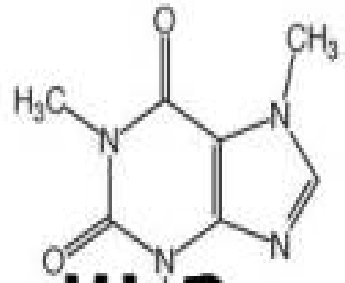
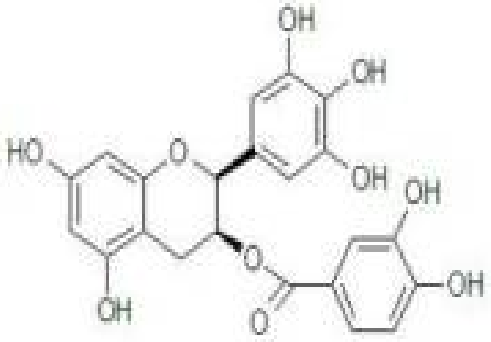
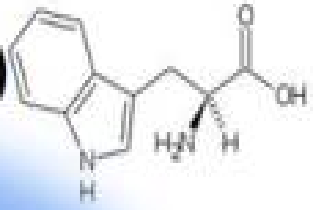




Health Benefits of Green Tea

Functional and Mechanistic Aspects

First Edition (2013)



http://dfns.u-shizuoka-ken.ac.jp/labs/tsc/

CONTENTS

Preface

Tea, a product of the leaves and buds of the plant *Camellia sinensis* (Theaceae), is consumed worldwide. Tea can be broadly classified according to the production method as unfermented (green tea), half-fermented (oolong tea), fully-fermented (black tea), or post-fermented (Pu-erh tea). Green tea was discovered in China around 3000 BC, and is reported to have various medicinal effects. Green tea is mainly consumed in Japan and China, whereas black tea is primarily consumed in Western countries, India, and other parts of the world. A search conducted in the PubMed database in November 2013 resulted in 5400, 2900, and 300 publications for the keywords “green tea”, “black tea”, and “oolong tea”, respectively. These results indicate that green tea is the primary target for investigation among the various teas, despite the fact that the global consumption of black tea is approximately 4 times higher than that of green tea.

Green tea contains various components with specific health-promoting effects. For example, the tea polyphenols, catechins (Figure 1), are believed to have protective effects against diseases such as cancer, obesity, diabetes, arteriosclerosis, dementia, and dental caries. They may also have anti-bacterial, anti-viral, and hepatoprotective effects. Of these catechins, epigallocatechin gallate (EGCG) displays the strongest bioactivity. Furthermore, caffeine induces wakefulness, decreases the sensation of fatigue, and has a diuretic effect. Theanine and gamma-aminobutyric acid act to lower blood pressure and regulate brain function. Vitamin C exhibits antiscorbutic activity, prevents cataracts, and strengthens the immune system.

A large body of scientific evidence has accumulated, which reveals how and why green tea and EGCG exhibit beneficial effects on health. The majority of evidence based on cellular and animal experiments indicates that green tea has positive effects; however, the evidence relating to its effects in humans remains inconclusive.

Obviously, it is important to summarize the present knowledge regarding the health benefits of green tea in order to develop further research in this field. This first edition provides the English counterpart to the majority of the Japanese version entitled —Health Benefits of Green Tea— Navigation to Functional and Mechanistic Aspects 2013 (in Japanese), as edited by the present editor, which is available on the website http://www.pref.shizuoka.jp/sangyou/sa-340/ryokucha_kenkou.html. It is hoped that general consumers have an opportunity to access evidence-based scientific information about the health-promoting effects of drinking green tea. When new information is available, the next edition will be published to provide the related updates.

The editor thanks all of the contributors and also The University of Shizuoka (President, Naohide KINAE), Shizuoka Prefecture (President, Heita KAWAKATSU) and Nippon Chagyo Chuoukai (President, Junichi SHINMURA) for their financial support.

Mamoru ISEMURA
Professor Emeritus and Invited Professor, University of Shizuoka

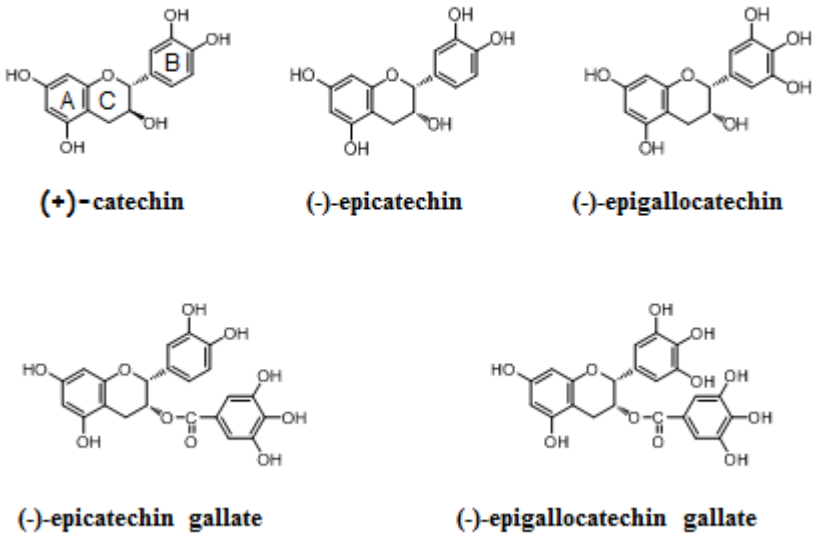


Figure 1. Chemical structures of (-)-catechin and 4 major green tea catechins. In the text, prefixes defining stereochemistry are removed for clarity.

1. ANTI-CANCER EFFECTS

1.1 ANTI-CARCINOGENIC EFFECTS OF GREEN TEA

Yoshiyuki NAKAMURA (Sugiyama Jogakuen University)

Abstract

As it develops, cancer progresses through a number of stages including initiation, promotion, progression, and metastasis. Green tea catechins show anti-cancer effects at each of these stages. Infusions of green and black, but also oolong and pu-erh teas have been shown to inhibit cancer promotion. The most effective component of those teas was found to be green tea catechins. The water-soluble high molecular weight components obtained from four kinds of teas by fractionation with various organic solvents also show anti-cancer activity. Various kinds of experiments in vitro and in vivo have mostly shown anti-cancer effects of green tea and catechins, and some human studies have supported these findings. One promising report from a human clinical study conducted in Italy noted that catechins were effective in preventing the development of prostate cancer.

Anti-cancer activity of green tea

The health-promoting effects of green tea have been known since ancient time. However, it was just started about 30 years ago that green tea was first subjected to evidence-based scientific investigation to determine its health-promoting effects. Okuda et al. (1984) and Kada et al. (1985) first reported the anti-mutagenic activity of catechins [1,2] and Oguni et al. (1989) found epidemiological evidence of a lower incidence of cancer-related deaths in tea production areas in Shizuoka Prefecture, Japan [3]. Interest in the anti-cancer activities of green tea has been growing in Japan ever since. The issue is now one of global interest and more than 200 papers related to the topic have been published during current decade [4]. Such numerous investigations of a single agricultural product are rather extraordinary and reflect the popularity of such studies.

Cellular and animal experiments have mostly confirmed the anti-cancer effects of green tea and catechins [4,5], and some human studies have supported these findings. One promising report from a human clinical study conducted in Italy noted that catechins were effective in preventing the development of prostate cancer [6].

Mechanism of anti-cancer activity of green tea components

Cancer progresses through several stages as it develops including initiation, promotion, progression, and metastasis (Figure 1). Green tea catechins have been shown

to exert anti-cancer effects at each of these stages. Several mechanisms of action have been proposed for catechins including some that involve their anti-oxidative effects and their ability to bind to cell surface receptor proteins on cancer cells [4,5].

Effect of tea ingredients on the process of cancer promotion

One of the most effective methods of cancer prevention may be to inhibit cancer development at the promotion stage since multiple factors are involved in cancer initiation. Infusions of green, black, oolong, and pu-erh teas have been inhibit cancer promotion in JB6 cells with the green and black tea infusions being the most effective [7]. The most effective component of green tea was found to be catechins. Other water-soluble high molecular weight components fractionated with various organic solvents have also shown anti-cancer activity.

The structures of these tea non-dialysates (TNDs) are not known at present, but they are considered to be a complex of condensed- and hydrolyzable-tannins, which includes catechins and carbohydrates. Their mechanism of action appears to involve inhibition of transcription factor AP-1 activity, which plays an important role during cancer promotion (Figure 2) [8]. Cancer preventing effects of teas are expected to owing to not only catechins but also these other components of various teas, because the inhibitory effect of TNDs on the AP-1 activation was rather stronger than a green tea catechin, epigallocatechin gallate.

It may be better to drink “tea” pleasantly, not to take catechin preparations as medication for health-promotion, such as cancer prevention.

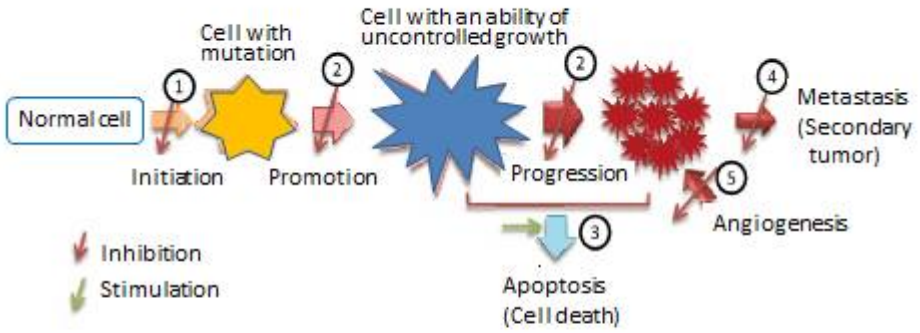


Figure 1. Cancer development and actions of tea catechins.

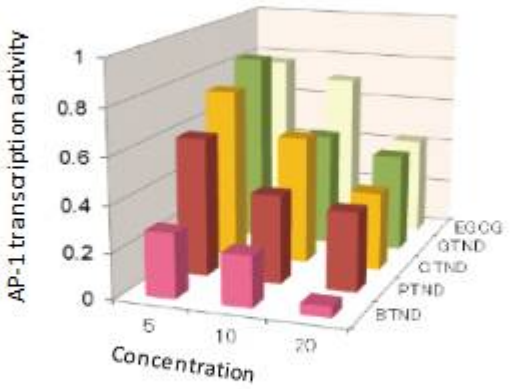


Figure 2. Effects of TNDs on AP-1 activity.

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1.2. ANTIMUTAGENESIS

Shuichi MASUDA (University of Shizuoka)

Abstract

Chemical, physical, and biological factors can modify DNA, leading to mutagenesis, i.e., the initiation step of carcinogenesis. Many components of foods and environmental substances can initiate carcinogenesis. Following the intake of acrylamide, a carcinogen contained in fried potatoes and potato chips, tea catechins prevented the formation of DNA adducts in mice. Nitrosamines are highly carcinogenic compounds that are generated in the stomach under acidic conditions when the secondary amine contained in fish and shellfish and nitrites contained in spinach and pickles are ingested simultaneously. Tea shows a strong inhibitory effect against nitrosamine formation. In vivo experiments conducted in rats and humans showed that a high-concentration green tea extract decreased the generation of the carcinogen nitrosomorpholine, while low-concentration green tea extract increased it. In addition, green tea catechins have been shown to weaken the effects of radiation through their antioxidative activity. Thus, green tea catechins reduce mutagenic activity through direct reactions with various mutagenic substances, resulting in anti-cancer effects.

Cancer causes

Genes are made from DNA, which is mainly comprised of 4 nucleotides: adenine, thymine, guanine, and cytosine. The nucleotide sequence carries the genetic instructions for the construction and operation of a living organism. Chemical, physical, and biological factors can cause modifications to the DNA, leading to mutagenesis, i.e., the initiation step of carcinogenesis. Tea catechins inhibit oxidative DNA damage induced by benzo[a]pyrene, a carcinogen contained in tobacco smoke. The next carcinogenic step, called promotion, occurs when cells grow aberrantly through unusual metabolism, including that of enzymes. In the progression stage, cells undergo malignant transformation to generate cancer cells. Many compounds, such as components of various foods and environmental substances, can initiate carcinogenesis. These chemical compounds have been estimated to constitute 80–90% of the total causes of carcinogenesis. It is well known that cancer incidence rates vary among countries that consume different foods, which is representative of the fact that chemical factors in food, water, tobacco smoke, and environmental substances are causative agents for cancer. Therefore, an inhibitor of cancer initiation is expected to exhibit anti-cancer properties,

and green tea has been studied as one such candidate.

Effects of tea catechins on chemical mutagenesis

Green tea, black tea, oolong tea, and their ingredients have been shown to reduce the mutagenic activity of heterocyclic amines which may be produced by heat cooking of meat and fish [1,2]. Pu-erh tea was shown to have the suppressive effect on mutagenesis due to aflatoxin B1, a fungal toxin to cause liver cancer [3]. In addition, Tea catechins have been shown to reduce the chromosomal aberration induced by chemical mutagens [4]. It has been reported that black tea polyphenols inhibit the chromosomal aberration induced by dimethylbenz[a]anthracene in hamster bone marrow cells [5]. It has also been shown that tea catechins inhibit oxidative DNA damage induced by benzo[a]pyrene, a carcinogen contained in tobacco smoke [6], and by tributyltin in mouse blood [7].

Following the intake of acrylamide, a carcinogen contained in fried potatoes and potato chips, the simultaneous intake of tea catechins strongly prevented the formation of DNA adducts in mice [8]. These anti-mutagenic activities of tea catechins are probably attributable to their ability to directly bind to activated mutagens, to inhibit the activity of drug-metabolizing enzymes, and to induce detoxifying enzymes (phase II enzymes) [9,10].

The aminocarbonyl reaction called the Maillard reaction generates heterocyclic amine and acryl amide [11,12]. Nitrosamines are highly carcinogenic compounds that are generated in the stomach under acidic conditions when the secondary amine contained in fish and shellfish and nitrites contained in spinach and pickles are ingested simultaneously. Tea shows a strong inhibiting effect against nitrosamine formation.

When secondary amine morpholine and sodium nitrite are mixed under acidic condition, nitrosomorpholine, a type of nitrosamine, is generated. A high-concentration green tea extract was shown to inhibit the generation of nitrosomorpholine, while low-concentration green tea extract promoted the reaction [13]. Similarly, in vivo experiments conducted in rats and humans showed that high-concentration green tea extract reduced the generation of nitrosomorpholine, but that low-concentration green tea extract increased it (Figure 1) [13]. These findings suggest that the ingestion of green tea at high concentrations would be beneficial for reducing the generation of carcinogenic nitrosamines.

Effects on mutagenic activity of radiation

Radiation exposure and the ingestion of foods exposed to radiation currently represent a serious problem. Several reports have described the inhibitory effect of tea

against the effects of radiation. When mice were irradiated with gamma rays and DNA damage was examined in blood cells, the inhibitory effects of tea catechins became clear [14]. Similarly, green tea, pu-erh tea, rooibos tea, and catechins have been shown to exert an inhibitory effect against the generation of chromosomal aberrations, as assessed by the formation of a small core in immature red corpuscles (Figure 2) [15].

Gamma radiation causes lipid peroxidation and cellular injury in the liver and other tissues, leading to apoptosis, which represents physiological cell death. Epicatechin and other catechins can reportedly prevent these events [16,17].

Protective effects of green tea against skin damage caused by exposure to ultraviolet rays have also been reported [18,19]. When humans and animals are exposed to gamma or ultraviolet rays, a water molecule will be converted to the hydroxyl radicals that are among the active oxygen radicals, damaging DNA. Green tea catechins were shown to weaken the effects of radiation through their antioxidative activity [20].

In brief, it can be said that tea is an important food material that can prevent carcinogenesis by inhibiting the mutagenic activity of compounds present in food and the environment.

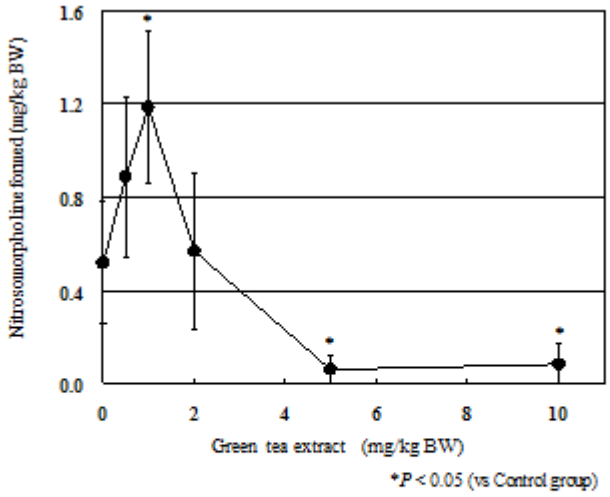

Figure 1. Effect of green tea extract on the formation of nitrosomorpholine in rats

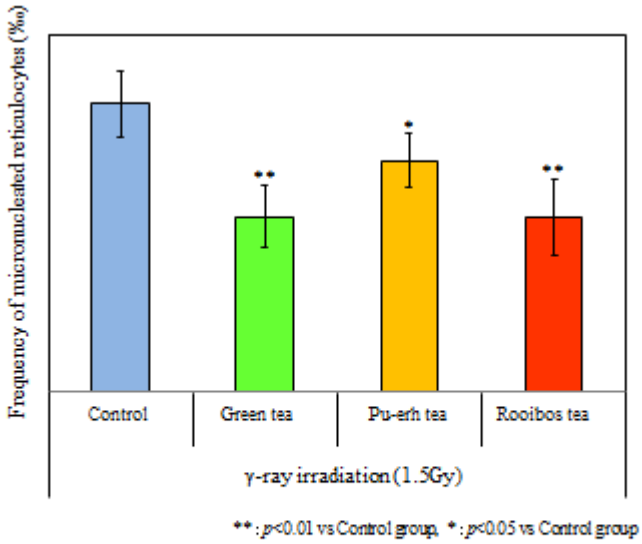

Figure 2. Inhibitory effect of tea extracts on the induction frequency of micronucleated reticulocytes in γ-ray irradiated mice

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1.3. EFFECTS ON CANCER-RELATED BACTERIA AND VIRUSES

Itaro OGUNI (Shizuoka Institute of Science and Technology)
Yuko SHIMAMURA (University of Shizuoka)

Abstract

Green tea catechins have been shown to protect against food poisoning-associated bacilli and other harmful bacteria in the intestines. Among these, the effect of epigallocatechin gallate (EGCG) is the strongest. These molecules also help neutralize various bacterial toxins including the cholera toxin, pertussis toxin, *Vibrio parahaemolyticus* heat-resistant hemolytic poison, *Staphylococcus aureus* toxins, and a verotoxin of *Escherichia coli* O-157.

Antibacterial and detoxification effects

Green tea catechins, which are abundant in green tea, have been shown to protect against food poisoning bacilli and harmful bacteria in the intestines [1,2]. Among these, the effect of epigallocatechin gallate (EGCG) is the strongest. The minimal inhibitory concentration of catechins required to inhibit the growth of disease-causing bacteria such as *Vibrio cholerae* and *Escherichia coli* O-157 as well as food poisoning-associated bacilli such as *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Clostridium welchii*, *Bacillus cereus*, and *Clostridium botulinum* is about one-tenth to one-half that contained in normally consumed quantities of green tea [1,2].

Catechins also help neutralize bacterial toxins including cholera toxins, pertussis toxins, *V. parahaemolyticus* heat-resistant hemolytic poisons, *S. aureus* toxins, and a verotoxin of *E. coli* O-157 [3,4]. Therefore, the green tea served in sushi restaurants may help protect against food poisoning.

The symptoms associated with cavity or periodontitis manifest as a result of the action of oral bacteria. Catechins are known to inhibit the growth of these bacteria. Furthermore, catechins prevent cavity formation by inhibiting the activities of the bacteria-derived enzymes involved in cavity formation [5].

Effects on *Helicobacter pylori*

In recent years, *H. pylori* infection has attracted attention as one of the main causes of chronic gastritis, gastric ulcers, and stomach cancer. Long-term *H. pylori* infection advances atrophic gastritis, which is a precancerous state preceding stomach cancer, and is considered to be associated with the development of its symptoms [6-8]. Although *H. pylori* infection is treated with antibiotics, current treatments have various problems

such as side effects and drug resistance, leading to the need for the development of safer and more effective treatments.

Studies on the anti-*H. pylori* actions of green tea and catechins, which have anticancer [9] and antibacterial activities, indicate that the growth of various *H. pylori* strains is inhibited by treatment with a 10-fold dilution of the catechin concentration in normal green tea (Table 1) [10-12].

A clinical study in which *H. pylori*-infected individuals were treated with capsules containing catechins (700 mg/d, which is the amount of catechins contained in 7 cups of green tea) for 1 month investigated the existence of *H. pylori*. The results show catechins have anti-*H. pylori* activity. Furthermore, studies on residents consuming large amounts of green tea in the area of Shizuoka, where the mortality rate due to stomach cancer is relative low, show that the *H. pylori* antibody positivity rate (i.e., index of infection) and the degree of gastric mucous membrane withering are lower than those of residents in areas with average stomach cancer mortality rates 10,11,13-15]. These findings suggest green tea consumption can help prevent stomach cancer by inhibiting the propagation of *H. pylori*.

