Inhibitory effect of black tea on the growth of established skin tumors in mice: effects on tumor size, apoptosis, mitosis and bromodeoxyuridine incorporation into DNA

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Female CD-1 mice were initiated with a single topical application of 7,12-dimethylbenz[a]anthracene and promoted with 12-O-tetradecanoylphorbol-13-acetate. Mice with established papillomas were then treated with black tea or decaffeinated black tea (4 mg tea solids/ml) as the sole source of drinking fluid for 11–15 weeks. In four separate experiments, oral administration of black tea inhibited the growth of papillomas (increase in tumor volume/mouse) by an average of 35%, 37%, 41% and 48%, respectively. Studies with decaffeinated black tea gave inconsistent results. In one experiment, administration of decaffeinated black tea inhibited papilloma growth (increase in tumor volume/mouse) by 27%, but in two additional experiments papilloma growth was stimulated by 14% and 193%, respectively. In a separate experiment, skin tumors were generated by treating SKH-1 female mice with ultraviolet B light (UVB; 30 mJ/cm²) twice weekly for 22 weeks, after which UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice were then treated with black tea (6 mg/ml tea solids) as the drinking fluid for 11 weeks. In this experiment, tumor growth (increase in tumor volume/mouse) was inhibited by 70%. Histological examination revealed that tea-treated mice had a 58% decrease in the number of nonmalignant tumors (primarily keratoacanthomas)/mouse and a 54% decrease in the number of squamous cell carcinomas/mouse. In addition, administration of black tea decreased the volume per tumor by 60% for nonmalignant tumors and by 84% for carcinomas. Mechanistic studies with tumors from these mice revealed that administration of black tea decreased the bromodeoxyuridine labeling index in squamous cell papillomas, keratoacanthomas and squamous cell carcinomas by 56%, 45% and 35%, respectively, and the apoptosis index was increased by 44%, 100% and 95%, respectively. Administration of black tea decreased the mitotic index in keratoacanthomas and squamous cell carcinomas by 42% and 16%, respectively. The results indicate that oral administration of black tea to tumor-bearing mice inhibited proliferation and enhanced apoptosis in nonmalignant and malignant skin tumors.

Materials and methods

Chemicals and tea
DMBA was obtained from Calbiochem—Behring (San Diego, CA), and TPA was obtained from LC Services Corporation (Woburn, MA). Bromodeoxyuridine was obtained from Sigma Chemical Company (St Louis, MO), 10% buffered formalin phosphate was purchased from Fisher Scientific (Springfield, NJ). Black tea and decaffeinated black tea were prepared from commercial grade tea leaves or lyophilized aqueous tea extracts from commercial grade tea leaves. Decaffeinated tea leaves were prepared by extracting tea leaves with supercritical CO2. All samples of tea were obtained from the US Tea Association (New York, NY).

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

Preparation of tea
In experiments 1–3, tea infusions were prepared using a commercial Bunn automatic basket tea brewer. Tea leaves (50 g) were placed in a filter paper-lined brewing basket, and 4 l of hot, deionized water were passed through the tea leaves in the brewing machine. The resulting tea brews contained 4 mg/ml tea solids and were similar to those consumed by humans. For experiments 4 and 5, lyophilized extracts of tea leaves were dissolved in water to produce reconstituted teas containing 4 or 6 mg/ml of tea solids, respectively. The compositions of the tea preparations (catechins, theaflavins, thearubigens, other polyphenols and caffeine) were described elsewhere (2) and were not significantly different for the hot water extract of tea leaves and for the lyophilized hot water extracts of tea leaves that were reconstituted with water.

UV light
UV lamps (FS72T12-UVB-HO) that emit UVB (280–320 nm; 75–80% of total energy) and UVA (320–375 nm; 20–25% of total energy) were obtained from the US Tea Association (Newburyport, MA).

For exposure to UV (UVA + UVB), 10 mice were housed in a 25.4 x 45.7 cm plastic box. Six boxes (without tops) were placed under eight 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 points.
positions under the lamps. The distance between the UV lamps and the backs of the mice or the UVB detector was ~43 cm. The amount of exposure to UVB was controlled by a Spectra 305 Dosimeter. The exposure time for a 30 mJ/cm² dose of UVB was ~25 s. Although all data are expressed as percent of 30 mJ/cm² UVB, some additional exposure to UVA also occurred as indicated above.

