Journal of Korean Association
of Cancer Prevention
1997; 2: 83-88

□심포지엄□

Japanese Green Tea as a Cancer Preventive in Humans

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Drinking green tea today is part of Japanese culture. The tea plant *Camellia sinenses* was imported by a Japanese Zen priest in the 12th century from China to Japan as a medicine. However, as a medicine, tea was not much studied. At present we drink tea during and after meals, that is, throughout the day. One cup of green tea infusion contains about 100~200 mg of tannins, the main constituent of which is (−)-epigallocatechin gallate (EGCG). Thus, EGCG is one of the tea polyphenols or tea tannins.

In 1983 we did the first scientific examination of EGCG as a cancer-preventive material in collaboration with Takuo Okuda, who was a professor of pharmacology at Okayama University at that time. We began with a study of the anti-carcinogenic effects of polyphenols derived from medicinal plants and drugs.[1,2] We first examined whether a polyphenol shared the phorbol ester receptor with a tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate(TPA).[3] Experimentally, EGCG inhibited in a dose-dependent manner the specific ^{3}H-TPA binding to the phorbol ester receptor in a membrane fraction of mouse skin. The median effective dose (ED_{50}) of EGCG for inhibition was about 300 times less than that ofTPA. Moreover, penta-*O*-galloyl-β-D-glucose isolated from a gall, *Shisandrea fructus,* pedunculagin, chebulinic acid, and buddledin A bound to the same receptors with similar ED_{50} values as EGCG did, although their structures are related neither to that ofTPA nor to each other.[1] These results raised a question: Does EGCG act as an antagonist, inhibiting the action ofTPA, or as an agonist, activating protein kinase C as TPA does?

We found that EGCG dose dependently inhibited the activation of protein kinase C induced by teleocidin, which is one of the TPA-type tumor promoters and a new activator of protein kinase C. In 1987 we first reported that EGCG acted as an antagonist and inhibited tumor promotion of teleocidin in a two-stage carcinogenesis experiment on mouse skin.[3]

EGCG AND JAPANESE GREEN TEA EXTRACT AS CANCER CHEMOPREVENTIVE AGENTS: A HYPOTHESIS

Tumor promotion is an important step in multistage carcinogenesis in humans and in developing benign tumors from initiated cells. This step can be reproduced in a two-stage carcinogenesis experiment on mouse skin. The study of chemical carcinogenesis revealed that initiation is mutation of a protooncogene by a carcinogen, resulting in an initiated cell. For example, a single application of about 50~100 μg of 7,12-dimethylbenz(a)-anthracene (DMBA), called an initiator, induces mutation in the second nucleotide of codon 61 of mouse c-Ha-*ras* gene.[4] Repeated topical applications of a tumor promoter (e.g., TPA, teleocidin, okadaic acid) result in tumors in a high percentage of mice, usually 90~100%.[5]

Because some of the cells in our body might already have been initiated, the step of tumor pro-

motion is probably already under way in middle-aged people. This concept is supported by the evidence that the occurrence of cancer in humans is associated with aging. However, cancer is avoidable with preventive measures, specifically, cancer chemoprevention, which has been defined by Michael Sporn at the National Cancer Institute (NCI) in the United States as "the prevention of the occurrence of cancer by administration of one or more compounds."[6] These compounds are now called cancer chemopreventive agents. On the basis of our evidence that EGCG inhibited tumor promotion on mouse skin, we think that EGCG and green tea extract have potential to be such agents.[7]

LABORATORY RESULTS

Since 1983, the Ministry of Health and Welfare of Japan has supported research on cancer chemoprevention within the framework of a cancer research program in Japan.[7] The Chemoprevention Branch, Division of Cancer Prevention and Control, NCI, in the United States presented the general rules for testing possible preventive agents in animal tumor models.[8] The Chemoprevention Branch of NCI found that a two-stage carcinogenesis experiment on mouse skin had numerous advantages as a tumor model, because this experiment gives quantitative and rapid results, and the mechanisms of tumor promotion in mouse skin have been well investigated.