Effects on influenza virus

EGCG and theaflavin gallate (TF3), components of black tea, are known to inhibit infections caused by the influenza virus [16,17]. This inhibitory effect is attributed to the inhibition of viral adsorption to cell surfaces [17]. EGCG or TF3 binds to the glycoprotein hemagglutinin (HA), which is present as a spike. Figure 1 shows the titer of antibodies recognizing influenza virus and the amount of EGCG that reduces the viral infectivity by 50% at 37°C. These results show that EGCG quickly inactivates the influenza virus and that its effect is as potent as that of the specific antibody [18].

Furthermore, a recent report describes that EGCG suppresses the activity of proteins indispensable for the growth of influenza virus [19]. In addition, TF3 and its derivatives suppress the production of inflammatory cytokines, which play a role in the development of various inflammatory conditions after viral infection [20].

Effects on other viruses

EGCG also exhibits antiviral activity against human immunodeficiency virus (HIV) [21]. Its mechanisms of action include the following: (1) direct action on virus particles, (2) inhibition of virus adsorption onto cell surfaces, (3) inhibition of viral RNA synthesis, and (4) suppression of viral propagation in the host's cells. In addition, EGCG can prevent HIV infection by neutralizing the seminal protein that mediates the viral infection [22]. Furthermore, EGCG and TF3 exhibit antiviral activities against the

poliomyelitis virus and other viruses [23].

Table 1 Minimum Inhibitory Concentration (MIC; μg/m1) of Catechins for Standard Strains and Clinical Isolates of *Helicobactor pylori*

	EC	EGC	ECG	EGCG
ATCC 43526	>200	200	50	50
ATCC 43629	>200	>200	50	50
ATCC 43579	>200	200	50	50
CAM (−)	200	200	50	50
CAM (+)	>200	200	50	50

ATCC: standard strain
CAM(−): clarithromycin resistant strain (n=8)
CAM(+): clarithromycin sensitive strain (n=12)
EC : Epicatechin , EGC : Epigallocatechin
ECG : Epicatechin gallate , EGCG : Epigallocatechin gallate

Bacterial susceptibility to catechins was tested by determining the minimum inhibitory concentration (MIC; μg/m1) for standard strains and 20 c1inically isolated strains of *H. pylori* using agar dilution method. All strains were tested on Brucella HK agar supplemented with 10% horse blood. A1iquots of *H. pylori* culture were transferred to the we1ls containing different concentrations of catechins and incubated at 35°C in microaerobic atomosphere (5% O_2, 10% CO_2) for 3 days.

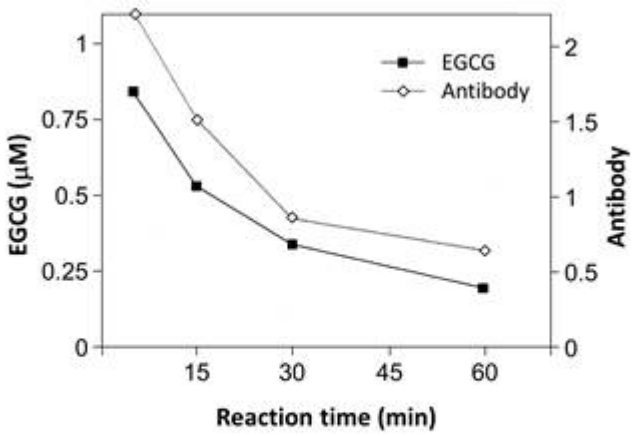

Figure 1. Kinetics of 50% inhibition of infectivity of the influenza virus by EGCG and specific antibody [18]

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1.4. ANTI-TUMOR EFFECTS

Mamoru ISEMURA (University of Shizuoka)

Abstract

A large number of experiments using cells and animals have demonstrated the anti-cancer activity of green tea and its catechins of which a major component EGCG is responsible for the activity. Apoptosis, or programmed cell death, is a physiological phenomenon which occurs when cells that are no longer useful to the body are eliminated. Tea catechin EGCG induces apoptosis in cultured cancer cells and inhibits the growth and metastasis of cancer in animal models. It is also known that EGCG exhibits anti-cancer effects through a variety of action mechanisms. For example, EGCG prevents the oxidative damage of DNA through the elimination of active oxygen. The results of several epidemiological studies indicate that intake of green tea reduces the risk of some kinds of cancer, although further studies are needed to reveal more clearly the anti-cancer activity of green tea and its components. There are now many clinical trials under way for a standardized green tea polyphenol preparation called Polyphenon E or sinecatechin, which is used as a medication for genital warts and has been approved by the United States Food and Drug Administration.

Introduction

About 25 years ago, a possibility was pointed out that green tea intake may reduce the risk of cancer on the basis of epidemiologic studies conducted in Japan [1]. Since then, the amount of research into the anti-cancer activity of tea has increased dramatically. Many experiments using mice and rats have revealed that the rate of carcinogen-induced carcinogenesis in the animals is much lowered by taking green tea or catechins and that the growth and metastasis of inoculated cancer cells is inhibited by the consumption of tea and tea catechins [2-6]. However, several animal experiments have failed to demonstrate the anti-cancer activity of green tea or catechins [3].

Apoptosis-inducing activity of green tea components

The major compound contributing to the anti-cancer activity of green tea is the polyphenolic compound epigallocatechin gallate (EGCG) [6], although in some cases epicatechin gallate has been shown to have stronger activity than EGCG [7]. To determine the molecular mechanisms by which EGCG exhibits its anti-cancer activity, experiments have been carried out using cultured cancer cells. When added to cancer

cells in the culture medium, EGCG inhibited growth and induced cell death. Several mechanisms have been proposed for this activity. One of the leading candidates involves apoptosis, or programmed cell death. Apoptosis is physiological cell death by which unnecessary cells are eliminated. The induction of apoptosis in cancer cells leads to the prevention of cancer development, and many anti-cancer drugs are known to induce apoptosis in cancer cells [6].

EGCG has been demonstrated to induce apoptosis by binding to a cell surface protein called Fas in cultured human leukemia cells [6] (Figure 1). This binding causes the activation of protease caspase 8, which activates a caspase-dependent deoxyribonuclease that in turn degrades DNA. The degradation of DNA results in the formation of apoptotic bodies to lead cell death. The involvement of Fas in EGCG-induced apoptosis has also been found in human adrenal cancer cells [8]. It is also known that a cell surface protein called 67kDa laminin receptor is involved in EGCG-induced apoptosis [5]. It should be pointed out that EGCG has a stronger apoptosis-inducing effect on cancer cells than on normal cells [9], an effect that is desired in anti-cancer drugs.

Recently, another possible mechanism for the action of EGCG has been proposed [10]. EGCG induces apoptosis in hepatoma cells leading to the cell death. A single strand RNA containing 20-25 nucleotides (microRNA) was found to be involved in the mechanism.

The involvement of apoptosis-inducing activity in the anti-cancer effect of EGCG has also been demonstrated in animal experiments. In a study examining EGCG's cancer-preventive activity towards rat colon carcinogenesis induced by azoxymethane, it was shown that peroral administration of EGCG significantly reduced the number of colonic aberrant crypt foci representing a precancerous lesion, together with an increase in apoptosis [11]. In experiments using the autochthonous transgenic adenocarcinoma of the mouse prostate model, which spontaneously develops metastatic prostate cancer, a dose of green tea catechins proportionally achievable by humans (six cups of green tea per day) significantly inhibited cancer development, increased survival rate, and induced the apoptosis of prostate cancer cells [6].

Other components of green tea have also been shown to have anti-cancer activity. These include aqueous non-dialyzable high molecular weight components derived from green tea, black tea, oolong tea and Pu-her tea which induce apoptosis [6,12]. There have been several trials investigating the enhancement of drug activity by combining the drugs with EGCG. Intraperitoneal injection of mouse lung carcinoma cells causes the formation of tumors after 2-3 weeks, but administration of EGCG

with the anti-inflammation drug sulindac induced apoptosis and reduced the growth of the tumors more effectively than administration of the drug alone [11] (Figure 2).

Chemical modification of catechins may provide drugs with an efficacy greater than EGCG. A peracetylated EGCG derivative has been shown to exhibit enhanced growth inhibitory activity to EGCG in both human esophageal and colon cancer cells [13,14]. Intragastric administration to mice resulted in higher bioavailability. The acylation of catechin may also be an effective chemical modification for improving its anti-cancer activity.

Other mechanisms of anti-cancer effects

There are several lines of evidence indicating that EGCG elicits anti-cancer activity by mechanisms other than apoptosis induction [15]. Also, cell cycle arrest and the prevention of oxidative DNA damage by anti-oxidative activity may contribute to the anti-cancer effects of EGCG [2-4].

Evidence in epidemiological and intervention studies

There are several epidemiological studies indicating that intake of green tea reduces the risk of cancer [6]. However, lack of an effect of green tea on cancer prevention has also been reported [6]. Thus, further studies are needed to more clearly investigate the anti-cancer activity of green tea and its components. Several human intervention trials have suggested that green tea catechins have anti-cancer activity [6]. It should also be noted that many clinical trials are now under way on the standardized green tea polyphenol preparation Polyphenon E or sinecatechins as a medication to treat genital warts approved by the United States Food and Drug Administration [16].

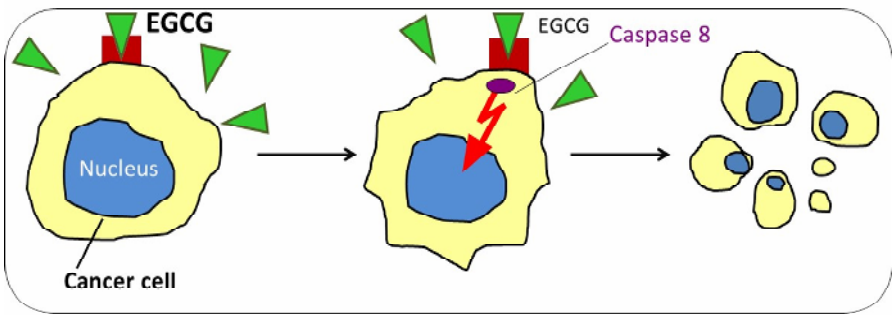

Figure 1. Apoptosis-inducing mechanism for EGCG.

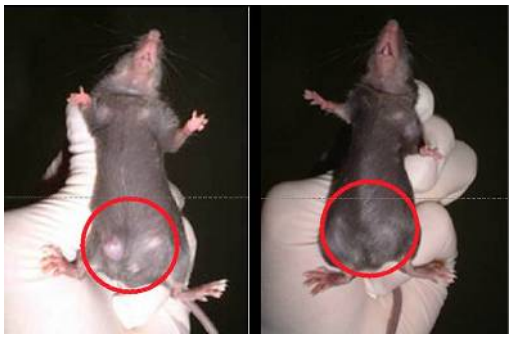

Figure 2. When mouse lung cancer cells ($1X10^6$) were inoculated into the peritoneal cavity, they propagated and formed tumor masses by 15 days after innoculation (left). Oral administration of EGCG together with intraperitoneal injection of sulindac, an anti-inflammatory drug, reduced formation of tumor masses (right).

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1.5. ANTI-METASTASIS

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Abstract

Metastasis is the major cause of morbidity and death in cancer patients. The inhibition of metastasis would markedly reduce the rate of cancer deaths. In a model depicting the blood-borne metastasis of tumor cells, metastatic cancer cells invade the circulation by degrading endothelial basement membranes. After adhering to the blood vessel wall, they extravasate by local degradation to form metastatic tumor colonies. Tumor-associated proteinases such as MMPs have critical roles in the degradation of basement membranes containing laminin, collagen IV and fibronectin. Therefore, any agent that inhibits one of these steps is expected to suppress cancer metastasis.

In both cellular and animal experiments, green tea and its catechins have been shown to inhibit the activities of MMPs, adhesion of cancer cells to the basement membranes of blood vessels, and angiogenesis. Several animal experiments have shown that green tea and its catechins inhibit metastasis. However, the efficacy of green tea and its catechins in the prevention of metastasis in humans is still unknown.

Cancer metastasis model

In a model depicting the blood-borne metastasis of tumor cells, metastatic cancer cells invade the circulation by degrading endothelial basement membranes (Figure 1) [1]. After adhering to the blood vessel wall, they extravasate by local degradation to form metastatic tumor colonies (Figure 1). Inhibition at any step of the metastatic process will be effectively inhibit metastasis and result in cancer prevention.

Inhibition of metastasis in animal experiments

Taniguchi et al. showed that the peroral administration of EGCG-rich catechins inhibited the metastasis of B16 melanoma cells and lung carcinoma cells in both experimental and spontaneous systems [2]. Similarly, Sazuka et al. detected green tea's inhibition of metastasis in lung tumor cells [3]. Since EGCG inhibited the adhesion of cancer cells to endothelial cell layers [1], and EGCG prevented cancer cells from attaching to fibronectin and laminin [4,5], these effects are considered to be involved in the anti-metastatic activity of green tea and catechins.

Inhibition of matrix metalloproteinases (MMPs)

MMPs are deeply involved at various steps of the metastasis process, including the invasion into surrounding tissues and degradation of endothelial basement membranes (Figure 1). EGCG was shown to be a strong inhibitor of MMPs, including MMP-2 and MMP-9 derived from cancer cells, and to suppress the protein and gene expression of MMPs [6-8]. Shankar et al. showed that EGCG inhibited growth, invasion, angiogenesis, and metastasis of human pancreatic cancer cells in a xenograft model, and that EGCG reduced MMP activities in the tumor, indicating that EGCG acts as an MMP inhibitor in vivo [9].

Maintenance of immune surveillance potential

Experimental tumor metastasis is enhanced in senescence-accelerated mouse prone 10 (SAMP10) strains of mice due to an age-related reduction in immune surveillance potential. In this system, it was demonstrated that natural killer cell activity reduced by aging was maintained by green tea catechins, and that catechins prevented the experimental tumor metastasis of melanoma cells in aged SAMP10 mice [10]. The finding suggests that catechins inhibit metastasis by inhibiting the age-related reduction in immune surveillance potential.

Inhibition of angiogenesis

Cancer cells produce several protein factors to stimulate angiogenesis for their own nutritional requirements. Several experiments have shown that green tea catechins inhibit angiogenesis. For example, when mouse corneas were stimulated by an angiogenesis-inducing factor, the degree of vascularization was lower in the group given green tea as compared to the group given water [11].

Experiments in a variety of intestinal cancer cells showed that EGCG inhibited the gene and protein expression and activities of various protein factors involved in angiogenesis. These factors include vascular endothelial growth factor (VEGF) and the VEGF receptor [12].

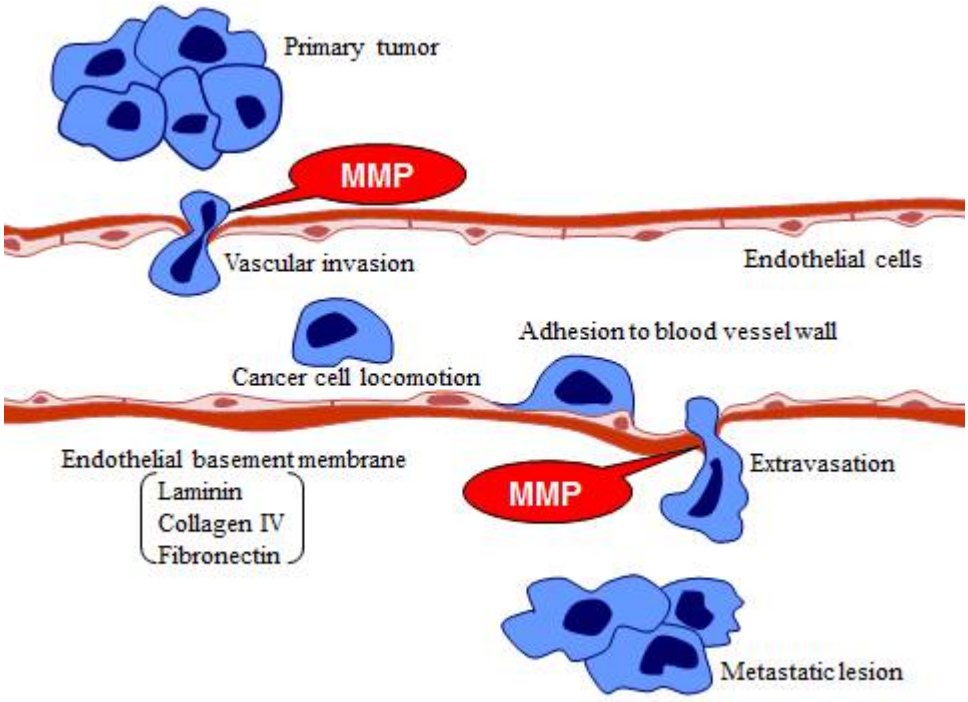

Figure 1. A model for the cancer metastatic processes [1].

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1.6. EFFECTS ON HUMAN CANCER
1.6.1. EPIDEMIOLOGICAL AND INTERVENTIONAL STUDIES

Mamoru ISEMURA (University of Shizuoka)

Abstract

Epidemiological and clinical studies have shown that green tea and tea catechins exert preventive effects against various cancers, including the stomach, colon, liver, mammary gland, and prostate cancers. However, other studies failed to demonstrate such effects. This may be due to several confounding factors, including the method of quantifying tea consumption, tea temperature, cigarette smoking, and alcohol consumption. Recently, catechin-based drugs have been developed and several lines of evidence have suggested that they are useful for preventive and therapeutic purposes in certain kinds of cancer.

Epidemiological and intervention studies

Prospective cohort and case-control studies are the two major types of epidemiological studies. The former is a cohort study that follows a group of similar individuals who differ with respect to certain factors under study to determine how these factors affect the rates of a certain outcome. In a case-control study, two existing groups differing in outcome are identified and compared on the basis of some supposed causal attribute.

In a clinical intervention study, participants are randomly divided into two groups. One group receives the test sample, such as a drug or foodstuff, while the other group receives a “placebo” that is similar in appearance but contains a non-effective ingredient, and then an outcome is compared.

Stomach cancer

Several epidemiological and intervention studies have shown the preventive effects of green tea and tea catechins on various cancers, including stomach cancer, while other studies do not support these findings. Yang et al. summarized the findings that had been reported by 2008 [1]. When more recent data are added, the preventive effects of green tea and tea catechins are suggested, but are not conclusive (Table 1).

Liver cancer

In a study that recruited 41,761 Japanese adults aged 40–79 years, the total incidence of liver cancer was 247 cases in over 9 years of follow-up [2]. In men, the multivariate-adjusted hazard ratios (HRs) for liver cancer incidence were 1.00

(reference) for <1 cup of green tea/day, 0.83 for 1–2 cups/day, 1.11 for 3–4 cups/day, and 0.63 for ≥5 cups/day (p for trend = 0.11). The corresponding data among women were 1.00 (reference), 0.68, 0.79, and 0.50 (p for trend = 0.04). Thus, green tea consumption was shown to be associated with a reduced risk of liver cancer incidence in the Japanese general population.

Ovarian cancer

A systemic review of 22 articles, including 5 epidemiological studies on ovarian cancer, found a significant association between green tea intake and both decreased ovarian cancer incidence and better prognosis [3]. The finding supports the clinical evaluation of the role of green tea or green tea components in the prevention and treatment of ovarian cancer.

Colon and prostate cancers

In prospective cohort studies with about 5 years of follow-up that included 60,567 Chinese men aged 40–74 years, 243 incident cases of colorectal cancer were identified. Regular green tea consumption was associated with a reduced risk of colorectal cancer in non-smokers (HR = 0.54). The risk decreased as the amount of green tea consumption increased. Each 2 g increment in the intake of dry green tea leaves per day (approximately equivalent to the amount of tea in one tea bag) was associated with a 12% risk reduction (HR = 0.88) [4]. No significant association was found among smokers.

A meta-analysis of 13 studies investigating the association between green tea and black tea consumption and prostate cancer risk found a significant association in Asian populations for highest green tea consumption vs. non-Asian populations/lowest green tea consumption (odds ratio [OR] = 0.62) [5]. The pooled estimate reached a statistically significant level for case-control studies (OR = 0.43), but not for prospective cohort studies (OR = 1.00). For black tea, no statistically significant association was observed (OR = 0.99).

Confounding factors

Epidemiological study results have been inconsistent. This may be due to several confounding factors, including the methods of quantifying tea consumption, tea temperature, cigarette smoking, and alcohol consumption. Future studies should incorporate the determination of biomarkers of tea polyphenols in blood and urine, as recent studies did [6-8]. Genetic polymorphisms may also influence the effects of tea consumption on cancer risk [1].

Intervention studies

In a recent study in Italy, 30 men with high-grade prostate intraepithelial neoplasias were given 600 mg of green tea catechins daily for 12 months, and only one patient developed prostate cancer, compared to 9 of the 30 patients in the placebo group [9]. This chemopreventive effect was also demonstrated 2 years later [9]. Protective effects of green tea extracts (Polyphenon E and EGCG) on human cervical lesions have also been reported [10].

Catechin-based drugs

The standardized green tea polyphenol preparation Polyphenon® E has been subjected to many clinical trials and approved for the treatment of genital warts by the United States Food and Drug Administration [11]. Randomized, double-blind, placebo controlled trials have demonstrated the efficacy and safety of Polyphenon E 15% ointment in the treatment of external anogenital warts [12]. Clearance rates with the ointment ranged from 54% to 65% with recurrence occurring in approximately 5.9% to 12% [13]. In another study, an oral 2,000 mg dose of Polyphenon E taken twice daily for up to 6 months was found to be well tolerated by patients with chronic lymphocytic leukemia [14]. Durable declines in the absolute lymphocyte count and/or lymphadenopathy were observed in the majority of patients.

