residue.

The composition of 1.25% green tea extract was determined by HPLC analysis using a Hewlett Packard Model 1090 system with a diode array ultraviolet detector. The HPLC method used a Hewlett Packard C-18 reverse-phase column (5 μm Hypersil octadecylsilane; 200 x 2.1 mm I.D.). The green tea water extracts were filtered through a 0.45 μm filter disk, and 20 μl were injected onto the column. The chromatography was monitored at 273 nm, and UV spectra were collected to confirm peak purity. The mobile phase contained three solvents (Solvent A, acetonitrile; Solvent B, 0.5% glacial acetic acid in water; and Solvent C, isopropanol) run by a step gradient method at 45°C as follows: 0 to 5 min (100% Solvent B); 5 to 15 min (5% Solvent A, 95% Solvent B); 15 to 22 min (1% Solvent A, 96% Solvent B, 3% Solvent C); 22 to 24 min (5% Solvent A, 90% Solvent B, 5% Solvent C); and 24 to 30 min (100% Solvent A). The retention times of gallic acid, theobromine, (−)-epigallocatechin, (+)-catechin, caffeine, (−)-epicatechin, (−)-epigallocatechin gallate, and (−)-epicatechin gallate were 4.0, 7.5, 8.7, 9.1, 12.1, 13.7, 14.5, and 24.2 min, respectively.

Green tea extracts were prepared every 2 to 3 days for use in animal experiments and were stored at 4°C until used. The stability of several polyphenols in the green tea extract upon storage in clear or dark brown water bottles at room temperature for 72 h was determined by the methodology described above.

Ultraviolet Light. Ultraviolet lamps (FS72T12-UVB-HO) that emit UVB (280 to 320 nm; 75 to 80% of total energy) and UVA (320 to 375 nm; 20 to 25% of total energy) were obtained from the Voltare Co. (Fairfield, CT). The dose of UVB was quantified with a UVB Spectra 305 dosimeter obtained from the Daavlin Co. (Bryan, OH). The radiation was further calibrated with a Model IL1700 research radiometer/photometer from International Light, Inc. (Neburgport, MA).

For exposure to UV (UVA + UVB), 10 mice were housed in a 25.4-cm x 45.7-cm plastic box. Six boxes (without tops) were placed under 8 UV lamps (50.8 x 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 to UVB was controlled by a Spectra 305 dosimeter. The exposure time for a 180-mJ/cm² dose of UVB was 130 to 160 s. Although all data are expressed as exposure to UVB, some additional exposure to UVA also occurred as indicated above.

UVB-TPA Protocol. In Experiment 1, female SKH-1 hairless mice (30 per group) were irradiated with UVB (180 mJ/cm²) once daily for 10 days, followed 2 wk later by twice weekly topical applications of TPA (10 nmol/application in 100 μl of acetone for 15 wk and 16 nmol/application for another 10 wk). A 1.25% green tea extract was placed in water bottles and given to the mice as their sole source of drinking water 2 wk before irradiation with UVB until 1 wk after the last dose of UVB. In Experiment 2, the mice were provided with green tea in their drinking water by a gradual stepwise increase in concentration (from 25 to 100% of the original extract), which allowed the animals to adapt to the green tea. The mice were provided with 25, 50, 75, and 100% of the full 1.25% green tea extract for 2 days at each concentration. The mice were continued on the full 1.25% green tea extract for an additional 7 days before treatment with UVB (180 mJ/cm²) once daily for 10 days. Tea administration was continued during the UVB treatment period and for 1 wk after discontinuation of UVB. One wk later, the mice were treated with twice weekly applications of 16 nmol of TPA for 25 wk. The gradual stepwise increase in the concentration of green tea described above prevented the decrease in body weight that was observed in Experiment 1. Body weight determinations were made periodically during Experiments 1 and 2. Skin tumors with a diameter greater than 1 mm were counted every 2 wk.

DMBA-UVB Protocol. Mice were initiated with DMBA followed 3 wk later by UVB treatment (30 to 180 mJ/cm²) twice weekly. The mice were given green tea extract just before and during the UVB treatment. In Experiment 1, 30 female SKH-1 hairless mice (6 to 8 wk old) were treated topically with 100 μl of acetone or 200 nmol of DMBA in 100 μl of acetone, followed 3 wk later by twice weekly applications of UVB (180 mJ/cm²) for 25 wk. The mice were given either water or a 1.25% green tea extract as their sole source of drinking water starting 1 wk after DMBA and 2 wk before UVB treatment. Tea was administered throughout the UVB treatment and until the end of the experiment. In Experiments 2, 3, and 4, 30 female SKH-1 mice were treated topically with 200 nmol of DMBA or vehicle, followed 3 wk later by twice weekly UVB treatment for 30 wk. At 2 wk before UVB treatment, the mice were given 25, 50, and 75% of the original green tea extract (2 days at each concentration), followed by the full extract which was administered for 1 wk before and throughout the UVB treatment interval until the end of the study. Body weights and skin tumors greater than 1 mm in diameter were recorded. At the end of each study, each skin was placed in 10% buffered formalin phosphate for histological examination. All tumors with a carcinoma-like appearance and several other representative tumors from each mouse were examined histologically. In Experiments 3 and 4, the number of each kind of tumor type was determined for each mouse.

DMBA-TPA Protocol. Female SKH-1 hairless mice (6 to 8 wk old; 30 mice/group) were treated topically with 200 μl of acetone or 200 nmol of DMBA in 200 μl of acetone. In Experiment 1, mice were treated with 1.25% green tea extract as their sole source of drinking water starting 1 wk after DMBA and continuing throughout the tumor promotion regimen until the end of the study. Two wk after the start of tea (3 wk after DMBA), the mice were treated twice weekly with 5 nmol of TPA for 25 wk. In Experiment 2, the mice were given 25, 50, and 75% of the original green tea extract (2 days at each concentration), followed by the full extract which was continued for 1 wk before and throughout the TPA treatment interval.

