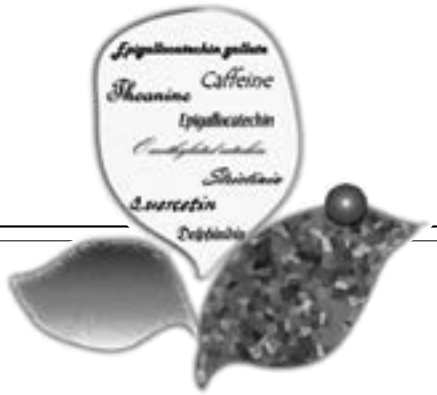
SCIENTIFIC EVIDENCE FOR THE HEALTH BENEFITS OF GREEN TEA

Japan Tea Central Public Interest Incorporated Association



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Preface

Junichi SHINMURA

Every year, the worldwide production of green tea is increasing, alongside the increasing awareness of its health benefits. A report by Dr. Tsuneo Kada from National Institute of Genetics triggered my interest in the functional roles of green tea. This report in March of 1981, indicated that, among 500 different types of food, the components of green tea effectively inhibited genetic mutation and malignant transformation of the cell. People engaged in science, business, and the industry of green tea received his findings with excitement. This discovery compelled me to go to the Institute twice for an interview with Dr. Kada.

In 1992, when UNESCO held The World Congress of Educational Cities in Göteborg, Sweden, I attended the meeting as mayor of Kakegawa city, representing Japanese municipalities practicing the "lifelong study program." There, a Swedish scholar asked me, "Japan is now an economically great nation with a world ranking of 2 in GNP, so I assume that the average life expectancy would be very short because of the nationwide 'workaholic' tendency. Contrary to this expectation, these days Japan has become the country with the longest life expectancy. What do you think has brought such improved life expectancy to your country?" I was puzzled initially, but answered at once "it is because Japanese people drink green tea." He appeared surprised by the reply and this conversation eventually motivated him to visit Japan and gather first-hand information.

One may ask questions; "Why do elderly Japanese people love to drink green tea? Why do they consider green tea to be so delicious? Why do they prefer green tea?" Three reasoned responses to these questions from Japanese people can be due to the effects of green tea. The first reason is that, when a person grows old, the mind and body ages and starts to feel spiritual, '*Wabi and Sabi*' (represent the comfort found in quietness, simplicity, and elegance in Japanese), and begins to enjoy the bitter taste of green tea. The second reason is that the mind and body craves certain elements from plants, which are possibly lost from the body due to aging, and green tea substitutes for such readily lost elements. The third reason is that habitual drinking of green tea tends to support the belief that green tea harbors elements which inhibit waste and toxin accumulation and that it protects from disease-causing agents by preventing and/or inhibiting their generation within the body.

By reading this book, one can become aware of the multiple functions of green tea in the prevention and treatment of various diseases. Upon understanding the explanations provided by the contributing authors, the reader can appreciate that modern science can explain what the Zen priest Eisai said in his book, "Kissa Youjouki (How to Prevent Diseases by Drinking Tea)", published in 1211. His saying was "Tea is a nature-given medicine to keep health and Tea is a medicine for all kinds of diseases."

One may think that green tea has too many effects. However, considering the saying "*Care will kill a cat*," there appears to be something more to green tea than just the association in the components analyzed and physical diseases. If the effects of the individual components in green tea, as described in this book, are studied further, synergies of the healing properties in the immune, physical, and mental systems may be found. Then, perhaps, the number of individuals depending on health insurance and nursing care insurance would decrease, thus reducing the National financial burden on health and welfare systems.

My "selfish strange theory," 30 years ago in Sweden, was that the major reason for a healthy life, increased life expectancy, and lowered incidence of dementia is to drink green tea. If the knowledge regarding the beneficial effects of green tea increases the number of the "green tea people" who drink more than 5 grams (two tea bags) of green tea every day, I shall be very happy. Then, I shall be able to recognize that what I answered to the Swedish scholar has become true.

This book has been written by the main members of The Society of Tea Science of Japan, which has been active for 30 years and, which is supported by The Chamber of Tea Association of Shizuoka Prefecture. This book has been published with contributors from researchers conducting cutting-edge research on various aspects of tea in Japan.

I express my heartfelt gratitude to all the contributors who spent their valuable time to write this book, and I would request them to continue undertaking appropriate steps in the future that will promote tea science. Furthermore, I thank deeply Dr. Zeno Apostolides of University of Pretoria, South Africa for his contribution to editing English for this book.

Junichi SHINMURA: President of Japan Tea Central Public Interest Incorporated Association

Efficacy of Tea in Human Health: Overview

Isao TOMITA

Abstract: Recent scientific findings on the effects of tea (*Camellia sinensis*) on human health are reviewed. Some mechanistic explanations are discussed. Though there are still some discrepancies between the results in animal and those of epidemiological studies, the reasons may be uncovered in the near future. The health effects of tea are summarized in Figure 2.

Keywords: efficacy, health effect, mechanistic explanation

How the physiological effect caused by tea drinking attracted humans

Many legends are told to explain why the people in ancient China began to drink tea. One of the stories told is about Wan Tu, the ancient Chinese emperor. He was banished to a remote southern part of China (Unnan district?) due to his cruel and tyrannizing governance. One day, when he was sitting in the shade of a large bush (the area where *Camellia sinensis* grew) to drink hot water, he found some leaves, which were floating in the water. After he drank the water with the leaves, he felt excited and freed from fatigue [1].

It is now known that the leaves of tea (*Camellia sinensis*) contain caffeine (2-4% in dry leaves) and theobromine (about 0.1%) both of which are soluble in hot water and show various physiological functions, such as stimulating the central nervous system.

It is also well known that the tea leaves contain a large amount of catechins (8-20% of the dry weight) of which the major one is (-)-epigallocatechin-3-*O*-gallate (EGCG) (Figure 1). The taste of tea are very unique, bitter and astringent because of the presence of the above substances. It may be worth knowing that their contents are quite different depending on the species of *Camellia* leaves. The leaves of *C. sinensis*, *C. taliensis*, and *C. irawaidiensis* are all known to contain caffeine, theobromine and

catechins, but other species such as *C. furfuraceae* and *C. sasanqua* have no such components [2]. Tea leaves are also known to contain theanine (0.5-3%) which is rarely found in plant kingdom.

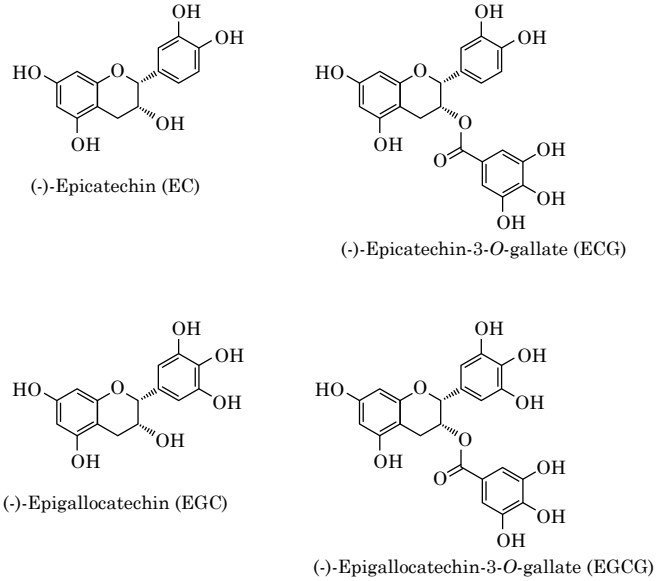

FIGURE 1: Chemical structures of tea catechins

Strong anti-oxidant properties of tea and its relation to disease prevention

Since tea drinking has a long history of more than 3,000 years, there has been much scientific research on the nature of the components and isolation of the responsible substances for their characteristic taste, color, aroma and physiological functions. However, it was not until the late 20th century that research on tea as a "functional food (beverage)" was carried out. It was at the time that it was discovered that oxygen radicals, such as the superoxide anion radical ($O_2^{-\cdot}$) and the hydroxyl radical ($\cdot OH$) formed from foreign stimulants, could cause degenerative disease and aging. The term "oxidant stress" became popular, and it was believed to be a main cause in developing diseases such as cancer, atherosclerosis, stroke, coronary heart disease and diabetes. The negative correlation between the mortality of such chronic diseases and the consumption of common vegetables and fruits containing various flavonoids as anti-oxidants seemed to accelerate this area of research [3]. Cao G *et al.* reported that tea has a very strong anti-oxidant activity compared to that of common vegetables in their ORAC (Oxygen Radical Absorbance Capacity

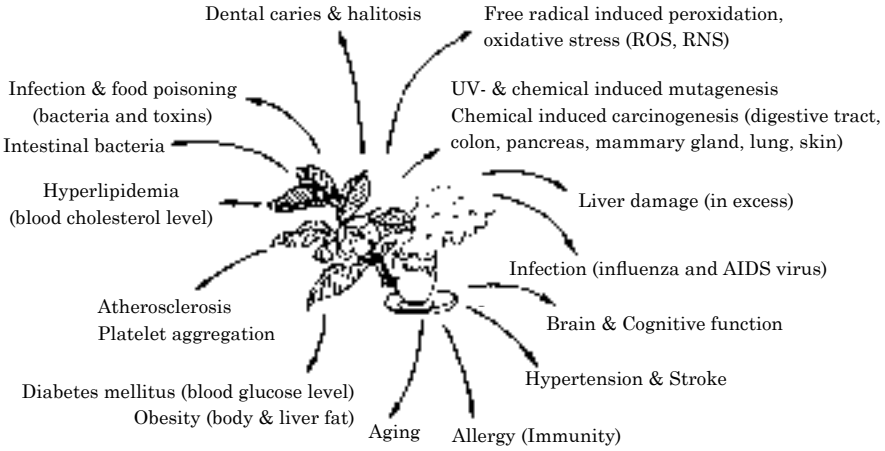

FIGURE 2: Possible effects of tea on health

Assay) systems [4]. We also recognized that the tea extracts as well as its main constituent, EGCG and its metabolites exerted strong anti-oxidant activities in rats [5]. The development of evaluating methods for detection of anti-oxidant activities using TBARS (thiobarbituric acid reactive substances), isoprostane (15-isoprostane F2t) and 8-OHdG (8-hydroxy-2'-deoxyguanosine) as biomarkers contributed highly to this area of research.

In another area of study, convenient methods using bacteria such as *Salmonella typhimurium* TA and *Escherichia coli* WP2 to detect mutagenic and anti-mutagenic substances were also employed, and pioneering works on the anti-mutagenic properties of tea extracts were reported in 1984-1985 (see review [6]). Their anti-carcinogenic effects in various assay systems at the stage of anti-initiation and anti-promotion were also demonstrated and reported. Mechanisms of anti-mutagenesis and anti-carcinogenesis were discussed in detail in the First International conference (ICMAA) which was held at the University of Kansas in October 1985. The presentation on the effects of tea seemed to attract successive research in different and diverse fields. Tea research done in the last 30 years has revealed that green as well as black tea are the most common and acceptable beverage to avoid or decrease the risk of chronic diseases (Figure 2). It has been well known that catechins in fresh tea leaves are easily oxidized to theaflavins, theasinensins, thearubigins and proanthocynidins by polyphenol oxidase in the process of black tea preparation. It is interested to know that theaflavin shows almost the same anti-oxidant activity as catechin (EGCG) *in vitro* experiment in molar basis. [5].

However, now, we have to respond to the questions: Why the anti-oxidant effects of catechins and theaflavines, for example, are so good, despite their limited absorption into the body. Their absorption is less than 2-3% of the intake, and the

maximum concentration in blood is only 0.03-0.38μmole/L for EGCG (T 1/2=2.5-5.1 hours) and it is far too low to expect direct anti-oxidant activity.

How does catechin exert effects on life-style-related diseases through the anti-oxidant activity?

In order to discuss the mechanistic explanation of catechins as a bio-anti-oxidant in connection to disease prevention, recent findings by several researchers on the effects of catechins for cell signaling or gene expression must be considered.

It is partly because catechin works in some experimental conditions as prooxidant, (not only as an anti-oxidant) and produces hydrogen peroxide *in vitro* and *in vivo* [4]. Hydrogen peroxide (H_2O_2) is now known to be an important 2nd messenger, transducing the oxidative signal into biological responses through post-translational protein modification. In case of excess H_2O_2 production, it might deteriorate vascular functions, for example, to promote vascular disease through multiple pathways [7]. Adverse effects, including liver damage by the administration of a high amount, the suppression of anti-oxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase), and H_2O_2 production might be enhanced.

As for the problems on EGCG triggered hepatotoxicity, and the safety of tea drinking in regular life, recent references discussed in detail should be referred [8, 9].

In recent years, on the other hand, the presence of many special binding molecules for EGCG (67kDa laminin receptor, vimentin, IGF1R, Fyn, PP2A) were reported. It is expected that they may explain the role of catechin as a powerful anti-oxidant even in its low level *in vivo*. That is a role of catechin to activate the nuclear factor erythroid 2 related factor 2 (Nrf2) and anti-oxidant response element (ARE) [10].

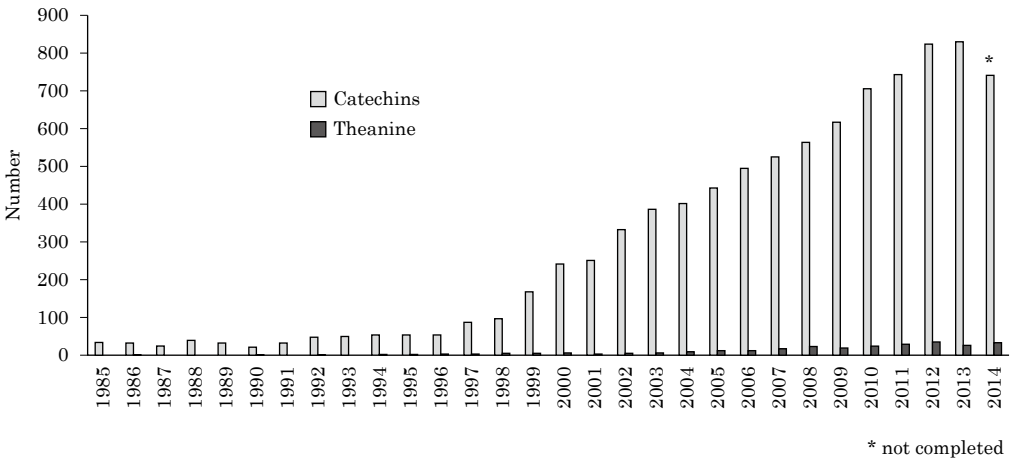

FIGURE 3: Number of articles containing tea catechins and theanine in the title/abstract from 1985

"Onko-chishin" - He that would know what shall be, must consider what has been -

Historically, tea (drinking) was introduced into Japan by several famous Buddhism priests who studied the doctrines in China. Eisai was one of them. He visited and stayed in China twice (AD 1167 and 1187) and learned Zen Buddhism deeply. Along with learning the religious discipline of Zen Buddhism, he devoted himself to tea, which always kept him awake and was good for his health. At the age 71 (1211), he wrote, "Kissa Youjouki" (The way to prevent diseases by drinking tea). By quoting Chinese literature, he described his belief about the effectiveness of tea, for human health. There is especially an important statement in the latter part of the above book telling us that the drug is only for one individual disease, while tea prevents all kinds of diseases (Panacea).

The importance of tea for human health, not only for physical but also for the mental, has been scientifically studied for the past 30 years. The number of research papers published in English on catechins, and theanine are shown in Figure 3. There still seems to be some discrepancy between the fundamental research results using cells and animals, for example, and those of epidemiological studies in several areas of investigation. The reason for this is unclear at present. However, it will undoubtedly be made clear in the near future. The interested reader is referred to several excellent reviews [11-18], for a better appreciation of tea for human health.

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Characteristics of Japanese Green Tea

Yoriyuki NAKAMURA

Abstract: Tea has been drunk long before the Christian era, as a non-alcoholic beverage for excitement, exhilaration and health. Tea is enjoyed as a beverage and also used for its health benefits. In the long history of tea production, many types of tea have been developed, depending on the variety of tea, harvest season, and methods of cultivation and manufacture. Japanese green tea is manufactured by a steaming process in order to inactivate the oxidizing enzymes of the leaves. This process is unique in the world. The manufacturing process by steam is important and essential for the formation of fresh aroma and good taste. Furthermore, the Japanese green tea ceremony (Cha-no-yu) is one of Japan's most traditional arts, and it has exerted a great influence not only upon the tea drinking habits of the people but also on the spiritual life of Japanese people. The content of chemical components is different from other teas. Recently, with the advance of modern chemistry, the components of tea and their specific health benefits have been analyzed, and a large body of scientific evidence is being accumulated.

Keywords: classification, cultivation, health benefits, history, Japanese green tea

The origins of the tea plant

Tea is one of the three most popular non-alcoholic beverages, next to cocoa and coffee. Tea is the national drink in the two most populated countries in the world, namely China and India. Tea is consumed by over two thirds of the global population.

Tea is an evergreen tree plant, belonging to the genus *Camellia* in family Theacea. The genus *Camellia* has over 90 species and is distributed from Nepal in the West to Japan in the East. The species are arranged in 12 sections [1]. The tea plant (*Camellia sinensis*) belongs to the Thea section along with four other species (*C. irrawadiensis*, *C. taliensis*, *C. gracilipes* and *C. pubicosta*). Varieties of tea plants

FIGURE 1: Old big tea plant in Vietnam and Chinese border region

are divided into two groups: *Camellia sinensis* var. *sinensis* is called China type. This has small leaves and a bush type tree. *C. sinensis* var. *assamica* is called Assam type, and has large leaves and is a tall type tree. These tea plants seem to have different origins depending on the plant morphology. However, they may be derived from the same parent species because hybridization between them occurs easily, resulting in viable hybrid seeds.

The origin of the tea plant is estimated to be around southwestern China. These provinces have a large number of centuries-old big tea plants (Figure 1), and these tea plants show a wide diversity in morphology and fermentability, etc. The climate of these provinces is warm temperature, ample rainfall and good drainage. This climate is suitable for tea cultivation. These provinces also have ideal soil for growing tea plants, and the influence of the glacial epoch did not affect the cultivation of tea plants [2, 3].

The history of tea consumption

The use of tea was known since before the Christian era. First, tea was drunk and eaten as a plant stimulant and an enjoyable drink. Tea drinking eventually became increasingly popular among ordinary people, despite its initial restriction to the aristocracy and members of the sacerdotal class. It is thought that tea was drunk as a very precious herb to help purify the body and preserve the mind.

In the Tang Dynasty era in China, Lu Yu (733-803), who is generally acknowledged as the founder of the tea culture, wrote a traditional book on tea called 'The Classic of Tea' or 'Tea Sutra (Cha-Kyou).' This book discussed the history of tea, the method of manufacture, the utensils of manufacture, the method of preparation, and the custom of drinking tea in China. Thereafter, tea was introduced not only to Japan by the Buddhists, but also to European countries by merchants. And now, tea is drunk

all over the world as one of the most favorite beverages.

Recently, with the advances of modern chemistry, components of tea have been analyzed. Some of the health benefits that were claimed for green tea in the past have been attributed to specific components of tea. Tea is accepted as the cultural beverage that has nutritional, sensory and body-modulating functionality.

Tea has specific chemical components: caffeine, catechins and a unique amino acid, namely theanine, that is not found in other plants. Caffeine is an alkaloid, and has a stimulatory effect. Drinking tea, therefore, helps relieve drowsiness and provide refreshment. Theanine, which helps relieve stress and promote relaxation, is the major amino acid of the tea plant, and is responsible for the Umami taste. Epigallocatechin gallate accounts for about 50 percent of the total amount of catechins, and may prevent lifestyle-related diseases. Gallated catechins have a bitter flavor. They confer an agreeable brisk and refreshing flavor.

Classification of tea

In the long history of tea production, many types of tea have been developed. These types differ in various respects, such as variety of tea, harvest season, and methods of cultivation and manufacture.

In general, tea is classified depending on the degree of fermentation (oxidation). In China, tea is classified into six types based on the degree of fermentation: the level of flavonoids, which is the precursor of the pigment in color (Figure 2); differences in catechin content; and the appearance of the infusion. Naturally, the components of the tea classified into six kinds differ, and a big difference is observed also in a flavor

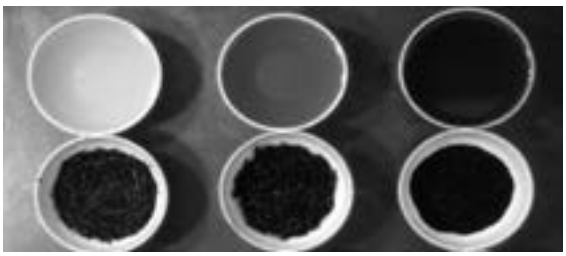

FIGURE 2: The tea made with the same tea-leaf
Left: Green tea, Center: Blue tea, Right: Black tea

TABLE 1: Characteristic constituents of six type teas

	Green tea	Yellow tea	Dark tea	White tea	Blue tea	Black tea
Amino acids mg/100g	4023	2599	161	4951	835	2355
Theanine mg/100g	875-3030	1667-2130	23-108	61-3828	186-873	1461
Chlorophyll mg/g	0.4-2.6	0.6-0.9	1.4-2.0	0.6-1.4	0.8-1.7	1.1
Carotene mg/g	0.019-0.051	0.02-0.03	0.001-0.005	0.004-0.01	0.016-0.03	0.017
Catechins mg/g	36.62-279.2	84.23-215.15	22.5-175.98	55.58-241.19	64.17-218.33	17.36

World Tea; International symposium on tea science in Japan(1991) p.30(Zenon Wang: Anhui agricultural college)

or functionality [4] as follows: (Table 1).

Green Tea: This type of tea includes both steamed tea (Japanese type) and pan-fired tea (Chinese type), drunk as unfermented and green-colored infusions. Green tea is the most common tea in Japan. However, it is gaining attention from all over the world, since its health benefits have been scientifically documented. Green tea consumption is increasing worldwide.

Yellow Tea: After withering, soft leaves are stacked to discolor the green chlorophyll. This tea has a characteristic yellowish infusion due to this process. Yellow tea is scented with phenolic methylsalicylic acid, and has a fresh aroma and plain taste.

Dark Tea: Dark tea is microbial fermented tea. The color of an infusion is brownish yellow or brownish red, and the taste is mellow. The aroma is musty, smoky and somewhat phenolic.

White Tea: White tea is made by gathering the tips of the leaf buds with white hairs and allowing them to wither and dry naturally. White tea which is silver-tipped and elegant in appearance, is regarded as one of the highest-grade teas in China.

Blue Tea: Oolong tea is a typical type of blue tea. After withering, the leaves are subjected to semi-fermentation. Blue tea has an excellent aroma.

Black Tea: Black tea, or fermented tea, is manufactured in four stages: withering, rolling, fermentation, and drying/roasting. Black teas account for approximately 70 percent of the global tea production.

Cultivation of tea in the world [5]

Tea is a luxury beverage, drunk all over the world. However, the tea-producing region is situated in a limited area enclosed by about forty degrees of latitude from 5° S to 35° N, and about seventy degrees of longitude from 67° to 140° E. More than 40 countries, including China, Japan, Vietnam, and old plantations in Southeast Asia, produce tea. In the early 20th century, the African nations Kenya, Uganda, Malawi and South Africa, as well as Iran, Turkey, and other small countries surrounding the Black Sea, became tea-producing countries. Furthermore, Brazil and Argentina in South America, and Australia have also recently begun to produce tea.

Recently, green tea has been paid attention too, since the health properties of tea have been scientifically examined. Global tea production will reach 5 million tons in two to three years.

Japanese Green Tea [5]

Japanese green tea has a very long history and has achieved unique developments. The Japanese custom of drinking green tea came from China in about AD 800. The

use of tea started when Buddhist monks, who had gone to China for study, returned to Japan bringing tea with them as a medicinal beverage. In the Kamakura period (1192-1333), the Zen monk Eisai wrote the beneficial effects of tea in his book 'Kissa Youjouki (Maintaining Health by Drinking Tea)': "Tea is a marvelous preventative medicine for maintaining people's health." Tea has strong power to prolong life.

Although tea had been used as a medicine and in religious offerings among the aristocracy and the sacerdotal class, it steadily gained popularity as a luxury drink among the warrior classes and the commoners.

On the production side of tea, Japanese green teas are adapted only to the manufacturing process by steam. The manufacturing process by steam is important and essential to the formation of aroma and taste of Japanese green tea. Compared to Chinese green tea (pan-fired tea), oolong tea and black teas, its chemical components are different. Furthermore, Japanese green tea production has achieved high quality and high yield due to the introduction of superior cultivars and the new mechanical cultivation systems, gaining interest from all over the world.

Cultivation of Japanese Green Tea [5]

Japanese green tea fields are managed by modern mechanical cultivation systems, and are very beautiful scenes (Figure 3). In Japan, commercial tea cultivation is carried out in the southern part of the country, where the average temperature is 11.5-18.0℃ and the average rainfall is 1500-2000 mm per year. Generally, the quality of tea is reduced by high temperature that increases yield.

Japanese green tea plants grow from March to November (spring to autumn) and remain dormant for the rest of the year. Leaves are harvested 2 to 4 times in a year, and each harvesting period is about 2 weeks long and 4-6 weeks apart. The

FIGURE 3: Japanese tea field and hand tea plucking landscape

first and second crops, called first flush and second flush, respectively, are harvested and processed from late April to the middle of May at intervals of 30 to 50 days. Tea production has been influenced by agro-technology, as well as by the section of suitable land, tea cultivars, plucking methods, pest controls, climatic factors and unique trading system. The first flush leaves are considered the highest quality, followed by the second flush, with the third flush producing the lowest quality tea.

After planting, the productivity in the tea field with fresh leaves accounts for about 7,000kg/ha in first flush, 6,000kg/ha in second flush, and 4000kg/ha in third flush. The buds of tea plants will continue to grow unless harvested. Therefore, delayed harvest is associated with a progressive decline in tea quality. Harvesting should be done at the optimal timing to avoid the deterioration of leaf quality. Thus, harvesting is an extremely important stage, which markedly affects both quality and production. Tea harvesting can be done by one of two methods: hand plucking and machine plucking. Normally, the machine plucking method is used because of its greater productivity. In Japan, hand plucking yields are approximately 10 to 15kg of tea per worker per day, while machine plucking yields are 6,900 to 7,600kg per worker per day.

Kinds of Japanese green tea [5]

Most Japanese green tea is 'Sencha.' In general, tea grades are classified according to size, color, aroma, flavor, and appearance of the leaves. Tea produced with the buds of younger leaves are acknowledged as the finest quality. First flush has a strong flavor and the amino acid content is high, whereas both second and third flush tend to be lightly astringent, and rich in catechins compared with first flush because the harvest season begins in the summer. First flush green tea harvested in the early spring, includes many aroma compounds, such as hexanoate, linalool oxides and dimethyl sulfide with a grassy aroma, and various esters [6], and the high content of amino acids make overall flavor palatable and highly acceptable (Figure 4).

'Sencha' is described as a refreshing, freshly aromatic infusion combining astringency with umami taste. To increase the 'Umami,' 'Tencha' and 'Gyokuro' are cultivated in the tea fields under a roof of straw or buffer muslin for 20 days, prior to harvesting. Shielding the plants from sunlight increases the content of the relaxing, health-promoting amino acid theanine in the leaves, resulting in a full-bodied tea. The leaves of 'Gyokuro' have a more defined needle shape than those of 'Sencha,' and are dark green in color. These are the best quality leaves for making Japanese green tea. Moreover, 'Tencha' which is used for the production of 'Matcha,' is unsuitable for drinking without grinding with a mortar (Figure 5).

Commercial green tea products with different taste, shape and quality have been developed and are consumed all the year round. 'Matcha' tea is widely used

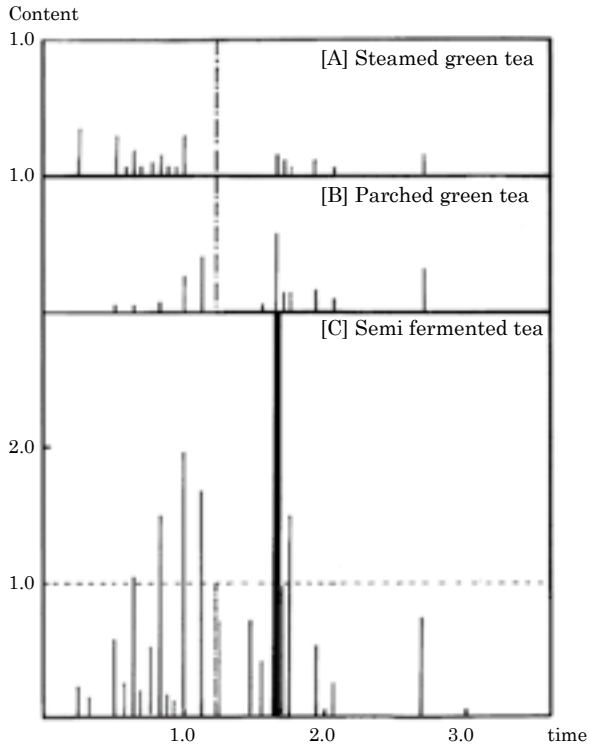

FIGURE 4: Gaschromatograms of steamed green tea, parched green tea and semi fermented tea made from fresh leaves plucked on the same day [6]

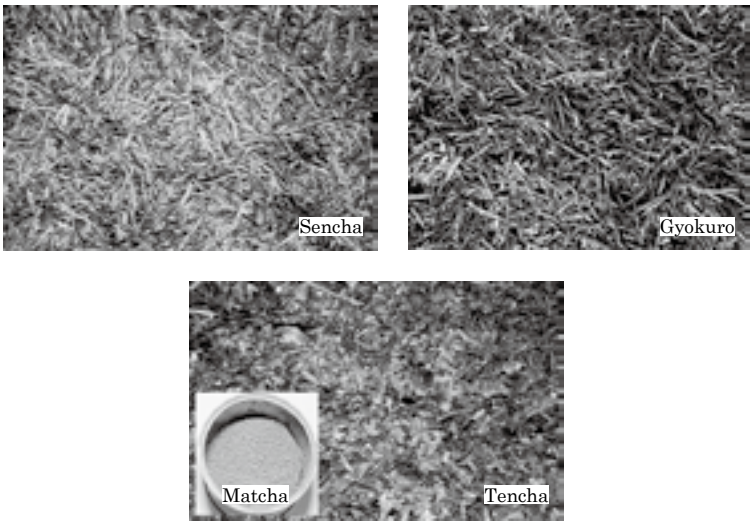

FIGURE 5: Three major Japanese green tea
Sencha: This is the most popular type of tea in Japan.
Gyokuro: This is the finest tea in Japan, and its taste is a tender sweet astringency and flavor.
Tencha: This is ground in a stone mortar into Matcha (powdered tea) and used in a tea ceremony.

as natural flavor or green colorant. Moreover, besides the daily use as a beverage, consumption is increasing for the various tertiary functions, e.g. effects like anti-microbial and anti-oxidant properties of catechins.

Characteristics of Japanese green tea contents

Recently, there has been a trend toward certain types of health claims in food science. Researchers have suggested the potential health benefits of food and classified them into three types: first, a nutritional function (the primary function) responsible for palatability; second, a sensory function (the secondary function); and third, a body-modulating function (the tertiary function) that controls physiological processes in the body, prevents and cures diseases, sustains physical performance, and delays aging.

Tea is a beverage characterized by all three of these functions, and Japanese green tea is regarded to be a high quality of tea from all over the world. Traditional Japanese cuisine, called "Wa-shoku" in Japanese, has gained in popularity in the world because of its healthy image. It is customary to serve Japanese green tea as part of a healthy meal. Japanese green tea is different in taste and aroma, etc. when compared with oolong tea or black tea. The differences are due to the volatile compounds, catechins, theanine, various amino acid, etc. Especially, Japanese green teas, made in Japan, contain a high content of theanine, arginine, chlorophyll and so on. Therefore, it is easy to distinguish it from green teas produced in other countries (Figure 6) [7].

Amino acids are the elements responsible for the 'Umami' taste and sweetness of Japanese green tea. Japanese green tea contains about 20 amino acids; theanine accounts for nearly 60 percent of the amino acid content, followed by glutamic acid, aspartic acid, arginine and serine. Theanine is responsible for the refined flavor and sweetness, and is a major flavor compound in green tea. The conversion of theanine to catechins, is catalyzed by sunlight. Cultivation in the shade can restrict this chemical reaction resulting in leaves rich in theanine. Gyokuro and Matcha, also known as shaded tea, are grown in shade, and have full-bodied, rich flavors. A recent study demonstrated that theanine helps relieve stress and promote relaxation, both of which are tertiary body-modulating functions. Interestingly, the relaxing properties of theanine counteract the stimulatory properties of caffeine.

Tea catechins are well known for their various biological activities, including anti-oxidative, anti-mutagenic, anti-tumor and anti-bacterial, etc. Catechins are responsible for the astringency in green tea. The content of catechins in Japanese green tea is estimated to be about 13 to 15 percent of dry weight of a tea leaf. Catechin content varies depending on the method of manufacture. Green tea that is usually unfermented during processing is rich in catechins, while black tea, which is fermented, contains lower levels, because of the enzymatic conversion of catechins

to theaflavins or thearubigins.

Japanese green tea is also a good source of beta-carotene, which is converted to vitamin A in the body. The body-modulating function of beta-carotene is also significant with regard to its health-promoting effects.

Green tea contains more vitamin B complex, C, E, P and U than black tea. Especially, the level of vitamin C (ascorbic acid) which has been shown to prevent scurvy and the common cold, is decreased during the fermentation stage (Figure 7) [8]. Black tea, therefore, does not contain vitamin C. In case of green tea, vitamin C is gradually degraded during the manufacturing process and storage term. In the case of 'Sencha,' the content of vitamin C averages 250mg per 100g.

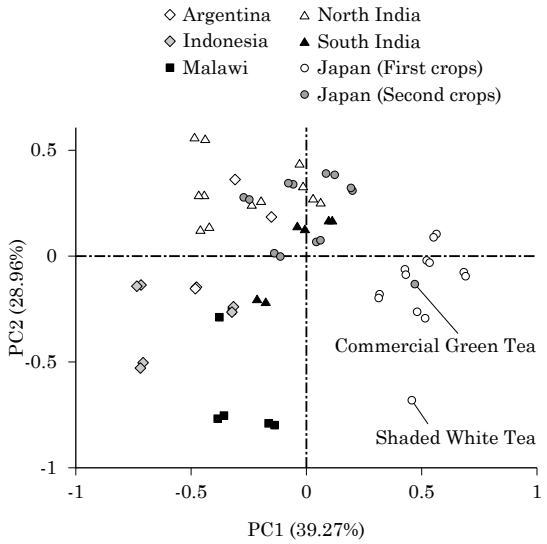

FIGURE 6: Principal Components Analysis score in the green tea produced in the various country

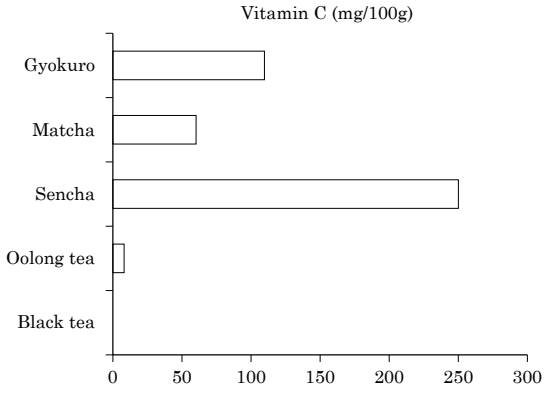

FIGURE 7: Vitamin C content of various tea

FIGURE 8: Tea ceremony (Courtesy of The Tea Museum Ochanosato)

The content of vitamin E which has been shown to prevent lifestyle-related diseases and to act as an inhibitor of aging, is higher than in other foodstuffs, and plays a characteristic role in the powerful physiological activity of alpha-tocopherol. Indeed, Japanese green tea is a super nutritional vegetable.

When we are exhausted, the pure green color of plants relieves us mentally. Tea infusions have an elegant and delicate appearance, clear jade green in color, especially when brewed with hot water. Plucked leaves rapidly undergo oxidation if left untouched or fermented immediately. The leaves become shriveled and dark brown in color. To make Japanese green tea, however, freshly harvested leaves are steamed in the steaming machine to inhibit enzymatic activities, and thus prevent the oxidation processes. Thus, the pale green color of green tea is largely dependent on this particular process. Chlorophyll, which is the pigment required for photosynthesis in plants, is responsible for the pale greenish color of green tea, while flavonol provides the infusion with a slightly yellow-green color. In contrast to the color of green tea, black tea gives a bright red infusion. These colors are unique to fermented teas due to the chemical reactions that occur during fermentation. In general, during fermentation, chlorophyll in the leaves is degraded, while the catechins are converted to various oxidized chemicals, such as the orange-colored theaflavins and red-purple thearubigins. These substances are responsible for the reddish color of the infusion.

In the cultural aspect of tea, tea ceremony 'Sado or Cha-no-yu' was completed in the 15th century, and it has exerted a great influence not only upon the tea drinking habits but upon the spiritual life of Japanese people (Figure 8). The Matcha used for the tea ceremony has the best taste and flavor in the world.

Japanese green tea, produced from the beautiful tea fields, achieves the highest quality and the highest yield in the world. This is due to the introduction of superior cultivars and the newly mechanized cultivation systems.

