

Cancer Prevention with Green Tea Polyphenols for the General Population, and for Patients Following Cancer Treatment

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Abstract: Green tea is now known to be the most effective beverage for cancer prevention, and evidence of this was recently extended to clinical applications. This paper briefly reviews several topics with regard to the development of our study on cancer prevention with green tea, and proposes a strategy for two-stages of cancer prevention with green tea. The topics are : 1) introduction of our initial work on (-)-epigallocatechin gallate (EGCG), the main constituent of Japanese green tea; 2) cancer preventive effects of EGCG and green tea extract, the lyophilized form of green tea infusion; 3) bioavailability of ³H-EGCG in mice; 4) delay of cancer onset by drinking 10 Japanese-size cups of green tea per day; 5) a prototype study for developing green tea beverage supplemented with green tea tablets by 102 healthy volunteers; 6) cancer prevention before cancer onset for the general population and high risk groups; 7) cancer prevention for patients following cancer treatment; 8) an example of a clinical trial looking toward prevention of cancer recurrence in the liver; and 9) possible prevention of human lung cancer. Considering all the above, green tea is a multi-, non-toxic cancer preventive for humans: It is nature's remedy.

Keywords: Green tea, EGCG, 10 Japanese-size cups, general population, cancer patients.

INTRODUCTION

In 1983 we did the first scientific examination of (-)-epigallocatechin gallate (EGCG), the main constituent of green tea and green tea extract, the lyophilized form of green tea infusion (Fig. 1). We studied their potential as cancer preventives in collaboration with Takuo Okuda, who was Professor of Faculty of Pharmaceutical Sciences at Okayama University at that time. EGCG inhibited both the phorbol ester receptor binding with a tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), in a membrane fraction of mouse skin, and activation of protein kinase C by the tumor promoter. The results suggested that EGCG acted as antagonist, inhibiting the action of tumor promoters. In 1987, we first reported that topical applications of EGCG inhibited tumor promotion of teleocidin, a TPA-type tumor promoter in a two-stage carcinogenesis experiment on mouse skin [1]. In addition, EGCG completely inhibited tumor promotion of okadaic acid, which is as potent as TPA, mediated through inhibition of protein phosphatases 1 and 2A [2]. Since EGCG inhibited both PKC pathway and okadaic acid pathway, we thought that EGCG treatment possibly inhibited the interaction of tumor promoters with their receptors: We named this the sealing effect [2]. The results encouraged us to move on to a significant project, cancer prevention with green tea, based on the fact that Japanese drink green tea all day long [3].

CANCER PREVENTIVE EFFECTS OF EGCG AND GREEN TEA EXTRACT

EGCG and green tea extract have demonstrated the following attributes as cancer preventives: They are non-toxic, for rodents and humans; they have a wide-range of target organs, such as digestive tract including esophagus, stomach, duodenum and colon, plus lung, liver, pancreas, breast, bladder, prostate, and skin; they have inhibitory effects on growth of human cancer cells associated with G₂/M arrest in PC-9 cells; and they showed inhibitory effects on lung metastasis of B16 melanoma cells, associated with reduction of various cytokine levels [4].

To look at the mechanisms of action of green tea polyphenols in cancer prevention, it is worthwhile to discuss evidence on TNF- α , the endogenous tumor promoter. We have obtained results showing that numerous inhibitors of tumor promotion and cancer preventive agents inhibited both TNF- α gene expression in the cells and TNF- α release from the cells induced by tumor promoters, resulting in a reduction of the amount of TNF- α in cancer cells and probably in their surrounding tissues [5]: The tea polyphenols (-)-epicatechin gallate (ECG), EGCG and (-)-epigallocatechin (EGC) dose-dependently inhibited TNF- α release from a human stomach cancer cell line, KATO III cells, treated with okadaic acid. The potency of these green tea polyphenols is closely associated with the potency of their growth inhibition [5].