Table 1. Epidemiological studies on correlation between green tea intake and the risk of human cancer*

Study type	Cohort	Case-control	Cohort	Case-control
	Risk reduction	No risk reduction	Risk reduction	No risk reduction
Colon	3	6	4	3
Lung	0	4	2	3
Stomach	2	6	8	8
Osophagus	0	2	4	5
Breast	3	5	3	0
Prostate	2	0	2	0
Ovaries	1	0	2	0
Pancreas	0	2	2	1
Kidney and	0	1	1	4

bladder				
liver	1	0	0	0
Endometrium	0	0	2	1
Thyroid	1	1	0	0
Blood	1	0	0	0

*Compiled from References [1], [2], [4], [15]-[24].

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1.6.2. EPIDEMIOLOGICAL STUDIES ON GASTRIC CANCER

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Abstract

Numerous cellular and animal studies have shown that green tea has a protective effect against cancer. However, epidemiological studies in humans have reported conflicting results. A systematic review of epidemiological evidence for the association between green tea consumption and the risk of gastric cancer in the Japanese population has suggested that green tea possibly decreases the risk of gastric cancer in women. However, current epidemiological evidence is insufficient to demonstrate any such association in men.

Prospective cohort and case-control studies

The results of database searches have identified 8 prospective cohort and 3 case-control studies as suitable for the systematic understanding of the relationship between green tea consumption and gastric cancer risk among the Japanese (Tables 1 and 2) [1-12]. In prospective cohort studies, various factors including dietary and lifestyle habits are investigated in people without diseases. A follow-up survey is carried out several years later to examine which factors are related to diseases found in the cohort. Case-control studies examine disease-related factors by comparing a group of patients with a specific disease with healthy groups matched for age and gender.

Results of prospective cohort studies

Of the 8 prospective cohort studies (Table 1), one showed that green tea consumption marginally increased the risk of gastric cancer [3], but the remaining studies did not show such an association. An inverse association between green tea consumption and distal gastric cancer was observed among women [5].

Results of case-control studies

The 3 case-control studies (Table 2) consistently showed a weak inverse association between green tea consumption and the risk of gastric cancer. In particular, Fujiki et al. [11] suggested marked protective effects of green tea in a general Japanese population.

Specific remarks

It should be noted that the protective effect of green tea is gender-dependent. When results are compared between men and women, a trend for gastric cancer risk reduction

by green tea is consistently observed in women [1,13]. Varying effects on organ subsites were also noted.

Table 1

Reference		Study	Subject					Magnitude of association*
First author	Year	period	Gender	Number	Age	Death/incidence	Number	
Nakachi	2000	1986-1999	Male and female	8,552	40+	Death	140	−
Tsubono	2001	1984-1992	Male	11,902	40+	Incidence	296	↑
			Female	14,409			123	−
Hoshiyama	2002	1988-1997	Male	30,370	40-79	Death	240	−
			Female	42,481			119	−
Sasazuki	2004	1990-2001	Male	34,832	40-59	Incidence	665	−
			Female	38,111			227	− (Distal region ↓↓)
Kahn	2004	1984-2002	Male	1524	40+	Death	36	−
			Female	1634			15	−
Sauvaget	2005	1980-1999	Male and female	38,576	34-98	Incidence	1270	−
Kuriyama	2006	1995-2001	Male	19,060	40-79	Death	138	−
			Female	21,470			55	−
Suzuki	2009	1999-2006	Male and female	12,251	65-84	Death	68	−

* ↑ ↑ ↑ or ↓ ↓ ↓ [Strong]: Relative risk, less than 0.5 or more than 2.0 (S); ↑ ↑ or ↓ ↓ [Moderate]: less than 0.5 or more than 2.0 (N) or less than 1.5 to 2.0 (S), or 0.5 to less than 0.67 (S); ↑ or ↓ [Weak]: more than 1.5 to 2 (N) or 0.5 to less than 0.67 (N), or 0.67−1.5 (N); − [No association]: 0.67−1.5 (N). (S) and (N) represent statistical significance and no statistical significance, respectively.

When tumors in the distal portion of the stomach were analyzed, the relative risk was 0.51 (95% confidence interval 0.30–0.86) in the highest category of green tea consumption (5 or more cups per day versus less than 1 cup per day) (p for trend = 0.01) [5]. A null association for upper-third gastric cancer was found for both sexes. These results were confirmed by pooling data from 6 cohort studies [13].

It can be concluded that green tea possibly decreases the risk of gastric cancer in women. However, current epidemiological evidence is insufficient to demonstrate any such association in men. Further detailed prospective studies are needed to confirm the impact of green tea on the occurrence of gastric cancer.

Table 2.

Reference		Study	Subject				
First author	Year	period	Gender	Age	Number of cases	Number of patients/Residents	Association*
Tajima	1985	1981-1983	Male and female	40-70	93	186	↓
Kono	1988	1979-1982	Male and female	20-75	139	Patients, 2547	↓
						Residents, 278	↓ ↓ ↓
Inoue	1998	1990-1995	Male and female	40+	893	21,128	↓

*See Table 1.

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1.6.3. INTERVENTION STUDY FOR COLORECTAL CANCER

Masahito SHIMIZU and Hisataka MORIWAKI (Gifu University)

Abstract

Basic research using cancer cells and laboratory animals has shown that green tea catechins inhibit the proliferation of colorectal cancer cells and carcinogenesis mediated by colonic inflammation and obesity. These findings suggest the potential of green tea catechins as preventive agents against colorectal cancer. When patients who had undergone endoscopic polypectomy were examined 1 year later to confirm a clean colon, they were divided into 2 groups, one of which received green tea extract supplementation for one year (1.5 mg/day). Follow-up colonoscopies conducted one year later indicated that green tea supplementation reduced the recurrence rate by about 50%. In addition, the size of relapsed adenomas was smaller in the supplemented group than in the control group. A reduced recurrence rate was also noted in patients who consumed 10 cups of green tea daily. The finding suggests that the daily consumption of 2.5–3.0 g of green tea extracts is effective for the prevention of recurrent colonic adenomas.

Introduction

Colorectal cancer is one of the cancer types familiar to the Japanese population, and the development of more effective methods of prevention, diagnosis, and therapy is urgently needed. Appropriate lifestyle choices, including healthful dietary decisions, refraining from smoking and excessive alcohol consumption, and engaging in regular moderate exercise are essential to the prevention of colorectal cancer.

In recent years, polyphenols, including green tea catechins, have drawn attention for their potential prevention of carcinogenesis and inhibition of cancer propagation [1-3]. Moreover, research findings have indicated that green tea catechins improve metabolic syndromes such as obesity and diabetes, which are risk factors for colorectal cancer [4-7].

Effects of green tea on colorectal cancer

Basic research using cancer cells and laboratory animals has shown that green tea catechins inhibit the proliferation of colorectal cancer cells and carcinogenesis mediated by colonic inflammation and obesity [8-12]. These findings suggest the potential of green tea catechins as preventive agents against colorectal cancer. To verify this possibility, clinical intervention studies were carried out to investigate whether or

not supplementation with green tea catechins suppresses the recurrence of colonic polyps (adenomas) after endoscopic excision.

Colorectal adenomas are the precursors to most sporadic colorectal cancers and their excision decreases the risk of colorectal cancer. It is also known that recurrence may be found within several years of endoscopic excision, necessitating periodic follow-up examinations.

In a clinical study [13], patients who had undergone endoscopic polypectomy were examined one year later to confirm a clean colon. They were then randomized into 2 groups while maintaining their usual green tea drinking habits. The supplemented group consumed 1.5 g/day of green tea extracts for one year and was compared with a non-supplemented control group. The results of follow-up colonoscopies conducted one year later indicated that supplementation with green tea extracts reduced the recurrence rate by about 50%. In addition, the size of relapsed adenomas was significantly smaller in the supplemented group than in the control group (Figure 1). No serious adverse events were observed in the supplemented group.

The recurrence rate was also reduced in patients who consumed 10 cups of green tea daily [13]. The finding suggests that daily consumption of 2.5–3.0 g of green tea extracts is effective for the prevention recurrent colonic adenomas. When drinking the daily amount of green tea necessary to achieve a protective effect is difficult, supplements may be used as an alternative method. Thus, these findings indicate the possibility that colonic adenomas and cancers may be prevented by a lifestyle that includes frequent green tea consumption.

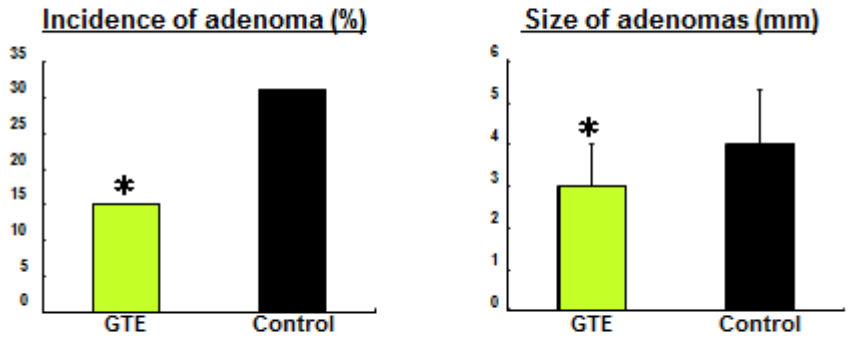

Figure 1. The group supplemented with green tea extracts (GTE) showed the reduced incidence and size of adenoma with a statistical significance.

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2. PREVENTIVE EFFECTS ON METABOLIC SYNDROME-RELATED DISEASES

2.1. EFFECTS ON BLOOD PRESSURE

2.1.1. BASIC STUDIES

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Hirofumi TACHIBANA (Kyushu University)

Abstract

The prevalence of lifestyle-related diseases such as hypertension has been increasing worldwide. In an experiment using stroke-prone spontaneously hypertensive rats, both green and black tea polyphenols attenuated blood pressure increases through their antioxidant properties. In in vitro experiments, epigallocatechin gallate and methylated catechins inhibited the activity of angiotensin I converting enzymes, which are involved in the production of angiotensin II and play a role in increasing blood pressure. Other tea components, such as theaflavin digallate and gamma-aminobutyric acid (GABA), also regulate blood pressure. In an experiment in which salt-sensitive hypertensive rats were given a diet containing 4% NaCl and received water, green tea, or gabaron tea (green tea containing a high content of GABA), for 4 weeks, the gabaron tea group showed the lowest blood pressure. Thus, tea consumption may be expected to be useful for the prevention of hypertension-related diseases such as hemorrhagic heart trouble, stroke, and renal failure.

Hypertension

The prevalence of lifestyle-related diseases such as hypertension has been increasing worldwide. The disease is of two main types: hypertension with unidentifiable causes and secondary hypertension accompanied by known causes such as renal disease, endocrine disease, and vascular disease. The former is caused by lifestyle and hereditary factors that interconnect in complicated ways, but the details are unclear.

Effects of green tea catechins on blood pressure

Several animal studies have indicated that the intake of green tea catechins reduces blood pressure [1-6]. For example, in an experiment using stroke-prone spontaneously hypertensive rats, it was shown that both green and black tea polyphenols attenuated blood pressure increases through their antioxidant properties [1]. The effective amount of green tea catechins corresponded to the amount contained in 10 cups [1]. The

depressive effects of green tea catechin intake were also noted in renal hypertensive rats [4].

Mechanism of action of green tea ingredients

The nervous system, endocrine (hormone) system, and kidney are mainly involved in the regulation of blood pressure. The kallikrein-kinin system and the renin-angiotensin system play important roles in the endocrine system. Agents with inhibitory activity against angiotensin I converting enzymes (ACE), which are involved in the production of angiotensin II, are used medically for the treatment of high blood pressure. In vitro experiments have shown that epigallocatechin gallate (EGCG) and methylated catechins inhibit the activity of ACE [6,7].

It is also known that kallikrein, which loosens vascular smooth muscle and causes blood pressure to drop, is involved in the mechanism of action of green tea catechins [4].

Other tea components, theaflavin digallate and gamma-aminobutyric acid (GABA), are also known to regulate the blood pressure. The green tea product called gabaron tea, which has a high content of GABA, is produced under conditions of very low oxygen concentration and low temperature, which result in a 20–30-fold increase in GABA content. In an experiment in which salt-sensitive hypertensive rats were given a diet containing 4% NaCl and received water, green tea, or gabaron tea for 4 weeks, the gabaron tea group showed the lowest blood pressure with the highest blood concentration of GABA [10].

Black tea also suppressed blood pressure increases in an experimental rat model of hypertension [1]. In addition, theaflavin digallate was shown to inhibit the activity of ACE in vitro [7].

In summary, since some experiments have shown that tea is effective in reducing high blood pressure [11,12], the consumption of tea may be expected to be useful in the prevention of hypertension-related diseases such as the hemorrhagic heart trouble, stroke, and renal failure.

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2.1.2. INTERVENTION STUDIES

Mari MAEDA-YAMAMOTO (National Food Research Institute, NARO)
Hirofumi TACHIBANA (Kyushu University)

Abstract

In clinical intervention studies, the intake of green tea and tea catechins was shown to improve high blood pressure. In addition, similar studies of adults with mild hypertension indicated that green tea containing methylated catechins had a stronger effect than green tea not containing methylated catechins.

Intervention studies on the effects of tea catechins on blood pressure

A clinical study that examined the effect of green tea drinking on blood pressure in 2,318 people who underwent a multiphasic health screening (human dock examination) showed that the higher the green tea consumption, the fewer the number of subjects with hypertension [1]. In addition, the results of an intervention clinical trial of the effect of tea catechin intake indicated improvement of hypertension by the dosage of 600 mg/day for 12 weeks [1].

An epidemiological study in Taiwan reported that the risk of developing hypertension was lower in the group that had habitual drinking of approximately one cup of green tea or oolong tea for at least 1 year than in the group of nonhabitual tea drinkers [2]. A Polish study demonstrated that blood pressure decreased when hypertensive patients took approximately 400 mg of green tea extract for 3 months [3]. Furthermore, when 88 English men with metabolic syndrome were divided into two groups: one group received a 400-mg capsule of epigallocatechin-3-gallate (EGCG) twice a day for 8 weeks and the other group was the control groups. The former group experienced a 2.6-mm Hg decrease in diastolic blood pressure, which was significantly different from that in the control group [4].

Clinical studies on green tea containing methylated catechins

When Benifuuki, a type of green tea containing methylated catechins, which are known to have anti-allergic properties [5,6], was administrated to patients with mild hypertension, systolic blood pressure was reduced after 8 weeks of consuming hot-water Benifuuki extracts containing 25 mg methylated catechins [7]. In another clinical study including 10 mild-hypertensive patients, intake of hot-water Benifuuki extracts containing 34 mg methylated catechin and 126 mg EGCG twice a day resulted in a decrease in blood pressure after 4 weeks and a significant decrease in systolic blood

pressure of 8.65 mm Hg in average (Figure 1) [7].

Among 20 adult men with mild hypertension, the patients who received a Benifuuki capsule containing 25 mg 3′-*O*-methylated EGCG (EGCG3 ″Me) and 122 mg EGCG showed decreases in systolic blood pressure by an average of 3.2 mm Hg and in diastolic blood pressure by an average of 6.2 mm Hg after 8 weeks, in comparison with those who received a capsule of the green tea Yabukita with 151 mg EGCG twice a day. The effect of Benifuuki was greater than that of Yabukita, which contained only small amounts of methylated catechins (Figure 2) [7].

Mechanistic aspects of the activities of the green tea components

EGCG3 ″Me and EGCG inhibited the activity of the angiotensin-converting enzyme, an enzyme associated with increased blood pressure; the activity of EGCG3″ Me was significantly stronger, at concentrations higher than 0.1 mM, than that of EGCG (Figure 3) [7]. Phosphorylation of a myosin light chain related to muscle shrinkage is known to cause essential hypertension (unidentified high blood pressure), and the inhibitory activity of EGCG3 ″Me was demonstrated to be greater than that of EGCG [8]. However, an inhibitory activity against phosphorylation was not detected in EGCG4 ″Me, a methylated catechin produced from EGCG in the liver of animals, including humans (Figure 4) [8]. Therefore, the mechanism of action on the blood pressure appears to vary according to the difference in chemical structure.

Theanine and caffeine were recently demonstrated to suppress an increase in blood pressure induced by high stress from intellectual work [9]. Various components seem to be involved in the anti-hypertension activity of green tea.

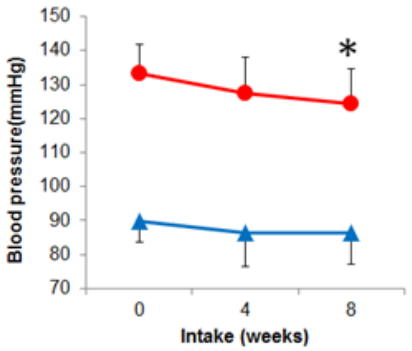
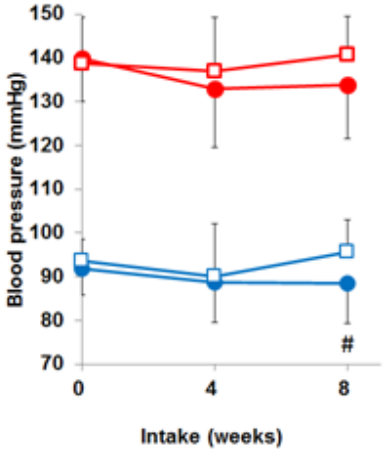
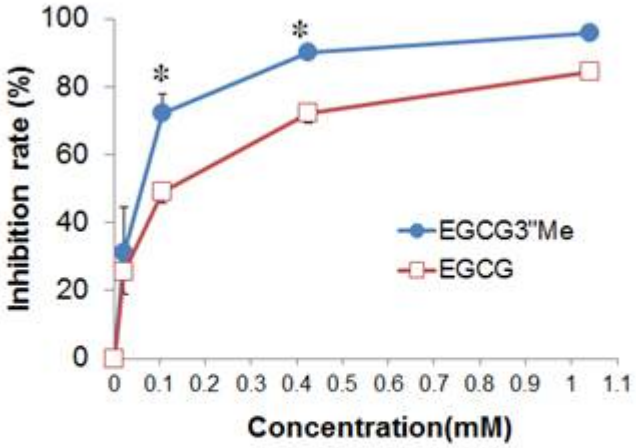


Figure 1. Changes in the blood pressure (●: systolic B.P. ▲:diastolic B.P.) of subjects taking 'Benifuuki' tea. Each point represents the mean ± S.D. Significantly different from the pre-intervension (0 week) value . *P<0.05



- Figure 2. Changes in the blood pressure of subjects taking 'Benifuuki' extract powder(●) and 'Green tea' extract powder(□). Red and blue represent systolic B.P. and diastolic B.P., respectively. Each point represents the mean±S.D. Significantly different from the 'Green tea' value. #P<0.1



- Figure 3. The inhibitory ratio on ACE activity by addition of EGCG3"Me(●) or EGCG(□). Data are expressed as mean± S.D. (n=3). *P<0.05 vs EGCG.

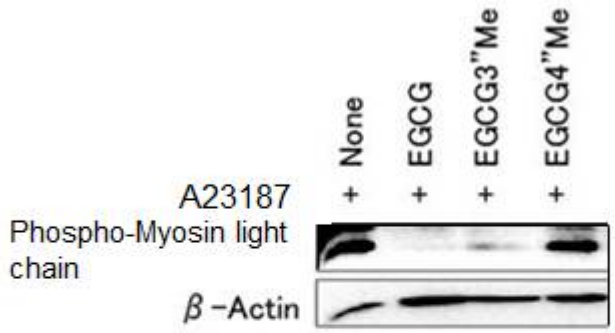


Figure 4. The effect of tea catechins on phosphorylation level of myosin light chain

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2.2. EFFECTS ON BLOOD CHOLESTEROL

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Abstract

Humans with high levels of blood cholesterol and triglycerides have a high risk of death from cardiovascular disease. Tea contains various components beneficial for health, including some that positively affect blood cholesterol levels. Animal experiments show green tea polyphenol catechins reduce the blood levels of cholesterol and triglycerides. The mechanism of action of catechins involves inhibiting the intestinal absorption of these fat components. Cholesterol is absorbed in the intestinal tract in microscopic particles called cholesterol micelles. Epigallocatechin gallate (EGCG) suppresses the absorption of cholesterol by inhibiting micelle formation. EGCG increases the uptake and degradation of low-density lipoproteins (LDL) by increasing LDL receptor expression, leading to decreased blood cholesterol levels. EGCG also decreases the synthesis of apolipoprotein B, which is indispensable for LDL biosynthesis. Thus, EGCG exhibits anti-atherosclerotic activity by both upregulating LDL expression and downregulating apolipoprotein B expression.

Cholesterol absorption

Cardio-cerebrovascular diseases are the greatest cause of death worldwide. Humans with high blood cholesterol and triglyceride levels have a high risk of the death from these diseases. It is well known that tea contains various components beneficial for health, including some positively affecting blood cholesterol levels [1].

Animal experiments show green tea polyphenol catechins reduce the blood levels of cholesterol and triglycerides. The ingestion of tea catechins is known to reduce cholesterol and neutral fat absorption in the intestinal tract [2,3]. Among the various tea catechins, epigallocatechin gallate (EGCG) is the most potent. Rats with hypercholesterolemia administered EGCG exhibit decreases in blood cholesterol levels. This decrease may be due to the mechanism by which EGCG reduces the blood cholesterol and triglyceride level, which involves an inhibition of their intestinal absorption [2-6]. Cholesterol is absorbed in the intestinal tract in the form of microscopic particles called cholesterol micelles (Figure 1) [4,6].