**Formation of established skin tumors in mice and treatment of tumor-bearing mice with tea**

Female CD-1 mice with skin papillomas were used for experiments 1–4, and female SKH-1 mice with skin papillomas, keratoacanthomas and carcinomas were used for experiment 5. After the generation of papillomas by topical DMBA/TPA treatment in experiments 1–4, the generation of papillomas, keratoacanthomas and carcinomas in SKH-1 mice by treatment with UVB in experiment 5, tumor-bearing mice were selected in each experiment and distributed into parallel groups with similar tumor characteristics (number of tumors/mouse, tumor size, etc.). The control group of mice were given water and the corresponding treated mice were given tea as their sole source of drinking fluid. In experiment 1, skin tumors were initiated by topical application of 200 nmol DMBA in 200 µl acetone, and 1 week later the mice were promoted with 5 nmol TPA (in 200 µl acetone) twice a week for 24 weeks. Four days after stopping TPA administration, water or black tea (1.25 g tea leaf/100 ml hot water; ~4 mg/ml tea solids) was given to the mice (28–29 animals/group) as their sole source of drinking fluid for 11 weeks. In experiment 2, skin tumors were initiated by topical application of 200 nmol DMBA, and 1 week later the mice were promoted with 5 mol TPA twice a week for 13 weeks. Twelve days after stopping TPA administration, water or black tea (1.25 g tea leaf/100 ml hot water; ~4 mg/ml tea solids) was given to the mice (27 animals/group) as their sole source of drinking fluid for 12 weeks. In experiment 3, mice were initiated by topical application of 20 nmol DMBA, and 1 week later the mice were promoted with 5 nmol TPA twice weekly for 17 weeks. At 3 days after stopping TPA, water or black tea (1.25 g tea leaf/100 ml hot water; ~4 mg/ml tea solids) was given to the mice (20 animals/group) as their sole source of drinking fluid for 12 weeks. In experiment 4, skin tumors were generated by initiating the mice with 200 nmol DMBA. One week later the mice were promoted with 5 nmol TPA twice a week for 14 weeks. Water or black tea (4 mg/ml lipoysylated tea solids) was given to the mice (28–29 animals/group) as their sole source of drinking fluid starting at 30 days after stopping TPA treatment, and tea administration was continued for 15 weeks. In experiment 5, skin tumors were generated by treating SKH-1 female mice (7–8 weeks old) with UVB (30 mJ/cm²) twice a week for 22 weeks, and UVB was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) were then treated with water or black tea (6 mg/ml lipoysylated tea solids) as their sole source of drinking fluid for 11 weeks. Fourteen to fifteen mice/group remained when the animals were killed. In all five studies, the mice were given 25%, 50% and 75% of full strength tea infusions (2 days at each concentration), followed by full strength tea until the end of each study.

**Tumor volume calculation, tissue section preparation and histopathological classification of tumors**

In all experiments, tumor volume was calculated by:

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

where \(r\) = radius. At the end of experiment 5, all skin samples with masses were placed in 10% buffered formalin phosphate for 24 h at 4°C. The tissue samples were dehydrated in ascending concentrations of ethanol, cleared in xylene, and embedded in Paraplast. Three 4 µm serial sections were cut through the center of each mass. One section was stained with eosin-philoxine-hematoxylin and examined by light microscopy for histopathological classification of tumors and focal hyperplastic areas according to the classification of Hui et al. (5) and in a kit from Oncogene Science (Cambridge, MA). Briefly, all the animals in experiment 5 were injected i.p. with BrdUr (50 mg/kg) and killed 1 h later. Sections from all skin masses were prepared as described above. Endogenous peroxidase was blocked by incubating the sections in 3% hydrogen peroxide in methanol for 10 min at room temperature. Specimens were then incubated in a moist chamber with 0.125% trypsin for 10 min at 37°C. Tissue sections were rinsed in distilled water and incubated at room temperature for 30 min with denaturing solution (Oncogene Science). The sections were incubated with blocking solution for 10 min at room temperature and covered with biotinylated mouse monoclonal anti-BrdUr antibody (Oncogene Science) for 30 min at room temperature for 90 min. Sections were rinsed with phosphate buffered saline and incubated with streptavidin-peroxidase for 10 min. Color development was achieved by incubation for 5 min at room temperature with a substrate solution containing 0.02% 3,3'-diaminobenzidine tetrahydrochloride and 0.02% hydrogen peroxide. The slides were weakly counterstained in Mayer’s hematoxylin (Sigma) for 2 min, cleared with xylene, mounted with a coverslip and scored under a light microscope. The method is described in more detail by Fukuda et al. (5) and in a kit from Oncogene Science. The BrdU labeling index was calculated from the number of stained BrdU positive cells/100 cells counted in each focal epidermal hyperplastic area and in each tumor. Based on the lesion size, 2–12 representative areas/lesion were measured.