Green tea extract contains at least four tea polyphenols: EGCG, ECG and EGC are active compounds, while (-)-epicatechin (EC) is inactive (Fig. 1). However, cotreatment with EC and EGCG, and EC and other tea polyphenols, synergistically enhanced cancer preventive activity such as induction of apoptosis and inhibition of TNF- α release [5]

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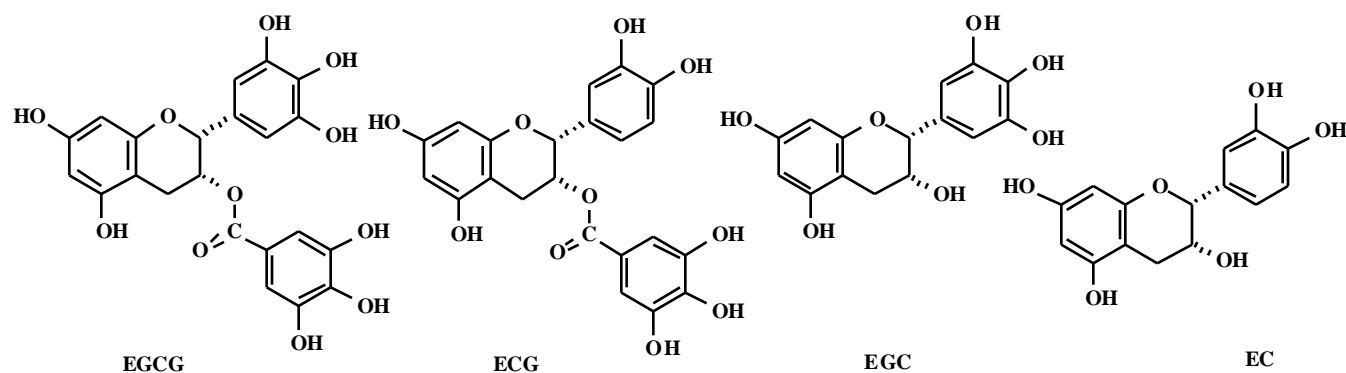


Fig. (1). Structures of (-)-epigallocatechin gallate (EGCG), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epicatechin (EC).

(Table 1). These results strongly indicate that whole green tea is a more efficient mixture of tea polyphenols for human cancer prevention than EGCG alone.

BIOAVAILABILITY OF ³H-EGCG IN MICE

From the systemic effects of EGCG and green tea extract, we assumed that EGCG and green tea polyphenols were easily distributed from the digestive tract to various organs where they induced anticarcinogenic effects. To prove this, we obtained ³H-EGCG with a specific activity of 48.1 GBq/mmol - fortunately a very stable compound. Whether ³H-EGCG could be incorporated into human lung cancer cell line PC-9 cells was first studied in the culture, and after treatment with ³H-EGCG for 1 and 24 hours, PC-9 cells were subjected to microautoradiography: Silver grains of ³H-EGCG appeared in the membrane, cytosol and nuclei, seeming to confirm that EGCG is incorporated into cancer cells [6]. We next administered ³H-EGCG into mouse stomach and studied its distribution: Within 24 hours after administration, 6.6% of total administered radioactivity was excreted in urine and 37.7% in feces. In addition, various amounts of the total administered radioactivity were found 24 hours after intubation in the digestive tract, liver, brain, kidney, lung, pancreas, and skin [7] (Table 2). Thus, the radioactivity was present in the organs where EGCG and

green tea extract had previously been shown to inhibit carcinogenesis (Table 2).

DELAY OF CANCER ONSET BY DRINKING 10 JAPANESE-SIZE CUPS OF GREEN TEA PER DAY

Green tea beverage as a cancer preventive has numerous advantages, and one scientific advantage is that results on the cancer preventive effects of green tea can be obtained from prospective cohort studies with humans consuming green tea. Kazuo Imai and Kei Nakachi surveyed 8,552 individuals aged over 40 in Saitama Prefecture, Japan, in 1986, on their living habits, including daily consumption of green tea. During the 10 years after 1986, they found a total of 419 cancer patients, 175 females and 244 males, among participants in the cohort study. Their study (1986 – 1996) revealed that drinking 10 Japanese-size cups (120 ml/cup) of green tea per day delayed cancer onset in humans by 7.3 years among females and by 3.2 years among males, compared with patients who had consumed less than three cups per day [8] (Table 3). The difference between females and males is partly due to higher tobacco consumption by males, but the results clearly indicated that drinking the equivalent of 10 Japanese-size cups per day, about 2.5 g green tea extract, prevents cancer in the general population. Furthermore, the reduction of relative risk of cancers was

Table 1. Synergistic Effects by Cotreatment with EC and Other Tea Polyphenols on Apoptosis and Growth Inhibition of Human Lung Cancer Cell Line PC-9, and on Inhibition of TNF- Release from BALB/3T3 Cells

	Induction of apoptosis ^a (A _{415 nm})		Growth inhibition ^b (% of control)		Inhibition of TNF- release ^b IC ₅₀ (μM)	
	without EC	with EC (200 μM)	without EC	with EC (200 μM)	without EC	with EC (100 μM)
		0.10 ± 0.03		97.8		>500 ^c
EGCG (100 μM ^d)	0.52 ± 0.22	0.98 ± 0.28	73.3	27.8	60	1 ^e
ECG (50 μM ^d)	0.27 ± 0.13	0.53 ± 0.18	62.2	43.3	30	7
EGC (200 μM ^d)	0.14 ± 0.03	0.43 ± 0.08 ^e	100.0	72.2	n.d.	n.d.

