The Prevention of Lung Cancer Induced by a Tobacco-Specific Carcinogen in Rodents by Green and Black Tea (44376)

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Abstract. **A growing body of evidence from studies in laboratory animals indicates that green tea protects against cancer development at various organ sites. We have previously shown that green tea, administered as drinking water, inhibits lung tumor development in A/J mice treated with 4-(methylnitrosamino)-1-(3-pyridyl)-l-butanone (NNK), a potent nicotine-derived lung carcinogen found in tobacco. The inhibitory effect of green tea has been attributed to its major polyphenolic compound, epigallocatechin gallate (EGCG), and, to a lesser extent, to caffeine. We have also demonstrated that while levels of O^6-methylguanine, a critical lesion in NNK lung tumorigenesis, were not affected in lung DNA. However, the levels of 8-hydroxydeoxyguanosine (8-OH-dG), a marker of oxidative DNA damage, were significantly suppressed in mice treated with green tea or EGCG. These studies underscore the importance of the antioxidant activity of green tea and EGCG for their inhibitory activity against lung tumorigenesis. Unlike green tea, the effect of black tea on carcinogenesis has been scarcely studied, even though the worldwide production and consumption of black tea far exceeds that of green tea. The oxidation products found in black tea, thearubigins and theaflavins, also possess antioxidant activity, suggesting that black tea may also inhibit NNK-induced lung tumorigenesis. Indeed, bioassays in A/J mice have shown that black tea given as drinking water retarded the development of lung cancer caused by NNK. However, data on the relationship of black tea consumption with the lung cancer risk in humans are limited and inconclusive. There is a need for additional tumor bioassays in animal models to better examine the protective role of black tea against lung cancer. The development of adenocarcinomas and adenosquamous carcinomas in F344 rats upon chronic administration of NNK provides an important and relevant model for lung carcinogenesis in smokers. Thus far, no information was previously available regarding the effects of tea on this model. We conducted a 2-year lifetime bioassay in F344 rats to determine whether black tea and caffeine are protective against lung tumorigenesis induced by NNK. Our studies in both mice and rats have generated important new data that support green and black tea and caffeine as potential preventive agents against lung cancer, suggesting that a closer examination of the roles of tea and caffeine on lung cancer in smokers may be warranted.**

[P.S.E.B.M. 1999, Vol 220]

A long-term goal in our research on tea and cancer is to conduct laboratory studies in animal models to strengthen and broaden the scientific database to characterize the relationship between tea consumption and human lung cancer caused by smoking. This area of research is of special importance because of the weak and inconclusive results that have been so far obtained from epidemiological studies on tea consumption and lung cancer (1, 2).

The carcinogen used in our animal studies is NNK, a nicotine-derived nitrosamine, which is formed during tobacco curing and smoking (3). NNK has been shown to be a potent lung carcinogen in rodents whose target organ specificity toward the lung is independent of the route of administration (3, 4). The two most commonly used animal models for studying NNK-induced lung tumorigenesis are the A/J mouse and the F344 rat (5–8). A comparison of these animal models is shown in Table I. The broader spectrum of tumor sites and the development of adenocarcino-

I would like to thank the National Cancer Institute (CA 46535) and the Tea Trade Health Research Association for their support.

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0037-9727/99/2204-0244$10.50/0
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mas and adenosquamous carcinomas upon chronic administration of NNK are the important features of the F344 rat model. Therefore, whereas the A/J mouse model is more economical and practical, the F344 rat appears to be a more important and relevant model for lung cancer in smokers.

This paper describes studies conducted in our laboratory in which the effects of tea on lung tumorigenesis in these animal models were examined. These studies demonstrated the protective effects and the possible mechanisms of green and black tea and their active compounds, such as EGCG and caffeine, against lung tumorigenesis induced by NNK.