Effect on low-density lipoproteins (LDL) receptor gene expression

The action of EGCG is not limited to the intestinal tract. Ingested EGCG is detected

in the blood, indicating it is transported throughout the body including the liver, which is the major site of lipid metabolism. When EGCG is added to human liver cancer cell cultures, it was found to increases the expression of LDL receptor, both at the mRNA and protein levels [8].

LDL receptor plays an important role in the process by which cells take up LDL to degrade it; LDL has arteriosclerosis-promoting activity. EGCG increases the uptake and degradation of LDL by increasing the quantity of LDL receptor, leading to decreased blood cholesterol levels.

In addition, EGCG inhibits the synthesis of apolipoprotein B, which is indispensable for LDL biosynthesis (Figure 2). These findings indicate EGCG exhibits anti-arteriosclerotic effects by both upregulating LDL receptor expression and downregulating apolipoprotein B expression [8].

Notably, several human studies demonstrate that increased green tea consumption lowers the risk of death from cardiovascular diseases [9-11].

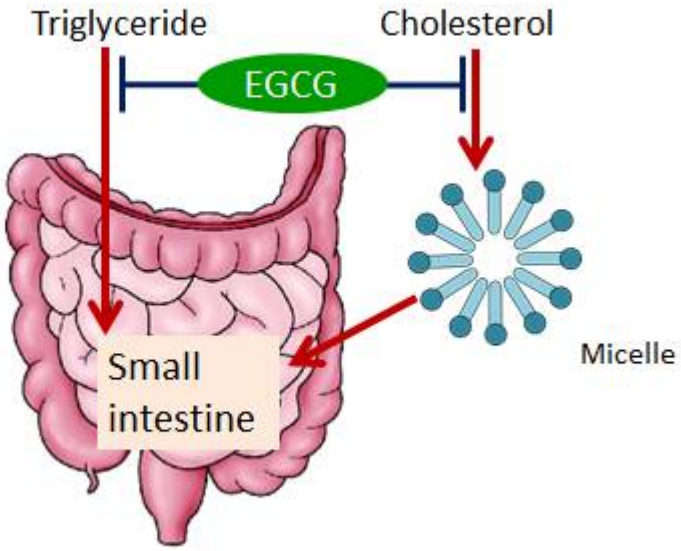

Figure 1. Cholesterol is absorbed in the intestinal tract in the form of cholesterol micelles. EGCG inhibits micelle formation and triglyceride absorption.

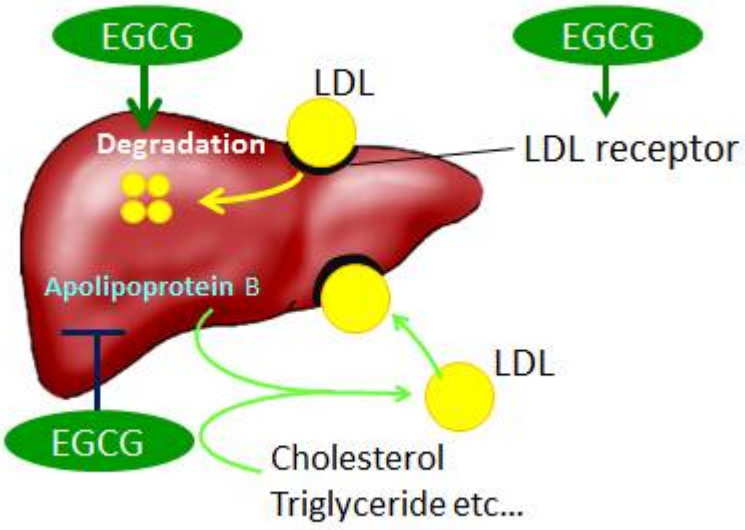

Figure 2. EGCG enhances the synthesis and degradation of LDL-receptor protein and inhibits the synthesis of apolipoprotein B.

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2.3. PROTECTION AGAINST ATHEROSCLEROSIS

Masahiko IKEDA (Fuji Tokoha University)

Abstract

When the blood concentration of cholesterol exceeds the physiological requirements, the blood level of low-density lipoprotein (LDL), a carrier of cholesterol, becomes high. This event causes the oxidation of LDL, generating oxidized LDL, which plays essential roles in the formation of atherosclerotic plaques. When the plaque reaches a certain size, it bursts, leading to myocardial and cerebral infarction. Green tea components such as epigallocatechin gallate and epigallocatechin are known to prevent the development of atherosclerosis by suppressing clot formation via the inhibition of platelet aggregation.

Atherosclerosis development

The human body comprises 60 trillion cells. Cholesterol is a component of the cell membrane and is an important compound from which steroid hormones, bile acid, and vitamin D, among others, are produced. Low-density lipoprotein (LDL), which is colloquially referred to as “bad cholesterol,” plays an essential role in the transportation of cholesterol from the liver to peripheral tissues via blood. When the peripheral tissues incorporate enough cholesterol, further incorporation is halted. If the amount of cholesterol exceeds the requirement, cholesterol will not be incorporated, consequently increasing the blood cholesterol level.

Excessive long-term elevations in blood levels of LDL cause LDL to infiltrate subendothelial tissues where it is oxidized. This oxidized LDL injures the endothelium of blood vessels and promotes platelet aggregation, which triggers clot formation. Monocytes are subsequently recruited around oxidized LDL and differentiate into macrophages, which engulf and thus eliminate oxidized LDL. A macrophage is a cell engaged in the removal of invading bacteria and viruses. In the presence of excessive cholesterol, these cells, along with intimal smooth muscle cells, become foam cells. The debris of dead foam cells deposits on subendothelial layers to initiate the formation of atherosclerotic plaques. Enlarged plaques may burst, leading to thrombogenesis by platelet recruitment and eventually to angina pectoris, myocardial infarction, and cerebral infarction [1,2].

Preventive effects of green tea against metabolic syndrome-related diseases

Tea catechins prevent plaque formation by suppressing LDL oxidation through their

antioxidant properties (Figure 1) [3]. Experiments on the oxidative impairment of porcine serum LDL in the presence of 5 μM Cu^{2+} show that oxidation is strongly inhibited by various tea catechins such as epigallocatechin gallate (EGCG) and epicatechin gallate [4,5]. These catechins have also been found to inhibit platelet aggregation in rabbits (Figure 1) [6].

In an intervention study, the tea group ingested 300 mg green tea catechin extract twice daily for 1 week. Plasma levels of oxidized LDL were then examined. The results indicate the tea group had a plasma EGCG concentration of 56 nmol/L at the end of the experiment. Moreover, no plasma EGCG was detected before the experiment, and the degree of LDL oxidation was lower in this group than the control group [7]. Therefore, these results suggest that the daily consumption of 7–8 cups green tea may reduce the oxidative susceptibility of LDL, reducing the risk of cardiovascular diseases.

Anti-atherogenic effects of tea catechins

When 10-week-old apoE-deficient mice were fed an atherogenic diet for 14 weeks, supplementation with a green tea extract (0.8 g/L) significantly reduced atheromatous areas and aortic weights compared to the water-treated group [8]. In addition, aortic cholesterol and triglyceride contents were 27% and 50% lower in the tea group than the control group, respectively. These results suggest a long-term ingestion of tea catechins prevents the development of atherosclerosis in apoE-deficient mice, probably through their potent antioxidative activity.

Concluding remarks

These findings suggest orally ingested tea catechins are absorbed in the digestive tract and enter blood circulation where they prevent the onset and development of atherosclerosis by inhibiting LDL oxidation and platelet aggregation, which lead to thrombus formation. Because most ingested catechins are excreted in urine [9,10], it is necessary to drink green tea several times per day at short intervals to maintain the blood concentration of catechins.

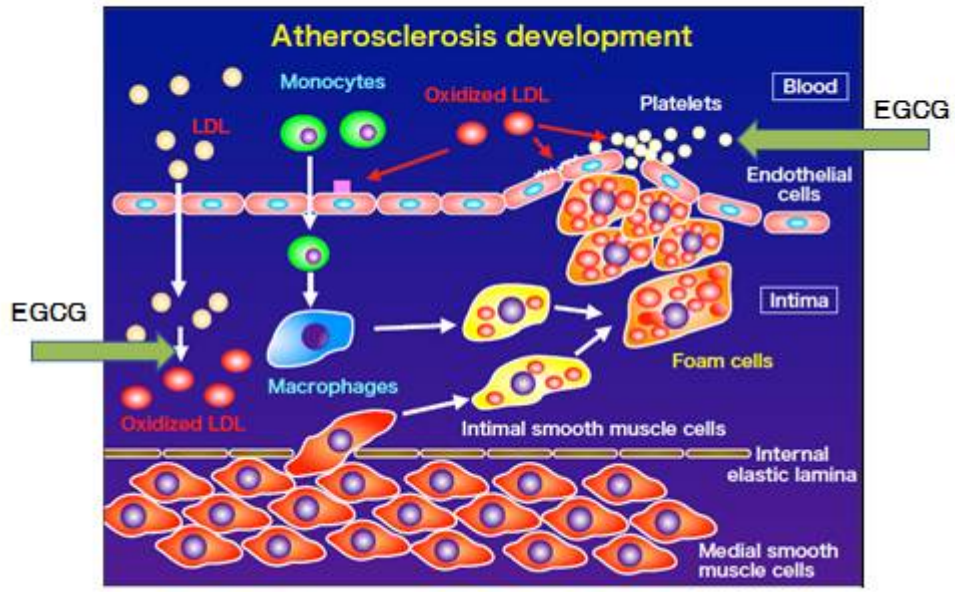

Figure 1. EGCG inhibits LDL-oxidation and platelet aggregation (green arrow).

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2.4. EFFECTS ON OBESITY
2.4.1. BASIC STUDIES

Kazutoshi SAYAMA (Shizuoka University)

Abstract

Green tea suppresses fat accumulation in the body by inhibiting adipocyte proliferation and differentiation, promoting lipolysis and improvement of lipid metabolism in liver. These effects are produced efficiently by a combination of catechins and caffeine, which are major components of green tea.

Obesity as a major cause of metabolic syndrome

Consumption of a western diet, which involves high fat intake, is now common in Japan. As a result, the number of patients with metabolic syndrome is consequently increasing [1]. It is known that the main cause of the disease is obesity. Obese patients frequently suffer from hyperlipidemia and/or hypertension, which promote the development of diabetes or could result in a myocardial infarction or stroke, all of which have life-threatening implications. Therefore, the prevention of obesity is important for living a healthy life.

Preventive effects of green tea components against obesity

Animal experiments showed green tea had an obesity-suppressing effect in that it inhibits fat accumulation. Weight of isceral fat in mice fed a diet containing 2% green tea powder for 4 months showed an approximately 60% reduction compared to the control mice fed a diet without green tea powder (Table 1). The lipid levels in the blood and liver decreased conspicuously in the green tea-fed group (Table 1) [2]. The combination of catechins and caffeine, which are both major components of green tea, also had similar effects [3].

Possible mechanism

The combinational action of catechins and caffeine may involve the suppression of lipid synthesis through the inhibition of enzymes involved in fatty acid synthesis and enhancement of the activity of lipid-degrading enzymes in the liver, a central organ of lipid metabolism [4]. Moreover, this combination has been demonstrated to effectively inhibit adipocyte proliferation as well as promote lipolysis and thermogenesis in adipocytes [4].

Concluding remarks

Fat consumption is efficiently promoted by exercise together with green tea intake, which could protect against obesity [5]. This and the above mentioned findings indicate daily green tea consumption with moderate physical activity is able to maintain the proper body weight without severe dietary restriction [6].

Table 1. Anti-obesity action by green tea

Treatment ratio		1 %	2 %	4 %
Body weight			↓	↓↓
Weight of IPAT			↓	↓↓
Food intake				↓
Lipid levels (Serum)	TC			
	TG		↓	↓
	PL			
	NEFA		↓	↓
Lipid levels (Liver)	TC		↘	↓
	TG		↘	↓
	PL			
Leptin level (serum)			↓	↓

IPAT : Intra-peritoneal adipose tissues

TC : Total cholesterol、TG : Triglyceride、

PL : Phospholipid、NEFA : Non-esterified free fatty acid

↘ : Tendency to decrease compare to the control.

↓ & ↓↓ : Significant difference compared with the control (P<0.05 & P<0.01)

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2.4.2. PREVENTIVE EFFECTS ON HUMAN OBESITY

Ichiro TOKIMITSU (R&D Research Fellow, Kao Corporation)

Abstract

A number of studies involving over 1,000 subjects have shown that the ingestion of green tea catechins causes a reduction in body fat. For example, the results of a human intervention study of 80 men and women with slight obesity showed that the group that consumed green tea with a high content of catechins had lower body weight, BMI (body mass index), and abdominal fat quantity as compared with the group that consumed green tea with a much lower catechin content. The results of several studies indicate that the long-term ingestion of tea catechins enhances energy expenditure and dietary fat oxidation in healthy subjects and that tea catechins stimulate lipid catabolism in the liver and muscle. These findings suggest that tea catechins reduce body fat by enhancing the consumption of fat preferentially as an energy source.

Effects of green tea containing a high concentration of catechins

It is known that the continuous intake of tea catechins at high concentrations is effective in reducing body fat through the enhancement of internal lipid metabolism and energy expenditure. Several studies have provided the following evidence based on the data obtained from over 1,000 subjects [1-13]:

1) Effective amounts of catechin intake are in the range of 500–600 mg/day per person.
2) Target subjects are males and females with slight obesity and a high visceral fat content.
3) There is no rebound phenomenon even after intake cessation.
4) There is no evidence indicating problems in physical findings and blood chemistry.

For example, in a human intervention study, 80 men and women with slight obesity were divided into 2 groups. One group ingested one bottle of green tea with a high content of tea catechins daily for 12 weeks while maintaining regular eating habits and physical activity and the second group similarly ingested regular green tea drink (control drink). The results showed that the former had lower body weight, BMI (body mass index), and abdominal fat quantity (sum of both visceral fat and subcutaneous fat) as compared with the latter (Figure 1) [4].

Mechanism of action of tea catechins

The results of several studies indicate that the long-term ingestion of tea catechins

enhances energy expenditure and dietary fat oxidation in healthy subjects and that tea catechins stimulate lipid catabolism in the liver and muscle [14-16]. These findings suggest that tea catechins reduce body fat by enhancing the consumption of fat preferentially as an energy source.

Lifestyle improvements such as undertaking an exercise regimen and developing healthy eating habits are important for the prevention of obesity and metabolic syndromes, but the practice is accompanied by difficulty. Daily ingestion of green tea can be undertaken with ease and lead to lifestyle improvement. Since scientific evidence for the prevention and improvement of obesity by tea drinking is currently accumulating, the habitual ingestion of green tea or tea catechins is expected to be helpful in reducing the risk of lifestyle-related diseases including hyperglycemia, hyperlipidemia, and hypertension, leading to the prevention of arteriosclerotic diseases.

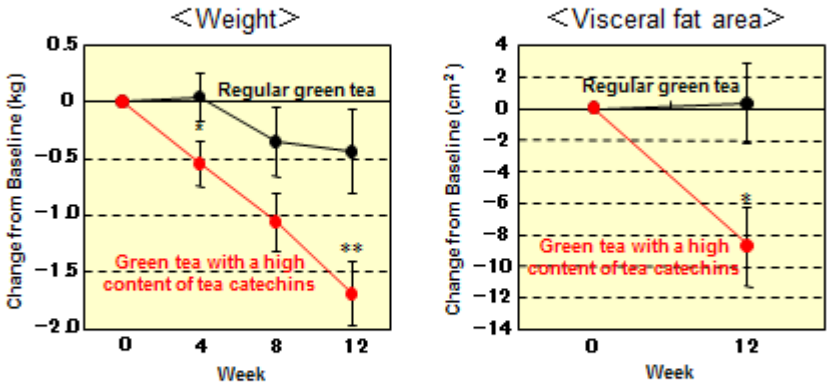

Figure 1. Effects of tea catechins on weight and visceral fat area.

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2.5. ANTI-DIABETIC EFFECTS

Noriyuki MIYOSHI (University of Shizuoka)

Abstract

Diabetes is a disease in which a person has chronically high blood sugar levels, and causes retinopathy, a kidney disease, as well as neuropathy. The ingestion of green tea is effective in preventing a rise in blood sugar levels. Several mechanisms of action are involved in this effect; these are as follows: 1. Inhibition of α-amylase activity, which is involved in the making of sugar from starch in the digestive juice, resulting in a reduction in glucose production and uptake in the digestive tract; 2. Promoting the glucose intake into skeletal muscle and adipose tissue; 3. Enhancement of insulin (hormone that lowers blood sugar levels) sensitivity, and protecting pancreatic β cells; and 4. Suppression of hepatic gluconeogenesis (glucose production from non-carbohydrates) to prevent a rise in postprandial blood glucose levels.

Introduction

Diabetes is the disease in which blood sugar levels are abnormally high after a meal and/or chronically. There are various types of diabetes, but approximately 90% of the cases of diabetes in Japan is type 2 diabetes caused by lifestyle factors stemming from eating and exercise habits. Long-term hyperglycemia will cause capillary disorders and lead to diabetes-related complications such as retinopathy, kidney diseases, and neuropathy. Green tea is expected to have beneficial effects in diabetes, a representative of the lifestyle-related diseases.

Experiments with cultured cells and animals

Studies using cultured cells and laboratory animals have clearly demonstrated the anti-diabetic activity of green tea and its ingredients, including epigallocatechin gallate (EGCG). Green tea and its ingredients exert anti-diabetic activity through the inhibition of α-amylase and α-glucosidase activities, inhibition of glucose absorption in the small intestine, protection of pancreatic β cells, the improvement of insulin sensitivity of peripheral organs, and inhibition of gluconeogenesis (making glucose from non-carbohydrate materials such as amino acids) in the liver (Figure 1).

Because α-amylase and α-glucosidase are enzymes necessary to produce glucose from dietary starch or sugar in the body, inhibition of these enzymes contributes to the prevention and suppression of the progress of diabetes by inhibiting the rise of blood

sugar levels [1,2]. Similarly, the inhibition of glucose absorption in the small intestine suppresses the rise of blood sugar levels [3].

The improvement of insulin sensitivity result in the rapid suppression of blood glucose level, which increases after meals by promoting glucose uptake by peripheral tissues. When ingredients from green tea, black tea, or an oolong tea were added to cultured fat cells derived from rats, they showed insulin-like activity by acting to increase the uptake of glucose [4]. A major ingredient exhibiting this activity in green tea and oolong tea is EGCG, while in black tea tannin and theaflavins exhibit this activity in addition to EGCG.

Insulin-secreting pancreatic β cells are sometimes injured by diabetes-associated factors, resulting in cell death in the worst case scenario, but EGCG is known to protect against this cellular damage [5]. The daily intake of green tea may have a protective effect against diabetes because the rise of blood sugar levels was suppressed in diabetic rats with drug-destroyed pancreatic β cells after they received EGCG for 8 weeks as compared with control rats given no EGCG [6].

An increasing number of reports describe the inhibitory effect of EGCG on gluconeogenesis. The administration of green tea or EGCG to cultured hepatocytes and hepatoma cells caused a decrease in the amount of gluconeogenic enzymes, glucose-6-phosphatase, and phosphoenolpyruvate carboxykinase through the suppression of their gene expression [7,8]. In this sense, EGCG has an insulin-like activity.

Animal experiments produced similar results [9]. One possible mechanism is that tea catechins including EGCG suppress the expression of transcription factors controlling the expression of gluconeogenic enzymes, leading to their reduced activities and resulting in diminished glucose synthesis [10].

Epidemiological and intervention studies

Several reports of epidemiological studies have described the anti-diabetic effect of green tea [11-15]. In an epidemiological study of Japanese subjects, the ingestion of green tea was shown to reduce the risk of type 2 diabetes [12]. The results of an intervention study on 60 male and female residents in Shizuoka Prefecture with mild hyperglycemia showed that the ingestion of green tea extracts decreased levels of hemoglobin A1c, one of the blood markers for the diagnosis of diabetes [14]. On the other hand, the ingestion of catechins or theaflavins for 3 months had no effects on the level of hemoglobin A1c in patients with a medical history of diabetes of more than 6 months in a study conducted in the United States [15].

Thus, the results of human studies are conflicting. This may be due to differences in

genetic and environmental factors such as race, sex, age, and lifestyle, and differences in the ingredients, concentrations, drinking frequency, and drinking period of tea. Therefore, it is necessary to clarify the antidiabetic effects of green tea by taking these factors into account in future studies. Because diabetes raises the risks of diseases such as liver and colon cancer in addition to obesity and arteriosclerosis, the habitual drinking of green tea would help in the primary prevention of various diseases.

