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Inhibition of *N*-nitrosomethylbenzylamine-induced esophageal tumorigenesis in rats by green and black tea

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In this study, we investigated the effects of green tea and black tea, when given either during or after carcinogen treatment, on esophageal tumorigenesis in male Sprague–Dawley rats. Rats were treated with *N*-nitrosomethylbenzylamine (NMBzA) (2.5 mg/kg, s.c., twice weekly) for 5 weeks; 39 weeks after the initial dose of NMBzA, 65% of the rats had esophageal tumors with an average of 1.4 ± 0.3 tumors per rat. In the groups of rats receiving 0.6% of decaffeinated green tea (DGT) or decaffeinated black tea (DBT) (6 mg tea solids/ml) as the sole source of drinking fluid during the NMBzA-treatment period, esophageal tumor incidence and multiplicity were reduced by ~70%. When the tea preparations were given after the NMBzA treatment period, the esophageal papilloma incidence and multiplicity were reduced by ~50%. The volume per tumor was much smaller in rats that received black tea after the carcinogen treatment period. In a second experiment, NMBzA was given to rats at a dose of 3.5 mg/kg (s.c., twice weekly) for 5 weeks; after 16 weeks, the tumor incidence was 82% and tumor multiplicity was 6.7 ± 1.2 tumors per rat. In the groups of rats receiving 0.9% regular green tea (RGT) or DGT after the NMBzA treatment period, tumor multiplicity was decreased by >55%. The volume per tumor was reduced by ~60% in the rats receiving 0.9% RGT. Histological analysis indicated that both the incidence and multiplicity of esophageal carcinoma was decreased by either RGT or DGT. The blood and urine levels of green tea polyphenols due to tea administration were determined in rats, and the levels were comparable to those in humans after tea ingestion. The above results indicate that both green tea and black tea can inhibit the tumorigenic action of NMBzA during the period of carcinogen treatment and the subsequent molecular events important for esophageal tumorigenesis.

Introduction

Tea (*Camellia sinensis*) is one of the most popular beverages consumed worldwide. An estimated 2.5 million metric tonnes of dried tea are manufactured annually. Of this amount, ~80% is black tea and ~20% is green tea. The relationship between tea consumption and esophageal cancer is a topic of extensive discussion (1–3). Based on correlative studies, it was suggested that excessive consumption of tea or tannin-containing foods, such as sorghum, may be a causative factor for the high incidence of esophageal cancer in many areas (4). For example, in the Caspian Littoral of Iran, individuals in the high-incidence area were reported to drink more tea than those in low-incidence areas (5). It was also suggested that consumption of very hot green tea-gruel is a causative factor for esophageal cancer in the Nara and Wekayama Prefectures in Japan (6). Several case-control studies, however, showed that there was no association between drinking of tea at normal warm temperatures and esophageal cancer, but ingestion of very hot tea was associated with a 2- to 3-fold increase in risk for esophageal cancer (7–11). It seems that the hot temperature of tea, rather than the components in tea, is an etiological factor in human esophageal cancer.

On the other hand, it was reported that oral administration of 2% tea infusion (2 g tea leaf /100 ml water) as the sole source of drinking fluid to rats during the entire experimental period inhibited *N*-nitrosomethylbenzylamine (NMBzA*)-induced esophageal tumor formation (12). Oral administration of green tea infusion also inhibited esophageal tumor formation induced by precursors (nitrite and corresponding amine) of NMBzA or nitrososarcosine in rats or mice, respectively (13–15). These interesting results also raise some important questions. Does tea inhibit the initiation of tumorigenesis or the post-initiation processes? Is the inhibitory action due to the caffeine in tea? Is the inhibitory action due to a direct interaction between tea components and the carcinogen when both are administered orally? Are the levels of tea components required to inhibit tumorigenesis much higher than those attained in humans due to tea consumption?

The present study was undertaken to answer these questions by investigating the effects of water extracts of decaffeinated green tea (DGT) and decaffeinated black tea (DBT), when administered to rats as drinking fluid either during or after the NMBzA treatment (s.c.) period, on esophageal tumorigenesis. The effects of RGT and DGT on NMBzA-induced esophageal tumorigenesis were compared. The levels of tea polyphenols in rat plasma and urine were analyzed and compared to those in humans after tea ingestion.

