Ingestion of Green Tea Rapidly Decreases Prostaglandin E_2 Levels in Rectal Mucosa in Humans[1]

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Abstract

The objective of this Phase I/II study was to assess the potential for green tea to be used as a colorectal cancer chemopreventive agent. This study measured the dose-related biological effects of administration of a single dose of green tea on the rectal mucosa of normal volunteers. Volunteers were admitted to the Robert Wood Johnson Medical School Clinical Research Center for 24 h. Baseline blood and rectal biopsy samples were obtained before the volunteers drank 0.6, 1.2, or 1.8 g of green tea solids dissolved in warm water. Blood samples were taken 2, 4, 8, and 24 h after the tea administration. Rectal biopsies were obtained at 4, 8, and 24 h. Prostaglandin E_2 (PGE_2) levels were analyzed by ELISA. Tea polyphenol levels in the blood, urine, and rectal tissue were measured by high-performance liquid chromatography using a Coulochem electrode array detection system. Statistical comparisons were made using ANOVA. Decreased levels of PGE_2 in rectal mucosa were observed at 4 and 8 h after consumption of green tea. There was no correlation between inhibition of PGE_2 and tissue or plasma levels of tea polyphenols. Ten of 14 subjects demonstrated a response to green tea, as evidenced by at least a 50% inhibition of PGE_2 levels at 4 h. We conclude that green tea constituents have biological activity in inhibiting PGE_2 synthesis. Given the 71% "response rate," we believe these data support the study of green tea as a colorectal chemopreventive agent in more long-term Phase II trials.

Introduction

In the United States, colorectal cancer is the third most common cancer in men and the second most common cancer in women; it is also the second leading cause of cancer deaths overall (1). Although the mortality rate has been falling over the past 5 decades, the incidence of colorectal cancer has increased slightly since the early 1970s (2). The frequency of colorectal cancer in the American population and the personal and economic impact of treatment and associated morbidity make it a potentially rewarding target for preventive strategies. Although early detection and treatment have been demonstrated to reduce colorectal cancer mortality, primary prevention potentially offers far greater benefits by reducing morbidity and the costs of colorectal cancer treatment (3).

Tea (*Camellia sinensis*) has been suggested as a possible cancer-preventive agent (4). Most tea is consumed in the form of black tea (78%, primarily in Western countries) or green tea (20%, primarily in Asian countries; Ref. 4). Green tea contains a variety of polyphenols, including flavanols, flavandiols, flavanoids, and phenolic acids. Flavanols (catechins) are the major tea polyphenols. The major components responsible for the putative anticarcinogenic activities of tea are not clearly known. In green tea, however, the catechins EGCG,[3] EGC, (-)-epicatechin-3-gallate, and EC are believed to be the active components because inhibition of carcinogenesis has been demonstrated with EGCG and mixtures of these polyphenols (4–9). Data from animal models of carcinogenesis demonstrate a protective effect of green tea and catechins against carcinogenesis in skin, lung, esophagus, stomach, duodenum, liver, and pancreas (4–19).

Several animal studies have demonstrated a protective effect of green tea on colon carcinogenesis (20–23). Rats that were chemically induced to form colon tumors were protected by low doses of green tea polyphenols (0.01% or 0.1% in the drinking water; Ref. 20). In another study using very low doses of green tea (0.05%, 0.01%, or 0.002%), rats ingesting the tea had a significantly lower incidence of colon carcinomas (21). Yet, a more recent study using a similar model did not find significant inhibition of colon carcinogenesis by black tea (24).

Epidemiological studies of the relationship between tea consumption and human cancer are plagued by multiple confounders and are not conclusive (25). The epidemiological evidence seems suggestive of a protective effect of tea consumption against the development of colorectal, uterine, and gastric cancers (4, 26, 27). Factors that confound the interpretation of these types of studies include influences of the temperature of the tea on the increased risk for esophageal cancer (26), type of tea consumed (green *versus* black), variations in methods of tea preparation, and lifestyle factors that may be associated with tea drinking.

A variety of mechanisms have been suggested for the anticarcinogenic effects of tea polyphenols. Tea polyphenols

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[3] The abbreviations used are: EGCG, (-)-epigallocatechin-3-gallate; EGC, (-)-epigallocatechin; ECG, (-)-epicatechin-3-gallate; EC, (-)-epicatechin; PGE_2, prostaglandin E_2; HPLC, high performance liquid chromatography.

are antioxidants with the ability to scavenge superoxide anions, singlet oxygen, and lipid peroxy-radicals, and to bind metal ions (4, 28–30). These antioxidant functions of tea polyphenols are generally considered to be important to the anticarcinogenic activities of tea. Inhibition of tumor promotion-related enzymes, including ornithine decarboxylase (31, 32), protein kinase C (33), lipoxygenase (34, 35), and cyclooxygenase (34, 35) by tea preparations may also be important. These activities may be particularly important when considering the potential use of tea preparations for prevention of colorectal cancer.