**Measurement of apoptosis**

All skin tumors and focal hyperplastic areas observed in animals from experiment 5 were studied for their apoptosis status by utilizing the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) method which detects digoxigenin-label 3'-OH ends of genomic DNA. Briefly, apoptotic cells were detected in situ using an immunoperoxidase Apoptag kit (Oncor, Gaithersburg, MD). Endogenous peroxidase was blocked by incubating the sections in 3% hydrogen peroxide in phosphate buffered saline for 5 min at room temperature and the specimens were incubated with 20 µg/ml proteinase K (Sigma) for 15 min at 37°C. After proteinase treatment, tissue sections were rinsed in phosphate buffered saline (pH 7.2) and incubated for 5 min with equilibrium buffer (Oncor). After equilibration, the sections were incubated in a humidified chamber with terminal deoxynucleotidyl transferase (TdT) enzyme for 1 h at 37°C (for the negative control, water was used instead of TdT enzyme). Sections were soaked in stop-wash buffer (Oncor) for 30 min and then rinsed in three changes of phosphate buffered saline. After rinsing, sections were covered with anti-digoxigenin-peroxidase (Oncor) and incubated at room temperature in a humidified chamber for 30 min. Sections were rinsed with phosphate buffered saline, and the brown color development was achieved by incubation for 6 min at room temperature with a substrate solution containing 0.224 mM 3,3'-diaminobenzidine with 0.02% hydrogen peroxide. The slides were counterstained in a methyl green solution (Oncor) for 10 min and visualized and scored under a light microscope. The details of this method are described by Wijsman et al. (6) and in the kit from Oncor. The apoptosis index is expressed as the amount of positive staining/lesion. This was semi-quantitatively assessed by estimation of the number and intensity of the stained cells/field. Based on the lesion size, 1.5–16 microscope fields (200-fold magnification/lesion) were evaluated and the average apoptosis index was calculated for each lesion. In this way, four grades of staining were observed: grade 0, no reaction; grade 1, weak reaction; grade 2, moderate reaction; and grade 3, strong reaction.

**Measurement of mitotic index**

Mitotic count is an established indicator of tumor proliferation. To measure the mitotic index (positive cells/microscope field), all mitotic figures including normal and atypical mitoses present in the entire tumor section were assessed with a 400-fold magnification. In this way, 3–32 microscope fields/tumor (representative of the entire tumor) were evaluated, and the average mitotic index was calculated for each lesion. Additional details of the methodology are given elsewhere (7).

**Results**

**Effect of oral administration of black tea on tumor growth in mice with established papillomas**

In four separate experiments with papilloma-bearing mice obtained by prior treatment with DMBA/TPA, oral administration of black tea (~4 mg/ml tea solids) as the sole source of drinking fluid inhibited growth of the tumors (increase in tumor volume/mouse) by 35%, 37%, 41%, and 48%, respectively (Figure 1, experiments 1–4). Comparable studies with decaffeinated black tea (~4 mg/ml tea solids gave inconsistent results. In one experiment, administration of decaffeinated black tea as the sole source of drinking fluid inhibited papilloma growth by 27% but in two additional experiments, papilloma growth was stimulated by 14 and 193%, respectively (data not shown).
Inhibition of tumor growth by black tea

Fig. 1. Effect of oral administration of black tea (BT) on the growth of established skin tumors in mice. Papilloma-bearing CD-1 mice previously initiated with DMBA and promoted with TPA were utilized in experiments 1–4. Tumor-bearing SKH-1 mice previously treated with UVB were utilized in experiment 5. In experiments 1–3, teas were prepared by extracting 1.25 g tea leaf/100 ml hot water (~4 mg/ml tea solids). In experiments 4 and 5, lyophilized tea solids were used (4 mg/ml tea solids in experiment 4 and 6 mg/ml tea solids in experiment 5). Tumor-bearing mice were treated with the tea preparations as their sole source of drinking fluid for 11–15 weeks. The details of these experiments are given in the Materials and methods section.