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Health Benefits of Green Tea

1

Anti-cancer Effects

Basic and Human Studies

Mamoru ISEMURA

Abstract: A large number of experiments using cells and animals have demonstrated the anti-cancer activity of green tea and its catechins, the major component of which, (-)-epigallocatechin-3-*O*-gallate (EGCG), is responsible for this activity. EGCG induces programmed cell death called apoptosis in cultured tumor cells and inhibits growth and metastasis of tumor cells 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 reactive oxygen species. The anti-metastatic property of green tea and catechins may be due to their inhibitory activity on tumor-associated proteinases and on cell adhesion of tumor cells. The results of several epidemiological studies have indicated that intake of green tea reduces the risk of various kinds of cancer. However, in other epidemiological studies, conducted on the intake of green tea, no benefit could be shown. These conflicting findings could be due to several confounding factors. Such factors are the method of quantifying tea consumption, tea temperature, cigarette smoking, and alcohol consumption. Further studies are needed to elucidate the anti-cancer activity of green tea and its components. Several clinical trials have shown that tea catechins are promising as an anti-cancer agent. A standardized green tea polyphenol preparation called Polyphenon® E or Sinecatechins has been approved as a medication for genital warts by the United States Food and Drug Administration. Several lines of evidence have suggested its effectiveness in certain cancer cases. These findings suggest that green tea and catechins are useful for preventive and therapeutic treatment of cancer.

Keywords: anti-cancer, anti-metastasis, apoptosis, clinical trials, EGCG, epidemiology

Introduction

About 25 years ago, a possibility was pointed out that green tea intake may reduce

the risk of cancer based on epidemiological studies conducted in Japan [1, 2]. There is an increasing number of research studies into the anti-cancer activity of tea. Many animal experiments have shown that the rate of carcinogen-induced carcinogenesis is reduced by taking green tea or catechins. The growth and metastasis of inoculated cancer cells is also inhibited by the consumption of green tea or tea catechins [2-5].

Apoptosis induction as an anti-tumor action of green tea components

The major compound contributing to the anti-cancer activity of green tea is the polyphenolic compound, epigallocatechin gallate (EGCG) [2-5]. To determine the molecular mechanisms by which EGCG exhibits its anti-tumor 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 programmed cell death called apoptosis. For example, the EGCG-treatment of MKN-50 cells induced a formation of DNA ladder at a nucleosome unit, one of the characteristic features of apoptosis (Figure 1). Apoptosis is physiological cell death by which unnecessary cells are eliminated. Many anti-cancer drugs are known to induce apoptosis in cancer cells, and its induction in cancer cells leads to the prevention of cancer development [2].

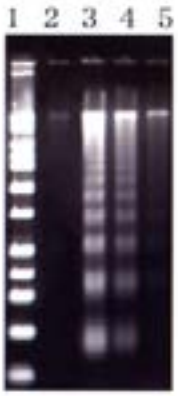

FIGURE 1: EGCG-mediated DNA ladder formation in human stomach cancer MKN-45 cells. DNAs extracted from the cultured cells treated with EGCG at 400 (3), 200 (4), and 100 (5) μM for 8 hours were electrophoresed on agarose and stained with ethidium bromide. DNA-size marker (1) and DNA from untreated cells (2) are also shown.

EGCG has been demonstrated to induce apoptosis by binding to a cell-surface protein called Fas in cultured human leukemia cells [2]. This binding causes the activation of protease caspase-8, which activates a caspase-dependent deoxyribonuclease that in turn degrades DNA at a nucleosome unit. It is also known that a cell-surface protein called 67kDa laminin receptor is involved in EGCG-induced apoptosis [2, 3, 5]. It should be pointed out that EGCG has a stronger apoptosis-inducing effect on cancer cells than on normal cells [2], an effect that is

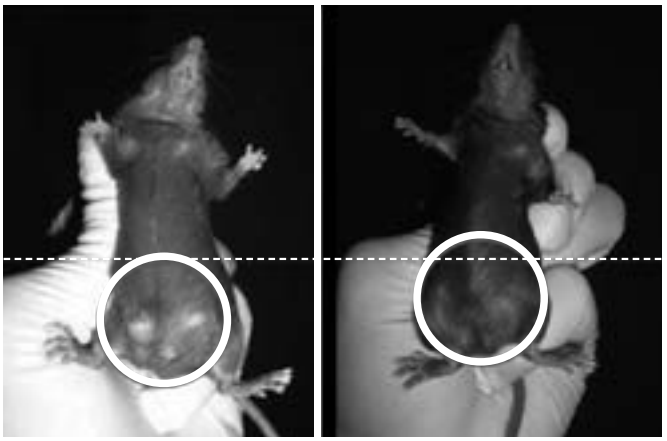

FIGURE 2: When mouse lung cancer cells (1×10^6) were inoculated into the peritoneal cavity, they propagated and formed tumor masses by 15 days after innoculation (left). Oral administration of EGCG together with intraperitoneal injection of Sulindac, an anti-inflammatory drug, reduced formation of tumor masses (right).

desired in anti-cancer drugs.

Another possible mechanism for the action of EGCG has also been proposed. EGCG induces apoptosis in hepatoma cells leading to the cell death. A single-strand RNA containing 20-25 nucleotides (microRNA) was involved in the mechanism [5].

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 [2]. In experiments using the autochthonous transgenic adenocarcinoma of the mouse prostate (TRAMP) model, which spontaneously develops metastatic prostate cancer, green tea catechins of the quantity achievable by humans (6 cups of green tea per/day) significantly inhibited cancer development, increased survival rate, and induced the apoptosis of prostate cancer cells [2-5].

There have been several trials investigating the enhancement of drug activity by combining the drugs with EGCG. For example, intraperitoneal injection of mouse lung carcinoma cells causes the formation of tumors after 2-3 weeks. However the 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 [2] (Figure 2).

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-erh tea, that induce apoptosis [6].

Other mechanisms of anti-tumor effects

There are several lines of evidence indicating that EGCG elicits anti-tumor activity by mechanisms other than apoptosis induction [3, 5]. These include cell cycle arrest, the prevention of oxidative DNA damage by anti-oxidative activity, and inhibitory activities in the reactive oxygen-mediated pathway.

Anti-metastatic action of green tea

Metastasis is the major cause of morbidity and death in cancer patients. The inhibition of metastasis would markedly reduce the rate of cancer deaths. The peroral administration of EGCG-rich catechins inhibited the blood-borne metastasis of B16 melanoma cells and lung carcinoma cells in both experimental and spontaneous systems [2]. Similarly, oral administration of green tea resulted in inhibition of metastasis of lung tumor cells [2]. In a TRAMP mouse model, it was demonstrated that the peroral administration of green tea catechins caused almost complete inhibition of metastasis. Because EGCG prevented cancer cells from adhesion to endothelial cell layers and from attaching to the basement membrane components such as fibronectin and laminin, these effects are involved in the anti-metastatic activity of green tea and catechins [2].

Matrix metalloproteinases (MMPs) are necessary in various steps of the metastasis process, including the invasion into surrounding tissues and degradation of endothelial basement membranes. EGCG was shown to be a strong inhibitor of MMP such as MMP-2 and MMP-9 derived from cancer cells, and to suppress the protein and gene expression of MMPs [2, 3, 5]. EGCG inhibited growth, invasion, angiogenesis, and metastasis of human pancreatic cancer cells in a xenograft model. The MMP activities were reduced in the tumor, indicating that EGCG acts as an MMP inhibitor *in vivo* [5].

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 lowered in the group given green tea as compared with the group given water [7]. 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 such as vascular endothelial growth factor and its receptor [8].

Catechins may inhibit metastasis by modulating the immune surveillance potential. In an experiment with a senescence-accelerated mouse prone-10 strain of mice, catechins were shown to inhibit metastasis by inhibiting the age-related reduction in this potential through maintenance of the natural killer cell activity [9].

Epidemiological studies

Epidemiological studies have shown that green tea and tea catechins exert preventive effects against various cancers, but other studies failed to demonstrate such effects [2-5]. Of 127 case-control studies and 90 cohort studies published from 1965 to 2008, 51 case-control studies and 19 cohort studies showed an inverse association between green/black tea consumption and cancer risk for various types of cancers. These included colon, lung, stomach, breast, prostate, ovarian, pancreatic, kidney, and bladder cancers [3]. Several recent findings are shown below.

Breast cancer: In a meta-analysis, including results from eight epidemiological studies on green tea intake and breast cancer risk, the relative risk of breast cancer based on three case-control studies was 0.70 for the highest green tea intake compared with the lowest or no green tea intake. No risk reduction was found in five prospective cohort studies [4]. In a cross-sectional study with 3,315 Chinese women in Singapore, daily green tea drinkers showed a significantly lower mammographic density percentage than the women who did not drink tea. This suggests that long-term exposure to green tea may be essential to exert its protective effect against breast cancer [4].

Colorectal cancer: In prospective cohort studies with about five 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 46% risk reduction of colorectal cancer in non-smokers. The risk decreased as the amount of green tea consumption increased. Each 2g 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 [4]. No significant association was found among smokers.

A similar cohort study on Chinese women showed a 44% risk reduction of colorectal cancer for women consuming ≥5g dry green tea leaves/day compared with non-tea drinkers, especially among women with long-term tea drinking [4]. In a study in which the urinary concentrations of specific tea catechins and their metabolites were examined, individuals with higher urinary catechin concentrations had a lower risk of colon cancer [4]. On the other hand, other epidemiological studies, especially prospective cohort studies, failed to detect an inverse association between green tea consumption and colorectal cancer risk [4].

Esophageal cancer: In a recent review of 15 epidemiological studies, six reported a significantly reduced risk of esophageal cancer associated with high amounts of tea consumption. Four of them reported a lower, but non-significant risk with green tea consumption, while three of them reported a significantly positive association between tea consumption and esophageal cancer risk. The remaining two studies reported a null association [4]. More recent meta-analysis on 24 case-control

and cohort studies with 7,376 cancer cases showed that green tea, but not black tea consumption, had protective effects [10].

Liver cancer: An analysis of green tea intake and liver cancer mortality in a cohort of 60,076 Chinese men and 29,713 Chinese women showed that regular green tea drinkers had a lower mortality relative to non-drinkers among women, but not among men [4]. In a study that recruited 41,761 Japanese adults aged 40-79 years, the total incidence of liver cancer was 247 cases in over nine years of follow-up [4]. In men, the multivariate-adjusted hazard ratios 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. The corresponding data among women were 1.00 (reference), 0.68, 0.79 and 0.50, respectively. Thus, green tea consumption was shown to be associated with a reduced risk of liver cancer incidence in the Japanese general population.

On the other hand, a study of 18,000 men and women in the Japanese Public Health Center-based Prospective Study Cohort II found an increased risk of liver cancer in women who drank 3-4 cups or ≥5 cups of tea compared with women consuming <3 cups of tea/day [4]. There was no association between green tea intake and liver cancer risk among men. Thus, studies that are more precise are needed to provide support for green tea consumption for the reduction of the risk of liver cancer.

Lung cancer: Of 12 recent studies, five studies found a significant inverse association between green tea intake and lung cancer risk. Three studies reported a non-significant lower risk of lung cancer in green tea drinkers than in non-drinkers. One study reported a significantly increased risk of lung cancer in green tea drinkers compared with non-drinkers. One study reported a positive but non-significant association between green tea intake and lung cancer risk. The remaining two studies reported a null association [4].

The inconsistent results of those studies could be partly a result of the potential confounding effect of smoking. For example, a study in Chinese women living in Shanghai showed that among non-smoking women, regular tea drinkers had a 35% reduction in lung cancer risk compared with those who did not drink tea regularly. However, there was no association between green tea intake and lung cancer risk among smokers [4]. Similarly, a more recent study revealed that an elevated risk of lung cancer was observed in smokers who never drank green tea as compared with those who drank more than 1 cup/day of green tea. [11].

Ovarian cancer: A systemic review of 22 articles, including five epidemiological studies on ovarian cancer, found a significant association between green tea intake and both decreased ovarian cancer incidence and better prognosis [12]. The finding supports the clinical evaluation of the role of green tea or green tea components in the prevention and treatment of ovarian cancer. Another analysis of six case-control studies and cohort studies on 3,842 cancer cases and 5,271 control cases

also indicated that drinking green tea can significantly decrease the risk of ovarian cancer (odds ratio (OR), 0.81) [13].

Prostate cancer: A meta-analysis of 13 studies investigating the association between green tea and black tea consumption and prostate cancer risk was undertaken. The analysis found a significant association in Asian populations for high versus no/low green tea consumptions (OR, 0.62) [14]. 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). In a prospective study conducted in Japan, green tea was not associated with localized prostate cancer, but consumption was associated with a dose-dependent decrease in the risk of advanced prostate cancer. The multivariate relative risk was 0.52 for men drinking ≥5 cups green tea/day compared with less than 1 cup/day [4].

On the other hand, in a prospective study of 19,561 Japanese men, green tea intake was not associated with a lower risk of prostate cancer [4]. Also, in a population-based, prospective cohort study of 27,293 Chinese men in Singapore, there was no association between daily green tea intake and prostate cancer risk, compared with no green tea intake [15].

Stomach cancer: Some epidemiological studies have shown an inverse association between green tea consumption and gastric cancer risk. In the analysis with 13 studies, the summary adjusted relative risk of gastric cancer for the highest compared with the lowest amount of green tea consumption was 18% lower. A pooled analysis of six cohort studies with >3500 incident gastric cancer cases found a significant inverse association between green tea consumption and gastric cancer risk in women [4]. Compared with those drinking <1 cup/day, women who consumed ≥5 cups green tea/day had a 20% lower risk of gastric cancer. No protective effect was seen in men [4]. A recent analysis for eight cohort and three case-control studies led to a similar conclusion [16].

In a case-control study of gastric cancer nested within a prospective cohort of Chinese men in Shanghai, urinary concentrations of tea catechins were significantly associated with a reduced risk of gastric cancer. Compared with the absence of epigallocatechin in urine, the OR of gastric cancer for the presence of urinary epigallocatechin was 0.52 after adjustment for confounding factors [4]. This suggests a 48% lower risk for tea drinkers.

More recently, Hou *et al.* reviewed ten case-control studies and seven cohort studies published between 1988 and 2010 to reveal the association between green tea consumption and the stomach cancer risk [17]. These reviewers found that seven studies suggested no association, eight an inverse association, and one a positive association, indicating that the evidence is insufficient to support the reducing effect of green tea in stomach cancer.

Other cancers: In a 9-years follow-up study with 41,761 Japanese adults aged 40-79, the multivariate-adjusted hazard ratio of hematologic malignancies for 5 cups/day or more compared with less than 1 cup/day of green tea was 0.58 [18]. This implies a 42% lower risk in the 5 cups/day group. The corresponding risk estimate was 0.52 for lymphoid neoplasms and 0.76 for myeloid neoplasms. These results imply 48% and 24% lower cancer risk, respectively.

In the Japanese Collaborative Cohort Study enrolled 50,221 Japanese men and women, a borderline significant inverse association between green tea intake and oral cancer risk was observed, with an hazard ratio of 0.44 (56% lower risk) for those consuming ≥5 cups green tea/day relative to <1 cup/day [4]. A significant inverse association between green tea consumption and the risk of endometrial endometrioid adenocarcinoma was reported. The multivariate-adjusted OR was 0.33 (67% lower risk) in the highest tea consumers (≥4 cups/day), compared with those in the lowest consumers (4 cups or less/week) [19].

In a case-control study, Wang *et al*. found that green tea intake (≥ 500mL/day) was associated with a decreased risk of clear cell renal cell carcinoma [20]. In a large-scale, population-based case-control study in urban Shanghai, it was demonstrated that regular green tea drinking was associated with 32% reduction of pancreatic cancer risk, compared with those who did not drink tea regularly in women [21].

The results of a prospective cohort study with 100,507 persons aged 40-69 indicated that high green tea consumption may be positively associated with premenopausal thyroid cancer risk, but inversely associated with postmenopausal thyroid cancer risk [22].

Confounding factors

Epidemiological study results have been inconsistent. This may be due to several confounding factors, including the quantity and quality of the tea consumed, tea temperature, cigarette smoking, and alcohol consumption [2-4]. For example, the high temperature of tea beverages may have some harmful effects on the stomach. A case-control study of gastric cancer in northeast China did not find an overall association between green tea intake and gastric cancer. When data were analyzed by tea temperature, a dose-dependent relation was observed between consumption of green tea at lukewarm temperatures and decreased gastric cancer risk. Conversely, no association was found between green tea consumed at a hot temperature and gastric cancer risk [4]. Another example is the above-mentioned study on lung cancer risk in Chinese women with a different smoking status.

Future studies should incorporate the determination of biomarkers of tea polyphenols in blood and urine, as recent studies did [2-4]. Genetic polymorphisms may also influence the effects of tea consumption on cancer risk [4, 11].

Intervention studies

In an experiment in which 42 patients with oral premalignant lesions were divided into one of four groups given 500, 750 or 1000mg green tea/m^2 body surface area/day or placebo, the favorable response rates were 58% in patients given 750 or 1000mg/m^2 green tea extract and 36.4% in those given 500mg/m^2 but it was only 18.2% in those assigned to the placebo arm [4]. The average body surface area for women is 1.6m^2 and for men 1.9m^2. These results encourage consumption of green tea extract against the progression of precancerous lesions in the oral cavity towards malignant transformation.

In a study in Italy, 30 men with high-grade prostate intraepithelial neoplasias were given 600mg of green tea catechins daily for 12 months. Only one patient developed prostate cancer, compared with nine of the 30 patients in the placebo group [4]. This chemopreventive effect was also retained when measured two years later [4].

In a clinical study for colorectal cancer, 71 patients were supplemented with 1.5g green tea extract (GTE)/day for 12 months and 65 control patients without supplementation. The results of follow-up colonoscopy demonstrated that the incidence of metachronous adenomas was 31% (20 of 65) in the control group and 15% (9 of 60) in the GTE group [23]. Thus, GTE appears useful for the chemoprevention of metachronous colorectal adenomas.

The standardized green tea polyphenol preparation Polyphenon® E or Sinecatechins has been subjected to many clinical trials [http://clinicaltrials.gov/] and approved for the treatment of genital warts by the United States Food and Drug Administration [24]. Randomized, double-blind, placebo-controlled trials have demonstrated the efficacy and safety of Polyphenon® E 15% ointment for the treatment of external anogenital warts [25]. Protective effects of Polyphenon® E on human cervical lesions were also demonstrated [5]. In another study, an oral 2,000 mg dose of Polyphenon® E taken twice daily for up to six months was found to be well tolerated by patients with chronic lymphocytic leukemia [26]. Durable declines in the absolute lymphocyte count and/or lymphadenopathy were observed in most patients.

These results are encouraging future studies on the usefulness of green tea catechins as an anti-cancer agent.

Conclusion

A large number of cell-based and animal experiments have demonstrated the anti-cancer activity of green tea and its catechins. The results of human studies such as epidemiological studies and clinical trials have also indicated that intake of

green tea reduces the risk of various kinds of cancer. However, a number of human studies failed to demonstrate such effects. The inconsistent results may have arisen from confounding factors such as cigarette smoking, alcohol drinking, and tea temperature. Although more precise experiments are needed, the current findings suggest the beneficial effects of green tea and its catechins on cancer.

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Green Tea Catechins for the Prevention of Colorectal Tumorigenesis: from Bench to Bed

Masahito SHIMIZU and Hisataka MORIWAKI

Abstract: Colorectal cancer (CRC) is a serious healthcare problem worldwide. Thus, effective prevention strategies are urgently required. The removal of adenomatous polyps, which are precancerous CRC lesions, may reduce the risk of CRC. Green tea catechins (GTCs) inhibit cell proliferation and induce apoptosis in CRC cells by blocking the activation of several receptor tyrosine kinases. GTC supplementation also prevents inflammation- and obesity-related colorectal tumorigenesis in animal studies. Furthermore, a preliminary human trial has shown that GTCs successfully prevent the development of colorectal adenomas. These studies suggest that GTC supplementation might be a promising strategy for the prevention of colorectal tumorigenesis.

Keywords: (-)-epigallocatechin-3-gallate, colorectal cancer, green tea catechins, receptor tyrosine kinase

Introduction

Colorectal cancer (CRC) is a major healthcare problem worldwide, due to its substantial morbidity and mortality. Therefore, the development of effective CRC chemopreventive strategies is necessary. It is generally accepted that most CRCs evolve from adenomatous polyps, and that the removal of these lesions reduces the risk for future CRC [1]. These findings suggest that inhibiting adenomatous polyp development using phytochemicals, including GTCs, may be a promising strategy to prevent CRC.

GTCs have received much attention for their beneficial effects, particularly

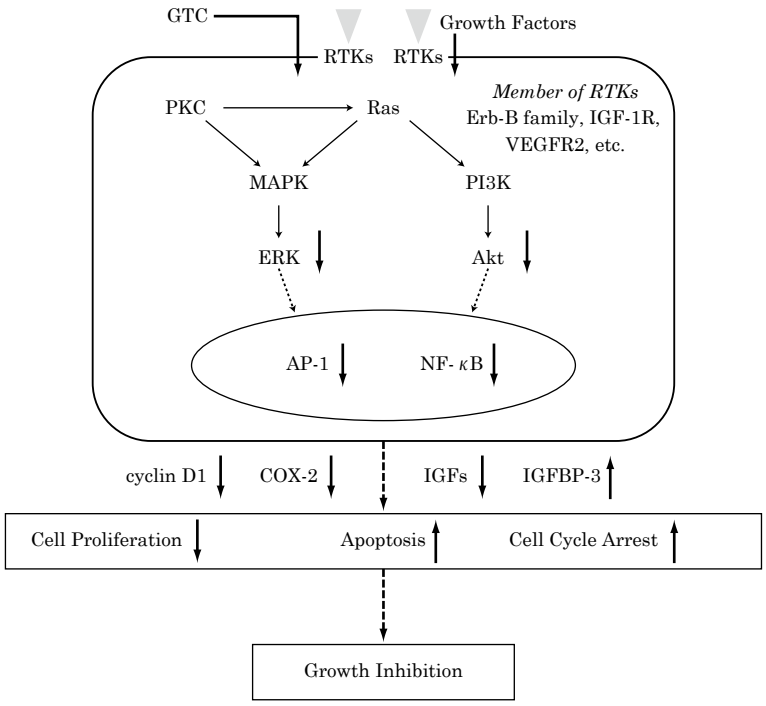

FIGURE 1: Effects of green tea catechins on receptor tyrosine kinases and their intracellular signaling pathways in cancer cells

their cancer chemopreventive activity. GTCs exert their chemopreventive effects via several mechanisms, including antioxidant and anti-inflammatory activity [2, 3]. GTCs also inhibit cell growth and induce apoptosis in human CRC cells. Recent studies have demonstrated that GTCs inhibit the activity of various receptor tyrosine kinases (RTKs) and specific signal transduction pathways, thereby inhibiting tumorigenesis [4-6] (Figure 1).

In this chapter, evidence is provided to show that GTCs suppress CRC cell growth by inhibiting RTK activation and downstream signal transduction pathways. The results from animal studies are also presented to show that GTC supplementation inhibits colorectal tumorigenesis associated with inflammation and obesity, both of which are critical risk factors for CRC. Furthermore, the results of a recent pilot trial are shown, which show the preventive effect of GTCs on colorectal adenoma recurrence after polypectomy.

Effects of GTCs on the RTKs in CRC cells

Abnormalities in the expression and function of RTKs and their multiple downstream signaling pathways, including the Ras/extracellular signal-regulated

kinase (ERK) and phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathways, play a critical role in colorectal carcinogenesis. Recent studies have revealed that GTCs exert antitumor activity by suppressing the activation of the epidermal growth factor receptor (EGFR) family of RTKs and their downstream effectors in CRC cells. We have reported that (-)-epigallocatechin-3-gallate (EGCG), the major biologically active component in green tea, inhibits the activation of EGFR, HER2 and HER3, and their multiple downstream signaling pathways in human CRC cell lines. Treatment with EGCG inhibits the activation of EGFR and HER2, the phosphorylation of Akt and ERK, and the transcriptional activity of the activator protein-1, nuclear factor-κB, and cyclin D1 promoters in HT29 human CRC cells. These effects were associated with the induction of apoptosis and cell cycle arrest in G_0-G_1 phase [7, 8].

It was also reported that EGCG inhibited the activation of insulin-like growth factor (IGF)-1 receptor (IGF-1R) in SW837 human CRC cells [9]. In this study, the inhibition of IGF-1R activation by EGCG was associated with a decrease in IGF-1/2 expression and an increase in IGF binding protein-3 expression, which negatively regulates the function of IGF-1/2 in CRC cells [9]. EGCG suppressed the growth of xenografts generated from human CRC cells by inhibiting the activation of vascular endothelial growth factor (VEGF) receptor-2 (VEGFR-2), ERK and Akt [10]. Because both the IGF/IGF-1R and VEGF/VEGFR-2 signaling axes play an important role in the development and growth of CRC, disruption of these loops by GTCs might be an effective strategy for the prevention of this malignancy.

Effects of GTCs on the inhibition of inflammation- and obesity-related colorectal tumorigenesis

Inflammation and obesity increase the risk of CRC. In an experiment to examine whether GTC supplementation prevents inflammation- and obesity-related colorectal tumorigenesis using animal models, treatment with GTCs significantly attenuated inflammation-related mouse colon carcinogenesis induced by azoxymethane (AOM) and dextran sodium sulfate by decreasing the expression of cyclooxygenase-2 (COX-2) and inflammatory cytokines, such as the tumor necrosis factor-α, in the colonic mucosa [11]. EGCG supplementation also suppressed AOM-induced colonic pre-neoplastic lesions by inhibiting the expression and activity of indoleamine 2,3-dioxygenase, which plays a critical role in the induction of immune tolerance [12]. Furthermore, drinking EGCG prevented obesity-related colorectal tumorigenesis by inhibiting the IGF/IGF-1R axis, improving hyperlipidemia, hyperinsulinemia and hyperleptinemia, and suppressing the expression of COX-2 and cyclin D1 [13]. EGCG, therefore, may be useful in colorectal tumorigenesis prevention in individuals who are obese or have chronic colitis.

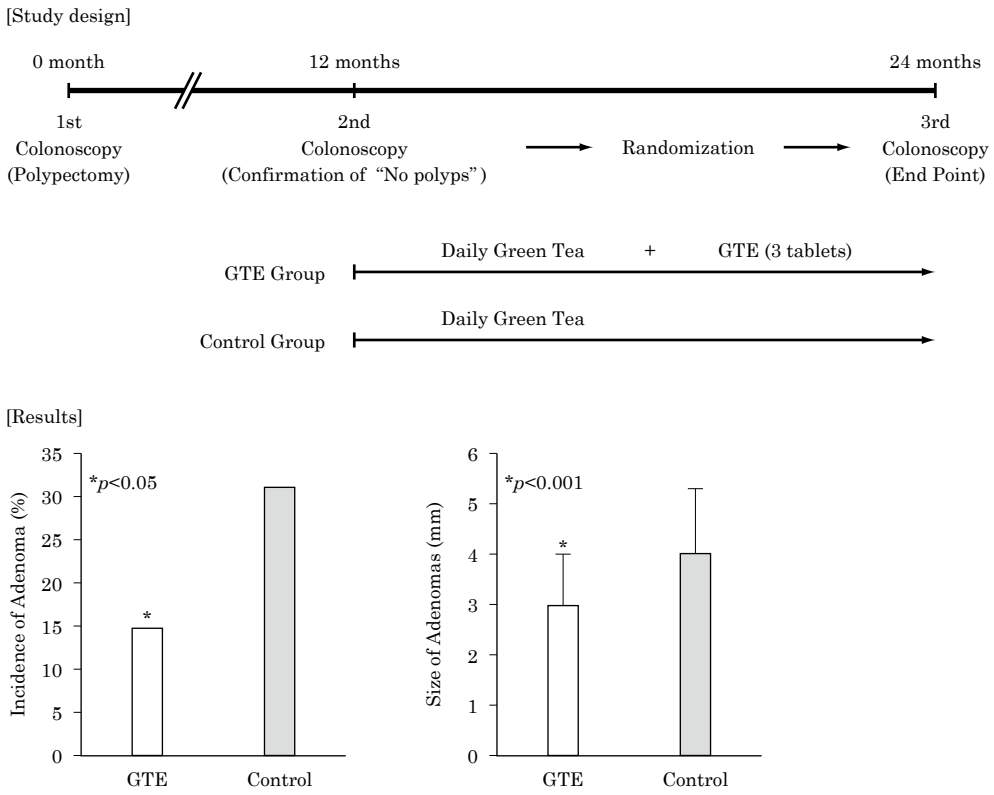

FIGURE 2: Pilot study revealing the preventive effect of green tea extract (GTE) on metachronous adenomas after polypectomy

Effects of GTCs on the prevention of colorectal adenomas

The successful prevention of colorectal adenoma development after polypectomy by GTC supplementation was shown in a pilot study [14]. In the study, the administration of green tea extract (1.5g/day for one year) in patients who had undergone polypectomy for colorectal adenomas reduced the development of metachronous colorectal adenomas, compared to patients who did not receive this supplement (incidence, 15% vs 31%; relative risk, 0.49; 95% CI, 0.24-0.99; $p<0.05$). The size of relapsed adenomas was also significantly smaller in the GTC supplement group, compared with the control untreated group ($p<0.001$) [14] (Figure 2). The absence of any serious adverse events as a consequence of GTC administration in the trial is a significant finding, and supports the use of GTCs as a "chemopreventive" in clinical practice.

Conclusion

The present review provides evidence that the effects of GTCs on the inhibition of colorectal carcinogenesis are mediated by the regulation of RTK activity, and their downstream signaling pathways. GTCs also suppress colorectal tumorigenesis by exerting anti-inflammatory activities and improving obesity-related metabolic abnormalities. The safety and efficacy of GTCs demonstrated in our intervention study could be crucial for the clinical application of GTCs as chemopreventive agents. GTCs might be an effective supplement for the chemoprevention of colorectal tumors.

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Green Tea Polyphenol EGCG Sensing Receptor

Hirofumi TACHIBANA

Abstract: The green tea catechin (-)-epigallocatechin-3-*O*-gallate (EGCG) is known to exhibit various biological and pharmacological properties. 67-kDa laminin receptor (67LR) has been identified as a cell-surface EGCG receptor that confers EGCG responsiveness to many cancer cells at physiological concentrations. Polyphenon® E is a clinical grade catechin mixture containing about 50% EGCG that is currently under investigation in multiple cancer trials. Polyphenon® E has been shown to exert anti-neoplastic effects by antagonizing tumor-induced myeloid derived suppressor cells though 67LR. MYPT1, eEF1A, protein phosphatase 2A, Akt, endothelial nitric oxide synthase, soluble guanylate cyclase, protein kinase Cδ, acid sphingomyelinase, and CGMP have been identified as EGCG-sensing related molecules for EGCG-induced cancer prevention *in vivo*. These factors mediate unique signaling for cancer prevention triggered by physiological concentrations of EGCG. Up-regulation of CGMP is a rate-determining process of this cell death pathway, and a cancer-overexpressed negative regulator of CGMP, phosphodiesterase 5 (PDE5) attenuates the cell death induced by EGCG. PDE5 selective inhibitors used for treating erectile dysfunction potentiate the anti-cancer effect of EGCG. Thus, a demonstraton of CGMP elevation caused by targeting the overexpressed 67LR and PDE5 in cancer cells may be a useful approach for cancer-specific chemotherapy.

Keywords: 67LR, cancer prevention, CGMP, EGCG, EGCG receptor

Introduction

Several beneficial health effects by green tea constituents have been documented. These include anti-carcinogenic, anti-oxidative, anti-allergic, anti-virus, anti-hypertensive, anti-atherosclerosis, anti-cardiovascular disease and

anti-hypercholesterolemic activities. The compounds responsible for these activities are a sub-group of polyphenols, namely catechins. The major green tea catechins are (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epicatechin. Because EGCG is found only in tea from *Camellia sinensis*, EGCG is regarded as a characteristic constituent of green tea. 67-kDa laminin receptor (67LR) has been identified as a cell-surface EGCG receptor that confers EGCG responsiveness to many cancer cells at physiological concentrations.

This chapter focuses the current understanding of EGCG sensing mechanisms through 67LR by which EGCG exerts biological and pharmacological properties.

Identification of EGCG sensing receptor

Pharmacokinetic studies in humans indicate that the peak plasma concentration after a dose of EGCG is <1.0 μM. It is noteworthy that most of the effects of EGCG in cell culture systems and cell-free systems have been obtained with considerably higher concentrations (10-100 μM) than those observed in the plasma or tissues of animals or in human plasma after administration of green tea or EGCG. Furthermore, the intracellular levels of EGCG are much lower than the concentrations observed in the extracellular levels. Searching for high-affinity proteins that bind to EGCG is the first step to understanding the molecular and biochemical mechanisms of the anti-cancer effects of tea polyphenols. Several proteins that can directly bind with EGCG have been identified with *in vitro* models [1]. All these proteins were demonstrated to be important for the inhibitory activity of EGCG in cell lines, but higher EGCG concentrations than the *K*d values were needed. To elucidate the detailed molecular basis for the action of EGCG, it is necessary to identify the molecular target, triggering a specific signaling of EGCG.

All-*trans*-retinoic acid (ATRA) was found to enhance the binding of EGCG to the cell surface of cancer cells when the binding was monitored on the basis of the increase in response units in a surface plasmon resonance assay. To identify candidates through which EGCG inhibits cell growth, a subtraction cloning strategy was used for cDNA libraries constructed from cells treated or untreated with ATRA. An analysis of the DNA sequence of an isolated single target that allows EGCG to bind to the cell surface identified this target as the 67LR [2]. The predicted *K*d value for the binding of EGCG to the 67LR protein is 39.9 nM. Most of the 67LR protein was found to exist in the raft fraction rather than the non-raft fraction [3]. This distribution pattern correlated well with the plasma membrane-associated EGCG concentration after treating the cells with EGCG [4]. Tumor growth was significantly retarded in EGCG-treated mice implanted with the B16 cells harboring a control shRNA, whereas tumor growth was unaffected by EGCG in the mice implanted with 67LR-ablated B16 cells, suggesting that 67LR functions as an EGCG receptor *in vivo* [5].

The 67LR has been implicated in laminin-induced tumor cell attachment and migration, in tumor angiogenesis, invasion and metastasis [6]. 67LR also acts as a receptor for pathogenic prion protein [7], Sindbis virus [8] and Dengue virus [9]. The 67LR extracellular domain corresponding to the 161-170 region has been identified as the EGCG binding site [10]. Caffeine and other tea polyphenols (C, EC, EGC, quercetin) were shown to be unable to affect the growth of 67LR-expressing cells, and also could not bind to the cell surface [2]. Galloylated catechins showed the cell-surface binding activities but their nongalloylated forms did not. Binding activities of pyrogallol-type catechins (EGCG and GCG) were higher than those of catechol-type catechins (ECG and CG) [11]. These patterns were also observed in their biological activities. Down regulation of 67LR expression caused a reduction of both activities of galloylated catechins. These results suggest that both the galloyl moiety and the B-ring hydroxylation pattern contribute to the exertion of biological activities of tea catechins and their 67LR-dependencies. Strictinin, an ellagitannins in green tea, is one of the hydrolysable tannins that can be described as esters of gallic acid with a polyol.

Strictinin has been shown to suppress IL-4 signaling [12]. Strictinin interacts with the non-lipid rafts of the plasma membrane and binds to non-lipid raft-associated IL-4R [13]. Cell surface binding and the IL-4 signaling inhibitory action of strictinin were unaffected by attenuation of 67LR expression. The *K*d value of strictinin binding to IL-4R was determined as 4.53 μM while that of EGCG, which was mainly located in the lipid-raft region of the cell surface, was 155 μM [13]. These findings suggest that both the galloyl moiety and the flavan-3-ol structure are involved in the interaction between 67LR and EGCG.