UVB-induced Sunburn Lesions. Female SKH-1 hairless mice (6 to 8 wk old; 10 mice/group) were administered 25, 50, 75, and 100% of a 1.25 or 2.5% green tea extract as their sole source of drinking water (2 days at each dose level), followed by full-strength green tea until the end of the study. The mice were irradiated with UVB (180 mJ/cm² once daily for 7 days) at 2 wks after the start of green tea administration. The incidence, area, and intensity of red color of the UVB-induced skin lesions were measured.

RESULTS

Composition and Stability of Compounds in Green Tea. A 1.25% green tea water extract (1.25 g of tea leaves/100 ml of water) similar in composition to that ingested by humans was prepared, and it contained 4.69 mg of tea solids per ml. Analysis of the green tea extract by HPLC indicated that the total green tea solids in the extract contained (−)-epigallocatechin gallate (15.1%), (−)-epigallocatechin (6.9%), (−)-epicatechin gallate (3.0%), (−)-epicatechin (1.8%), (+)-catechin (0.5%), caffeine (8.1%), and theobromine (0.4%) (Table 1). The compounds that were measured accounted for 36% of the total green tea solids present in the water extract. Storage of the green tea water extract in water bottles for 72 h at room temperature resulted in only a small decrease (8 to 12%) in the concentration of tea catechins (data not presented). Little or no difference between the storage of 1.25% green tea extract in clear or dark brown water bottles was observed.

Table 1 *Composition of 1.25% green tea water extract*

Chinese gunpowder green tea leaves (12.5 g) were extracted twice with 500 ml of water. The clear filtrates were combined and analyzed by HPLC. The filtrate contained 4.69 mg of green tea solids per ml.

Component	μg/ml	% of solids in extract
Total catechins	1280	27.3
Catechins		
(−)-Epigallocatechin gallate	708	15.1
(−)-Epigallocatechin	324	6.9
(−)-Epicatechin gallate	141	3.0
(−)-Epicatechin	84	1.8
(+)-Catechin	23	0.5
Caffeine	380	8.1
Theobromine	19	0.4

Inhibitory Effect of Orally Administered Green Tea on UVB-induced Skin Lesions. Application of 180 mJ/cm² of UVB to SKH-1 mice once daily for 7 days resulted in the formation of red skin lesions. The formation of these skin lesions by UVB light was inhibited in a dose-dependent manner by administration of green tea in the drinking water for 2 wk prior to and during application of UVB (Fig. 1). This inhibition was characterized by a decreased area and intensity of red color of the lesions in green tea-treated animals. Treatment of the mice with green tea extract did not influence the body weight (data not shown).

Inhibitory Effect of Orally Administered Green Tea on UVB-induced Initiation of Tumorigenesis in Mouse Skin. Although topical application of UVB (180 mJ/cm²) once daily for 10 days followed by topical application of TPA twice weekly for 25 wk resulted in the development of skin tumors (Fig. 2), treatment of parallel groups of mice with UVB or TPA alone did not result in any tumors (data not presented). The time of appearance of tumors was delayed, and the number of tumors per mouse was decreased in this UVB/TPA model when green tea extract was given in the drinking water for 2 wk before and until 1 wk after UVB treatment (Fig. 2). In Experiment 1, the animals were given 1.25% green tea extract as their sole source of drinking water starting 2 wk before UVB treatment, and this resulted in a decrease in fluid consumption and a drop in body weight (Fig. 2, *top*). In a second experiment, the mice were administered 25, 50, 75, and 100% of the full concentration of the 1.25% green tea extract as their sole source of drinking water starting on Days 1, 3, 5, and 7 (2 days at each dose), and full-strength green tea was then administered for a week prior to and until 1 wk after UVB treatment. Green tea was discontinued 1 wk after the last dose of UVB, and the animals were promoted with TPA twice weekly for 25 wk. No significant effect of green tea on body weight was observed in this second study. Administration of a 1.25% green tea extract as the sole source of drinking water decreased the number of tumors per mouse by 82% in Experiment 1 and by 64% in Experiment 2. The incidence of tumor-bearing mice was decreased by 77% in Experiment 1 and 38% in Experiment 2.

Inhibitory Effect of Green Tea on UVB-induced Tumorigenesis in DMBA-initiated SKH-1 Mice. Although the topical application of 200 nmol of DMBA to SKH-1 mice did not cause the formation of skin tumors (data not presented), subsequent treatment of these mice with UVB twice weekly produced large numbers of tumors. The formation of these tumors was inhibited by administration of green tea just prior to and during the UVB treatment period.

In four separate experiments, we treated SKH-1 mice with 200 nmol of DMBA. One wk later, the mice were given green

