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Subject: Comments on Epigallocatechin-3-gallate (ACS 989-51-5) nominated by the ICCEC on May 8, 2001

Dear Dr. Masten:

Based on current knowledge of the properties of the epigallocatechin-3-gallate, EGCG, I firmly believe that this naturally occurring chemical should not undergo any of the tests proposed.

One third of the dry weight of green tea is EGCG. Green tea has been consumed safely without adverse effects by billions of people world wide. Biomedical research shows that green tea and EGCG actually inhibit human cancer formation. For example, an explanation for the lower lung cancer rate in Japan despite a higher smoking rate is that the Japanese consume appreciable amount of green tea a day of the order of 5-10 cups. In addition, there has been extensive laboratory research on the mechanisms of action of EGCG and of green tea in lowering the risk of cancer in animal models, those caused by tobacco, or others caused by genotoxic carcinogens, such as the heterocyclic amines formed during cooking. In fact, I have shown that green tea and EGCG prevents the formation of heterocyclic amines in appropriate, realistic model.systems.

Furthermore, under an NCI contract N01-CN-35569, we performed studies on tea, tea polyphenols and specifically EGCG in a model of colon cancer. We did perform preliminary toxicology and we found that we could administer 9000 ppm green tea without toxicity, or 4500 ppm polyphenols without toxicity. I am attaching the relevant publication (Proc. Soc. Exptl. Biol. Med. 217:104-108, 1998).

In addition, we found that EGCG inhibited the mutagenicity of genotoxic carcinogens. The control EGCG, of course, displayed no mutagenicity. In addition, in the DNA repair bioassay in hepatocytes, in which genotoxic carcinogens are powerfully active, tea polyphenols inhibited the effect.

Tea has been the subject of review in IARC monograph number 51, 1991.

Thus, it is my considered opinion, based on my extensive knowledge in the field of carcinogenesis and chemopreventive mechanisms, especially by tea and the tea polyphenols, that it would be an improper use of the NTP resources to perform any tests bearing on the safety of EGCG . There is an enormous literature that EGCG is safe.

By the way, with my colleagues, Dr. Michael Shimkin and Dr. Elizabeth Weisburger, I developed the NCI Bioassay Program beginning in 1961. It is my group that selected the F344 rat and the B6C3F1 hybrid mouse for *in vivo* bioassays. Beginning in 1968, we conducted workshops, chaired by Dr. Alexander Hollander of Oak Ridge, on *in vitro* approaches to carcinogen bioassay and safety testing. When I came here in 1972, these programs were transferred from NCI to NIEHS and now constitute the NTP.

I should be glad to work with you in the future in reviewing the suitability to test chemicals or products nominated to your activities at the NTP.

With all good wishes,

John H. Weisburger, PhD, MD(hon)
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JHW/nr
Attachment

Effect of Tea Extracts, Polyphenols, and Epigallocatechin Gallate on Azoxymethane-induced Colon Cancer (44211)

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Abstract. **Studies were conducted to determine the chemopreventive efficacy of several types of tea extracts on azoxymethane-induced colon cancer in male F344 rats. After determining the maximally tolerated dosage of the tea products, their effect in a colon cancer model was investigated. Groups of 36 male F344 rats received 2 subcutaneous doses of 15 mg/kg azoxymethane (AOM) at Weeks 6 and 7. Experimental groups also received as drinking fluids 3600 ppm of black or green tea extracts, 1800 ppm of EGCG, or 1800 ppm of black or green tea polyphenols beginning at 5 weeks of age. Additional groups drank a lower dose of 360 ppm of the five tea products. The experiments were terminated 43 weeks after the first tea exposure. No evidence of toxicity was observed since the body weight gain of all groups was similar. The rats given AOM had carcinoma of the small intestine and of the colon, classified histologically as *in situ* carcinoma, exophytic, invasive, and Peyer's patch carcinoma. In the small intestine, most of the neoplasms were classified as invasive, but in the colon, most were exophytic. The various tea products failed to produce a significant difference in the incidence of the several types of colon and small intestine carcinoma. The multiplicity of colon cancers ranged from 1.2–2.8 in all groups. The group on 3600 ppm of green tea had a significantly higher tumor multiplicity than the control group on AOM and water. Also, the group on 3600 ppm of green tea had a significantly higher tumor multiplicity than the group on 360 ppm. The tea products did not affect the development aspects of the tumors in most groups. The mechanisms underlying these findings rest on the fact that azoxymethane is metabolized mainly by cytochrome P450 2E1, and this enzyme system is not affected by tea.**

