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A Very Low Dose of Green Tea Polyphenols in Drinking Water Prevents N-Methyl-N-nitrosourea-induced Colon Carcinogenesis in F344 Rats

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The effect of tea polyphenols, major constituents of tea, on colon carcinogenesis was investigated. A total of 129 female F344 rats were given an intrarectal instillation of 2 mg of N-methyl-N-nitrosourea 3 times a week for 2 weeks, and received a water solution of green tea extract (GTE) as drinking water throughout the experiment. Autopsies at week 35 revealed significantly lower incidence of colon carcinomas in rats ingesting 0.05%, 0.01% or 0.002% GTE solution than in controls ingesting 0% GTE solution: 43%, 40% and 33% vs. 67%. The data suggest that GTE, even at a very low dose (0.002% solution), has a potent inhibitory effect on colon carcinogenesis.

Key words: Colon cancer — Colon carcinogenesis — Tea polyphenol — Green tea — Cancer chemoprevention

Tea is the most widely consumed beverage in the world, and green tea is favored in Japan. Some epidemiologic studies on cancer incidence have suggested an inhibitory effect of green tea on stomach cancer and colorectal cancer.[1,2] In addition, a green tea extract, of which the main constituents are tea polyphenols, given in drinking water inhibited the development of azoxymethane-induced colon cancer in rats.[3] Tea solution also has antitumorigenic activity in other organs such as the skin, lung, forestomach, and esophagus in rats and mice.[4–7]

In the present study we have demonstrated that even a small dose of green tea extract in drinking water has an inhibitory effect on colon cancer development induced with a different carcinogen, N-methyl-N-nitrosourea (MNU), in rats. The powdered solids of green tea extract were obtained from Dr. Douglas A. Balentine, U.S. Tea Association, Englewood Cliffs, NJ. Its constituents (% of solids) were gallic acid, 0.2%; theobromine, 0.1%; caffeine, 6.7%; (−)-epigallocatechin, 10.3%; (+)-catechin, 0.5%; (−)-epigallocatechin gallate, 11.2%; (−)-epicatechin, 2.4%; (−)-epicatechin gallate, 2.2%. The total catechins were 26.6%.

A total of 129 female F344/NSlc rats (Shizuoka Laboratory Animal Center, Hamamatsu), 7 weeks of age at the start of experiment, were housed, 5 rats per cage, in plastic cages with sterilized wood-chip bedding in a specific-pathogen-free animal room under constant conditions (12-h light-dark cycle, a temperature of 22°C, a relative humidity of 50%). They had free access to standard laboratory chow CE-2 (CLEA Japan, Tokyo) and drinking water. All animals received an intrarectal instillation of 0.5 ml of 0.4% MNU (Nacalai Tesque, Kyoto) aqueous solution 3 times a week at weeks 1 and 2 by the procedure described previously.[8] Thirty rats each in 3 experimental groups were given tap water solutions of green tea extract at a concentration of 0.05%, 0.01% or 0.002% (w/v) (hGTE, mGTE and lGTE groups, respectively) as drinking water during the entire period of the experiment from week 1. The control group of rats had plain tap water. The drinking water was changed every other day, and the consumed volume was recorded. Body weight was measured once a week. The experiment was terminated at week 35, when all the rats were autopsied and inspected grossly. The colon was excised and cut open longitudinally. The location, size and shape of tumors were recorded. They were fixed with 4% formaldehyde solution and processed for histological examination.

The significance of differences among data from the control and the experimental groups was tested by using the χ^2 test and Student's t test. Differences were considered statistically significant when the P value was 0.05 or less.

The body weight gain and the volume of drinking water consumed were comparable in all groups of rats. The mean body weight was 110 g at the start of experiment and 231 g at the termination. The mean volume of drinking water was 16–18 ml/rat/day during the first 5 weeks and 13–15 ml/rat/day thereafter. At week 35, the incidence of colon tumor development was significantly lower in the 3 experimental groups, hGTE, mGTE and lGTE groups, than in the control group (Table I). The mean number of tumors per rat was smaller in the experimental groups than in the control group, and the difference was significant in the mGTE and lGTE groups.

