Effects of Green Tea and Black Tea on 4-(Methylnitrosamo)-1-(3-pyridyl)-1-butanone Bioactivation, DNA Methylation, and Lung Tumorigenesis in A/J Mice

Stephanie Tao Shi, Zhi-Yuan Wang, Theresa J. Smith, Jun-Yan Hong, Wu-Feng Chen, Chi-Tang Ho, and Chung S. Yang

Laboratory for Cancer Research, College of Pharmacy, Rutgers University, Piscataway, New Jersey 08855-0759; S. T. S., Z.-Y. W., T. J. S., J.-Y. H., W.-F. C., C. S. Y., and Department of Food Science, Cook College, Rutgers University, New Brunswick, New Jersey 08903

ABSTRACT

Previous studies in our laboratory showed that decaffeinated green tea and black tea extracts inhibited 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced tumorigenicity in A/J female mice. In order to understand the mechanism of the inhibitory action, we examined the effects of decaffeinated green tea, black tea, and tea components on the metabolic activation of NNK in vitro and in vivo in this animal model. When added to incubation mixtures containing mouse lung microsomes, decaffeinated green tea and black tea extracts and their fractions, at concentrations up to 0.4 mg/ml, inhibited NNK oxidation and NNK-induced DNA methylation. Among the tea components examined, (−)-epigallocatechin-3-gallate was the most potent inhibitor with 50% inhibitory concentrations of about 0.12 mM for both NNK oxidation and DNA methylation. At these concentrations, (−)-epigallocatechin-3-gallate inhibited the catalytic activities of several P450 enzymes and was more potent against P450 1A2 and 2B1 than 2E1. When decaffeinated green or black tea extracts were given to female A/J mice as the sole source of drinking fluid before an i.p. injection of NNK (100 mg/kg body weight), a statistically significant inhibition of lung DNA methylation, however, was not observed, although a significant reduction in lung tumor multiplicity was observed. The results suggest that, although inhibition of the metabolic activation of NNK and the subsequent DNA alkylation by tea extracts can be demonstrated in vitro, this mechanism may not be important for the inhibitory action of tea against lung tumorigenesis.

INTRODUCTION

Lung cancer is one of the leading causes of death in the United States, and cigarette smoking is the most important etiological factor for this disease. NNK is a potent tobacco-specific carcinogen formed from the nitrosation of nicotine during tobacco processing and cigarette smoking. Various studies have pointed to the possible importance of NNK in tobacco-related human cancers. In experimental animals, NNK has been shown to induce tumors in the lung, liver, and nasal cavity of rats, the lung and skin of mice, and the lung, trachea, and nasal cavity of hamsters. NNK is metabolized in many organs such as the liver, lung, and nasal cavity, mainly by cytochrome P450 enzymes. The metabolic activation pathways involve α-oxidation of either the methylene carbon, leading to the formation of keto aldehyde and methylidiazohydroxide, or the methyl carbon leading to the formation of formaldehyde.
**MATERIALS AND METHODS**

**Tea and Tea Components.** Instant decaffeinated green tea and black tea were dehydrated water extracts from decaffeinated tea leaves and were supplied by the Thomas J. Lipton Co. (Englewood Cliffs, NJ). The decaffeinated tea leaves were prepared by extracting tea leaves with supercritical CO₂. EGCG, EGC, ECG, and EC were the major catechins in decaffeinated green tea powder accounting for 10.1, 8.0, 2.3, and 2.3% of the dry weight, respectively. Caffeine was decreased to 0.3% of the dry weight. In the decaffeinated black tea powder, the amounts of EGCG, EGC, ECG, EC, and caffeine were 2.6, 1.2, 1.8, 2.7, and 0.4% of the dry weight, respectively. The amount of theaflavins was 1.5%, and thearubigins were estimated to be 24.8% of the dry weight of black tea leaves. To make 0.6% decaffeinated tea extracts, decaffeinated green or black tea was dissolved in 1 liter of warm (55°C) water and then freeze-dried to obtain DGTF-1, DGTF-2, DGTF-3 and DBTF-1, DBTF-2, DBTF-3. The relative weight ratios of DGTF-1, DGTF-2, DGTF-3 were 1:0.5:1.7. The relative weight ratios of DBTF-1, DBTF-2, DBTF-3 were 1:1.2:3.1.

EC, EGC, ECG, and EGCG were purified from DGTF-1 by column chromatography using a Sephadex LH-20 column (19). The appropriate fractions were identified by UV spectra with a characteristic peak at 275 nm (25). After elution, each fraction was pooled and freeze dried. The purity of EGCG was 98% as determined by HPLC.