[P.S.E.B.M. 1998, Vol 217]

The leaves of the tea plant, *Camellia sinensis*, have been used for several thousand years for the preparation of beverages used by mankind. Originally discovered in China, the leaves contain specific polyphenols and the enzyme polyphenol oxidase. When the leaves were processed by partial drying, immediate heating, and maceration, the polyphenol oxidase was inactivated and under these conditions, the polyphenols in tea remained in their native state, typical of green tea. The main polyphenol in green tea is epigallocatechin gallate (EGCG). A partial oxidation, for about 30 min, of the polyphenols by the polyphenol oxidase yields oolong tea. More complex tea polyphenols, theaflavin gallates, and polymeric thearubigins, typical of black tea, arise when the oxidation is allowed to operate for about 2 hr (1, 2).

In recent years, research has begun on the possible health-promoting effects of tea. The initial impetus for such activities was the epidemiological observation that the customary intake of tea by populations may lead to a lower incidence of several types of cancer and of coronary heart disease. Data were obtained in the Orient where the cus-

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tomary tea is green tea, and in Europe or the Near East, where the usual tea consumed is black tea (3–6).

These epidemiological observations were the basis for detailed laboratory research in animal models for several types of cancer including cancer of the skin induced by UV light or cancer of the lung, esophagus, and mammary gland, using appropriate carcinogens (3, 7).

Cancer of the colon is an important type of neoplasm in the Western world and of increasing importance in Japan, as the dietary traditions in that country become Westernized (8). An early model for cancer of the colon in rats was induction with 2',3-dimethyl-4-aminobiphenyl that was extensively used to study the mode of action and its enhancement or inhibition by various dietary and other environmental components (9). The application of that model decreased after Druckrey (10) discovered in the late 1960s an inexpensive and readily available colon-specific chemical carcinogen, 1,2-dimethylhydrazine, and its metabolite, azoxymethane (AOM). AOM served as the carcinogen for numerous investigations on its mechanism of action, as well as the modification of its effect by diet, drugs, and other chemicals (8, 9). This carcinogen was, therefore, used in the current effort to determine whether green tea or black tea extracts and the corresponding tea polyphenols would modify colon cancer induction by AOM.

Materials and Methods

Chemicals. Azoxymethane was purchased from Ash Stevens, Inc., Detroit, MI. The tea extracts, tea polyphenols, and EGCG were prepared under standardized conditions by the research laboratories of Thomas J. Lipton, Inc., Englewood Cliffs, NJ. Each batch obtained over the course of these studies was analyzed by the Lipton staff and by McKesson Bioservices (Rockville, MD) the repository for test chemicals for the National Cancer Institute. All solutions were prepared with deionized water, purified with a Millipore Milli-Q Water System.

Stability studies show that solutions of all tea products were stable at room temperature for at least 2 days (data not shown). Therefore, fresh solutions were made three times a week for administration to the experimental animals.