Effect of oral administration of black tea on tumor growth, mitosis, BrdUr incorporation into DNA and apoptosis in mice with established papillomas, keratoacanthomas and squamous cell carcinomas

In an additional study utilizing UVB pretreated SKH-1 mice with papillomas, keratoacanthomas and squamous cell carcinomas (experiment 5), oral administration of black tea (6 mg/ml tea solids) for 11 weeks had no effect on body wt but inhibited tumor growth (increase in tumor volume/mouse) by 70% when measured in living animals (Figure 1).

Histological examination of the profile of all tumors in the animals from experiment 5 revealed that tea-treated mice had a 58% decrease in the number of nonmalignant tumors (primarily keratoacanthomas)/mouse and a 54% decrease in the number of carcinomas per mouse (Tables I and II). In addition, the administration of black tea for 11 weeks decreased the volume/tumor by 60% for nonmalignant tumors and by 84% for carcinomas (Table III). Tumor volume/mouse was decreased by 83% for nonmalignant tumors and 93% for squamous cell carcinomas (Table III). These results indicate that administration of black tea to tumor-bearing mice inhibits the formation of new skin tumors and the growth of existing skin tumors.

In water-treated control mice, the BrdUr labeling index and mitotic index in nonmalignant tumors (papillomas and keratoacanthomas) was substantially higher than in areas of focal epidermal hyperplasia, and these indices of cell proliferation were increased to an even greater extent in squamous cell carcinomas (Tables IV and V). The apoptosis index was lower in nonmalignant tumors and in squamous cell carcinomas than in areas of focal epidermal hyperplasia (Table VI). Similar results were also obtained in mice treated with black tea.

Administration of black tea for 11 weeks inhibited the incorporation of BrdUr into DNA by 46% in nonmalignant tumors and by 35% in squamous cell carcinomas (Table IV). The mitotic index was decreased 39% in nonmalignant tumors and by 16% in squamous cell carcinomas (Table V). These results are the first demonstration of an inhibitory effect of oral administration of tea on the proliferation of malignant and nonmalignant tumors in tumor-bearing animals.

Examination of apoptosis in all tumors from the animals of experiment 5 revealed that administration of black tea for 11 weeks increased apoptosis by 89% in nonmalignant tumors and by 95% in squamous cell carcinomas (Table VI). This is the first demonstration of a stimulatory effect of oral...
administration of tea on apoptosis in malignant and nonmalignant tumors in tumor-bearing animals.

**Discussion**

In the present study, we investigated the possibility that oral administration of black tea may have an inhibitory effect on the growth of established epidermal papillomas, keratoacanthomas and squamous cell carcinomas. We treated tumor-bearing mice with black tea as the sole source of drinking fluid for 11–15 weeks. In four separate experiments, oral administration of black tea (~4 mg/ml tea solids) to CD-1 mice inhibited the growth of papillomas by 35–48% (Figure 1, experiments 1–4). These results are similar to the inhibitory effect of oral administration of green tea on skin papilloma growth observed earlier (3). The composition and strength of the green and black tea brews used in these studies were similar to those commonly ingested by humans. However, given as the total drinking fluid of mice in our experiments, this administration represents a higher intake level than ordinary tea drinking by humans. Studies with decaffeinated black tea gave inconsistent results. In one experiment, administration of decaffeinated black tea (~4 mg/ml tea solids) inhibited papilloma growth (increase in tumor volume/mouse) by 27%, but in two additional experiments papilloma growth was stimulated by 14% and 193%, respectively. Further studies are needed to determine the effects of decaffeinated black tea and caffeine on the growth of epidermal tumors.

Although oral administration of regular green or black tea...
Inhibition of tumor growth by black tea

Table IV. Effect of oral administration of black tea on BrdUr incorporation into the DNA of established papillomas, keratoacanthomas and squamous cell carcinomas in SKH-1 mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Apoptosis index (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Focal epidermal hyperplasia</td>
<td>Squamous cell papillomas</td>
</tr>
<tr>
<td>Water</td>
<td>15</td>
<td>14.1 ± 1.5</td>
</tr>
<tr>
<td>Black tea</td>
<td>14</td>
<td>14.3 ± 1.9</td>
</tr>
</tbody>
</table>

Female SKH-1 mice (7-8 weeks old) described in Tables I-III and in the Materials and methods section for experiment 5 were irradiated with UVB (30 mJ/cm²) twice weekly for 22 weeks, and UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) were then treated with lyophilized black tea (6 mg/ml tea solids) or water as the drinking liquid for 11 weeks. BrdU (50 mg/kg) was injected i.p., and the animals were killed 1 h later. BrdU positive cells in each area of local epidermal hyperplasia and in each tumor described in Table I (total of 147 lesions) were counted and expressed as the number of positive cells/100 cells counted. Each value is the mean ± SE and the numbers in parentheses represent percent inhibition.

