Green Tea Extracts for the Prevention of Metachronous Colorectal Adenomas: A Pilot Study

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Abstract

Background: **Experimental studies indicate the chemopreventive properties of green tea extract (GTE) on colorectal cancer. Epidemiologically, green tea consumption of >10 cups daily reduced colorectal cancer risk in Japanese. Because colorectal adenomas are the precursors to most sporadic colorectal cancers, we conducted a randomized trial to determine the preventive effect of GTE supplements on metachronous colorectal adenomas by raising green tea consumption in the target population from an average of 6 cups (1.5 g GTE) daily to ≥10 cups equivalent (2.5 g GTE) by supplemental GTE tablets.**

Methods: **We recruited 136 patients, removed their colorectal adenomas by endoscopic polypectomy, and 1 year later confirmed the clean colon (i.e., no polyp) at the second colonoscopy. The patients were then randomized into two groups while maintaining their lifestyle on green tea drinking: 71 patients supplemented with 1.5 g GTE per day for 12 months and 65 control patients without supplementation. Follow-up colonoscopy was conducted 12 months later in 125 patients (65 in the control group and 60 in the GTE group).**

Results: **The incidence of metachronous adenomas at the end-point colonoscopy was 31% (20 of 65) in the control group and 15% (9 of 60) in the GTE group (relative risk, 0.49; 95% confidence interval, 0.24-0.99; $P < 0.05$). The size of relapsed adenomas was also smaller in the GTE group than in the control group ($P < 0.001$). No serious adverse events occurred in the GTE group.**

Conclusion: **GTE is an effective supplement for the chemoprevention of metachronous colorectal adenomas.** (Cancer Epidemiol Biomarkers Prev 2008;17(11):3020–5)

Introduction

Colorectal cancer is a major health care problem worldwide due to its substantial morbidity and mortality. Therefore, the early detection and prevention through screening and other strategies, such as chemoprevention, have recently received more attention with regard to comprehensive colorectal cancer management. It is generally accepted that most colorectal cancers evolve from adenomatous polyps, and the removal of these lesions has been shown to reduce the risk for future colorectal cancer (1, 2). Several lines of evidence support the use of colorectal adenomas as clinical end points for colorectal cancer chemoprevention trials (3). Among the several agents under evaluation, aspirin and cyclooxygenase (COX)-2 inhibitors seem to have a beneficial effect to prevent the development and/or recurrence of adenomas (4-7). However, the daily use of these agents needs careful follow-up to evaluate any side effects or adverse effects such as gastrointestinal hemorrhage and cardiovascular toxicity (8).

Epidemiologic and preclinical evidence suggests that certain types of polyphenolic phytochemicals possess cancer chemopreventive properties and thus might be useful for clinical chemoprevention trials (9, 10). Among these phytochemicals, green tea catechins have been shown to inhibit carcinogenesis or the growth of chemically induced cancers in various tissues, including the colorectum (11). Our previous studies showed that (−)-epigallocatechin gallate (EGCG), one of the major constituents of green tea, inhibits cell growth and induces apoptosis in human colorectal cancer cells by inhibiting the expression of COX-2 and the activation of epidermal growth factor receptor family of receptor tyrosine kinases (12, 13). EGCG can also inhibit the activation of insulin-like growth factor-I receptor, which belongs to a separate family of receptor tyrosine kinases in human colorectal cancer cells (14). These findings are important because an increase in the COX-2 expression and an abnormality in the receptor tyrosine kinase activity have been shown to play significant roles in colorectal cancer development, and these factors might thus be one of the critical targets for colorectal cancer chemoprevention and/or treatment (15, 16). These findings also suggest that green tea catechins may inhibit the development and/or recurrence of colonic adenomas and, as a result, they may be beneficial for suppressing

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subsequent cancer development in the colorectum in humans.

Previous epidemiologic studies suggested that there might be an effective dose of green tea catechins per day for cancer prevention. Prospective cohort studies conducted in Saitama, a tea-producing and tea-consuming area in eastern Japan, indicated the delay of cancer onsets in a population taking ≥10 Japanese-size cups (120 mL/cup) of green tea per day (equivalent to ≥2.5 g green tea extract), whereas the average consumption was 6 cups (17). To supplement this gap, green tea extract (GTE) tablets were prepared by the Green Tea Local Products Laboratory, Saitama Prefecture, Agriculture and Forestry Research Center, Saitama, Japan (18). To test a hypothesis if supplementation with GTE inhibits the colorectal cancer risk, we conducted a randomized trial of GTE tablets for the prevention of metachronous colorectal adenomas in subjects of a high-risk group (i.e., those with post-polypectomy state of their preceding colorectal adenomas).