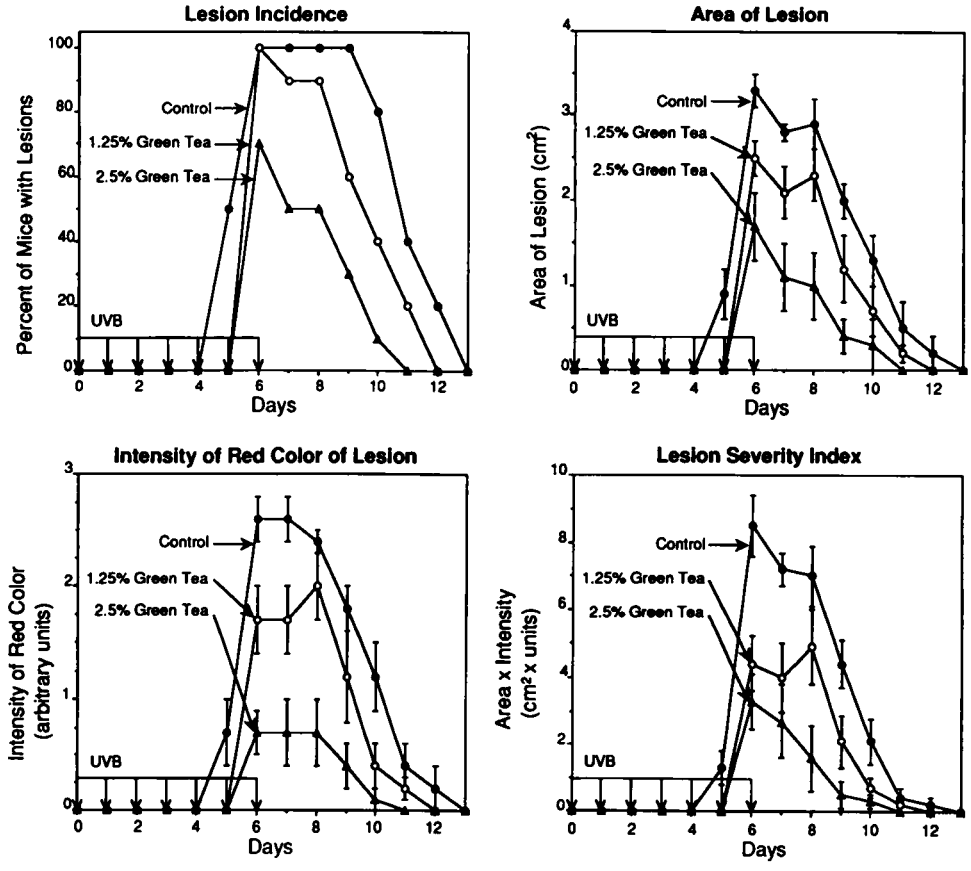

Fig. 1. Inhibitory effect of p.o. administration of green tea on UVB-induced skin lesions. Female SKH-1 mice (10 per group) were given green tea as their sole source of drinking water for 2 wk before, during, and for 7 days after treatment with UVB (180 mJ/cm²) once daily for 7 days as described in "Materials and Methods." The area (cm²) and intensity (arbitrary units of red color) of the UVB-induced skin lesions were measured daily for each mouse. A subjective scale for the measurement of the intensity of red color of the lesions was as follows: *0*, no lesion; *1* barely detectable red lesion; *2*, moderate red lesion, and *3*, bright red lesion. *Points*, mean from 10 mice; *bars*, SE.

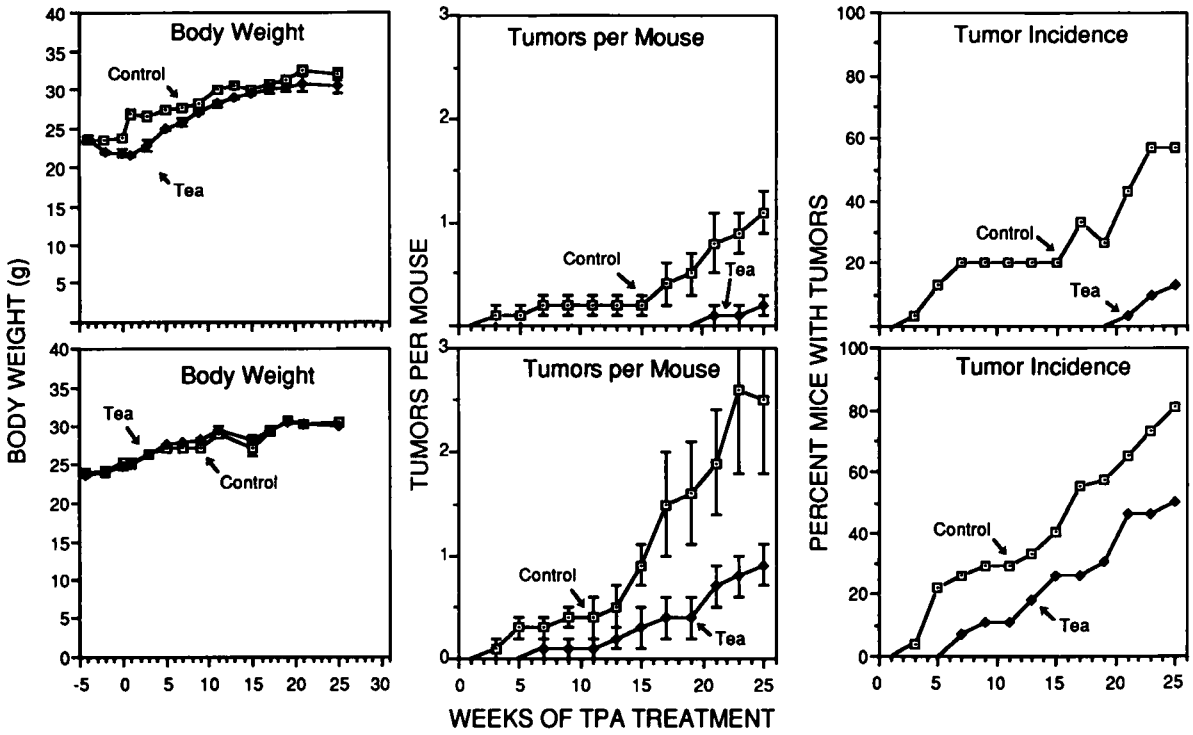

Fig. 2. Inhibitory effect of p.o. administration of green tea on initiation of skin tumors by UVB. In Experiment 1 (*top*), female SKH-1 mice were given 1.25% green tea extract as their sole source of drinking water for 2 wk prior to and during 10 days of UVB treatment (180 mJ/cm^2/day) and for 1 wk after UVB treatment. The mice were then given only water and treated topically with TPA twice weekly for 25 wk as described in "Materials and Methods." In Experiment 2 (*bottom*), female SKH-1 mice (30 per group) were given water or green tea extract in the drinking water with a stepwise increase in concentration for 1 wk, followed by full-strength 1.25% green tea extract for an additional week prior to and during 10 days of UVB treatment (180 mJ/cm^2/day) and for 1 wk after UVB treatment. The mice were then given only water and treated topically with TPA twice weekly for 25 wk as described in "Materials and Methods." *Points*, mean from 26 to 30 mice; *bars*, SE.