*<p< 0.01.

**<p< 0.05.

***<p< 0.10.

Table V. Effect of oral administration of black tea on the mitotic-index in established papillomas, keratoacanthomas and squamous cell carcinomas in SKH-1 mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Mitotic index (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Focal epidermal hyperplasia</td>
<td>Squamous cell papillomas</td>
</tr>
<tr>
<td>Water</td>
<td>15</td>
<td>0.31 ± 0.10</td>
</tr>
<tr>
<td>Black tea</td>
<td>14</td>
<td>0.25 ± 0.06</td>
</tr>
</tbody>
</table>

Female SKH-1 mice (7-8 weeks old) described in Tables I-IV and in the Materials and methods section for experiment 5 were irradiated with UVB (30 mJ/cm²) twice weekly for 22 weeks, and UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) were then treated with lyophilized black tea (6 mg/ml tea solids) or water as the drinking liquid for 11 weeks. Tissue sections from animals in experiment 5 were examined for mitoses using a microscope with a 400-fold magnification. The entire area of each focal epidermal hyperplasia and each tumor described in Table I (total of 147 lesions) were evaluated for mitoses, and the data are expressed as the number of cells in mitosis/microscopic field (mitotic index). The number of fields examined per lesion depended on the size of the lesion and varied from 3-32 fields. Each value is the mean ± SE and the numbers in parentheses represent percent inhibition.

*<p< 0.01.

**<p< 0.05.

***<p< 0.10.

Table VI. Effect of oral administration of black tea on apoptosis in established papillomas, keratoacanthomas, and squamous cell carcinomas in SKH-1 mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Apoptosis index (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Focal epidermal hyperplasia</td>
<td>Squamous cell papillomas</td>
</tr>
<tr>
<td>Water</td>
<td>15</td>
<td>1.37 ± 0.22</td>
</tr>
<tr>
<td>Black tea</td>
<td>14</td>
<td>2.15 ± 0.24b</td>
</tr>
</tbody>
</table>

Female SKH-1 mice (7-8 weeks old) described in Tables I-V and in the Materials and methods section for experiment 5 were irradiated with UVB (30 mJ/cm²) twice weekly for 22 weeks, and UVB administration was stopped. Tumors were allowed to develop during the following 13 weeks, and tumor-bearing mice (17/group) were then treated with lyophilized black tea (6 mg/ml tea solids) or water as the drinking liquid for 11 weeks. The apoptosis index was determined for each of the 147 lesions described in Table I by evaluating the number of cells stained and the intensity of staining as described in the Materials and methods section. Each value is the mean ± SE, and the numbers in parentheses represent percent increase.

*<p< 0.01.

**<p< 0.05.

***<p< 0.10.

inhibited complete UVB-induced carcinogenesis and UVB-induced carcinogenesis in DMBA-initiated mice, administration of decaffeinated green or black tea inhibited carcinogenesis in the DMBA/UVB model but had no effect or stimulated carcinogenesis in the complete UVB-induced carcinogenesis model (2,8). In these studies, oral administration of caffeine was found to be an effective inhibitor of UVB-induced complete carcinogenesis (8). Additional studies indicated that topical application of caffeine inhibited TPA-induced tumor promotion in DMBA-initiated mice (8,9).

The results of our studies indicated that treatment of mice with black tea had a small inhibitory effect on body wt
increases in some but not all experiments. A possible effect of black tea on body wt gain is important because earlier studies have shown an inhibitory effect of calorie or diet restriction on TPA-induced tumor promotion and tumor size (10,11).

In experiment 5, we evaluated the effect of oral administration of black tea (6 mg/ml tea solids) to SKH-1 mice with established papillomas, keratoacanthomas and carcinomas (in mice previously treated with UVB). In this study, oral administration of black tea for 11 weeks to tumor-bearing mice had no effect on body wt but decreased by 58% the number of nonmalignant tumors (primarily keratoacanthomas)/mouse and decreased by 54% the number of squamous cell carcinomas/mouse (Table II). The total number of histologically identified tumors/mouse was decreased by 57% in the black tea-treated animals (Table II). Administration of black tea decreased the volume/tumor by 60% for nonmalignant tumors and by 84% for carcinomas. These decreases in tumor number and in the size of the tumors were caused by a combination of an inhibitory effect of black tea administration on the formation of new tumors and an inhibitory effect of black tea on the growth of existing tumors.