Patients, Materials, and Methods

Materials. The GTE tablets were kindly provided by the Green Tea Union of Saitama (Iruma Kumiai Seicha), Saitama, Japan. One tablet of GTE (500 mg) contained 52.5 mg EGCG, 12.3 mg (−)-epicatechin, 34.6 mg (−)-epigallocatechin, 11.1 mg (−)-epicatechin gallate, and 15.7 mg caffeine. One tablet of GTE is approximately equivalent to 2 Japanese-size cups of green tea.

Study Design. The present study was conducted as a randomized trial to investigate whether GTE supplementation inhibits the metachronous adenomas after total endoscopic resection of preceding colorectal adenomas because such mucosa provides a field with a higher risk for the development of second primary adenomas (19). We evaluated the antitumor efficacy and safety of 1.5 g GTE (3 tablets) per day. The eligibility criteria were ages between 20 and 80 y and good health except colorectal polyps. The exclusion criteria were invasive colorectal cancers and any condition that required treatment with aspirin or other nonsteroidal anti-inflammatory drugs. All patients provided written informed consent before enrollment. The participants answered the questionnaire about their habitual tea consumption (including green tea, black tea, and oolong tea). Our supplementation strategy was to raise green tea consumption by giving 3 GTE tablets daily, from a baseline average of 6 cups estimated by questionnaire survey to ≥10 cups, because the latter level of green tea consumption has been shown to reduce colorectal cancer risk in a preceding cohort study (17).

Initially, the required sample size was calculated as 60 patients in each group, based on the incidence of metachronous adenomas (19) and estimated risk reduction by GTE as 0.5 (11). We actually recruited 136 participants who underwent endoscopic resection of one or more colorectal adenomas. Twelve months later, they received total colonoscopy again to confirm that no endoscopically detectable polyp remained (''clean colon''). The participants were then randomized into two groups: the GTE group was given 3 GTE tablets per day for 12 mo and the control group received no supplement. Otherwise, they kept their lifestyle on green tea drinking. We did not use placebo because green tea is a daily beverage of Japanese, and we aimed to raise the consumption to a target level. Thus, the evidence level of this study sits in the grade 2 of Agency for Health Care Policy and Research classification.[7]

The patients were instructed to visit their outpatient clinic every 3 mo and were checked by the study physicians for any subjective changes during the course of the given treatment. At 12 mo after beginning the GTE supplements, follow-up colonoscopy was done to observe the occurrence of any new adenomas. The endoscopists were not informed on which group the participants belonged to in the trial to rule out any bias in the evaluation. This trial involved four clinical centers (Gifu University Hospital, Gifu Municipal Hospital, Gifu Prefectural General Medical Center, and Gihoku Kousei Hospital), and the ethical committee at each center approved the study protocol.

Statistical Analysis. All statistical analyses were done using the SAS Release 6.12 or 8.1 software (SAS Institute, Inc.).

Results

The composition of participants is shown in Fig. 1. A total of 136 patients were initially enrolled and randomized following the second colonoscopy. There was no difference in tea consumption between the two groups (mean of 6.6 Japanese-size cups of green tea per day in both groups). The assigned treatment was initiated in 133 patients (68 in the GTE and 65 in the control groups), whereas 3 patients in the GTE group were excluded as shown in Fig. 1. Eight in the GTE group were lost before the end-point colonoscopy. Thus, the data from a total of 125 patients (60 in the GTE group and 65 in the control group) were subjected to statistical analysis. The baseline characteristics of the patients are summarized in Table 1. No significant difference was noted between the GTE and control groups for sex or age. There was also no significant difference in the number or the size of removed adenomas between the two groups.