Anti-inflammatory actions of EGCG through EGCG sensing receptor 67LR

Lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria, is one of the most powerful activators of TLR4 signaling. LPS is also a well known inducer of inflammatory mediators, leading to death from endotoxic shock [14]. EGCG has been shown to rescue mice from LPS-induced lethal endotoxemia and down regulate inflammatory responses in macrophages [15]. Anti-67LR antibody treatment or RNAi-mediated silencing of 67LR resulted in abrogation of the inhibitory action of EGCG on LPS-induced activation of downstream signaling pathways and target gene expressions in murine macrophages [16]. Additionally, EGCG reduced the TLR4 expression through 67LR. Interestingly, EGCG induced a rapid up-regulation of Tollip protein, a nagative regulator of TLR-signaling, and this EGCG action was prevented by 67LR silencing or anti-67LR antibody treatment. These findings demonstrate that 67LR plays a critical role in mediating anti-inflammatory action of a physiologically relevant EGCG and Tollip expression

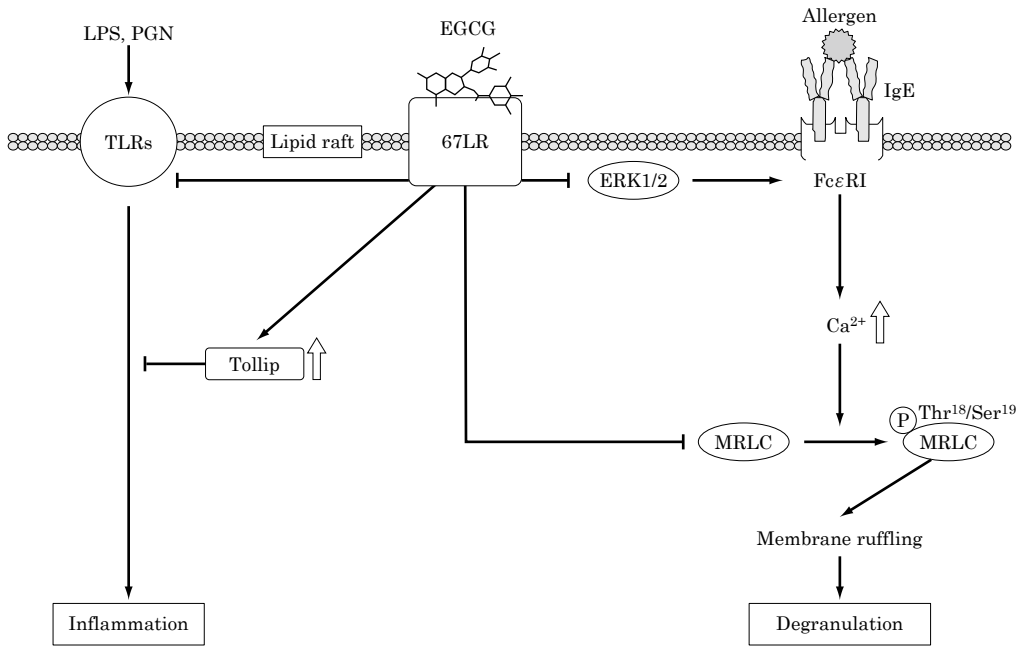

FIGURE 1: EGCG sensing pathway for the anti-allergic and anti-inflammatory actions through 67-kDa laminin receptor (67LR)
The suppression of MRLC phosphorylation through the cell-surface binding to the 67LR contributes to the inhibitory effect of EGCG on the histamine release from basophils.
The 67LR also mediates the EGCG-induced suppression of FcεRI expression in basophils by reducing ERK1/2 phosphorylation.
67LR and Tollip are indispensable for mediating anti-inflammatory action of EGCG on TLRs signaling induced by LPS and PGN.

67LR: 67-kDa laminin receptor
TLRs: toll-like receptors
LPS: lipopolysaccharide
PGN: peptidoglycan
Tollip: toll-interacting protein
ERK1/2: extracellular signal-regulated kinase1/2
MRLC: myosin regulatory light chain

could be modulated through 67LR in macrophages [16] (Figure 1). Peptidoglycan (PGN), a major component of the cell wall of Gram-positive bacteria, is one of the most powerful activators of TLR2 signaling. 67LR and Tollip are indispensable for mediating anti-inflammatory action of EGCG on TLR2 signaling induced by PGN [17].

Cancer cell growth inhibition of EGCG through EGCG sensing receptor 67LR

By performing functional genetic screening, eukaryotic elongation factor 1A

(eEF1A) has been identified as a critical factor in the suppression of melanoma cell proliferation [5]. eEF1A is an important component of the eukaryotic translation apparatus and is also known as a multifunctional protein that is involved in a large number of cellular processes. Tumor growth was significantly retarded in EGCG-treated mice implanted with the B16 cells harboring a control shRNA, whereas tumor growth was unaffected by EGCG in the mice implanted with eEF1A-ablated B16 cells, indicating that eEF1A is involved in EGCG-induced cancer prevention.

EGCG-induced cell growth inhibition may result from the reduction of the phosphorylation of MRLC at Thr-18/Ser-19 through 67LR [18]. The activity of myosin phosphatase is known to be inhibited by phosphorylation of its targeting subunit MYPT1 at Thr-696. EGCG induced the dephosphorylation of MYPT1 at Thr-696. Further, this effect correlated with EGCG-induced reduction of the MRLC phosphorylation, suggesting that EGCG activates myosin phosphatase by reducing the MYPT1 phosphorylation level at Thr-696. Tumor growth was significantly retarded in EGCG-administered mice implanted with the B16 cells harboring a control shRNA, whereas tumor growth was unaffected by EGCG in the mice implanted with MYPT-1-ablated B16 cells, suggesting that MYPT1 is indispensable for EGCG-induced cancer prevention. The involvement of MYPT1 in downstream EGCG-triggered signaling from both 67LR and eEF1A was further documented by confirming abrogation of 1μM EGCG-induced reduction of the MYPT1 phosphorylation level at Thr-696 and the MRLC phosphorylation in 67LR- or eEF1A-ablated B16 cells. These results suggest that MYPT1 is involved in downstream EGCG signaling from both 67LR and eEF1A (Figure 2).

Mutational activation of *BRAF* is the most prevalent genetic alteration in human melanoma, resulting in constitutive melanoma hyperproliferation. A selective *BRAF* inhibitor showed remarkable clinical activity in patients with mutated-*BRAF*. Unfortunately, most patients acquire resistance to the *BRAF* inhibitor, highlighting the urgent need for new melanoma treatment strategies. EGCG inhibits cell proliferation independently of *BRAF* inhibitor sensitivity. By performing functional genetic screening, protein phosphatase 2A (PP2A) has been identified as a critical factor in the suppression of melanoma cell proliferation [19]. 67LR activates PP2A through adenylate cyclase/cAMP pathway eliciting inhibitions of oncoproteins and activation of tumor suppressor Merlin (Figure 2). Activating 67LR/PP2A pathway leading to melanoma-specific mTOR inhibition shows strong synergy with the *BRAF* inhibitor PLX4720 in the drug-resistant melanoma. Moreover, SET, a potent inhibitor of PP2A, is overexpressed on malignant melanoma. Silencing of SET significantly enhanced EGCG-induced anti-melanoma activity by potentiating 67LR/PP2A signaling *in vivo*.

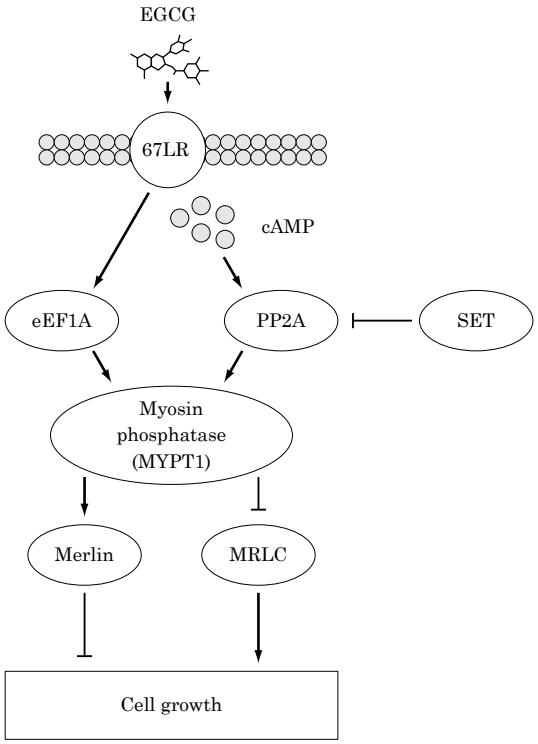

FIGURE 2: EGCG sensing pathway for eliciting cancer cell growth inhibition through 67LR
After EGCG binding to 67LR, EGCG activates myosin phosphatase through adenylate cyclase/cAMP/PP2A or eEF1A pathway. The myosin phosphatase activates tumor suppressor Merlin and dephosphorylates its substrates (e.g. MRLC), and actin cytoskeleton rearrangement is induced.

67LR: 67-kDa laminin receptor
PP2A: protein phosphatase 2A
MYPT1: myosin phosphatase target subunit 1
MRLC: myosin regulatory light chain
SET: Suvar3-9, Enhancer-of-zeste, Trithorax

Cancer cell killing activities of EGCG through EGCG sensing receptor 67LR

EGCG has been shown to induce growth arrest and subsequent apoptotic cell death in multiple myeloma (MM) cells, while having no significant effect on growth of normal cells such as peripheral blood mononuclear cells (PBMCs) [20]. The expression of 67LR was significantly elevated in MM cells compared to normal PBMCs. RNAi-mediated inhibition of 67LR expression resulted in abrogation of EGCG-induced apoptosis in myeloma cells, indicating that 67LR plays an important role in mediating EGCG activity in MM while sparing PBMCs. EGCG also induces cell death in acute myeloid leukaemia (AML) patient samples. AML cells express the 67LR while normal controls do not express the receptor [21].

Increases in membrane fluidity and clustering of lipid rafts play crucial roles in apoptosis. EGCG increased lipid-raft clustering, whereas treatment with EC, which lacks biological activity, did not induce lipid-raft clustering in MM cells [22]. Pretreatment with the anti-67LR antibody could block EGCG-induced lipid-raft clustering, whereas pretreatment with the control antibody did not. Exposure of myeloma cells to cholesterol inhibited lipid-raft clustering and apoptosis, suggesting that the apoptotic activity of EGCG is caused by lipid-raft clustering.

Lipid-raft clustering occurs after generation of ceramide by acid sphingomyelinase (ASM). The expression of ASM was abnormally elevated in MM cells relative to normal PBMCs. EGCG activated ASM in MM cells, but did not affect normal PBMCs [22]. Moreover, pretreatment with an anti-67LR antibody blocked EGCG-induced activation of ASM, suggesting that 67LR mediates ASM activation by EGCG. These observations show that EGCG modulates the sphingolipid pathway through activating ASM via 67LR and that ASM is necessary for EGCG-induced lipid-raft clustering, leading to apoptotic cell death in MM cells.

Protein kinase Cδ (PKCδ) is critical for the induction of apoptosis. EGCG led to phosphorylation at Ser664. EGCG increased PKCδ phosphorylation at Ser664 in MM cells, but not in normal PBMCs [22]. EGCG-induced PKCδ phosphorylation at Ser664 was not observed in cells pretreated with the anti-67LR antibody, suggesting that 67LR mediates EGCG-induced phosphorylation of PKCδ at Ser664. Furthermore, treatment with the PKCδ-specific inhibitor abolished the EGCG-induced activation of ASM. Silencing of ASM in MM cells did not affect EGCG-induced PKCδ phosphorylation at Ser664. Overall, these results suggest that EGCG-induced ASM activation is a secondary event that occurs after activation of PKCδ. Oral administration of EGCG promoted the cleavage of caspase 3, a key mediator of apoptosis, in tumor cells. Moreover, EGCG induced PKCδ phosphorylation at Ser664 and enhanced ASM activity in tumors, indicating that EGCG activates PKCδ and ASM in MM cells *in vivo* [22].

67LR has been shown to be involved in shear stress-induced endothelial nitric oxide synthase (eNOS) expression in normal endothelial cells [23]. EGCG induced NO production in MM cells, but had no effect on PBMCs from healthy donors [24]. EGCG elicited eNOS phosphorylation at Ser1177, which is involved in eNOS activation, and that this phosphorylation was attenuated by pretreatment of MM cells with an anti-67LR monoclonal antibody. Akt mediates the activation of eNOS by phosphorylation at Ser1177, leading to an increase in NO production. EGCG increased Akt kinase activity, which was attenuated by pretreatment of MM cells with an anti-67LR monoclonal antibody [24]. These findings indicate that EGCG induces NO production through 67LR-dependent activation of Akt and eNOS.

EGCG elevated the amount of CGMP in MM cells, but had no effect on normal PBMC [24]. Anti-67LR antibody inhibited EGCG-induced CGMP elevation. NO increases the intercellular CGMP concentration by activating soluble guanylate cyclase (sGC). The sGC inhibitor prevented the CGMP up regulation induced by EGCG. The inhibitor also attenuated EGCG-induced cell death and ASM activation. Taken together, these results suggested that the 67LR/Akt/eNOS/NO/sGC/CGMP pathway mediates EGCG-induced cell death (Figure 3). Other tea catechins did not affect the intracellular CGMP concentration.

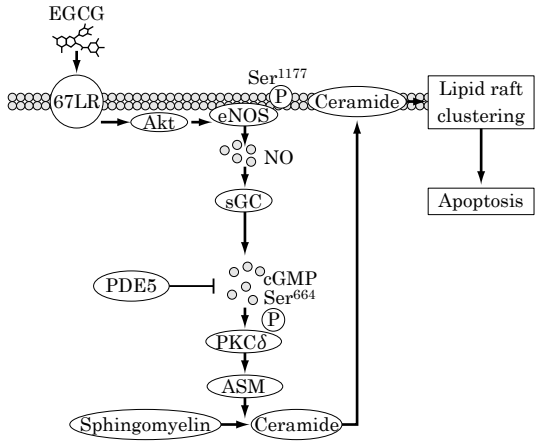

FIGURE 3: EGCG sensing pathway for inducing cancer cell death through 67LR
67LR activates the peculiar apoptotic signaling Akt/eNOS/NO/sGC/CGMP/PKCδ/ pathway.

67LR: 67-kDa laminin receptor
eNOS: endothelial nitric oxide synthase
sGC: soluble guanylate cyclase
PDE5: phosphodiesterase 5
PKCδ: protein kinase Cδ
ASM: acid sphingomyelinase

67LR as a methylated EGCG sensing receptor

FcεRI plays a central role in the induction and maintenance of IgE mediated allergic responses such as atopic dermatitis and food allergy. EGCG was able to decrease the cell-surface expression of FcεRI in human basophilic cells. The suppressive effect of EGCG was inhibited by the knockdown of 67LR [3]. The *O*-methylated derivatives of EGCG, (-)-epigallpcatechin-3-*O*-(3-*O*-methyl)-gallate (EGCG3"Me) has been shown to inhibit allergic reactions *in vitro* [25, 26]. EGCG3"Me can suppress the FcεRI expression in human basophilic cells the same as EGCG [27]. The 67LR also mediated the EGCG3"Me-induced suppression of FcεRI expression [28].

Regulation of EGCG activity by modulation of 67LR expression

To obtain the anti-cancer effects of EGCG when consumed at a reasonable concentration in daily life, the combination effect of EGCG and food ingredients that may enhance the anti-cancer activity of EGCG have been investigated.

ATRA, the active derivative of vitamin A, enhanced the expression of 67LR on B16 cells and increased the binding of EGCG to the cell surface. The growth of the cells treated with a control antibody was inhibited by the combined EGCG and ATRA treatment [29]. This growth-suppressive effect was eliminated upon treatment with an anti-67LR antibody. To determine the *in vivo* efficacy of the combined treatment, mice were implanted with B16 cells and treated with EGCG and/or ATRA. Compared to treatment with a vehicle control, the combined treatment significantly reduced the tumor volume over the duration of the study. The 67LR levels in the tumor were increased upon oral administration ATRA, or combination of EGCG and ATRA.

RAR that binds to ligand ATRA form a heterodimer with RXRs and regulate the expression of specific genes. Knockdown of RAR attenuated the ATRA-induced enhancement of 67LR expression [29]. TTNPB, a pan-RAR agonist enhanced the protein levels and cell-surface levels of 67LR. Moreover, treatment with TTNPB enhanced EGCG-induced cell growth inhibition. Collectively, these findings indicate that any compounds which activate RAR may be a candidate to enhance the anti-tumor activity of EGCG.

Low O_2 conditions have a profound impact on malignant progression and response to therapy. Activation of hypoxia-inducible factor 1 (HIF-1) alpha in cancer cells induces the expression of various genes responsible for resistance to chemotherapy. 67LR protein levels were reduced by exposure to low O_2 levels (5%), without affecting the expression of HIF-1 [30]. EGCG-induced anti-cancer activity is abrogated under low O_2 levels (5%) in various cancer cells. Notably, treatment with the proteasome inhibitor, prevented down regulation of 67LR and restored sensitivity to EGCG under 5% O_2. In summary, 67LR expression is highly sensitive to O_2 partial pressure, and the activity of EGCG can be regulated in cancer cells by O_2 partial pressure.

Potentiation of EGCG activity by modulation of EGCG sensing pathway via 67LR

CGMP has a crucial role in EGCG-induced MM-specific cell death. EGCG at physiologically achievable levels could induce NO production but could not up regulate the concentration of CGMP sufficiently to induce MM cell death [24]. These results suggested that up regulation of CGMP may be a “choke point” of the EGCG-induced apoptotic signaling pathway. PDEs are enzymes that inactivate CGMP signaling by hydrolyzing the 3,5’-phosphodiester bond. Significant inhibition of cell proliferation was observed when EGCG was combined with the PDE5-selective inhibitors [24]. The protein levels of PDE5 and 67LR increased substantially in the MM cells compared with those in normal PBMCs. The PDE5 inhibitor vardenafil, which is used for treating erectile dysfunction, had no effect on the number of viable normal PBMCs from healthy donors, but significantly enhanced the killing activity of EGCG on primary MM cells from patients. The combination of EGCG and vardenafil significantly suppressed the tumor growth in the mice. The combination of EGCG and vardenafil inhibited the proliferation of the gastric cancer cell line MKN45, the pancreatic cancer cell line PANC-1, the prostate cancer cell line PC3, and acute myeloid leukaemia cells but did not affect normal human diploid fibroblasts or normal HUVECs [24]. Levels of 67LR and PDE5 were elevated in various types of human cancers (gastric, pancreatic, prostate, and breast) compared with their normal counterparts. To evaluate the *in vivo* activity of EGCG and vardenafil in combination on MDA-MB-231 cells, the cells were injected subcutaneously into

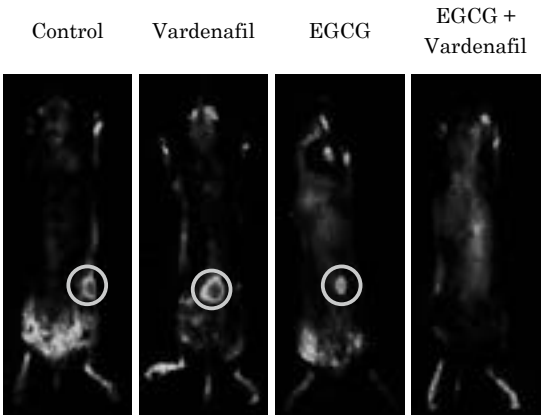

FIGURE 4: PDE5 inhibition potentiates the anti-cancer effect of EGCG.
Human breast cancer cell line MDA-MB-231-RFP cells were injected subcutaneously into nude mice and the mice given EGCG (15 mg/kg i.p.) and/or vardenafil (5 mg/kg i.p.). The combination suppressed tumor growth (red signal) significantly.

female nude mice; mice were given i.p. injections of EGCG and/or vardenafil. EGCG and vardenafil in combination significantly suppressed tumor growth (Figure 4).

Conclusions

67LR is a critical sensor molecule to respond to EGCG and mediates the beneficial activities of this phytochemical such as anti-cancer, anti-atherosclerosis, insulin sensing modulation, anti-allergic, and anti-inflammatory activities (Figure 5). MYPT1, eEF1A, PP2A, CGMP, and ASM are EGCG-sensing relating molecules for EGCG-induced cancer prevention *in vivo*. These factors mediate unique signaling for cancer prevention triggered by physiological concentrations of EGCG. These findings suggest that these are "master factors," which determine the efficacy of cancer-preventive activity of EGCG and have important implications for development and use of EGCG as a cancer-chemopreventive agent. Probably, only a tumor with a high expression level of these "master factors" has sensitivity to physiological concentrations of EGCG, while lower expression of those molecules causes "EGCG-resistance."

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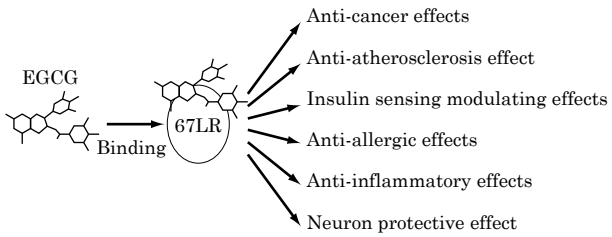

[67LR as a EGCG sensing receptor]

FIGURE 5: 67LR is a critical sensor molecule to respond to EGCG and mediates the beneficial activities of this phytochemical.
Anti-cancer effects (Nat Struct Mol Biol. 2004; 11: 380, Blood. 2006; 108: 2804, J Biol Chem. 2008; 283: 3050, Proc Natl Acad Sci USA. 2012; 109: 12426, Clin Cancer Res. 2013; 19: 1116, J Clin Invest. 2013; 123: 787, J Bio Chem. 2014; 289: 32671.)
Anti-atherosclerosis effect (J Mol Cell Cardiol. 2010; 149: 1138.)
Insulin sensing modulating effects (Planta Med. 2010; 76: 1694, Am J Physiol Cell Physiol. 2009; 297: C121, Mol Nutr Food Res. 2009; Mol Nutr Food Res. 2012; 56: 580.)
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Clinical Trials and New Drug Development

Yukihiko HARA

Abstract: After a brief description of the chemistry of tea catechins and their purification from green tea, various examples of their applications are summarized. These are based on their physiological functions, and in tumor suppression. The promulgation of the "Botanical drug" in 2004 by United States Food and Drug Administration (US FDA) was essential to allow enable clinical trials with tea catechins. Successful completion of extensive safety/toxicity tests from microbial to human PK (pharmacokinetic) tests by US NCI (National Cancer Institute), permitted clinical trials with the crude extract of green tea, termed as "Polyphenon® E". This was administered orally or topically in various Randomized Controlled Trial (RCT) clinical trials for chemoprevention or for benign tumors. EGCG, the most abundant catechin in tea, is scheduled for several RCTs therapies for various disorders.

Keywords: clinical trial, polyphenol, tea catechin

Introduction

Studies on the effects of tea catechins on physiological functions have been conducted extensively over the last 30 years since around 1980. This chapter covers studies ranging from *in vitro* and animal studies to epidemiological research. Several clinical trials have verified these efficacies in humans. The anti-microbial properties of tea catechins were proven to help against influenza infection and for the improvement of intestinal flora. Improvement of various parameters of metabolic syndrome have been verified, including the reduction of visceral fat, blood lipid, blood sugar and blood pressure. These anti-metabolic syndrome trials, in several clinical trials, were conducted to verify efficacies and meet the requirements of the regulatory authorities. Ready-to-Drink tea beverages are now registered in the Food for Specified Health Uses (FOSHU) system of Japan.

Recently, more emphasis has been placed on Randomized Controlled Trial studies to prove various efficacies of tea catechins on human subjects for the development of new products for the dietary supplements and pharmaceutical industries.

This chapter focuses on the topics relating to prevention or suppression of tumors, or cancerous growths, by tea catechins. This follows a brief description of what tea catechins are and how they have come to be regarded as essential elements in "tea and health".

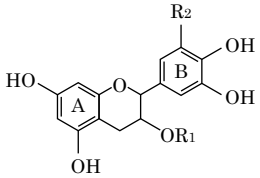
The clinical studies described here are divided into four topics. Firstly, a 15-year study of cancer prevention, measuring specific biomarkers, with oral Polyphenon® E, a defined crude tea catechin. Secondly, a catechin ointment approved by the US FDA that treats benign tumors in genital warts of humans, called *Condyloma acuminata*. Thirdly, a clinical trial done in Italy on the prevention of pre-cancerous conditions of the prostate developing into prostrate cancer, by administering crude tea catechins. Fourthly, a range of clinical trials under preparation for the "treatment" of diseases using specific tea catechins or the combination of catechins with other agents.

What and why tea catechins

The properties and beneficial health effects of tea were reviewed in two books by Hara [1] and Kuroda and Hara [2]. The topics include: the history of tea, catechins and extraction methods, anti-oxidative action [3], anti-bacterial action [4], anti-viral action [5], prevention of cancer [6], hypolipidemic action [7], hypoglycemic action [8], hypotensive action [9], effects on intestinal flora [10], and practical applications of these functions.

Chemistry and natural history of tea catechins

Plants synthesize a group of biochemical compounds called polyphenols. Polyphenols are used as chemical defense agents against viruses, bacteria, fungi and insects. In other words, they keep plants healthy. Polyphenols also deter herbivores. These polyphenols are concentrated in the young tender leaves that are most vulnerable to insects. Tea catechins are a subgroup of the polyphenols.



	R_1	R_2
Epicatechin	H	H
Epicatechin-3-gallate	Gallate	H
Epigallocatechin	H	OH
Epigallocatechin-3-gallate	Gallate	OH

FIGURE 1: Molecular structure of tea catechins

Catechins are synonymous to bioflavonoids. In the tea plants, they form about 15 to 30% of the dry weight of young tea shoots. These flavonoids consist of two phenolic or aromatic rings, A and B, with several hydroxyl groups (Figure 1). There is a variety of catechins such as: (+)-catechin, (+)-gallocatechin, (-)-epicatechin (EC) and (-)-epigallocatechin (EGC) as well as galloyl catechins: (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG) and (-)-gallocatechin gallate (GCG). EGCG is the most abundant and comprises more than 50% of the total catechins.

Oxidation of catechin leads to the formation of dimers and polymers; this is the major chemical reaction involved in the manufacturing of black tea. The resulting dimers and polymers, called theaflavins and thearubigins, respectively, retain many of the biological properties of the catechins.

Purification of catechins

Catechins are extracted from green tea leaves, along many other compounds, by hot water. The catechins are separated from the other compounds by aqueous ethanol on column of a special resin. The resulting catechin powder, called Polyphenon® E (or Theaphenon® E), with a total catechin content of about 90%, is composed of EGCG>65%, EC>10%, ECG<10%, EGC<10% and traces of other catechin derivatives. Polyphenon® E is a trademark of Mitsui Norin Co., Ltd. and Theaphenon® E is a trademark of Tea Solutions, Hara Office Inc.

Development of Polyphenon® E as a chemopreventive formula

Polyphenon® E is a heterogeneous botanical extract of green tea, manufactured under current Good Manufacturing Practice (CGMP). Polyphenon® E is stable under normal storage and readily packaged into capsules, which are also stable, according to the complete stability tests supported by the Department of Chemoprevention/ National Cancer Institute, USA (NCI/DCP). Mitsui Norin Co. prepared a voluminous Drug Master File (DMF) showing Chemistry, Manufacturing and Controls of the manufacturing facility, filed with the Food and Drug Administration, USA (FDA) and was approved as a CGMP facility for the manufacturing of Polyphenon® E. This DMF should be available for investigators conducting chemoprevention trials as well as for other clinical applications of Polyphenon® E. Although Mitsui Norin Co. ceased to supply Polyphenon® E to other investigators except one particular topical drug use, Theaphenon® E (manufactured under food-grade GMP) has been supplied instead of Polyphenon® E. Theaphenon® E is chemically equivalent to Polyphenon® E and is readily available from the present author upon request by investigators, for research purposes.

Botanical drug

In 2004, the US FDA issued a guideline for "Botanical Drug Products" wherein the active component, i.e. Active Pharmaceutical Ingredient of a Botanical Drug, should be the crude extract of plants without being purified [11]. The critical feature of a botanical drug is that no particular effective component is necessarily assumed. An agent will be approved as a botanical drug if the defined crude extract in the drug formulation shows efficacy in clinical trials. As crude extract of green tea, Polyphenon® E is composed of more than ten different catechins (including isomers and derivatives) as well as other miscellaneous components, including trace amounts of unknowns.

In the manufacturing of Polyphenon® E under CGMP, certain criteria must be met in the specification, such as "consistency", "stability", "absence of adulterants" and "traceability". In other words, as an FDA official put it, "process is the product." From 1997 to 2008, NCI/DCP has supported almost 25 Phase 1 and 2 clinical trials of chemoprevention in the United States. These clinical trials are in collaboration with several investigators as well as Mitsui Norin Co. using Polyphenon® E capsules/ placebos. A list of these clinical Phase 2 trials are shown in Table 1.

The difficulty in these trials is to identify biomarkers that accurately predict the agent's clinical benefit or cancer incidence-reducing effect. Another difficulty in the clinical trials is the recruiting of subjects, because the subjects are essentially healthy individuals, with little motivation to join in the trials.

Condyloma Ointment Project - an FDA approved botanical drug

The following is an overview of the successful registration of a tea extract product with the US FDA. The initial fact finding trial was successfully done in a clinical setting in China. Application of Polyphenon® E ointment on genital warts (*Condyloma acuminata*) eliminated the warts effectively at the Beijing Cancer Center Hospital in 1990, and in the subsequent trials [12]. This study demonstrated the therapeutic effect of tea catechins on benign tumors.

On this basis, a German pharmaceutical company, MediGene AG, spent time and money on Phase 2 and Phase 3 clinical trials internationally. In 2006, the US FDA approved the marketing of the ointment as a prescribed Botanical drug in the United States [13]. The marketing of this drug is under way in EU countries as well as in the United States under the trademark of Veregen®. The inventors of the patent, which include Yukihiko Hara, and the manufacturer of the active pharmaceutical ingredient spent more than 16 years to bring this product to the clinic. According to the US FDA, Veregen® is the first-ever botanical drug approved under the FDA

TABLE 1: Clinical Trials with Tea Catechins: Polyphenon® E

	Status	Study
1	Recruiting	Study of Polyphenon® E in Men With High-grade Prostatic Intraepithelial Neoplasia Condition: **Prostatic Hyperplasia** Interventions: Drug: Polyphenon® E, 200mg EGCG bid; Drug: placebo
2	Completed	Erlotinib and Green Tea Extract (Polyphenon® E) in Preventing Cancer Recurrence in Former Smokers Who Have Undergone Surgery for Bladder Cancer Condition: **Bladder Cancer** Interventions: Dietary Supplement: Polyphenon® E; Drug: erlotinib hydrochloride; Other: Erlotinib placebo; Other: Polyphenon® E
3	Recruiting	Study of Polyphenon® E in Addition to Erlotinib in Advanced Non Small Cell Lung Cancer Condition: **Advanced Non-Small Cell Lung Cancer** Intervention: Drug: Polyphenon® E +Tarceva (Erlotinib)
4	Recruiting	Safety of Polyphenon® E in Multiple Sclerosis Pilot Study Condition: **Multiple Sclerosis** Intervention: Drug: Polyphenon® E
5	Completed	Efficacy and Safety Study of Polyphenon® E to Treat External Genital Warts Condition: **Condylomata Acuminata** Intervention: Drug: Polyphenon® E Ointment 10%, Polyphenon® E Ointment 15%
6	Active, not recruiting	Green Tea Extract in Preventing Esophageal Cancer in Patients With Barrett's Esophagus Conditions: **Esophageal Cancer; Barrett's Esophagus** Interventions: Dietary Supplement: Polyphenon® E (Poly E); Other: placebo
7	Recruiting	Treatment of Epidermolysis Bullosa Dystrophica by Polyphenon® E (Epigallocatechin 3 Gallate) Condition: **Epidermolysis Bullosa Dystrophica** Interventions: Drug: Polyphenon® E before placebo; Drug: placebo before treatment
8	Recruiting	Pilot Study of Green Tea Extract (Polyphenon® E)in Ulcerative Colitis Condition: **Mild to Moderately Active Ulcerative Colitis** Intervention: Drug: Polyphenon® E
9	Recruiting	A Phase I Study of Chemoprevention With Green Tea Polyphenon® E (PPE) and the Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase Inhibitor Erlotinib (OSI-774, Tarceva) in Patients With Premalignant Lesions of the Head and Neck Conditions: **Cancer of Head and Neck; Neoplasms** Intervention: Drug: Erlotinib and Green Tea Polyphenon® E
10	Active, not recruiting	Green Tea or Polyphenon® E in Preventing Lung Cancer in Former Smokers With Chronic Obstructive Pulmonary Disease Conditions: **Lung Cancer; Pulmonary Complications** Interventions: Dietary Supplement: defined green tea catechin extract; Dietary Supplement: green tea; Other: placebo
11	Active, not recruiting	A Study of the Effect of Polyphenon® E (Green Tea Extract) on Breast Cancer Progression Condition: **Breast Cancer** Intervention: Drug: Polyphenon® E (EGCG)
12	Active, not recruiting	Green Tea Extract in Treating Women With Hormone Receptor-Negative Stage I, Stage II, or Stage III Breast Cancer Condition: **Breast Cancer** Interventions: Dietary Supplement: Polyphenon® E; Other: placebo
13	Completed	Green Tea Extract (Polyphenon® E) in Preventing Cancer in Healthy Participants Condition: **Unspecified Adult Solid Tumor, Protocol Specific** Intervention: Dietary Supplement: defined green tea catechin extract
14	Completed	Pharmacokinetic Study of Topically Applied Veregen® 15% Compared With Oral Intake of Green Tea Beverage Conditions: **Genital Warts; Perianal Warts** Interventions: Drug: Polyphenon® E (Veregen®) 15% Ointment; Other: Green Tea Beverage with defined catechin content

	Status	Study
15	Not yet recruiting	Treatment of the Recessive Nonbullous Congenital Ichthyosis by the Epigallocatechine Cutaneous Condition: **Lamellar Ichthyosis** Intervention: Drug: apply Veregen® 10% on a randomized area and the moisturizing cream of the other side
16	Recruiting	Green Tea Extract in Treating Patients With Nonmetastatic Bladder Cancer Condition: **Bladder Cancer** Interventions: Dietary Supplement: defined green tea catechin extract; Other: placebo
17	Terminated	Green Tea Extract in Treating Patients With Actinic Keratosis Condition: **Non-melanomatous Skin Cancer** Intervention: Drug: kunecatechins ointment
18	Recruiting	Green Tea Extract in Treating Current or Former Smokers With Bronchial Dysplasia Conditions: **Lung Cancer; Precancerous Condition; Tobacco Use Disorder** Interventions: Dietary Supplement: defined green tea catechin extract; Other: placebo
19	Active, not recruiting	Green Tea Extract and Prostate Cancer Condition: **Prostate Cancer** Intervention: Drug: Polyphenon® E (EGCG)
20	Active, not recruiting	Green Tea Extract in Preventing Cervical Cancer in Patients With Human Papillomavirus and Low-Grade Cervical Intraepithelial Neoplasia Conditions: **Cervical Cancer; Precancerous Condition** Interventions: Dietary Supplement: defined green tea catechin extract; Other: placebo
21	Recruiting	A Pilot Study of Chemo-prevention of Green Tea in Women With Ductal Carcinoma *in situ* Condition: **Ductal Carcinoma *In Situ*** Intervention: Drug: Polyphenon® E
22	Active, not recruiting	Green Tea Extract in Preventing Cancer in Former and Current Heavy Smokers With Abnormal Sputum Conditions: **Lung Cancer; Tobacco Use Disorder** Interventions: Dietary Supplement: defined green tea catechin extract; Other: placebo
23	Active, not recruiting	Defined Green Tea Catechins in Treating Patients With Prostate Cancer Undergoing Surgery to Remove the Prostate Condition: **Prostate Cancer** Interventions: Dietary Supplement: defined green tea catechin extract; Other: placebo
24	Recruiting	Green Tea Extract in Treating Patients With Monoclonal Gammopathy of Undetermined Significance and/or Smoldering Multiple Myeloma Conditions: **Multiple Myeloma and Plasma Cell Neoplasm; Precancerous Condition** Interventions: Dietary Supplement: defined green tea catechin extract; Genetic: gene expression analysis; Genetic: protein analysis; Other: laboratory biomarker analysis
25	Active, not recruiting	Green Tea Extract in Treating Patients With Stage 0, Stage I or Stage II Chronic Lymphocytic Leukemia Condition: **Leukemia** Intervention: Dietary Supplement: defined green tea catechin extract

Guideline, as described by Chen *et al.* 2008, in the Commentary section of Nature Biotechnology [14].

Prostate cancer Prevention

Bettuzzi *et al.* discovered that the intake of tea catechins (GTC: Green Tea Catechins) prevented prostate cancer by arresting the progress of pre-cancerous condition into cancer [15]. Sixty volunteers were recruited who were diagnosed with high-grade prostatic intraepithelial neoplasia (HGPIN). These volunteers were at high-risk to develop prostate cancer within a year or two. By oral administration of 600mg GTC per day for twelve months, the development of prostate cancer was reduced to only one out of 30 subjects in the catechin-therapy group, whereas nine out of 30 subjects developed cancer in the placebo group.

All relevant data was favorable for the catechin-therapy group, such as keeping a high quality of life with no adverse events. Two years follow up observation confirmed no development of prostate cancer in the catechin-therapy group. The daily dose of 600mg tea catechin corresponds to five to six cups of green tea per day. Future clinical trials on a much larger number of subjects are desired to confirm the efficacy of tea catechins in prostate cancer prevention.

Cancer treatment by combined agents and other clinical programs

Registration has been made for the trademark of Theaphenon® with the US Patent and Trademark Office, aiming to follow a similar to, but slightly different path from that taken by, Polyphenon® E. Since Polyphenon® E established itself as a Botanical drug, given the United States Adopted Names, “sinecatechins”, by the Committee, no similar compounds have been allowed for pharmaceutical use. One possible way was to register Theaphenon® as health supplement, with no health claims on the label. There is heavy market competition among similar products. Another option was to make a CGMP compliant, very pure EGCG product from the major component in tea, as a kind of commodity.

As for the anti-cancer activities of EGCG, many possible mechanisms have been reported (reviewed in [16]). Tachibana *et al.* found that there is a specific receptor on cancerous cells for EGCG, that is the 67-kDa laminin receptor (67LR) which upon EGCG binding, induces cancer cells into apoptosis [17, 18]. They further found that this apoptosis-inducing effect was greatly enhanced by co-administering a phosphodiesterase-5 inhibitor (PDE-5 Inhibitor) [19]. Clinical confirmations of these findings are highly warranted and collaborating studies are in progress to bring these findings into clinical use with due regulatory process. The first target will be the treatment of CLL (chronic lymphocytic leukemia).