**Animals and Microsomes.** Female A/J mice, 5 weeks of age, were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained on an AIN-76A semipurified pellet diet (ICN Biochemicals, Cleveland, OH) for 1 week. Mice were housed 10/cage in polycarbonate cages on corn cob bedding and were maintained in an air-conditioned room with a 12-h light and dark cycle. Tea extracts (0.6%) were made fresh every other day and were given as the sole source of drinking fluid. Microsomes were prepared and isolated as described previously (26) and stored at −70°C. The protein concentration was determined according to Lowry et al. (27).

**Chemicals.** Unlabeled NNK, [5-³H]NNK (2.20 Ci/mmol; purity, >98%) and [methyl-³H]NNK (0.836 Ci/mmol; purity, >95%) were purchased from Chemsyn Science Laboratories (Lenexa, KS), and the radiolabeled NNK was further purified by HPLC before use. NNK metabolite standard was generously supplied by Dr. Stephen S. Hecht of the American Health Foundation (Valhalla, NY). Glucose 6-phosphate, glucose-6-phosphate dehydrogenase, NADP⁺, calf thymus DNA (type I), HEPES, and p-nitrophenol were purchased from Sigma Chemical Co. (St. Louis, MO). p-Aminosalicylic acid and 2-ethoxylethanol were from VWR Scientific (Philadelphia, PA). m-Cresol and 8-hydroxyquinoline were obtained from Aldrich Chemical Co. (Milwaukee, WI). Pentoxysresorufin, ethoxyresorufin, and resorufin were from Pierce Chemical Co. (Rockford, IL). RNase A was purchased from Pharmacia (Uppsala, Sweden), and protease K was from Bethesda Research Laboratories (Gaithersburg, MD).

**NNK Metabolism in Vitro.** NNK metabolism in rodent microsomes was carried out as described by Smith et al. (28) with minor modifications. Unless otherwise stated, the incubation mixture contained 100 mM sodium phosphate buffer (pH 7.4), 1 mM EDTA, 3 mM MgCl₂, 0.1 mg lung microsomal protein, 10 µM NNK (containing 1 µCi of [³H]NNK), 5 mM NaHSO₃, NADPH generating system I (2.5 mM NADP⁺, 5 mM glucose 6-phosphate, and 0.6 unit glucose-6-phosphate dehydrogenase), and the desired amount of tea components in a total volume of 0.4 ml. Sodium bisulfite was added to trap the keto aldehyde formed in the incubation (29). After preincubation at 37°C for 30 min, the reaction was terminated with saturated barium hydroxide and 25% zinc sulfate. The sample was centrifuged and filtered, and 200 µl were injected with 5 µl NNK metabolite standard onto a reverse-phase HPLC system equipped with a Radiometric Flo-One/Beta radioflow detector (Radiometric Instruments and Chemical Co., Tampa, FL). It was eluted with a linear gradient of 92% solvent A (0.02 M Tris-HCl containing 1 mM sodium bisulfite, pH 6.0, freshly prepared every day) and 8% solvent B (95% methanol) at 37°C for 30 min. The reaction was carried out at 37°C for 20 min and terminated with 2 ml methanol. The fluorescence (excitation at 550 nm and emission at 585 nm) was measured in a Perkin-Elmer LS-5B luminescence spectrometer.

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**Fig. 1. Metabolic pathways of NNK. Modified from Hecht and Hoffmann (1).**

<table>
<thead>
<tr>
<th>NNK-N-oxide</th>
<th>NNK</th>
<th>NNAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Metabolic Pathways of NNK" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
p-Nitrophenol hydroxylase was determined by the method of Reinke and Moyer (31) with minor modifications. The reaction mixture contained 100 mM sodium phosphate buffer (pH 7.4), NADPH-generating system II, 0.1 mM p-nitrophenol, and 0.2 mg mouse lung microsomes in a total volume of 0.5 ml. The reaction was carried out at 37°C for 15 min and terminated with 0.25 ml sodium phosphate buffer (pH 7.4), NADPH-generating system II, 0.1 mM phenol/chloroform/isoamyl alcohol (25:24:1, v/v). DNA was again precipitated with an equal volume of phenol, followed by extraction with an equal volume of chloroform/isoamyl alcohol (24:1, v/v). DNA was then purified by extraction with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, v/v) and precipitation with an equal volume of ethanol followed by redissolution in TE buffer. The DNA methylation level was determined by liquid scintillation counting in the purified DNA sample and is expressed as pmol methyl/mg DNA.