Preliminary Toxicology. Preliminary toxicological studies were performed utilizing groups of six male F344 rats, 6 weeks of age, that received solutions in drinking water containing 9000, 4500, 2250, 1125, and 663 ppm of the black and green tea extracts, or 4500, 2250, 1125, 663, and 332 ppm of black and green tea polyphenols, and EGCG. A control group of 12 rats received the deionized water. The weight gain of all rats was followed over a 7-week period. The data showed that even the highest dose level of each product gave little change in the rate of body weight gain, albeit in the groups on the highest level, 9000 ppm of the black or green tea extract, or 4500 ppm of EGCG, but not on the highest level of the black and green tea polyphenols, the mean weights were 275 grams, compared to 295 grams of the rats that drank water. Based on this information, as well as the report of Narisawa and Fukaura (12), showing that a similar, good inhibition of colon cancer, induced by intra-rectal infusion of MNNG, was obtained over a range of dosages of green tea, including the lowest level, it was decided to use 3600 ppm of the black and green tea extract, and 1800 ppm of the black and green tea polyphenols, and of EGCG, as the high dose level. The low dose level selected for all tea products was 360 ppm.

Treatment of Rats. Male F344/N TacBR rats were procured at age 4 weeks from Taconic Germantown, NY. They were maintained for 2 weeks in the quarantine unit of the Research Animal Facility of this Institute. During that time, eight rats were sacrificed and analyzed for the absence of viral, bacteriological, and pathological contaminants as indication of excellent health. Upon certification of health status, the rats at the end of that period were distributed, using a table of random numbers, into experimental and control groups. The experimental groups each contained 36 rats, and the control groups contained 12 rats. All of the experimental groups were placed on the high- and low-dose levels of black and green tea extracts, EGCG, and black and green tea polyphenol at age 6 weeks. The administration of the tea products continued for the duration of the experiment, namely 43 weeks. Each experimental rat received two doses of 15 mg/kg body weight of AOM in 1 ml/kg saline solution by subcutaneous injection at Week 7 and Week 8. The control groups received the subcutaneous injection of normal saline solution twice at 7 and 8 weeks of age. They also received the high dose levels, 3600 ppm, of the black and green tea extracts only, or 1800 ppm of EGCG and black and green tea polyphenols, or water. All rats were fed a modified pelleted AIN-76 semi-purified diet, No. 112,174 (Dyets, Inc., Bethlehem, PA). The drinking fluids were available *ad libitum*. Fluid intake was measured over two 3-day periods during Weeks 10 and 11. The groups drinking water consumed 17–18 ml, and the groups on tea products consumed 15–16 ml, but groups on 3600 ppm black or green tea took in 21–23 ml/day.

All rats were weighed every 2 weeks for the first 10 weeks, and monthly thereafter. They were housed in groups of two per cage and examined carefully for any adverse effects or tumor development. If an animal appeared weak, it was isolated and observed twice per day. If survival seemed threatened, the rat was sacrificed by CO_2 euthanasia. All rats were sacrificed in this manner 42 weeks after the first dose of AOM, and each rat underwent careful necropsy. All grossly abnormal tissues were harvested, washed with saline, and fixed in 10% buffered formalin. The intestinal tract, from cecum to anus, was obtained and rinsed with saline to eliminate intestinal contents and feces. The tract was mounted on a corkboard, sectioned longitudinally, and examined for the presence of grossly visible abnormalities, including tumors. Any tumors present were measured with calipers in three dimensions. After fixation, tissues

were prepared for histopathological study by preparation of blocks and sections that were usually stained with hematoxylin and eosin (H&E). All sections were studied by A. Rivenson, MD, board-certified in pathology.

Statistical Evaluation. The gross and pathologically reviewed results were tabulated, and differences between groups and the control reference group were evaluated statistically by the chi-square test. For multiple comparisons, the Dunn-Sidak (13) correction of a Type I error rate was applied. Tumor multiplicity was evaluated with a single classification analysis of variance (ANOVA), and by Tukey's adjustment (14) for multiple comparisons.

Results

Preliminary Toxicology. During the 7-week, multiple-dose administration of the black and green tea extracts, the highest dosage, 9000 ppm, yielded a slightly lower but not significant decreased weight gain. This was also found for the highest level, 4500 ppm of EGCG, but not for the green and black tea polyphenols, given at 4500 ppm.

