Black tea and mammary gland carcinogenesis by 7,12-dimethylbenz[a]anthracene in rats fed control or high fat diets

Adrienne E. Rogers1, Laurie J. Hafer, Yvette S. Iskander and Shi Yang

Mallory Institute of Pathology and Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, MA 02118, USA

1To whom correspondence should be addressed
Email: aerogers@bu.edu

Epidemiological studies suggest that tea may reduce cancer risk, and in laboratory rodents, chemopreventive effects of tea or purified extracts of tea have been demonstrated in lung, gastrointestinal tract and skin. There is some evidence of chemoprevention by tea in the mammary gland, but the data are not conclusive. In order to evaluate more fully the possible influence of black tea on 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary gland tumors in the female S–D (Sprague–Dawley) rat, three large studies were performed: experiment 1, tumorigenesis in rats fed AIN-76A diet and given 25 mg/kg DMBA and 1.25 or 2.5% whole tea extract or water to drink; experiment 2, tumorigenesis in rats given 15 mg/kg DMBA and the same diet and fluids as in experiment 1; and experiment 3, tumorigenesis in rats fed control or HF (high fat) diet and given 15 mg/kg DMBA and 2% tea or water to drink. Tea was given throughout the experiment; DMBA was given by gastric gavage at 8 weeks of age. There was no consistent effect of tea on tumorigenesis in rats fed AIN-76A diet; there was, however, evidence in experiment 3 of a reduction of tumorigenesis by tea in rats fed the HF diet. In experiment 3, rats fed the HF diet and given water showed the expected increase in tumor burden (number and weight) compared with rats fed control diet. However, rats fed the HF diet and given 2% tea showed no increase in tumor burden; their tumor burden was significantly lower than in rats fed the HF diet and given water (P < 0.01) and was not different from rats fed control diet and given water or tea. In addition, in experiment 3, the number of malignant tumors per tumor-bearing rat was increased by the HF diet in water-drinking rats (P < 0.01) but not in tea-drinking rats. Therefore, it appears that tea partially blocked the promotion of DMBA-induced mammary tumorigenesis by the HF diet.

Introduction

Epidemiological studies strongly suggest that diet components are responsible in part for geographic and cultural differences in cancer site and incidence. For example, fruits and vegetables, soybeans, grains and tea are thought to contain nutrient and non-nutrient substances that reduce cancer risk in the breast, prostate and gastrointestinal tract; in contrast, fats are postulated to increase cancer risk at the same and other sites (1–5). These and other diet components have been evaluated in laboratory animal tumor models (1,5–8). The diet components considered here in relation to breast cancer, namely black tea and fats high in N-6-polyunsaturated fatty acids (N-6-PUFA*), are consumed in large amounts by people in many parts of the world and may influence cancer risk.

Recent publications of epidemiological studies indicate a possible reduction of esophageal, rectal, pancreatic and colon cancer risk associated with green tea consumption in China; studies in western populations have not yielded consistent results on effects of black tea on cancer risk at any tissue site (9–16). Epidemiological data on tea and cancer risk have been extensively reviewed recently by Yang et al. (9) who concluded that the data are suggestive of cancer risk reduction by tea at some sites but are not consistent. Kohlmeier et al. (11) concluded that there may be some protection by tea in high cancer risk groups, but the evidence is weak.

In laboratory rodents, extracts of green or black tea given in place of drinking water or added to feed reduce carcinogenesis by certain nitrosamine and polycyclic aromatic hydrocarbon carcinogens in the lung, gastrointestinal tract, liver and skin, and by UV light in the skin (9,10,17–21). Whole aqueous extracts, decaffeinated extracts and purified components of tea have been found effective to varying degrees in the different tumor models. The most extensively studied preparations reported have been aqueous extracts of green tea and its major polyphenol, (–)-epigallocatechin-3-gallate (EGCG), both of which have chemopreventive activity in the organs listed. Mechanisms postulated for the anti-carcinogenic effects of tea extracts include antioxidant activity and alteration of xenobiotic metabolism (9,10).