Materials and methods

Materials

NMBzA was obtained from Ash Stevens Inc. (Detroit, MI). β-Glucuronidase and sulfatase were purchased from Sigma Chemical Co. (St Louis, MO). DGT and DBT powders (dehydrated water-extracts of tea leaves) were supplied by Thomas J.Lipton Company (Englewood Cliffs, NJ). Decaffeination was accomplished by extracting tea leaves with CO_2 at the supercritical temperature. The major components in DGT and DBT were determined previously (16,17). The contents of (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG) and (–)-epicatechin (EC) in the DGT powder were 10.34, 8.82, 2.54 and 2.03%, respectively (17). For the preparation of 0.6 or 0.9% tea extract, 0.6 or 0.9 g of tea powder respectively was added to 100 ml of warm (50–55°C) deionized water. Fresh tea extracts were

*Abbreviations: NMBzA, *N*-nitrosomethylbenzylamine; DGT, decaffeinated green tea; DBT, decaffeinated black tea; RGT, regular green tea; EGCG, (–)-epigallocatechin-3-gallate; EGC, (–)-epigallocatechin; ECG, (–)-epicatechin-3-gallate; EC, (–)-epicatechin.

prepared on Mondays, Wednesdays and Fridays, and were given to animals as the sole source of drinking fluid.

Animals

Male Sprague–Dawley rats, 75–90 g body wt, were purchased from Taconic Inc. (Germantown, NY). The animals were housed two per plastic cage, and were maintained at 20 ± 2°C, 50 ± 10% relative humidity, and on a 12 h light/dark cycle. All animals were fed laboratory chow 5012 from Purina Mills Inc. (St Louis, MO). Experiments were initiated after a 1 week acclimation period.

NMBzA-induced tumorigenesis

In the first experiment, male Sprague–Dawley rats were divided into eight groups: group 1 (15 rats, water alone), group 2 (10 rats, 0.6% DGT alone) and group 3 (10 rats, 0.6% DBT alone) were negative control groups. Animals in groups 4–8 were treated with NMBzA (2.5 mg/kg body wt, s.c., twice weekly) for 5 weeks. Group 4 was the positive control (25 rats, water). In groups 5 and 6, the rats (20 in each group) were given 0.6% DGT and DBT respectively, starting 2 weeks before and until 1 week after the NMBzA treatment period. In groups 7 and 8, the rats (20 in each group) were given 0.6% DGT and DBT respectively, starting 1 week after the last dose of NMBzA and until the end of the experiment. In all of the tea treatment groups, the concentration of the tea in the drinking water was increased stepwise from 25% of the full dose on day 1 to 50, 75 and 100% of the full dose on days 3, 5 and 7, respectively. The experiment was terminated at 39 weeks after the first dose of NMBzA. One or two rats from each group were killed 2–5 weeks before the termination time point to check tumor formation; these animals were not included in the final data analysis.

Similar conditions were used in the second experiment, except that a higher dose of NMBzA (3.5 mg/kg) and 0.9% of RGT or DGT were used. Male Sprague–Dawley rats were divided into six groups: group 1 (10 rats, water alone), group 2 (10 rats, 0.9% RGT alone) and group 3 (10 rats, 0.9% DGT alone) were negative control groups. Animals in groups 4–6 were given NMBzA (3.5 mg/kg, s.c., twice weekly) for 5 weeks. Group 4 was the positive control (45 rats, water). In groups 5 and 6, the 45 rats in each group were given 0.9% RGT and DGT, respectively, as the drinking fluid starting 1 week after the last dose of NMBzA and until the end of the experiment. The experiment was terminated 16 weeks after the first dose of NMBzA treatment.

Pathological analysis

Animals were killed by CO_2 asphyxiation. The entire length of the esophagus was removed, opened longitudinally and placed flat on a piece of filter paper. The entire esophageal tissue was then fixed in 80% alcohol. Tumors ≥1 mm in diameter were counted under a dissecting microscope. The tumor volume was determined by measuring the three-dimensional size (height, length and width) of all tumors and by using the average of the three measurements as the radius. Tumor volume was calculated by: volume = $4\pi r^3/3$. The whole esophageal tissue was then made into a 'Swiss roll', cut in half, and embedded into a paraffin block. Serial sections (5 μm) were made. Twelve sections (each contained the whole length of the esophagus) from ~50 sections were mounted on slides, and stained with H&E. Four slides out of the 12 slides from each rat were picked randomly (independent of the visible tumors) for histopathological analysis, and the average score (lesions per slide) of a rat was treated as one sample. Microscopic lesions of the esophagus were classified into four main categories: basal cell hyperplasia (both the volume and number of the basal cells increased, but the normal cell morphology was still maintained), dysplasia (cells with nuclear atypia), papilloma (benign projections of esophageal mucosa into the lumen with interstitial stalks; the papilloma cells were similar to those of basal cell hyperplasia, and occasionally similar to those of dysplastic cells) and carcinoma (invasive cells with squamous pearl formation and polymorphous cells with hyperchromatic nuclei prominent).