The effect of green tea on human colon carcinogenesis is unknown. To explore the potential of green tea as a colorectal cancer preventative, we initiated a Phase I trial of the effect of a single ingestion of green tea on a set of colorectal carcinogenesis biomarkers—basal PGE_2 levels, cyclooxygenase activity, crypt cell proliferation, and apoptosis—on the rectal mucosa of normal volunteers.

Materials and Methods

Subjects. A preliminary study to assess tissue penetration of tea polyphenols was carried out in 10 volunteer subjects scheduled to undergo abdominal surgery with resection of a portion of the intestine. Each of these preliminary subjects ingested 1.2 g of standardized green tea ~12 h before surgery. At the time of surgery, 1–10 g of full-thickness intestinal tissue (small bowel, colon, or rectum) was harvested, flash frozen in liquid nitrogen, and stored at –70°C for subsequent determination of tissue polyphenol levels.

For the formal Phase I trial, 15 normal volunteers (ages 22–57) were admitted to the Robert Wood Johnson Medical School Clinical Research Center. They were instructed to start a clear liquid diet devoid of fruit and vegetable juices and tea 36 h before the study and to cease all food and beverage intake, except water, 12 h before the study. None of the volunteers were pregnant or lactating; had renal, hepatic, cardiac, or pulmonary dysfunction, or a coagulopathy; had a history of inflammatory bowel disease or colorectal cancer; were taking nonsteroidal anti-inflammatory drugs or steroids at any time in the 4 weeks before the study; were strict vegetarians; were abusing illicit drugs or alcohol; or had drank greater than two 8-ounce portions of tea per day for 2 weeks before the study. Written, informed consent was obtained from all subjects. These studies were carried out with the approval of the Institutional Review Board of the University of Medicine and Dentistry of New Jersey/Robert Wood Johnson Medical School (protocol number 2095).

Treatment Protocol. After a tap water enema, baseline blood and rectal biopsies were obtained at 8:00 a.m. Rectal biopsies were obtained with biopsy forceps introduced through an anoscope. A single dose (0.6, 1.2, or 1.8 g) of standardized green tea powder (courtesy of Thomas J. Lipton Co., Englewood Cliffs, NJ) containing ~10% by weight of polyphenols (36) was mixed in warm water and drunk by the volunteers at 9:00 a.m. Pooled urine samples were collected between 0–4 h, 4–8 h, and 8–24 h (hour 0 being the time of tea ingestion). Blood samples were drawn at 2, 4, 8, and 24 h, and rectal biopsies were obtained at 4, 8, and 24 h. Four to six rectal biopsies (10–20 mg of full-thickness samples of rectal mucosa) were collected at each time point; half were snap frozen in liquid nitrogen and half were fixed in 10% formalin. All rectal biopsies were obtained in a zone from 4–6 cm centimeters above the dentate line. Each biopsy specimen was obtained from fresh mucosa not previously sampled.

Analytic Methods. Blood collected for clinical laboratory studies was analyzed in the Clinical Pathology Laboratory of Robert Wood Johnson University Hospital. Blood for tea polyphenol assay was centrifuged, and the plasma fraction was separated and immediately frozen at –70°C. Urine was collected with each void, pooled into appropriate time periods, and was stored frozen at –70°C.

The levels of tea polyphenols in plasma, urine, and rectal biopsies were determined by a HPLC procedure (37). In brief, the plasma, tissue, or urine was incubated with β-glucuronidase and sulfatase to convert conjugated polyphenol species to the free forms. The resulting free tea polyphenols were extracted into ethyl acetate, dried under nitrogen, and redissolved in buffer. The individual catechins were analyzed by HPLC using a Coulochem electrode array detection system. To determine basal PGE_2 levels, frozen colonic biopsy samples were homogenized at ice temperature in 0.3 ml of 0.1 M Tris-HCl buffer containing 5.6 μM indomethacin (pH 7.4) with a microtube pestel and vortexed thoroughly for 2 min. The homogenate was immediately analyzed for basal PGE_2 levels by ELISA according to the manufacturer's instructions (Cayman Chemical).