tea in the drinking water, and 2 wk after starting green tea administration the mice in Experiments 1, 2, 3, and 4, respectively, were treated with 180, 180, 60, or 30 mJ/cm^2 twice weekly for 25 to 30 wk. Green tea administration was continued until the termination of the study. The results of these studies are shown in Fig. 3. In Experiment 1, administration of 1.25% green tea extract reduced body weight 5 to 20% during the first 4 to 6 wk and caused an 87% decrease in the number of tumors per mouse that resulted from treatment with 180 mJ/cm^2 of UVB twice weekly for 25 wk. The percentage of tumor-bearing mice was decreased by 45%. In Experiments 2, 3, and 4, the dose of green tea was gradually increased during the first week of tea treatment, and full-strength tea was administered for 1 wk prior to twice weekly application of UVB and until the end of each study. No significant effect of green tea on body weight was observed using this dosing regimen. Under these conditions, 1.25% green tea extract (4.69 mg of tea solids/ml) as the sole source of drinking water decreased by 41% the number of tumors per mouse that were induced by 180 mJ/cm^2 of UVB twice weekly for 30 wk. In Experiment 3, the administration of 0.63 or 1.25% green tea extract as the sole source of drinking water decreased by 53% and 67%, respectively, the number of tumors per mouse that resulted from treatment of DMBA-initiated mice with 60 mJ/cm^2 of UVB twice weekly. In Experiment 4, administration of these same green tea extracts decreased by 38% and 59%, respectively, the number of tumors per mouse that resulted from 30 mJ/cm^2 of UVB twice weekly in DMBA-initiated mice. The data obtained from Experiments 2 to 4 indicate that administration of green tea delays by 2 to 5 wk the time required for the appearance of tumors. The results obtained from Experiments 3 and 4 indicate that the 0.63% green tea extract is somewhat less effective than 1.25% green tea extract in inhibiting UVB-induced tumorigenesis in DMBA-initiated SKH-1 mice.

Administration of green tea not only decreased the number of UVB-induced tumors per mouse in DMBA-initiated SKH-1 mice (Fig. 3), but tumor size was also markedly decreased (Table 2). In Experiment 1, we observed that the tumors in tea-treated animals were smaller than in the water control group, but the magnitude of the effect was not quantified. In Experiment 2, 1.25% green tea extract as the sole source of drinking water decreased tumor size by 75%. In Experiment 3, 0.63% and 1.25% green tea extract as the sole source of drinking water decreased tumor size by 85% and 77%, respectively. In Experiment 4, these same green tea extracts decreased tumor size by 55% and 84%, respectively.

Mice that were initiated with DMBA and treated twice weekly with 180, 60, or 30 mJ/cm^2 of UVB for 30 wk had an average tumor size of 932 mm^3, 750 mm^3, and 121 mm^3 per mouse, respectively, and the spleen weights were increased by 97, 118, and 31%, respectively (Table 2). Treatment of the mice with 1.25% green tea just prior to and during 180 mJ/cm^2, 60 mJ/cm^2, or 30 mJ/cm^2 of UVB twice weekly inhibited the increase in tumor volume per mouse by 85, 93, and 93%, respectively, and the increase in spleen size was inhibited 69, 82, and 100%, respectively (Table 2). Treatment of the mice with 0.63% green tea extract as their sole source of drinking water just prior to and during 60 mJ/cm^2 or 30 mJ/cm^2 of UVB twice a week for 30 wk decreased the tumor volume per mouse by 93 and 72%, respectively, and the increase in spleen size was inhibited 53 and 45%, respectively (Table 2). We evaluated the relationship between tumor size and spleen weight in all of our tumor-

