Chemopreventive Effects of Green and Black Tea on Pulmonary and Hepatic Carcinogenesis

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The chemopreventive effects of decaffeinated green and black tea treatment on liver and lung tumorigenesis were examined in carcinogen-treated mice. Male C3H mice were given decaffeinated green or decaffeinated black tea in their drinking water prior to, during, and after treatment with diethylnitrosamine (50 μg/kg bw, ip, once per week for 8 weeks). After 40 weeks of tea treatment, mice were sampled and examined for pulmonary and hepatic tumors. Mice treated with both DENA and tea displayed a significant decrease in the mean number of lung and liver tumors compared to DENA-only treated animals. Mice that received 0.63 or 1.25% green tea or 1.25% black tea showed a reduction in liver tumor numbers from 54, 50, and 63%, respectively, from that seen in the DENA-only treated mice. Tea treatment also significantly decreased the multiplicity of lung adenomas. Mice receiving DENA and either 0.63 or 1.25% green tea or 1.25% black tea showed a decrease in the mean number of lung tumors of 40, 46, and 34%, respectively, from DENA-only treated mice. While a possible association between the chemopreventive activity of tea on lung tumor response and the concentration of (-) epigallocatechin gallate (EGCG) in the tea was suggested, no apparent relationship between EGCG concentration and liver tumor response was seen, however. These results show a dose-dependent chemoprevention of both lung and liver tumors by both black and green tea in diethylnitrosamine-treated C3H mice.

A number of natural compounds have been shown to possess chemopreventive activity to chemically induced carcinogenesis. Tea, a preparation from the dried leaves of Camellia sinensis, and one of the most widely consumed beverages in the world, has been shown to have cancer chemopreventive activity. Depending on the manufacturing technique, tea can be found in one of two forms: green and black. Green tea is made by either steaming fresh tea leaves or roasting with dry heat, thereby avoiding oxidation of the polyphenolic components (Graham, 1992). In the manufacture of black tea, the fresh tea leaves are crushed and undergo fermentation and oxidation (Graham, 1992). Several epidemiological studies have suggested that consumption of green tea might be related to lower risk of cancer in humans (Yang and Wang, 1993; Gao et al., 1994). A significant inhibition of genotoxicity by an extract of green tea has been reported in several mutagenicity assays (Chen et al., 1986; Wang et al., 1989; Liu et al., 1989; Mukhtar et al., 1992). These studies have demonstrated that tea not only inhibited the mutagenicity of a wide spectrum of individual chemical carcinogens but also the mutagenicity of less defined carcinogenic mixtures, such as cigarette smoke condensate, coal tar, and fried fish extract (Wang et al., 1989; Liu et al., 1989).

The chemopreventive effects of green tea or its major polyphenols on chemical carcinogenesis have been studied in several rodent tumor models, including skin (Mukhtar et al., 1992; Katiyar et al., 1992; Conney et al., 1992; Wang et al., 1994), forestomach (Conney et al., 1992), lung (Xu et al., 1992; Castonguay et al., 1991; Wang et al., 1992), liver (Klaunig, 1992; Li et al., 1991), duodenium (Fujita et al., 1989), esophagus (Han and Xu, 1991), intestine (Imaida et al., 1992), and colon (Yamane et al., 1991). Most of the cancer chemopreventive experiments have been performed using green tea and/or its major catechin component, (-) epigallocatechin gallate (EGCG), as the protective agent. Much less is known about the potential chemopreventive effects of black tea (Wang et al., 1992, 1993), this despite the fact that black tea is the most commonly consumed form of tea in the United States and Europe (Graham, 1992). The present investigation examined and compared the chemopreventive effects of decaffeinated black and decaffeinated green tea on diethylnitrosamine (DENA)-induced hepatic and lung cancer in the rodent in an effort to further elucidate the mechanism(s) by which tea exerts its chemopreventive activity.