Immunohistochemical studies with tumors from the mice in experiment 5 revealed that black tea decreased the incorporation of BrdUrd into the DNA of squamous cell papillomas, keratoacanthomas, and squamous cell carcinomas by 56%, 45% and 35%, respectively, and the apoptosis index was increased by 44%, 100% and 95%, respectively. Administration of black tea decreased the mitotic index in keratoacanthomas and squamous cell carcinomas by 42% and 16%, respectively. The results presented here indicate that oral administration of black tea had a strong/moderate inhibitory effect on the proliferation of nonmalignant skin tumors and a somewhat smaller inhibitory effect on the proliferation of malignant skin tumors. Oral administration of black tea had a marked stimulatory effect on apoptosis in both nonmalignant and malignant tumors. Our data represent the first observations indicating that the inhibitory effect of black tea administration on tumor growth in vivo results from a combination of decreased cell proliferation and enhanced programmed cell death. The mechanism(s) of the effects of black tea administration on proliferation and apoptosis of tumor cells requires additional research on the expression of *bax*, *bcl* 2, wild-type *p53*, *p21* and other genes that regulate the cell cycle and apoptosis. Studies by other investigators have indicated that oral administration of (−)epigallocatechin gallate to rats together with N-methyl-N'-nitro-N-nitrosoguanidine inhibited BrdUrd-labeling in the gastric mucosa when compared with labeling in the gastric mucosa of animals only treated with N-methyl-N'-nitro-N-nitrosoguanidine (12). Oral administration of a green tea polyphenol fraction or (−)epigallocatechin gallate inhibited 4-(methylN-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung hyperplasia (13), and oral administration of decaffeinated green tea inhibited salt-induced gastric hyperproliferation (14). In a recent in vitro study a green tea polyphenol fraction (80 µg/ml) stimulated apoptosis in cultured epidermal carcinoma cells A431 (15). Although the present study with black tea and our earlier study with green tea (3) are the first investigations on the effects of tea administration on the growth of non-transplanted established tumors, studies by others have indicated an inhibitory effect of orally or i.p. administered green tea or a green tea polyphenol fraction on the growth of transplanted Ehrlich ascites tumor cells, sarcoma 180 cells, human mammary cancer MCF 7 cells or human prostate cancer cells (12,14,15). In one study, oral administration of a green tea infusion (5 g tea leaves/100 ml water) twice a day starting 1 week before i.p. injection of Ehrlich ascites tumor cells and continuing for an additional 8 days resulted in a 41% inhibition of tumor growth (16). In another study, oral administration of 400–800 mg/kg of a decaffeinated instant green tea powder once a day for 4 days starting 24 h after s.c. inoculation of mice with sarcoma 180 cells resulted in a 50–59% inhibition of tumor growth during a 21-day interval (17). In another study, i.p. or s.c. injections of a green tea polyphenol fraction or (−)epigallocatechin gallate (EGCG) into mice that were inoculated s.c. with cells from a solid Ehrlich tumor or with cells from a sarcoma 180 tumor inhibited tumor growth by 20–56% (18). Feeding a green tea polyphenol fraction in the diet was also reported to inhibit the growth of sarcoma 180 cells that were inoculated into mice (18). In a recent study, daily i.p. injections of EGCG inhibited the growth of transplanted MCF 7 human mammary cancer cells or LNCaP 104-R human prostate cancer cells in immunodeficient mice (19). The results of our earlier study (3) and the present study with established tumors as well as the studies with transplanted tumors described above indicate that green tea, black tea and constituents of these teas have an inhibitory effect on tumor growth and may also cause tumor regression. Recent studies suggest that green tea can inhibit the progression of epithelial papillomas to carcinomas (20) and that oral administration of EGCG can inhibit the metastasis of B16 melanoma cells in mice (21). Although additional research is needed to investigate more fully whether green and black tea can inhibit the growth or enhance the regression of carcinomas, the present study suggests that oral administration of black tea inhibits proliferation and enhances apoptosis both in established nonmalignant and malignant epidermal tumors in mouse skin.

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**References**

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