DNA Methylation in Vitro. DNA methylation was performed as described previously (32) with several modifications. Unless otherwise stated, [methyl-^3H] NNNK (20 µM) and 0.2 mg calf thymus DNA were incubated with 0.2 mg lung microsomal protein or 0.1 mg liver microsomal protein in the presence of 100 mM sodium phosphate buffer (pH 7.4), 1 mM EDTA, and 3 mM MgCl₂ in a total volume of 0.2 ml. The reaction was initiated with NADPH-generating system I after a 3-min preincubation. The incubation was carried out at 37°C for 30 min and was terminated by the addition of 100 µl 7.5 mM ammonium acetate. After the removal of the microsomes by centrifugation, DNA was precipitated by 2.5 volumes of 2-ethoxyethanol and redissolved in TE buffer (30 mM Tris-HCl-1 mM EDTA, pH 7.5). DNA was then purified by extraction with an equal volume of phenol, followed by extraction with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, v/v). DNA was again precipitated by 1 l M sodium acetate and 2.5 volumes of 2-ethoxyethanol and redissolved in TE buffer. DNA content and purity were determined spectrophotometrically (1 unit of A₂₆₀ correspond to 50 µg DNA/ml). All of the DNA samples prepared in this study had an A₂₆₀/A₂₅₄ ratio larger than 1.8. The DNA methylation level was determined by liquid scintillation counting in the purified DNA sample and is expressed as pmol methyl/mg DNA.

DNA Methylation in Vivo. Decaffeinated green tea or black tea extracts (0.6%) were given as the sole source of drinking fluid to groups of 5–7 mice for 2 weeks, 2 days, or 2 h before an i.p. injection of 100 mg NNK containing 3.75 mCi [methyl-^3H]NNK per kg body weight. The mice were sacrificed 4 h after the NNK treatment, and the lung and liver were removed. Isolation and purification of DNA was accomplished by a modification of the method of Smart et al. (33). The mouse tissue was homogenized in TE buffer. Sodium dodecyl sulfate was added to the homogenates to a final concentration of 1%. DNA was treated with 100 µg/ml RNase A for 1 h followed by extraction with an equal volume of phenol solution (phenol:m-cresol:8-hydroxyquinoline:water, 500:70:0.5:55, w/w). DNA was precipitated with 2 volumes of ethanol and washed once with 80% ethanol and once with ethyl ether. DNA was then redissolved in TE buffer at 55°C in a shaking water bath. Once in solution, DNA was treated with 100 µg/ml RNase A for 1 h followed by 50 µg/ml proteinase K treatment for an additional h. DNA was extracted again as outlined above, starting with the addition of the p-aminoacillic acid/sodium chloride solution. Finally, DNA was dissolved in TE buffer, and the DNA content and methylation level were determined as described above. Statistical analysis was performed by using the Dunnett t test.

NNK Tumorigenesis. The effect of tea on NNK-induced tumorigenesis was studied using a model established by Hecht et al. (34). The A/J mice were administered a single i.p. dose of NNK (100 mg/kg body weight). Group 1 (water alone) and Group 2 (NNK alone) were negative and positive control groups, respectively. Decaffeinated green tea (0.6%) was given as the sole source of drinking fluid in Groups 3–7. In Group 3, tea was given starting 2 weeks before and until 1 week after NNK treatment. In Group 4, tea was given for 2 days just before the time of NNK treatment. Mice in Group 5 started drinking tea at the time of NNK treatment and continued for 1 week. Mice in Groups 6 and 7 were also given tea starting 1 week and 5 weeks after NNK treatment until the end of the experiment, respectively. The body weight of the mice was determined once a week throughout the experiment. Sixteen weeks after the NNK treatment, the animals were sacrificed to remove the lung. Tumors on the lung surface were examined under a dissecting microscope. Tumors with a diameter greater than 0.5 mm were scored. Histopathological examination was performed to ascertain the nature of the tumors.