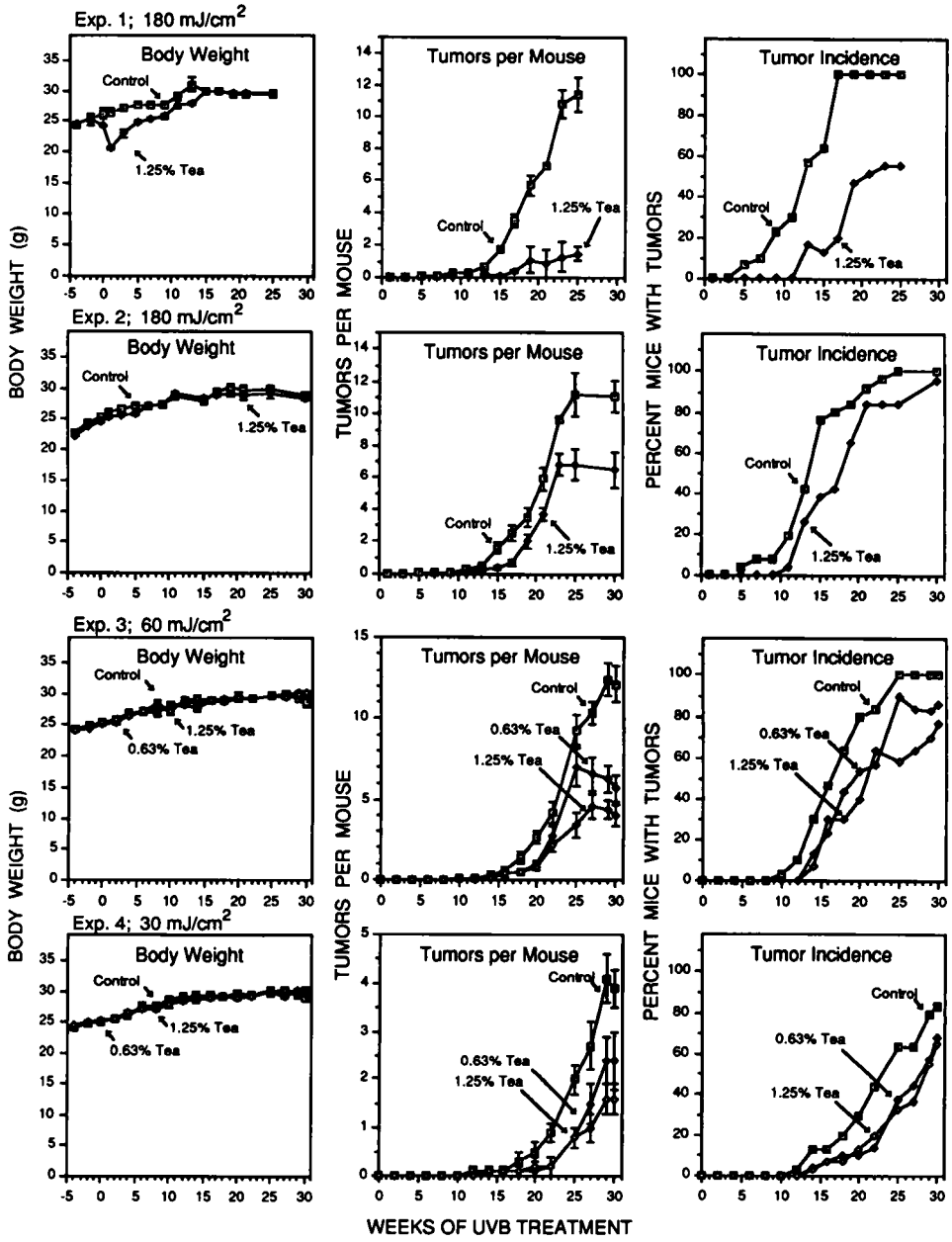

Fig. 3. Inhibitory effect of p.o. administration of green tea on UVB-induced skin tumors in mice previously initiated with DMBA. Female SKH-1 mice were initiated with 200 nmol of DMBA, and p.o. administration of green tea was started 1 wk later. In Experiment 1, the mice were given 1.25% green tea extract as their sole source of drinking water for 2 wk prior to UVB treatment and during the UVB treatment period as described in "Materials and Methods." In Experiments 2, 3, and 4, mice were given gradually increasing amounts of green tea extract in the drinking water for 1 wk, followed by full-strength tea for an additional week prior to and during treatment with UVB for 30 wk as described in "Materials and Methods." *Points*, mean from 25 to 30 mice; *bars*, SE.

bearing and non-tumor-bearing animals from Experiments 2, 3, and 4 (Fig. 3). When we plotted the total tumor volume per mouse *versus* spleen size for individual animals, a positive relationship was observed ($r = 0.535$, $P < 0.01$).

Histological examination of the tumors that resulted from treating DMBA-initiated mice with UVB for 25 to 30 wk (Fig. 3, Experiments 1 to 4) revealed the presence of papillomas, keratoacanthomas, squamous cell carcinomas, and basal cell carcinomas (Table 3). Treatment of the mice with green tea decreased the percentage of mice with papillomas in all four experiments, and the percentage of mice with keratoacanthomas and squamous cell carcinomas was decreased in 3 of the 4 experiments (Table 3). Basal cell carcinomas were only observed in Experiments 1 and 3, and green tea administration completely prevented the formation of basal cell carcinomas in both experiments (Table 3). In Experiments 3 and 4, we evaluated the effect of green tea administration on the total number and kinds of neoplasms that were induced in DMBA-initiated mice that were treated with UVB. These results indicate that treatment of mice with 1.25% green tea extract as their sole source of drinking water had a strong inhibitory effect on the formation of papillomas, keratoacanthomas, and basal cell carcinomas, but a smaller inhibitory effect was observed on the formation of squamous cell carcinomas (Table 4).

Inhibitory Effect of Green Tea on TPA-induced Tumor Promotion in Mouse Skin. Administration of green tea in the drinking water for 2 wk prior to and during twice weekly topical application of 5 nmol of TPA for 25 wk inhibited tumorigenesis in SKH-1 mice previously initiated with 200 nmol of DMBA. The administration of 1.25% green tea extract as the sole source of drinking water for 2 wk prior to and during TPA promotion resulted in a 5 to 20% decrease in body weight during the first 2 to 6 wk of tea administration, and there was an 84% decrease in the number of tumors per mouse after 25 wk of TPA promotion (Fig. 4, Experiment 1). In Experiment 2, the dose of tea was gradually increased during the first week and given at full strength during the second week and during TPA administration. No effect on body weight was observed. In this study,

Table 2 *Effect of p.o. administration of green tea on tumor size and spleen weight during skin tumorigenesis initiated by DMBA followed by treatment with UVB in SKH-1 mice*

Female SKH-1 mice were treated topically with DMBA or vehicle and given green tea in the drinking water for 2 wk before and during treatment with UVB as described in "Materials and Methods."