Plasma and urine levels of green tea polyphenols

The plasma or urine sample was incubated with β-glucuronidase and sulfatase to convert the conjugates of polyphenols to the free form. The free forms of green tea polyphenols were then extracted with ethyl acetate and assayed by a method using HPLC and a coulochem electrode array system for detection (18).

Results

Effects of DGT and DBT on NMBzA-iduced esophageal tumorigenesis (experiment 1)

Oral administration of 0.6% DGT and DBT as the drinking fluid did not significantly affect the body weights of rats in groups 2 and 3 (Table I). However, the body weights of rats in groups 7 and 8, which consumed tea after the NMBzA-treatment period, were significantly higher than those of group 4 ($P < 0.05$). For rats in groups 5 and 6, which consumed 0.6% tea during the NMBzA treatment period and then switched to water, the average body weights were not significantly different from those of group 4.

Thirty-nine weeks after the administration of the first dose of NMBzA, 65% of the rats in the positive control group developed visible esophageal tumors (≥1 mm) with an average of 1.4 ± 0.3 tumors per rat (Table I). Oral administration of 0.6% DGT or DBT as the sole source of drinking fluid during the NMBzA treatment period significantly decreased the tumor incidence and tumor multiplicity by ~70%. Administration of 0.6% DGT or DBT as the sole source of drinking fluid after the NMBzA-treatment period inhibited esophageal tumor multiplicity ($P < 0.1$). It also decreased tumor incidence by ~50%, but the difference was not statistically significant. The volume per tumor was drastically decreased due to administration of DBT (by >90%) after the NMBzA treatment period. Histopathological examination of the esophageal

Table I. Effects of decaffeinated green tea and decaffeinated black tea on NMBzA-induced esophageal tumors in male Sprague–Dawley rats (experiment 1)

Treatment	No. of animals	Body wt (g)	Tumor incidence (%)	Tumor multiplicity	Volume per tumor (mm^3)
1 Water alone	15	647.7 ± 19.0	0	0	0
2 DGT alone	10	679.2 ± 27.9	0	0	0
3 DBT alone	10	702.2 ± 12.1	0	0	0
4 NMBzA	23	641.3 ± 13.2	65.2	1.4 ± 0.3	16.7 ± 6.2
Given during the NMBzA treatment period					
5 DGT (0.6%)	19	645.1 ± 9.0	17.2[a]	0.4 ± 0.2[c]	5.8 ± 3.8
6 DBT (0.6%)	19	637.5 ± 14.6	17.2[a]	0.4 ± 0.2[c]	25.5 ± 9.6
Given after the NMBzA treatment period					
7 DGT (0.6%)	20	683.5 ± 13.7[a]	30.0	0.7 ± 0.3[d]	6.1 ± 2.4
8 DBT (0.6%)	19	690.9 ± 10.3[a]	31.6	0.6 ± 0.3[d]	1.5 ± 0.5[b]

Male Sprague–Dawley rats were divided into eight groups. Groups 1–3 were negative control groups (water, 0.6% DGT, or 0.6% DBT as the sole source of drinking fluid, respectively). Animals in groups 4–8 were given NMBzA (2.5 mg/kg body wt, s.c., twice weekly) for 5 weeks. Group 4 was the positive control (water). Rats in groups 5 and 6 were given 0.6% DGT and DBT, respectively as the drinking fluid starting 2 weeks before and until 1 week after the last dose of NMBzA. Rats in groups 7 and 8 were given 0.6% DGT and DBT, respectively as the drinking fluid starting 1 week after the last dose of NMBzA and until the end of the experiment. The experiment was terminated at 39 weeks after the first dose of NMBzA treatment. The number of visible tumors of the esophagus (≥1 mm) were counted and expressed as the mean ± SE.