The epidemiological evidence for an effect of total dietary fat intake on breast cancer risk is not consistent, but a recent review (2) concluded that the weight of evidence is that post-menopausal breast cancer risk is associated with increased dietary fat intake. Attempts to detect age, endocrinological and body size characteristics as well as tumor characteristics that might clarify a relationship between dietary fat and breast cancer risk or mortality have been suggestive of increased risk of mortality with higher fat intakes (22,23) or have yielded negative results (24,25). Further studies of interactions among menopausal status, body mass and serum hormones (26,27) may contribute to clarification of the effects of dietary fat on breast cancer risk.

Diet high in N-6-PUFA are consistent and relatively powerful promoters of mammary gland tumorigenesis in laboratory rodents. Other types of fat also may be promoters. N-6-PUFA and other fats may enhance initiation of tumors as well as promotion, but their greatest and most consistent effects are as promoters (1,7,8,28).

Effects of tea have not been evaluated fully in rodent breast cancer models. The experiments that have been reported.
generally have yielded data that suggest protection by tea or its components but have not shown statistically significant effects. Hirose et al. (29) reported that female Sprague–Dawley (S–D) rats given 50 mg/kg 7,12-dimethylbenz[a]anthracene (DMBA) at 7 weeks of age and, beginning one week later, fed a natural product diet containing 1% green tea catechins (of which 53.9% was EGCG) survived longer and showed somewhat increased tumor latency and decreased tumor size compared with controls not fed tea. They reported a similar result in female F344 rats fed 2% 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) plus 1% green tea catechins in a natural product diet (30). Weisburger et al. (31) reported that female S–D rats fed a high N-6-PUFA (23.5% corn oil) diet that promotes mammary tumorigenesis, given 1.25% black tea extract to drink from 6 weeks of age, and given 5 mg DMBA (equivalent to ~25 mg/kg) by gavage at 7 weeks of age, had fewer mammary fibroadenomas than rats given water; both groups had similar numbers of adenocarcinomas. In the same laboratory in the 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) mammary tumorigenesis model, tea-drinking rats developed more tumors than controls, but the tumors were smaller and had longer latencies than in controls. Consumption of 1% black tea in a purified diet for 2 weeks before DMBA exposure reduced DMBA–DNA adducts in the mammary glands of S–D rats (32), which would predict an effect of tea on initiation of carcinogenesis.

Fujiki et al. (33) found no effect of 0.1% EGCG in drinking water on murine mammary tumor virus (MMTV) tumorigenesis in SHN mice; Sakata et al. (34) found no effect of green tea extract (0.1 and 0.05%) in drinking water in the same model.

Liao et al. (35) reported that i.p. daily injection of EGCG (1 mg) inhibited growth of tumors from human breast cancer MCF-7 cells implanted subcutaneously in BALB/c female nude mice that carried 17β estradiol implants. Komori et al. (36) reported that green tea catechins (EGCG 85%) and whole green tea extract inhibited growth of two human breast cancer cell lines (MCF-7 and BT20) in culture.

In summary, there is suggestive evidence that green tea catechins or whole green or black tea extracts reduce DMBA-or PHIP-induced mammary tumorigenesis in female rats and that similar preparations inhibit growth of human breast cancer cell lines transplanted into mice or cultured in vitro. Similar tea preparations did not reduce tumorigenesis by IQ in rats or by MMTV in SHN mice.

In order to evaluate more fully the possible influence of black tea on DMBA-induced mammary gland tumors in the female S–D rat, three large studies were performed: experiment 1, comparison of tumorigenesis in rats fed AIN-76A diet and given 25 mg/kg DMBA and 1.25% of 2.5% tea extract or water to drink; experiment 2, comparison of tumorigenesis in rats given 15 mg/kg DMBA and the same diet and drinking fluids as in experiment 1; and experiment 3, comparison of tumorigenesis in rats fed control or high fat (HF) diet, and given 15 mg/kg DMBA and either 2% tea or water to drink.

Materials and methods
Female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA), 4–5 weeks of age, were housed individually in environmentally controlled animal quarters and handled according to the NIH guidelines. They were fed AIN-76A diet throughout the experiment (experiments 1 and 2) or fed AIN-76A diet before DMBA exposure and then, 96 h later, divided into groups and fed either AIN-76A diet, or an HF diet that was nutritionally equivalent to a calorically based to the AIN-76A diet (experiment 3). The HF diet contained 24% vitamin-free casein, 24% corn oil, 30.8% sucrose, 9.3% corn-starch, 1.2% AIN vitamin mix, 4.2% AIN mineral mix, 0.24% choline bitartrate, 0.36% α-methionine and 5.9% α-cellulose.