Effect of Green Tea, Epigallocatechin Gallate (EGCG), and Caffeine on Lung Cancer in the A/J Mouse

In a previous bioassay, we examined the effects of green tea and its major constituents, EGCG and caffeine, on lung tumor development in A/J mice treated with NNK (9). The treatment protocol included chronic administration of NNK at a dose of 11.7 mg/kg b.w. by gavage three times per week from Week 2 to Week 12. Animals were divided into groups shown in Table II. Green tea (2%) and EGCG and caffeine at the concentration found in tea, 560 and 1120 ppm, respectively, were given in drinking water to the NNK-treated Groups 2, 3, and 4, from Week 0 to Week 13. The bioassay was terminated in Week 18. The average body weights for Groups 2 and 5 given green tea were consistently lower than those in Groups 1 and 3 that drank water or water containing EGCG, respectively. This loss in body weight gains was likely due to caffeine in green tea because similar weight losses were observed in the groups given caffeine (Groups 4 and 7) in drinking water. The results of this bioassay (Table II) showed that the 2% green tea inhibited NNK-induced lung tumors per mouse by approximately 45% as compared to the NNK group given only water. EGCG appeared to be the major active ingredient in green tea since it reduced lung tumor multiplicity by 30%. Interestingly, caffeine also inhibited lung tumor formation slightly, but significantly. The inhibitory effect of caffeine may be, in part, associated with its negative effect on body weight.

A similar protocol was used for studying the mechanism of inhibition of lung tumorigenesis (9). We demonstrated that green tea and EGCG did not affect the levels of O^6-methylguanine in lung DNA, a critical lesion of NNK lung tumorigenesis; however, both treatments suppressed the formation of 8-OH-dG, a benchmark of oxidative damage, in the lung DNA of the NNK-treated mice (Table III). These results underscore the potentially important roles of green tea and EGCG as antioxidants for the inhibition of lung tumorigenesis. It should be noted that the small, but significant, protective effect by caffeine in A/J mice should not be ignored in view of its widespread consumption by humans. The data obtained from this and other laboratory animal studies have provided impetus for epidemiologic investigations to examine the potential of green tea consumption in reducing the risk of lung cancer in smokers (10).

Table I. Comparison of the Two Commonly Used Animal Models for NNK-Induced Lung Tumorigenesis

Bioassay features	A/J mouse	F344 rat
NNK Dosing	Single dose[a]	Multiple chronic doses
Time needed	16–20 weeks	2 years
Tumor site	Only lung tumors	Broad spectrum of tumor sites
Lung tumor type	Adenomas	Adenocarcinomas Adenosquamous carcinomas

[a] A single-dose regimen is usually used in this model. However, multiple dosing has also been used as described here and in Ref. 9.

Table II. Effects of Green Tea, EGCG and Caffeine on NNK-Induced Lung Adenomas in A/J Mice

Treatment group	% of mice with tumors	Number of animals	Tumors/mouse (± SD)
1. NNK	100	30	22.5 ± 4.7
2. Tea + NNK	100	25	12.2 ± 4.3[a]
3. EGCG + NNK	100	25	16.1 ± 5.3[a]
4. Caffeine + NNK	100	15	19.2 ± 4.8[b]
5. Tea	7	15	0.1 ± 0.2
6. EGCG	20	15	0.3 ± 0.6
7. Caffeine	20	15	0.3 ± 0.6

[a] $P < 0.001$ as compared to group 1
[b] $P < 0.05$ as compared to group 1

Table III. Effects of Green Tea and EGCG on the 8-OH-dG Levels in Lung and Liver DNA of A/J Mice 2 hr After Treatment with NNK[a]

Treatment	Number of mice	8-OH-dG/10^5 dG	
		Lung	Liver
1. Control	11	1.7 ± 1.2[b]	3.9 ± 0.6
2. NNK	10	3.2 ± 1.7[c]	4.7 ± 1.1
3. NNK + tea	11	1.9 ± 1.0[d]	4.3 ± 1.4
4. NNK + EGCG	12	2.1 ± 1.1[e]	4.3 ± 1.2
5. EGCG	10	1.8 ± 0.9[f]	4.1 ± 1.2
6. Tea	11	1.8 ± 0.7[g]	4.0 ± 1.4

[a] Mice that drank water, green tea, or EGCG solution as drinking water were administered NNK in corn oil (23 mg/kg b.w.) by gavage 3 times weekly for 3 weeks. Mice were sacrificed 2 hr after the last NNK treatment.
[b] Mean ± SD.
[c] $P < 0.01$ as compared to Group 1.
[d] $P < 0.05$ as compared to Group 2.
[e] $P < 0.05$ as compared to Group 2.
[f] $P < 0.01$ as compared to Group 2.
[g] $P < 0.01$ as compared to Group 2.