[a]$P < 0.05$, significantly different from those in group 4 based on the chi-squared test.

[b]$P < 0.01$, [c]$P < 0.05$, [d]$P < 0.1$, significantly different from those in group 4 based on Student's t-test.

samples showed the formation of hyperplasia, dysplasia and papilloma, but no carcinomas were observed. Oral administration of DGT or DBT during or after NMBzA treatment significantly reduced the incidence (by >54%) and multiplicity (by >70%) of papilloma. A significant reduction on the formation of hyperplastic or dysplastic lesions was not, however, observed (Table II).

Effects of RGT and DGT on NMBzA-induced esophageal tumorigenesis (experiment 2)

In order to induce esophageal carcinomas and to shorten the experimental period, the dose of NMBzA was increased from 2.5 to 3.5 mg/kg body wt (s.c. twice weekly for 5 weeks). The effects of RGT and DGT, when administered after the NMBzA treatment period, were studied. There were no statistically significant differences in body weight, food consumption or fluid consumption in the various groups of rats during the entire experimental period (data not shown). From 10 to 15 weeks after the first NMBzA treatment, ~13% of the rats in the positive control group died and 80% of them bore esophageal tumors. The experiment was terminated during week 16. In the positive control group, 82% of the rats had visible esophageal tumors (≥1 mm) with an average of 6.7 ± 1.2 tumors per rat (Table III). Oral administration of 0.9% RGT or DGT after the NMBzA treatment period significantly inhibited esophageal tumor multiplicity by 55 or 67%, respectively. RGT and DGT appeared to inhibit tumor incidence by 20%, but this inhibition was not statistically significant. Oral administration of 0.9% RGT or DGT also significantly decreased esophageal volume per tumor by 57 and 35, respectively.

Histopathological examination of the esophageal samples showed the formation of hyperplasia, dysplasia, papilloma and carcinoma (Figure 1). In this experiment, 40% of the rats developed carcinomas (Table IV). Oral feeding of 0.9% RGT or DGT significantly decreased the carcinoma incidence and multiplicity as well as the multiplicity of papillomas. Tea administration also appeared to decrease the incidence of hyperplasia and dysplasia, but only the DGT group showed a statistical significance in decreasing the number of dysplastic lesions.

Urinary and plasma levels of tea polyphenols in the rats

When rats were given 0.9% DGT as drinking fluid for 3 weeks, EGCG, EGC and EC (mainly as glucuronide and sulfate conjugates) were detected in the plasma (Table V). The concentrations of total EGCG, EGC and EC in the plasma were 0.08, 0.18, and 0.07 μM, respectively. The concentrations of total EGC and EC in the urine were 14.7 and 35.5 μM, respectively. When tea was not administered to rats, the levels of EGCG, EC and EGC in either the plasma or urine samples were all below the limit of detection.

Discussion

Throughout the experiments, there was no indication of increased tumorigenesis or signs of toxicity due to tea administration. The inhibitory action of tea preparations against NMBzA-induced esophageal tumorigenesis was clearly demonstrated. In the first experiment, both the incidence and multiplicity of esophageal papilloma were reduced by the

Table II. Effects of decaffeinated green tea and decaffeinated black tea on the histopathology of esophageal lesions in Sprague–Dawley rats induced by NMBzA (experiment 1)

Treatment	No. of animals	Incidence of lesion (%)			No. of lesion per slide (mean ± SE)		
		Hyperplasia	Dysplasia	Papilloma	Hyperplasia	Dysplasia	Papilloma
4 NMBzA alone	23	100	39	65	6.0 ± 1.1	0.6 ± 0.2	1.0 ± 0.2
Given during the NMBzA treatment period							
5 DGT (0.6%)	19	100	39	17[a]	7.1 ± 0.4	0.4 ± 0.2	0.2 ± 0.1[b]
6 DBT (0.6%)	19	94	47	24[a]	6.1 ± 0.9	0.7 ± 0.2	0.2 ± 0.1[b]
Given after the NMBzA treatment period							
7 DGT (0.6%)	20	100	30	30[a]	5.3 ± 0.8	0.4 ± 0.2	0.3 ± 0.1[b]
8 DBT (0.6%)	19	100	30	22[a]	3.8 ± 0.5	0.4 ± 0.2	0.2 ± 0.1[b]

The samples were from the same experiment as in Table I.
[a]$P < 0.05$, significantly different from those in group 4 alone based on the chi-squared test.
[b]$P < 0.05$, significantly different from those in group 4 based on Student's *t*-test.