The present author has been involved in other investigations for the therapeutic efficacy of tea catechins in clinical settings in addition to CLL, including multiple sclerosis, inflammatory bowel diseases, diabetes, neuroblastoma, endometriosis, uveites and sarcopenia. These disorders are expected to be treated by EGCG alone or with Theaphenon® E. Each of these will take years for the preparation of the protocol and will demand large funding before the Institutional Review Board (IRB)'s approval. Theaphenon® E should be useful for these clinical trials.

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2

Effects on Metabolic Syndrome and Related Diseases

Preventive Effects of Obesity by Green Tea and Its Components

Kazutoshi SAYAMA

Abstract: Obesity is one of the cause of metabolic syndrome in humans and the increase is a serious social problem even in Japan. Recently, it was clarified that obesity could suppress by intake of green tea and its component and the suppressive action attract the attention all over the world. As a basic research by using mouse, it was reported that fat accumulation was suppressed by oral administration of a diet containing 2% green tea powder and the active constituents were green tea catechins and caffeine. Moreover, the suppressive mechanisms were suppression of lipid absorption from intestine, improvement of lipid metabolism in liver and adipocytes, and promotion of calorigeneic action in adipose cells. Furthermore, clinical studies suggested that combination of a moderate exercise and intake of green tea catechins and caffeine suppressed fat accumulation even in humans. On the other hand, other kinds of tea such as oolong, pu-erh and black tea also have suppressive action of obesity by the intake with meal.

Keywords: caffeine, catechins, obesity

Introduction

Eating habits of Western style diets with high fat are commonly used even in East Asia. For example, animal fat intake in Japan increase to approximately three times compared to it before 40 years. As a result, patients of the metabolic syndrome increase and it becomes the recent social problem [1]. The main cause of metabolic syndrome is obesity. Serious diseases such as fatty liver, high blood pressure, hyperlipidemia, sleep apnea syndrome which developed by obesity increase year by year and progress to diabetes. Moreover, obesity leads to arteriosclerosis and

finally, the diseases such as myocardial infarction and stroke which lead to death are developed. It has been already said that one of 4-5 people of the adult is obese in Japan. Thus, it goes without saying that the prevention of obesity is important to enjoy a healthy life. When it is thought that health damage may be brought about by extreme temperance in eating and by deflection of the nourishment by being on a diet, it might be ideal to prevent obesity by taking foods or food components which have anti-obesity actions by eating normal diets.

From a long time ago, it was suggested that green tea and the components have anti-obesity action by some research results showing their inhibitive action of lipid absorption and induction of lipid metabolism in liver [2-4]. Then, it was reported that green tea has strong anti-obesity action in the study which green tea powder was orally administered to mice [5, 6]. Starting from this study, anti-obesity action by green tea beginning to attract attention and elucidate the action by the green tea constituents and the mechanism is pushed forward.

In this chapter, at first, anti-obesity actions by catechins, caffeine and theanine, green tea components, and the suppressive mechanisms is amplified. Moreover, the inhibiting actions of fat accumulation by other kinds of tea such as oolong, pu-erh and black tea are also described.

Experimental studies of anti-obesity effects by green tea

Previously, Sano *et al.* [7] reported that the drinking of water extracts of green tea had no effects on the body weight, weight of intraperitoneal adipose tissue and lipid metabolism in rats. However, Sayama *et al.* reported that the increase of body weight and fat accumulation in mice was suppressed by the administration of diet

TABLE 1: Effects of green tea on weights of body and adipose tissues and levels of several lipids in serum and liver

Concentration of Green Tea powder		1%	2%	4%
Body weight			⇓	⇓⇓
IPAT			⇓	⇓⇓
Food intake				⇓
Lipid levels (serum)	TC			
	TG		⇓	⇓
	PL			
	NEFA		⇓	⇓
Lipid levels (Liver)	TC		↘	⇓
	TG		↘	⇓
	PL			
Leptin level			⇓	⇓

IPAT: Intraperitoneal adipose tissues, TC: Total cholesterol, TG: Triglyceride, PL: Phospholipid, NEFA: non-esterified fatty acid

↘ : Tendency to decrease vs control

⇓ , ⇓⇓ : Significantly decrease vs control (p<0.05, 0.01)

containing green tea powder (Table 1) [6]. Green tea powder (GTP) was mixed with a commercial powder diet for mice at concentrations of 1, 2 and 4% and administered to four weeks-old female ICR mice for 16 weeks. As a result, the body weight increase was significantly suppressed by 2 and 4% GTP diets and the reduction ratios were 65% in the 2% treatment group and 87% in the 4% treatment group, respectively, compared with the control. Moreover, the serum levels of triglyceride (TG) and non-esterified fatty acids (NEFA) were significantly lower than the control. Levels of TG and total cholesterol (TC) in the liver were also decreased by GTP feeding. These results indicated that GTP remarkably suppressed the body weight increase and fatty accumulation in mice when added to the diet at 2% and higher concentrations and the suppressive effects were suggested to be due to the improvement action of GTP on the lipid metabolism. In particular, it was noteworthy that administration of 2% GTP diet suppressed fat accumulation without the reduction of food intake.

Anti-obesity action by green tea components

The previous section demonstrated that green tea has anti-obesity action. However, it has never been clarified which component of green tea is responsible for the anti-obesity effects of green tea. Among many green tea components, catechins are main components of green tea and many physiological functions such as anti-cancer and suppression of lipid absorption, were already reported. Caffeine is a main constituent on the anti-obesity action of oolong tea and promotes thermogenesis *in vivo*. Theanine is a main amino acid and a peculiar component of green tea. Thus, the effects by these three major components on the weights of body and several

TABLE 2: Effects of catechins, caffeine and theanine on weights of body and adipose tissues and levels of several lipids in serum and liver in mice

		GTP	Caffeine	Catechins	Theanine	Caffeine + Catechins	Caffeine + Theanine	Catechins + Theanine	Caffeine + Catechins + Theanine
Body weight		⇓⇓	⇓		⇓	⇓⇓	⇓		⇓
IPAT		⇓⇓	⇓		⇓	⇓⇓	⇓		⇓
Food intake									
Lipid levels (Serum)	TC								
	TG	⇓		⇓	⇓	↘			↘
	PL								
	NEFA	⇓		⇓	⇓	⇓	⇓	↘	⇓
Lipid levels (Liver)	TC								
	TG	↘		⇓		↘	↘	⇓	↘
	PL								

GTP: Green tea powder, TC: Total cholesterol, TG: Triglyceride, PL: Phospholipid, NEFA: non-esterified fatty acid
↘ : Tendency to decrease vs control
⇓ , ⇓⇓ : Significantly decrease vs control (p<0.05, 0.01)

organs, food intake and lipid levels in mice. As a result, caffeine and theanine have suppressive effects of fat accumulation by the single administration [8] (Table 2) and theanine exerted the action in limited treatment dose [9]. Moreover, it was demonstrated that catechins acted synergistically with caffeine in manifestation of anti-obesity activities and the action was almost equal compared to it by green tea [8]. Furthermore, epigallocatechin gallate (EGCG), main component of catechins, also has anti-obesity action by the single administration to mice [10].

Inhibition mechanism of fat accumulation by green tea components

The main organs of lipid metabolism in the body are liver and adipose tissues (cells). In a recent study, it is reported that lipid accumulation is suppressed because the lipid metabolism in those organs is improved by catechins alone or the combination of catechins and caffeine.

Effects of Green Tea Components on the Lipid Metabolism in Liver

Improvement mechanism of lipid metabolism in mouse liver by catechins and caffeine : The activities, protein concentrations and mRNA expression levels of several enzymes involved in lipid metabolisms and the related mRNA expression levels in liver were analyzed in the mice treated with diets containing 0.3% catechins and 0.05% caffeine, singly or in combination. As a result, enzymatic activity of fatty acid synthase (FAS) was decreased and those of acyl-CoA oxidase (ACO) and carnitine palmitoyltransferase-II (CPT-II) contrarily increased

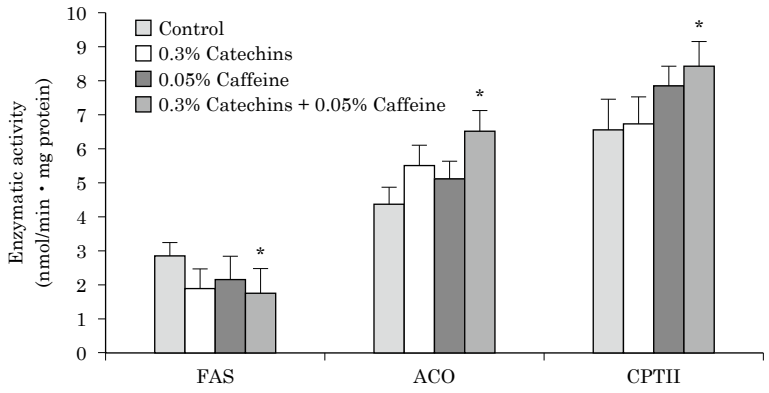

FIGURE 1: Effects of catechins and caffeine on the enzymatic activities involved with lipid metabolism in liver in mice.
The means and SE for 6 mice are plotted.
*: Significant difference at $p<0.05$ compared to the control.

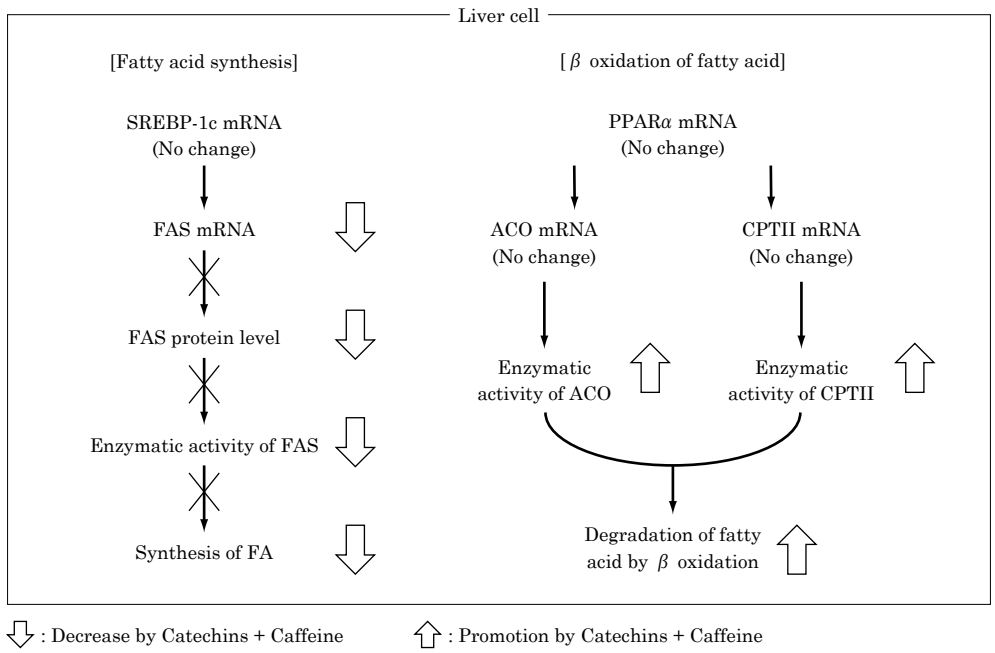

FIGURE 2: Improvement mechanisms of fatty acid synthesis and β oxidation by catechins and caffeine in mice
SREBP-1c: Sterol regulatory element-binding proteins-1c, PPARα: peroxisome proliferator-activated receptor α, FAS: Fatty acid synthase, ACO: acyl-CoA oxidase, CPTII: carnitine palmitoyl transferase II

in liver by administration of diet containing 0.3% catechins + 0.05% caffeine (Figure 1) [11]. The expression level of mRNA and protein concentration of FAS were also lowered. However, mRNA expression of ACO, CPT-II, PPARα and SREBP-1c were not affected by the treatment. These results indicate that strong suppressive action of fat accumulation by catechins and caffeine might result from the inhibition of fatty acid production by suppression of FAS mRNA expression and the protein production and the activation of fatty acid oxidation by promotion of the enzymatic activities in liver (Figure 2).

Improvement mechanism of lipid metabolism in mouse liver by EGCG and caffeine : In the same administration experiments as 3-1-1 by using diets containing EGCG and/or caffeine, there were no effects on any activities and mRNA expression of fatty acid synthesis and oxidation in the liver. Therefore, it was suggested that improvements of fatty acid synthesis and oxidation by catechins and caffeine in the liver might be caused not solely by ECGC but by the synergistic action of all catechins including EGCG.

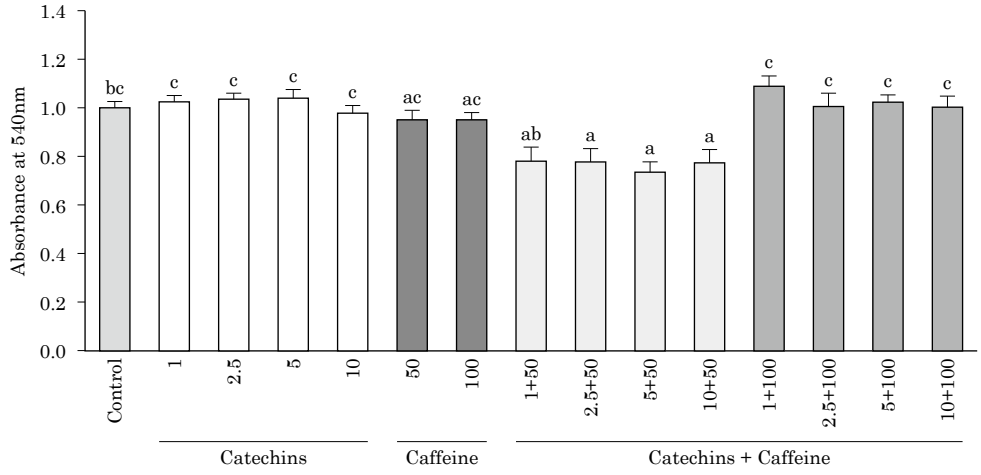

FIGURE 3: Effects of catechins and caffeine on lipid accumulation in 3T3-L1 adipocytes *in vitro*
Values are means ± SE on 9 wells by 3 experiments.
Values not sharing common letters are significantly different from each other at $p<0.05$.

Effects of Green Tea Components on the Lipid Metabolism in Adipocytes

3T3-L1 mouse pre-adipocyte cell line was used for elucidation of suppressive action of lipid accumulation in adipose cells by catechins and caffeine.

Suppressive mechanism of lipid accumulation in 3T3-L1 mouse adipocyte: After the differentiation of 3T3-L1 cells from the pre-adipocyte to adipocyte, the differentiated cells were cultured in mediums containing catechins at 1, 2.5, 5 and 10μg/mL and caffeine at 50 and 100μg/mL, singly and in combination, during eight days. Then, the levels of lipid accumulation and triglyceride and enzymatic activity of glycerol-3-phosphate dehydrogenase (GPDH), a marker of lipid synthesis in adipocyte, in the cultured cells were analyzed. As a result, lipid accumulation in the cells was significantly suppressed by addition of catechins and caffeine, but not affected by catechins or caffeine alone (Figure 3) [12]. Moreover, the GPDH activity was also suppressed by both catechins and caffeine.

Additionally, mRNA expression of PPARγ, GLUT4, HSL, P53, UCP-1, CD137 and TMEM26 which are involved in lipid metabolism and calorigenic action in the cells was also analyzed. In the culture in the medium containing catechins and caffeine, the expression levels of PPARγ, GLUT4, HSL, UCP-1 and TMEM26 increased and the levels of p53 and CD137 decreased. These results indicated that lipid accumulation in adipocytes was suppressed by catechins and caffeine. One of the suppressive mechanisms was considered to be the involvement of the reduction of glycerol-3-phosphate synthesis and following suppression of triglyceride production

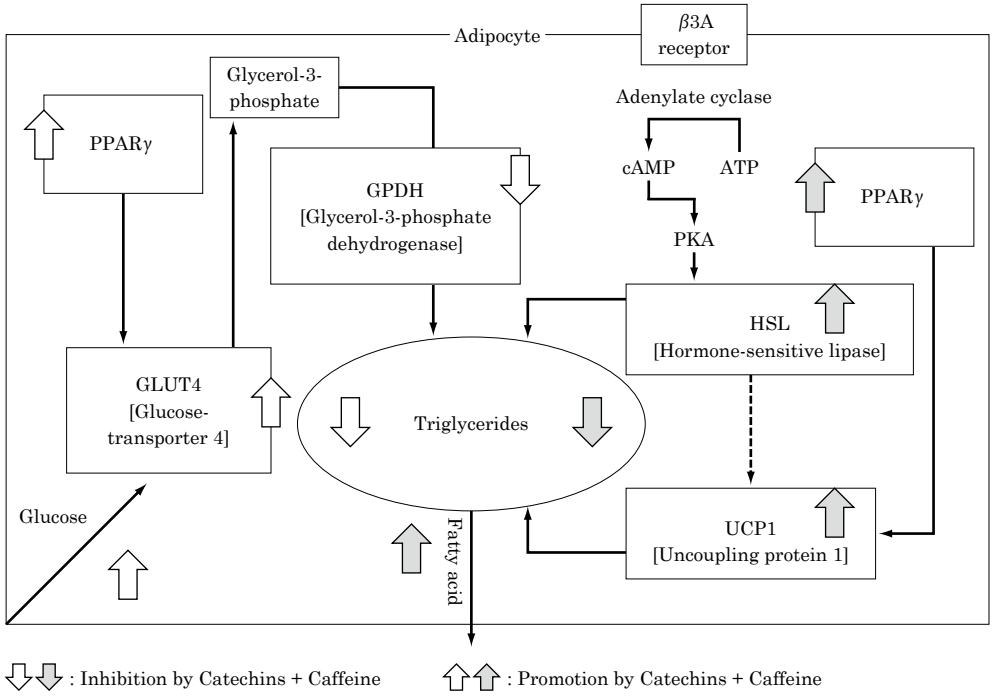

FIGURE 4: Improvement mechanisms of lipid metabolism in 3T3-L1 adipocytes by catechins and caffeine
PPARγ: Peroxisome proliferator-activated receptor γ

in adipocytes (Figure 4). Moreover, it is noteworthy that catechins and caffeine might be involved in not only the suppression of lipid synthesis and accumulation but also promotion of the calorigenic action by conversion of adipocyte from white adipocyte to beige adipocyte through the gene expression of UPC-1 and TMEM26 [13].

Anti-obesity action by combination of green tea components ingestion and physical exercise

On an experiment by using mice, it was clarified that whole-body fat was burned up efficiently by combinational treatment of intake of a special green tea bevarage with rich in catechins and physical exercise (swimming) [14]. This result was also demonstrated in human trials. Thus, these results show that it may be possible to keep the proper body weight without dietary restriction by daily adequate physical exercise and green tea intake.

Inhibitory effects of fat accumulation by other teas

Oolong Tea

As well as green tea, oolong tea is also traditionally known to have anti-obesity

and hypolipidaemic effects. Recently, it was clarified that anti-obesity action by oolong tea was due to caffeine [15] and the specific polymerized polyphenols [16]. The action was confirmed in human [17] and released a specified health oolong tea drink based on the action in Japan.

Pu-erh Tea

Recent research clarified that pu-erh tea has inhibitory actions of serum lipid levels and body weight gain [18]. Moreover, it suppressed proliferation and fat accumulation in the cultured adipocytes *in vitro* [19].

Black Tea

Black tea contains many polymerized polyphenols produced by strong fermentation. It was reported that administration of black tea extract containing high concentration of them or the highly purified polyphenols mixture reduced serum and liver lipid levels and also suppressed body weight gain in rats [20, 21]. When rats were given sucrose-rich diet and a drink of black tea extracts (1% w/v), black tea extracts significantly decreased body weight gains and food efficiency. Moreover, the hypertriglyceridemia and hypercholesterolemia induced by sucrose-rich diet were normalized by black tea.

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The Effects of Tea Galloyl Catechins on the Reduction of Body Fat

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Abstract: It is known that obesity by the accumulation of abdominal visceral fat causes hyperglycemia, high blood pressure, and lipid profile abnormalities, and raises the risk factors such as coronary heart disease and cerebrovascular disease. In an intervention study conducted in subjects with borderline high body mass index (BMI), drinking tea catechins with a galloyl moiety showed decreased body fat and BMI. Furthermore, elevation of postprandial plasma triacylglycerol (TG) levels in subjects with mild and borderline hypertriacylglycerolemia were inhibited by consumption of tea catechins, when consumed along with fat. Tea catechins may suppress postprandial plasma TG by slowing down TG absorption through the inhibition of pancreatic lipase activity. It is currently thought that when postprandial serum TG levels are elevated, insulin promotes accumulation of body fat. Therefore, suppression of postprandial TG by tea catechins is thought to be one of the mechanisms underlying their anti-obesity effects.

Keywords: intervention study, obesity, tea catechins with a galloyl moiety, triacylglycerol, visceral fat

Introduction

Since TG is important for energy storage and maintenance of temperature *in vivo*, it is one of the important nutrients of the body. However, excessive accumulation of TG in adipose tissue induces obesity, and the enlarged fat cells show excessive secretion of cytokines such as the tumor necrosis factor-α and resistin. On the other hand, secretion of adiponectin is inhibited. An adiponectin promotes AMP kinase activity, which induces uptake of glucose and fatty acid combustion. These

obesity-related changes in cytokines and adiponectin secretion combine to increase insulin resistance [1-3]. Thus, obesity causes hyperglycemia, high blood pressure, and lipid profile abnormalities such as hypertriglyceridemia, and subsequently increasing risk for coronary heart disease (CHD) and cerebrovascular disease [4]. Since an excessive accumulation of visceral fat is closely related to metabolic syndrome, measurement of abdominal circumference is necessary for a diagnosis of abdominal visceral obesity, a diagnostic criterion for metabolic syndrome in Japan [5]. In addition, the measurement of abdominal circumference is a diagnostic criterion for metabolic syndrome in Adult Treatment Panel III of the National Cholesterol Education Program in the USA [6]. Therefore, it is thought that suppressing excessive accumulation of visceral fat is very important for preventing metabolic syndrome and improving quality of life.

Tea catechins are known to reduce body fat [7-9]. Furthermore, body fat reduction by consumption of beverages containing tea catechins with a galloyl moiety has been reported in a human intervention study of subjects with mildly elevated and/or borderline high BMI [10]. Multiple mechanisms of that action have also been reported for tea catechins [11-13]. On the basis of these reports, the Food for Specified Health Use approved by the Japanese government has permitted labeling of beverages containing tea catechins with a galloyl moiety as “Suitable for a person concerned about body fat” [14]. This chapter reviews the effects of tea catechins, especially those with a galloyl moiety, on body fat in obesity and discusses their action mechanisms.

Tea catechins with a galloyl moiety

Tea catechins are present at a concentration of 12-16% by weight, in dried green tea leaves (*Camellia sinensis*) [15]. There are four naturally-occurring catechins, including (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG), with EGCG accounting for about 50% of all the tea catechins. When manufactured green tea beverages are canned or bottled in polyethylene terephthalate (PET) bottles, they are typically sterilized by heating. Catechins are epimerized by the sterilization process and are changed to (-)-catechin (C), (-)-gallocatechin (GC), (-)-catechin gallate (CG) and (-)-gallocatechin gallate (GCG). Therefore, eight species of catechins exist in the sterilized beverages. When Longjing green tea catechin and purified EGCG were autoclaved at 120℃ for 20 min, epimerization of EGCG to GCG was observed [16]. Further, when the catechin concentration of green tea in cans and PET bottles was measured, eight species of catechins were noted, including EC, EGC, ECG, EGCG, C, GC, CG, and GCG [10]. ECG, EGCG, CG, and GCG had a galloyl moiety, and CG and GCG were named heat-treated catechins with a galloyl moiety. The biological activities of the tea catechins with a galloyl moiety are particularly interesting.

Effects of tea catechins with a galloyl moiety in reducing body fat in intervention studies

Obesity is a condition in which excessive fat accumulates in the adipose tissue of the body. The fat that accumulates in the subcutaneous layer is called subcutaneous fat, while that which accumulates around internal organs is called visceral fat, and these two together are called body fat. When discussing a variation of body fat, we evaluate mainly the BMI, total fat area (TFA) which is the sum of visceral fat area (VFA) and subcutaneous fat area (SFA) measured by X-ray computer tomography (CT).

Kajimoto *et al.* reported the effects of consumption of a beverage containing tea catechins with a galloyl moiety on body fat level, BMI, and body weight in adults [10]. This report described a double-blind study of three parallel groups of healthy or moderately obese subjects (98 men and 97 women) aged 20 to 65 years, with BMI between 22.5 and 30kg/m^2. The subjects received a beverage (250mL/bottle) containing 215.3mg of tea catechins, mostly with a galloyl moiety and including more than 90% gallate esters of tea catechins, or a placebo beverage. The subjects consumed either three bottles of placebo beverage (control group), two bottles of catechin-containing beverage and one bottle of placebo beverage (low-dose group), or three bottles of tea catechin-containing beverage (high-dose group), per day at mealtimes for 12 weeks. The groups that consumed either two or three bottles of the catechin-containing beverage showed a significant decrease in BMI at 4, 8 and 12 weeks compared to the initial value (Figure 1A). Abdominal circumference also significantly decreased in both catechin groups compared to their initial measurements (0 week) and those of the placebo group at 12 weeks. Measurement of the abdominal fat area by X-ray CT at 12 weeks indicated a significant reduction in both TFA and VFA in the catechin group, compared to the placebo group (Figure 1B). These results showed that consumption of a beverage rich in tea catechins with a galloyl moiety was useful for reducing body fat in subjects with mild or borderline adiposity.

The effects of consumption of a beverage containing tea catechins with a galloyl moiety on body fat and serum cholesterol level have also been reported in a separate intervention study conducted in healthy adults [17]. A double-blind placebo-controlled study was conducted by dividing 73 healthy adult subjects (BMI between 23 and 30kg/m^2, total serum cholesterol levels from 200 to 260mg/dL) into two groups. Each subject in the test group was given two bottles per day of a beverage (250mL/bottle) containing 169.7mg of catechins with a galloyl moiety, and each subject in the placebo group was given two bottles per day of a placebo beverage at lunch and dinner time for 12 weeks. Consumption of tea catechins with a galloyl moiety resulted in a significant decrease in both body weight and BMI at 12 weeks compared to 0

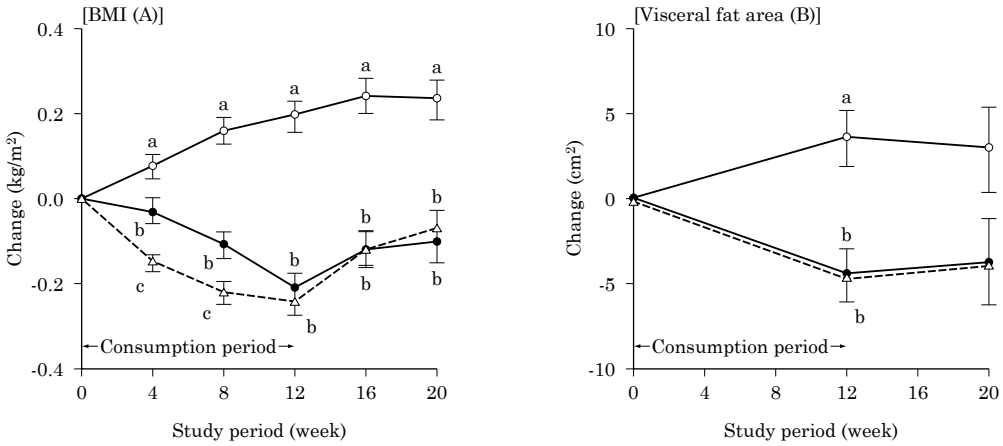

FIGURE 1: Change of BMI (A) and visceral fat area (B) in men and women consuming tea catechins for 12 weeks

○, control group (n=66); ●, low-dose group (n=65); △, high-dose group (n=64)

Each point represents the mean ± SEM.

Those in the same study period not sharing a letter differ, $p<0.05$. (Reference [10], altered partially)

week. At 8 and 12 weeks, body weight and BMI were significantly decreased in the catechin group compared to the placebo group. Measurement of abdominal fat areas by X-ray CT at 12 weeks indicated a significant reduction in both total fat and VFA in the catechin group, compared to the placebo group. This study also showed that at 12 weeks, serum LDL cholesterol levels significantly decreased in the tea catechin group compared to the placebo group. These results showed that consumption of a beverage rich in tea catechins with a galloyl moiety was useful for reducing body fat in subjects with mild or borderline adiposity, as well as for lowering serum cholesterol level in those with mild or borderline hypercholesterolemia.

A 12-week randomized double-blind placebo-controlled study was conducted in order to evaluate the effect of consumption of tea catechins with a galloyl moiety on body fat level in healthy women [18]. A total of 41 women (BMI between 23 and 30kg/m^2) were divided into two groups. Each subject in the test group was given two bottles/day of the test beverage (340mL/bottle) containing 169.9mg of tea catechins with a galloyl moiety, and each subject in the placebo group was given two bottles/day of a placebo beverage at breakfast and dinner time for 12 weeks. Measurement of abdominal fat area by X-ray CT examination at 12 weeks after beverage consumption indicated a significant reduction in VFA in the catechin group compared to the placebo group. These results suggested that the consumption of tea catechins with a galloyl moiety may also be useful for prevention of obesity-related diseases in women.

Suppressive effects of tea catechins with a galloyl moiety on postprandial blood TG level in intervention studies

Postprandial plasma TG level significantly reduced in human subjects with mild or borderline hypertriacylglycerolemia on consumption of a test beverage containing 674mg of tea catechins (rich in tea catechins with a galloyl moiety) and a piece of bread spread with 20g of butter [19]. Furthermore, postprandial plasma TG levels in male subjects with mild or borderline hypertriacylglycerolemia were significantly reduced by consumption of 215.3mg tea catechins (rich in catechins with a galloyl moiety) and a piece of bread spread with 20g of butter [20]. In these reports, it was shown that tea catechins, especially tea catechins with a galloyl moiety, reduced the elevation of postprandial blood TG level.

Suppressive effects of tea catechins with a galloyl moiety on hypertriacylglycerolemia

The effect of tea catechins on hypertriacylglycerolemia has been studied in rats [12]. Wistar rats were orally dosed with tea catechins rich in EGCG and ECG, or heat-treated tea catechins rich in GCG and CG (100mg/kg body weight), as well as a lipid emulsion containing 200g/L soybean oil, 12g/L egg lecithin, and 22.5g/L glycerin, at a dose of 10mL/kg. Serum TG was measured sequentially, showing that tea catechins with a galloyl moiety suppressed postprandial hypertriacylglycerolemia by delaying lymphatic transport of dietary fat. In addition, these tea catechins inhibited the activity of pancreatic lipase *in vitro* in a dose-dependent manner. The authors also showed that when purified catechins were used, GCG and CG inhibited the activity of pancreatic lipase more than EGCG and ECG.

A lipid emulsion administered orally to rats with EGCG at a dose of 100mg/kg resulted in the increase in plasma TG level being significantly inhibited after one hour and two hours compared to without EGCG [21]. This study also showed that 200mg/kg tea catechin, rich in EGCG and ECG, administered alone (without lipid emulsion) had no effect on plasma TG level. These findings strongly suggested that tea catechins with a galloyl moiety suppressed absorption of dietary fat through the small intestine.

Action mechanisms of tea catechins in reducing body fat

The fat ingested in a meal mainly exists as TG, containing three molecules of fatty acid bound to glycerol. TG is emulsified by bile salts in the duodenum, and is digested successively to free fatty acids and monoglyceride by pancreatic lipase prior to absorption across the epithelium of the small intestine. These are then

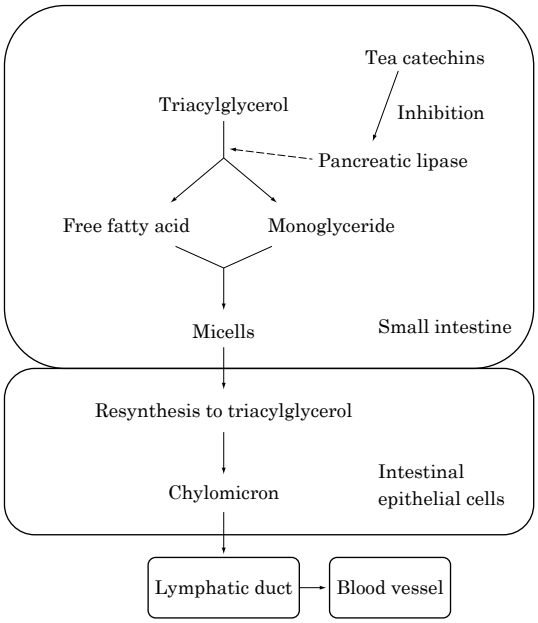

FIGURE 2: Schematic representation of pancreatic lipase activity inhibition by tea catechins in the small intestine

resynthesized to form TG in the epithelial cells of the small intestine and released into the lymph, which then enters the blood stream (Figure 2). Therefore, postprandial blood TG levels increase temporarily after a meal, resulting in chylomicronemia. This TG is mainly present in the chylomicrons; however, it can be hydrolyzed to free fatty acids and glycerol by lipoprotein lipase on the vascular endothelial surface in muscle and/or adipose tissue. These free fatty acids are used as an energy source in muscle cells, or are stored as TG in fat cells.

It is possible that when postprandial blood TG level is continuously elevated, the actions of insulin promotes the accumulation of subcutaneous fat and visceral fat [22, 23]. Insulin increases glucose uptake by promoting expression of glucose transporter 4 (GLUT4) in skeletal muscles and adipose tissue. In addition, it is known that insulin regulates the blood glucose level by promoting glycogenesis and inhibiting gluconeogenesis in the liver, and other actions. Since insulin promotes activity of lipoprotein lipase in the adipose tissue [24], the hydrolysis of TG of very-low-density lipoprotein (VLDL) and chylomicrons in plasma to free fatty acids is promoted, and these are taken up into adipocytes, and are resynthesized to TG and accumulated in adipose tissue. In addition, since insulin reduces the activity of hormone-sensitive lipase in adipocyte, it inhibits hydrolysis of TG in adipocytes [25]. In this way, TG is taken into adipocytes by the action of lipoprotein lipase, glucose is converted to fat in the liver, and fat accumulation increases in adipose tissue.

The first approach to preventing obesity is dietary control and exercise. However, when an excessively fatty diet is ingested, methods can be employed to reduce fat digestion and absorption in the small intestine. In Europe and America, it has been reported that orlistat, a gastrointestinal lipase inhibitor that markedly reduces fat

absorption, is well tolerated and offers a promising new approach for the long-term management of obesity [26].

It has been suggested that tea catechins, particularly those with a galloyl moiety, inhibited pancreatic lipase activity and delayed fat absorption from the intestinal tract, thereby suppressing the elevation of postprandial serum TG level (Figure 2) [12, 13]. As a similar example, manno-oligosaccharides from coffee mannan were shown to reduce the elevation of postprandial serum TG level by inhibiting lipid absorption [27]. In addition, compared to a placebo, manno-oligosaccharides were shown to reduce human abdominal visceral and subcutaneous fat [28]. This study also suggested that manno-oligosaccharide-induced attenuation of the increase in postprandial blood TG level, via inhibition of fat absorption from the small intestine, was one of the mechanisms involved in the reduction in body fat [28]. On the basis of these results, it was suggested that one of the mechanisms by which tea catechins reduced body fat was the suppression of the increase of postprandial TG by delaying the absorption of fat from the small intestine.

Ikeda *et al.* reported that compared to the control animals, rats treated with tea catechins rich in EGCG and ECG, or heat-treated tea catechins rich in EGCG, ECG, GCG and CG showed significantly lower visceral fat deposition and concentration of hepatic TG [13]. The authors of this study also reported that the activities of fatty acid synthase and the malic enzyme in the liver cytosol were significantly lower in both groups of rats treated with catechins than in control rats. However, fatty acid β-oxidation enzyme activities were not significantly different among the three groups. It is reported that fatty acid synthase and acetyl-CoA carboxylase-1 mRNA levels were markedly decreased in adipose tissue of mice who received EGCG supplements [9]. In addition, dietary supplementation of EGCG resulted in a dose-dependent attenuation of body fat accumulation. Leptin and stearoyl-CoA desaturase-1 (SCD-1) gene expression in white fat was reduced, and gene expression of SCD-1, malic enzyme, and glucokinase in liver was reduced [29]. These findings suggest that reduction of fatty acid synthesis by EGCG may reduce accumulation of TG in liver and adipose tissue.

Furthermore, it is possible that insulin promotes subcutaneous and visceral fat accumulation, when postprandial blood TG is at a high level [22, 23]. In an intervention study, consumption of dietary tea catechins with a galloyl moiety resulted in attenuation of the postprandial increase in blood TG levels [19, 20]. This is thought to be one of the mechanisms by which tea catechins reduces body fat. Long-term EGCG treatment has been reported to reduce the development of obesity and fatty liver in mice fed with a high-fat diet [30]. In another study, short-term EGCG treatment in mice with obesity induced by a high-fat diet resulted in decreased mesenteric fat and blood glucose levels, compared to control mice fed with a high-fat diet [30]. EGCG treatment was also reported to attenuate insulin resistance in mice fed with a high-fat diet, and these effects may be mediated by

decreased lipid absorption and other mechanisms [30]. Furthermore, it was reported that administration of green tea reduced adipose tissue weight, plasma cholesterol levels, and free fatty acids in rats [31]. Green tea also reduced glucose uptake, accompanied by a decrease in translocation of GLUT4 in adipose tissue, while it stimulated glucose uptake with GLUT4 translocation in skeletal muscles. Both of these effects would reduce obesity [31].