Effect of Black Tea and Caffeine on Lung Cancer in F344 Rats

Most animal studies on tea and cancer reported in the literature involve the study of the effects of green tea. Relatively few studies have investigated the potential of black tea as an inhibitor against carcinogenesis, even though black tea production accounts for more than 75% of total tea production worldwide (11). The chemical composition of black tea differs significantly from that of green tea due to extensive oxidation of catechins during manufacturing (12). The oxidation products found in black tea, thearubigins and theaflavins, possess antioxidant activity (13), suggesting that black tea may also inhibit NNK-induced lung tumorigenesis. Indeed, bioassays in A/J mice have shown that black tea given as drinking water retarded the development of lung cancer caused by treatment with NNK (14). However, there has been a lack of data on the effect of tea in lung tumorigenesis in F344 rats. Recently, we completed a 2-year lifetime bioassay in F344 rats in which the effects of black tea and caffeine on the lung tumorigenesis induced by NNK were examined (15).

In this bioassay, animals were divided into six groups, as shown in Table IV. Beginning at 7 weeks of age, animals in Group 1 were given only deionized water, those in Groups 2a, 2b, and 2c were given tea as drinking water at three dose levels, 2%, 1%, and 0.5%, respectively. Rats in Groups 3a and 3b were given water containing caffeine at 680 ppm and 170 ppm, respectively; these are concentrations identical to those found in 2% and 0.5% tea infusions. One week later, animals in Groups 1 to 3 were administered NNK at a dose of 1.5 mg/kg body weight by subcutaneous injection three times weekly for 20 weeks for a total of 90 mg/kg body weight. Animals received tea or caffeine for 22 weeks, beginning 1 week before to 1 week after the NNK treatment. In Groups 4 and 5, rats were given 2% tea or 680 ppm caffeine in drinking water, respectively, without carcinogen. Group 6 served as the control without any treatment. The bioassay was terminated during Week 101; all major organs and gross lesions were harvested during necropsy and fixed in 10% buffered formalin solution. Representative sections were obtained and processed for microscopic examination. Tumor incidence was determined by dividing the number of animals with tumors by the number of animals in each group.

Contrary to the loss of body weight gains observed in A/J mice treated with caffeine or 2% green tea, the growth curves of the caffeine- and black tea–treated groups in this study were similar to that in the control group (Group 1). The lung cancer incidence results are summarized in Table IV. The NNK-treated group that drank 2% black tea (Group 2a) developed only a 19% lung tumor incidence ($P < 0.05$) as compared to 47% in NNK-treated groups that drank only water (Group 1). Only one animal showed a lung adenocarcinoma in this group, whereas seven occurred in Group 1 ($P < 0.05$). Groups 2b and 2c, receiving 1% and 0.5% black tea, showed no differences in lung cancer incidence as compared to Group 1. The most remarkable observation was that only 10% of the rats in Group 3a, given caffeine in drinking water at a concentration identical to that found in the 2% tea, developed lung tumors. This reflects an 80% reduction in tumor incidence ($P < 0.01$). The tumor incidence of this group is comparable to the spontaneous incidence of tumors commonly seen in the control group. At the lower concentration (170 ppm, Group 3b), caffeine also appeared to reduce the lung tumor incidence from 47% to 20%; however, this reduction in incidence was not statistically different from that in Group 1.

Although the lung is the major target organ, NNK also induces tumors in the liver and nasal cavity in F344 rats. In Group 1, 11 of 32 animals developed hepatic tumors (34%). All of the tumor-bearing animals had adenomas, and only two developed hepatocellular-carcinomas. The only group that showed a significant decrease in liver tumor incidence was Group 2a, with a 12% incidence ($P < 0.05$). A total of 11 animals developed liver adenomas in Group 1, whereas only two rats developed liver tumors in Group 2a ($P < 0.05$). In the nasal mucosa, 19% of the animals in Group 1 developed tumors, all of which were benign. The incidences of

Table IV. Incidence of Lung Tumors After Treatment with NNK and/or Test Substances