MATERIALS AND METHODS

Chemicals. DENA was purchased from Sigma Chemical Co. (St Louis, MO). Decaffeinated green tea and black tea were kindly provided by...
Chemopreventive Effects of Green and Black Tea

Preparation of tea solutions. Green tea and black tea solutions (1.25% (w/v)) were chosen as the highest concentration of tea to be studied based on previous experimental information and on the known daily consumption of tea by tea drinkers in the general population (Graham, 1992). One-half of this dose (0.63%) was also used in order to examine whether any observed effects of tea were dose-dependent. Fresh tea solutions (tea) were prepared once a week by adding 1.25 or 0.63 g of tea in 100 ml boiling water and standing for 0.5 hr (Wang et al., 1992) and stored at 4°C. Fresh tea or drinking water was given to the mice daily. Mice treated with tea received tea as their only source of water. The polyphenol content of the tea solution was determined by high-pressure liquid chromatography (HPLC) (Ho et al., 1992). The stability of the tea solutions both at 4°C and in the drinking water was examined by sampling tea solutions daily for 7 days and measuring the concentration of the major polyphenol, EGCG, in the tea solution.

Experimental design. After a 2-week quarantine period, C57/H mice were randomly divided into eight groups of 15 mice per group. Mice in groups 1, 2, and 3 were injected with saline (0.05 ml, ip) once a week for 8 weeks from the beginning of the experiment. Mice in groups 4, 5, 6, 7, and 8 were injected with DENA in saline (50 mg/kg bw, ip) once a week for 8 weeks from the beginning of experiment. During the entire study period (40 weeks) mice in groups 1 and 4 (control groups) were given deionized drinking water, mice in groups 2 and 6 were given 1.25% decaffeinated green tea, mice in groups 3 and 8 were given 0.63% decaffeinated black tea, mice in group 5 were given 0.63% decaffeinated green tea, and mice in group 7 were given 0.63% decaffeinated black tea. In order to reduce the number of animals used in this study, only the high-dose tea treatment was examined in the tea control groups (Groups 2 and 3). Addition of tea to the drinking water was started 2 weeks prior to DENA treatment and continued until the termination of the experiment (40 weeks). Mice were acclimated to the tea solutions by the increasing the concentration of the tea solutions stepwise from 25 to 100% strength during this first 2 weeks of the study.

Histopathological analysis. At the termination of the experiments, the mice were sacrificed by carbon dioxide asphyxiation and necropsied. Grossly visible lung and liver tumors were detected, counted, and mapped for subsequent histopathologic confirmation. The livers were weighed, separated by lobe, sectioned into 2-mm-thick strips, and fixed with 10% neutral buffered formalin. Any previously uncounted tumors uncovered during this sectioning were added to the total gross tumor count for each mouse. All lung and liver tissue was processed for paraffin embedding and subsequent staining with hematoxylin and eosin (H & E). Hepatic lesions were classified histologically into hepatocellular foci, hepatocellular adenomas and hepatocellular carcinomas by using criteria defined previously (Frith and Ward, 1979; Lipsky et al., 1981; Ward, 1980). Pulmonary lesions were classified as defined by Shimkin and Stoner (1975).

Stereological analysis was used to quan...
FIG. 1. Monthly body weight measurements during the experimental period. DENA-treated mice showed a significant decrease in body weight compared to non-DENA-treated mice. No significant difference was seen, however, in body weights of mice receiving tea compared to those not receiving tea. Deionized water; 1.25% green tea; 1.25% black tea; DENA only; DENA and 0.63% green tea; DENA and 1.25% green tea; DENA and 0.63% black tea; DENA and 1.25% black tea.

nomas after histopathologic evaluation. Subgrouping of the hepatic adenomas by size was performed (Table 2). A large portion of the hepatic adenomas (>60%) in all of the DENA-treated groups were less than 2 mm in diameter. Both 0.63 and 1.25% green tea treatment and 1.25% black tea significantly reduced the number of adenomas in this size category compared to that seen in the DENA-only treated mice. The mean number of adenomas 2–5 mm in diameter was decreased in all mice treated with tea but only statistically significant in Group 5 (0.63% green tea) and 8 (1.25% black tea). Mice from all tea treatments showed a statistically significant decrease in the mean number of adenomas that were greater than 5 mm in diameter.