Chronic Study. Based on an interpretation of the results obtained in the preliminary toxicology of the tea products, it was decided to use dosages of 3600 ppm as the high dose level for the black and green tea extracts and 1800 ppm as the high dose levels for the black and green tea polyphenols as well as EGCG. The low dose level selected for all five tea products was 360 ppm. This dose selection appeared appropriate since the body weight gain of all groups of rats followed a similar trend over the entire experimental period of 42 weeks. The control groups not given AOM had no tumors during these tests.

Rats treated with AOM had some cancers in the small intestine (not tabulated), but most neoplasms were found in the colon, particularly in the descending colon (Table I). The lesions were classified as *in situ*, exophytic, invasive, and Peyer's patch carcinomas. At the level of the small intestine, most of the cancers were of the invasive type, whereas in the colon, the majority of neoplasms were of the exophytic type. Irrespective of the type and amount of tea product consumed as drinking fluid, there was no significant difference in the occurrence of the types of colon and small intestinal cancer, compared to those found in animals drinking water (Table I).

Tumor multiplicity provides another dimension to explore modifying effects in colon carcinogenesis. Little difference in tumor multiplicity was evident, except there seemed to be an increased multiplicity of exophytic colon carcinoma in the group on the high dose of green tea, which in turn was statistically different from the multiplicity at the low dose of the green tea extract (Table II). If the data are restricted to multiplicity to tumor-bearing animals only, the rats at the high dosage of black tea and green tea extract displayed more exophytic colon carcinoma, compared to the animals drinking the corresponding low dosages of the tea extracts.

Differential tumor volumes can be an indication of specific distinct effects. A statistical difference, with a higher tumor volume, was observed in the rats on the high dose level versus the low dose level of green tea (data not shown). When adjusted for the number of comparisons made, and applying the Dunn-Sidak correction, this difference was not significant. The tumor volume was somewhat lower with the low dosage of the tea products compared to the high dosage. These findings may parallel, in part, the results obtained by Narisawa and Fukaura (12) who seemed to find the best protection against MNU-induced

Table I. Effect of Tea Products on AOM-induced Colon Cancer

Group	Treatment ppm	Tumor incidence[a] N (%)			
		Colon			
		In Situ	Exophytic	Invasive	Peyer's Patch
1.	Water	1 (2.8)	25 (69)	4 (11)	4 (11)
2.	BL, 3600	4 (11)	25 (69)	5 (14)	4 (11)
3.	BL, 360	4 (11)	30 (83)	6 (17)	3 (8.3)
4.	GR, 3600	5 (14)	32 (89)	5 (14)	4 (11)
5.	GR, 360	1 (2.8)	25 (69)	0 (0.0)	2 (5.6)
6.	EGCG, 1800	4 (11)	23 (64)	5 (14)	7 (19)
7.	EGCG, 360	3 (8.3)	26 (72)	3 (8.3)	4 (11)
8.	BL Poly, 1800	5 (14)	24 (67)	3 (8.3)	2 (5.6)
9.	BL Poly, 360	2 (5.6)	25 (69)	2 (5.6)	5 (14)
10.	GR Poly, 3600	3 (8.3)	24 (67)	4 (11)	4 (11)
11.	GR Poly, 360	2 (5.6)	22 (61)	4 (11)	7 (19)

[a] All groups of rats were injected twice subcutaneously at Weeks 6 and 7 with 15 mg/kg AOM. BL, black tea extract, GR, green tea extract, EGCG, epigallocatechin gallate, BL poly, black tea polyphenol fraction, and GR, green tea polyphenol fraction; concentrations as ppm in deionized water, freshly prepared 3 times per week. Data show number (N) of rats with tumor and percent in groups of 36. AOM also induced neoplasms in the small intestine in low incidence (data not shown). In contrast to colon, where the tumors were mostly of the exophytic type, in the small intestine, they were more of the invasive type.