Recently, our research group identified a new tumor promoter, okadaic acid, which was isolated from the black sponge, Halichondria okadai. Okadaic acid is as potent a tumor promoter as TPA on mouse skin, but okadaic acid tumor promotion is mediated through different mechanisms of action from those mediating TPA tumor promotion.[5]

Fig. 1 shows that repeated topical applications of EGCG completely inhibited tumor promotion of okadaic acid in a two-stage carcinogenesis experiment on mouse skin, with tumor initiation by DMBA. Specifically, initiation was done with a single topical application of 100 μg of DMBA, and tumor promotion was achieved by repeated applications of 1 μg of okadaic acid twice a week. In the control group, which received DMBA and okadaic acid, 73.3% of the mice developed tumors, with an average of 4.2 tumors per mouse in week 20. In the experimental group, 5 mg of EGCG was applied topically 15 minutes before each treatment with okadaic acid. Repeated applitations of 5 mg of EGCG completely inhibited tumor promotion by okadaic acid on mouse skin.[9] Thus, EGCG inhibited tumor promotion of two compounds associated with different mechanisms of action on mouse skin: a TPA-type tumor promoter, teleocidin, and a non- TPA-type tumor promoter, okadaic acid (Table 1).

Table 1 also shows that EGCG and green tea extract in water inhibited carcinogenesis of various organs in rodents,[7] including the glandular stomach,[10] duodenum,[11] colon,[12,13] liver,[14] and pancreas (Satake et al., unpublished observations, 1996). In the United States, similar results were also reported: green tea extract taken in water inhibited development of tumors on mouse skin induced by either ultraviolet B light alone or ultraviolet B light plus TPA[15] and lung carcinogenesis in mice induced by a nico-tine-derived nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).[16] Moreover, EGCG and green tea extract dose dependently inhibited growth of stomach, lung, and mammary cancer cell lines. EGCG and green tea extracts in cell culture systems were about 200 times less effective than potent anticancer agents, such as adriamycin, on the basis of their ED_{50} values.[17] Recently, Shun- ichiro Taniguchi at Kyushu University was the first to report that EGCG in drinking water inhibited metastasis of B16 melanoma cells into the lung.[18]

As for the mechanisms of action of EGCG and

green tea extract, we previously reported that a single application of EGCG to mouse skin immediately inhibited the specific binding of ^{3}H-TPA and ^{3}H-okadaic acid, suggesting that the interaction of a tumor promoter with its receptor was inhibited by EGCG in both cases. This was named the "sealing effect."[9] However, it is not yet clear whether the anticarcinogenic effects are induced only by the sealing effect. Because EGCG has systemic effects, it might have additional mechanisms of action, such as an antioxidant or a free radical scavenger.[19]

We assume that EGCG is easily absorbed from the digestive tract and induces anticarcinogenic effects in various organs. Our preliminary results with ^{3}H-EGCG showed that radioactivity administered by intubation into mouse stomach was found after 24 hours in various organs, including the digestive tract, liver, lung, brain, skin, and serum (Suganuma et al., unpublished observations, 1996).

EPIDEMIOLOGICAL STUDIES

After we first reported the anticarcinogenic effects of EGCG, in 1989 Itaro Oguni at Hamamatsu College, University of Shizuoka, cited vital statistics revealing that the mortality rates from total cancer and stomach cancer were significantly lower in Shizuoka Prefecture, the leading tea-producing area in Japan.[20] In separate studies, Japanese epidemiologists found a significantly decreased risk of gastric cancer among patients whose green tea consumption was more than 10 cups per day.[21]

Kazue Imai and Kei Nakachi, Division of Epidemiology of our research institute in Saitama, analyzed all cancer patients observed in a cohort study by two variables: numbers of deaths and ages at death of males and females, depending on green tea consumption. They first found that cancer patients whose green tea consumption was more than 10 cups per day died 4.5 years (males) to 6.5 years (females) later than did the cancer patients who drank fewer than 3 cups of green tea per day.[22] The results indicated that drinking green tea attenuated development of cancers in both sexes, and it also was associated with a later onset of cancer. In addition to these exciting results, they also found that drinking green tea prevents cardio-vascular disease and disorders of the liver in humans by reducing total serum cholesterol, triacylglycerol levels, and the atherogenic index, as well as reducing levels of glutamic-oxaloacetic transaminase (aspartate aminotransferase) and glutamic- pyruvic transaminase.[23] Nakachi's group made tablets of green tea extracts (two tablets are equivalent to 1 cup of green tea) and they are now administering green tea tablets to a healthy population.