In mice fed with a high-fat diet, administration of tea catechins was reported to increase β-oxidation of fatty acids activity in liver mitochondria [11]. In addition, green tea extract was reported to improve running endurance in mice by stimulating lipid utilization during exercise, and β-oxidation activity was increased [32]. Green tea extract was reported to prevent increased body fat accretion in rats fed on a high-fat diet. This body fat suppression resulted in part from reduction in fat digestion, and to a greater extent from an increase in brown adipose tissue thermogenesis through β-adrenoceptor activation [33]. Furthermore, chemokines, such as monocyte chemoattractant protein (MCP)-1, derived from mesenteric adipose tissue, plays a crucial role in activation of macrophage migration into adipose tissue in obese mice [34]. EGCG treatment attenuated the elevation of MCP-1 expression in mice fed on a high-fat diet, and it was suggested that these effects may be mediated by decreased lipid absorption, decreased inflammation, and other mechanisms [30].

As mentioned above, since tea catechins appear to function via multiple mechanisms of action to reduce body fat deposition, more studies are required to elucidate these mechanisms in more detail.

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Protective Effects of Green Tea Catechin on Cardio- and Cerebral Vascular Diseases

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Abstract: In order to confirm the epidemiologic data, anti-atherogenic and cerebral vascular protective effects of green tea catechin were experimentally examined. Lag time of Cu^{2+}-mediated oxidation of LDL drawn from young volunteers before and after 1-week ingestion of the green tea catechin extract (Polyphenon®) 300mg twice daily, was significantly prolonged by 15 min compared with that of before experiment. Apoprotein E (apo-E)-deficient mice susceptible to atherosclerosis, were given a cholesterol diet and drinking water with and without supplement of Polyphenon® 0.8mg/mL for 14 weeks. Atheromatous area in the aorta and aortic weights were both significantly attenuated by 23% in the catechin group compared with control group. Aortic cholesterol and triglyceride content were 27% and 50% lower, respectively, in the catechin group. Male malignant SHRSP rats at five weeks of age were maintained on a regular chow and water with and without supplement of 0.5% Polyphenon®. The catechin ingestion significantly delayed stroke onset by 10 days compared with control. Plasma EGCG concentration greatly decreased at post-stroke compared with that of pre-stroke. Ameliorative effects of catechin were evaluated in middle cerebral artery occluded male Wistar rats. They were given drinking water with and without supplement of 0.25 and 0.5% Polyphenon® for five days prior to operation and during the experiment. Right middle cerebral artery was occluded for 2 hours, then reperfused for 22 hours. Catechin ingestion dose-dependently reduced the brain infarct area and volume. Infarct volume was inversely correlated with plasma EGCG concentration. Dark staining of iNOS, neutrophils and peroxynitrite observed in vessel wall of small arteries in control rats, was not observed for the catechin group. Catechin ingestion blocked 3-fold increase of serum NOX concentration in the jugular vein, and reduced by 35% a 2-fold increase of plasma lipid peroxide level seen in control rats after reperfusion. Neurological

deficit was alleviated by 0.5% catechin ingestion. These results strongly support the epidemiological data that daily intake of green tea exerts cardio- and cerebral vascular protective effects. These health effects are mainly based on the potent radical scavenging properties and inhibition of nuclear factor activation that are regulated by the intracellular redox-state.

Keywords: apo-E deficient mouse, atherosclerosis, humans, LDL oxidation, M-SHRSP, MCAO-rats, stroke

Introduction

Several investigations, including Huxley *et al.* [1] reported that flavonoid intake is inversely associated with coronary heart disease mortality. Wang *et al.* [2] found that one cup of green tea/day decreased the incidence of coronary artery disease by 10%. Epidemiologic studies on cerebral vascular disease showed that daily tea drinking [3-5] and flavonoid intake [6] reduced the risk of stroke.

The leaves of tea contain anti-oxidative polyphenols consisting of various flavan 3-ols. Among them, (-)-epigallocatechin gallate (EGCG) is a principal component. We showed that these polyphenols have a variety of pharmacological effects: anti-oxidative [7], anti-mutagenic [8], anti-cancer promoting [9] and potent inhibitory effects on Cu^{2+}-mediated oxidative modification of low-density lipoproteins (LDL) [10].

Various unknown factors might be involved in those epidemiologic data, which sometimes lead to wrong conclusions. Based on several *in vitro* data of green tea catechin so far found in our laboratory, therefore, we aimed to confirm experimentally the results from epidemiologic studies of green tea on cardio- and cerebral vascular diseases. The protective effects of green tea catechins on the development of atherosclerosis were examined in apo-E deficient mice susceptible to atherosclerosis while those on stroke onset were examined using malignant stroke-prone spontaneously hypertensive rats (M-SHRSP), and the ameliorative effects using middle cerebral artery occlusion (MCAO)-rats.

Anti-atherogenic effects in humans and apo-E deficient mice

In subendothelial space, LDL are converted to oxidized forms through contact with macrophages, endothelial and smooth muscle cells. Oxidatively modified LDL have chemotactic properties and recruit blood monocytes developing into tissue macrophages. In addition, modified LDL is taken up into macrophages through scavenger receptors to convert to lipid-laden foam cells. Thus, the prevention of LDL oxidation is assumed to be one of the initial and critical measures of anti-atherogenesis.

TABLE 1: Effect of 1-week ingestion of green tea catechins on Cu^{2+}-mediated LDL oxidation in young volunteers

Group	Control	Catechin	Significance vs Control
Lag time (min)			
Before*	68.1±2.9	64.9±2.4	
After*	68.3±2.0	79.6±5.9	
Individual difference	0.2±2.8	13.7±6.1[a]	$p<0.05$[b]

Data represent mean ± SE (n=11)
*Before and after the experiment period
[a]$p<0.05$ by paired Student's t-test for before vs after within group
[b]$p<0.05$ by paired Student's t-test for before vs after between groups

Ex vivo anti-oxidative effects on LDL in humans [11]

Twenty-two male volunteers at an average of 25 years were recruited to participate in the experiment. They had meals under the same dietary regimen during two weeks. After 1-week washout period, they were divided equally into two groups. Catechin group had 300mg of Polyphenon®: ((-)-EGCG 58.4%, (-)-ECG 11.7%, (-)-EC 6.6%, (+)-GCG 1.6%, (-)-ECG 0.5%, caffeine 0.4%) twice daily, before breakfast and dinner for one week. Blood was withdrawn before breakfast prior to and at the end of experiment. Plasma of catechin group contained 56.0nM of total concentration of EGCG on an average after the experiment in the catechin group while EGCG was neither detected in plasma before nor after the experiment in the control group. Plasma levels of ascorbate and α-tocopherol were similar for both groups, whereas β-carotene level was significantly higher in the catechin group. LDL was prepared from plasma of each person, and after dialysis, subjected to Cu^{2+}-mediated oxidation at 37℃. The oxidation curve of LDL from the catechin group obtained at the end of experiment was shifted toward the right compared with the respective curve 1 week before of catechin. The lag time was significantly prolonged by 15 min (before: 64.6 ± 2.4 min vs after: 79.6 ± 5.9 min: mean ± SE (11 samples) $p<0.02$ by paired t-test). In contrast, the lag time for the control group was unchanged before and after experiment. Propagation rate was similar for both groups. (Table 1)

Ingestion of 300mg Polyphenon® twice daily is equivalent to drinking 7 to 8 Japanese size of tea cups of green tea per day, also equivalent to drinking 2 to 3 cups of American size of coffee mug. These results imply that daily drinking of ordinary amounts of green tea may exert preventive effects on *in vivo* LDL oxidation.

In vivo anti-atherogenic effects in apo-E deficient mice [12]

It was examined whether a long-term ingestion of green tea catechin exerts anti-atherogenic effects in apo-E deficient mice susceptible to atherosclerosis [13]. Lack of the functions of apo-E leads to elevation of atherogenic lipoprotein levels in the circulation and progression of atherosclerosis in the deficient mice.

In this experiment, 10-week old male apo-E deficient mice C57BL/6J(-) were fed for 14 weeks an atherogenic diet containing 1.25% cholesterol, 0.5% sodium cholate, 12.5% cocoa butter in a regular chow. The catechin group had drinking water supplemented with Polyphenon® 0.8mg/mL during the experimental period, while the control group received drinking water without the supplement. Blood samples were withdrawn from retro-orbital puncture every four weeks and from the abdominal aorta at the end of experiment.

There was no difference in serum cholesterol level between the two groups through the whole period. TBARS (thiobarbituric acid reactive substances) level in plasma and in Cu^{2+}-oxidized β-VLDL were significantly lower in the catechin group than in control. This indicates that plasma lipids from the catechin group are less susceptible to *in vivo* and *in vitro* oxidation. The aorta from the arch to the femoral bifurcation was dissected at the end of experiment. The weight of the cleaned aorta from the catechin group was reduced by 33% compared with that from control, and atherosclerotic area analyzed by an NIH image was significantly 27% less in the catechin group compared with control. Cholesterol and triglyceride contents in the aorta were both 25% lower for the catechin group compared with those in the control group. These results clearly indicate that long-term tea consumption prevents the development of atherosclerosis in apo-E deficient mice (Figure 1).

Hayek *et al.* [14] fed red wine or its polyphenols quercetin or catechin, to apo-E deficient mice maintained on a regular diet, and observed the reduced susceptibility to oxidation of LDL and attenuation in the development of the lesion in the aortic arch in mice fed red wine or quercetin, and to a lesser extent in mice fed catechin. Our experiment was carried out in a more severe condition than that by Hayek.

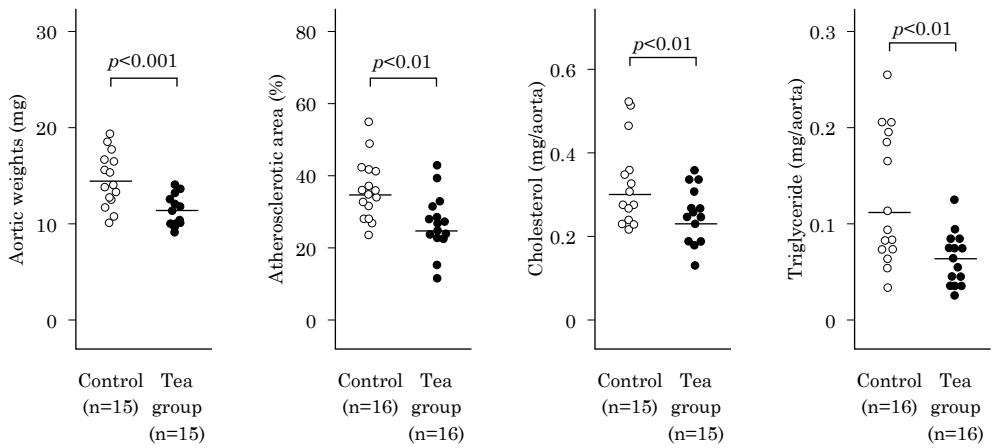

FIGURE 1: Reduction by catechin ingestion of aortic weights, atherosclerotic lesion and aortic lipid contents in apo-E deficient mice fed an atherogenic diet for 14 weeks with water supplemented with/without 0.8mg Polyphenon/mL

Nevertheless, green tea catechins prevented *in vivo* lipoprotein oxidation and development of atherosclerosis.

Prevention of onset of spontaneous stroke in M-SHRSP, and protection of cerebral ischemic damage in rats

In stroke, the region of ischemic penumbra is metabolically compromised, but still potentially viable for a few hours after the onset. Therefore, early reperfusion by thrombolytic therapy is assumed for the most effective measure to reduce infarct size and improve neurological function. However, reperfusion can exacerbate the brain damage in the penumbra by generation of oxygen-free radicals from activated neutrophils and by excessive production of nitric oxide (NO) which immediately forms highly toxic peroxynitrite by reaction with superoxide. Thus, the augmentation of superoxide, NO and peroxynitrite production during ischemia appears to be involved in cerebral ischemic damage. The cerebral protective effects of green tea catechin were evaluated in M-SHRSP that develop stroke spontaneously at their early ages, and in rats submitted to MCAO and reperfusion.

Preventive Effects of Onset of Spontaneous Stroke in M-SHRSP [15]

M-SHRSP develop severe hypertension and spontaneous stroke at early ages [16]. They were given water supplemented with and without 0.5% Polyphenon® beginning at five weeks of age, and blood pressure, heart rate, and locomotive activity were continuously monitored from eight weeks of age using a telemetry system. Stroke onset was assessed by the appearance of neurological symptoms, body weight loss, and circadian rhythm disturbances in heart rate, blood pressure, and locomotive activity.

Catechin ingestion significantly delayed stroke onset by 10 days compared with the control group (Table 2). Blood pressure was similar at 10 weeks of age in both groups, but the rate of the increase was smaller in the tea group than in the control group. Plasma NO^{2-} and NO^{3-} concentrations rapidly increased after the onset of stroke in both groups without significant difference between both groups. In catechin group, plasma EGCG concentration significantly decreased at post-stroke compared with that of pre-stroke.

TABLE 2: The effect of green tea catechins on the median latency to stroke onset
Male M-SHRSP at 5 weeks of age were given drinking water supplemented with or without 0.5% green tea catechins.

Group	n	Stroke day (mean ± SD)
Control	9	79.7±2.9
Catechin	8	90.1±5.2*

*$p<0.01$ vs control by Student's t-test

Ameliorative Effects on Stroke in Transient MCAO Rats [17]

Male Wistar rats at eight weeks of age were divided into three groups: control, low and high-dose catechin groups; they took drinking water supplemented with and without 0.25 or 0.5% Polyphenon®, from five days prior to the operation and throughout the experimental period. The operation of middle cerebral artery occlusion was performed on day 0 by inserting an occluder filament through the right internal carotid artery as far as the bifurcation of the middle cerebral artery [18]. The artery was occluded for 2 hours and reperfused for 22 hours before they were killed. The brain was sliced in 2mm thick and stained with triphenyl tetrazolium chloride.

Intake of water with and without the supplement for five days was similar in the three groups. Body weights were not significantly changed during experiment. Cerebral infarction was examined in 2mm-thick slices of the cerebrum. Figure 2A shows typical photographs of control and 0.5% catechin group. Infarct areas (colorless) are apparently smaller in the catechin group than in control. Figure 2B shows the infarct area of the three groups at various distances from the bregma. The largest area at -0.3mm was approximately 35% of the entire area in the control group while it was 29% in the low-dose group and 21% in the higher dose group. Figure 3 shows an inverse correlation between infarct volume and total amount of catechin ingested for five days/rat before operation (Figure 3A), or EGCG concentration in

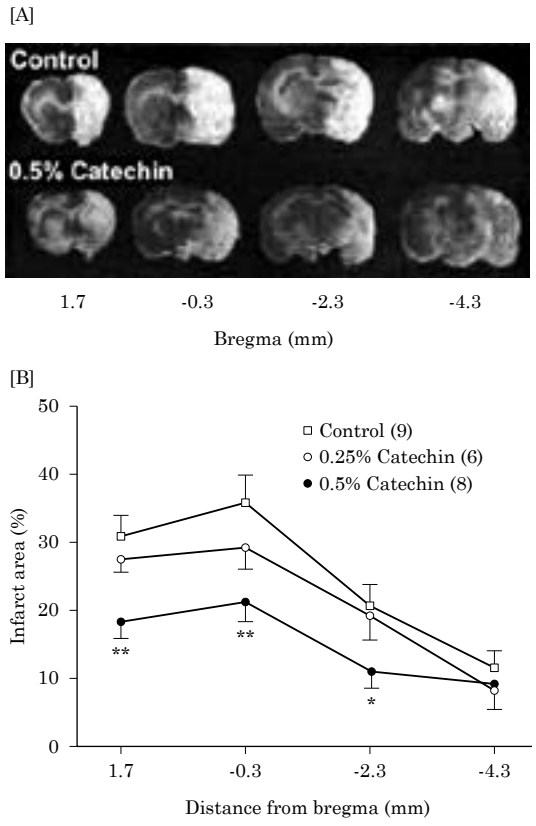

FIGURE 2: Typical photographs of cerebral infarction (A), and reduction of infarct area by catechin ingestion (B) in MCAO-reperfused rats
Rats were given *ad libitum* water supplemented with/without, catechin 5 days before MCAO and throughout the experiments.
Each point and vertical bar indicate mean ± SE for the number of rats shown in parentheses.
Significance: **p*<0.05, ***p*< 0.01 (Dunnett's test) as compared to control group

plasma taken at 30 min after the operation (Figure 3B). Cerebral infarct volume decreased linearly with plasma EGCG concentration. Infarct volume was 40% lower on average in rats with plasma concentration 0.8 to 1.5 micromol /L compared with control rats. This high plasma concentration was attained by high doses of catechin ingestion. The expression of iNOS, infiltration of neutrophils (MPO as its marker), and the formation of peroxynitrite (nitrotyrosine as its marker) were examined by immunohistochemical staining in the coronal section at -0.3mm distance from the bregma. The dark brown stains of iNOS, MPO and nitrotyrosine were observed in control rats, whereas in the brain from the high-dose catechin group, a small amount of dark brown iNOS stain was found in vessel walls of small arteries, but the stain of MPO and nitrotyrosine was not observed.

Before rats were killed at the end of experiment, their neurological deficit was assessed by a posture reflex score according to Longa [19]. Control rats showed severe neuronal damage with a mean score of 3.2 ± 0.2. Catechin at a high dose significantly decreased the neurological deficit (mean score rats 2.1 ± 0.3, $p<0.05$) compared with that of control rats. The effect of the low-dose was not statistically apparent.

Preventive effects of tea catechins on cerebral ischemic damage in MCAO-reperfused rats are assumed to be mediated by the inhibition of iNOS expression, infiltration of neutrophils and formation of peroxynitrite. This is mainly through the potent scavenging property of oxygen radicals produced in the affected area and inhibition of activation of nuclear factors that are regulated by the intracellular redox-state.

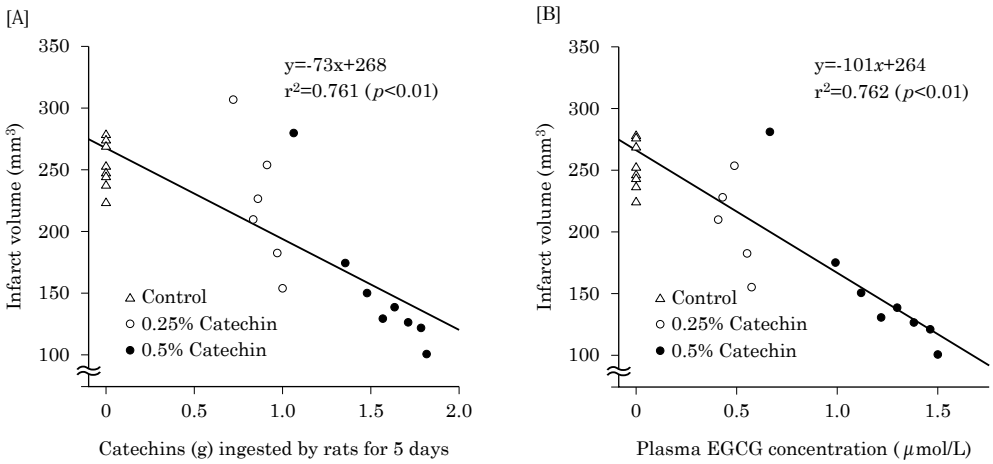

FIGURE 3: Inverse correlation between cerebral infarct volume and catechin ingestion (A), or plasma EGCG concentration (B)

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Preventive Effects on Human Obesity

Ichiro TOKIMITSU

Abstract: Obesity is a risk factor for several chronic diseases, including heart disease, liver disease, diabetes, cancer, and arthritis. Tea catechins are polyphenolic compounds present in the unfermented dried leaves from the plant, *Camellia sinensis*. Results from some randomized, controlled intervention trials have shown that the consumption of tea catechins (>540mg/day) may cause a reduction in body weight and body fat. Moreover, several human and animal studies have indicated that the long-term ingestion of tea catechins enhanced energy expenditure and lipid oxidation in the liver and muscle. These findings suggest that tea catechins may reduce body fat, at least in part, by enhancing the lipid consumption preferentially as an energy source.

Keywords: energy expenditure, intervention, lipid oxidation, obesity, tea catechins

Introduction

Obesity, especially visceral fat type obesity, is closely associated with lifestyle-related diseases such as hyperlipidemia, hypertension, and hyperglycemia. These symptoms are defined as metabolic syndrome, and this syndrome is considered to increase the risk some chronic diseases, including heart disease, liver disease, cancer, arthritis, and others. Some epidemiological studies have examined the impact of tea on body weight and other markers related to obesity [1, 2].

A longitudinal analysis within The Netherlands Cohort study of 4,280 adults over the 14-year study period found an inverse relationship between catechin consumption and an increase in body mass index (BMI) in the general female population [3]. The BMI increases for the lowest and highest quintiles of catechin consumption were 0.77 and 0.31kg/m^2, respectively. Recently, there have been several trials in humans, showing favorable effects of tea catechins on obesity [4-6],

but not all the studies have found positive results for obesity-related measures [7, 8]. As the doses of tea catechins used in these negative studies were somewhat lower than those used in the studies that reported positive effects, the observed differences may be dose related. A meta-analysis of 11 human trials reported an average body weight loss of 1.31kg for subjects in the catechin-therapy group relative to controls, with most intervention periods being approximately 12 weeks [9].

There are excellent reviews reporting obesity-related health effects of tea catechins [10, 11]. In this chapter, results of recent studies are described with emphasis on those of the present authors in the hope of stimulating additional studies on the preventive effects of green tea for human obesity.

Anti-obesity effects of tea catechins

The efficacies of tea catechins on obesity have been investigated in the C57BL/6J mice. This mouse model is used to study dietary-induced obesity. In this model, obesity was absent when mice were fed a standard diet containing 5% fat. A high-fat and high-sucrose diet containing 30% fat and 13% sucrose, however, led to a significant increase in body weight and body fat (visceral fat tissue) weight compared with a standard diet. The addition of tea catechins to a high-fat and high-sucrose diet (0.1%, 0.2% and 0.5% wt/wt) over a period of 11 months, significantly inhibited body weight and body fat weight gains in a dose-dependent manner compared with a high-fat and high-sucrose diet alone [12].

Several studies have reported the anti-obesity effects of tea catechins in humans. The following findings have been obtained from over 1,000 subjects [13-16].

(1) The effective dose is >540mg/day (equivalent to 2-3 cups of tea per day, each made with 250mL water and 1 teabag (2-2.5 grams of tea)).

(2) Tea catechins are effective in obese people (with a high BMI).

(3) Tea catechins have similar effects in both sexes.

(4) Rebound phenomena are not observed after discontinuation.

(5) Physical and hematologic findings are normal.

In one study, 240 adults with a mean age of 41.7±9.9 years and a mean BMI of 26.8±2.0kg/m^2, were divided into two groups in a randomized double-blind, controlled parallel multicenter trial. The control group was 49 women and 68 men; consuming tea catechins 96.3mg/day, while the catechin group was 51 women and 72 men; consumed tea catechins 582.8mg/day [14]. Test beverages (340mL) containing tea catechins were given for 12 weeks. After 12 weeks, the BMI values, abdominal fat areas (visceral and subcutaneous fat areas) on abdominal computer tomographic images, and total fat area (calculated by adding the visceral fat area (VFA) to the subcutaneous fat area) were significantly lower in the catechin group compared with those in the control group. Changes in abdominal fat areas are shown in Figure 1. In sub-class analysis with respect to sex, the effects were similar between men and

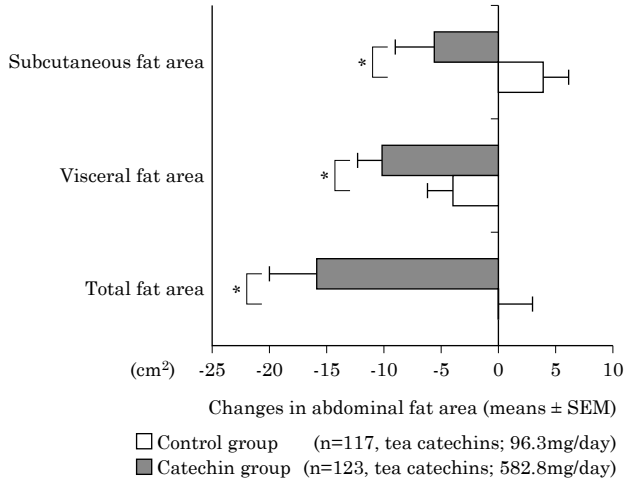

FIGURE 1: Effect of tea catechin ingestion for 12 weeks on abdominal fat in humans
Significantly different from control group by *t*-test (*p<0.05)

women. Continuous ingestion of the tea catechin beverage significantly reduced the body weight and body fat content. Surprisingly, the systolic blood pressure (initial value: 130 or higher) and serum LDL cholesterol levels, were reduced, suggesting that tea catechins contribute to reducing the risks of obesity and cardiovascular diseases [14].

Several studies of tea catechins in clinical use have been reported. Patients with type 2 diabetes who were not receiving insulin therapy ingested a catechin beverage (n=23, 582.8mg of tea catechins/day) or a control beverage (n=20, 96.3mg of tea catechins/day) for 12 weeks [17]. The reduction in waist circumference was significantly greater in the catechin group than in the control group. Adiponectin, which is negatively correlated with visceral adiposity, increased significantly only in the catechin group. In patients treated with insulinotropic agents, the increase in the insulin concentration and reduction of the HbA1c concentration were significantly greater in the catechin group than in controls. These findings suggest that tea catechins might be therapeutically beneficial in patients with type 2 diabetes who do not yet require insulin therapy.

Anti-obesity mechanisms of tea catechins

The mechanism by which tea catechins cause a reduction in body weight and body fat remains an active area of investigation. Much of the work in humans and animals have focused on the effects of tea catechins on thermogenesis and lipid oxidation. Other potential mechanisms include modifications in appetite, decreased nutrient absorption, and alteration of gut microflora composition.

The results of one human study indicated that the continuous ingestion of tea catechins enhanced energy expenditure and dietary fat oxidation. In this experiment, 14 healthy men aged 26 to 42 years were divided into a high-concentration tea

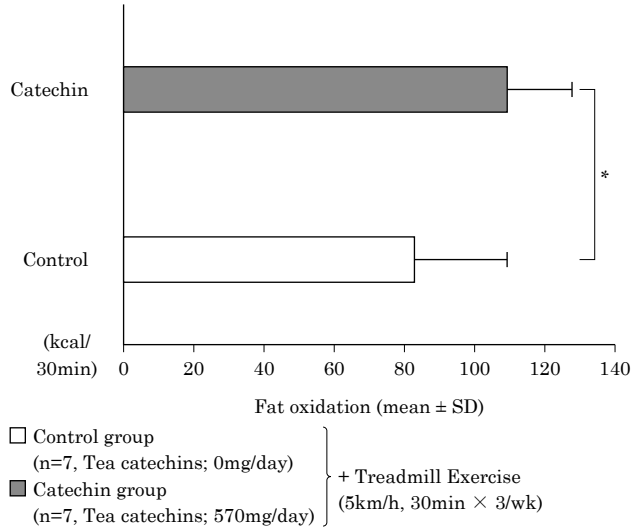

FIGURE 2: Effect of tea catechin ingestion for 8 weeks on fat oxidation during treadmill exercise
Significantly different from control group by *t*-test (*p<0.05)

catechin beverage group (n=7) and a control group consuming a beverage containing no tea catechin (n=7) [18]. Both groups drank 500mL of a beverage containing 570mg or 0mg of tea catechins daily for eight weeks, and performed periodic 30-minute treadmill exercise at 5km/hour, three times a week during this period. After eight weeks, breath analysis was performed, and energy expenditure was measured in the resting state and during the treadmill exercise. The amount of fat combustion during exercise was significantly increased when continuous tea catechin ingestion was combined with periodic exercise, compared to that without tea catechin ingestion (Figure 2).

In a randomized controlled study, 12 subjects consumed a beverage containing 77.7mg of tea catechins/day (control group) or 592.9mg of tea catechins/day (catechin group) for 12 weeks [19]. Diet-induced thermogenesis (DIT) was measured based on oxygen consumption during the eight hours following ingestion of a standard diet (800kcal) before and after continuous ingestion of the test beverages. Dietary lipid oxidation based on the excretion of $^{13}CO_2$ after the injection of dietary ^{13}C-labeled lipids during the DIT measurement was also examined. The control group showed no increase in DIT after 12 weeks of ingestion, compared with the baseline levels. The catechin group, after 12 weeks of ingestion, showed a significantly (p<0.05) higher DIT than the control group. The mean rate of increase in DIT over 12 weeks in the catechin group was 38.9kcal. Furthermore, the increase was more marked in subjects with a higher initial VFA value. Fat oxidation was also significantly enhanced only in the catechin group and these parameters were significantly higher than those in the control group after 12 weeks of ingestion. These findings suggest that continuous ingestion of tea catechins increases DIT and postprandial fat oxidation, and that these effects may, at least in part, contribute to body fat reduction during continuous ingestion of tea catechins.

Several animal studies suggested that tea catechins stimulate lipid catabolism in the liver and muscles [13, 20]. Tea catechins up-regulated the expression of several genes involved in hepatic beta-oxidation of fatty acids in mice following one month of high-fat feeding, including acyl-CoA oxidase and medium chain acyl-CoA dehydrogenase [13]. Moreover, tea catechins and regular exercise, when combined, stimulate whole-body fat utilization under sedentary and exercise conditions [20]. These findings suggest that tea catechins reduce body fat, at least in part, by preferentially enhancing the lipid utilization as an energy source.

Lifestyle improvements such as undertaking an exercise regimen and developing healthy eating habits are essential for the prevention of obesity and metabolic syndrome, but the practice is accompanied by difficulty. Daily ingestion of green tea can be undertaken with ease and leads 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 heart disease, liver disease, diabetes, cancer, arthritis, and others.

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Epidemiological Studies on the Effects of Green Tea - An Intervention Study on Lipid Metabolism

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Abstract: Cardiovascular disease is one of the primary causes of mortality, globally. Traditional Japanese diets have been gaining attention for the prevention of cardiovascular disease, particularly the benefits of green tea. A meta-analysis published in 2011 reported that consumption of green tea beverages or green tea extract had a statistically significant effect on lowering serum triglyceride levels by 7.20mg/dL (95% CI: -8.19, -6.21) and serum low-density lipoprotein cholesterol levels by 2.19mg/dL (95% CI: -3.16, -1.21). On the other hand, no statistically significant change was observed in serum high-density lipoprotein cholesterol levels. This result was also confirmed in the subgroup analysis, as well as sensitivity analysis stratified by beverage/extract, dose, intervention period, health status of participants, or study quality. The same results were obtained in an intervention study, which has been conducted in Kakegawa city, Shizuoka Prefecture.

Keywords: epidemiological study, green tea, lipid metabolism

Introduction

Cardiovascular disease is one of the primary causes of mortality, globally. The age-adjusted mortality rate for cancer in 2010 was 121.4 per 100,000 people, and 194.1 per 100,000 people for cardiovascular diseases, including ischemic heart disease and cerebrovascular disease [1]. Thus, improving lipid metabolism, which is one of the causes of cardiovascular disease, has a major effect on promoting human health.

Traditional Japanese meals have been attracting attention for the preventive effects on cardiovascular disease. In particular, polyphenols found in green tea, mainly (-)-epigallocatechin-3-gallate, have been reported to have a protective effect for cardiovascular diseases in various studies/reports on cells or animals. However, only a few studies in humans are available, and no consistent conclusions have been obtained. In a large-scale prospective cohort study, all-cause and cardiovascular disease mortality risks were found to be lowered in people who were consuming high amounts of green tea, and the results were domestically and internationally spotlighted [2].

The preventive benefit for cardiovascular disease found in the observational studies need to be investigated in intervention studies.

This chapter introduces some of the results obtained from epidemiological studies on green tea, which have been conducted in Kakegawa city in Shizuoka Prefecture in conjunction with the current trend of intervention studies examining the association between green tea and lipid metabolism in humans.

Summary of intervention studies

The meta-analysis examining the association between the consumption of flavonoids and cardiovascular disease risk factors reported in a 2008 issue of the American Journal of Clinical Nutrition (AJCN) included four studies. In this meta-analysis, the consumption of green tea was associated with a significant decrease in serum low-density lipoprotein cholesterol (LDL-C) levels, while no association was found for serum high-density lipoprotein cholesterol (HDL-C) levels [3].

The results of a number of intervention studies, particularly randomized controlled studies, were reported following the meta-analysis; in 2011, two meta-analyses were reported consecutively [4, 5]. One was published in American Dietetic Association [4], and the other was in AJCN [5]. Study quality of the latter article is critically evaluated using either the Jadad score [6] or other methods as follows.

A literature search identified 14 studies that were included in the analysis. After pooling the results of those studies, it was determined that the consumption of green tea beverages or green tea extract significantly lowered serum triglyceride levels by 7.20mg/dL (95% CI: -8.19, -6.21) and serum LDL-C levels by 2.19mg/dL (95% CI: -3.16, -1.21). On the other hand, no statistically significant changes were observed in the serum HDL-C level. These results were also confirmed in the subgroup analysis, as well as sensitivity analysis stratified by beverage/extract, dose, intervention period, health status of participants, or study quality.

Kakegawa study

Since 2009, an epidemiological study called “Kakegawa study” has been

conducted in Kakegawa city, Shizuoka Prefecture, Japan, in which the following four topics have comprehensively been examined:

(1) A cohort study on the association between green tea consumption and life-style-related diseases

(2) A green tea intervention study

(3) An analysis of the effect of green tea types on absorption

(4) An analysis of catechin receptor expression levels

The major findings of the green tea intervention study are summarized below.

Similar to the findings published in AJCN in 2011 [5], the results of Kakegawa study indicated that the consumption of green tea may be associated with lipid metabolism. The effects of green tea extract powder was investigated by using a randomized controlled trial design, with an intervention period of 12 weeks. The randomized block design of 2 by 4 was employed to assign 150 subjects to three groups. Group A was administered 1.8g/day of "Yabukita" green tea extract powder, group B was administered 1.8g/day of "Benifuuki" green tea extract powder, and group C was given a placebo similar in appearance and taste to green tea extract powder. The results of the study will appear in a scientific journal.

Conclusion

Consumption of green tea may contribute to lowering serum LDL-C levels among various lipid metabolism pathways. However, the study period of these previously reported intervention studies is as short as three months. Although longitudinal intervention studies are necessary to evaluate the effect of certain substances on human health, maintaining the study quality in an intervention study on daily beverages such as green tea may be challenging because of frequent non-compliance or other issues. Thus, it may be best to evaluate the effect of consuming green tea on human health by both longitudinal cohort studies and short-term intervention studies. So far, consumption of green tea suggests a preventive benefit for cardiovascular disease or improvement of lipid metabolism in both longitudinal cohort and short-term intervention studies.

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3

Effects of Green Tea on Influenza Infection and the Common Cold

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Abstract: Influenza and the common cold are acute infectious illnesses of the respiratory tract. Influenza is a severe, highly infectious disease caused by the influenza virus; when aggravated, it may become life-threatening. Both illnesses are highly infectious, making prevention very important.

In basic studies, tea catechins have been found to inhibit influenza viral adsorption and suppress replication. Catechins are also effective against some cold viruses. In addition to catechins, green tea contains theanine and vitamin C, which enhance immunity against viral infection, suggesting green tea may prevent influenza and/or the common cold.

Although the anti-viral activity of tea components has been demonstrated, there have been few clinical studies to support their utility. Epidemiological studies suggest regular consumption of green tea decreases rates of influenza infection and some cold symptoms. Gargling with green tea catechin extracts and consumption of catechins and theanine may protect against the development of influenza. Further studies are needed to confirm their clinical efficacy.

Keywords: common cold, gargling, infection, influenza, respiratory tract

Introduction

Influenza and the common cold are acute infectious illnesses of the respiratory tract, including the nose and throat. Most cases are viral. Influenza is a severe disease caused by the influenza virus; it is highly infectious and may progress to life-threatening diseases such as pneumonia and or encephalitis when aggravated. Because both influenza infections and colds spread easily via droplets and contact, public prevention measures are important. Prophylaxis includes hand washing, facial masks, gargling, and vaccination (for influenza). However, none of these methods is completely effective.

Traditional wisdom holds that tea prevents the common cold; thus, gargling with green tea has been recommended for influenza prophylaxis in elementary schools in some districts in Japan. Indeed, the concept of gargling and drinking green tea to prevent influenza and the common cold is becoming more common [1].

Experimental evidence for the effects of tea components on influenza and cold viruses

Tea components exhibit antiviral activities against pathogens of the respiratory tract. These viral pathogens include influenza and parainfluenza viruses, adenovirus, and respiratory syncytial virus (RSV) [1-8]. Tea catechins bind to the spikes on the surface of the influenza virus and inhibit viral adsorption onto the host cell surface, thus preventing infection. Green tea extract (GTE) inhibits growth of influenza virus by preventing its adsorption and hemagglutination. Nakayama *et al.* showed that epigallocatechin gallate (EGCG) and theaflavin digallate inhibit the *in vitro* infectivity of influenza A and B viruses in Madin-Darby canine kidney (MDCK) cells [3]. Black tea extract at beverage concentrations also inhibits the infectivity of influenza virus in mice [4]. Imanishi *et al.* found that tea extract inhibits the acidification of intracellular compartments such as endosomes and lysosomes, suppressing the growth of influenza A and B viruses in MDCK cells [6]. Catechins also suppress the replication of influenza virus [5-7].