Table III. Effects of regular green tea and decaffeinated green tea on NMBzA-induced esophageal tumors in Sprague–Dawley rats (experiment 2)

Treatment	No. of animals	Body wt (g)	Tumor incidence (%)	Tumor multiplicity	Volume per tumor (mm^3)
1 water alone	10	481.3 ± 6.9	0	0	0
2 RGT alone	10	476.1 ± 5.1	0	0	0
3 DGT alone	10	485.6 ± 6.0	0	0	0
4 NMBzA	39	462.5 ± 10.2	82.1	6.7 ± 1.2	8.7 ± 1.5
5 NMBzA and RGT (0.9%)	42	457.8 ± 7.0	64.9	3.0 ± 0.7[b]	3.7 ± 1.0[a]
6 NMBzA and DGT(0.9%)	42	476.4 ± 8.8	66.7	2.2 ± 0.4[b]	5.7 ± 1.1[c]

The experimental conditions were similar to those in Table I except that a higher dose of NMBzA (3.5 mg/kg) was given and 0.9% of RGT or 0.9% of DGT was used. Male Sprague–Dawley rats were divided into six groups. Groups 1–3 were negative control groups (water, 0.9% GT or 09% DGT respectively as the drinking fluid). Animals in groups 4–6 were given NMBzA. Group 4 was the positive control. Rats in groups 5 and 6 were given 0.9% RGT or DGT respectively as the drinking fluid starting 1 week after the last dose of NMBzA and until the end of the experiment. The experiment was terminated at 16 weeks after the first dose of NMBzA. The number of visible tumors of the esophagus (≥1 mm) were counted and expressed as the mean ± SE.
[a]$P < 0.01$, [b]$P < 0.05$, [c]$P < 0.1$, significantly different from those in group 4 based on Student's *t*-test.