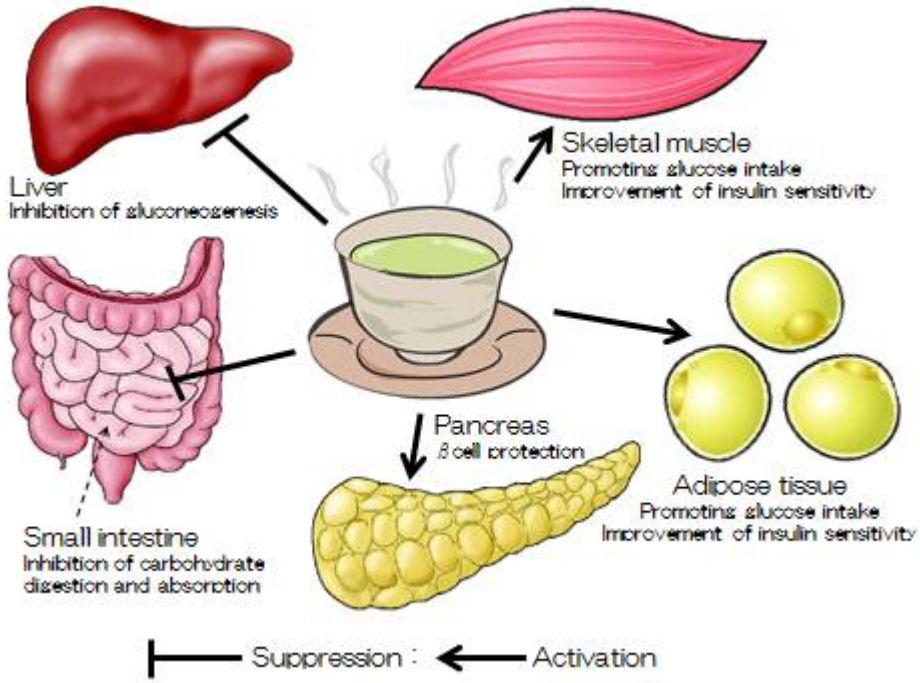

Figure 1. Effects of green tea and its ingredients.

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2.6. EPIDEMIOLOGICAL STUDIES ON METABOLIC SYNDROME

Mamoru ISEMURA (University of Shizuoka)

Abstract

Epidemiological studies to date have so far failed to show that consumption of green tea prevents metabolic syndrome. However, clinical intervention studies have suggested that green tea has a positive effect on this condition.

Metabolic syndrome

The diagnostic criteria for metabolic syndrome in Japan reported in 2005 include a waist circumference at the height of the navel of ≥85 cm for males or ≥90 cm for females as well as two or more of the following:

1) a plasma HDL cholesterol concentration <40 mg/dL or a plasma triacylglyceride concentration >150 mg/dL or both.
2) diastolic blood pressure >85 mmHg or systolic blood pressure >130 mmHg or both.
3) a fasting blood sugar concentration >110 mg/dL.

The transition from obesity to metabolic syndrome is associated with arteriosclerosis, and the condition also increases the risks for myocardial and cerebral infarction.

Clinical intervention studies

Improvement of each of the variables including body weight, body mass index (BMI), body fat, waist circumference, blood pressure, triglycerides, LDL-cholesterol, HDL-cholesterol, blood glucose concentration, and hemoglobin A1c may prevent the development of metabolic syndrome. Green tea, which was already reported to reduce fat accumulation in the book 'Yojyokun' written by Ekiken Kaibara during the Edo period, has been subjected to numerous analyses to determine its effects on human health [1]. A number of animal experiments [1-4] and clinical intervention studies [3-8] have confirmed the fat-suppressing effects of green tea and green tea catechins. For example, an intervention study of 195 male and female subjects aged 20–65 years with a high BMI indicative of obesity revealed that subjects with high catechin intake had lower body weight, BMI, abdominal circumference, total abdominal fat area, and visceral fat area values after 12 weeks compared with the placebo group [4,5].

Another study of patients with metabolic syndrome in the United States reported that body weight and BMI were decreased after 8 weeks among subjects who consumed green tea and green tea catechins compared with those who consumed water alone [7].

In addition, the blood lipid oxidation index was decreased among subjects in the green tea group. In Brazil, a study of elderly patients with metabolic syndrome revealed that green tea consumption for 60 days led to reductions in body weight, BMI, and abdominal circumference [8].

On the other hand, a study in the U.K. showed that 40–65-year-old obese men who ingested a capsule containing 400 mg of *epigallocatechin gallate* twice a day for 8 weeks experienced a reduction in diastolic blood pressure, but no other significant effects on metabolic syndrome-related indices such as the insulin resistance index were noted [9].

Epidemiological studies

An epidemiological study of 1,902 Japanese men and women aged 40 or older found no correlation between green tea intake and metabolic syndrome since green tea consumption did not appear to have any influence on blood pressure, abdominal circumference, fasting plasma glucose, or lipid indices [10]. In addition, a Japanese cohort study of 554 adults in Tokushima reported similar findings. Both studies, however, found that coffee consumption lowered the risk of metabolic syndrome [11].

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3. HEPATO-PROTECTIVE EFFECTS

3.1. EFFECTS ON HEPATITIS AND LIVER FIBROSIS

Takuji SUZUKI (Yamagata University)

Abstract

Severe and sustained inflammation may induce liver fibrosis, the stage before cirrhosis and liver cancer. Green tea catechins inhibit the biosynthesis of inflammation-promoting proteins and collagen and suppress the onset and development of hepatitis and liver fibrosis. The increase of reactive oxygen species (ROS) in the liver causes cellular damage that leads to hepatitis. Epigallocatechin gallate has antioxidant activity that removes ROS and prevents hepatitis. Ingestion of excessive amounts of green tea catechins may decrease liver function. Therefore, it is necessary to be aware of excessive intake of green tea ingredients.

Hepatitis and liver fibrosis

Viral hepatitis is the most common hepatitis type in Japan. There are three types, depend upon virus type, including A, B and C, respectively. As the other, hepatitis can be subdivided into alcoholic and non-alcoholic types. Acute or chronic inflammation of the liver is a symptom of all hepatitis types. When this inflammatory state lasts long-term or when high levels of inflammation occur in the short term, hepatocytes are injured and collagen and other connective tissue components accumulate in the injured area causing liver fibrosis. Liver fibrosis may progress to cirrhosis and eventually to liver cancer. Therefore, preventing inflammation and relieving liver fibrosis are key factors in the prevention of hepatitis.

Hepatoprotective effects of green tea

To examine the hepatoprotective effects of green tea, several studies have used an animal model in which viral hepatitis, similar to that in humans, was induced in animals [1-7]. Rats that consumed the green tea beverage had reduced hepatic gene expression of inflammation-promoting proteins such as tumor necrosis factor-α and interleukin-1β; as a result, their blood protein concentrations decreased [3]. Consistent with the finding, histochemical observations indicated that the degree of hepatic injury was suppressed in the green tea group treated with a hepatitis inducing drug, galactosamine (Figure 1).

In a similar experiment in which the chronic effects of green tea were examined, green tea effectively prevented the progression of hepatitis to liver fibrosis. The ingestion of green tea reduced the genetic expression of fibrosis-promoting factors,

collagen, and transforming growth factor-β, a finding that suggested the mechanism of the anti-fibrotic action of green tea [5].

Anti-oxidative action of green tea

Among green tea catechins, epigallocatechin gallate (EGCG) in particular has very strong anti-oxidant activity that immediately removes reactive oxygen species (ROS) produced in the body. When ROS are generated in large quantities in the body, oxidative stress causes serious damage in various kinds of cells. Excessive ROS-induced oxidative stress in the liver may cause hepatitis. The hepatitis-preventive and relieving actions of catechins may result from their strong anti-oxidant ability [8.9]. Thus, green tea intake is expected to prevent the development of hepatitis and cirrhosis.

Side effects of green tea ingredients

The ingestion of excessive amounts of polyphenols, including green tea catechins, may not only put a burden on the liver's detoxification system but also negatively affect liver function [10,11]. In one study, when animals ingested highly concentrated EGCG (1500 mg/kg), serum concentrations of alanine aminotransferase, an index of liver damage, increased markedly, while the indexes of oxidative stress also increased. The findings of that study suggest that humans should be careful to avoid excessive intake of green tea ingredients.

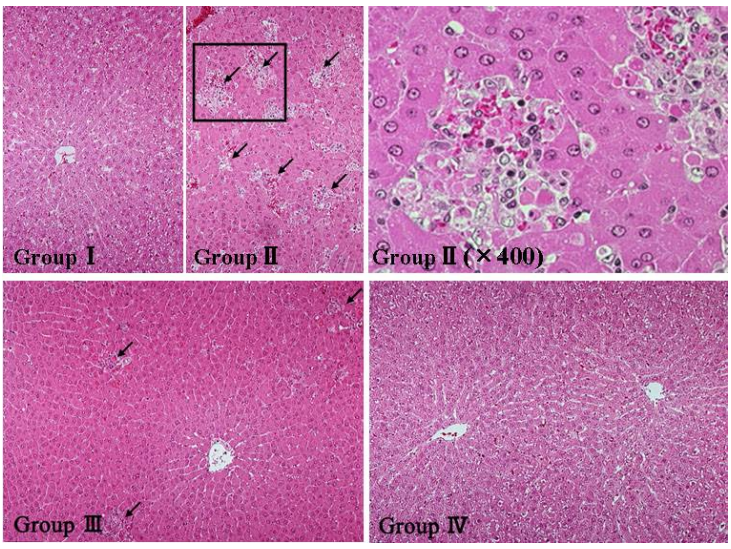

Figure 1. Preventive effect of green tea against drug-induced hepatitis in rats [3]
Group I, control; Group II, galactosamine -treated; Group III, galactosamine/green tea-treated; Group IV, green tea-treated.

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3.2. PREVENTIVE EFFECTS ON CHRONIC HEPATITIS C

Yoichi SAMESHIMA (Kakegawa Municipal General Hospital)

Abstract

When a patient suffers from chronic hepatitis C, iron accumulates in the body. Excessive quantities of iron may cause insulin resistance (diabetes) and liver steatosis, and their progression reduces the curative effect of interferon, which is used to treat hepatitis. Green tea inhibits the absorption of iron and the onset of fatty liver and may prevent an iron-induced decrease in the drug's curative effect. Green tea catechins have anti-viral properties and may prevent invasion of the hepatitis C virus into hepatocytes, blocking the onset and aggravation of chronic hepatitis C by suppressing viral propagation in hepatocytes.

Chronic hepatitis C and iron surplus

Iron accumulates in patients with chronic hepatitis C [1]. An iron surplus increases oxidative stress, which leads to resistance against therapeutics and promotes carcinogenesis within various lesions [2]. These patients begin to accumulate fat and develop insulin resistance (diabetes) [3]. The available causal treatment of chronic hepatitis C is currently the only interferon (IFN) treatment, but the iron surplus makes the disease resistant to IFN and decreases its curative effect [4]. Bloodletting is an effective method for IFN-resistant chronic hepatitis C because it can remove iron [5]. An iron-limited diet is also useful. Thus, the active intake of green tea is recommended since it inhibits the absorption of iron [6].

Effects of green tea catechins

One report states that catechins are effective against hepatitis C, the main cause of liver cancer in Japanese patients [7]. According to the statistical report from the Ministry of Health, Labor, and Welfare of Japan, the death rate of liver cancer is relatively low in the western areas of Shizuoka Prefecture [8]. These areas are among the major production centers of green tea, and their inhabitants habitually ingest green tea. Thus, the frequent intake of green tea may be one of the reasons for the low death rate of patients with liver cancer.

It was recently reported that catechins prevent the invasion of the hepatitis C virus into hepatocytes [9] and suppress the viral propagation within hepatocytes [10]. Green tea has anti-viral properties that reduce hepatic lesions induced by factors such as iron toxicity and oxidative stress in chronic hepatitis C. It also decreases IFN resistance, which may increase its curative effect.

Intervention studies of green tea catechins

In a clinical trial of intractable chronic hepatitis C with the quantity of type 1 super high virus (>850 KIU/mL) and histological diagnosis verified by liver biopsy, the patients received a combination chemotherapy regimen of 6 g of green tea powder and IFN/ribavirin, the latter of which is used in conventional therapy. The results indicated no serious side effects, and all of the patients completed the treatment. The complete recovery rate prior to this clinical trial was 16%, whereas that after this therapy was 56%. The green tea expense was <0.5% of the total treatment costs [11].

For the treatment using pegylated-IFN/ribavirin/green tea, patients received green tea powder 4 weeks before and during the treatment. The results indicated an increase in the plasma level of adiponectin (also known as the 'longevity hormone'), which has anti-metabolic syndrome action. Patients with adiponectin concentrations <15 μg/mL showed increased rates of complete recovery, whereas those with no increase in adiponectin had lower recovery rates (Figure 1) [12]. The histopathological examination results indicated that the higher the oxidative stress marker levels in the hepatocyte nuclei, the more resistant the hepatitis C was to the IFN therapy, suggesting that the degree of oxidative stress is largely associated with the curative effect [13].

These findings indicated that the combined use of green tea increased the safety and effectiveness of IFN/ribavirin therapy for chronic hepatitis C. We speculate that the green tea combination improves IFN therapy by the following mechanisms: relieving iron toxicity and oxidative stress, increasing adiponectin levels, improving lipid metabolism and insulin resistance, and suppressing the invasion and propagation of hepatitis C virus.

Concluding remarks

Recent studies in the United States and in European countries have shown that coffee intake reduces the risk of liver cancer, increases the IFN curative effect, and decreases the risk of death from all causes [14-16]. It will be necessary to perform randomized controlled clinical trials and large-scale nourishment epidemiological studies to confirm the effect of green tea in Japan, where green tea is markedly consumed.

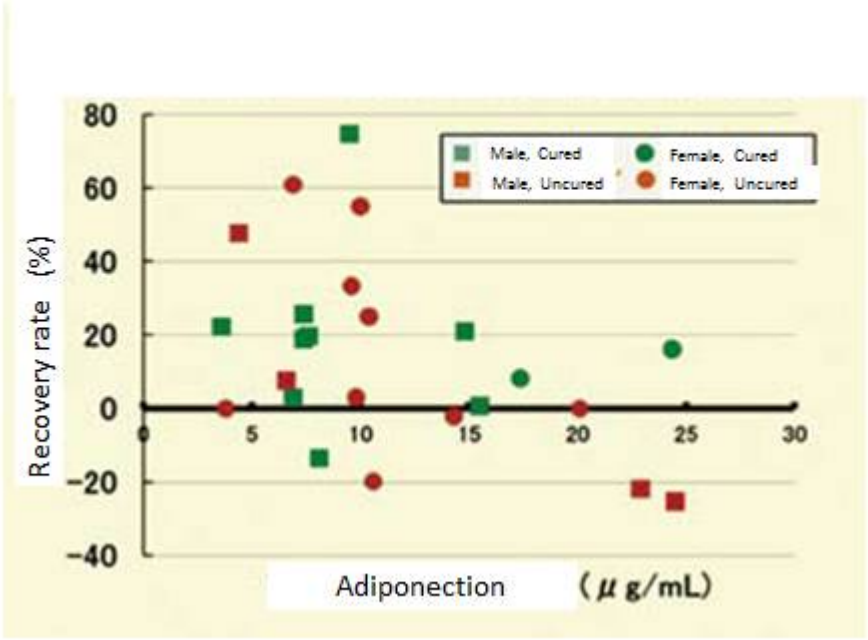

Figure 1. Patients with adiponectin concentrations <15 μg/mL showed increased rates of complete recovery, whereas those with no increase in adiponectin had lower recovery rates.

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4. ANTI-SENESENCE EFFECTS

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Abstract

Reactive oxygen species (ROS) are generated as a natural byproduct of normal metabolism of oxygen in the body, but these are usually degraded immediately by antioxidative enzymes. When this balance collapses, leading to the accumulation of ROS, the oxidative injury accelerates senescence processes, including brain atrophy and cognitive dysfunction. As tea catechins have antioxidant activities, they are able to suppress oxidative injury and senescence. Mental stress is also known to accelerate senescence. Theanine, a component of green tea, can reduce stress by suppressing an excessive release of glutamate, which is the principal excitatory neurotransmitter in the brain.

Anti-senescence and healthy longevity

Recently, the Ministry of Health, Labor and Welfare of Japan reported that the Japanese healthy life expectancy (healthy longevity), i.e., the duration for which one can live independently without requiring routine care, was 70.4 years for men and 73.6 years for women as of 2010. Because the average Japanese lifespan is 79.4 years for men and 85.9 years for women, as reported by the Japanese Ministry of Health, Labor and Welfare in 2011, shortening the 9–12-year difference between the average lifespan and healthy life expectancy is important.

In general, senescence is not considered an illness, but in age-related diseases such as cancer, lifestyle-related diseases, cardiovascular disease, and dementia, senescence is an important risk factor. Therefore, the suppression of senescence is expected to contribute to the extension of healthy life expectancy. The progress of senescence mainly depends on physical dysfunction, but various environmental or nutritional factors can also influence the aging process. For example, the old Japanese saying that “moderate eating keeps the doctor away” is proven by experiments in monkeys in which a restriction of food intake extended lifespans and decreased the occurrence of diseases including cancer, cardiovascular disease, and diabetes [1]. It has been proposed that the activation of the gene called “the longevity gene” is one of the mechanisms by which limited caloric intake prevents senescence [2,3].

Anti-senescence activity of green tea

The accumulation of oxidative injury by ROS is an important promoter of

senescence. Small quantities of ROS are generated in the mitochondria when a cell produces energy, but these are usually reduced to water immediately by antioxidative enzymes. However, when this balance collapses, oxidative stress accumulates to injure cells. Because catechins contained in green tea have strong antioxidative activities, they are expected to be able to suppress the accumulation of oxidative injury in the living body. Animal experiments have shown that green tea catechins suppressed the age-associated atrophy of the brain and functional declines in learning and memory (Figure 1) [4-6].

Because it has been found that the ingestion of green tea catechins suppresses oxidative injury not only in the brain but also in the liver and kidney, it appears useful for the overall improvement of the physical oxidation-reduction balance. Since ROS are constantly generated in the body, drinking green tea frequently at meal times or during breaks between meals may be effective for the prevention of oxidative injury accumulation.

Anti-senescence activity of green tea

These days many people feel stress. Moderate stress may have positive effects, but it is possible that long-term stress not only triggers depression and cardiovascular diseases, but also accelerates senescence. Experimental results showed that placing laboratory animals under conditions of long-term psychosocial stress related to social relationships, as in human society, caused life shortening and acceleration of brain senescence, including brain atrophy and reduced learning ability [7].

These findings indicate that mental stress accelerates senescence. The ingestion of theanine is now known to suppress the shortening of life and the functional decline of the brain even under stressful conditions [7]. Thus, theanine is able to suppress senescence by reducing stress. Theanine has been reported to act to the glutamine transporter and show anti-stress action by inhibiting an excessive release of glutamate, thereby decreasing the concentration of glutamate, an excitatory neurotransmitter, in the brain [8].

Studies in human subjects

Because the effect of theanine is partly inhibited by green tea catechins and caffeine [9], it might be useful to ingest theanine-rich tea as well as theanine supplements to obtain an expected effect of theanine. In fact, it has been reported that improvement was observed in the cognitive function of elderly people who took a capsule of theanine-rich green tea powder [10].

Because various factors participate in "senescence", the mechanism has yet to be

completely elucidated. Although the progress and causes of senescence might vary in different organs, catechins and theanine are expected to be able to suppress the senescence of the brain.

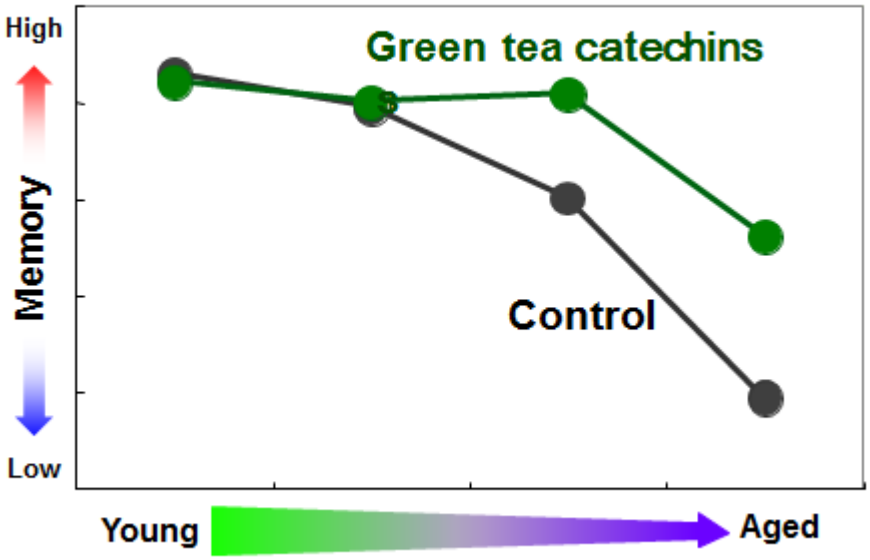

Figure 1. Memory retention in mice ingested green tea catechins

Senescence-accelerated mouse (SAMP10) shows memory decline with aging. As mice prefer a dark place, mice move into the dark box when placed in the light box. However, when mouse was given a weak electric shock through the floor of the dark box, mouse learned not to enter the dark room. Memory retention was tested one month later using same test. Memory decline was much suppressed in mice ingested green tea catechins than in control mice that ingested water.

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5. EFFECTS ON COGNITIVE FUNCTION

5.1. PREVENTIVE EFFECTS ON ALZHEIMER DISEASE

Keiko UNNO (University of Shizuoka)

Abstract

In Alzheimer disease, β-secretase is abnormally activated in brain protein metabolism and the amyloid-β protein is accumulated. Epigallocatechin-3-gallate, a major green tea catechin, prevents dementia by inhibiting the accumulation of amyloid-β protein via the activation of α-secretase, which functions normally in cerebral protein metabolism.