The preventive effects of 12-month supplementation with GTE on metachronous adenomas are summarized in Table 2. At end-point colonoscopy, at least one colorectal adenoma was diagnosed in 31% (20 of 65) of the patients in the control group. In contrast, the supplementation with 1.5 g GTE significantly prevented metachronous adenomas to 15% (9 of 60 patients; relative risk, 0.49; 95% confidence interval, 0.24-0.99; $P < 0.05$). GTE also inhibited the size of adenomas (Table 2).

Regarding the baseline clinicopathologic characteristics, we found that the inhibitory effect of GTE tablets depended significantly on the daily green tea drinking (number of cups per day; Table 3). Supplemental GTE reduced the recurrence rate to 13% (6 of 47) in the subjects with 4 to 9 cups of tea intake, whereas the rate was still 60% (3 of 5) in those who drink ≤3 cups of green tea per day. Stratification with other characteristics such as number, size, and histologic grade of initial adenomas did not produce significant result (data not shown),

[7] http://www.ahrq.gov/

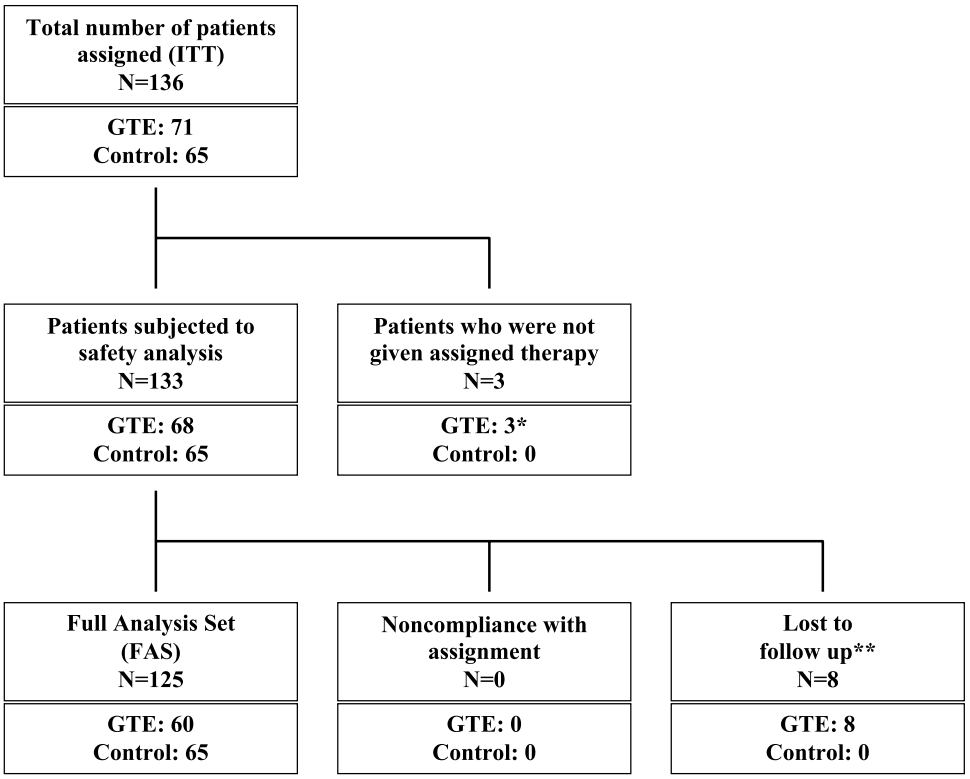

Figure 1. Details of analysis. ITT, intention-to-treat. FAS, full analysis set. *, due to withdrawal; **, could not be reached from the day after the initiation of the trial.

presumably due to the small number of patients in each classified category.

For adverse effects of GTE, three patients complained of digestive symptoms such as abdominal fullness and epigastric discomfort. However, they continued to take GTE tablets.