Hepatic foci, predominately basophillic in phenotype, were observed in all DENA-treated mice (Table 3). No foci were observed in saline injected mice. Using stereological techniques, the effects of tea treatment on the number and mean volume of hepatic foci were evaluated. A significant decrease in the number of hepatic foci and mean focal volume was seen in tea-treated mice compared to DENA-only treated mice (Table 3).

Lung adenomas were found in all DENA treated animals (Table 4). Tea administration significantly inhibited lung adenoma multiplicity. Mice treated with DENA and 0.63% green tea, 1.25% green tea, or 1.25% black tea showed an inhibition in the number of lung adenomas of 40, 46, and 34%, respectively, from that seen in the DENA-only treated mice. Black tea (0.63%) (Group 7) produced a 22% of reduction in lung adenoma formation, albeit not statistically different from that seen in the DENA only treated mice (Group 4).

The observed greater chemoprotective effects of green tea seen compared to black tea effects were further examined by comparing the EGCG concentration (mg/ml) in the tea solutions with the mean number of lung tumors per mouse and mean number of liver tumors (adenomas) per mouse. While a close relationship between the concentration of EGCG and average number of lung tumors was found (Fig. 2), no such correlation was seen with liver tumors (Fig. 3).

### TABLE 1

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>No. of mice</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Relative liver weight*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Deionized water only</td>
<td>15</td>
<td>36.39 ± 1.05</td>
<td>1.85 ± 0.06</td>
<td>0.051 ± 0.001</td>
</tr>
<tr>
<td>2</td>
<td>1.25% Green tea only</td>
<td>15</td>
<td>35.59 ± 1.24</td>
<td>1.84 ± 0.06</td>
<td>0.052 ± 0.001</td>
</tr>
<tr>
<td>3</td>
<td>1.25% Black tea only</td>
<td>15</td>
<td>35.63 ± 0.73</td>
<td>1.80 ± 0.05</td>
<td>0.051 ± 0.002</td>
</tr>
<tr>
<td>4</td>
<td>DENA only</td>
<td>15</td>
<td>30.77 ± 0.62*</td>
<td>2.36 ± 0.18</td>
<td>0.077 ± 0.006*</td>
</tr>
<tr>
<td>5</td>
<td>DENA + 0.63% Green tea</td>
<td>15</td>
<td>29.91 ± 0.50&quot;</td>
<td>1.86 ± 0.22</td>
<td>0.062 ± 0.007&quot;</td>
</tr>
<tr>
<td>6</td>
<td>DENA + 1.25% Green tea</td>
<td>15</td>
<td>30.93 ± 0.57&quot;</td>
<td>1.80 ± 0.10</td>
<td>0.058 ± 0.004&quot;</td>
</tr>
<tr>
<td>7</td>
<td>DENA + 0.63% Black tea</td>
<td>15</td>
<td>30.65 ± 0.85&quot;</td>
<td>2.03 ± 0.17</td>
<td>0.067 ± 0.006</td>
</tr>
<tr>
<td>8</td>
<td>DENA + 1.25% Black tea</td>
<td>15</td>
<td>31.36 ± 0.67&quot;</td>
<td>1.98 ± 0.29</td>
<td>0.063 ± 0.009&quot;</td>
</tr>
</tbody>
</table>

* Values represent the mean ± SEM.
* Relative liver weight equals liver weight/body weight.
* Value is statistically significant from the groups treated without DENA (p < 0.01).
* Values is statistically significant from DENA only group (p < 0.05).
CHEMOPREVENTIVE EFFECTS OF GREEN AND BLACK TEA