After fixation, rectal biopsies were embedded in paraffin and serially sectioned at 5 mm and stained with H&E for histological analysis. The proliferation pattern was analyzed by immunostaining with anti-K_i67 (MIB-1) antibody according to a previously published procedure (38). The pattern of apoptotic cells was also analyzed. Apoptotic cells were visualized in histopathological sections that maintain crypt architecture by use of terminal deoxynucleotidyl transferase-mediated nick end labeling of apoptotic DNA strand breaks (39).

All biomarker parameters were assayed and interpreted by an observer blinded to the treatment dose and to the timing of the biopsy (baseline *versus* some time point after ingestion of tea).

Statistical Analysis. Percent inhibition of PGE_2 was analyzed by a Student's *t* test comparing baseline with the other time points. Time- and dose-dependent correlations and polyphenol concentration in tissue and plasma to PGE_2 levels correlations were determined by χ^2 analyses.

Results

Preliminary Study of Tea Polyphenol Levels in Intestinal Mucosa. Ten subjects (ages 28–80, five females) received 1.2 g of standardized green tea ~12 h before laparotomy. Grossly normal intestinal tissue was harvested from the operative specimens and analyzed for tea polyphenol content. The mean ± SE of the polyphenol tissue concentration (ng/g wet tissue) was 382 ± 146 for EGCG, 89 ± 28 for EGC, and 39 ± 23 for EC. These data indicate that significant levels of tea polyphenols were detectable after ingestion of a single, moderate "dose" of tea, equivalent to two to three cups of brewed tea.

Phase I Study. Fifteen subjects (ages 22–57, two females) participated in the Phase I study. A total of 60 anoscopic biopsy procedures (4 per subject) were carried out. The only complication that occurred was one episode of persistent bleeding from a biopsy site requiring transport of the subject to an operating room for better visualization and suture ligation.

Tea Polyphenol Pharmacokinetics. Plasma concentration *versus* time curves for EGCG and EC are shown in Fig. 1, *A* and *B*, respectively. Due to an interfering peak, HPLC results for EGC were obscured. Maximal plasma concentration (C_{max}) was clearly dose dependent. Maximum plasma polyphenol concentrations occurred at 2 h after tea ingestion. The mean plasma