Treatment	No. of mice	Body wt (g)	Spleen wt (mg)	Tumor vol/ mouse (mm^3)	Av. vol/tumor (mm^3)
180 mJ/cm^2 twice weekly					
Control	20	28.7 ± 0.3[a]	159 ± 8[b]	0	0
DMBA + UVB + water	25	28.9 ± 0.4	313 ± 28	932 ± 324	84 ± 24
DMBA + UVB + tea (1.25%)	26	28.4 ± 0.2	206 ± 17[c]	136 ± 29[c]	21 ± 5[c]
60 mJ/cm^2 twice weekly					
Control	44	30.4 ± 0.2[c]	140 ± 4[b]	0	0
DMBA + UVB + water	30	28.5 ± 0.7	305 ± 38	750 ± 313	62 ± 24
DMBA + UVB + tea (0.63%)	29	29.9 ± 0.3	218 ± 19[c]	51 ± 15[c]	9 ± 2[c]
DMBA + UVB + tea (1.25%)	30	30.1 ± 0.4	169 ± 12[d]	56 ± 18[c]	14 ± 4[c]
30 mJ/cm^2 twice weekly					
Control	44	30.4 ± 0.2[c]	140 ± 4[d]	0	0
DMBA + UVB + water	30	28.9 ± 0.4	184 ± 19	121 ± 56	31 ± 13
DMBA + UVB + tea (0.63%)	28	29.7 ± 0.3	164 ± 11	34 ± 19	14 ± 7
DMBA + UVB + tea (1.25%)	29	30.2 ± 0.3[c]	120 ± 6[d]	8 ± 4[c]	5 ± 2[c]

[a] Mean ± SE, obtained from Experiments 2, 3, and 4 that are described in Fig. 3.
[b] Statistically different from the DMBA + UVB + water group ($P < 0.001$).
[c] Statistically different from the DMBA + UVB + water group ($P < 0.05$).
[d] Statistically different from the DMBA + UVB + water group ($P < 0.01$).

Table 3 *Effect of green tea on the histopathology of tumors in mice initiated with DMBA followed by treatment with UVB*

Female SKH-1 mice were treated as described in the legend to Fig. 3. Histological studies were done with all tumors having a carcinoma-like appearance and with several other representative tumors from each tumor-bearing animal.

Experiment	Treatment	No. of mice	% of mice with			
			Papilloma	Keratoacanthoma	Squamous cell carcinoma	Basal cell carcinoma
1	UVB (180 mJ/cm^2) twice weekly for 25 wk					
	Water	30	67	97	47	10
	Tea (1.25%)	29	7	66	3	0
2	UVB (180 mJ/cm^2) twice weekly for 30 wk					
	Water	25	48	92	60	0
	Tea (1.25%)	26	12	100	73	0
3	UVB (60 mJ/cm^2) twice weekly for 30 wk					
	Water	30	33	97	40	13
	Tea (0.63%)	29	31	93	31	0
	Tea (1.25%)	30	3	73	33	0
4	UVB (30 mJ/cm^2) twice weekly for 30 wk					
	Water	30	20	80	20	0
	Tea (0.63%)	28	0	64	14	0
	Tea (1.25%)	29	7	48	14	0

administration of 1.25% green tea extract as the sole source of drinking water decreased the number of tumors per mouse by 55% (Fig. 4, Experiment 2).

DISCUSSION

The data presented here indicate that p.o. administration of an aqueous green tea extract (4.69 mg of green tea solids/ml) as the sole source of drinking water to SKH-1 mice inhibited UVB-induced sunburn lesions (Fig. 1), UVB-induced initiation of skin tumors (Fig. 2), UVB-induced skin tumorigenesis in mice previously initiated with DMBA (Fig. 3), and TPA-induced tumor promotion in mice previously initiated with DMBA (Fig. 4). In addition to inhibiting the formation of tumors in mice, the p.o. administration of green tea caused a marked decrease in tumor size (Table 2). The concentration of total tea solids and of individual polyphenolic compounds in the green tea used for our studies (Table 1) is similar to the concentration of polyphenols and total tea solids present in some green tea brews used by humans. Treatment of SKH-1 mice with UVB (180 mJ/cm^2) once daily for 10 days followed by topical application of TPA in acetone twice weekly for 25 wk produced skin tumors in 55 to 80% of the mice (Fig. 1). In a parallel group of control mice, topical application of TPA twice weekly for 25 wk or treatment with UVB (180 mJ/cm^2) once daily for 10 days followed by topical application of only acetone twice weekly for 25 wk did not cause the formation of any tumors (data not presented). Our results indicate that brief exposure of mouse skin to UV light causes permanent cellular changes that do not result in skin tumors unless the mice are then treated with TPA for several weeks. These observations indicate that UV can function as an initiator and TPA as a promoter of tumorigenesis in mouse skin. An advantage of this animal model of photocarcinogenesis is the short duration of UV irradiation that is needed.

In a second animal model, treatment of DMBA-initiated SKH-1 mice with UV twice weekly for 30 wk resulted in large numbers of papillomas, keratoacanthomas, and squamous cell carcinomas as well as some basal cell carcinomas (Tables 3 and 4). No tumors were observed in DMBA-initiated mice that were not subsequently treated with UV light.[5] In animals treated

[5] Z-Y. Wang, M-T. Huang, T. Ferraro, C. S. Yang, and A. H. Conney, unpublished observations.

Table 4 *Effect of green tea on the number of papillomas, keratoacanthomas, squamous cell carcinomas, and basal cell carcinomas in mice initiated with DMBA followed by treatment with UVB*

Female SKH-1 mice were treated as described in the legend to Fig. 3. Histological studies were done with all tumors having a carcinoma-like appearance and with several other representative tumors from each tumor-bearing animal.