RESULTS

Effects of Tea Components on NNK Metabolism in Vitro. NNK metabolism mediated by the lung microsomes from the A/J mouse resulted in the formation of keto aldehyde (determined as its bisulfite
adduct), keto alcohol (α-hydroxylation products), NNK N-oxide (pyridine N-oxidation product), and NNAL (from carbonyl reduction). The oxidation of NNK was inhibited by decaffeinated green tea extracts and DBTF-1, DBTF-2, DBTF-3 in a concentration-dependent manner (Fig. 3). The IC_{50} of decaffeinated green tea extracts for keto aldehyde formation was approximately 0.20 mg/ml. All three DBTFs had stronger inhibitory effects on NNK oxidation than decaffeinated green tea extracts. The IC_{50}s of DBTF-1 and DBTF-2 for keto aldehyde formation were approximately 0.05 mg/ml, which was 25% of the IC_{50} of decaffeinated green tea extracts. DBTF-3 was relatively less potent than DBTF-1 and DBTF-2, showing an IC_{50} of 0.15 mg/ml for keto aldehyde formation. Decaffeinated green tea and black tea fractions had no effect on NNAL formation. When tea fractions were added to the incubation mixture after the reaction was completed, it did not decrease the oxidation of NNK.

Relative Inhibitory Potencies of Different Epicatechin Derivatives in Vitro. The inhibitory effects of purified epicatechin derivatives on NNK metabolism are shown in Fig. 4. Among the four components tested, EGCG was the strongest inhibitor, whereas the inhibitory potencies of the other three were similar. EGCG had an IC_{50} of approximately 0.12 mM for keto aldehyde formation, and the IC_{50}s were even lower for the formation of NNK N-oxide and keto alcohol. Similar differences in IC_{50}s were also seen in the inhibitory pattern of decaffeinated green tea extracts, DBTF-1, DBTF-2, DBTF-3 (Fig. 3), and other purified compounds (Fig. 4), suggesting that different metabolic pathways have different sensitivities to the inhibition by tea. EGCG also inhibited NNK oxidation in mouse liver microsomes with an IC_{50} of 0.13 mM for the formation of keto aldehyde (data not shown).

Mechanisms and Specificities of the Inhibition by EGCG. When 0.1 mM EGCG was added to the incubation mixtures containing mouse lung microsomes and 0.25–20 μM NNK, changes in the apparent K_{m} were not appreciable, and the apparent V_{max} decreased (Fig. 5; Table 1) suggesting a mechanism of noncompetitive inhibition for EGCG. For the formation of keto aldehyde, NNK N-oxide, and keto alcohol, EGCG had apparent K_{i} values of 178, 46.9, and 80.7 μM, respectively (Table 1).

In order to assess the specificity of the inhibitory action of EGCG, several enzyme activities of the lung microsomes were measured (Fig. 6). Ethoxyresorufin deethylase activity, which is mainly due to P450s 1A1 and 1A2, was more sensitive to the inhibition by EGCG (estimated IC_{50} 0.08 mM) than pentoxyresorufin dealkylase activity, which is mainly due to P450 2B1 (IC_{50} 0.12 mM). p-Nitrophenol hydroxylase activity, which is mainly due to P450 2E1, was relatively insensitive to the inhibition by EGCG with an IC_{50} of 0.62 mM.
**Table 1 Kinetic parameters of NNK metabolism and its inhibition by EGCG**

Mouse lung microsomes (0.1 mg protein) and NNK (0.25, 0.5, 1, 5, 10, and 20 μM) were incubated in 0.4 ml in the absence or presence of 0.1 mM EGCG. The data were analyzed by curve-fitting to the Michaelis-Menton equation followed by nonlinear regression analysis. The apparent Ke values were calculated as follows: Ke = Vmax [inhibitor] / (Vmax - Vmax').

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control Vmax (pmol/min/mg)</th>
<th>EGCG Vmax (pmol/min/mg)</th>
<th>Ke (μM)</th>
<th>Ke' (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keto aldehyde</td>
<td>4.6</td>
<td>42.3</td>
<td>4.3</td>
<td>27.1</td>
</tr>
<tr>
<td>NNK N-oxide</td>
<td>1.0</td>
<td>62.6</td>
<td>0.7</td>
<td>20.0</td>
</tr>
<tr>
<td>Keto alcohol</td>
<td>1.4</td>
<td>45.9</td>
<td>1.6</td>
<td>20.5</td>
</tr>
</tbody>
</table>

Inhibition of DNA Methylation by Tea Components in Vitro.

When added at concentrations of 0.05 to 0.4 mg/ml, decaffeinated green tea and black tea extracts and their fractions caused a concentration-dependent inhibition of the NNK-induced DNA methylation in lung microsomes from A/J mice (Fig. 7). Decaffeinated green tea and black tea extracts had similar inhibitory activities. However, DBTF-2 showed stronger inhibitory effects than decaffeinated green tea extracts and its fractions. It is consistent with the results on the inhibition of NNK oxidation in vitro. As shown in Fig. 8, EGCG inhibited NNK-induced DNA methylation in lung and liver microsomes in a concentration-dependent manner with IC50s of about 0.12 mM and 0.25 mM, respectively.