Basic studies also show catechins are effective against cold-induced viruses [1, 2]. Weber *et al.* studied the effects of green tea catechins on adenovirus infection in cell culture [8]. They reported the anti-adenoviral activity of EGCG via extra- and intracellular mechanisms, with the suppression of virus assembly and maturation cleavage carried out by the viral protease adenain [8]. In addition to catechins, green tea contains theanine and vitamin C, which enhance immunity against viral invasion, supporting the efficacy of green tea in prevention of influenza and the common cold.

Clinical effects of green tea on influenza and/or the common cold

Though experimental studies have revealed the anti-viral activity of tea extracts, there is limited evidence for their clinical efficacy. Iwata *et al.* studied the prevention of influenza infection by gargling with black tea [9]. They found the hemagglutination inhibition (HI) titers of influenza viruses were significantly higher in the control group than in the black tea group. In our interventional study of residents living in a nursing home for the elderly, gargling green tea catechins three times a day for three months (at a concentration equivalent to about half that of a commercially available green tea beverage with 200μg/mL total catechins) decreased the incidence of influenza compared to gargling with water (Figure 1) [10]. Additional confirmatory randomized controlled trials are ongoing, but the results are inconclusive; although promising trends have been observed, the results have not reached statistical significance [11-13]. In an epidemiological study of the common cold, Noda *et al.* reported that green tea gargling reduced fever periods in children [14].

In our interventional study, the incidence of influenza was lower in adult volunteers who took green tea components (378mg total catechins and 210mg theanine) daily for five months [15]. In another epidemiological study of elementary school children in Kikugawa City, Shizuoka (one of the major tea production areas of Japan), the incidence of influenza was lower in children drinking 1-5 cups of green tea per day than in children drinking less than 1 cup per day (Figure 2) [16]. An interventional study also showed that three months intake of a green tea component reduced the number of people with cold onset and symptoms [17].

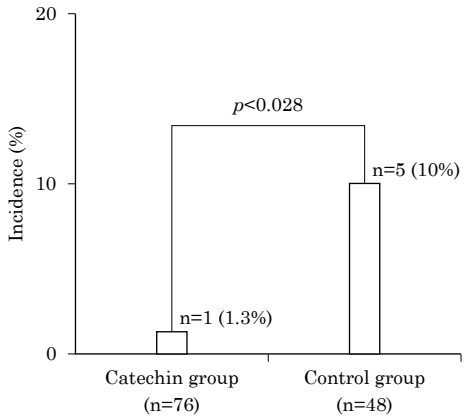

FIGURE 1: The incidence of influenza infection among the elderly nursing home residents gargling with tea catechins or water [10]

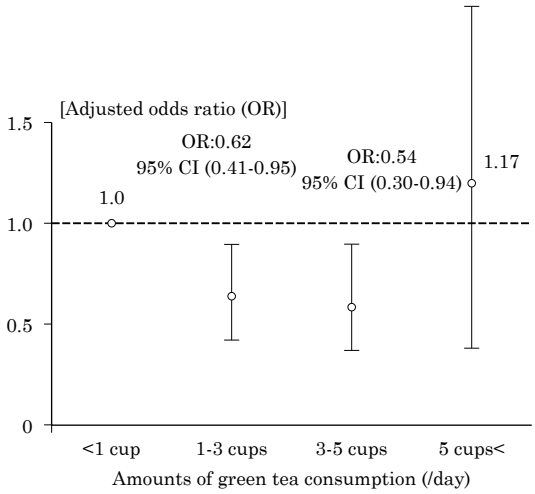

FIGURE 2: Relationship between the incidence of influenza infection and green tea consumption among elementary school children [16]
OR: odds ratio, CI: confidence interval

Conclusions

Although experimental studies have demonstrated the antiviral activity of tea components, there is little clinical evidence to support their efficacy in influenza and the common cold. Further studies are needed to confirm the clinical efficacy of tea.

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4

Immunomodulating Effect of Green Tea

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and Manami MONOBE

Abstract: The anti-allergic effect of epigallocatechin-3-*O*-(3-*O*-methyl) gallate (EGCG3"Me) and epigallocatechin-3-*O*-(4-*O*-methyl) gallate (EGCG4"Me) isolated from Japanese or Taiwanese tea (*Camellia sinensis* L.) leaves. These *O*-methylated catechins strongly inhibited mast cell activation and histamine release after FcεRI cross-linking through the suppression of tyrosine phosphorylation (Lyn) of cellular protein kinase, and the suppression of myosin light chain phosphorylation and high-affinity IgE receptor expression via the binding to the 67kDa laminin receptor. A double-blind clinical study on subjects with Japanese cedar pollinosis or perennial allergic rhinitis was carried out. On the eleventh week after starting intake, the most severe cedar pollen scattering period, symptoms i.e. blowing nose and itching of eyes were significantly relieved from 'Benifuuki' green tea containing 34mg/day of EGCG3"Me compared with placebo 'Yabukita' green tea that did not contain EGCG3"Me. One consecutive month intake of 'Benifuuki' green tea was useful for the reduction of some symptoms derived from Japanese cedar pollinosis, and did not affect any normal immune responses in subjects with Japanese cedar pollinosis. In addition, the green tea 'Benifuuki' was found to significantly relieve the symptoms of perennial rhinitis compared with a placebo 'Yabukita' green tea. From the investigation that the effects of cultivars, tea seasons of crops and manufacturing methods, green or semi-fermented teas, made from fully-matured 'Benifuuki' in second crop season, should be consumed.

The green tea components strictinins and theogallin showed anti-allergic action by inhibiting histamine release through suppressing the biosynthesis of immunoglobulin E. It was reported that epigallocatechin (EGC) and polysaccharide in tea leaves had the immunostimulating activities. Oral administration of the mixture with a high EGC ratio (1:2-3 = epigallocatechin gallate (EGCG)/EGC) resulted in greater IgA production by murine Peyer's patch cells. The EGCG/EGC ratio in the 4℃ green tea extract was around 1:3-4, whereas in the 100℃ extract, it was around 1:0.7. It was identified that EGC induced phagocytosis can be blocked by catalase and an inhibitor of transient receptor potential melastatin 2. Moreover, it was found that a crude tea polysaccharide fraction from immature tea leaves containing many RNAs, as compared with that from mature tea leaves, was related to increases in phagocytic activity using macrophage-like cells. The crude tea polysaccharide increased phagocytosis through toll-like receptor 7.

Keywords: anti-allergic action, clinical trials, epicatechin-3-*O*-(3-*O*-methyl) gallate (EGCG3"Me), epidemiological studies, epigallocatechin (EGC), immunostimulating effect

Introduction

Tea (*Camellia sinensis* L.) is consumed all over the world, and in large quantities in Japan and China, where it has been used for medicinal purposes for thousands of years. Tea has been found to exhibit various bioregulatory activities, such as being anti-oxidative, anti-hypertensive, anti-hypercholesterolemic and anti-bacterial, and has immune regulatory effects. Catechins, a group of polyphenolic compounds, have been shown to be largely responsible for these activities. Allergy has been defined as a disease of excessive immune activity, and in Japan, the morbidity of allergy is estimated to be about 30%. Many Japanese are reluctant to use of anti-allergic medicine as a result of side effects and mounting medical expenses, so there is a demand for the development of physiological-functional foods for allergy prevention. Immune regulatory effect, anti-allergic effect or immunostimulating effect, is one of these functional properties in which tea polyphenols apparently play a significant role.

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].

Action mechanism of *O*-methylated EGCG

O-Methylated catechins show the allergy-relieving effects by inhibiting the release of histamine from mast cells and basophils [5, 6]. EGCG3"Me strongly inhibited mast cell activation through the prevention of tyrosine phosphorylation (Lyn, Syk, and Btk) of cellular protein [5], the expression of FcεRI [7], and myosin light chain phosphorylation [6]. So, it is suggested that mast cell degranulation (histamine/leukotriene release and interleukin secretion after FcεRI cross-linking) was inhibited by these preventive effects (Figure 1).

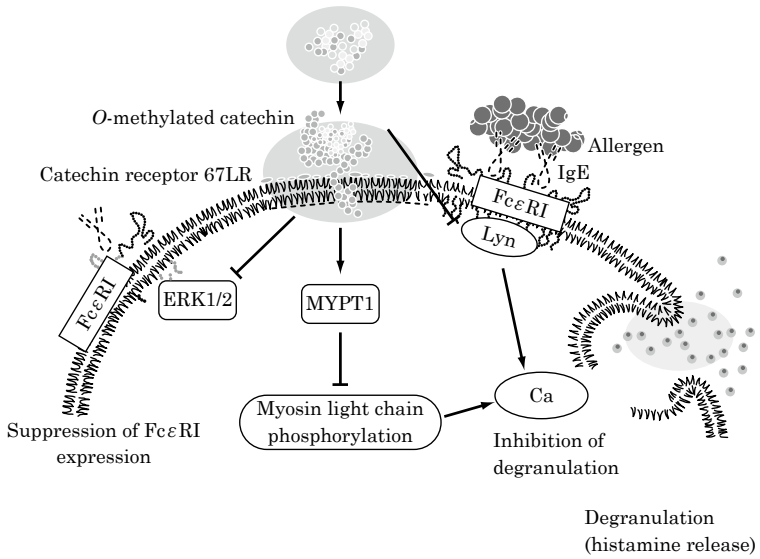

FIGURE 1: Anti-allergic model of mast cell activation by EGCG3"Me

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 >34mg of total methylated catechins, compared to patients in the placebo group [8].

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 as compared with those who began to drink it after pollen administration [9].

Furthermore, the efficacy and safety of 'Benifuuki' green tea in patients with mild perennial allergic rhinitis were evaluated in a double-blind, randomized parallel-group study. Seventy-five patients with mild perennial allergic rhinitis meeting the predetermined criteria for subjects were assigned to either the 'Benifuuki' green tea or 'Yabukita' green tea beverage group. The subjects took 700mL of tea beverage (34mg of EGCG3"Me contained in 700mL), recorded their nasal and ocular symptoms every day for 12 weeks, and visited the hospital every six weeks for consultation and blood collection. As a result, the scores for nasal and ocular symptoms in the 'Benifuuki' group were lower than those of the 'Yabukita' group, with a significant difference in the 7th-12th weeks for nasal scores and 4th-12th weeks for ocular scores [10, 11] (Figure 2). No adverse effect was observed in physiological, hematological, and biochemical parameters, with normal immune responses of peripheral blood leukocytes, and no subjective symptoms throughout the experiment. An additional study involving nine healthy subjects without any allergic symptoms was also conducted. The subjects were given 700mL of 'Benifuuki' green tea daily for 12 weeks, and no adverse effect was noted throughout the study. These results suggest that 'Benifuuki' green tea beverage containing *O*-methylated EGCG is useful for the treatment of mild perennial allergic rhinitis [10].

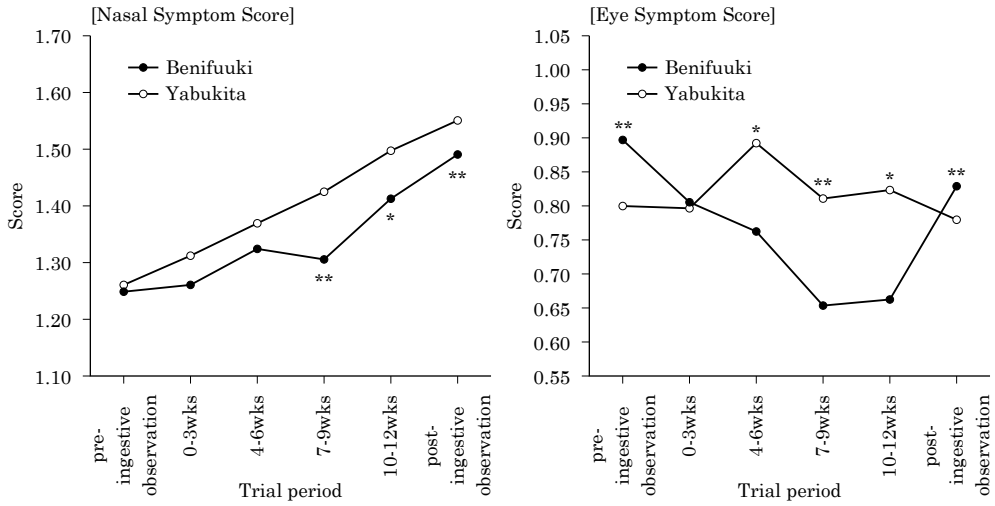

FIGURE 2: Changes in the nasal symptom score and the eye symptom score after 'Benifuuki' green tea in perennial allergic rhinitis patients
*p<0.05, **p<0.01: Significantly different between groups

Topical application of the cream-mixed 'Benifuuki' extract to infants with atopic dermatitis for eight weeks significantly reduced the consumption of the steroid hormone compared with the application of green tea cream containing no methylated catechins [12]. It is possible to develop functional articles such as beverage or food with this 'Benifuuki' green tea.

Action of other tea ingredients

Compounds that suppress the biosynthesis of IgE, which contributes to allergic reactions, have been found in tea. IgE plays a key role in the pathogenesis of allergic disease. Interleukin (IL) 4 is a potent and critical stimulator of immunoglobulin class switching from IgM to IgE in B cells and induces the expression of a germline transcript (εGT), which is critical to initiate IgE production. While searching for molecules that inhibit εGT expression induced by IL-4, it was found that polyphenol strictinin, which was isolated from tea leaves, could inhibit the IL-4-induced εGT expression in the human B cell line DND39. Strictinin also acted on human peripheral blood mononuclear cells obtained from healthy donors to inhibit IL-4-induced εGT expression [13]. Strictinin demonstrated similar inhibitory activity in peripheral blood mononuclear cells obtained from atopic donors. Interestingly, strictinin decreased ovalbumin-induced IgE production in mice, whereas the production of IgG and IgM was not affected. Furthermore, it was found that the IL-4-induced STAT6 tyrosine phosphorylation, which is essential for IL-4-induced εGT expression, was inhibited in DND39 cells upon treatment with strictinin.

Taken together, these results suggest that strictinin could inhibit IgE production through the inhibition of IL-4-mediated signaling in B cells. In addition, galloyl strictinin or theogallin also inhibited IgE production in human B cells [14]. It will be necessary to clarify in the future whether such action is applicable to humans.

Immunostimulating effect

The phagocytosis-enhancing activity (immunostimulating activity) of green tea polyphenols, such as EGCG, EGC, (-)-epicatechin gallate (ECG), (-)-epicatechin (EC), (+)-catechin (+C) and strictinin, was investigated, using VD3-differentiated human promyelocytic leukemic HL60 cells. EGCG, EGC, ECG and strictinin, but not EC and +C, increased the phagocytic activity of macrophage-like cells, and a caspase inhibitor significantly inhibited the phagocytic activities. These results suggest that the pyrogallol-type structure in green tea polyphenols may be important for enhancement of the phagocytic activity through caspase signaling pathways [15].

It was found that the EGCG/ EGC ratio in a green tea extract was affected by the extraction temperature. The EGCG/EGC ratio in the 4℃ extract was around 1:3-4, whereas in the 100℃ extract, it was around 1:0.7. In addition, oral administration

of the mixture with a high EGC ratio (1:2-3 = EGCG/EGC) resulted in greater IgA production by murine Peyer's patch cells [16].

The major polyphenols in green tea, EGC and EGCG, have been shown to enhance the phagocytic activity of macrophage-like cells; however, the mechanism involved was not clarified. Recently, it was found that the catechin-induced phagocytosis could be blocked by catalase and an inhibitor of transient receptor potential melastatin 2 [17].

Moreover, in an attempt to identify the immunostimulants contained in green tea extract, it was found that a crude tea polysaccharide fraction increased phagocytic activity in macrophage-like cells and that the crude tea polysaccharide from young tea leaves contained many RNAs as compared with that from mature tea leaves. Furthermore, the crude tea polysaccharide was shown to increase phagocytosis through toll-like receptor 7 (TLR7) [18]. TLR7 senses viral single-stranded RNA (ssRNA), induces the production of type I interferons (IFNs), IFN-α and β, in macrophages such as dendritic cells, and its immune system protects the host from virus infection. It was also found that iTPS, a crude extract from young green tea leaves containing a macromolecule with ssRNA fragments, induced IFN-α production in human macrophage-like cells [19]. In addition IFN-α production was inhibited by treatment with TLR7 inhibitors or a phagocytosis inhibitor [19].

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5

Anti-diabetic Effects of Green Tea

Noriyuki MIYOSHI

Abstract: Diabetes mellitus (DM) is a disease in which a person has chronically high blood sugar levels. There are various types of DM, but approximately 90% of the cases in Japan are type II DM caused by lifestyle factors stemming from eating and exercise habits. Long-term hyperglycemia can cause capillary disorders and lead to DM-related complications such as retinopathy, kidney diseases, and neuropathy. A number of studies have indicated that the ingestion of green tea or tea catechins is effective in preventing a rise in blood sugar levels. Several mechanisms of action are involved in this effect; these are: 1) inhibition of α-amylase activity in the digestive juice, which is involved in producing sugar from starch, resulting in a reduction in glucose production and uptake in the digestive tract, 2) promotion of the glucose intake into skeletal muscle and adipose tissue, 3) enhancement of sensitivity of insulin, a hormone that lowers blood glucose levels, and protection of pancreatic β cells, and 4) suppression of hepatic gluconeogenesis, *e.g.* glucose production from non-carbohydrates to prevent a rise in postprandial blood glucose levels. Recent cellular and animal studies revealed that molecular mechanisms underlying gluconeogenesis were suppressed by green tea catechins in which epigallocatechin gallate, a main constituent of green tea catechins, inhibits gene and protein expressions of transcriptional factors involved in gluconeogenesis. In human studies, amelioration of insulin resistance by green tea and catechins has been published. Several epidemiological studies have suggested that the habitual drinking of green tea reduces the morbidity risk of DM. Although further detailed analyses are required to evaluate the beneficial

effects on humans, drinking green tea appears to prevent and improve DM through multiple activities of its constituents. Because DM increases the risk of colon and liver carcinogenesis in addition to obesity and arteriosclerosis, habitual drinking of green tea would be a promising strategy for the primary prevention of not only DM but also these related disorders.

Keywords: diabetes, gluconeogenesis, green tea, hyperglycemia, insulin resistance

Introduction

DM is a disease that shows abnormal elevation of chronic and/or postprandial blood sugar levels. Under physiological conditions, peptide hormones, including insulin and glucagon are capable of controlling blood glucose levels. After the postprandial degradation of carbohydrates by digestive enzymes, generated glucose is absorbed in the small intestine and enters the blood stream leading to the elevation of blood glucose levels. The blood glucose is transported and incorporated into the liver, muscle, and adipose tissue, resulting in the regulation of normal blood glucose levels by the action of insulin secreted from pancreatic β cells. On the other hand, glucagon secreted from pancreatic α cells facilitates the hepatic glycogen degradation to up-regulate and maintain the blood glucose level under the hypoglycemic condition induced by, for example, fasting.

The pathology of DM has shown that various factors induce abnormal glycemic control, including postprandial and/or chronic hyperglycemia. DM is evaluated using diagnostic criteria, including fasting plasma glucose, casual plasma glucose, glucose tolerance test, hemoglobin A1c, and other symptoms such as thirst, polydipsia, polyuria, and loss of body weight. DM is roughly divided into type I and type II DM. Type I DM is caused by the destruction of pancreatic β cells resulting in impaired insulin secretion. Pathogenesis of type II DM is complicated, but known to involve genetic and environmental factors causing insulin resistance. Except for the usage of insulin formulation in type I diabetic patients, several classes of anti-diabetic drugs are clinically prescribed. They target pancreatic β cells to enhance insulin secretion, the liver to reduce gluconeogenesis, the small intestine to reduce glucose absorption, and a transcription factor, PPAR γ to improve insulin resistance. Other types of anti-diabetic drugs are dipeptidyl peptidase-4 inhibitors and incretin mimetics, including glucagon-like peptide-1, glucose-dependent insulinotropic peptide and their analogues.

Consumption of green tea is generally believed to be beneficial for human health. Several *in vitro* and *in vivo* studies have elucidated that green tea, and the constituent epigallocatechin gallate (EGCG) have multiple anti-diabetic activities. This chapter reviews recent results from some basic research of molecular mechanisms for

anti-diabetic activities of green tea/EGCG and human studies for anti-diabetic effect of green tea/EGCG ingestion.

Experiments in cultured cells and animals

Studies using cultured cells and laboratory animals have demonstrated the anti-diabetic activity of green tea and catechins, including EGCG. They 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, *e.g.* glucose production from non-carbohydrates such as amino acids in the liver (Figure 1).

Because α-amylase and α-glucosidase are enzymes necessary to produce glucose from dietary starch sugars within the body, inhibition of these enzymes contributes to the prevention and suppression of the progress of DM 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 would result in the rapid suppression of blood glucose levels, which increases after meals by promoting glucose uptake by peripheral tissues. When ingredients from green tea, black tea, or oolong tea were

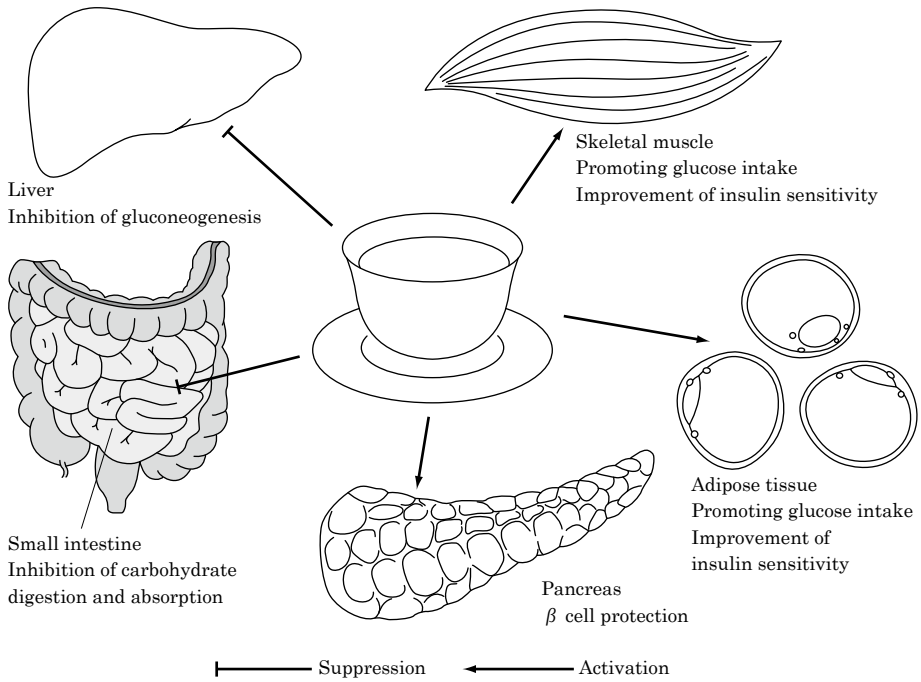

FIGURE 1: DM-related effects of green tea and EGCG

added to cultured fat cells, 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 tannins and theaflavins also contribute to this activity in black tea.

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

An increasing number of reports describe the inhibitory effect of EGCG on gluconeogenesis. In cultured hepatocytes and hepatoma cells, EGCG had an insulin-like activity, in that it caused a decrease in the protein expression of gluconeogenic enzymes, glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, by suppressing their gene expression [7, 8].

Animal experiments gave similar results [9]. One possible mechanism is that tea catechins, including EGCG, suppress the expression of transcription factor, hepatocyte nuclear factor 4α, mediating the expression of these gluconeogenic enzymes, leading to their reduced activities and resulting in diminished glucose synthesis (Figure 2) [10].

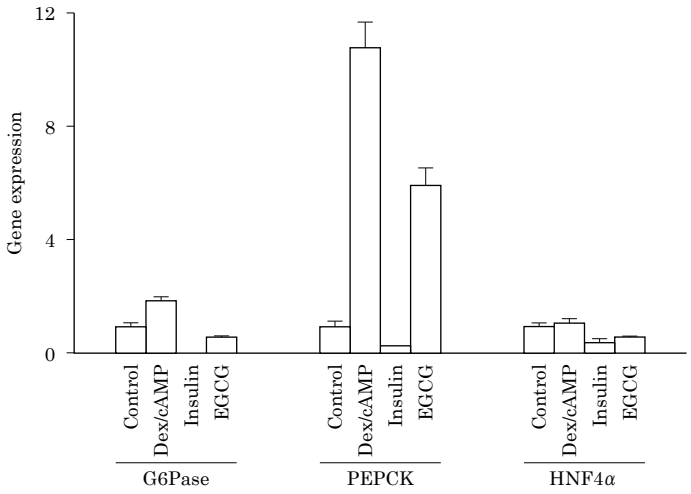

FIGURE 2: Effects of EGCG on gene expression of glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), and hepatocyte nuclear factor 4α (HNF4α) EGCG and insulin suppressed the elevated levels of gene expression of G6Pase and PEPCK which were induced by the treatment with dexamethasone/cyclic AMP (Dex/cAMP) in rat hepatoma H4IIE cells. EGCG and insulin also inhibited the gene expression of HNF4α in these cells.

Epidemiological studies

In a cohort study of 17,413 Japanese subjects, the ingestion of green tea was shown to reduce the risk of type II DM, in which multivariable odds ratio for DM among participants who frequently drank green tea (≥6 cups of green tea per day) was 0.67 (95% CI, 0.47 to 0.94) compared with those who drank less than one cup per week [11]. Huang *et al.* found that green tea consumption was associated with a lower risk of impaired fasting glucose in a study on 4,808 Chinese subjects [12].

A meta-analysis on 12 eligible studies found, overall, no statistically significant relationship between tea consumption and risk of type II DM. However, results showed that daily tea consumption (≥3 cups/day) was associated with a lower type II DM risk with a relative risk of 0.84 compared with the lowest/non-tea group [13]. In contrast, Pham *et al.* found a positive association between green tea consumption and insulin resistance in a study on 1,151 men and 289 women aged 18-69 years [14].

Taken together, these epidemiological studies suggest that ingestion of green tea has a beneficial effect on DM, although some data do not support this notion.

Intervention studies

Several reports of intervention studies have described the anti-diabetic effect of green tea. A randomized controlled trial conducted on type II DM patients (age; 32-73, 53 male and 13 female) in Shizuoka, Japan. This study showed that daily ingestion of green tea extract containing 544mg catechins for two months caused a significant reduction in hemoglobin A1c level and a borderline significant reduction in diastolic blood pressure. However, several other parameters, including body weight, body mass index, body fat, systolic blood pressure, and fasting serum glucose level did not differ significantly from those in the control (non-intervention) group [15]. Similarly, another intervention study on 60 patients with mild hyperglycemia showed that the daily ingestion of green tea extracts decreased levels of hemoglobin A1c, but not those of fasting serum glucose, after the cross-validation test, two month intervention [16].

Results of an intervention study with Japanese type II DM patients showed that the increase in insulin was significantly greater in the catechin group than in the control group, although no apparent difference was noted between the two groups in blood levels of glucose and hemoglobin A1c [17]. In a randomized, double-blinded, and placebo-controlled trial performed in Taiwan (n=92), the ingestion of 500mg green tea extract three times a day for 16 weeks, levels of an insulin resistance marker and the secretion of glucagon-like peptide-1 were ameliorated in type II DM patients, suggesting the favorable effects of green tea extract on DM [18].

In a randomized clinical trial in which 100 mildly hypertensive patients with diabetes, Mozaffari-Khosravi *et al.* found that type II diabetic individuals who consumed three glasses of green tea daily for four weeks showed significant decreased systolic and diastolic blood pressures [19]. Venables *et al.* showed that the acute ingestion of green tea extracts increased fat oxidation during moderate-intensity exercise and improved insulin sensitivity and glucose tolerance in healthy young men [20].

On the other hand, several intervention studies have found no beneficial effects of green tea on DM. A double-blind, placebo-controlled, randomized multiple-dose (0, 350, or 750mg catechins and theaflavins for three months) study conducted in the United States demonstrated no effects on the level of hemoglobin A1c in patients with a medical history of DM of more than six months [21]. A double-blind randomized intervention study on non-diabetic overweight or obese male subjects in the United Kingdom showed that twice daily ingestion of 400mg EGCG for eight weeks had no significant effects on glucose tolerance, insulin sensitivity, and insulin secretion, although reduced diastolic blood pressure was observed [22]. Crossover randomized control trial without blinding on 14 healthy volunteers in southern Sweden (aged 22-35 years, seven male and seven female, $20<BMI<30.8kg/m^2$) showed that there were no glucose or insulin-lowering effects after 300mL of green tea or water were consumed together with breakfast [23].

Thus, human studies have shown conflicting results. This can be caused due to the differences in genetic and environmental factors, such as race, sex, age, lifestyle, and differences in the ingredients, concentrations, drinking frequency, and drinking period of tea. Therefore, it is necessary to clarify the anti-diabetic effects of green tea by taking these factors into account in future studies. Nevertheless, we now have several lines of evidence, suggesting that the habitual drinking of green tea has beneficial effects in the primary prevention of DM and related diseases such as obesity, arteriosclerosis, liver cancer, and colon cancers.

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6

Protective Effects of Catechins on Hepatitis and Liver Fibrosis

Takuji SUZUKI

Abstract: Severe and sustained inflammation may induce liver fibrosis, the stage before cirrhosis and liver cancer. The results of cellular and animal experiments have shown that 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 anti-oxidant activity that removes ROS and prevents hepatitis. Thus, green tea and catechins may have hepatoprotective effects. Many epidemiological studies have demonstrated the protective effect of green tea on liver diseases, although some studies found no such effects. Results of a clinical trial on nine patients of intractable chronic hepatitis C suggested that a combination of green tea powder and interferon/ribavirin is useful as a therapy regimen. On the other hand, ingestion of excessive amounts of catechins may exhibit hepatotoxicity. Humans should be careful of excessive intake of green tea ingredients.

Keywords: hepatitis, hepatitis virus, inflammation, liver fibrosis, reactive oxygen species (ROS)

Introduction

Viral hepatitis is the most common type of hepatitis in Japan. There are three types, depending on virus type, namely A, B and C, respectively. 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 proteins accumulate in the injured area to cause 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. Several studies have suggested that green tea and its catechins have hepatoprotective effects.

Effects of green tea in animal models of hepatitis

Animal models of hepatitis can artificially be made using drugs, which induce inflammation specifically in the liver. These animal models have often been used in assessment of hepatitis because its pathology is similar to human viral hepatitis. In 2005, Abe *et al.* demonstrated that green tea had hepatoprotective effects in D-galactosamine (GalN)-induced hepatitis model animals [1]. The result of histochemical observations indicated that the degree of hepatic injury induced with GalN was suppressed in rats given a green tea beverage (Figure 1). The green tea beverage caused reduced hepatic gene expression of inflammation-promoting proteins such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and their blood protein concentrations were decreased (Table 1). In a similar experiment, the green tea beverage was shown to prevent chronic development of liver fibrosis accompanied by reduced gene expression levels of collagen α1 and transforming growth factor-β [2].

Wang *et al.* showed that (-)-epigallocatechin-3-gallate (EGCG) suppressed protein expression of inflammation chemokines such as monocyte chemoattractant protein-1 and macrophage inflammation protein-1a in both liver and serum of mice with concanavalin A-induced hepatitis [3]. These studies revealed that serum protein levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) decreased, indicating that hepatic injury was suppressed by green tea. This suggest that the effects of green tea involves relieving inflammation to suppress hepatitis and liver fibrosis.

Removing effects of ROS by green tea

One of the effective compounds in green tea extracts is catechins, among which

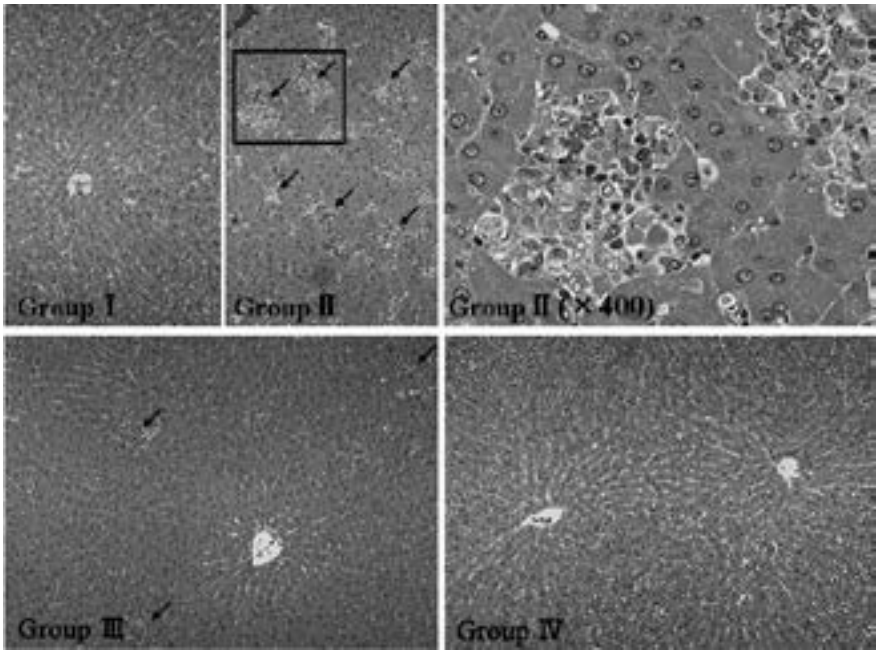

FIGURE 1: Protective effects of green tea against drug induced hepatitis
Liver sections were stained by hematoxylin-eosin staining. Group I, control; Group II, rats given GalN (500mg/kg), Group III; rats given GalN (500mg/kg) and catechin-rich beverage; Group IV, rats given catechin-rich beverage. Necrosis of hepatocytes and infiltration of leukocytes into the liver were observed in Group II, whereas these were prevented by ingestion of beverage containing high catechin in Group III. Arrows indicate leucocyte's infiltration.

TABLE 1: mRNA expression levels and serum protein levels of inflammatory cytokines (TNF-α and IL-1β)

	Group	TNF-α	IL-1β
mRNA expression levels	I	100 ± 28^{a}	100 ± 16^{a}
	II	603 ± 102^{b}	281 ± 46^{b}
	III	366 ± 112^{a}	171 ± 26^{a}
	IV	166 ± 67^{a}	89 ± 8^{a}
Serum protein concentration (ng/mL)	I	$<0.7^{a}$	$<0.7^{a}$
	II	23.7 ± 5.5^{b}	68.3 ± 5.9^{b}
	III	13.0 ± 1.7^{b}	30.3 ± 8.3^{b}
	IV	$<0.7^{a}$	$<0.7^{a}$

Group I, control; Group II, rats given GalN (500mg/kg); Group III, rats given GalN (500mg/kg) and catechin-rich beverage; Group IV, rats given catechin-rich beverage
Values indicate mean ± SD from three different determinations.
Significant differences indicate superscript between different alphabet ($p<0.05$ by ANOVA).

EGCG possesses the highest anti-oxidant activity. ROS can cause hepatitis and liver fibrosis leading to liver cirrhosis and hepatocellular carcinoma. Thus, green tea may be useful to prevent these diseases.

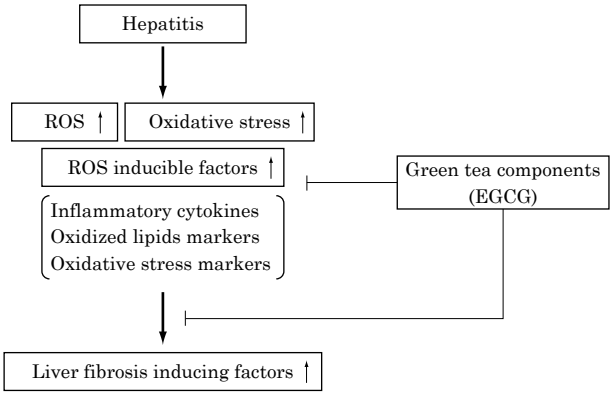

FIGURE 2: Effects of green tea components against development of reactive oxygen species leading to hepatitis
When hepatitis occurs, ROS develop in liver which give oxidative stress to hepatocytes leading to secretion of inflammatory cytokines and production of oxidized substance that cause hepatic disorders. Green tea catechins remove ROS and inhibit production of ROS-mediated factors.

Lin *et al.* demonstrated that green tea extracts prevented generation of ROS in liver, bile, and blood in GalN-induced hepatitis animals. It also inhibited expression of activator protein-1 enhancing ROS-mediated liver fibrosis and nuclear factor kappa B-mediating inflammation [4]. Kobayashi *et al.* found that green tea decreased the levels of 4-hydroxynonenal, a marker for lipid oxidation, and 8-oxo-2'deoxyguanosine (8-OHdG), a marker for oxidative stress, in animals with liver fibrosis induced by bile duct ligation [5].

Zhen *et al.* demonstrated that EGCG improved CCl_4-induced liver fibrosis by suppressing the level of glutathione and increasing the level of thiobarbituric acid reactive substances [6]. These findings demonstrated that green tea suppressed hepatitis through decreasing ROS production and hepatitis-related factors, including inflammation mediators (Figure 2).