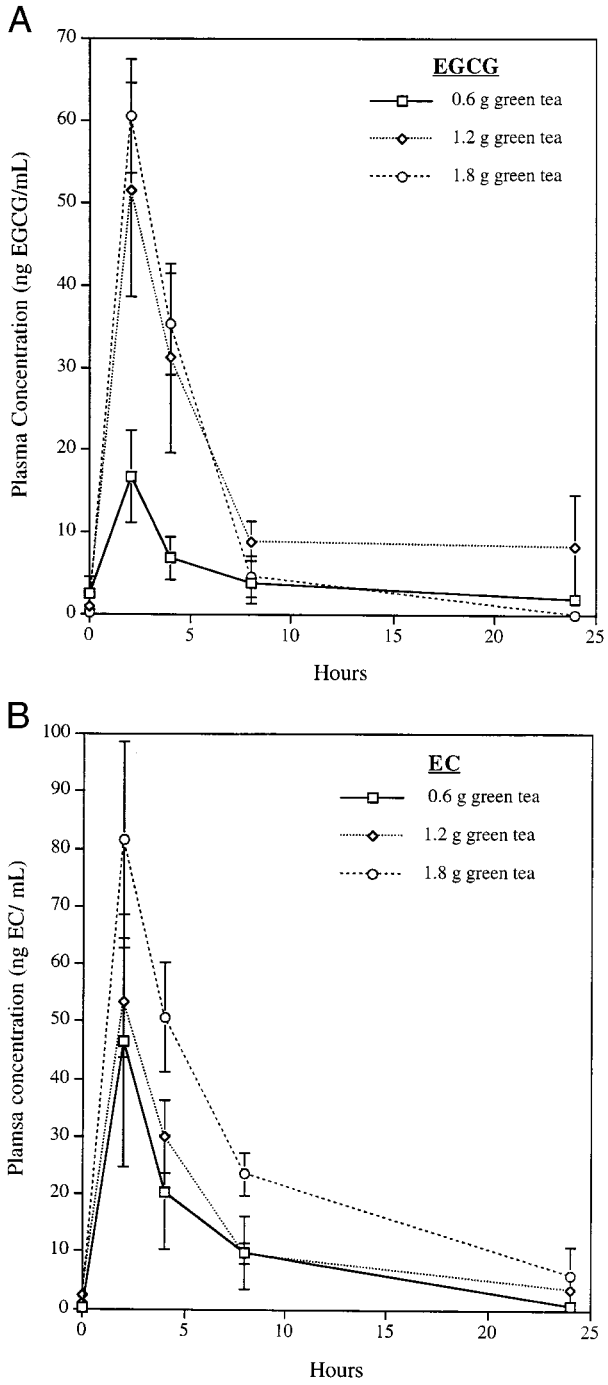

Fig. 1. Plasma polyphenol concentration *versus* time curves. Green tea solids were given at three different doses (0.6 g, 1.2 g, and 1.8 g). The plasma concentrations (ng/ml) of EGCG and EC at 0, 4, 8, and 24 h are shown.

half-life for each of the polyphenols was ~4 h for both EGCG and EC.

The highest EGC and EC levels were observed in urine samples collected between 0 and 4 h (Table 1). EGCG was not excreted in the urine, consistent with the previous results (37). Tissue concentration/time data for the tea polyphenols in rectal mucosa were variable and not clearly dose dependent (Table 2).

Table 1 Mean EGC and EC values[a] per green tea dose in urine over time

Dose	Time interval (h)	EGC	EC
0.6	0–4	1310 ± 561	1310 ± 465
	4–8	552 ± 392	492 ± 276
	8–24	189 ± 154	304 ± 130
1.2	0–4	2680 ± 864	3100 ± 996
	4–8	300 ± 84	480 ± 147
	8–24	600 ± 383	660 ± 320
1.8	0–4	4440 ± 1580	7760 ± 2560
	4–8	600 ± 205	1140 ± 330
	8–24	440 ± 93	560 ± 144

[a] Values are mean ng polyphenol/ml of urine ± SE of five subjects. EGCG is not excreted in the urine.

Table 2 Mean EGC, EC, and EGCG values[a] per green tea dose in rectal biopsies over time

Dose	Time point	EGC	EC	EGCG
0.6[b]	0	1.2 ± 0.7	0.9 ± 0.5	5.0 ± 2.7
	4	103 ± 31	4.4 ± 3.5	37.0 ± 30.9
	8	16.0 ± 6.7	5.3 ± 4.3	25.4 ± 20.3
	24	0.8 ± 0.5	0.6 ± 0.3	3.05 ± 1.7
1.2[b]	0	3.4 ± 1.9	2.0 ± 1.1	10.2 ± 5.4
	4	11.1 ± 6.4	7.0 ± 3.8	41.3 ± 25.1
	8	4.2 ± 0.2	2.9 ± 0.5	14.8 ± 2.8
	24	2.3 ± 1.2	1.8 ± 0.9	8.2 ± 5.2
1.8	0	0 ± 0	0 ± 0	0.6 ± 0.6
	4	7.8 ± 6.9	6.8 ± 4.9	31.2 ± 23.9
	8	4.2 ± 2.6	2.2 ± 1.2	28.5 ± 18.0
	24	1.8 ± 1.5	1.6 ± 0.8	7.1 ± 4.4

[a] Values are mean ng polyphenol/mg protein in rectal biopsy homogenate ± SE of three, three, and five subjects for doses 0.6 g, 1.2 g, and 1.8 g, respectively.
[b] Due to problems with some samples, two subjects were excluded from the analysis from groups 0.6 and 1.2 doses. Thus, $n = 3$ for these groups.

There was no evident correlation between the dose of tea ingested and the time-dependent concentration of polyphenols in the rectal mucosa.

Levels of PGE_2. Ingestion of green tea decreased rectal mucosal concentrations of PGE_2 at 4 and 8 h after tea administration (Fig. 2 and Table 3). At 24 h, the PGE_2 concentrations returned to the baseline levels. Although the high dose of tea (1.8 g) was more effective in lowering the PGE_2 levels than the low (0.6 g) and moderate (1.2 g) doses, a good dose-dependent effect was not observed because the moderate dose did not produce lower PGE_2 levels than the low dose. Seventy-one percent (10 of 14) exhibited a "response" to tea, as evidenced by a >50% reduction in basal PGE_2 levels at 4 h after ingestion.

Crypt Cell Proliferation and Apoptosis. Because of wide variability in the histological appearance of immunohistochemical stains of rectal biopsies, presumably reflecting the wide biological variability in individuals (40), there was no clearly evident effect of green tea ingestion on crypt cell proliferation or apoptosis (data not shown).