Black tea (World Blend Tea, Southern Tea Co., Marietta, GA) was formulated and supplied under the auspices of the Tea Trade Health Research Association. The tea was a mixture of leaves grown and processed in the major tea growing countries, the percent composition from each source was constant in all lots. A different lot was used in each of the three experiments; only one lot was used for an experiment The tea (2.5% in experiments 1 and 2, 2% in experiment 3) was brewed three times per week in a Bunn® automatic basket tea maker using deionized water and was supplied fresh in calibrated bottles to the rats at that concentration or diluted to 1.25% (experiments 1 and 2). Two days later the remaining tea was measured, and fresh tea was given. Controls were given water from the same deionizing system on the same schedule. The rats were introduced to tea in increasing concentrations over a two-week period beginning at their entry into the laboratory in experiment 1.

The groups are summarized in Table I. In experiments 1 and 2, there were four DMBA-treated groups: two drinking water (C2 and C3) and two drinking tea (T2 and T3). Group T2 rats drank 1.25% tea, and they and C2 rats were given unlimited access to feed. Group T3 rats drank 2.5% tea and had unlimited access to feed; group C3 rats were individually matched by weight to T3 rats and pair-fed three times/week to the matched rat throughout the experiment. There were, in addition, two groups, 10 rats each, not given DMBA and given water (C1) or 2.5% tea (T1) and free access to feed. In experiment 3 there were four DMBA-treated groups, two fed AIN-76A diet and given either 2.0% tea (T4) or water (C4) and two fed the HF diet and given either 2.0% tea (TF4) or water (CF4).

In all experiments, the rats were weighed weekly. DMBA (25 mg/kg in experiment 1, or 15 mg/kg in experiments 2 and 3) in 0.2 ml sesame oil, was administered by gastric gavage in a single dose to rats 8 weeks of age. Beginning 4 weeks later, rats were palpated weekly for tumors. Rats were administered by gastric gavage in a single dose to rats 8 weeks of age. Beginning 4 weeks later, rats were palpated weekly for tumors. Rats were killed and necropsied when they bore tumors that were 3–4 cm in diameter or were ulcerated; all rats remaining were killed by CO2 inhalation and necropsied 16–18 weeks after DMBA administration.

All mammary glands and tumors were rapidly excised; tumors were weighed and sectioned; sections were fixed in 10% neutral buffered formalin (experiment 1) or 4% paraformaldehyde (experiments 2 and 3) or fixed on dry ice and held at ~80°C for histochemical, biochemical and molecular studies (to be reported separately). Mammary glands were similarly fixed or frozen. Fixed tissues were processed, embedded, cut and stained with hematoxylin and eosin using routine methods.

Table I. Treatment groups

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group</th>
<th>No. of rats</th>
<th>Tea</th>
<th>Water</th>
<th>DMBAa</th>
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<tr>
<td>1 and 2b</td>
<td>C1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td></td>
<td>C2</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td></td>
<td>C3</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td></td>
<td>T1</td>
<td>10</td>
<td>2.5%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>40</td>
<td>1.25%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>40</td>
<td>2.5%</td>
<td>0</td>
<td>0</td>
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<tr>
<td>3c</td>
<td>C1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td></td>
<td>C4</td>
<td>20</td>
<td>0</td>
<td>0</td>
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<td></td>
<td>CF4</td>
<td>30</td>
<td>0</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>10</td>
<td>2%</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>T4</td>
<td>20</td>
<td>2%</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>TF4</td>
<td>30</td>
<td>2%</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

a Experiment 1, 25 mg/kg in 0.2 ml sesame oil by gastric gavage at 8 weeks of age; experiments 2 and 3, 15 mg/kg in 0.2 ml sesame oil by gastric gavage at 8 weeks of age.

b All rats fed AIN-76A diet throughout with free access except C3 rats; they were matched and individually pair-fed to T3 rats.

c All rats were fed AIN-76A diet before and until 96 h after DMBA administration; from that time until termination of the experiment, CF4 and TF4 rats were fed the high N-6-PUFA diet. All rats had free access to feed.