^aValues represent the mean + SD of two separate experiments performed in duplicate.

^bValues are representative of two separate experiments performed in duplicate.

^cIC₅₀ of EC

^dConcentration for experiment on apoptosis and growth inhibition of human lung cancer cell line PC-9.

^eP<0.01.

achieved by high consumption of green tea in all organs, such as lung, colorectum, liver and stomach [8].

questionnaires, and their blood samples were examined. We obtained the following answers from the questionnaires: 93% of the participants were able to continue drinking green

Table 2. Incorporation of ³H-EGCG into Target Organs and Associated with Reduction of Tumor Incidence

Organs	% of total administered radioactivity (24 h after)	% of tumor-bearing animals	
		without	with EGCG
Stomach	3.93	62.2	31.0
Duodenum	0.35	63.0	20.2
Small intestine	5.69	n.d.	
Colon	4.52	77.3	38.1
		67.0	33.0*
Liver	0.89	83.3	53.2
Brain	0.32	n.d.	
Kidney	0.28	n.d.	
Lung	0.16	96.3	65.5
Pancreas	0.07	54.0	28.0
Skin	1.9 x 10 ⁴ /100 mg	65.0	28.0*

*: Green tea extract
n.d.: Not determined

A PROTOTYPE STUDY FOR DEVELOPING GREEN TEA BEVERAGE SUPPLEMENTED WITH GREEN TEA TABLETS BY 102 HEALTHY VOLUNTEERS

We have determined that the effective cancer preventive amount is 10 Japanese-size cups of green tea per day, based on both the results of the cohort study mentioned above [8], and the results of a case control study on gastric cancer and diet by Kono’s group, conducted in southern Japan in 1988 [9]. One Japanese-size cup contains roughly 80 – 120 ml, and 10 cups contain about 2.5 g green tea extract. We now recommend taking this amount daily using a combination of green tea beverage and tablets of green tea extract [10].

Before going on to clinical study, we asked some 100 healthy volunteers to consume daily 10 Japanese-size cups of green tea for three months, with informed consent. Group A of 51 volunteers took green tea beverage and green tea tablets for the first 3 months, and group B, the same number, took green tea beverage and the tablets for another 3 months. After six months trial, all 102 volunteers answered

tea and taking green tea tablets, and over 90% of the participants found their living habits or meals unaffected by green tea. Our conclusion is that most Japanese can easily take the recommended 10 cups of green tea per day, or 2.5 g green tea extract per day [11].

Table 3. Age of Cancer Onset According to Green Tea Consumption

	Green tea consumption (cups/day)		
	≤ 3	4 - 9	≥ 10
Females (175) ^a	67.0 + 1.7 (49)	66.4 + 1.3 (102)	74.3 + 2.2 (24)
Males (244)	65.0 + 1.5 (59)	67.2 + 1.0 (114)	68.2 + 1.1 (71)

^aNumber of cancer cases

About 50% of the volunteers experienced very mild temporary disorders, such as abdominal bloating, heartburn,

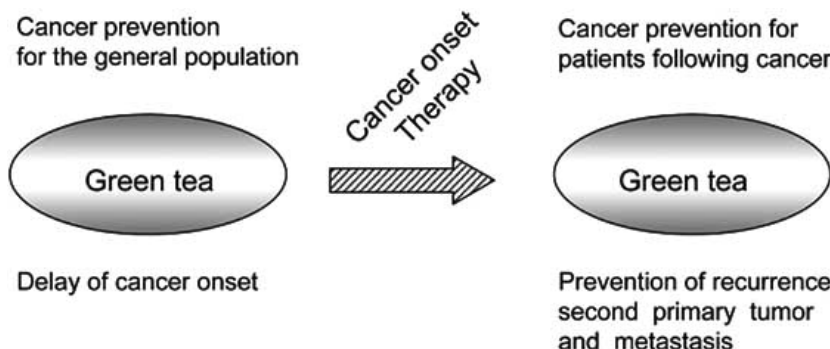


Fig. (2). Two-stages of cancer prevention with green tea: for the general population before cancer onset and for patients following cancer treatment.