Effects of green tea against viral hepatitis

There are three major types (A, B and C) of viral hepatitis depending upon the virus type. Several studies have shown inhibitory effects of green tea or its components against the hepatitis virus infection *in vitro*. Xu *et al.* found that green tea extracts, and EGCG had inhibitory effects on production of hepatitis B virus (HBV) antigens and extracellular and intracellular viral DNA in HepG2-N10 cells expressing HBV stably [7].

Ciesek *et al.* showed that EGCG had the anti-hepatitis C virus (HCV) infection effect at 1mg/mL and that the effect was concentration dependent, while other catechins such as epigallocatechin, epicatechin gallate and epicatechin had no effect. In this case, EGCG inhibited cell-to-cell spread of HCV but had no effect on RNA replication and release of HCV particle [8]. Calland *et al.* demonstrated that EGCG had an anti-viral effect against HCV, whereas EGCG did not inhibit infection with other viruses such as bovine viral diarrhea virus, yellow fever virus, and sindbis

virus [9]. Fukazawa *et al.* showed that EGCG exhibited an anti-HCV activity by inhibiting the viral entry into cells [10].

A recent review article also described anti-infective properties of EGCG [11]. These results suggest that intake of green tea and catechins may prevent viral hepatitis.

Effects of green tea components against fatty liver disease

There are three major types of fatty liver; alcoholic, non-alcoholic, and drug-induced fatty liver. Alcoholic fatty liver is caused by excess ingestion of alcohol, which induces hepatocyte disorders. The major cause of non-alcoholic fatty liver

TABLE 2: Reports of preventive effects by green tea extraction or green tea catechins in model animals of metabolic disorder

Disease stage	Metabolic disorder model	Treatment	Period (week)	Observed effects	References
Hepatic steatosis	Leptin mutation model (ob/ob mice)	1-2% GTE*	6	Decrease of ALT and AST in serum and total lipids and triacylglycerol in liver	[13]
	Leptin mutation model (ob/ob mice)	0.5-1% GTE*	6	Decrease of serum ALT and total lipids, triacylglycerol and choresterol in liver	[14]
	High fat diet (60%) fed mice	3.2% EGCG	16	Decrease of triacylglycerol in liver	[17]
	SREBP-1c excess expression in adipocytes model mice	0.05-0.1% EGCG	12	Supression of hepatocytes hypertrophy	[16]
	High fat diet (60%) fed rats	1g/L EGCG	6	Supression of hepatocytes hypertrophy	[20]
	Choline deficient high fat diet+nitrite (30mg/kg/day) intravenous injection	Fermented GTE (containing 3% EGCG)**	14	Decrease of triacylglycerol in liver	[19]
Non-alcoholic steatohepatitis (NASH)	High fat diet (60%) fed rats	1g/L EGCG	6	Supression of infiltration of inflammatory cells in liver	[20]
	SREBP-1c excess expression in adipocytes model mice	0.05-0.1% EGCG	12	Supression of hepatocytes hypertrophy and decrease of mallory body	[16]
	Choline deficient high fat diet+nitrite (30mg/kg/day) intravenous injection	Fermented GTE (containing 3% EGCG)	14	Supression of hepatic fibrosis	[19]
	Leptin mutation model (ob/ob mice)	0.5-1% GTE	6	Supression of infiltration of inflammatory cells in liver	[15]

* 30% total catechins (wt/wt) [48% EGCG, 31% epigallocatechin, 13% epicatechingallate and 8% epicatechin]
** 19% total catechins [54% epigallocatechin, 37% gallocatechin, 6% epicatechingallate, 3% EGCG]
Abbreviation: GTE: green tea extract, SREBP-1c: sterol regulatory element binding protein-1c

disease (NAFLD) is disturbed lifestyle and dietary habit taking a high carbohydrate and fat diet. NAFLD may induce chronic fatty liver disease such as non-alcoholic steatohepatitis (NASH) caused by a long-term lifestyle. Masterjohn and Bruno summarized the effects of green tea against NAFLD or NASH (Table 2) [12].

In a study using ob/ob mouse, an obesity model due to leptin deficiency, accumulation of lipids and the levels of hepatic functional markers such as serum ALT and AST were significantly suppressed by a diet containing 1-2% (w/w) green tea extracts [13]. Park *et al.* demonstrated that fatty liver was improved by ingestion of a diet containing 1% (w/w) green extracts in ob/ob mice and that anti-ROS enzymes such as superoxide dismutase, catalase and glutathione peroxidase were increased in ob/ob mice given green tea extracts [14]. Chung *et al.* showed that a green tea diet reduced the levels of oxidative stress markers and nitric oxide mediated by the inducible nitric oxide synthase in the liver of ob/ob mice [15].

Ueno *et al.* demonstrated that EGCG improved fatty liver, hypertrophic liver, and hepatitis in sterol regulatory element binding protein-1C transgenic mice and that EGCG markedly mitigated insulin resistance and oxidative stress [16]. They also observed that mice ingesting EGCG showed decreases in blood levels of ALT, glucose, cholesterol and triglycerides.

Bose *et al.* showed that a high-fat diet containing EGCG reduced not only body weight gain, but also levels of blood insulin, cholesterol and hepatic triglycerides in high-fat diet NAFLD mice [17]. Park *et al.* demonstrated that in a rat model of high-fat diet NAFLD/NASH, green tea extracts improved developmental of NAFLD by decreasing the binding activity of NF-κB which is a major transcriptional factor to regulate inflammatory cytokine expression [18]. Nakamoto *et al.* and Kuzu *et al.* also found that green tea components improved diet-induced NAFLD and NASH [19, 20].

EGCG has an inhibitory effect on absorption of dietary cholesterol in the small intestine [21]. Therefore, these results show that the inhibition of dietary cholesterol absorption in the small intestine and the attenuation of oxidative stress in the liver by green tea components, contributed to the suppression of NAFLD/NASH development.

Epidemiological studies of green tea ingestion in hepatic disorders

Jin *et al.* reviewed ten credible reports related to the protective effect of green tea consumption against liver diseases such as liver cancer, liver cirrhosis, and fatty liver diseases and found eight reports to show that ingestion of green tea or green tea components prevented the onset of several liver diseases [22]. Imai and Nakachi found that the intake of green tea was associated with the decrease of liver disease incidence in a study on 1,371 men over 40 years old [23].

Ui *et al.* have revealed that ingestion of green tea is associated with a decreased

liver cancer incidence in the Ohsaki Cohort study, which was conducted on 41,761 Japanese people between 40 to 79 years old [24]. Li *et al.* found that risk of hepatocellular carcinoma incidence was lowest in Chinese tea drinkers who drank green tea for over 30 years [25]. They also show that although the risk of hepatocellular carcinoma was markedly high in alcohol-drinkers who did not drink green tea, the risk was lowered by drinking green tea habitually. They suggested that ingestion of green tea was effective for prevention of alcohol-induced hepatitis and infection by hepatitis virus. In a meta-analysis using 13 epidemiological studies, Fon Sing *et al.* found an association of green tea intake with a reduced risk of primary liver cancer development in both men and women [26].

On the other hand, a study on Singapore Chinese demonstrated that green tea consumption was not associated with the risk of liver cirrhosis, while coffee consumption had a protective effect on non-viral hepatitis [27]. In a survey with 18,815 Japanese adults from 1993 to 2006, green tea ingestion was not associated with liver cancer incidence caused by the hepatitis virus [28].

Thus, many epidemiological studies have demonstrated the protective effect of green tea on liver diseases, although some studies found no such effects.

Clinical trials

A clinical trial was performed on nine cases of intractable chronic hepatitis C with very high viral load, more than 850 kilo international unit/mL. The patients received a combination therapy regimen of 6g of green tea powder per day and interferon/ribavirin. The therapy with green tea was 3.5 times more effective than treatment without green tea, a standard treatment in Japan [29]. These results warrant further study on clinical use of green tea and its extracts.

A randomized, placebo-controlled, Phase IIa clinical trial supported a protective role of green tea catechins in liver damage by HBV [30]. Individuals seropositive for HBV antigen and aflatoxin-albumin adducts were randomly assigned to a 500mg catechins/day group, a 1000mg group and a placebo group. After three months, these catechin groups showed significantly decreased urinary concentrations of 8-OHdG as compared with those in the placebo group. These results suggest that green tea catechins are effective in reducing oxidative DNA damage, which would lead to hepatitis development and liver cancer in patients with infected HBV.

Liver disorders by excessive ingestion of green tea components

The ingestion of excessive amounts of green tea catechins may not only put a burden on the liver's detoxification system but also negatively affect liver function. The unfavorable effects of supplements related to green tea were reviewed by

Mazzanti *et al.* [31]. Recently, Patel *et al.* presented a case of acute impending liver failure in a male using a weight loss product containing green tea extract [32]. Therefore, people should exercise caution before ingesting excessive amounts of green tea ingredients.

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7

Effects on Aging and Dementia

Keiko UNNO

Abstract: As the number of elderly rapidly increases in Japan, the number of patients with dementia is also increasing. The most important risk factor for dementia is "aging," and "senescence" acts as a promoting factor. Prevention of "brain senescence" is an important strategy for the prevention of dementia. The accumulation of oxidative damage is considered to be a major cause of senescence. Reactive oxygen species (ROS) are generated during many physiological processes. An increased production or reduced scavenging of ROS induces the accumulation of oxidative damage and the malfunction of biological systems that may induce senescence, neurodegenerative diseases and lifestyle-related illness. Catechins in green tea have powerful anti-oxidative activity. Oxidative damage, brain atrophy and cognitive decline have been shown to be suppressed in aged mice which ingested green tea catechins, and when ingested from a middle age, the age-related cognitive decline is significantly suppressed. In addition, many studies of green tea catechins using experimental animals suggest that they can protect the brain from Alzheimer's disease. In addition, theanine, an amino acid in green tea, suppresses stress-induced senescence caused by chronic psychosocial stress, which has been demonstrated to induce shortened lifespan and accelerated brain senescence in experimental animals. Daily consumption of several cups of green tea is thus expected to be effective in the prevention or reduction of brain senescence and dementia.

Keywords: aging, Alzheimer's disease, brain, cerebral atrophy, learning, memory, senescence, stress

Introduction

The number of elderly is rapidly increasing in Japan, with a parallel increase in the number of dementia patients. The most important risk factor for dementia is "aging," while "senescence" acts as a promoting factor. Dementia is incurable; therefore, prevention of "brain senescence" is an important strategy to prevent dementia. Aging is a physiological phenomenon affecting all living animals, but with considerable inter-individual differences. There is social responsibility to study the mechanism of senescence and explore treatments that prevent senescence. Components in green tea demonstrate, with accumulating scientific evidence, that they prevent brain senescence in animal models. The effect of green tea components on Alzheimer's disease (AD) has been studied using a mouse model. The function of green tea in the maintenance of brain health is well documented. Recent basic studies of green tea catechins on aging and dementia are discussed in this chapter.

Senescence and oxidative stress

Senescence, defined as "aging after maturing" is a universal phenomenon that all species undergo. Therefore, senescence is a natural process and hence excluded from any list of diseases. However, senescence is a promoting factor of age-related diseases such as cancer, lifestyle-related diseases, cardiovascular disease and dementia. The main cause of senescence is a decrease in biological functions with aging, but the degree of progression of senescence is different among individuals. Hereditary and environmental factors are involved in senescence. Such factors include the accumulation of damage caused by reactive oxygen species (ROS), decreased immune capacity, changes in metabolism, mutation or chemical modification of biopolymers or their deposition in tissues and psychological stress. Various factors affect the progression of senescence. The degree of senescence may differ between individuals, of the same age.

The accumulation of oxidative stress is an important factor of senescence. Organisms produce energy efficiently by using oxygen. Although ROS are a normal byproduct of cellular metabolism, their concentration is kept low by the presence of anti-oxidants in the body. Even though ROS constantly damages proteins and DNA, the latter two are rapidly metabolized, repaired or removed. However, during senescence, the balance between generation and removal of ROS, repair of damaged DNA, proteins and fatty acids, becomes gradually skewed towards less removal. Hence, damage accumulates with age. Even though excess ROS brings about

oxidative damage, it is an important player in normal signal transduction. When an abnormality occurs in the expression and regulation of ROS, normal biological functions are altered, resulting in senescence, neurodegenerative diseases and lifestyle-related diseases.

Brain senescence

To evaluate biologically age-related changes in the brain, changes in gene expression were investigated. The genetic information of an individual is unchanged from birth until death. However, the required genetic information is different among organs such as the brain and liver, and different between young and old individuals. Therefore, age-related changes in the brain could be studied by examining changes in gene expression in the brain.

An extensive analysis of changes in gene expression in the brains of humans between 26 and 106 years of age, confirmed that gene expression changed significantly with age [1]. Some genes that were highly expressed at a young age decreased with age. While other genes that were weakly expressed at a young age increased with aging. Genes whose expression levels are low in the elderly are involved in synaptic plasticity and vesicle transportation, and in mitochondrial function where energy is produced. On the other hand, genes whose expression level was high in the elderly were involved in stress response, anti-oxidative activity and DNA repair. These results suggest that increases in damage to the elderly brain are involved in the decrease of cognitive function with aging.

Some gene expression patterns are similar in young and old people, suggesting that almost all age-related changes in gene expression are biologically determined. However, the patterns of gene expression of middle-aged individuals, 40 to 70 years old, are very different. Gene expression data in the brain indicate that some individuals have a youthful brain relative to their chronological age, while others have an aged brain relative to their chronological age. The lifestyle that a person leads in their middle age is considered to affect, in part, the rate of senescence when aged.

Aging mouse model and ROS

Brain atrophy in the prefrontal cortex, and a decrease in learning and memory abilities, are observed in many elderly people. However, the degree of atrophy is less in elderly people who are active and healthy, suggesting a possible therapy for preventing brain atrophy. Studies on brain atrophy using animal models are very limiting. Since the lifespan of a mouse or rat, which are frequently used, is as short as 2-3 years, atrophy in the brain is difficult to study in these animal models.

The senescence-accelerated mouse (SAM) model, developed by a group at

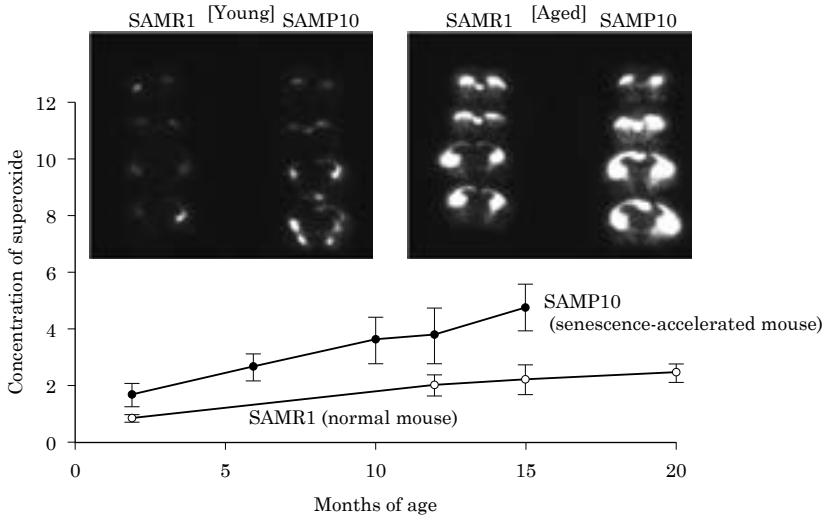

FIGURE 1: Superoxide in the brain
The generation of superoxide in the brain increased with aging. The concentration of superoxide was higher in SAMP10 than in SAMR1 (a normal mouse) [3]

Kyoto University in Japan, is inbred mice exhibiting a short lifespan with various senescence-related symptoms. A clone of the SAMP10 mouse exhibits brain atrophy in the frontal region and cognitive dysfunction with aging, showing characteristics close to physiological brain senescence of humans [2]. The level of superoxide, one ROS, was measured in the brain of SAMP10 mice and was compared with the level in SAMR1 (a normal mouse) [3]. Superoxide was detected as a white area in a photo of the brain sections (Figure 1). The results showed that ROS generation was higher in the aged mice brain than in the young mice brains. ROS increases with age. Increased ROS concentrations accelerate senescence. This suggests that ingestion of anti-oxidants suppresses brain senescence.

Green tea catechin

Green tea exhibits strong anti-oxidant activity. Green tea has 20-30 times higher anti-oxidative activity than blueberry and strawberry [4]. Catechins, bitter compounds in green tea, have potent anti-oxidative activities. About 10-20% w/w of green tea consists of catechins, although the content varies depending on the season, geography and growth conditions. Catechins are a group of several molecules. Epigallocatechin gallate (EGCG) is the most abundant, accounting for 5-10% w/w of green tea. The other abundant catechins are epigallocatechin (EGC; 1-5%), epicatechin gallate (ECG; 1-2%), and epicatechin (EC; 0.5-1.5%). Caffeine is normally 1-2% w/w of green tea.

When green tea is prepared with hot water, the concentration of catechins is about 60mg/100mL (0.06%). The content of catechins in a commercially available pet bottle of green tea, is about 50mg/100mL (0.05%). Some commercial beverages are with the increased content of catechins. Black tea is processed differently from green tea, even though the same tea leaves are used as the raw material. Thus, the content of catechins in black tea is about 1/3-1/5 of that in green tea. Instead, theaflavins and thearubigins, oxidatively polymerized catechins, are higher in black tea. Theaflavins are important contributors to the bright-red color of black tea. Catechin content in oolong tea is about half than that of green tea. However, the sum of polyphenols is similar in the green, oolong and black tea, since all three are manufactured from the same leaves, by different processing methods.

Anti-senescence effect of green tea

The learning and memory abilities of SAMP10 mice that had ingested green tea catechin in their drinking water were investigated. Control mice ingested water without catechin. The learning ability of the mice was examined by utilizing the natural behavior of mice, which prefer the dark. The mice were placed in a light room. After a while, the entrance to the dark room was opened. The mice entered the dark room immediately. A weak electric shock was delivered to the mice, teaching them to avoid the dark room. The mice returned to the light room. The mice were repeatedly trained (maximum of five times) until they learned to avoid the dark room. The time it took for the mice to learn was measured. Longer learning time represents lower learning ability. One month later, the mice were tested to assess whether memory had been retained.

The learning ability and memory retention, in aged mice that had ingested green tea catechin, was better than the controls (Figures 2 and 3). Brain atrophy was suppressed in mice that had ingested green tea catechin (Figure 4). In addition,

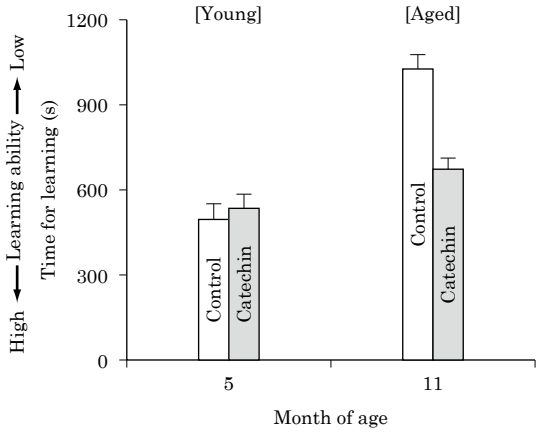

FIGURE 2: Suppression of learning decline
Longer learning time represents lower learning ability. Although the learning ability was lower in aged SAMP10 mice than in young mice, the decline with aging was suppressed in mice that had ingested green tea catechin [5] (Unno K, *et al.*, 2004, with modification).

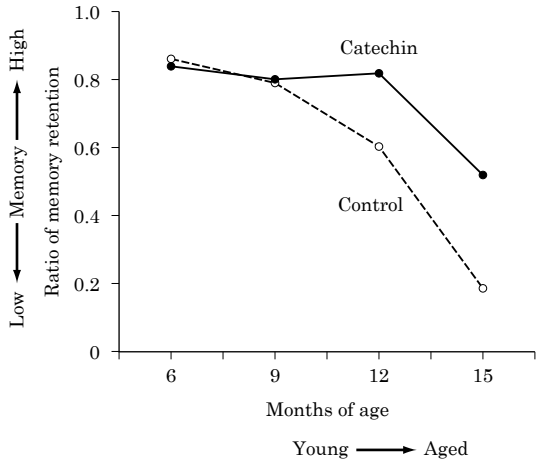

FIGURE 3: Suppression of memory decline
The ratio of the mice that retained memory to avoid the dark room was examined. Although this was lower than in aged control mice, it was higher in age-matched mice that had ingested green tea catechins [6] (Unno K, *et al.*, 2007, with modification).

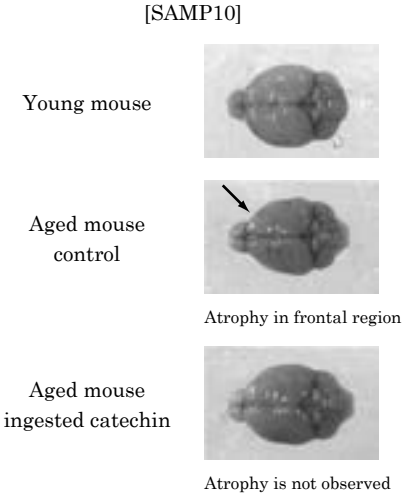

FIGURE 4: Suppression of brain atrophy
While brain atrophy in the frontal region was observed in aged SAMP10 control mice, it was lower in aged mice that had ingested green tea catechin.

oxidative damage in the brain DNA in the mice that had ingested green tea catechin was lower than in the control mice. These results suggest that brain atrophy and cognitive dysfunction was suppressed through decreased oxidative damage by green tea catechin.

Prevention of brain senescence from middle age

Senescence of the brain in mice can be suppressed by ingesting green tea catechin. In general, middle-aged humans become aware of their senescence. Therefore, influence of the starting time of green tea catechin intake was investigated. Learning ability was investigated in mice that started to drink green tea catechin from a middle age. The results demonstrated that the decline in learning was suppressed in aged mice that had ingested green tea catechin from a middle age [7]. These results suggest that the suppression of brain senescence in humans is expected, even if

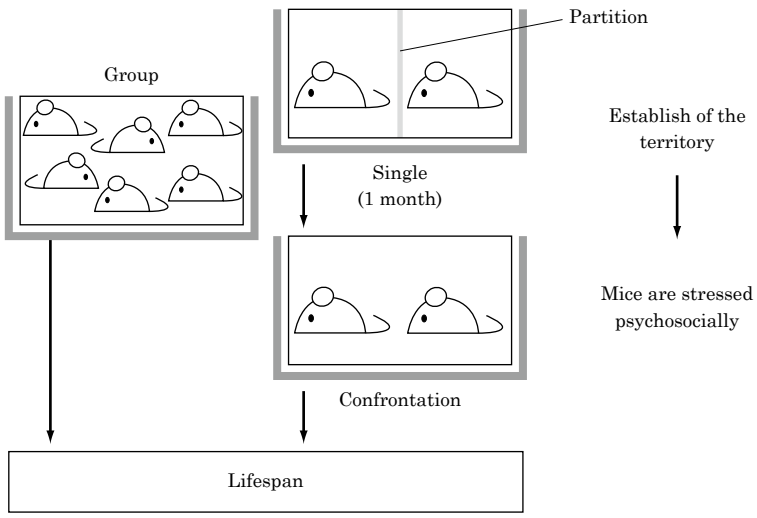

FIGURE 5: Group and confrontational housing
After the two mice were housed separately, for one month, in a cage with a partition, they were housed confrontationally by removing the partition. Mice in group-housing were housed at six mice per cage.

green tea catechin intake is started from middle age.

While the amount of green tea catechins that was effective in the mouse experiments was calculated to be equivalent to ten cups of green tea per day in humans, less green tea may be effective in humans. Green tea catechin is excreted relatively quickly (3 hours) from the body, while ROS are produced continuously. Therefore, it is believed that the benefits of green tea catechin are valid if ingested frequently. To avoid the diuretic and stimulant effects of caffeine in green tea, a supplement of caffeine-free green tea catechin is recommended.

Psychosocial stress and senescence

Modern life causes stress in many people. Moderate stress sometimes exerts a good effect; however, long-term stress is believed to trigger depression and cardiovascular diseases, and accelerate senescence. Psychosocial stress was applied to mice, and the effect was investigated. Two mice were housed separately in a cage with a physical partition for establishing territories. Then, the partition was removed to create confrontation between them. They experience psychosocial stress when an intruder enters their territory (Figure 5). On the other hand, group-housed mice have no territorial boundaries because they recognize each other as group members. The results demonstrated that the lifespan of mice housed confrontationally was significantly shorter than that of group-housed mice (Figure 6). In addition, brain atrophy and cognitive decline were accelerated in confrontational housing. These

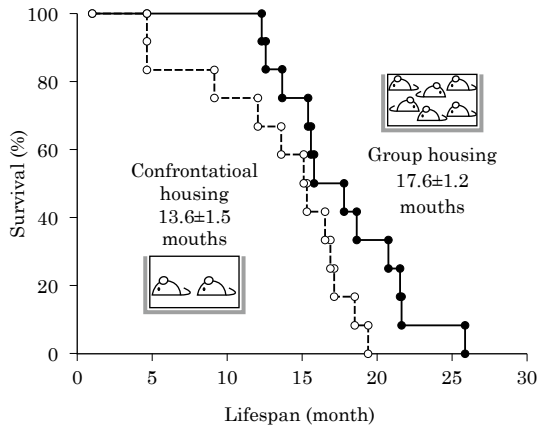

FIGURE 6: Shortened lifespan in stressed mice
The mean survival time of mice in confrontational housing was significantly shorter than of mice in group-housing [8] (Unno K, *et al.*, 2011, with modification).

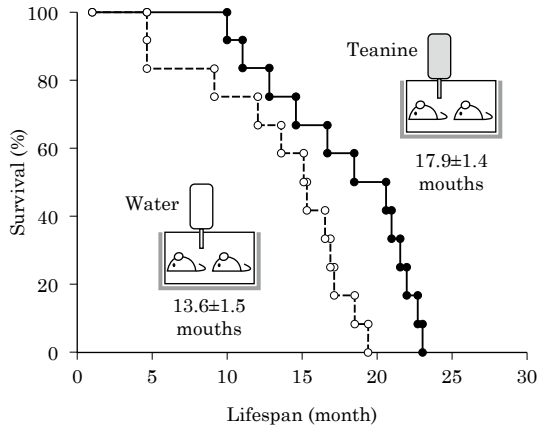

FIGURE 7: Prolonged lifespan of mice by theanine intake
The mean survival time of mice in confrontational housing was significantly prolonged by theanine intake. Survival time was similar to that of group-housed mice [8] (Unno K, *et al.*, 2011, with modification).

results indicate that brain senescence is accelerated by chronic stress. However, the shortened lifespan and cognitive decline were suppressed in stressed mice that had ingested theanine, an amino acid unique to tea (Figure 7). Theanine suppresses senescence by reducing psychosocial stress.

Theanine has been reported to influence the level of glutamic acid, an excitatory neurotransmitter in the brain, by acting on glutamine receptors [9]. Inhibition of the excess release of glutamate is believed to exert an anti-stress effect. The anti-stress effect of theanine is, in part, abolished by the catechins and caffeine in tea [10]. Thus, the ingestion of theanine-rich tea or a theanine supplement would have an effective anti-stress effect. In fact, elderly people who consumed capsules of theanine-rich green tea powder showed improved cognitive function [11].

Healthy life expectancy and dementia

Healthy life expectancy is defined as “the time that people can live independently without requiring daily care.” In Japan, this is 70.4 years for men and 73.6 years

for women, as indicated by the Ministry of Health, Labour and Welfare in 2010. The average life span for men is 79.4 years and that for women is 85.9 years in Japan (2011, the Ministry of Health, Labour and Welfare). The challenge to extend the healthy life expectancy to the average life span is very important. "Suppressing senescence" is considered to significantly contribute to the extension of healthy life expectancy.

The Japanese Ministry of Health, Labour and Welfare estimated that the global burden of elderly with dementia exceeded 460 million people in 2012. This number is expected to exceed 700 million people by 2025. It is much higher than previous estimates; therefore, a therapy for dementia is needed urgently. Cognitive function, once developed normally, is significantly reduced in dementia due to atrophy and cerebral vascular disease of the brain. The disease progresses from memory disorder to disorientation and psychiatric disorders. It can be roughly classified into cerebrovascular and denatured types. Denatured type dementia is categorized by AD, Parkinson's disease and Lewy body dementia. Although vascular dementia was the major type in Japan, about 60% of current dementia is thought to be AD-type dementia. Therefore, the next section introduces the effects of green tea components on AD that were obtained from animal experiments.

Effect of green tea catechin on Alzheimer's disease

AD is a brain disease associated with aging. Because nerve cells die in AD, the patient has difficulty to make correct decisions or retain memories. There are several hypotheses as to the cause of AD. The leading hypothesis is that nerve cells are injured by β-amyloid protein deposits, resulting in cell death. A successful treatment is still being sought.

In AD patients, a protein, amyloid β (Aβ), accumulates in the brain. Aβ is a fragment of the amyloid precursor protein (APP) that penetrates into the cell membrane. The Aβ monomer is soluble, but at a high concentration Aβ forms insoluble amyloid fibrils that are believed to exhibit cytotoxicity [12]. In normal conditions, APP is cleaved first by α-secretase, and soluble sAPPα is produced. Subsequently, a fragment called p3, is cut out by γ-secretase. During pathology, APP is cleaved first by β-secretase, and then Aβ cleaved out by γ-secretase [13-14]. Although Aβ presence is low in normal people, the level increases in AD patients. There are two kinds of Aβ; Aβ40 composed of 40 amino acids, and Aβ42 composed of 42 amino acids. Because Aβ42 has a higher aggregation property than Aβ40, accumulation of Aβ42 is thought to be involved in the onset of AD. Attempts to treat AD have been studied by inhibiting the production of Aβ. Substances that inhibit the action of β- and γ-secretases, and substances that increase the degradation of Aβ have been studied.

The effect of tea components on AD have been studied. EGCG, an abundant

catechin in green tea, has been demonstrated to be effective for treating AD in animal experiments. EGCG reduced the accumulation of Aβ in the AD mice model. EGCG reportedly suppresses the production of Aβ by activating the α-secretase pathway [15]. Because the activation of α-secretase will increase the metabolism of APP in the normal direction, it is considered to be important. However, the effective dose of EGCG in animal experiments is higher than that found in human serum after green tea consumption. To enhance EGCG uptake into the body, EGCG nanoparticles have been studied [16]. The combination of EGCG and fish oil that reduces the risk of dementia epidemiologically has been studied [17]. EGCG is also considered to have a chelating action with iron, an increasing effect on neurogenesis and a stabilizing effect on mitochondria for preventing AD [18]. To prevent senescence and dementia, further studies into the mechanism of green tea catechins are needed in the future.

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8

Green Tea and Brain Function

Hidehiko YOKOGOSHI

Abstract: Green tea infusion gives a specific taste and flavor, and it is now one of the most popular drinks in the world. The infusion is characterized as containing such compounds as tea polyphenols, caffeine, theanine, vitamins, etc. These compounds may also affect brain functions. Green tea contains anti-oxidant vitamins such as vitamins A, C and E. These anti-oxidants reduce excessive anti-oxidative stress, contributing toward 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.

Keywords: Alzheimer's disease, brain dysfunction, cognition, Parkinson's disease, theanine

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

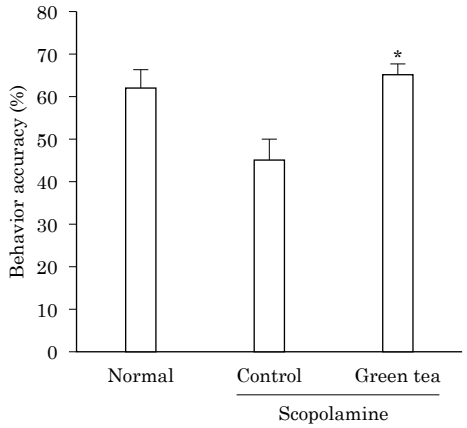

FIGURE 1: The intake of green tea catechins improves drug-induced behavioral dysfunction $p<0.05$ vs control group

(Figure 1) [1]. Among the components of green tea, anti-oxidant 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.

Anti-oxidant vitamins

Vitamins with anti-oxidant activities, such as vitamins A, C and E, are abundant in green tea. Commercial green tea leaves contain vitamin C of about 280mg per 100g dried leaves. These vitamins are believed to be effective for 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 one 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 polyphenols (catechins)

One of the noticeable components of green tea leaves are tea polyphenols, which attribute to the slight astringent and bitter taste of green tea infusions. A number

of papers have been published on the anti-mutagenic activity [5], suppressive effect of chromosome aberration [6], anti-oxidant activity [7], depressor effect on renal hypertension, inhibitory effect on lipid peroxidation, or inhibitory effects on arteriosclerosis of green tea polyphenols. The effects of polyphenols on brain functions are described below.

Oxidative stress-induced by nitric oxide may cause neuronopathy. An experiment examining the effect of epigallocatechin gallate (EGCG) on nitric oxide generation showed that the nitric oxide concentration increased in nerve cells in the hippocampus, which is a part of the cerebrum concern 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 [8]. In addition, several reports have described the cranial nerve protection by EGCG [9, 10] and the effect of EGCG on neuropathy in experiments using Parkinson's disease rat models [11].

Caffeine

Caffeine content of coffee beans is usually 1.5% w/w, while that of green tea leaves reached 5% w/w at the maximum. The content of caffeine is generally high in the tea leaves evaluated as high quality by sensory test. Caffeine has been applied as a cardiac stimulant and a diuretic. It also stimulates the cerebral cortex to induce excitation in the central nervous system. 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 [12]. Rats induced to have experimental epilepsy after consuming a low concentration of caffeine ad libitum for 15 days 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 six years revealed that caffeine reduced disease development [13]. Caffeine is also known to improve the neurotransmission efficiency of dopaminergic neurons [14].

Theanine (γ-glutamylethyl amide)

The content of total nitrogen in green tea extracts ranges from 4.5 to 6.0% w/w, and about half of it is free amino acids. Theanine and glutamic acid are the major amino acids in green tea infusions, and aspartic acid and arginine are the next [15].

Theanine is a very unique amino acid and is known to be produced by the tea plant and certain species of genus *Camellia*. The rate of metabolism of theanine in tea leaves is slow, but its transport from root to leaf is so rapid that this amino acid is accumulated in tea leaves [16].

When theanine was administered intragastrically, absorbed theanine was distributed to various organs and incorporated into the brain through the blood-brain barrier via the leucine-preferring transport system [17]. Administration of theanine caused a significant increase in dopamine concentrations within the brain, especially in the striatum, hypothalamus, and hippocampus [17]. Direct administration of theanine into brain striatum using the microdialysis technique caused a significant increase of dopamine release in a dose-dependent manner [18]. On the other hand, some behavioral effects of theanine have been researched. Cognition was influenced by long-term intake (3 months) of theanine and a passive or active avoidance test, performed by using step-through cages, showed that the avoidance learning ability was significantly improved in animals given theanine. The memory ability estimated by the transfer test using Morris water maze apparatus was also improved by the administration of theanine, as compared to the control [18].

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 without theanine, died.

In an experiment in which theanine was directly administered to the lateral ventricle of gerbils and the carotid artery was bound 30-minutes later by a clip to stop blood flow for three minutes to cause ischemia, the results obtained seven days later indicated that the ischemia-induced hippocampal cranial nerve cell death was suppressed by theanine administration [19]. Electroencephalographic measurement of alpha waves showed 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 [20].

To evaluate the psychosocial effect on lifespan and cognitive function, a study investigated the effect of confrontational housing on mice because conflict between male mice is a psychosocial stress [21]. Two male mice were separately housed in the same cage with a partition for establishing the territorial imperative in each mouse. Then, the partition was removed and mice were co-housed confrontationally (confront-housing) using a model mouse of accelerated-senescence (SAMP10) that exhibited cerebral atrophy and cognitive dysfunction with aging. The level of oxidative damage in cerebral DNA was also higher in mice housed confrontationally, than group-housed control mice. On the other hand, theanine (20μg/mL, 5-6mg/kg) suppressed the shortened lifespan, cerebral atrophy, learning impairment, behavioral depression and oxidative damage in cerebral DNA [21]. These results suggest that psychosocial stress accelerates age-related alterations such as oxidative

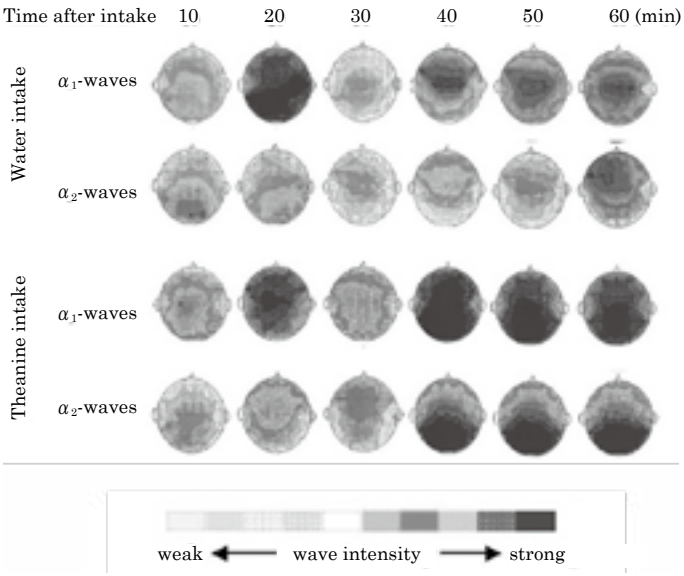

FIGURE 2: Electroencephalographic measurement of alpha waves shows higher frequencies among human subjects taking theanine as compared to those taking water.

damage, lifespan, cognitive dysfunction and behavioral depression. The intake of theanine might be a potential candidate for suppression of disadvantage, such as oxidative damage, lifespan, cognitive dysfunction and behavioral depression, under psychosocial stress.