Effect of Oral Treatment with Tea on NNK-induced DNA Methylation in A/J Mice. Decaffeinated green tea or black tea extracts (0.6%) were given as the sole source of drinking fluid to the mice starting 2 weeks, 2 days, or 2 h prior to an i.p. injection of [methyl-3H]NNK (100 mg/kg). In mouse lung, oral treatment of the mice with decaffeinated green tea produced about 6–13% inhibition in different treatment groups, but they were not statistically significant (Table 2). Similar results were obtained in mouse liver with 10–20% inhibition of DNA methylation, although intubation of decaffeinated green tea 2 h before NNK injection showed a statistically significant inhibition. Decaffeinated black tea had no appreciable effect on DNA methylation.

Inhibition of NNK-induced Lung Tumorigenesis by Decaffeinated Green Tea. Treatment of A/J mice with a single i.p. dose of NNK (100 mg/kg) resulted in 100% of mice bearing lung adenomas with 9.6 ± 1.1 adenomas per mouse after 16 weeks (Table 3). When 0.6% decaffeinated green tea extracts were given in drinking water either during or after the NNK-treatment period, tumor multiplicity was significantly reduced. In Group 3, tea treatment, starting 2 weeks before and until 1 week after NNK injection, inhibited tumor multiplicity by 56%. The inhibitory action was weaker (31%) when the period of tea treatment was shortened to 2 days (Group 4). One week of tea drinking after NNK injection (Group 5) also showed some (20%) inhibition on tumor multiplicity. When the tea extracts were given starting 1 week (Group 6) and 5 weeks (Group 7) after the
NNK-treatment period until the end of the experiment, tumor multiplicity was reduced by 65 and 54%, respectively (Table 3). Tumor incidence, however, was not as sensitive to tea treatment as tumor multiplicity. A 20% decrease in the number of mice bearing adenomas was observed only in Group 3. Decaffeinated green tea did not affect the body weight of the mice in this experiment.

**DISCUSSION**

Decaffeinated tea extracts were used in this work to eliminate the possible complication introduced by the presence of caffeine. Previously, our laboratory demonstrated that green tea extracts decreased the body weight of mice used in a carcinogenesis experiment. Subsequent studies with decaffeinated tea extracts showed that the effects of tea on NNK tumorigenesis were not due to caffeine (24).

Although most of the previous studies have focused on green tea, our results suggest that decaffeinated black tea and its fractions also have strong inhibitory effects on NNK oxidation and DNA methylation in vitro (Figs. 3 and 7). This indicates that, in addition to monomeric flavanols, theaflavins, thearubigins, other oligomeric flavanols could be strong inhibitors of NNK bioactivation. Various hepatic P450 enzymes have been shown to be inhibited by (-)-epicatechin derivatives from green tea (35). The presently demonstrated differences in the inhibition of pentoxysresorufin dealkylase, ethoxyresorufin deethylase, and p-nitrophenol hydroxylase activities by EGCG in mouse lung microsomes suggest that EGCG and perhaps other tea components inhibit the activities of different P450 enzymes to different extents. P450 2B1 or an immunorelated P450 form has been shown to be partially involved in the oxidation of NNK in mouse lung microsomes (28). The inhibition of the pentoxysresorufin dealkylase activity (an indicative enzyme activity of P450 2B1) by EGCG suggests that the decrease in NNK oxidation by tea and tea components is in part due to the binding of EGCG or other tea components to P450 2B1.

Tea polyphenols are known as tannins which have high affinity to biological macromolecules such as proteins, lipids, carbohydrates, and nucleic acids. The inhibition of P450 enzymes by EGCG may be a result of its binding property due to the polyphenolic structures. Under the present experimental conditions, the results of the kinetic study in Fig. 5 and Table 1 show that EGCG and possibly other tea polyphenols may be noncompetitive inhibitors of NNK oxidation. An alternative explanation could be that tea polyphenols bind and inactivate the NNK-metabolizing P450 enzymes irreversibly as a result of further oxidation to the corresponding O-quinones. Such an irreversible inhibition was apparent when the incubation time was prolonged to 30 min (data not shown).