Table II. Multiplicity of AOM-induced Tumors Among All Animals

Group	Treatment ppm	Colon							
		In Situ		Exophytic		Invasive		Peyer's Patch	
		X	SD	X	SD	X	SD	X	SD
1	Water	0.03	0.17	1.50	1.38	0.11	0.32	0.11	0.30
2	BL, 3600	0.11	0.32	2.06	2.12	0.17	0.45	0.11	0.32
3	BL, 360	0.11	0.32	1.61	1.10	0.17	0.38	0.08	0.28
4	GR, 3600	0.19	0.52	2.78	1.99	0.17	0.45	0.14	0.42
5	GR, 360	0.06	0.33	1.28	1.16	0.00	0.00	0.08	0.37
6	EGCG, 1800	0.14	0.42	1.39	1.38	0.19	0.58	0.22	0.48
7	EGCG, 360	0.08	0.28	1.25	1.13	0.11	0.40	0.11	0.32
8	BL Poly, 1800	0.14	0.35	1.19	1.12	0.08	0.28	0.06	0.23
9	BL Poly, 360	0.06	0.23	1.36	1.31	0.06	0.23	0.19	0.58
10	GR Poly, 3600	0.14	0.49	1.50	1.54	0.11	0.32	0.17	0.51
11	GR Poly, 360	0.06	0.23	1.22	1.38	0.11	0.32	0.22	0.48

Statistical Analysis: X is mean, SD is standard deviation. Groups 2, 4, 6, 8, and 10 were compared to the control Group 1 using a one-way analysis of variance (ANOVA) followed by Dunnett's procedure (13, 14) for comparing several treatment groups to a control. The comparisons 2 vs. 3, 4 vs. 5, 6 vs. 7, 8 vs. 9, and 10 vs. 11 were made using a t-test. The analysis showed exophytic colon carcinoma; Group 1 vs. 4 was significant at $P < 0.01$ and Group 4 vs. 5 was significant at $P < 0.01$. All other differences were not significant.

colon cancer in the groups of rats drinking the lower dosage of tea.

Discussion

In parallel studies on the effect of tea at the standard dose of 1.25% as consumed by humans, or over a range of tea dosages, it was found that the carcinogenicity of AOM in the colon was not affected significantly (15). The current study demonstrates that the effects of black tea are now also true for green tea, for the corresponding black and green tea polyphenol concentrates, and even for pure epigallocatechin gallate, the major green tea polyphenol. It would seem that the underlying mechanism for the failure of tea product intake to affect the carcinogenicity of AOM to the colon rests on the inability of tea to block the metabolic activation of AOM. Indeed, Sohn *et al.* (16) found that AOM undergoes metabolism through the influence of cytochrome P450 2E1 in the liver, yielding methylazoxymethanol (MAM), which undergoes terminal activation to a reactive alkylating agent, the methyl carbonium ion in the colon itself. The limiting event is the conversion of AOM to MAM in the liver with the specific cytochrome isozyme noted.

In parallel studies, we have discovered that black or green tea sharply alters a number of the cytochrome P450 isozymes, specifically the CYP450 1A1, 1A2, and 2B1 (17). Significantly, the key cytochrome enzyme required for the metabolism of AOM, namely P450 2E1, is not at all affected by tea, which accounts for these findings (17). Based on this knowledge, it can be predicted that carcinogens, metabolized and detoxified by both the cytochromes noted above, which were induced by tea, and glucuronyltransferase, which was also found to be induced, would be affected. Xu *et al.* (18) reported recently that black tea and green tea both sharply lowered the carcinogenicity of the food mutagen and carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline, in agreement with this hypothesis. Indeed, this carcinogen is metabolized by CYP450 1A1, 1A2, and the phase II enzyme glucuronyltransferase. Therefore, it would seem that failure of tea products to modify colon carcinogenesis with the classic carcinogen, AOM, stems from the fact that tea does not affect the biochemical systems required for AOM metabolism.

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