CLINICAL TRIALS

It is difficult to conduct a clinical trial with green tea in Japan, because people have a background level of green tea in their daily lives. However, basic clinical studies with green tea have enabled researchers to develop intermediate biomarkers for cancer development. Tetsuro Yamane, Kyoto Prefectural University of Medicine, reported that the oral administration of EGCG reduced the level of orni-thine decarboxylase in the gastric mucosa as well as in the colon, suggesting that ornithine decarboxylase is a suitable biomarker for the digestive tract.[10] Tadashi Ikeda at the First Department of Surgery, Keio University, recently reported that the combination of green tea extract and tamoxifen completely inhibited development of mammary tumors in C3H/Ouj mice, whereas green tea extract alone was not significantly effective and tamoxifen alone reduced mammary tumor development by 50%.[24] It is worthwhile to teat whether tamoxifen is more effective in preventing the recurrence of mammary cancer in Japanese than in Westerners.

FUTURE DEVELOPMENT

Cancer prevention is becoming one of the most important areas of cancer research, but it normally requires large amounts of funding. We are fortunate to have discovered that EGCG is a natural cancer preventive agent and to have extended this discovery to Japanese green tea extract-a nontoxic chemopreventive agent for humans. Finding out just how EGCG and green tea extract inhibit cancer development is our next task.

On the basis of the study on tumor promotion with various types of tumor promoter, we found a new tumor promotion pathway mediated through tumor necrosis factor-α (TNF-α).[25] TNF-α was originally found to be a cytokine-inducing hemorrhagic necrosis of transplanted solid tumors in mice. EGCG dose dependently inhibited TNF-α release from BALB/3T3 cells that were induced by a tumor promoter, okadaic acid.[7] Because TNF-α acts as an endogenous tumor promoter in the body, the sealing effect of EGCG reduced the amount of tumor promoter in the tissues. Thus, EGCG opens a new pathway toward the goal of inhibiting the growth of cancer cells in vivo.

For many centuries, Asian people have believed in the benefits of driking green tea. We would now like to use its benefits for cancer chemoprevention in humans worldwide. Such beneficial agents may also be present in other traditional foods. Green tea is just one example revealed by basic cancer research.

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Fig. 1. Inhibition by (-)-epigallocatechin gallate (EGCG) of tumor promotion induced by okadaic acid on mouse skin, with tumor initiation by 7,12-dimethylbenz(a)anthracene (DMBA). Groups were treated with DMBA and okadaic acid (○) and with DMBA and okadaic acid plus EGCG (●).

Table 1. Anticarcinogenic effects of EGCG and green tea extract in various organs*

Organs	Species	Carcinogens/ Tumor Promoters	Reduction in		References
			Tumor Incidence (%)	Average No. of Tumors per Animal	
Skin	Mouse CD-1	DMBA/teleocidin	53.0 → 13.0	2.1 → 0.1	3
		DMBA/okadaic acid	73.3 → 0	4.2 → 0	9
Glandular stomach	Rat Wister	MNNG	62.0 → 31.0	0.88→ 0.43	10
Duodenum	Mouse	ENNG	63.0 → 20.0	1.2 → 0.3	11
	C57BL/6		63.0 → 20.0	0.8 → 0.3	11
Colon	Rat	AMO	77.3 → 38.1	1.5 → 0.6	12
	Rat	MNU	67.0 → 33.0†	1.2 → 0.5†	13
Liver	Mouse C3H/HeN	Spontaneous	83.3 → 52.2	1.8 → 0.9	14
Pancreas	Hamster	BOP	54.0 → 33.0†	1.0 → 0.5†	‡

*Abbreviations: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; EGCG, (-)-epigallocatechin gallate; ENNG, *N*-ethyl-*N'*-nitro -*N*-nitrosoguanidine; DMBA, 7,12-dimethylbenz(a)anthracene; AOM, azoxymethane; MNU, methylnitrosourea; BOP, *N*-nitrosbis- (2-oxopropyl)amine.
†Grean tea extract
‡Satake et al., Manuscript in preparation.