TABLE 2
Liver Adenoma Incidence and Multiplicity in DENA and/or Tea Treated Mice

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Adenoma incidence*</th>
<th>Mean no. of adenomas/mouse</th>
<th>Gross size distribution of adenomas†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 mm</td>
</tr>
<tr>
<td>1</td>
<td>Deionized water only</td>
<td>0/15 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.25% Green tea only</td>
<td>0/15 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1.25% Black tea only</td>
<td>0/15 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>DENA only</td>
<td>15/15 (100)</td>
<td>15.2 ± 2.71</td>
<td>9.4 ± 1.9 (61)</td>
</tr>
<tr>
<td>5</td>
<td>DENA + 0.63% Green tea</td>
<td>15/15 (100)</td>
<td>7.0 ± 1.24* (54%)</td>
<td>4.8 ± 0.5% (69)</td>
</tr>
<tr>
<td>6</td>
<td>DENA + 1.25% Green tea</td>
<td>15/15 (100)</td>
<td>7.6 ± 0.56* (50%)</td>
<td>4.6 ± 0.5% (60)</td>
</tr>
<tr>
<td>7</td>
<td>DENA + 0.63% Black tea</td>
<td>15/15 (100)</td>
<td>11.8 ± 1.46* (22%)</td>
<td>8.2 ± 1.1 (69)</td>
</tr>
<tr>
<td>8</td>
<td>DENA + 1.25% Black tea</td>
<td>15/15 (100)</td>
<td>5.7 ± 1.37* (63%)</td>
<td>3.4 ± 0.8% (59)</td>
</tr>
</tbody>
</table>

* Values represent the mean ± SEM.
† Percentage of mice exhibiting grossly visible hepatic adenomas in parentheses.
‡ Average number of adenomas per mouse.
§ Number of adenomas of the total adenomas detected less than 2 mm in diameter (<2 mm), between 2 and 5 mm in diameter, (2-5 mm) and greater than 5 mm in diameter (>5 mm). Numbers in parentheses represent the percentage of total adenomas for that treatment in the respective size class.

DISCUSSION

Tea has been reported to possess a variety of beneficial pharmacological and physiological effects (Cheng et al., 1986). Brewed tea contains a number of chemical components, specifically the polyphenols, which have been suggested to be important for the anticarcinogenic activity of tea (Mukhtar et al., 1992; Xu et al., 1992; Fujita et al., 1989; Khan et al., 1992). Because of the difference between green and black tea in the amount polyphenols, we examined the relative chemopreventive effects of both green and black teas on DENA-induced liver and lung tumorgenesis in the mouse. The use of decaffeinated tea in the present study eliminated the previously reported adverse effects of caffeine on animal growth (Xu et al., 1992; Wang et al., 1992).

In the present study, 0.63 and 1.25% green tea and 1.25% black tea, while not decreasing tumor incidence, did significantly reduce both liver and lung tumor multiplicity. The lack of an effect on the tumor incidence may be in part due to the high dose of DENA used. Wang and co-workers (Wang et al., 1992) found that the protective effect of green tea against lung tumor development in A/J mice was DENA

TABLE 3
Mean No. and Volume of Hepatic Foci in DENA and/or Tea Treated Mice

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Foci incidence*</th>
<th>Number of foci/cm³ liver</th>
<th>Mean volume of foci (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Deionized water only</td>
<td>0/15 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.25% Green tea only</td>
<td>0/15 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1.25% Black tea only</td>
<td>0/15 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>DENA only</td>
<td>15/15 (100)</td>
<td>27.2 ± 6.96</td>
<td>0.42 ± 0.12</td>
</tr>
<tr>
<td>5</td>
<td>DENA + 0.63% Green tea</td>
<td>15/15 (100)</td>
<td>15.8 ± 2.89 (47)</td>
<td>0.16 ± 0.13 (62)</td>
</tr>
<tr>
<td>6</td>
<td>DENA + 1.25% Green tea</td>
<td>15/15 (100)</td>
<td>16.9 ± 4.54 (38)</td>
<td>0.42 ± 0.19 (0)</td>
</tr>
<tr>
<td>7</td>
<td>DENA + 0.63% Black tea</td>
<td>15/15 (100)</td>
<td>13.4 ± 3.15 (51)</td>
<td>0.30 ± 0.06 (29)</td>
</tr>
<tr>
<td>8</td>
<td>DENA + 1.25% Black tea</td>
<td>15/15 (100)</td>
<td>20.7 ± 1.64 (24)</td>
<td>0.21 ± 0.05 (50)</td>
</tr>
</tbody>
</table>