Discussion

One strategy to reduce cancer-related deaths is through chemoprevention. Chemoprevention can be defined as controlling the occurrence of cancer by slowing, blocking, or reversing the development of the disease by the administration of naturally occurring or synthetic compounds. Chemopreventive compounds should ideally have: (*a*) little or no toxic effect; (*b*) high

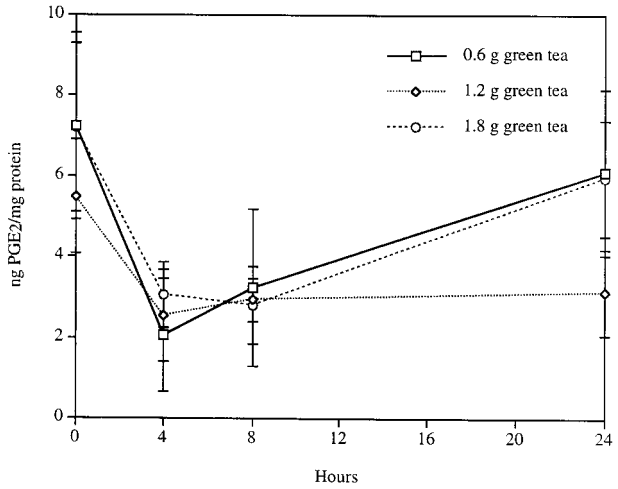

Fig. 2. Basal PGE_2 levels (ng/mg of protein) at 0, 4, 8, and 24 h for three doses (0.6 g, 1.2 g, and 1.8 g) of green tea.

efficacy against multiple types of cancer; (*c*) oral administration; (*d*) known mechanisms of action; (*e*) low cost; and (*f*) human acceptance (41–44). On the basis of these criteria, it is logical that compounds found naturally in foods and drinks have been investigated. Tea is thought to meet these criteria because it is widely consumed, has no known toxicity, and has demonstrated inhibitory activity against carcinogenesis in animal models (4, 22–25).

Our preliminary study demonstrated that measurable amounts of polyphenols are present in the gastrointestinal tissues. Our present and previous results (37) on plasma and urine concentrations of tea catechins indicated that these compounds are absorbed. The effect of p.o. administered tea was rapid; at 4 and 8 h after tea administration, PGE_2 levels were significantly lower at all doses of tea. Although the 1.8-g dose seemed to be more effective in lowering PGE_2 formation than the 0.6-g dose, a good dose-response relationship was not observed. This was probably due to the variability of the individuals and sampling procedure. There were no correlations between the inhibition of PGE_2 formation and the tea polyphenol levels in the plasma or rectal tissue. This lack of correlation may be explained by individual variations and the difficulties caused by repeated rectal biopsies interfering with each other. The biopsy-induced inflammation may mask some of the effects of the tea. Of interest, however, is that although increased inflammatory changes were seen in the immunohistochemical slides over time, lowering of PGE_2 levels was observed. Whereas an increase in inflammation may be explained by serial anoscopies, inhibition of PGE_2 may be the result of tea administration. This is believed to be due to the inhibition of cyclooxygenase-2 activity in the rectum. Preliminary experiments, however, indicate that the level of cyclooxygenase in the rectal mucosa, as determined by Western blot analysis, was not significantly affected by the tea treatment (data not shown).

The immunohistochemical slides regarding proliferation and apoptotic rates were difficult to interpret. Individual variations in these rates were very high and make it difficult to determine the effect of tea on these biomarkers. Recent analysis of the variability in rectal mucosal proliferation measurements calls into question the ability of proliferating cell nuclear antigen to show treatment effects (40). Potential sources of variation include the effect of bowel preparation, age, gender, diet, time of day, fluctuation over time, and biopsy location. Similar difficulties are likely to occur with the measurement of apoptotic cells, as with proliferation indices (45). Because subjects were able to act as their own control over time, certain variables were likely to play a role in this study's difficulty in assessing proliferation and apoptosis. Proliferation indices are known to have circadian rhythm (46) that could affect our ability to demonstrate tea treatment changes over a 24-h period.

Table 3 Percent inhibition of PGE_2 formation over time after green tea consumption in colorectal tissue

Dose	4 h[a]	8 h[a]	24 h[a]	Response rate[b]
0.6	58.3 ± 12[c]	63.1 ± 12[c]	17.2 ± 1[d]	4/5
1.2	49.6 ± 14[c]	41.0 ± 11[c]	17.0 ± 43[d]	3/5
1.8	78.2 ± 10[c]	60.5 ± 16[c]	0 ± 25[d]	3/4[e]

[a] Values are the mean percent inhibition compared with the baseline (0 h) time point ± SE for four or five subjects.
[b] Response rate is described as at least 50% inhibition at 4 h (responsers/n).
[c] Inhibition is significantly different from the baseline time point.
[d] Inhibition is significantly different from the other time points, but not the baseline point.
[e] PGE_2 levels were not determined in one subject because of lack of biopsy material.