Treatment	No. of mice	Papilloma		Keratoacanthoma		Squamous cell carcinoma		Basal cell carcinoma	
		No.	No./mouse	No.	No./mouse	No.	No./mouse	No.	No./mouse
UVB (60 mJ/cm²) twice weekly for 30 wk									
Water	30	35	1.17	294	9.80	25	0.83	5	0.17
Tea (0.63%)	29	29	1.00	189	6.52	16	0.55	0	0
Tea (1.25%)	30	1	0.03	138	4.60	17	0.57	0	0
UVB (30 mJ/cm²) twice weekly for 30 wk									
Water	30	9	0.30	136	4.53	7	0.23	0	0
Tea (0.63%)	28	0	0	61	2.18	6	0.21	0	0
Tea (1.25%)	29	2	0.07	37	1.28	5	0.17	0	0

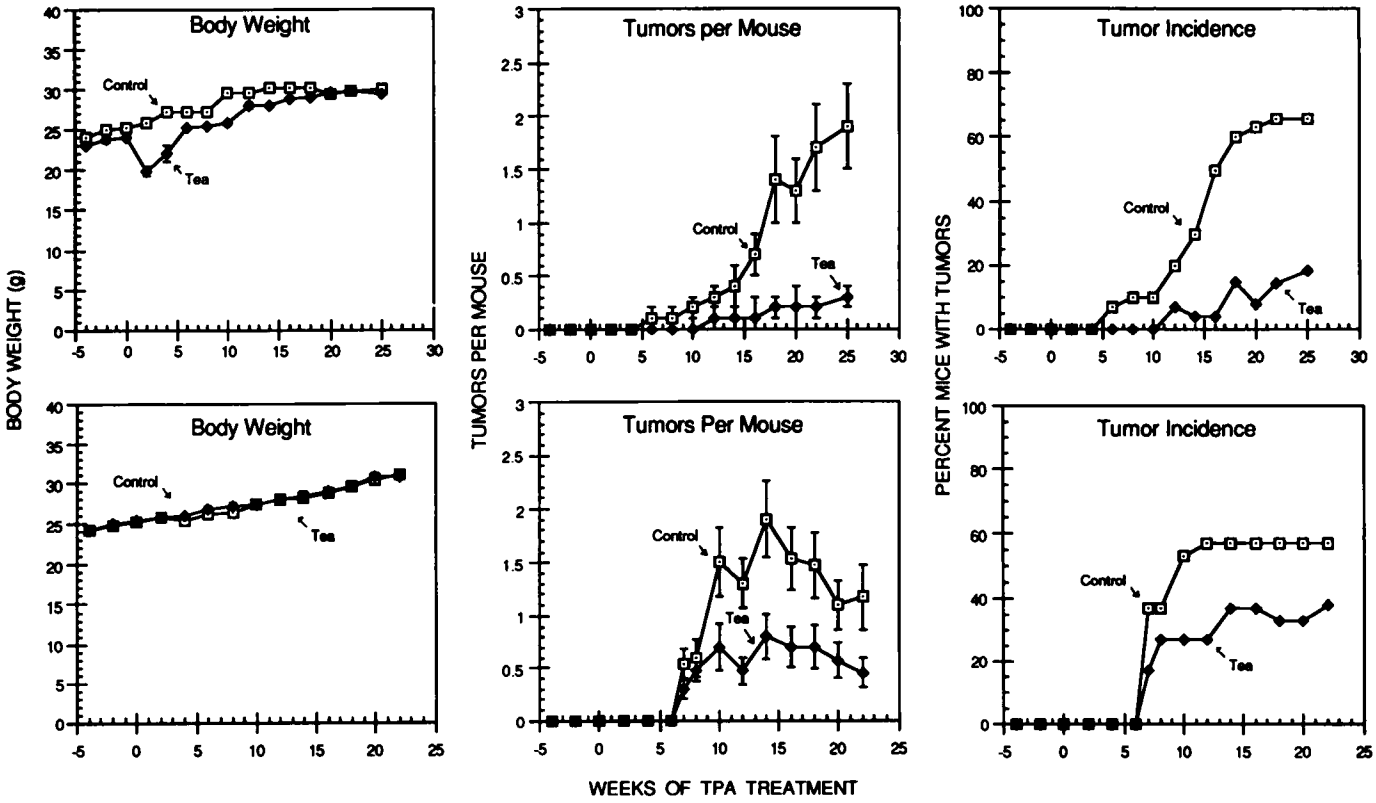

Fig. 4. Inhibitory effect of p.o. administration of green tea on TPA-induced tumor promotion in mice previously initiated with DMBA. Female SKH-1 mice were initiated with 200 nmol of DMBA. A week later, the mice were given water or green tea extract in the drinking water. In Experiment 1 (*top*), the mice were given 1.25% green tea extract as the sole source of drinking water. In Experiment 2 (*bottom*), the mice were given green tea extract in the drinking water with a stepwise increase in concentration for 1 wk followed by full-strength 1.25% green tea extract for an additional week prior to and during promotion with 5 nmol of TPA twice weekly for 22 to 25 wk as described in "Materials and Methods." *Points*, mean from 29 to 30 mice; *bars*, SE.

topically with 200 nmol of DMBA followed by 30 mJ/cm² of UVB twice weekly, the first tumor was observed at 10 wk whereas animals treated with only UVB twice weekly had their first tumor at 25 wk.[5] We believe the tumorigenic action of UVB in this DMBA/UVB animal model results from UVB-induced repetitive and cumulative DNA damage in mouse epidermis that was already damaged from prior DMBA treatment. In addition to the well-known DNA-damaging effect of UV irradiation, it is likely that UV also exerts additional modulation of cellular function that is not a direct DNA-damaging effect and that UV irradiation may have some "tumor-promoting" activity. UV light has been shown to induce epidermal ornithine decarboxylase activity (8, 9), inflammation (10), and the production of hydrogen peroxide (11), and these effects may contribute to the tumorigenic action of UV light in DMBA-initiated mice.