* Values represent the mean ± SEM.
† Foci incidence is the number of mice in that group that exhibited hepatic foci of the total number of mice treated in that group. Percentages in parentheses.
‡ Value is statistically significant from that of DENA only group (p < 0.05).
§ Value is very statistically significant from that of DENA only group (p < 0.01).
dosage-dependent. In that study, green tea significantly decreased the lung tumor incidence at a low dose of DENA (10 mg/kg, once a week for 8 weeks), but did not lower the incidence of lung tumors in mice that were treated with a high dose of DENA (20 mg/kg, once a week for 8 weeks) (Wang et al., 1992). The chemopreventive effects of tea on DENA-induced lung tumorigenesis in our study were consistent with these results. Both green and black tea produced their inhibitory effects on DENA-induced lung adenomas in a concentration dependent manner. While green tea inhibited lung tumorigenesis at both concentrations studied, black tea exerted its inhibitory effect only at the high concentration (Table 4). A major water-soluble component of tea

![FIG. 2. The relationship between the EGCG concentration of the tea (log of concentration; mg/ml) and mean number of lung tumors. A correlation between the number of lung adenomas and EGCG concentration in the tea was seen.](https://academic.oup.com/toxsci/article-abstract/29/2/244/1623514/103514)”

![FIG. 3. The relationship between the EGCG concentration (log of concentration; mg/ml) of the tea and mean number of liver adenomas was examined. No correlation was seen between EGCG concentration and number of observed gross liver tumors.](https://academic.oup.com/toxsci/article-abstract/29/2/244/1623514/103514)
EGCG, has been shown to possess chemopreventive properties (Mukhtar et al., 1992; Xu et al., 1992; Fujita et al., 1989; Imaida et al., 1992) and is approximately five times higher in green tea than in black tea. While the present study did not specifically study the chemopreventive effects of EGCG, by comparing the lung tumor multiplicity data against our experimentally determined concentration of EGCG in the black and green tea, a linear relationship between EGCG concentration and the number of lung tumors appears to be present (correlation coefficient of 0.987, p = 0.007) (Fig. 2). This suggests that at least for lung tumors a possible strong association between the presence of EGCG and the lung tumor response. This is supported by previous work by Xu et al. (1992), who reported that EGCG was the major protective agent in green tea against tobacco-specific nitrosamine-induced lung tumorogenesis in A/J mice.

Green and black tea also inhibited DENA-induced liver adenoma multiplicity. While there was a significant lower number of hepatic adenomas in tea-treated mice, all DENA-treated mice (with or without tea administration) had a similar tumor size distribution. Tea treatment also decreased the number and size of liver foci (Table 3). The results obtained in the liver are consistent with the fact that tea treatment may inhibit the formation of hepatic tumors at one or several stages of the cancer process. Tea may decrease the total number of initiated cells induced by DENA during this first stage of carcinogenesis, may block or slow the selective clonal expansion (promotion) of the initiated cells, and may prevent the progression of preneoplastic lesions to neoplastic tumors. Further investigation is needed to define which stage or stages of the liver carcinogenesis process is influenced by the tea treatment. In contrast to those seen with lung carcinogenesis, no association was seen between EGCG concentration in the tea and the inhibition of liver tumor multiplicity. A recent study has shown that EGCG given to C3H mice over their life span significantly reduced spontaneous hepatic tumor incidence (Nishida et al., 1994).

In summary, the present results showed that both black tea and green tea exhibited chemopreventive activity against DENA-induced lung and liver carcinogenesis. While the exact component of tea responsible for this activity is not known, the concentration of EGCG, a water-soluble component of tea, appears to be associated with the observed reduction in pulmonary tumorogenesis. No such relationship was seen between the EGCG concentration in tea and liver tumor inhibition by the tea. In addition, the present study also showed that liver carcinogenesis can be inhibited by tea treatment. The stages of experimental rodent hepatic carcinogenesis have been well studied and characterized, thus the present findings will allow for the subsequent dissection of the stage or stages of hepatic cancer development modified by the tea treatment. And finally, this study has confirmed that black tea (the major form of tea used in Europe and North America) exhibits significant chemopreventive activity in chemically induced lung and liver carcinogenesis.

REFERENCES