Dementia

The population of elderly people is increasing rapidly in Japan, and the Ministry of Health, Labour and Welfare estimated that the number of elderly persons with dementia was more than 3 million in 2012. The estimate suggests that patients with dementia will exceed 4 million people in 2020; therefore, establishing preventive measures for dementia need to be immediately implemented. Dementia is a progressive disease in which normal cognitive function is markedly impaired by atrophy and cerebrovascular disorder. Patients with dementia have defective abilities of learning a new experience and recognizing a person, time, and place, and develop mental disorders. This disease can be classified into cerebral blood vessel and denaturation types. The latter includes Alzheimer disease, Parkinson disease, and Levy corpuscle disease. Alzheimer's dementia currently accounts for approximately 60% of dementia cases in Japan.

Alzheimer disease

In Alzheimer disease, the amyloid-β (Aβ) protein is accumulated in the brain [1]. It is a fragment of the β-amyloid precursor protein (APP), which is formed by the proteolytic cleavage of APP. When accumulated at a high concentration, Aβ forms amyloid fibers and shows cytotoxicity [2]. Normally, APP undergoes degradation by the enzyme α-secretase, and water-soluble sAPPα is produced as a result; the peptide p3 is successively generated by the activity of the γ-secretase enzyme (Figure 1).

In pathological conditions, APP undergoes degradation by two kinds of enzymes, namely β-secretase and γ-secretase; as a result, Aβ is generated [3,4]. In patients with Alzheimer disease, this process is enhanced, as compared to that in normal conditions, so that an excessive amount of Aβ is generated. Aβ has two types, namely Aβ40 and Aβ42. Accumulation of Aβ42 is deeply associated with Alzheimer disease because it

aggregates faster than Aβ40. Therefore, Aβ42 is expected to contribute to the treatment of Alzheimer disease by controlling Aβ production. In addition, compounds that enhance Aβ degradation or inhibit the activities of β-secretase and γ-secretase are being studied as potential therapeutic agents for the prevention and treatment of Alzheimer disease.

Action of tea catechins

Among green tea catechins, EGCG has been used in animal experiments. For example, EGCG was shown to decrease the Aβ accumulation in an Alzheimer disease model mouse via a mechanism in which EGCG activates an α-secretase pathway to suppress the production of Aβ [5]. Enhancing α-secretase activity would normalize APP metabolism, suggesting the importance of the EGCG's mechanism of action.

However, the effective EGCG dose in mice is considered to be too high for use in humans. Therefore, methods have been developed to improve the efficacy by using EGCG nanoparticles (ultrafine particles) for enhancing in vivo uptake, or by using EGCG with fish oil which is epidemiologically suggested to reduce a risk for dementia [6,7].

As for the preventive action of EGCG against Alzheimer disease and Parkinson disease, various activities such as stabilization of mitochondria, iron chelation, and neurogenesis have also been proposed [8].

Therapeutic drug for Alzheimer disease

The medicines used for Alzheimer disease include donepezil (brand name, Aricept). This drug can prevent memory failure by inhibiting the enzyme degrading acetyl choline, which participates in memory formation. EGCG may be effective for Alzheimer disease by a different mechanism from donepezil and other Alzheimer's drugs. In addition, tea components other than catechins might have a potential for use in the treatment of the disease; thus, future studies are warranted.

Epidemiological studies

An epidemiological research with Japanese aged ≥70 years showed that the prevalence of dementia in the group who drank more than two cups of green tea a day is half of that of the group that consumed less than three cups a week of green tea. The results suggest the preventive action of green tea against dementia [9]. Meanwhile, the results of a study of 716 Chinese residents in Singapore, aged ≥55 years, indicated that the drinkers of green tea, black tea, and oolong tea were superior in terms of perception, memory, executive ability, and information processing power [10]. A more detailed

study in future should clarify whether green tea intake is effective for dementia prevention.

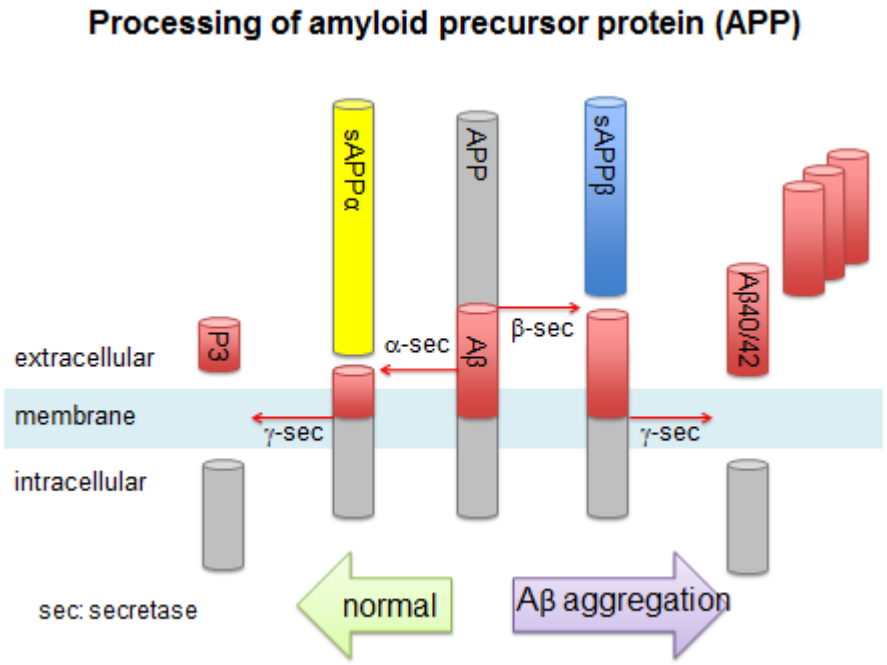

Figure 1.

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5.2. PREVENTIVE EFFECTS ON BRAIN STROKE

Masaki TABUCHI (Kinki University)

Abstract

Brain disorder is caused by arterial temporary confinement (ischemia) and cerebral infarction following reperfusion. The intake of tea catechins reduces the degree of ischemia and the nerve symptom associated with brain disorder. The intake of tea catechins suppresses onset of cerebral hemorrhage and is effective in protecting the brain functional disorder to occur after a brain damage.

Characteristics of stroke

A stroke is the disease in which there are the nerve symptoms such as the paralysis of hands and feet and disturbance of consciousness due to necrosis of brain cells and their defective function after brain ischemia (lack of blood flow) caused by blockage (thrombosis) or a hemorrhage. Once the stroke was the first in the causes of death but now is the fourth next to cancer, cardiovascular disease, and neumonitis by the progress of the cure and management of the blood pressure. However, the number of patients of the stroke may increase with aging in future.

A characteristic of the stroke is that a physical disability and mental disorder are left as aftereffects, and the stroke is the first place in the diseases that nursing care is necessary for. In other words, it is a big burden for not only a patient but also the family of a patient. Therefore, it is very important to prevent and reduce brain disorder at the time of the onset.

Green tea and the risk of the stroke

In cohort studies, it was shown that green tea consumption was inversely associated with death from stroke [1,2]. The study conducted with 40,530 Japanese adults aged 40 to 79 years for 7 years found that the multivariate hazard ratios of mortality due to stroke associated with different green tea consumption frequencies in males were 1.00 (reference) for less than 1 cup/day, 0.85 for 1 to 2 cups/day, 0.97 for 3 to 5 cups/day, and 0.65 for 5 or more cups/day. The values in females were 1.00 for less than 1 cup/day, 0.79 for 1 to 2 cups/day, 0.61 for 3 to 5 cups/day, and 0.58 for 5 or more cups/day. It should be noted that the consumption of roasted tea was not associated with a stroke risk reduction [2].

Animal model of cerebral infarction

In the study of the cerebral infarction, a method is often used in which bloodstream is temporarily stopped by bundling of rat middle cerebral artery to cause ischemia followed by **reperfusion** [3,4]. For a patient who had the cerebral infarction, the initial treatment may be performed to recanalize by the thrombotic dissolution or removal to reduce the extent of the cerebral damage. Therefore, this rat cerebral infarction model is reflective of the human case.

In this animal model, cerebral infarction (neuronal necrosis) is observed 22 h after **reperfusion** of 2h-ischemia. In addition, the neurologic symptoms such as the paralysis of the foreleg and the loss of the self-motion were observed. It is now believed that the increase in the oxidative stress by the excessive production of reactive oxygen species such as nitric oxide and superoxides is involved in the mechanism by which brain disorder is aggravated [5].

Actions of tea catechins

Among various components contained in green tea, catechins, in particular, epigallocatechn gallate (EGCG) attract attention as a component to prevent the stroke onset. When rats received 0.5% water solution of green tea catechins (Polyphenone E® containing 58.4 % EGCG) for 5 days, the volume of cerebral infarction induced by the ischemic- **reperfusion** was approximately 40% smaller than that of rats given water. In addition, the oxidative stress was reduced and the nerve symptom lightened in the catechin group, indicating that tea catechins were effective for prevention of cerebral infarction [6].

A recent study using the rat model showing the brain damages similar to those in the stroke indicated that in the young rats which had received 0.1% solution of catechins (Teavigo® with a EGCG content of 94%) for 4 weeks, the oxidative stress induced by the brain damage was reduced, neuronal apoptosis (cell death) decreased, and the brain functional disorder reduced as compared with water-given rats [7,8]. Furthermore, it was revealed that neuronal reproduction in damaged brain tissue was promoted [9].

Effect of tea catechins on cerebral hemorrhage

In the study of cerebral hemorrhage, the spontaneously hypertensive stroke-prone (SHRSP) rats in which hypertension develops hereditarily and cerebral hemorrhage develops in 3 months after birth is frequently used [10]. Recently, Malignant SHRSP (M-SHRSP) rats have also been developed in which a rise in blood pressure is higher and the cerebral hemorrhage develops in earlier than SHRSP [11]. The experiments using M-SHRSP rats also provided the similar findings [12]. Young M-SHRSP rats which received a 0.5% water solution of Polyphenone E®, showed a mild rise of the

blood pressure and reduced frequency to have cerebral hemorrhage as compared with the water-given rats [13].

Concluding remarks

The detailed mechanism by which tea catechins exhibit protective effects on the cerebral hemorrhage is not known at present, but there are several candidates. It may be that EGCG removes the reactive oxygen species which are generated in association with a stroke and suppresses the synthesis of a hormone raising the blood pressure. Thus, it appears possible that the onset of stroke and the brain disorder after the stroke may be prevented by keeping a lifestyle to intake a green tea from youth.

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5.3. GREEN TEA AND BRAIN FUNCTION

Hidehiko YOKOGOSHI (Chubu University)

Abstract

Green tea contains antioxidant vitamins such as vitamins A, C, and E. These antioxidants reduce excessive antioxidative stress, contributing to the maintenance of blood vessel integrity and reduction of brain disorders. Tea catechins suppress nitric oxide generation, reduce the oxidative stress caused by nitric oxide, and protect cranial nerve cells. The daily consumption of low concentrations of caffeine reduces damage due to epilepsy. Theanine, a tea component, reduces cell death due to various factors and helps prevent cranial nerve cell death.

Green tea and brain function

The actions and reactions of animals are influenced by cranial neuron cell communication. Several compounds involved in this communication, including acetylcholine, glutamic acid, and dopamine, are termed neurotransmitters. Diseases caused by decreased brain function include dementia and schizophrenia. For example, when rats are given a drug that interrupts the acetylcholine nerve communication, they exhibit cognitive impairment resembling that seen in Alzheimer's disease. However, the intake of green tea catechins improves such behavioral dysfunction (Figure 1) [1]. Among the components of green tea, antioxidant vitamins, catechins, caffeine, and theanine are significantly associated with brain function. Studies on the influence of green tea in the prevention of these brain disorders are currently in progress.

Antioxidant vitamins

Vitamins with antioxidant activities, such as vitamins A, C, and E, are abundant in green tea. These vitamins are believed to be effective in the prevention of brain function disorders and cerebropathy. An important causative factor of Alzheimer's disease is oxidative stress. In fact, Alzheimer's disease patients exhibit decreased vitamin C and E concentrations in cerebrospinal fluid [2]. Subjects consuming vitamin E alone or vitamins C and E every day for 1 month exhibit significantly increased cerebrospinal fluid concentrations of vitamins C and E, possibly delaying the development of Alzheimer's disease [2]. In addition, aged stroke-prone experimental rats administered vitamins C and E exhibit a decrease in high blood pressure development, active oxygen production, and oxidative stress, resulting in improved maintenance of the function and structure of blood vessels [3]. Furthermore, in an experiment using a mouse model of

middle cerebral artery confinement, vitamin E effectively reduced the occurrence of brain disorders, with the infarcted portion of the middle cerebral artery being reduced in size in the vitamin E-treated group [4].

Tea catechins

Oxidative stress induced by nitric oxide may cause neuronopathy. An experiment examining the effect of epigallocatechin gallate (EGCG) on nitric oxide generation shows that the nitric oxide concentration increases in nerve cells in the hippocampus, which is a part of the cerebrum part concerned with memory and space perception. When the blood supply to the brain was temporally stopped as a model of ischemia, intraperitoneal EGCG administration decreased the nitric oxide concentration and relieved brain neuronopathy due to ischemia [5]. In addition, several reports describe cranial nerve protection by EGCG [6,7] and the effect of EGCG on neuropathy in experiments using Parkinson's disease model rats [8].

Caffeine

Excessive caffeine ingestion can cause acute convulsions and exacerbate ischemia-related neuropathy and epilepsy. On the other hand, long-term caffeine intake may have different effects on these diseases [9]. Rats induced to have experimental epilepsy after consuming a low concentration of caffeine ad libitum for 15 d exhibited significant suppression of cellular disorder in the hippocampus, which is the part of the brain concerned with memory; moreover, cellular disorders tended to be suppressed in the pear cortex, which is the part of the brain that distinguishes scents. Therefore, the habitual intake of low concentrations of caffeine may reduce brain damage due to epilepsy.

Caffeine has been shown to protect against Parkinson's disease. An epidemiological study examining the effect of caffeine intake on male Parkinson's disease patients for 6 years revealed that caffeine reduced disease development [10]. Caffeine is also known to improve the neurotransmission efficiency of dopaminergic neurons [11].

Theanine

Cell death due to neurotoxicity was significantly suppressed with the simultaneous addition of a large quantity of glutaminic acid and theanine to rat cerebrum-derived nerve cell cultures. Meanwhile, approximately 50% of the cells died without theanine.

In an experiment in which theanine was directly administered to the lateral ventricle of gerbils and the carotid artery was bound 30-min later by a clip to stop blood flow for 3 min to cause ischemia, the results obtained 7 d later indicated that the

ischemia-induced hippocampal cranial nerve cell death was suppressed by theanine administration [12]. Electroencephalographic measurement of alpha waves shows higher frequencies among human subjects taking theanine (Figure 2). Therefore, theanine exerts a relaxation effect and may also have a protective effect on nerve cells [13].

Concluding remarks

Green tea contains various components in addition to those described herein. There may include components that improve brain and neurological functions. Despite the limited findings, green tea appears to greatly contribute to maintaining normal brain function. Drinking green tea results in relaxation, protects nerve cells of the brain, and maintains brain function. Thus, green tea appears to be an essential drink for maintaining health, particularly in Japan, which is dealing with the effects of population aging and high-stress lifestyles.

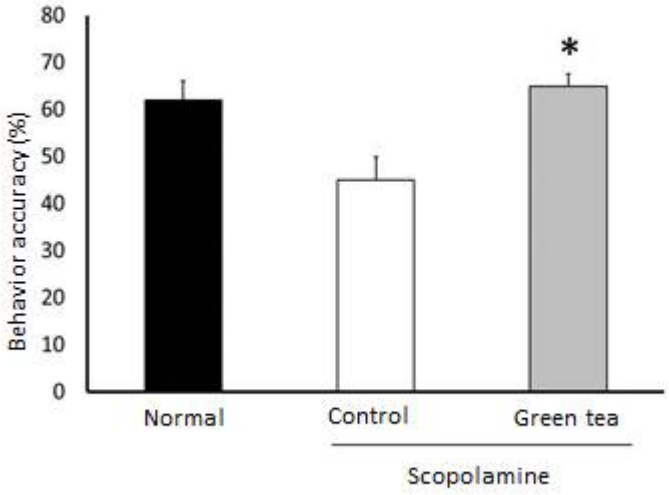

Figure 1. The intake of green tea catechins improves drug-induced behavioral Dysfunction.

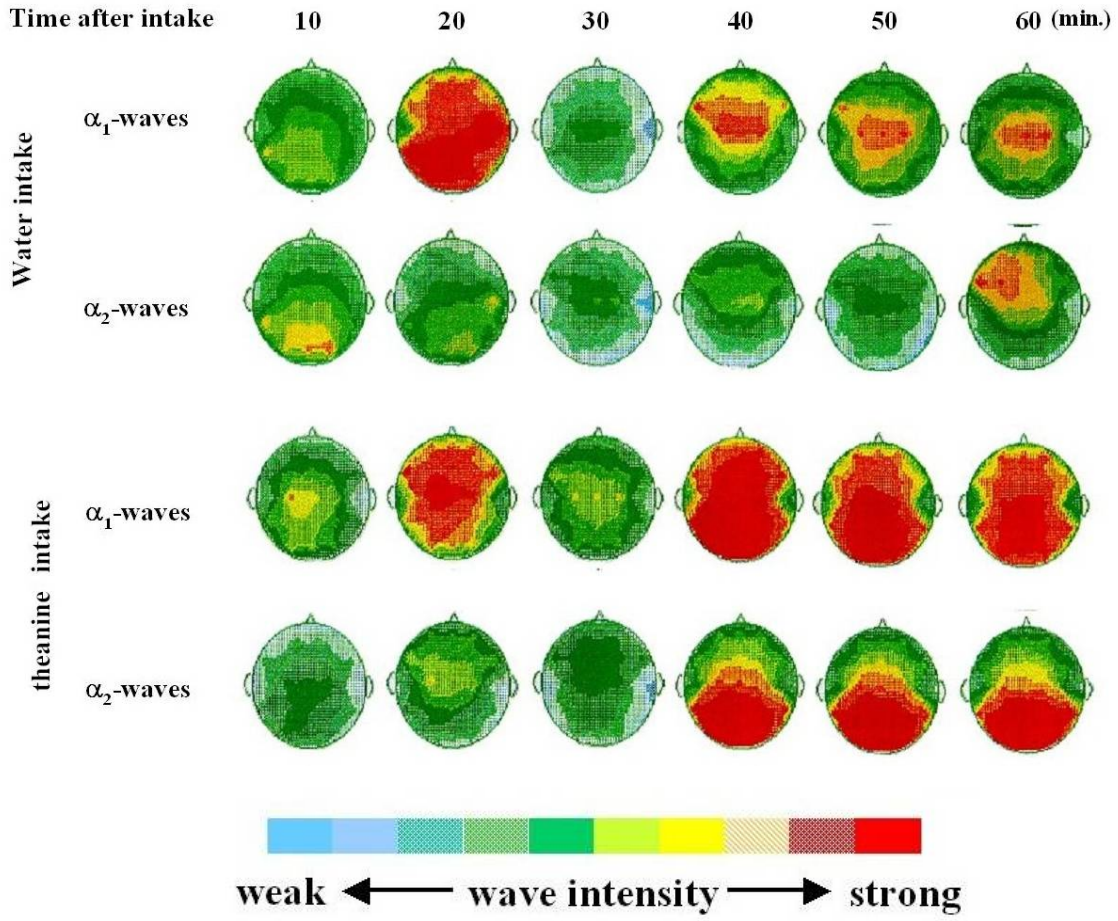

Figure 2. Electroencephalographic measurement of alpha waves shows higher frequencies among human subjects taking theanine as compared to those taking water.

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6. ANTI-ALLERGIC EFFECTS

Mari MAEDA-YAMAMOTO (National Food Research Institute, NARO)

Abstract

In human clinical trials, the green tea brand Benifuuki, containing 3″-epigallocatechin-3-*O*-(3-*O*-methyl) gallate (EGCG3″Me), was found to significantly relieve the symptoms of perennial or seasonal rhinitis compared with a placebo green tea that did not contain EGCG3″Me. This action of EGCG3″Me was due to inhibition of the release of histamine, which causes allergic symptoms. The green tea components strictinins and theogallin show anti-allergic action by inhibiting histamine release through suppressing the biosynthesis of immunoglobulin E.

Anti-allergic ingredients of green tea

Allergies are caused by excessive immunoreactions that are triggered by chemical mediators such as histamine and leukotrienes, which are released when mucosal mast cells and basophilic leukocytes in the blood are activated by the cross-linking of specific allergens and immunoglobulin E (IgE) on the cell surface. Among the various components of tea, methylated catechins and epigallocatechin gallate (EGCG) are known to have anti-allergic properties that prevent excessive immunoreactions [1-3]. *O*-Methylated catechins are derivatives of EGCG and epicatechin gallate, in which a hydroxyl group in a galloyl residue is methylated to form an ester. Tea cultivars such as Benifuuki and Benihomare are rich sources of this type of catechin [4]. *O*-Methylated catechins show the allergy-relieving effects by inhibiting the release of histamine from mast cells and basophils [5,6].

Intervention studies

In a human clinical trial, clinical symptoms such as rhinitis and itchy eyes were reduced in patients with symptoms of cedar pollinosis who drank Benifuuki containing 1.5–2.5% methylated catechins (dry weight), the equivalent of a daily consumption >34 mg of total methylated catechins, compared to patients in the placebo group (Figure 1) [7].