The inhibitory effect of p.o. administered green tea on UV- and TPA-induced tumorigenesis may be due to the antioxidant properties of green tea polyphenols (12, 13). Several compounds with antioxidant activity inhibit UV light-induced skin carcinogenesis (14–19). Ascorbic acid (14), α-tocopherol (15), β-carotene (16), selenium (17), butylated hydroxytoluene (18), and a mixture of dietary antioxidants (ascorbic acid, α-tocopherol, reduced glutathione, and butylated hydroxytoluene) have been reported to inhibit UV-induced skin carcinogenesis (19). (−)-Epigallocatechin gallate, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-epicatechin, and (+)-catechin are some of the polyphenolic compounds in green tea, and all of these compounds possess antioxidant activity (13). Caffeine is another component of green tea that may contribute to the inhibitory effect of this beverage on UV-induced tumorigenesis. Topical application of caffeine has been shown to inhibit UV-induced

tumorigenesis on mouse skin (20), and caffeine has also been shown to inhibit breast tumorigenesis in rats (21, 22).

The molecular mechanisms of UV-induced skin sunburn lesions and skin tumor initiation are unknown. UV light has been reported to cause oxidative damage in cellular DNA (23–30), presumably because of the ability of UV to generate reactive oxygen-free radicals (31) and to cause oxidative DNA modification (23–30). Exposure of cultured cells or hairless mice to UV causes mutations and the formation of a cyclobutane-pyrimidine dimer (major photoproduct), pyrimidine-pyrimidone (6–4) photoproducts, thymine glycol, 5-hydroxymethyl-2′-deoxyuridine, and 8-hydroxy-2′-deoxyguanosine in DNA as well as DNA-protein cross-links (23–30). In addition to these UV-induced DNA lesions, UV has also been shown to inhibit DNA repair and to damage the immune system (32–37). The importance of high levels of DNA repair for the prevention of UV-induced carcinogenesis was emphasized in studies with xeroderma pigmentosum patients who are deficient in the excision repair of UV-induced pyrimidine dimers (38). These patients and cultured cells from these patients are particularly sensitive to UV-induced mutagenesis and carcinogenesis (39). It is of interest that certain polyphenols in green tea inhibit UV-induced mutagenesis in a repair-proficient strain of *Escherichia coli*, but no inhibitory effect was observed in a repair-deficient strain (40). These results suggest that green tea polyphenols can protect the bacterial DNA repair system from UV-induced damage or that the tea polyphenolic compounds enhance the activity of the DNA repair system in *E. coli* (40). Additional studies are needed to determine whether administration of green tea enhances the repair of UV-induced DNA lesions in mouse epidermis.

UV light and TPA have been shown to damage the immune system (32–37, 41–43), possibly by increasing the cellular production of active oxygen species that reduce immune function (44). Antioxidants such as ascorbic acid, α-tocopherol, and glutathione can protect the immune system from active oxygen species (44). Topical application of α-tocopherol was found to inhibit UV-induced immunosuppression in the skin of mice (15), and it is possible that the antioxidants in green tea may exert a similar effect. The marked increase in spleen size observed in mice treated chronically with UV (Table 2) suggests that UV treatment (or the tumors generated by UV treatment) may have an inhibitory effect on the immune system. It is of interest that p.o. administration of green tea inhibited UV-induced increases in spleen size (Table 2). Studies on the effect of green tea on the immune system are needed.

In some animal models, the administration of high doses of certain tea components or related substances may have tumor-enhancing effects. Repeated s.c. injections of a chloroform-isolated polyphenolic fraction of black tea (8 mg once a week for 45 to 77 wk) resulted in fibrous histiocytomas in rats (45). Repeated topical applications of a black tea extract were reported to increase the incidence of skin tumors in mice that were previously treated with benzo(*a*)pyrene (46). In contrast, using a similar model, the incidence of skin tumors was unchanged, but the latent period for tumor appearance was decreased (47). Although we have not studied the effect of topical administration of black tea extracts, recent studies in our laboratory demonstrated a potent inhibitory effect of a green tea polyphenol fraction on TPA-induced tumor promotion in DMBA-initiated mice (Footnote 4; Ref. 5). Recently, in a different model, a decreased incidence of *N*-nitrosomethylbenzylamine-induced esophageal tumors was found in rats given black tea (48). In other studies, multiple injections of tannic acid in rats were reported to cause liver tumors (49), but it should be noted that tannic acid is not a known component of any tea beverage (1). In our studies, administration of a 1.25% green tea extract as the sole source of drinking water for 30 wk did not cause liver tumors or signs of liver toxicity in SKH-1 mice.[5]

In addition to the inhibitory effect of p.o. administered green tea on the UV- and chemically induced skin tumors described here, p.o. administration of green tea also inhibits the formation of chemically induced tumors in several other organs. Administration p.o. of green tea to rodents inhibits *N*-nitrosodiethylamine-induced forestomach and lung tumors (50), 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone-induced lung tumors (51), and *N*-nitrosomethylbenzylamine-induced esophageal tumors (48, 52). In addition to these studies, p.o. administration of (−)-epigallocatechin gallate (the major polyphenol in green tea) inhibits ENNG-induced duodenal tumors (3). Epidemiology studies on the effects of green tea on human cancer have been contradictory (1), and more extensive epidemiology studies are needed.

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