Treatment group	Total number of rats	Number of rats with tumors			Total number of rats with tumors	Tumor incidence (%)
		Adenoma	Adenocarcinoma	Adenosquamous carcinoma		
1. NNK	32	10	7	1	15	47
2a. NNK/2.0% black tea	26	4	1	0	5	19[a]
2b. NNK/1.0% black tea	26	9	2	1	11	42
2c. NNK/0.5% black tea	26	7	5	1	13	50
3a. NNK/caffeine (high)	20	1	1	0	2	10[b]
3b. NNK/caffeine (low)	20	3	1	0	4	20
4. 2.0% black tea	10	1	0	0	1	10
5. Caffeine (high)	10	0	0	0	0	0
6. Control	10	0	0	0	0	0

[a] $P < 0.05$ as compared to Group 1.
[b] $P < 0.01$ as compared to Group 1.

nasal cavity tumors in all treatment groups were not significantly different from one another.

Discussion

Both bioassays described here were designed to mimic, to some extent, the human situation in that tea was given as drinking water for the period of carcinogen exposure. However, these protocols did not allow us to distinguish whether the inhibitory effect of tea is on the initiation or postinitiation stage of NNK-induced carcinogenesis. It appeared that green tea and EGCG did not alter the levels of O^6-methylguanine, but instead, these treatments inhibited oxidative damage induced by NNK. Similarly, decaffeinated black tea inhibited lung tumor formation in A/J mice treated with NNK but did not significantly alter the levels of DNA methylation by NNK in the lung DNA (14). Also, the black tea polyphenols inhibited NNK-induced lung tumorigenesis in A/J mice after NNK dosing (14). These results seem to support the mechanism of inhibition by tea polyphenolic compounds during the postinitiation stages of lung carcinogenesis.

The induction of hepatic cytochrome P450 enzymes, such as 1A2, 1A1, and 2B1, has been described in rats given either green or black tea (16–18). Caffeine has been identified as the active compound in tea responsible for enzyme induction in the liver (16, 18). Although the mechanism of inhibition of lung tumorigenesis by caffeine is not yet known, we have postulated that the induction of cytochrome P450 enzymes in the rat liver may, in part, account for the inhibition, as the increased metabolism of NNK in the liver will result in its decreased bioavailability to the lung (15).

The bioassay studies in A/J mice and F344 rats both suggested that, in addition to tea polyphenols, caffeine appeared to play a role and, in some cases, a major role toward the inhibition of lung tumorigenesis induced by NNK. It is well documented that caffeine modulates carcinogenesis at various organ sites, including liver, skin, lung, and mammary gland, in animals treated with carcinogens (19). Caffeine can either stimulate or inhibit carcinogen-induced mammary gland tumorigenesis, depending on the species and strains and the phases during which it is administered (20). Caffeine was shown to inhibit gastric tumor promotion by NaCl in rats (21), but it enhanced the pancreatic tumorigenesis caused by N-nitrosobis(2-oxopropyl)amine in hamsters when administered during the postinitiation phase (22). Caffeine also protected against UV light–induced skin tumorigenesis and seemed to be actively involved in the protection of UV-induced skin tumorigenesis by green and black tea (23, 24). Treatment with caffeine significantly decreased lung tumor formation in mice treated with the precursors of N-nitrosomorpholine, and with N-nitrosodiethylamine, 4-nitroquinoline-1-oxide, and urethane (25–28). Interestingly, caffeine seems to invariably protect against lung tumorigenesis induced by different carcinogens.

As mentioned before, most of the available information from epidemiologic studies indicates that there is no apparent relationship between tea consumption and lung cancer risk. Furthermore, the relationship of caffeine intake (e.g., coffee consumption) on human lung cancer is yet to be clarified. In this regard, the protective effect of tea and caffeine against lung cancer demonstrated in our animal studies provides some basis to warrant large scale, well-designed epidemiologic studies or intervention trials to further assess the role of tea and caffeine in lung cancer among smokers.

I am indebted to all my associates at the AHF and to other collaborators outside the AHF and, especially to Dr. C. T. Ho at the Department of Food Sciences of Rutgers University, who analyzed the content of caffeine in both green and black tea in the studies described.

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