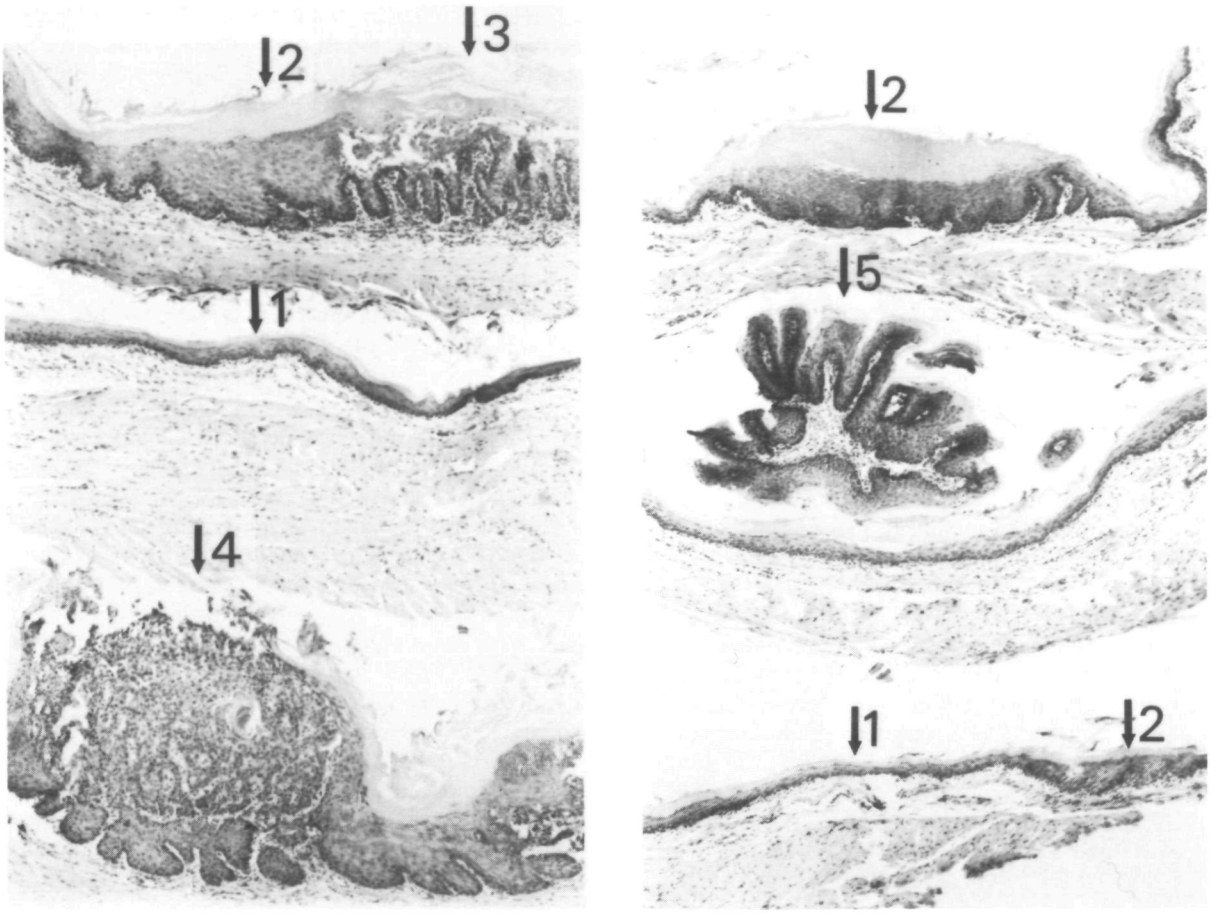

Fig. 1. Histology of the esophageal tissues from a NMBzA-treated rat in 'Swiss rolls' (H&E, ×120). ↓1, Normal esophageal epithelium. ↓2, Basal cell hyperplasia—both the volume and the number of the basal cells increased, but the normal cell morphology was still maintained. ↓3, Dysplasia—papillae with atypical epithelial cells were prominent and the keratin layer over dysplastic lesions were interrupted ↓4, Squamous cell carcinoma—invasive tumor cells were prominent with squamous pearl formation in the tissues. ↓5, Papilloma—benign projections of esophageal mucosa into the lumen with interstitial stalks.

Table IV. Effects of green tea administration on esophageal lesions induced by NMBzA (experiment 2)

Treatment	No. of animals	Incidence of lesion (%)				No. of lesion per slide (mean ± SE)			
		Hyperplasia	Dysplasia	Papilloma	Carcinoma	Hyperplasia	Dysplasia	Papilloma	Carcinoma
4 NMBzA alone	39	95	70	75	40	18.1 ± 2.0	2.3 ± 0.4	3.3 ± 0.5	1.1 ± 0.3
5 NMBzA and RGT (0.9%)	42	98	50	60	18[a]	14.0 ± 1.2	1.5 ± 0.4	2.1 ± 0.4[c]	0.3 ± 0.1[c]
6 NMBzA and DGT (0.9%)	42	98	58	65	23[b]	11.5 ± 0.9	1.1 ± 0.2[c]	2.1 ± 0.4[c]	0.5 ± 0.2[d]

The samples were from the same experiment 2 as described in Table III.
[a]$P < 0.05$, [b]$P < 0.1$, significantly different from those in group 4 based on the chi-squared test.
[c]$P < 0.05$, [d]$P < 0.1$, significantly different from those in group 4 based on Student's *t*-test.

Table V. Urinary excretion and plasma concentration of EC, EGC and EGCG in Sprague–Dawley rats consuming DGT in the drinking fluid

Treatment	Plasma (μM)			Urine (μM)		
	EGCG	EGC	EC	EGCG	EGC	EC
Water	<0.02[a]	<0.03[a]	<0.002[a]	<0.02[a]	<0.03[a]	<0.002[a]
0.9% DGT	0.08 ± 0.01	0.18 ± 0.05	0.07 ± 0.01	<0.02	14.7 ± 3.8	35.5 ± 8.6

Ten rats (body wt 83.6 ± 0.6 g) were divided into two groups; five rats were given water and five rats were given 0.9% DGT as the sole source of drinking fluid. Three weeks later, the rats were placed in metabolism cages (1 rat/cage) for urine collection into plastic bottles containing ascorbic acid and EDTA as preservatives. Blood samples were collected in heparin-containing blood collection tubes when the animals were killed. Each value represents the mean ± SE from five rats.
[a]Below the level of detection.

administration of DGT and DBT either during or after the NMBzA-treatment period (Table II). When the dose of NMBzA was increased to 3.5 mg/kg, higher tumor incidence (75% papilloma and 40% carcinoma) was observed. RGT and DGT, when given after the carcinogen treatment period, decreased both the incidence and multiplicity of carcinoma and the multiplicity of papilloma (Table IV). A decrease in tumor size by tea administration was also observed in some experiments (Tables I and III), and this is consistent with the results in our previous studies with other animal models (17,19–21). In these experiments, the effects of tea administration on hyperplasia and dysplasia were not clear. One possibility is that tea administration decreased the progression of these lesions to tumors and, therefore, would not significantly reduce the number of hyperplastic and dysplastic lesions.