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Table I. MNU-induced Colon Tumors in F344 Rats Ingesting Green Tea Extract (GTE)

Groups[a]	No. of rats examined	No. of rats with tumors	No. of tumors per rat	No. of tumors per tumor-bearing rat
Control	39	26 (67%)	1.2±0.2[b]	1.8±0.2[b]
hGTE	30	13 (43%)[c]	0.8±0.2	1.8±0.3
mGTE	30	12 (40%)[c]	0.5±0.1[c]	1.2±0.1[c]
lGTE	30	10 (33%)[c]	0.5±0.2[c]	1.5±0.4

a) All rats were given an intrarectal dose of 2 mg of MNU 3 times a week for 2 weeks, and received either 0% (control group), 0.05% (hGTE group), 0.01% (mGTE group) or 0.002% (lGTE group) water solution of GTE as drinking water throughout the experiment. The experiment was terminated at week 35.
b) Mean±SEM.
c) Significantly different from the control group: $P<0.05$ or less.

Also, the mean number of tumors per tumor-bearing rat was smaller in the mGTE and lGTE groups, though the difference was only significant in the mGTE group. The data clearly demonstrate the inhibitory effect of green tea extract in drinking water on MNU-induced colon carcinogenesis. Interestingly, the very low dose of green tea extract (0.002%) showed the same or greater potency as compared with the other doses.

The tumor-bearing rats had 1 to 5 tumors, and all the tumors were located in the distal half of the colon from the anus to 13 cm proximal to the anus. They were plaque-shaped or polypoid, and most of them were from 1 to 6 mm in diameter. Histologically, the tumors were well-differentiated adenocarcinomas except for 2 poorly differentiated adenocarcinomas. They mostly extended within the mucosa and submucosa, but 3 extended into the proper muscle to the serosa with metastases to the lymph nodes, peritoneum and lungs. The macroscopic and microscopic findings of the tumors were not different among the groups. No other tumors were observed in the gastrointestinal tract or other organs.

The results of the present investigation are consistent with a previous report, in which green tea extract in drinking water at a concentration of 0.1% or 0.01% affected promotion of colon carcinogenesis induced with azoxymethane in rats.[3] Topical application of (−)-epigallocatechin gallate, a main constituent of tea polyphenols in green tea extract, inhibited teleocidin-induced tumor promotion on mouse skin initiated with 7,12-dimethylbenz[*a*]anthracene.[9] A recent study demonstrated that green tea extract inhibited protein kinase C activation *in vitro*, as did (−)-epigallocatechin gallate, and it was hypothesized that the polyphenolic compounds have a sealing effect on the receptors within the cell membrane, resulting in blocking of the interaction of tumor promoters, hormones and growth factors.[10]

In the present study, green tea extract was given throughout the entire period of the experiment as a model of the human situation. Thus, green tea extract could be expected to affect not only the promotion phase but also the initiation phase of carcinogenesis, since green tea extract had an inhibitory effect on chemically induced tumorigenesis in the skin, lungs, forestomach and esophagus in rats and mice which received green tea extract over the period of carcinogen administration.[4–7] Furthermore, green tea extract and its main constituents, tea polyphenols, have potent antioxidant properties and inhibit the mutagenic activity of tryptophan pyrolysates, Trp-p-1 and -2, and of benz[*a*]pyrene-diol epoxide in the Ames test.[11, 12]

It is of special interest that the lower the concentration of green tea extract in drinking water, the smaller the number of colon tumors found in the present study. In fact, 0.05% green tea extract solution is green-yellow in color, like regular green tea, but 0.002% green tea extract solution is colorless like tap water. Thus, a test with an even lower dose would be of interest. Most polyphenols in green tea extract escape digestion in the intestine, and 80% of the amount is excreted in the feces.[12] It would appear that intraluminal polyphenols above a minimum effective dosage may act directly on the colonic mucosa and show antitumorigenic activity. This could be the reason why a very low dose of green tea extract given orally was effective against colon tumor development.

It is very interesting to note that daily consumption of hot green tea was found to be associated with a decreased risk of adenomatous polyps of the colorectum in one report,[2] and the risk of adenomatous polyps of the sigmoid colon decreased somewhat with increasing amount of green tea consumption.[13] However, another study failed to show any difference in the risk of colorectal cancer between two groups with high and low consumption of green tea.[14] Our present finding that a very low dose of green tea extract appears to have considerable activity for the prevention of colon carcinogenesis points up the need for more extensive epidemio-

logic studies on humans and further experimental investigations on animal models, including dose-response tests and examination of the antitumorigenic mechanisms of green tea extract.

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