The patients who started to ingest Benifuuki 1.5 months before pollen dispersal showed reduced symptoms, such as frequency of nose-blowing, tear quantity, and sore throat (Figure 2) [8] as compared with those who began to drink it after pollen administration. A similar effect was also found in the case of chronic allergic rhinitis [9]. Topical application of the cream-mixed Benifuuki extract to infants with atopic dermatitis for 8 weeks significantly reduced the consumption of the steroid hormone compared with the application of green tea cream containing no methylated catechins [10].

Action of other tea ingredients

Compounds that suppress the biosynthesis of IgE, which contributes to allergic reactions, have been found in tea. These compounds include tannins called strictinins (strictinin and galloyl strictinin) and theogallin [11,12]. IgE is generated by the recombination of immunoglobulin M, and animal experiments have shown that these tannins inhibited this process to decrease the quantity of IgE in the blood [11]. However, it will be necessary to clarify in the future whether such action is applicable to humans.

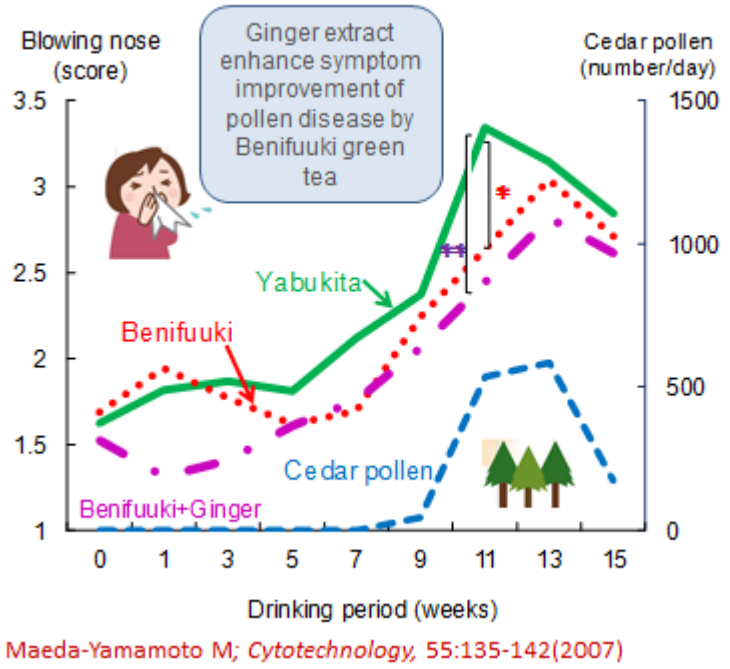

Figure 1.

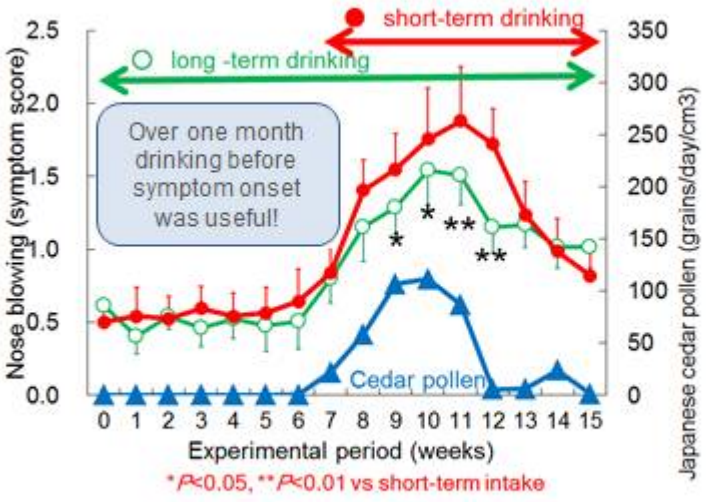

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Figure 2.

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7. ANTI-BACTERIAL AND ANTI-VIRAL EFFECTS

7.1. EEFFECTS ON INFLUENZA

7.1.1. BASIC STUDIES

Takashi SYZUKI (Universiry of Shizuoka)

Abstract

Two proteins, hemagglutinin (HA) and neuraminidase (NA), are present as spikes on the surface of influenza virus particles. They participate in the establishment of infection, which involves the mucous membrane cells of the nose and the release and propagation of the virus. Epigallocatechin gallate (EGCG), one of the major tea catechins, directly acts on HA and NA to suppress the adsorption of influenza virus onto human mucous membrane cells and viral release. Strictinin, a tea polyphenol, inhibits the fusion of viruses with the cell membrane when an influenza virus particle penetrates a cell, preventing the replication of the virus. Thus, at least 2 components of green tea may protect against influenza virus infection.

Infection and replication of the influenza virus

Influenza is a disease in which influenza virus infects the respiratory organs including the nose, throat, and trachea. Influenza viruses are classified into 3 types: types A, B, and C. Infection with type A or B virus results in symptoms such as fever, muscular pain, chills, and cough [1]. In contrast, the more severe symptoms are not observed in type C virus infection. Type A and B influenza viruses infect mucous membrane cells in the nose through 2 proteins, hemagglutinin (HA) and neuraminidase (NA), which are present as spikes on the surface of the virus particle. The infection starts when HA is adsorbed onto the cell surface (Figure 1) [2]. Then, the viral RNA moves into the cell nucleus where it replicates. After viral proteins are synthesized, they assemble together to form new virus particles. NA has sialidase enzymatic activity; the new viruses are released from infected cells by this enzyme [3]. In addition, NA plays a role in viral invasion [4,5].

Activities of green tea components against influenza virus

Green tea contains components that directly act on influenza virus to prevent infection. These include catechins such as epigallocatechin gallate (EGCG) and strictinin. EGCG and epicatechin gallate (ECG) act on HA and NA to suppress virus adsorption and replication [6]. EGCG and ECG inhibit the activity of viral RNA polymerase, suppressing virus propagation [7,8]. Unlike catechins, strictinin does not

interfere with the functions of HA and NA, but rather inhibits the fusion of the virus with the cell membrane when a virus invades a cell (Figure 1) [9]. An experiment using chickens shows diet and water containing green tea components suppresses the replication of influenza virus [10]. These results are helping to clarify the effects of green tea components against influenza virus.

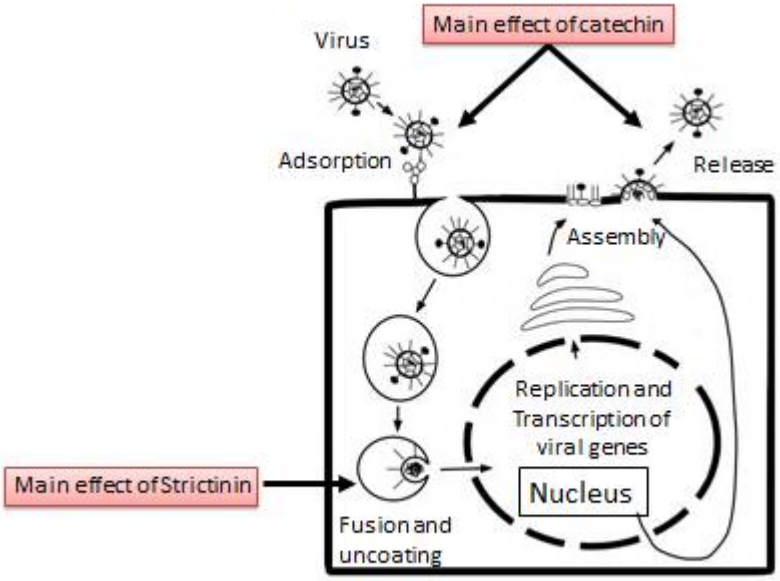

Figure 1. Effects of catechin and strictinin on infection and replication of the influenza virus.

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7.1.2. EPIDEMIOLOGICAL STUDIES ON INFLUENZA INFECTION

Hiroshi YAMADA (University of Shizuoka)

Abstract

Epidemiological studies suggest the conventional intake of green tea decreases influenza infection. Gargling with green tea catechin extract and the intake of the catechins and theanine may protect against the development of influenza or colds.

Prevention of cold and influenza

The common cold is an acute infectious disease of the nose and throat. Most cases are due to viruses. Meanwhile, influenza is a severe disease caused by influenza virus; it is highly infectious and may progress to pneumonia and encephalitis when aggravated. Because both colds and influenza infections spread via droplets and contact, prevention is very important. Prophylaxis includes hand washing, mask wearing, gargling practice, and vaccination (for influenza). However, none of these methods is completely effective. Gargling green tea catechin and drinking green tea to prevent cold and influenza have recently attracted much attention.

Protective efficacy of tea catechin gargling against influenza

Tea catechins bind to the spikes on the surface of influenza virus and inhibit viral adsorption onto the host cell surface, thus preventing infection (Figure 1) [1,2]. In addition, catechins suppress the replication of influenza virus [3-5]. Basic science studies show catechins are also effective against cold viruses [6].

In a clinical interventional study of people living in elderly nursing homes, gargling tea catechins 3 times a day for 3 months (at a concentration equivalent to about half that of a commercially available green tea beverage with 200 μg/mL total catechins) decreased influenza onset compared to gargling with water (Figure 2) [7].

Protective efficacy of green tea intake against influenza

In addition to catechins, green tea contains theanine and vitamin C, which enhance immunity, suggesting green tea intake is effective in preventing influenza infection. In an interventional study in which adult volunteers took green tea components (378 mg total catechins and 210 mg of theanine per day for 5 months), the onset of influenza was lower in the group taking the green tea components [8]. In addition, an epidemiological study conducted on elementary school children in Kikugawa City, Shizuoka, which is one of the major tea production areas of Japan, shows the frequency of influenza onset

in children drinking 1–5 cups of green tea per day was less than that children drinking less than 1 cup per day [9]. An overseas interventional study shows that the 3-month intake of a green tea component reduced the frequency of cold onset and symptom appearance [10].

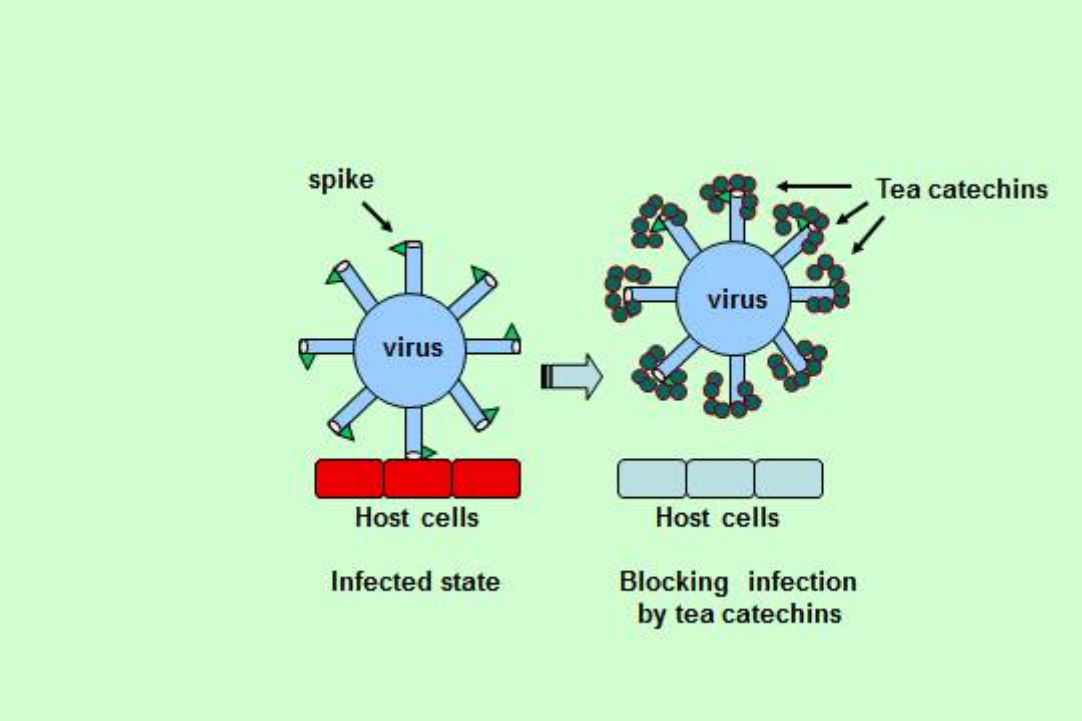

Figure 1. Anti-viral effect of tea catechins against influenza (schema)

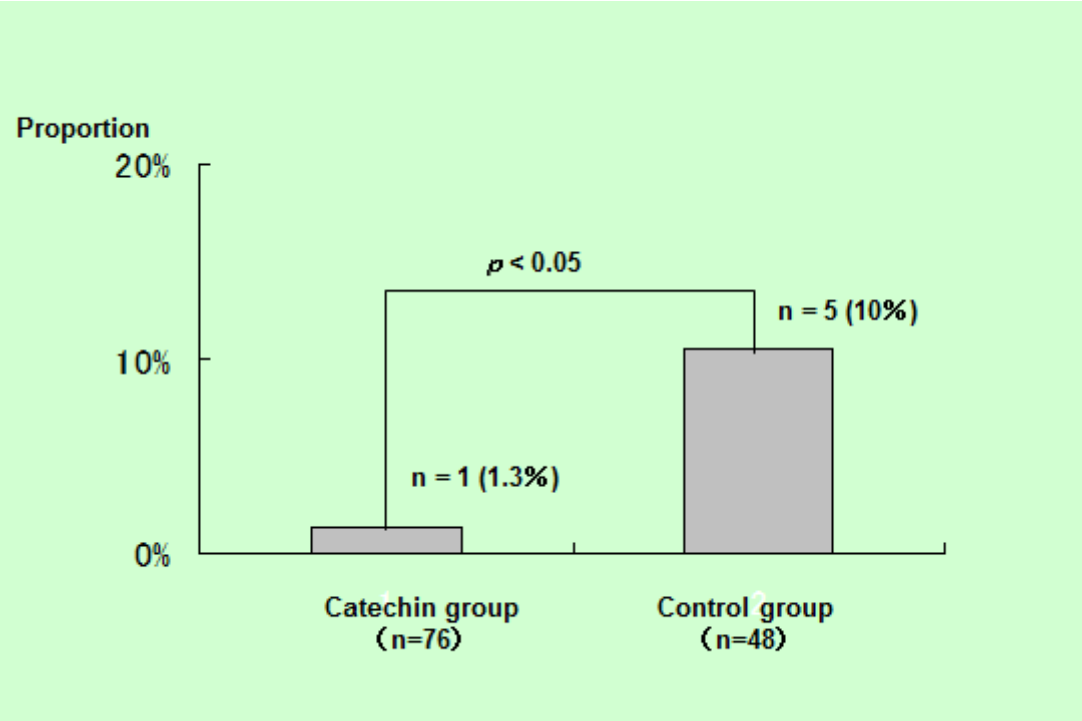

Figure 2. Comparison of the incidence of influenza infection

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7.2. EFFECTS ON DENTAL DISEASES

Tsutomu OKUBO (Taiyo Kagaku Co., Ltd.)

Abstract

Green tea catechins have antibacterial properties against bacteria causing cavities and periodontal disease. Itprevents the development of cavities by inhibiting the activity of enzymes related to plaque formation. A green tea component, fluorine, strengthens teeth and prevents cavity formation. A case–control study reported in 2006 involving 12,019 men and 13,059 women between 40 and 64 years of age revealed that frequent green tea consumption decreased the rate of tooth loss.

Preventive activity of green tea catechins against cavities

Japanese people customarily drink green tea, particularly after meals. This cleans the inside of the mouth and also removes food odors. Various studies including animal and human experiments show catechins contained in green tea have antibacterial effects, and can prevent cavity formation and periodontal disease [1-5]. A rat experiment shows cavities generated by feeding sugar can be prevented by catechin administration (Figure 1).

Cavity-causing bacteria convert sugar into glucans, which are insoluble and adhesive; glucans attach to a tooth with other microbes, forming plaques. Acid generated by the plaque dissolves the tooth enamel,thus forming a cavity. Green tea catechins have antibacterial properties against cavity-causing bacteria and inhibit the activity of glucosyltransferase, which is involved in glucan formation (Figure 2) [6]. Therefore, catechins prevent or act against cavity development. In addition, animal experiments show the combined intake of sweets (e.g., caramel, chocolate, and candy) with catechins lowers the risk of cavity formation [7].

Preventive action of green tea catechins against periodontal disease

Periodontal disease is an oral illness in which teeth are lost along with cavities. Prophylaxis including healthy eating habits is important in both old and middle age. During the development of periodontal disease, plaque accumulates in periodontal pockets between the teeth and gums, bacteria propagate to cause periodontitis, alveolar bone is absorbed, the gums degrade, and eventually the teeth are lost. Therefore, catechins have antibacterial effects against the bacteria causing periodontal disease. Itsuppresses the activity of collagen-degrading enzymes, which are involved in the resorption of alveolar bone. These effects of catechins have been observed in human

studies with periodontitis patients [8].

Improvement of halitosis by green tea catechins

Catechins improve halitosis (i.e., bad breath) [9]. There are several kinds of halitosis, including physiological and pathological bad breath as well as that caused by eating and drinking certain foods,as well as smoking. The former is caused by the quality and quantity of metabolism, and the secretion of the body fluids, and diseases of the oral cavity such as cavities and pyorrhea. While, the latter is caused by smoking, alcohol and garlic consumption, and occasionally the retention of rotten food residues in the mouth. Catechins combat halitosis by binding directly to odorous components and inhibiting the growth of bacteria causing cavities and periodontal disease as well as preventing the oxidation of oils and fats.

Action of fluorine

Fluorine, which is contained in green tea, strengthens teeth. In experiments using cow teeth as well as human studies, teeth pretreated with tea fluorine exhibited resistance against acid decalcification, which is a form of the dissolution of calcium salt crystals [10]. Thus, green tea components such as catechins exert positive oral effects including the prevention of cavities, periodontal disease, and halitosis. In addition, fluorine strengthens teeth and prevents cavity formation. Therefore, green tea extract is now beenused in various sweets, toothpastes, and wet tissues for babies. It can be said that casual daily tea breaks aid oral health.

Epidemiology of tooth loss

In a case-control study reported in 2006 involving 12,019 men and 13,059 women between 40 and 64 years of age, the odds ratio (i.e., the relative degree of risk) of losing a tooth was 0.82 for male subjects drinking 2–3 or 3–4 cups of green tea daily and 0.77 for those drinking 5 cups or more vs. 1.00 for those with less than 1 cup per day [11]. Similar results were obtained for women. These findings demonstrate that frequent green tea consumption decreases the risk of tooth loss. Wheres, future studies are required to confirm whether green tea is effective for preventing tooth loss.

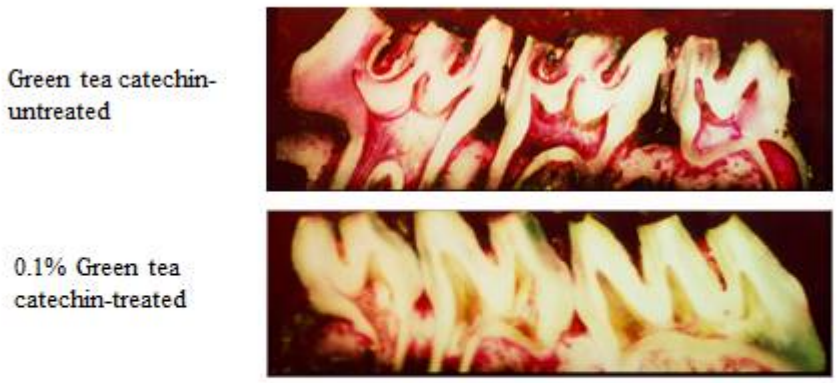

Figure 1. Preventive effect of green tea catechins on dental caries.

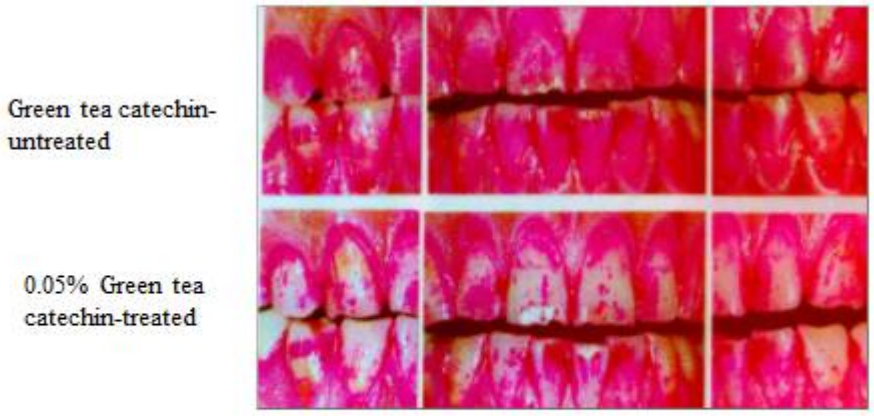

Figure 2. Inhibitory effect of green tea catechins on the adhesion onto dental plaque

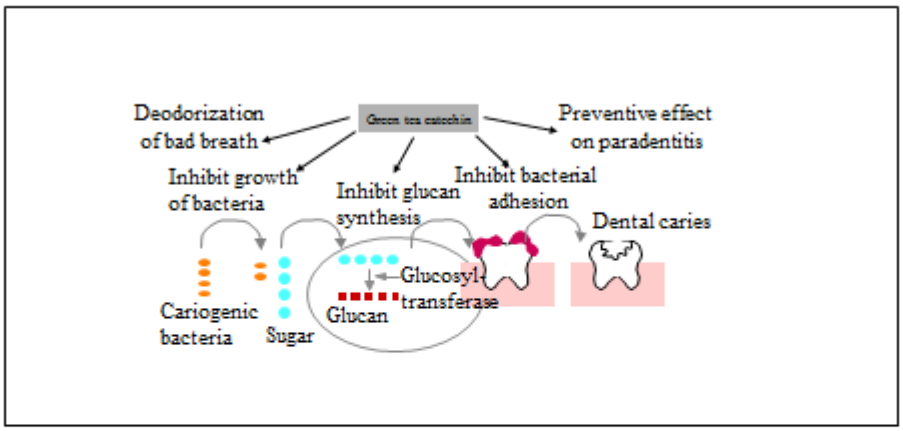

Figure 3. Inhibitory effect of green tea catechins on dental caries formation.