In addition to confirming the conclusion by Han and Xu (12), our studies provide the following new information. (i) The inhibitory action of tea was not due to the direct interaction of tea components with the carcinogen NMBzA, because tea was given orally and NMBzA was administered by s.c. injection. (ii) We demonstrated the inhibitory effects of tea, when given either during or after the carcinogen treatment period. This observation suggests that tea components can inhibit both the initiation and post-initiation events in esophageal tumorigenesis. (iii) We demonstrated that caffeine was not essential for the inhibitory activity, because decaffeinated tea preparations were effective in the inhibition of tumorigenesis. The activities of RGT and DGT were comparable, even though RGT appeared to be slightly more effective in reducing the tumor volume and inhibiting carcinoma formation (Tables III and IV). (iv) DBT had a similar inhibitory activity to DGT against esophageal tumorigenesis, and when given after the NMBzA treatment period, DBT was more effective in reducing the tumor volume (Tables I and II).

When tea was administered during the carcinogen treatment period, the protection against tumorigenesis might have been a result of the inhibition of carcinogen activation, alkylation of DNA by reactive intermediates, or oxidative damage of DNA due to reactive oxygen species produced during carcinogen metabolism. In a preliminary experiment, green tea and black tea polyphenol preparations were found to inhibit the activation of NMBzA in rat esophageal microsomes (unpublished results). The inhibition of NMBzA-induced DNA methylation in the rat esophagus, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced 8-hydroxydeoxy-guanosine formation in mouse lung have been reported previously (22,23). In addition, the antioxidative, anti-proliferative and anti-inflammatory activities of the tea preparations (16,24–31) may be closely related to their anticarcinogenic activities when given after the carcinogen treatment period.

Consistent with our previous results with other animal models (17,19), the present study demonstrates that green and black tea preparations have comparable inhibitory activities against tumorigenesis. The major components responsible for the anti-carcinogenesis activities in green tea are believed to be the tea polyphenols (flavanols), EGCG, EGC, EC and ECCG. The levels of monomeric flavanols in black tea are only 10–37% of those in green tea. It is likely that theaflavins and thearubigins in black tea also have inhibitory actions against carcinogenesis. More research is needed to identify the active constituents in both green and black tea as well as to determine the possible synergistic or antagonistic effects of the tea constituents.

The present study demonstrates the presence of substantial amounts of green tea polyphenols in rat urine and plasma. When rats were given 0.9% DGT as the sole source of drinking fluid, the concentrations of EGCG, EGC and EC were 0.07–0.18 μM in the plasma. In the urine, EGCG was not detected, but EGC and EC were much higher than in the plasma (from 15 to 36 μM). These levels appeared to be comparable to plasma and urinary levels in humans who consumed tea. Our recent human study indicated that after ingesting 1.2 g of DGT (in 200 ml warm water), the concentrations of EGCG, EGC and EC were 0.1–0.7 μM in the plasma after 1–4 h, and the concentrations of EGC and EC were 4–20 μM in urine samples collected in the first 6 h (18). Further comparative studies of blood and urinary levels of tea polyphenols between animals and humans are needed to provide quantitative information on the possible cancer preventive effect of tea in humans.

Epidemiology studies on the effects of tea consumption on human cancer have not yielded clear conclusions (1). However, a recent population-based case-control study of esophageal cancer in Shanghai by Gao *et al.* (32) indicated that after accounting for cigarette smoking, alcohol drinking, dietary factors and other variables, tea consumption was strongly associated with reduced risk for esophageal cancer. The present study demonostrates the biological plausibility for such an association and provides a basis for additional case-control or prospective studies in different populations and for intervention studies on the possible preventive effect of tea against esophageal cancer.

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