Discussion

This is the first report indicating that human metachronous colorectal adenomas were significantly prevented by daily green tea consumption supplemented with GTE. As numerous scientists reported, green tea catechins have cancer-preventive activity in cell culture systems and in animal experiments, and their activity was much more ''potent'' than those of calcium, β-carotene, and vitamins C and E (11, 20). However, definitive clinical prevention trials with green tea catechins were not much conducted due to the difficulty in choosing a suitable surrogate biomarker for each cancer and in raising funds because green tea is a beverage but not a pharmaceutical. However, we think that green tea beverage is worthwhile to be tested for its cancer preventive activity in human trials because clinical application of chemopreventive agents requires extreme safety and effectiveness as green tea. Recently, a double-blind, placebo-controlled study with green tea in Italian patients showed a successful prevention: The progression of prostate cancer in men with high-grade prostate intraepithelial neoplasia, the main premalignant lesion of prostate cancer, was significantly prevented by oral administration of green tea catechins, 600 mg/d for 1 year (21). This clinical trial shows cancer prevention with green tea in a high-risk group (i.e., the cancer prevention of the first-stage; ref. 18). Following also our own successful experience in preventing human second primary liver cancer (22, 23), we decided to start the present study with the clean colon after polypectomy because such colonic mucosa provides a high-risk field for metachronous

Table 1. Base-line characteristics of the patients

Characteristic	GTE group	Control group	*P*
No. of patients	60	65	—
Sex (male/female)	40/20	41/24	NS*
Age (y), median (range)	62 (39-79)	63 (38-72)	NS†
No. of removed adenomas at the 1st colonoscopy/person, median (range)	2 (1-5)	2 (1-5)	NS†
Size of adenomas (mm), median (range)	6 (2-16)	5 (2-12)	NS†
No. of adenomas with high-grade dysplasia (%)	10 (9)	14 (13)	NS*

Abbreviation: NS, not significant ($P > 0.05$).
*χ^2 test.
†Kruskal-Wallis test.

Table 2. Characteristics of patients with metachronous adenomas at end-point colonoscopy

Characteristic	GTE group	Control group	*P*
Total no. of patients	60	65	—
No. of patients with metachronous adenomas (%)	9 (15)	20 (31)	<0.05*
Sex (male/female)	6/3	14/6	NS*
Age (y), median (range)	62 (38-71)	59 (38-74)	NS†
No. of metachronous adenomas/person, median (range), mean ± SD	1 (1-2), 1.3 ± 0.5	1 (1-4), 1.5 ± 1.0	NS†
Size of relapsed adenomas (mm), median (range), mean ± SD	3 (2-4), 3.0 ± 1.0	4 (2-10), 4.0 ± 1.3	<0.001†
No. of adenomas with high-grade dysplasia (%)	0 (0)	1 (4)	NS*

*χ^2 test.
†Kruskal-Wallis test.

(i.e., second primary colorectal adenoma; ref. 19), which we targeted as the end-point biomarker. The rate of metachronous tumorigenesis in the present control group was similar to that in our preceding study (19), but seems to be higher than those in other previous studies (4, 5). We emphasize that the rate observed in this study was the one from the exact clean colon confirmed twice by total colonoscopy. Thus, this report strongly suggests that GTE effectively prevents the tumorigenesis that arises from a high-risk field in humans. In addition, serious side effects or adverse effects were not observed with daily green tea consumption supplemented with GTE. This observation is important because the long-term use of aspirin and COX-2 inhibitors had substantial risk of toxicity, although they were effective in colorectal cancer prevention (8).

Molecular mechanisms of chemoprevention with green tea catechins are suggested as follows (11, 24-26): EGCG inhibits *COX-2* gene expression; the biosynthesis of prostaglandin E_2, a major product of COX-2 enzyme; and the activation of the Ras/mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt signaling pathways in colorectal cancer cells (13, 27). Clinical studies also showed that the ingestion of green tea extract inhibited the COX-dependent arachidonic acid metabolism and decreased the level of prostaglandin E_2 in colorectal mucosa (28, 29). Thus, we think that GTE prevented metachronous colonic adenoma, in part, by interfering with the COX-2/prostaglandin E_2 axis in the mucosa because the aberrant activation of this axis is an essential prerequisite to the early development of adenoma (30, 31). In addition, there is increasing evidence for a complex positive feedback circuitry between epidermal growth factor receptor–related signaling, COX-2, and prostaglandin E_2, and this seems to be associated with the early stage of colonic tumorigenesis (30-33). The insulin-like growth factor/insulin-like growth factor-I receptor system is also involved in the COX-2/prostaglandin E_2 axis and, therefore, plays a role in colorectal carcinogenesis (34, 35). These reports, together with our recent finding that EGCG can inhibit epidermal growth factor-epidermal growth factor receptor binding via its sealing effects (36), suggest that some types of receptor tyrosine kinases, including epidermal growth factor receptor family and insulin-like growth factor-I receptor proteins, are suitable targets of green tea catechins (12-14).