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8. PREVENTIVE EFFECTS ON OSTEOPOROSIS

Shinichi IWAI (Showa University)

Abstract

Osteoporosis develops when the balance of bone-formation (osteoplasty) and bone-dissolution (resorption) that comprises bone metabolism collapses. Epigallocatechin gallate (EGCG) prevents osteoporosis by inhibiting 1) the generation and activity of osteoclast cells participating in bone resorption and 2) the activity of enzymes (matrix metalloproteinases) that degrade scaffold components including collagen fibers.

Osteoporosis

Osteoporosis is a disease in which bone becomes easily fractured as bone density decreases and the bone becomes fragile and is prevalent among postmenopausal woman [1]. The decreased bone density develops when the bone metabolic activity of bone-dissolution (resorption) exceeds that of bone formation (osteoplasty) (Figure 1). Giant osteoclasts are formed by the cellular fusion of precursor osteoclasts derived from bone marrow cells. The activation of these cells causes bone absorption [2].

It is expected that the inhibition of bone resorption by osteoclasts leads to the prevention of osteoporosis, and medical drugs such as bisphosphonate have been developed along these lines [3]. Bone density is an index of the content of minerals such as calcium and magnesium in bones, and a drop in bone density may cause osteoporosis.

Ingestion of green tea and bone density

The results of several case-control studies examining the effect of tea, particularly black tea, on bone density have shown that tea ingestion increases bone density, although some reports indicate that tea has no effect or even deceases bone density [4-6]. In a case-control study of 632 Japanese women aged 60 years or older, green tea intake was shown to increase bone density [7]. A clinical intervention study showed that in 171 postmenopausal American women, the catechin group, in which 500 mg of green tea was consumed daily, had higher levels of plasma alkaline phosphatase as compared to the group that did not consume catechins, suggesting higher bone formation activity in the former group [6]. However, the study showed no changes in indexes of bone resorption and bone density, indicating the necessity of future studies to evaluate the role of tea intake in osteoporosis.

Cellular and animal investigations of the effects of green tea

Green tea contains various components including epigallocatechin gallate (EGCG), a member of the tea catechins, which presumably has the most potent biological activity. Black tea contains theaflavin digallate (TFDG), which is generated by the dimerization of EGCG and has activities similar to those of EGCG (Figure2) [10,11].

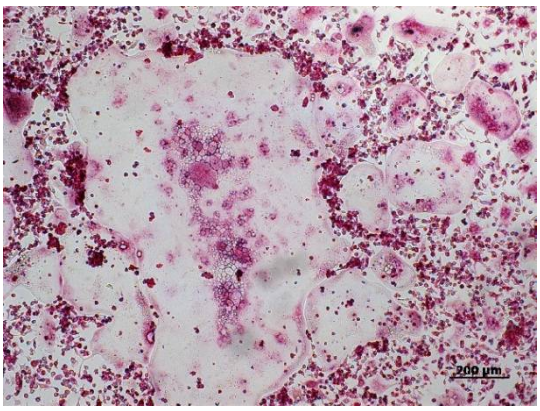
In cellular experiments, EGCG inhibited the promotion of the differentiation of myeloblasts into activated osteoclasts via precursor osteoclast cells and osteoclasts. EGCG at 10 μM added to the cell culture medium inhibited the formation of giant osteoclasts, which were generated in the control cultures with no added EGCG (Figure 1).

EGCG and TFDG had similar effects on the process of cell fusion and osteoclast activation. It has been reported that the ingestion of green tea containing 400 mg of EGCG results in a plasma concentration of about 2 μM [12]. Recent results show that EGCG is effective at 1 μM concentrations, indicating that EGCG may function in vivo.

EGCG and TFDG also have the ability to inhibit matrix metalloproteinases (MMPs) of osteoclasts and chondrocytes [8,13]. MMPs are enzymes that degrade matrix components such as collagens, of which the scaffolding for bone is made. Scaffold protection is now known to be important in the prevention of osteoporosis, suggesting that tea is useful in the prevention of the disease through its inhibitory effect on MMPs.

On the basis of its EGCG and TFDG content, it is speculated that green tea has the highest protective activity against osteoporosis among the 3 kinds of tea. Since the ingestion of the green tea named Benifuuki results in a higher blood concentration of these components, it may have a more beneficial effect than other varieties of green tea.

A



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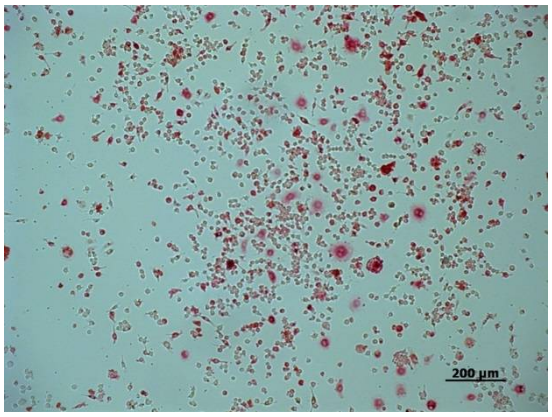

Figure 1. A, Giant osteoclasts. There were the giant osteoclasts at 6 days. B, The osteoclast formation was inhibited by 10 μM EGCG. No giant osteoclasts were observed at 6 days.

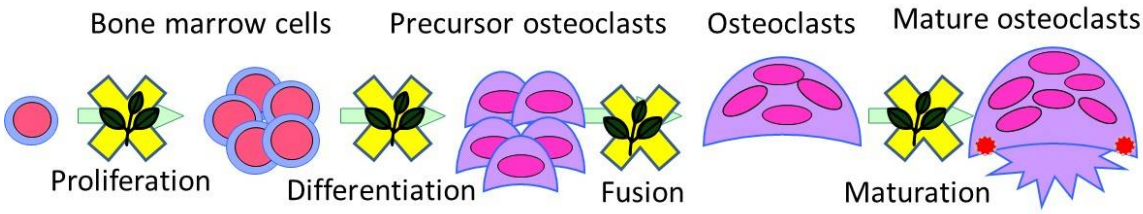

Figure 2. The process of osteoclasts maturation.

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9. EFFECTS ON PERIODONTAL DISEASES

Takashi MATSUO (Tokushima University)

Abstract

Fibroblasts are involved not only in the metabolism of matrix components such as collagens, but also in the modulation of immune systems by producing cytokines, protein factors that are mainly released from the immune cells to mediate various intercellular interactions. Experiments have shown that catechins such as epigallocatechin gallate suppress the production of cytokine IL-6 of gingival fibroblasts. Catechins also interfere with the fibroblast production of CCL20, which is one of the chemokines that promotes leukocyte chemotaxis, as well as that of the chemokine CXCL10. Since these activities of catechins are closely related to the inhibition of an inflammatory reaction, it can be said that tea catechins act on gingival fibroblasts to suppress an inflammatory reaction and to halt the development of periodontosis. It is also known that theaflavin contained in black tea has a similar effect.

Periodontal disease and inflammation

Periodontal disease, contracted by around 80% of the Japanese population, is caused by plaque accumulated between the tooth and gingivae. In 1 g of plaque, there are more than 100 million bacteria, including the notorious *Porphyromonas gingivalis*. These bacteria cause sustained inflammation, and, as a result, the periodontium, including the alveolar bone (bone supporting a tooth), is destroyed (Figure 1). Thus, the pathological state of periodontal disease is chronic inflammation due to bacteria, and is affected profoundly by the body's immune system. Immune cells such as T cells, B cells, and macrophages play a role in preventing bacterial invasion, but cause damage if they are deployed excessively.

Anti-inflammatory activity of tea catechins

Tea is considered to be safe to drink and contains catechins such as epigallocatechin gallate (EGCG). Tea catechins are known to have anti-inflammatory and anti-bacterial effects [1-4]. Therefore, it is expected that catechins may be useful in the prevention of chronic inflammation of the periodontium caused by bacteria such as *P. gingivalis*. Immune cells play an important role in the onset and development of chronic gingival inflammation and fibroblasts do so in the reproduction and restoration of the periodontium. These aspects have led to the study of the effects of catechins on

fibroblasts.

Action of catechins on fibroblasts

Fibroblasts are involved not only in the metabolism of matrix components such as collagens in the extracellular matrix that fills spaces between cells and cell masses, but also in the modulation of immune systems by producing cytokines, protein factors that are mainly released from the immune cells to mediate various intercellular interactions. For example, the gingival fibroblasts produce a cytokine called IL-6 by various stimuli, and are concerned with the progress of inflammation.

Experiments have shown that catechins such as EGCG suppress the production of IL-6 [5]. Catechins were also found to interfere with the fibroblast production of CCL20, which is one of the chemokines that promotes leukocyte chemotaxis, as well as that of the chemokine CXCL10 [6,7]. Since these activities are closely related to the inhibition of an inflammatory reaction, it can be said that tea catechins act on gingival fibroblasts to suppress an inflammatory reaction and to halt the development of periodontosis. It is also known that theaflavin contained in black tea has a similar effect [8].

Although plaque removal by tooth brushing is indispensable to the prevention and medical treatment of periodontosis, topical application of catechins appears effective for the prevention of the disease by sterilizing residual bacteria that are not removed completely by brushing and reducing gingival inflammation.

Effects on cavity and pulpitis

The two major dental diseases are periodontosis and cavities. When stimulated by bacteria, fibroblasts in pulpitis produce IL-6 and the chemokine IL-8, in addition to proteins called ICAM-1 and VCAM-1. Catechins were found to suppress the production of these [9,10]. ICAM-1 and VCAM-1 are cell adhesion proteins and are deeply associated with the development of symptoms and progress of pulpitis.

In addition, it was found that catechins suppress the production of inflammatory cytokines and chemokines by suppressing the activation of transcription factor NF-kappa B, which controls genetic information, by inhibiting the phosphorylation of an enzyme called MAPK to reduce its activity [11]. Inhibition of the activation of NF-kappa B would suppress the gene expression of these proteins, which in turn would result in the reduced production of these proteins.

As mentioned above, tea polyphenols including catechins can act on fibroblasts and exhibit anti-inflammatory effects. The safety of catechins is high and their antibacterial properties are also expected to be applicable to dental problems and to have clinical applications as anti-inflammatory agents.

The plaque bacteria present in a periodontal pocket causes gingival inflammation and the infiltration of immune cells. The immune response by these cells promotes the gradual absorption of alveolar bone, eventually leading to dental loss. Thus, catechins are expected to prevent the development of various dental diseases, including periodontosis.

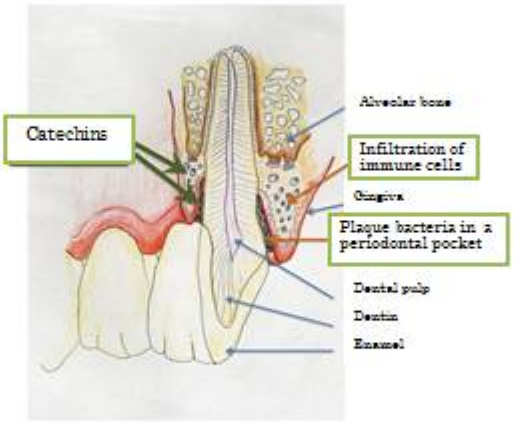

Figure 1. Anti-inflammatory and anti-bacterial effects of tea catechins.

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10. EFFECTS OF ENTEROBACTERIAL FLORA

Yukihiko HARA (University of Shizuoka)

Abstract

More than 100 species of bacteria inhabit the human bowel, including *Lactobacillus* and *Bacillus bifidus,* which are assumed to be favorable enteric bacteria that decrease the number of deleterious bacteria including *Clostridium* species. Tea catechins have been shown to increase the number of *Lactobacillus* and *Bacillus bifidus* and to reduce the quantities of compounds such as ammonia, sulfide, and indole, the cause of bad fecal smells.

Enterobacterial flora

There are more than 100 trillion bacteria in the human bowels that range from the distal end of the small intestine to the large intestine. These enterobacterial flora affect human health including various geriatric diseases, aging, and immune functions. Bacteria found in the feces of healthy adults include *Bacteroides*, *Eubacteria*, *Peptococcus*, *Clostridium*, and *Bacillus bifidus*; with age, quantities of *B. bifidus* decrease and quantities of enteral *Micrococcus* and *Welsh* bacteria (*Clostridium perfringens*), which produce bad fecal odor, increase. Since tea catechins have anti-bacterial effects on bacteria that cause food poisoning and bad odors but have no effect on favorable bacteria like *Lactobacillus* species, and since animal experiments have shown remarkable fecal odor reduction by catechins, attention has been paid to determining the effect of catechins on human feces. However, it is difficult to examine the effect of tea catechins alone because of the extensive factors involved in everyday human life including varying meal contents and exercise in healthy individuals as well as the wide range of changes in enterobacterial flora. For these reasons, it is necessary to study a human group that is maintained under controlled nourishment conditions.

Interventional studies

There is a nursing facility that meets the study conditions described above. In such an environment, fecal smells are a significant problem, and reducing such odors is important to caregivers. A research group of doctors of a medical clinic, the Gotemba Jyuji-no-sono Shinryosho, with cooperation from the nourishment department of the Seirei Mikatahara General Hospital, examined whether tea catechins could help reduce fecal odors in bedridden elderly people taking liquid nourishment through a gastrostomy tube [1].

Subjects included 15 residents of six facilities, including those mentioned above, and each subject received liquid nourishment three times a day through either a nasogastric tube or a gastrostomy tube. The patients included 10 females and 5 males with an average age of 70.3 years (range, 51–93 years) and an average weight of 41 kg (range, 28–56 kg). Underlying conditions included stroke in 10 patients, subarachnoid hemorrhage in 2 patients, and Parkinson's disease, cerebral contusion, and spinocerebellar degeneration in one patient each.

Each patient received 1000 kcal per day in the form of Ensure liquid, 30 mL of soy sauce, and 1300–1600 mL of water. They also received 100 mg of tea catechins (160 mg of Polyphenon 60 powder containing >60% catechins) in the nutritive liquid at every meal. This daily tea catechin dose corresponded to 300 mg of tea catechins, the equivalent catechins quantity of 5–6 cups of common green tea. The intervention was continued for 3 weeks and fecal samples were collected at five suitable stages and stored at the appropriate temperature.

Careful examination of the fecal samples yielded the following findings:

1) Favorable enteric bacteria, including *Lactobacillus* and *B. bifidus*, increased during the tea catechin supplementation, while deleterious bacteria, such as *Clostridium*, decreased (Figure 1);
2) As a result, organic acids in the feces increased, and pH decreased;
3) Quantities of ammonia, sulfide, indole, and skatole that give feces their bad smell decreased;
4) These effects tended to be restored by stopping the tea catechin supplementation;
5) Reduced fecal odor was clearly recognized during the tea catechin supplementation.

On the basis of the above results, the facility is using the catechin supplementation continuously and analyzing its effect on the feces of 35 subjects with fluid food intake. The results obtained so far are as expected. In addition, the results of interviews of more than 20 healthy adults who ingested tea catechins every day for 3 months showed that they had good enteric condition and favorable bowel movements (Figure 2).

These findings indicate that the habitual intake of green tea and catechins increases quantities of enteral lactic acid bacteria, reduces unfavorable bacteria, maintains a favorable enteric condition, and reduces fecal odors [2,3]. It appears that an important method for maintaining a healthy physical condition involves drinking green tea during and after a meal.

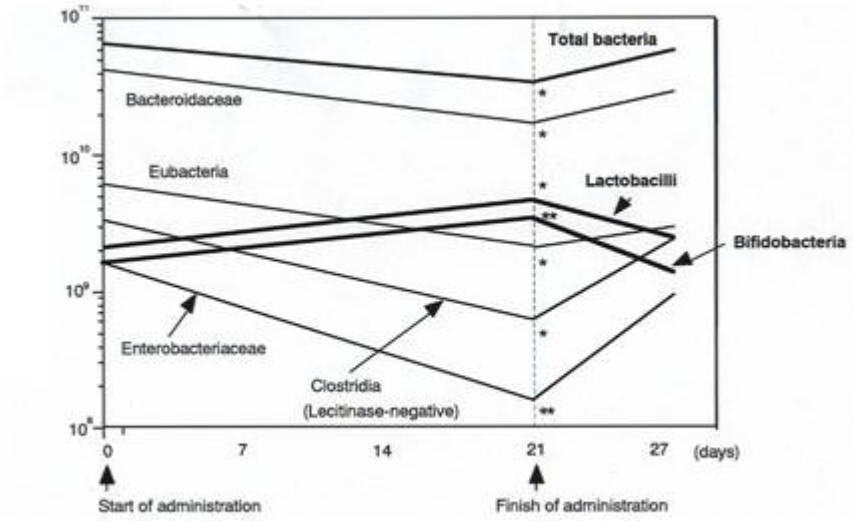

Figure 1. Change in the number of intraintestinal bacteria in the feces of elderly bed-ridden patients taking 300 mg tea catechins a day.
Ordinate: No. of bacteria (colony-forming activity)

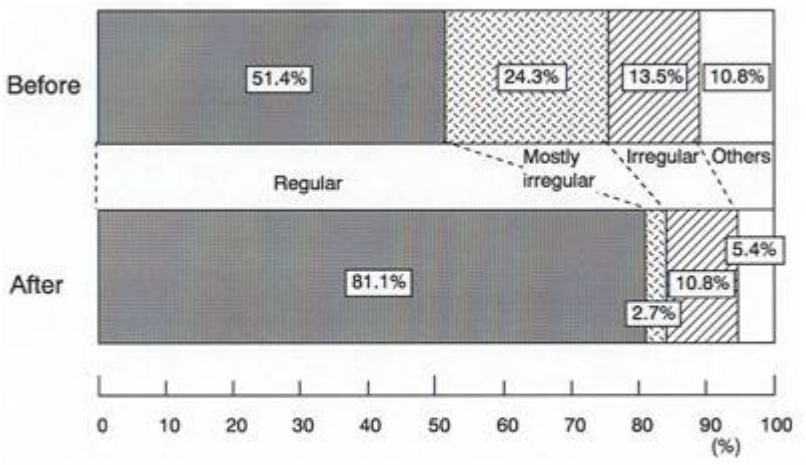

Figure 2. Ingestion of catechins resulted in good enteric condition and favorable bowel movements.（N=37）

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11 ADVERSE EFFECTS OF GREEN TEA INGREDIENTS

Mamoru ISEMURA (University of Shizuoka)
Hiroshi YAMADA (University of Shizuoka)

Abstract

In most cases, green tea and its ingredients are seemingly helpful for disease prevention and treatment; however, this should be treated with caution, as the intake of green tea has rather unfavorable health effects when taken in large quantities. For example, the caffeine content of green tea may cause a sleep disorder, and green tea supplemented with epigallocatechin gallate may cause hepatic injury. Although these cases are rather rare, individual sensitivity to these components should be taken into consideration. In addition, it should be noted that there are many epidemiological studies in which the usefulness of green tea was not demonstrated.

Green tea supplementation and liver damage

Intake of a green tea supplement was reported to be associated with a liver injury. A survey by Mazzanti and others from 1999 to 2008 reported liver injury cases in 6 men (27–45 years old) and 28 women (19–69 years old), of whom 15 took a supplement only containing green tea extract and 9 of them consumed Exolise, a product made in France [1]. This product was removed in April 2003 from the market. Because of the reported cases of acute liver injury associated with green tea supplementation, the intake of green tea containing high concentrations of catechins should be taken with caution [1-3].

Epidemiological studies

An epidemiological research study in Japan reported that green tea drinking in men was associated with a rather high risk of stomach cancer [4].

In the case of thyroid cancer, among postmenopausal women, those who drank one cup or less a day of green tea had a risk for thyroid cancer of 1.00, which was reduced to 0.47 in those who drank more than five cups of green tea, whereas premenopausal women had 1.66 times higher risk for thyroid cancer [5]. In addition, drinking hot tea may increase the risk for esophageal cancer [6]. Among smokers, green tea intake increased the risk for colon cancer [7].

Asthma

Three patients with asthma who worked in a processed tea factory in 1994 were reported to have shown skin and bronchial reactions to EGCG; however, no such reaction was observed in 5 physically unimpaired individuals and 5 persons with asthma who had not been exposed to green tea powder [8]. Based on these criteria, the causative agent was judged to be EGCG. Release of allergy-causing histamine was observed when EGCG was added to blood samples obtained from patients with asthma, and some cases showed an intracutaneous reaction to EGCG [9]. These findings suggest that the antibody immunoglobulin E is associated with and is a causative factor of asthma. Furthermore, 21 cases of asthma were reported to be attributable to green tea intake [10].

In addition, hypersensitivity pneumonitis was reported in a 51-year-old man who underwent tea catechin inhalation therapy for 1 month and tuberculosis treatment for 3.5 months [11].

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