Statistical analysis of results was performed using the programs SPSS 7.0 and SAS. The cumulative probability of bearing a palpable tumor over time was analyzed by Wilcoxon and Log-Rank tests. Chi-squared statistics were calculated to compare tumor incidences among groups. ANOVA statistics with appropriate post-hoc tests (Scheffe’s and Tukey’s B) were used to analyze and compare body weight, tumor number and tumor weight by treatment group and by pathology.
Mammary gland carcinogenesis in rats fed high fat diet and black tea

Fig. 1. Cumulative probability of bearing a mammary tumor in DMBA-treated rats in experiment 1. ■, C2; ▲, C3; ●, T2; *, T3.

Fig. 2. Cumulative probability of bearing a mammary tumor in DMBA-treated rats in experiment 2. ■, C2; ▲, C3; ●, T2; *, T3.

Fig. 3. Cumulative probability of bearing a mammary tumor in DMBA-treated rats in experiment 3. ■, C4; ▲, CF4; ●, T4; *, TF4.

Results

The rats readily accepted tea as their fluid source and ate and gained weight normally. In experiments 1 and 2, neither concentration of tea was associated with a statistical change in weight gain (data not shown). In experiment 3, rats fed the HF diet (TF4 and CF4) had identical weight gains that were slightly, but not statistically, greater than water-drinking rats fed the control AIN-76A diet (C4); tea-drinking rats fed the AIN-76A diet gained weight normally until 15 weeks of age but then showed reduced weight gain and weighed about 10% less than the C4 rats at termination of the experiment (data not shown).

Fluid intake was highly variable; in experiment 1 the tea-fed rats’ daily average intake increased from 30 ml at 10 weeks of age to 38 ml at 16 weeks of age. Total tea intake over the entire experiment represented 58 ± 13 (T2) or 106 ± 22 (T3) g extracted tea leaf. The C2 and C3 groups had identical water intakes that increased on average from 41 ml at 10 weeks to 45 ml at 16 weeks of age. The fluid intakes in experiment 2 were similar in all respects to the intakes in experiment 1; intake was not measured in experiment 3.

In the three experiments, there was no consistent effect of tea on tumorigenesis in rats fed the AIN-76A diet; there was some evidence of a reduction of tumorigenesis by tea in experiment 3 in rats fed the HF diet.

Cumulative probability of tumor in experiment 1 was higher in the T2 group than in the C2 group ($P = 0.04$) and was greater, but not statistically so, in C3 than in T3 (Figure 1). In experiment 2, cumulative probabilities of tumor in both water control groups (C2 and C3) were higher than the corresponding tea-drinking groups (T2 and T3); there were no statistically significant differences. The lower dose of DMBA in experiment 2 induced a lower cumulative probability of tumor in all groups compared with experiment 1 (Figure 2).

In experiment 3, rats fed the HF diet showed the expected increase in cumulative probability of tumor compared with rats fed the control AIN-76A diet (C4 versus CF4, $P = 0.003$; T4 versus TF4, $P = 0.05$). Tea did not statistically have an effect on the cumulative probability of tumor, although TF4 was somewhat lower than CF4 (Figure 3).

In experiments 1 and 2, tumor incidence, number and weight did not differ consistently or statistically between tea-fed and water-fed rats (Table II). However, in rats fed the HF diet and given 2% tea (TF4) in experiment 3, tumor burden and total tumor weight per tumor-bearing rat, was statistically reduced ($P < 0.01$) compared with rats fed the HF diet and given water (CF4). Tumor number also was reduced in TF4 rats compared with CF4 rats, but the reduction was not significant (Table II).

In experiment 1, 85% of tumors were malignant, 62% were malignant in experiment 2, and 76% were malignant in experiment 3. In experiments 1 and 2, there was no effect of tea on the incidence of benign or malignant tumors. In experiment 3, the number of tumors and of malignant tumors per tumor-bearing rat was increased by the HF diet in water-drinking rats (C4 versus CF4, $P < 0.01$) but not in tea-drinking rats which failed to show the increase associated with the HF diet. Therefore, tea partially blocked the promotion of tumorigenesis by the HF diet.