Green tea has biological activity in the colorectal mucosa. Because PGE_2 inhibition in other settings is related to a lower risk of certain cancers and the response rate among the subjects was 10 of 14 (71%), these data suggest a potential role for green tea as a chemopreventive agent against colorectal cancer. The data support proceeding with a longer-term Phase II study.

References

1. Boring, C. C., Squires, T. S., Tong, T., and Montgomery, S. Cancer statistics. CA Cancer J. Clin., *44:* 7–26, 1994.

2. Garfinkel, L. Cancer statistics and trends. *In:* G. P. Murphy, W. Lawrence, and R. E. Lenhard (eds.), Textbook of Clinical Oncology, pp. 1–9. Atlanta: American Cancer Society, 1995.

3. Byers, T., and Gorsky, R. Estimates of costs and effects of screening for colorectal cancer in the United States. Cancer (Phila.), *70:* 1288–1295, 1992.

4. Yang, C. S., and Wang, Z. Y. Tea and cancer: a review. J. Natl. Cancer Inst., *58:* 1038–1049, 1993.

5. Xu, Y., Ho, C. T., Amin, S. G., Han, C., and Chung, F. L. Inhibition of tobacco-specific nitrosamine induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. Cancer Res., *52:* 3875–3879, 1992.

6. Landau, J. M., and Yang, C. S. The effect of tea on health. Chem. Ind., *17:* 904–906, 1997.

7. Fujiki, H., Yoshizawa, S., Horiuchi, T., Suganuma, M., Yatsunami, J., Nishiwaki, S., Okabe, S., Nishiwaki, M. R., Okuda, T., and Sugimura, T. Anticarcinogenic effect of (-)-epigallocatechin gallate. Prev. Med., *21:* 503–509, 1992.

8. Yang, C. S., Chen, L., Lee, M-J., and Landau, J. M. Effects of tea on carcinogenesis in animal models and humans. *In:* (Edited under the auspices of the American Institute for Cancer Research), Dietary Phytochemicals in Cancer Prevention and Treatment, pp. 51–61. New York: Plenum Press, 1996.

9. Wang, Z. Y., Khan, W. A., Ather, M., Bickers, D. R., and Mukhtar, H. Protection against polycyclic aromatic hydrocarbons induced skin tumor initiation in mice by green tea polyphenols. Carcinogenesis (Lond.), *10:* 411–415, 1989.

10. Wang, Z. Y., Huang, M-T., Ferraro, T., Wong, C-Q., Lou, Y-R., Reuhl, K., Iatropoulos, M., Yang, C. S., and Conney, A. H. Inhibitory effect of green tea in the drinking water on tumorigenesis by ultraviolet light and 12-O-tetradecanoylphorbol-13-acetate in the skin of SKH-1 mice. Cancer Res., *52:* 1162–1170, 1992.

11. Katiyar, S. K., Agarwal, R., Wang, Z. Y., Bhatia, A. K., and Mukhtar, H. (-)-Epigallocatechin-3-gallate in *Camellia sinensis* leaves from Himalayan region of Sikkim: inhibitory effects against biochemical events and tumor initiation in Sencar mouse skin. Nutr. Cancer, *18:* 73–83, 1992.

12. Wang, Z. Y., Huang, M. T., Lou, Y. R., Xie, J. G., Reuhl, K. R., Newmark, H. L., Ho, C. T., Yang, C. S., and Conney, A. H. Inhibitory effects of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B

light-induced skin carcinogenesis in 7,12-dimethylbenz[α]anthracene-initiated SKH-1 mice. Cancer Res., *54:* 3428–3435, 1994.

13. Han, C., and Yong, X. The effect of Chinese tea on the occurrence of esophageal tumor induced by *N*-nitrosomethylbenzylamine in rats. Biomed. Environ. Sci., *3:* 35–42, 1990.

14. Wang, Z. Y., Wang, L. D., Lee, M. J., Ho, C. T., Huang, M. T., Conney, A. H., and Yang, C. S. Inhibition of *N*-nitrosomethylbenzylamine-induced esophageal tumorigenesis in rats by green and black tea. Carcinogenesis (Lond.), *16:* 2143–2148, 1995.