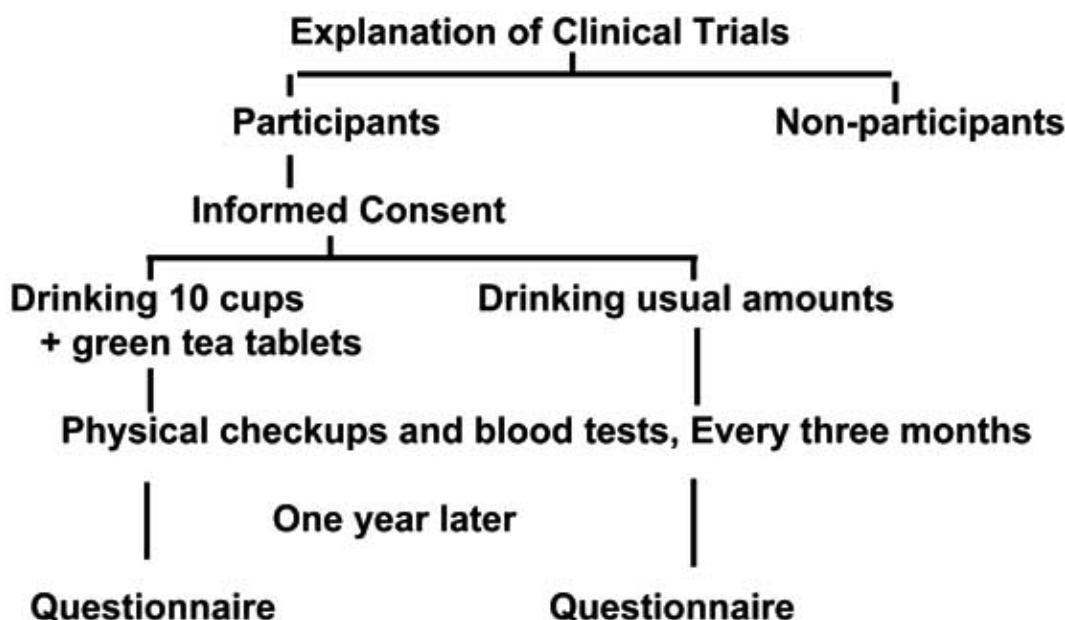


Fig. (3). Prevention of cancer recurrence in the liver at a University clinic.

nausea, and insomnia, due to the caffeine in green tea extract. Since we thought that tablets containing 5% caffeine might have caused some disorders, the caffeine content was reduced from 5% to less than 3% [11]. We chose not to make completely decaffeinated tea, since Conney's group had demonstrated that decaffeinated teas were inactive or less effective inhibitors of tumor formation [12]. New green tea tablets containing less than 3% caffeine recently went on sale in Japan, under the commercial name of G.T.E. and produced by Iruma-Kumiai Seicha in Saitama Prefecture [11]. These tablets now make it possible for both the general population and high risk groups to easily consume the recommended amount of green tea polyphenols. We call this The First stage of cancer prevention with green tea, leading to delay of cancer onset, as our cohort study already indicated [13] (Fig. 2).

CANCER PREVENTION FOR PATIENTS FOLLOWING CANCER TREATMENT

Due to advances in diagnosis and treatment of cancers, there are many healthy cancer patients in Japan. Specifically, there are 2.7 million surviving cancer patients, and 1.6 million have survived over 5 years. These patients are seriously looking for drugs that will prevent recurrence, second primary tumors and metastasis. In collaboration with

Japanese clinicians, we are making progress toward prevention of cancer recurrence in various organs, including relapse of prostate cancer, polyp development after colorectal polypectomy, and recurrence of breast, liver and esophagus cancers [10, 11]. We call cancer prevention for both the general population and cancer patients "The two-stages of cancer prevention with green tea" (Fig. 2) [13].

Recently we obtained some exciting results on prevention of polyp development after colorectal polypectomy, by consuming 10 Japanese-size cups of green tea supplemented with green tea tablets (Moriwaki, personal communication).