The fact that tea administration effectively decreased tumor multiplicity but was less effective in reducing tumor incidence (Table 3) is consistent with the published results (22, 24). Treatment of decaffeinated green tea starting 2 weeks before and until 1 week after NNK injection was more effective than 2 days of tea treatment in the reduction of tumor multiplicity (Table 3). When tea was administered just after NNK injection for a period of 1 week, tumor multiplicity was also slightly decreased. The inhibitory effect was still evident when tea was administered 1 week or 5 weeks after NNK. These results indicate that tea is effective during initiation, postinitiation, and even later stages of lung tumorigenesis.

It is generally believed that metabolic activation of NNK leads to the formation of electrophiles that can subsequently alkylate DNA, resulting in the activation of cellular protooncogenes (12). The inhibitory action of decaffeinated green tea and black tea on NNK oxidation and NNK-induced DNA methylation in vitro appears to be consistent with their inhibition of NNK-induced tumorigenesis. In the DNA methylation experiment in A/J mice, however, decaffeinated green tea only showed marginal inhibitory effects but was not statistically significant. Decaffeinated black tea did not seem to have any effects on DNA methylation, either in the lung or in the liver. The discrepancy between the in vitro and in vivo results is probably due to the higher dosage of tea used in the experiments in vitro. Xu et al. (22) also showed that neither green tea nor EGCG inhibited NNK-induced O°-methylguanine formation or stimulated its repair in the lung of A/J mice, although both treatments inhibited the lung tumor induction by NNK. These authors observed the inhibition of 8-hydroxydeoxyguanosine by tea treatment and suggested that tea protects against oxidative damages of DNA (22). Protection against DNA single-strand breakage has also been suggested (36). Other mechanisms.

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**Table 2** Effect of oral administration of decaffeinated tea on NNK-induced DNA methylation in mouse lung and liver

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Adduct level (pmol/mg DNA)</th>
<th>Inhibition (%)</th>
<th>Adduct level (pmol/mg DNA)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNN alone</td>
<td>7</td>
<td>568.3 ± 79.9</td>
<td>635.4 ± 446.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green tea (2 wk)</td>
<td>6</td>
<td>532.3 ± 53.2</td>
<td>632.8 ± 344.6</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Green tea (2 days)</td>
<td>6</td>
<td>496.6 ± 72.3</td>
<td>362.4 ± 477.2</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Green tea (2 h)</td>
<td>6</td>
<td>501.7 ± 121.7</td>
<td>2905.6 ± 742.4</td>
<td>20°</td>
<td></td>
</tr>
<tr>
<td>Black tea (2 wk)</td>
<td>6</td>
<td>579.2 ± 49.5</td>
<td>4391.8 ± 524.8</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different from the positive control (NNK alone) group (*P < 0.05) as determined by the Dunnett t test.

**Table 3** Effects of oral administration of decaffeinated green tea on NNK-induced mouse lung tumorigenesis in different stages

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>% animals bearing adenomas</th>
<th>No. of adenomas/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) Water alone</td>
<td>10</td>
<td>10</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td>2) NNK alone</td>
<td>24</td>
<td>100</td>
<td>9.6±1.1</td>
</tr>
<tr>
<td>Green tea given during the NNK treatment (at 0 hour period)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) −2 wk to 1 wk</td>
<td>25</td>
<td>80° (20%)</td>
<td>4.2±0.7° (30%)</td>
</tr>
<tr>
<td>4) −2 days to 0 h</td>
<td>25</td>
<td>100 (0%)</td>
<td>6.6±0.6° (31%)</td>
</tr>
<tr>
<td>5) 0 h to 1 wk</td>
<td>20</td>
<td>100 (0%)</td>
<td>7.7±0.8° (20%)</td>
</tr>
<tr>
<td>Green tea given after the initiation period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6) 1 wk to 16 wk</td>
<td>24</td>
<td>80° (20%)</td>
<td>3.4±0.5° (65%)</td>
</tr>
<tr>
<td>7) 5 wk to 16 wk</td>
<td>24</td>
<td>100 (0%)</td>
<td>4.4±0.3° (54%)</td>
</tr>
</tbody>
</table>

*P < 0.05, significantly different from Group 2 using χ² test.

bP < 0.05, significantly different from Group 2 using the Dunnett t test.
including antioxidative activities (13), induction of phase II enzymes (37), and inhibition of DNA synthesis and cell proliferation (38) are also likely to be involved in the anticarcinogenic activities of tea. Although these mechanisms may also be applicable to human situations, the protective effect of tea against human tobacco-related carcinogenesis remains to be studied (13).

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