Table II. DMBA-induced mammary tumor incidence, number and burden in female S–D rats drinking tea or water and fed control or high N-6-PUFA diets

<table>
<thead>
<tr>
<th>Group</th>
<th>% tumor incidence</th>
<th>No. of tumors&lt;sup&gt;b&lt;/sup&gt; (per tumor-bearing rat)</th>
<th>Total tumor weight (g)&lt;sup&gt;b&lt;/sup&gt; (per tumor-bearing rat)</th>
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<tr>
<td></td>
<td></td>
<td>(per tumor-bearing rat)</td>
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<tr>
<td>Experiment 1&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
<td>C2</td>
<td>60 (58)</td>
<td>2.9 ± 1.7 (2.4 ± 1.4)</td>
<td>4.0 ± 4.5</td>
</tr>
<tr>
<td>T2</td>
<td>75 (75)</td>
<td>3.4 ± 2.3 (3.0 ± 2.1)</td>
<td>5.6 ± 7.5</td>
</tr>
<tr>
<td>C3</td>
<td>42 (28)</td>
<td>2.8 ± 1.9 (2.2 ± 1.4)</td>
<td>4.7 ± 6.3</td>
</tr>
<tr>
<td>T3</td>
<td>68 (62)</td>
<td>3.2 ± 2.3 (2.7 ± 2.0)</td>
<td>5.4 ± 5.9</td>
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<tr>
<td>Experiment 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>62 (50)</td>
<td>2.0 ± 1.2 (1.4 ± 1.1)</td>
<td>3.0 ± 3.4</td>
</tr>
<tr>
<td>T2</td>
<td>40 (22)</td>
<td>1.9 ± 1.2 (1.1 ± 1.2)</td>
<td>3.1 ± 4.1</td>
</tr>
<tr>
<td>C3</td>
<td>42 (28)</td>
<td>1.7 ± 0.8 (1.0 ± 1.0)</td>
<td>1.7 ± 3.1</td>
</tr>
<tr>
<td>T3</td>
<td>40 (32)</td>
<td>2.1 ± 1.8 (1.2 ± 1.1)</td>
<td>2.7 ± 3.1</td>
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<tr>
<td>Experiment 3</td>
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<tr>
<td>C4</td>
<td>70 (45)</td>
<td>1.6 ± 0.9 (1.0 ± 1.0)</td>
<td>1.0 ± 0.9</td>
</tr>
<tr>
<td>T4</td>
<td>35 (20)</td>
<td>2.0 ± 1.8 (1.3 ± 1.8)</td>
<td>1.9 ± 1.4</td>
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<tr>
<td>CF4</td>
<td>80 (70)</td>
<td>4.1 ± 2.6 (3.1 ± 2.2)</td>
<td>6.0 ± 4.1&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>TF4</td>
<td>67 (50)</td>
<td>2.6 ± 1.5 (1.9 ± 1.6)</td>
<td>2.9 ± 2.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Malignant tumor incidence and number in parentheses.
<sup>b</sup>Mean ± standard deviation.
<sup>c</sup>Forty rats per group; C2, water; T2, 1.25% tea; C3, water; T3, 2.5% tea.
<sup>d</sup>C4 and T4, 20 rats each; CF4 and TF4, 30 rats each; C4, water; T4, 2% tea; CF4, high fat diet + water; TF4, high fat diet + 2% tea.
<sup>e</sup>Significantly greater than C4 (P < 0.01) for all tumors and for malignant tumors.
<sup>f</sup>Significantly greater than C4 (P = 0.001) and than TF4 (P = 0.01).

Discussion

In the three bioassay experiments, the model responded as expected to DMBA at the two doses given and to the amount of corn oil in the diet (7.8). The ingestion of black tea had no consistent effect on mammary gland carcinogenesis in rats fed the AIN-76A diet, but tea did reduce tumor burden compared with rats drinking water. The group had also a somewhat reduced cumulative probability of bearing a tumor, but the reduction was not statistically significant. The effects of tea on DMBA tumorigenesis in rats reported previously in other laboratories have also been detected as a reduction of tumor multiplicity or size, and the Weisburger et al. report (31) of reduced DMBA incidence or latency when coffee or caffeine is given at initiation and no effect on promotion (40,41). Since caffeine may be present in polyphenol extracts of tea (21), it could contribute to the reported effects of tea and its extracts.

Interactions of tea or tea extracts with the estrogen receptor (ER) in the mammary glands of rats in the experiment reported here and with the ER of calf uterus in vitro have been found (42). Such effects have been suggested by other studies (35).

Further studies in this model, using both tea preparations and high N-6-PUFA diets, should yield information useful in chemoprevention and in understanding the basic mechanisms in mammary gland tumorigenesis.

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References