AN EXAMPLE OF A CLINICAL TRIAL

A study on prevention of cancer recurrence in the liver was conducted at a Japanese University clinic (Fig. 3). First, after liver cancer patients had cancer lesions removed, they listened to an explanation of clinical trials with green tea supplemented with green tea tablets. Patients who chose to join the trials agreed by informed consent and were divided into two groups: those drinking 10 cups of green tea supplemented with green tea tablets for one year or drinking just the usual amount (less than 10 cups). During the year, they were given physical checkups and blood tests every three months, and at the end of the year, they answered a

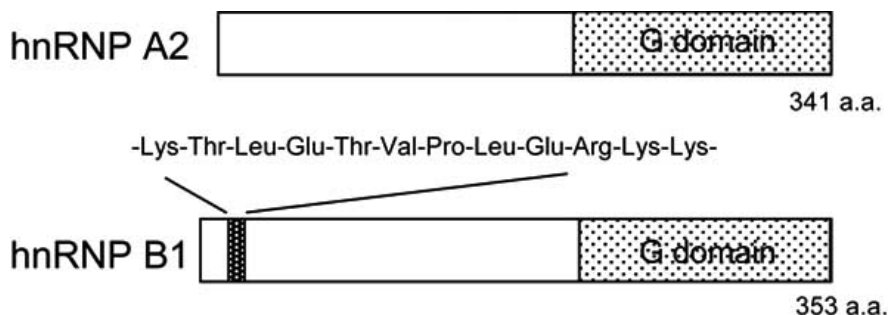


Fig. (4). Structures of heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2) and hnRNP B1 proteins.

Table 4. Inhibition of A549 Cell Growth and hnRNP B1 Gene Expression by EGCG, ECG or Genistein

	Inhibition of cell growth IC ₅₀ (μM)	Inhibition of hnRNP B1 gene expression	
		mRNA expression IC ₅₀ (μM)	Transcriptional regulation IC ₅₀ (μM)
EGCG	70.0	25.0	29.0
ECG	60.0	50.0	n.d.
Genistein	27.0	36.0	66.0

n.d.: Not determined

questionnaire. At present, 15 patients are drinking 10 cups of green tea supplemented with green tea tablets, and do not complain any adverse effects, while 10 patients drink usual amounts of green tea without the supplements. Results are encouraging.

POSSIBLE PREVENTION OF HUMAN LUNG CANCER

Lung cancer prevention is a worthy challenge for green tea, since large-scale lung cancer chemoprevention trials with various compounds have been unable to demonstrate any preventive effects on lung cancer in humans [14, 15]. It has already been reported that tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice was inhibited by green tea [16], and green tea consumption in Japan may be responsible, in part, for the lower cancer mortality rate among Japanese cigarette smokers [17]. As previously reported, radioactivity of ³H-EGCG was distributed into the lungs, and silver grains derived from ³H-EGCG, as determined by microautoradiography, were found in the cells of the lungs, clearly indicating that the lungs are target organs [7]. In addition, we have demonstrated that duplicate administrations of ³H-EGCG at 6 hour intervals enhanced incorporation of the radioactivity in the lungs 4 times. All the results show that the more green tea we drink, the higher concentrations of green tea polyphenols we get in the lungs [7].

To achieve successful cancer prevention in the lungs, we have to establish a significant biomarker for lung cancer prevention in humans. In 1988, Melvyn S. Tockman and James L. Mulshine first identified heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1) protein as an early biomarker of human lung cancer [18]. Although hnRNP A2/B1 protein was intriguing, its antibody seemed unable to well differentiate lung cancer cells from normal lung cells. Looking at the structures of A2 and B1 proteins carefully (Fig. 4), we found that it would be possible to raise a specific antibody against the additional 12 amino acids of B1 protein, which A2 lacks, and to identify B1 protein only [19].

It is generally accepted that the key targets of cancer prevention are the very early stages of the disease, while the targets of cancer treatment or therapy are the late stages of cancer associated with metastasis. Our anti-B1 antibody specifically stained an early stage of lung cancer, bronchial dysplasia, and did not stain adjacent noncancerous tissue [20]. Furthermore, we found that EGCG and ECG, like genistein as a control, dose-dependently inhibited the constitutively elevated expression of hnRNP B1 gene [21]. Treatments with EGCG and genistein also dose-dependently

inhibited both transcriptional regulation of *hnRNP A2/B1* gene and production of hnRNP B1 protein in human lung cancer cell lines (Table 4). Thus, lung is a reasonable and efficient target organ for human cancer prevention with green tea, using monitoring by a new specific biomarker, hnRNP B1.

CONCLUSION

Since we established green tea beverage as cancer preventive, we have proposed our definition of cancer prevention as follows: the administration of cancer preventives, such as green tea, to delay the carcinogenic process in humans - no matter when the carcinogenesis starts - thereby blocking the appearance of clinical symptoms.

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