15. Wang, Z. Y., Hong, J-Y., Huang, M-T., Reuhl, K. R., Conney, A. H., and Yang, C. S. Inhibition of *N*-nitrosodiethylamine- and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced tumorigenesis in A/J mice by green tea and black tea. Cancer Res., *52:* 1943–1947, 1992.

16. Chung, F-L., Wang, M., Rivenson, A., Iatropoulos, M. J., Reinhardt, J. C., Pittman, B., Ho, C-T., and Amin, S. G. Inhibition of lung carcinogenesis by black tea in Fischer rat treated with a tobacco-specific carcinogen: caffeine as an important constituent. Cancer Res., *58:* 4096–4101, 1998.

17. Shi, S., Wang, Z. Y., Smith, T. J., Hong, J-Y., Chen, W-F., Ho, C-T., and Yang, C. S. Effects of green tea and black tea on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone bioactivation, DNA methylation, and lung tumorigenesis in A/J mice. Cancer Res., *54:* 4641–4647, 1994.

18. Fujita, Y., Yamane, T., Tanaka, M., Kuwata, K., Okuzumi, J., Takahashi, T., Fujiki, H., and Okuda, T. Inhibitory effect of (-)-epigallocatechin gallate on carcinogenesis with *N*-ethyl-*N*-nitrosoguanidine in mouse duodenum. Jpn. J. Cancer Res., *80:* 503–505, 1989.

19. Yamane, T., Takahashi, T., Kuwata, K., Oya, K., Inagake, M., Kitao, Y., Suganuma, M., and Fujiki, H. Inhibition of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-induced carcinogenesis by (-)-epigallocatechin gallate in rat glandular stomach. Cancer Res., *55:* 2081–2082, 1995.

20. Yamane, T., Hagiwara, N., Tateishi, M., Akachi, S., Kim, M., Okuzumi, J., Kitao, Y., Inagake, K., Kuwata, K., and Takahashi, T. Inhibition of azoxymethane-induced colon carcinogenesis in rat by green tea polyphenol fraction. Jpn. J. Cancer Res., *82:* 1336–1339, 1991.

21. Narisawa, T., and Fukaura, Y. A very low dose of green tea polyphenols in drinking water prevents *N*-methyl-*N*-nitrosourea-induced colon carcinogenesis in F344 rats. Jpn. J. Cancer Res., *84:* 1007–1009, 1993.

22. Pingzhang, Y., Jinying, Z., Shujun, C., Hara, Y., Qingfan, Z., and Zhengguo, L. Experimental studies of the inhibitory effects of green tea catechin on mice large intestinal cancers induced by 1,2-dimethylhydrazine. Cancer Lett., *79:* 33–38, 1994.

23. Weisburger, J. H., Rivenson, A., Garr, K., and Aliaga, C. Tea, or tea and milk, inhibit mammary gland and colon carcinogenesis in rats. Cancer Lett., *114:* 323–327, 1997.

24. Weisburger, J. H., Rivenson, A., Aliaga, C., Reinhardt, J., Kelloff, G. J., Boone, C. W., Steele, V. E., Balentine, D. A., Pittman, B., and Zang, E. Effect of black tea on azoxymethane-induced colon cancer. Carcinogenesis (Lond.), *19:* 229–232, 1998.

25. Blot, W. J., Chow, W. H., and McLaughlin, J. K. Tea and cancer—A review of the epidemiological evidence. Eur. J. Cancer Prev., *5:* 425–438, 1996.

26. Gao, Y. T., McLaughlin, J. K., Blot, W. J., Ji, B. T., Dai, Q., and Fraumeni, J. J. Reduced risk of esophageal cancer associated with green tea consumption. J. Natl. Cancer Inst., *86:* 855–858, 1994.

27. Ji, B. T., Chow, W. H., Hsing, A. W., McLaughlin, J. K., Dai, Q., Gao, Y. T., Blot, W. J., and Fraumeni, J. F. Green tea consumption and the risk of pancreatic and colorectal cancers. Int. J. Cancer, *70:* 255–258, 1997.

28. Roback, J., and Gryglewski, R. J. Flavonoids are scavengers of superoxide anions. Biochem. Pharmacol., *37:* 83–88, 1988.

29. Sorota, Y., Takahama, U., and Kimura, M. Protective effect of quercetin and rutin on photosensitized lysis of human erythrocytes in the presence of hematoporphyrin. Biochim. Biophys. Acta, *799:* 313–317, 1984.