Because supplementation with 3 tablets of GTE raised the daily consumption of green tea up to ≥10 cups equivalent in total and significantly prevented metachronous colorectal adenomas in Japanese patients, we think that the supplemental GTE is an effective and promising tool for chemoprevention of Japanese colorectal cancer. However, it is important to note that the present trial was randomized but not placebo controlled. We did not use placebo because green tea is a daily beverage of Japanese and we aimed to raise the consumption to a target level, with supplemental GTE tablets, although it was assumed to cause some limitations in this trial. Actually, there would be many ways that the treatment arm could be known by endoscopists, regardless of how well we try to keep the treatment blinded. Thus, it would be recommended to use placebo, if this study would be conducted again in other countries where people do not drink green tea, like the case of Italian patients (21).

Another study limitation of the present trial is the small number of participants and the short (1 year) supplementation period. Although the full analysis set of patients (see Fig. 1) showed a significant effect of GTE tablets (Table 2), we should note that the withdrawals and drop-outs were observed only in the GTE arm. Thus, the intention-to-treat analysis makes the difference between the two groups not significant (data not shown). To strengthen this concern in the study, larger patient number and longer supplementation period would be required.

It is still difficult to estimate how many grams of green tea catechins are consumed per day per person because the amount of daily consumption of green tea varies depending on lifestyle. However, the questionnaire

Table 3. Prevention of metachronous adenomas by daily green tea consumption plus GTE supplementation

	Total	Green tea consumption (cups/d)		
		≦3	4∼9	≧10
No. of patients with GTE supplementation	60	5	47	8
No. of patients with metachronous adenomas	9	3	6	0
No. of patients without metachronous adenomas	51	2	41	8

NOTE: $P_{overall} < 0.01$ by contingency table analysis (3×2 χ^2 test).

survey of this study revealed that there were no significant differences in the daily amounts of green tea among all participants: The patients of both groups took 6.6 cups per day, which is equivalent to 1.5 g GTE. Therefore, the patients of the GTE group who were supplemented with 3 tablets of GTE (1.5 g green tea extract) were assumed to consume ≥2.5 g GTE (>10 cups) per day. This estimation is well consistent with the epidemiologic data that the consumption of ≥10 cups of green tea per day significantly reduced the relative risk of cancers in several organs, including the colorectum (17, 37). In addition, it is important to note that green tea is ingested in Japan many times a day, and the multiple drinking of green tea directly promotes cancer-preventive effects. This statement is experimentally supported by the results that multiple treatments with green tea catechins synergistically inhibited cell growth and induced apoptosis in human lung cancer A549 cells (38).

We should, however, note that the epidemiologic observations about the effect of green tea on cancer incidence are not consistent with recent reviews (39). Thus, our present results prompt precise characterization of the appropriate target population to receive GTE supplements for cancer prevention. With this regard, Table 3 suggests that the best candidates would be the subjects who take 4 to 9 cups of green tea daily because they would easily reach or exceed the target amount of 10 cups by supplemental GTE tablets. However, for those with ≤3 cups tea intake, supplementation with more GTE tablets or other approaches to improve lifestyle itself would be required. In addition, we have recently reported that EGCG inhibits the obesity-related colorectal carcinogenesis by improving hyperlipidemia, hyperinsulinemia, and hyperleptinemia (40). Therefore, obese people, who are at high-risk for colorectal cancer (41), might also be an appropriate target population for GTE supplements to prevent colorectal cancer development.

Finally, we have to emphasize that GTE is preferable to EGCG alone for the practical application because induction of apoptosis, growth inhibition, and inhibition of *TNF-α* release in cancer cells were synergistically brought by the combination of active catechins, such as EGCG, with the inactive catechin (−)-epicatechin (12, 42). The synergistic preventive effects of EGCG with COX-2 inhibitors, such as sulindac and celecoxib, were also found in both induction of apoptosis and inhibition of intestinal tumor formation in Min mice (43). Therefore, combination cancer prevention with green tea catechins and COX-2 inhibitors will open a new strategy for humans (43, 44).

In conclusion, we presented for the first time that green tea supplemented with GTE was safe on the regular basis and significantly prevented metachronous colorectal adenomas in Japanese patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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