30. Takahama, U. O2-dependent and independent photo-oxidation of quercetin in the presence and absence of riboflavin and effects of ascorbate on the photooxidation. Photochem. Photobiol., *41:* 149–152, 1985.

31. Huang, M. T., Ho, C. T., and Wang, Z. Y. Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin. Carcinogenesis (Lond.), *13:* 942–954, 1992.

32. Wang, Z. Y., Agarwal, R., Bickers, D. R., and Mukhtar, H. Protection against ultraviolet B radiation-induced photocarcinogenesis in hairless mice by green tea polyphenols. Carcinogenesis (Lond.), *12:* 1527–1530, 1991.

33. Yoshizawa, S., Horiuchi, T., and Sugimura, M. Penta-o-galloyl-β-D-glucose, and (-)-epigallocatechin gallate: cancer prevention agents. *In:* M. T. Huang, C. T. Ho, and C. Y. Lee (eds.), Phenolic Compounds in Foods and Health II: Antioxidants and Cancer Prevention, pp. 316–325. Washington, DC: American Chemical Society, 1992.

34. Katiyar, S. K., Agarwal, R., and Wood, G. S. Inhibition of 12-*O*-tetradecanoyl phorbol-13-acetate caused tumor promotion in 7,12-dimethylbenz(a)anthracene-initiated SENCAR mouse skin by a polyphenolic fraction isolated from green tea. Cancer Res., *52:* 6890–6897, 1992.

35. Agarwal, R., Katiyar, S. K., Zaidi, S. I., and Mukhtar, H. Inhibition of skin tumor promoter caused induction of epidermal ornithine decarboxylase in SENCAR mice by polyphenolic fraction isolated from green tea and its individual epicatechin derivatives. Cancer Res., *52:* 3582–3588, 1992.

36. Balentine, D. A. Manufacturing and chemistry of tea. *In:* C. T. Ho, M. T. Huang, and C. Y. Lee (eds.), Phenolic Compounds in Foods and Their Effects on Health I: Analysis, Occurrence, and Chemistry, pp. 102–117. Washington, DC: American Chemical Society, 1992.

37. Lee, M. J., Wang, Z. Y., Li, H., Chen, L. Sun, Y., Gobbo, S., Balentine, D. A., and Yang, C. S. Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol. Biomark. Prev., *4:* 393–399, 1995.

38. Wang, L. D., Shi, S. T., Zhou, Q., Goldstein, S., Hong, J. Y., Shao, P., Qiu, S. L., and Yang, C. S. Changes in p53 and cyclin D1 protein levels and cell proliferation in different stages of human esophageal and gastric-cardia carcinogenesis. Int. J. Cancer, *59:* 514–519, 1994.

39. Gavrieli, Y., Sherman, Y., and Ben-Sasson, S. A. Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. J. Cell Biol., *119:* 493–501, 1992.

40. McShane, L. M., Kulldorff, M., Wargovich, M. J., Woods, C., Purewal, M., Freedman, L. S., Corle, D. K., Burt, R. W., Mateski, D. J., Lawson, M., Lanza, E., O'Brien, B., Lake, W., Moler, J., and Schatzkin, A. An evaluation of rectal mucosal proliferation measure variability sources in the polyp prevention trial: can we defect informative differences among individuals' proliferation measures amid the noise? Cancer Epidemiol. Biomark. Prev., *7:* 605–612, 1998.

41. Morse, M. A., and Stoner, G. D. Cancer chemoprevention: principles and prospects. Carcinogenesis (Lond.), *14:* 1737–1746, 1993.

42. Sporn, M. B. Carcinogenesis and cancer: different perspectives on the same disease. Cancer Res., *51:* 6215–6218, 1991.

43. Stich, H. F. The beneficial and hazardous effects of simple phenolic compounds. Mutat. Res., *259:* 307–324, 1991.

44. Boone, C. W., Kelloff, G. J., and Malone, W. E. Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials (a review). Cancer Res., *50:* 2–9, 1990.

45. Einspahr, J. G., Alberts, D. S., Gapstur, S. M., Bostick, R. M., Emerson, S. S., and Gerner, E. W. Surrogate end-point biomarkers as measures of colon cancer risk and the use in cancer chemoprevention trials. Cancer Epidemiol. Biomark. Prev., *6:* 37–48, 1997.

46. Marra, G., Anti, M., Percesepe, A., Armelao, F., Ficarelli, R., Coco, C., Rinelli, A., Vecchio, F. M., and D'Arcangelo, E. Circadian variations of epithelial cell proliferation in human rectal crypts. Gastroenterology, *106:* 982–987, 1994.

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