Concluding remarks

Green tea contains various components in addition to those described herein. These may include components that improve brain and neurological functions. Despite the limited findings, green tea appears to contribute toward 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.

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9

Anti-aging and Tea

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Toshikazu YOSHIKAWA

Abstract: Tea has anti-oxidative effects due to its polyphenol compounds, especially the catechins. This anti-oxidative effect of catechins is responsible for the anti-aging properties. Catechins exhibit their anti-oxidative effect by direct quenching of free radicals and reactive oxygen species, and increasing of the anti-oxidative enzymes. Although many beneficial functions of catechins depend on their powerful anti-oxidant properties, catechins also possess other functions, not related to their anti-oxidant properties, such as anti-aging, anti-metabolic syndrome, anti-cancer, and anti-inflammatory functions. The increase of the life span due to catechins still needs definite proof. However, there is evidence that catechins can improve the quality of life. We should first clarify the exact mechanism/s of aging.

Keywords: HUVEC, ICAM-1, MKN45, PMN

Introduction

The anti-aging effect and other functions of catechins are summarized in this chapter.

Ingredients of tea

Tea can be classified into three main types according to the manufacturing

TABLE 1: Polyphenol and caffeine composition of the main tea types as % of their dry weight

	Green Tea (Sencha)	Black Tea (Assam)	Oolong Tea
EGCG	7.33	2.79	1.18
EGC	3.21	0.39	0.73
ECG	1.04	1.15	0.31
EC	1.41	0.74	0.35
Gallic Acid	0.07	0.25	0.12
Caffeine	3.83	4.74	2.91

process; non-fermented tea (green tea), semi-fermented tea (oolong tea), and fermented tea (black tea) (Table1). All three types of tea contain abundant amounts of anti-oxidants. The largest fraction (10-20% w/w) of green tea, is the astringent polyphenols called catechins. Tea also contains other anti-oxidants, e.g. water-soluble flavonoids, vitamin C, E, B2 and lipophilic carotenoids. Furthermore, the components of anti-oxidative enzymes, such as zinc, manganese and selenium are also found in tea. Zinc or manganese are components of the superoxide dismutase (SOD), and selenium is a component of glutathione peroxidase enzyme.

Catechins

The major catechins are (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG). Among these catechins, EGCG exhibits the strongest anti-oxidant power, which is reported to be 200 times stronger than that of vitamin E. EGC has second strongest anti-oxidant power. The content of these four catechins varies depending on the type of tea. EGCG and EGC are known to be abundant in green tea (Table 1) [1].

The function of catechins

Free radicals and reactive oxygen species (ROS) (superoxide, hydroxyl radicals, or singlet oxygen) are generated in small amounts in all living cells during the process of energy production required to sustain life. However, excessive free radical or ROS production can be a harmful oxidative stress to the living body. A decrease in anti-oxidant defense mechanism is related to the pathogenesis of various diseases e.g. arteriosclerosis, ischemic heart disease, cardiovascular injury [2] or skin aging [3]. Catechins, due to their anti-oxidant properties, have protective effects against many diseases. Catechins exhibit their anti-oxidant effects by 1) direct quenching of ROS, 2) by chelating oxidative stress-causing transition metals, 3) inhibiting the activation of transcription factors or oxidative stress-promoting enzymes, or 4) inducing anti-oxidant enzymes [4-6].

Although many beneficial functions of catechins depend on their powerful

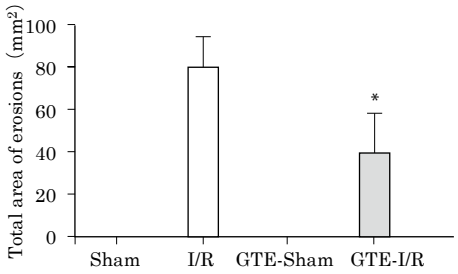

FIGURE 1: Gastric mucosal protection effect of green tea extract (GTE) in rat gastric I/R model
*p<0.01 vs control rats receiving double distilled water (sham)

anti-oxidant effects, they also possess other functions. Longevity due to green tea has been claimed, since antiquity. Recent scientific studies have revealed important properties, e.g., anti-aging, anti-metabolic syndrome, cancer preventing, and anti-inflammatory effects.

In several experiments to elucidate the mechanism of anti-inflammatory effects of catechins, it was found that the green tea extract significantly suppressed auto-oxidation of rat brain tissues. This was detected when these tissues were exposed to air in the presence of green tea extract, compared with those without green tea extract [7]. An *in vitro* experiment using human gastric mucosal epithelial cells (MKN45; human gastric cancer cell line) and human umbilical vascular endothelial cells isolated from umbilical cords (HUVEC) showed that upon stimulation with interleukin 1β (IL-1β), an inflammation-inducing cytokine, these cells produced significant amounts of interleukin-8 (IL-8), a neutrophil chemotactic factor. However, catechins significantly suppressed IL-1β induced IL-8 production. However, there were no differences between the individual catechins, that have different anti-oxidant strengths (Table 2A). This suggests that the anti-inflammatory actions of catechins are unrelated to their anti-oxidant properties. In addition, the examination of the expression of adhesion molecules on neutrophils (polymorphonuclear neutrophils; PMN, CD11b/CD18) and vascular endothelial cells (intercellular adhesion molecule-1; ICAM-1 and E-selectin), which were expressed at the early stage of the inflammation, showed that catechins strongly suppressed the expression of CD11b/CD18 (Table 2C), and ICAM-1 (Table 2B) [8]. Furthermore, in the study using a rat ischemia-reperfusion (I/R) model, oral or intravenous administration of green tea extract significantly suppressed I/R-induced lipid peroxidation and erosion in the gastric mucosa (Figure 1) [7].

Besides the above-described actions, catechins have been reported to have a unique property, a bactericidal function. In August 1996, a severe epidemic, caused by enterohemorrhagic *Escherichia coli* (EHEC) O157: H7, occurred in Japan [9]. This disease is known to cause bloody diarrhea in addition to hemolytic uremic syndrome and acute encephalopathy with central nervous system disorders [10], in which the main pathogenic factor is a Vero Toxin. Interestingly, 5% green tea in saline killed O-157: H7 and neutralized Vero Toxin production [11].

TABLE 2: Anti-inflammatory effect of catechins as described in the text

[A]			IL-8 (pg/mL)
	(μM)	MKN45	HUVEC
Unstimulated		45.0±0.63	433±67
Control		68.0±3.10#	2464±195#
EC	0.2	57.4±0.18*	2228±277
	2.0	54.8±3.70*	2275±308
	20.0	58.8±3.60*	2271±299
EGC	0.2	51.3±4.40*	2240±191*
	2.0	59.4±1.35*	2264±198*
	20.0	59.9±0.63*	2277±186*
ECG	0.2	61.6±1.37	2354±193
	2.0	57.4±0.63*	2231±231
	20.0	52.0±1.43*	1804±461
EGCG	0.2	59.9±3.50*	2238±261
	2.0	57.6±1.00*	2111±242*
	20.0	56.4±2.20*	2180±189*

Effects of catechins on IL-8 production from MKN45 and HUVEC

[B]			O.D. at 492nm (% of Control)
	(μM)	ICAM-1	E-selection
Unstimulated		1.0±0.2	0.413±0.1
Control		100±11.3#	100±2.3#
EC	0.2	70.7±1.2*	101±6.4
	2.0	71.8±2.7*	91±4.3*
	20.0	73.7±2.2*	95±4.8
EGC	0.2	78.0±1.3*	93±0.8
	2.0	81.8±2.6*	92±4.4
	20.0	77.9±6.2*	95±5.5
ECG	0.2	65.9±0.8*	93±9.0
	2.0	63.7±2.0*	94±7.9
	20.0	50.15±2.1*	90±4.7*
EGCG	0.2	56.36±2.4*	94±2.9
	2.0	62.76±1.3*	95±1.6
	20.0	64.39±0.5*	94±1.3

Effects of catechins on surface expression of adhesion molecules on HUVEC

[C]			O.D. at 492nm (% of Control)
	(μM)	CD11b	CD18
Unstimulated		25.8±5.5	55±7.9
Control		100±33.6#	100±1.0#
EC	0.2	15.9±1.8*	45±1.4*
	2.0	13.8±0.9*	39±5.5*
EGC	0.2	14.1±0.8*	56±4.2*
	2.0	30.0±7.0*	57±2.1*
ECG	0.2	16.0±21.0*	45±5.7*
	2.0	31.5±2.7*	55±5.8*
EGCG	0.2	35.5±2.7*	79±2.8
	2.0	17.5±5.3*	50±2.1*

Effects of catechins on surface expression of adhesion molecules on PMN

Data are presented as mean ± SE.
#$p<0.001$ when compared with the data of unstimulated cells
*$p<0.05$ when compared with the control

In summary, green tea (mainly catechins) can exhibit anti-aging effects by combining various properties.

Catechins and anti-aging

A direct relationship between catechins and anti-aging is difficult to prove scientifically. In this section, we summarize the three reports that might explain the direct relationship between catechins and anti-aging.

Firstly, Zhang *et al.* [12] have reported that EGCG can significantly extend the life span of *Caenorhabditis elegans* under a certain stress. More specifically, although the exposure of *C. elegans* to cold stress (35℃) reduced the life span of *C. elegans*, the addition of EGCG at 0.1, 1.0 and 10.0μg/mL extended its life span by 13.1%, 8.0%, and 11.8% respectively under the same stress (Figure 2A). Similarly,

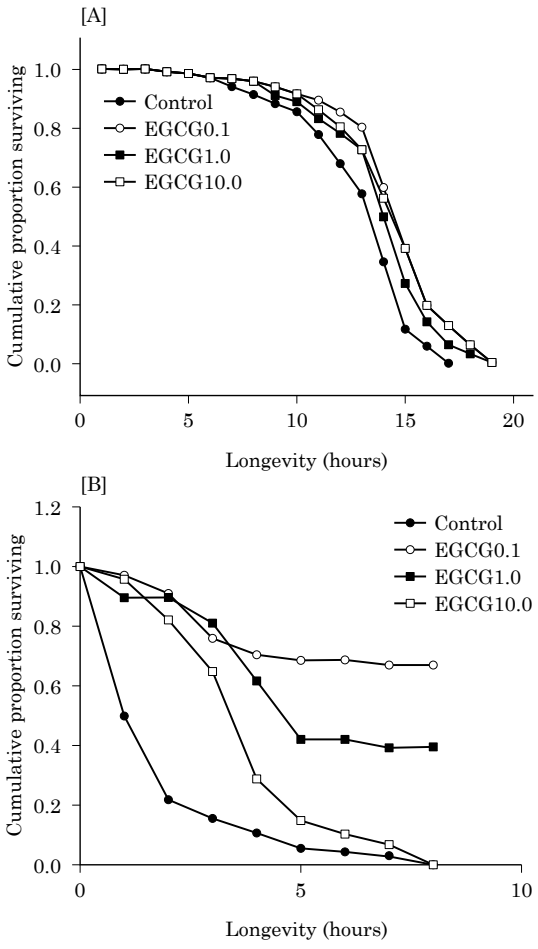

FIGURE 2: Anti-aging effect of EGCG in *C. elegans* under [A] cold- or [B] oxidative- stress
The data were processed with the Kaplan-Meier survival analysis.

the exposure of *C. elegans* with oxidative stress by adding juglone reduced the life span of *C. elegans*, whereas the addition of EGCG at 0.1, 1.0 and 10.0μg/mL extended its life span to 172.9%, 177.7% and 88.5% respectively under the same stress (Figure 2B). To further investigate the mechanism by which EGCG extends life span of *C. elegans*, these authors created a green fluorescent protein transgenic *C. elegans* with superoxide dismutase-3 (SOD3) and heat shock protein-16.2 (HSP16.2), and found that EGCG induced anti-oxidant capacity via the up-regulation of the expression of both genes. Besides these genes, EGCG caused upregulating of aging-associated genes i.e. daf-16, sod3, skn1. The authors postulated that EGCG may extend the average life expectancy in humans by protection against environmental stress.

Secondly, Meng *et al*. [13] have reported that EGCG extends the lifespan of human fibroblast cells via the regulation of mitochondrial integrity and the enhancement of the activity of anti-oxidant enzymes. Specifically, they have reported that in human fibroblasts subjected to hydrogen peroxide stimulation, EGCG at a concentration of 25μM or 50μM for 24 hours increases the gene expression and enzymatic activity of anti-oxidant enzymes e.g. catalase, SOD1, SOD2, and glutathione peroxidase, reduces the intracellular oxidative stress, and maintains the function of mitochondria. They measured the senescence-associated β-galactosidase activity, the indicator of cellular senescence, by measuring the emitted fluorescent intensity by the senescent cells (relative fluorescence unit: RFU) and the area occupied by the labeled senescent cells (Relative area), and found that EGCG did not increase the number of senescent cells (Figure 3), suggesting the favorable effect of EGCG on life extension.

Thirdly, in addition, due to the fact that the life span of diabetic patients is 7-8 years shorter than the life span of healthy people, Si *et al*. [14] investigated the life prolongation effect of EC on obese diabetic mice (db/db mice) and the *Drosophila*

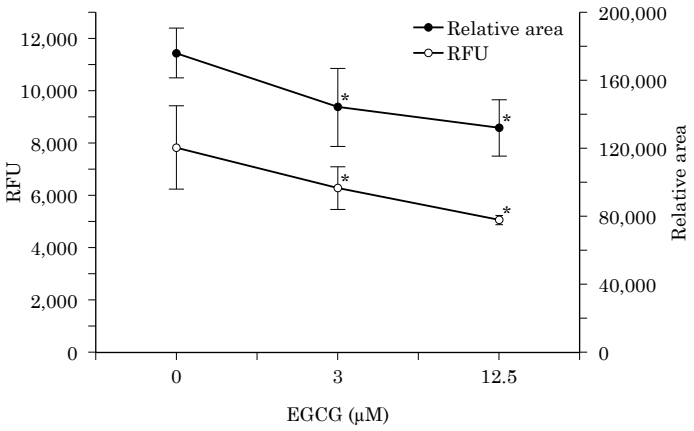

FIGURE 3: Effects of EGCG on cellular senescence of the middle-aged human diploid fibroblasts
*p<0.05, compared to the untreated group as determined by one-way ANOVA (analysis of variance)

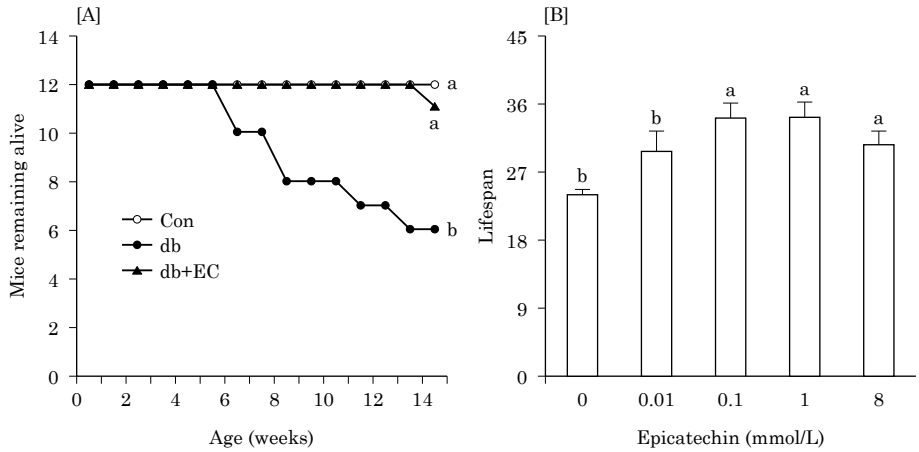

FIGURE 4: Life extending effect of EC
[A] Dietary supplementation of epicatechin promotes survival of diabetic mice.
Curves without a common letter differ, $p<0.01$
Con; control, db; diabetic mice, EC; epicatechin
[B] Mean lifespan of *Drosophila* treated with epicatechin.
Columns without a common letter differ, $p<0.05$

melanogaster. They found that EC administration to obese diabetic mice is effective to reduce the mortality rate from 50% to 8.4% after 15 weeks (Figure 4A). These effects were unrelated to the change in blood pressure, blood sugar, food intake or body weight gain. They also found that EC extends the life span of *D. melanogaster* (Figure 4B).

Conclusions

As described above, catechins have anti-aging effects in a variety of cell types and species, most of which depend on the anti-oxidant effects of catechins. There has been no definite proof that catechins directly control life span of living beings. To prove this issue, we should first clarify the precise mechanism of aging.

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10

Radioprotective Effects of Green Tea

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Abstract: Green tea contains mainly four catechins, namely epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), and non-polyphenol compounds such as vitamins that are known to be strong anti-oxidants. Biological adverse effects induced by radiation are caused by radicals and active oxygens from water radiolysis *in vivo*. In some scientific reports, radioprotective activity of green tea and its components against the adverse effects induced by ionizing radiation was demonstrated. *In vitro* tests, with green tea and its components such as catechins and vitamins, show the protective effects against the adverse damages (DNA breakage and lipid peroxidation, etc.) of irradiated cells or DNA samples. *In vivo* tests on experimental animals administered catechins, gallic acid, vitamins and caffeine exhibit various radioprotective effects, such as inhibition activities against DNA damage and lipid peroxidation, protective effect of hematological parameters and elevating effect of anti-oxidant defense systems. Some reports demonstrated the inhibitory effects of tea on the absorption of radioactive materials and how this mechanism is induced by the astringency action of green tea tannins in the mucosa of the gastrointestinal tract. From these reports, green tea, which Japanese people drink routinely, might be a useful candidate for reduction of radiation damage. This chapter provides evidence on the biological effects induced by radiation exposure and radioprotective activity of green tea.

Keywords: DNA damage, epigallocatechin gallate, radiation, radioprotective effect, vitamins

Introduction

A nuclear accident occurred on March 11, 2011, in Fukushima, Japan and large amounts of radioactive materials were unfortunately released into the environment. Air, soil, sea, river in our environment and foods were contaminated by many radioactive materials. Radiation is used for therapy of cancer; however, exposure to radioactive materials is a serious problem to human health. Radiation exposure produces free radicals and reactive oxygen species by water radiolysis *in vivo*. Induction of damage to living cells is thought to be the result of interaction of free radicals with DNA or other cellular molecules, e.g. proteins and lipids, leading to cell death or genomic instability and possibly cancer. Therefore, it is very important to establish and develop effective radioprotective methods with natural products.

Radioactive materials

We are routinely exposed by many environmental mutagens, which produce DNA damage and cause mutation or chromosomal abnormality. It is well known that these mutagens contribute toward the development of cancer (Figure 1). On

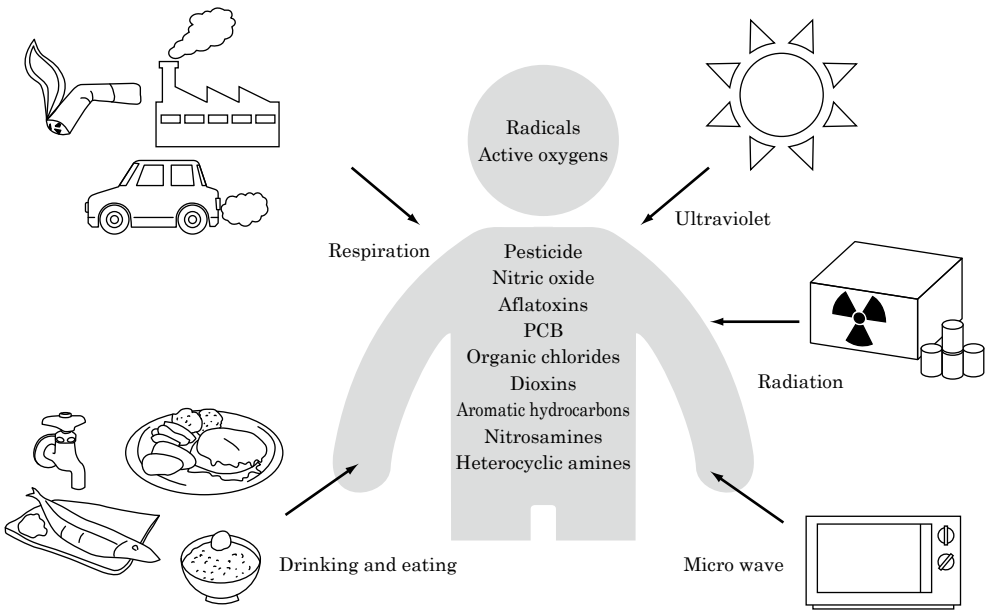

FIGURE 1: Environmental carcinogens

March 11, 2011, a tremendous earthquake occurred in northeast Japan, causing a large accident at a nuclear power plant in Fukushima. This accident led to the emission of many radioactive nuclides from the power plant, and radioactive materials contaminated not only the power plant but also a large area in our living environment, including air, soil, sea, river, foods and so on.

Radiation exposure causes serious problems to human health. There are four main types of ionizing radiation: α particles, β particles, photons (γ- or X-rays) and neutrons. The stable and unstable elements occur naturally and unstable elements give off ionizing radiation to change the physical stable state of the elements. These are known as radioactive materials, such as ^{131}I, ^{137}Cs, ^{134}Cs, ^{226}Ra and ^{238}Ra. Radioactivity is a spontaneous process by which unstable radioactive atoms decay to a different state and emit excess energy in the form of radiation. Half-life is the time required for radioactive material to decrease by one half. Each radioactive material has a unique half-life time period (^{131}I: 8 days; ^{137}Cs: 30 years; ^{238}U: 4.47 billion years). When radiation occurs, it passes through space or materials. In particular, passing radiation through a human body results in various adverse effects. Sievert (Sv) is used as the unit to estimate the degree of radiation effect against the human body [1]. If one stands close to or far from a radiological material, an exposed radiation dose expressed as Sv can be high or low, respectively. Becquerel (Bq) is used as the unit which estimates radioactive intensity (the ability of release radiation [1]) and varies according to the kind of radioactive materials.

When we are exposed to radiation, various biological effects are induced by ionizing radiation. Irradiation induces different damages to the human body on exposed body parts [1]. Skin and mucosa injury, the decrease of white and red blood cell amounts induced by bone marrow damages, and spinal cord disorder result from exposure to radiation. High-dose radiation increases the risk of death. The ionization effect of radiation affects the proteins or nucleic acids in cells and changes cell functions by loss of enzyme function. High-dose radiation causes damage to genetic material and cell death. The effects of radiation on human health include “physical effects” and “genetic effects.” The physical effects include acute injuries such as erythema, acomia and nausea, and late injuries such as cataract and cancer. Genetic effects mean passing on the damage to genes in germ cells, such as sperm or ovum, to the next generation. The effects of radiation on the human body are classified to deterministic effects and stochastic effects. The deterministic effects are caused not at a low-dose radiation but at a high-dose radiation more than a certain dose, and include acomia and cataracts. The intensity of radiation is associated with a high probability of inductions of the stochastic effects. The stochastic effects include cancer and genetic effects, and are assumed to be induced at low-dose radiation. Therefore, it is especially important to deal with the damage to genetic material in the study of biological radiation effects.

Radiation damage

Genetic effects, such as leukemia and other cancers, are particularly problematic among various biological radiation effects. Radiation attacks DNA as target site and induces DNA strand breaks and base lesions [1]. In living systems, the direct radiation action causes breaking chemical bonds of biological molecules by ionization. Water (H_2O) is an essential component for all living organisms, making up about 70% of the weight of most organisms. Radiolysis of water by ionizing radiation ionizes or excites the water molecule. The ionized water molecule is highly unstable and immediately changes to hydroxyl radical ($\cdot OH$), followed by super oxide anion or hydroxyl peroxide by the decomposition of water molecules [1].

These radicals react with biogenic constituents such as proteins, lipids and nucleic acids and impair their functions *in vivo*. This effect is described as the indirect radiation effect and induces various damages such as single or double-strand break, base lesion, sugar lesion, oxidation and hydration in genetic material. Single-strand break can be repaired accurately. However, double-strand break cannot be corrected or be repaired at the error site, and is associated with mutation and cell mortality. Radicals generated from radiation react with the site of unsaturated double bond such as 5, 6-site in pyrimidine and 7, 8 or 4, 5-site in purine, and add hydroxy group or hydrogen to these sites. Amino groups in cytosine or adenine are also oxidized by radicals to be subjected to deamination, dimer formation, *N*-oxide formation from adenine, cross-linking with protein and so on.

These gene damages are generally repaired spontaneously; however, when the amount of damages exceed the repair capacity, various adverse effects occur in the organism. Especially, hematopoietic cells and epithelial cells of the small intestine lining or the eye lens, have the short cell division cycles and are susceptible to damage by exposure to radiation. If fertilized human eggs are exposed to radiation during cell division, teratogenesis or mental delays are induced. Radiation exposure during any part of the gestation period induces carcinogenesis or genetic effects.

Recently, it was pointed out that electromagnetic waves from microwave ovens, mobile phones and personal computers are also related to the cancer occurrence or genetic effects. The details of these reports need to be examined carefully. Therefore, it is very important to prevent and control the biological effects induced from ionizing radiations for a healthy lifestyle.

Radioprotective effects of green tea catechins

Now, various functions of food ingredients and materials have received a lot of attention. The inhibitory effects that they have on the biological adverse effects induced by radiation have been reported. Especially, the effects of green tea, which Japanese people drink daily, have been brought to international attention. Green tea has various functions, such as anti-oxidative, anti-bacterial, anti-obesity, anti-mutagenic, anti-diabetic and anti-carcinogenic activities. The inductions of these functions are attributed to catechins contained in green tea. Green tea contains four catechins, such as epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), and other polyphenols, too. Because the biological effects of radiation are induced by radicals from water radiolysis *in vivo*, the radioprotective effect of green tea, which has a wide variety of anti-oxidants and radical scavengers, is now highly anticipated.

Previously, some researchers reported the radioprotective effects of catechins and other polyphenols in green tea. It was reported that the treatment of human HaCaT keratinocytes with EGCG alone suppressed cell growth and induced apoptosis in these cells, and EGCG inhibited irradiation-induced apoptosis by inactivating the caspase pathway in HaCaT cells [2]. EC showed the DNA protection effects against the damage by ionizing radiation. Under *in vitro* conditions of radiation exposure, plasmid pBR322 DNA was protected by EC in a concentration dependent manner [3]. Certain reports also demonstrated that pBR322 DNA and the splenocytes pretreated with EGCG prevented DNA strand break, the lipid peroxidation, membrane damage and DNA damage induced by γ-radiation [4].

Additionally, the inhibitory effect of black tea on radiated DNA damage was observed in pBR322 DNA, calf thymus DNA and normal lymphocytes [5]. It was reported that green tea and EGCG showed the protecting effect against DNA scission in γ or X-ray-irradiated pUC18 plasmid DNA prepared from *E. coli*. It is considered that the protecting effect of EGCG against DNA scission may be derived from the scavenging reaction of EGCG against hydroxyl radical [6, 7]. When radiation is used for therapy of oral cancer patients, salivary gland cells are damaged by exposure to radiation. So, normal salivary gland cells treated with EGCG for 24 hours were γ-irradiated to investigate whether EGCG can protect normal salivary gland cells. The result demonstrated that EGCG increased the number of surviving salivary gland cell and protected cells from γ-irradiation-induced damaged by stimulating DNA synthesis [8]. Other studies demonstrated that the number of granulocyte and erythrocyte colonies that were generated in X-ray irradiated hematopoietic stem $CD34^+$ cells decreased. However, EGCG addition yielded a 2-fold increase in the proliferation of each hematopoietic progenitor [9]. Treatment with gallic acid (GA) significantly inhibited the lipid peroxidation and DNA damage in rat liver

microsomes or plasmid pBR322 exposed to γ-radiation compared with untreated controls [10]. Oncogenic transformation in mouse C3H10T1/2 cells induced by X-rays was suppressed by addition of rooibos tea (*Aspalathus linealis*) extract, which contains many polyphenols, and the transformation incidence was similar to the spontaneous level [11]. The radioprotective effects of catechins and polyphenols can be attributed to their activities of scavenging radicals produced by radiation. The scavenging activities of EGCG against hydroxyl radical (·OH) were demonstrated by using Electron Spin Resonance [12].

Radioprotective effect of catechins or polyphenols was demonstrated *in vivo* tests with experimental animals. It was found that administration of GA to mice prior to whole-body radiation exposure reduced lipid peroxidation in the liver and brain, and cellular DNA damage in peripheral blood leukocytes using the alkaline comet assay [10]. Administration of EC to mice 1 hour prior to exposure to γ-radiation significantly protected cellular DNA against radiation-induced strand breaks in peripheral blood leukocytes in alkaline comet assay studies [3]. Administration of tea polyphenols or EGCG after γ-radiation inhibited the reduction of spleen atrophy, hematological parameters (RBC, WBC and PLT), activity of superoxide dismutase (SOD) and increase of malondialdehyde level in 28 days [13].

Administration of green tea extracts and the four main catechins before irradiation to mice resulted in an increase of jejunal crypt cell survival, formation of endogenous spleen colonies and inhibition of apoptosis in crypt cells [14]. Pre-administration with catechins before X-ray exposure inhibited the body weight loss, shortening of survival time and the decrease of peripheral white blood cells of irradiated mice [15]. Treatment with green tea polyphenols and four individual catechins prior to irradiation of mice, significantly suppressed the decline in hematological parameters (RBC, WBC, Hb), and protected the anti-oxidant defense system, as evidenced by decrease of serum lipid peroxidation (malonyldialdehyde) and elevation of the anti-oxidant enzyme SOD. EGCG showed the strongest effects in these test systems and significantly reduced the elevated serum inflammatory cytokines [16].

Administration of EGCG to mice for one month prevented radiation-induced lipid peroxides formation in liver and significantly prolonged the life span after lethal whole-body X-irradiation [17]. Radiotherapy is used in the treatment of head and neck carcinomas, but a high-dose of radiation has resulted in the damage to salivary glands and auditory conduction pathway. Peng *et al.* confirmed that the intragastrical administration of tea polyphenols for 14 days before radiation, daily, decreased the lesion and the apoptosis index in the cells of submandibular glands [18]. EC markedly attenuated the radiation-induced embryo toxicity and protected against radiation-induced loss and changes of auditory neuromast in the zebrafish. The intratympanic administration of EC was protective against radiation-induced hearing loss in the rat model [19]. Radioprotective effects were also demonstrated for the extracts of Japanese green tea, Chinese pu-erh tea and rooibos tea produced

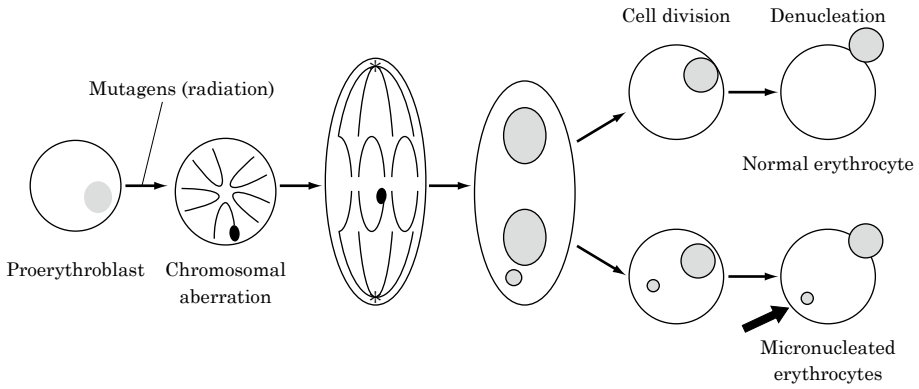

FIGURE 2: Induction of micronuclei by radiation

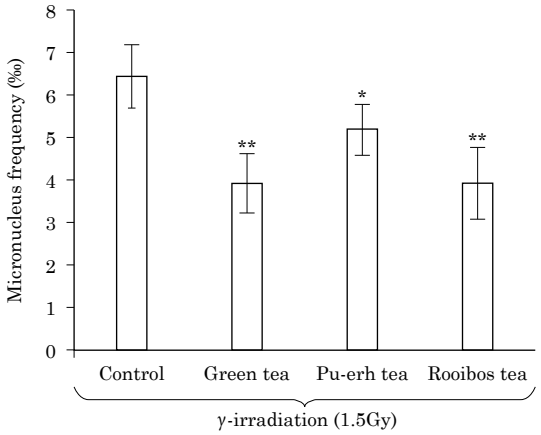

FIGURE 3: Inhibitory effects of tea extracts on the micronucleus frequency induced by γ-irradiation
*: $p<0.05$ vs Control **: $p<0.01$ vs Control

in South Africa [20, 21]. Radiation exposure to mice, induces chromosomal damage (the frequency of micronuclei) in bone marrow or peripheral blood cells. Micronuclei are produced in damaged nucleus and remain behind in the otherwise anucleated cytoplasm. An increase in the frequency of formation of micronucleated polychromatic erythrocytes in animals administered with tea extracts is estimated as chromosomal aberration induced by irradiation (Figure 2). Administration of tea extract to mice for 28 days before γ-rays exposure inhibited the formation of micronucleated peripheral reticulocytes peripheral blood cells (Figure 3). It was also reported that pretreatment of luteolin, which is a flavonoid contained in rooibos tea, to mice prior to γ-rays irradiation showed the inhibitory effects on lipid peroxidation of bone marrow and spleen [22].

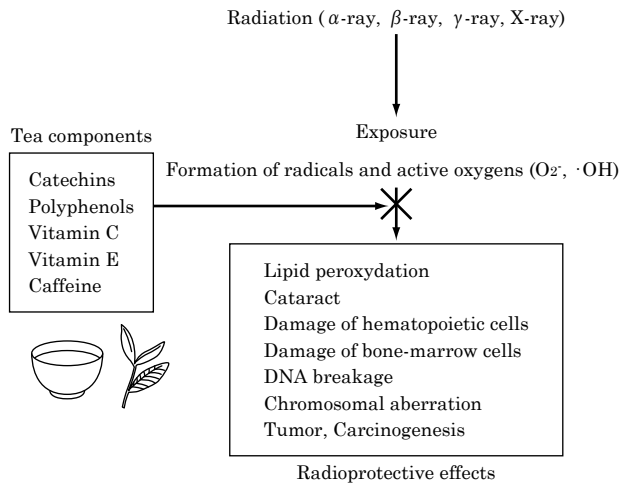

FIGURE 4: Inhibitory effect of tea components on biological effects of radiation

Radioprotective effects of non-green tea catechins

Tea contains various anti-oxidants, such as vitamin C and vitamin E, other than polyphenols including catechins. These anti-oxidants also show the radioprotective effects. *In vitro* systems, the addition of vitamin C to γ-irradiated calf thymus DNA reduced the DNA damage [23], and a vitamin E treatment before γ-irradiation decreased induction of micronuclei in human lymphocytes [24]. Other experiments reported that vitamin C suppressed γ-radiation-induced apoptosis in human peripheral blood leukocytes when vitamin C was added before γ-irradiation [25]. When human T-lymphoblastic MOLT-3 cells were treated with vitamin E prior to γ-irradiation, the amount of radiation-induced apoptosis was significantly reduced. Vitamin C alone did not show any protective effect. However, the combination of vitamin E and C reduced radiation-induced apoptosis [26].

Daily pretreatment with vitamin C before γ-irradiation exposure resulted in the elevation of mice survival numbers, the decrease of inflamed sites and augmentation of the collagen synthesis [27, 28]. Administration of the vitamin C and E to mice immediately after irradiation decreased the radiation-induced frequency of micronucleus induction [29]. Testicular weight and spermatogonia ratio of γ-irradiated mice pretreated with vitamin E were significantly higher than those without vitamin E treatment [30]. Prior supplementation with vitamin E showed the reduction of lipid peroxidation in salivary glands of mice exposed to ^{131}Iodine [31]. Pretreatment with caffeine, as a non-vitamin compound in tea, reduced the induction of micronuclei in human peripheral blood lymphocytes by radiation [32]. These results indicate that radioprotective effects of tea and its components are attributable to the radical scavenging activities of tea.

However, some researchers reported the effects of tea on the absorption of radioactive material *in vivo*. It is demonstrated that the administration of green tea (Sencha and Matcha) prior to the administration of ^{90}Sr decreased the level of ^{90}Sr in bone [33]. This mechanism of tea was induced by astringency action of green tea tannins in the mucosa of the gastrointestinal tract.

As mentioned above, tea and its components show strong radioprotective effects (Figure 4). Because we usually drink tea beverages, tea is a useful candidate for protecting against damage by ionizing radiation, and it is hoped that additional effective activities of tea will be demonstrated in this field.

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Abbreviations

67LR	67kDa laminin receptor
+C	(+)-catechin
8-OHdg	8-hydroxy-2'-deoxyguanosine
ACO	acyl-CoA oxidase
AJCN	American Journal of Clinical Nutrition
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOM	azoxymethane
apo-E	apoprotein E
APP	amyloid precursor protein
ARE	anti-oxidant response element
ASM	acid sphingomyelinase
AST	aspartate aminotransferase
ATRA	all-trans-retinoic acid
Aβ	amyloid β
BMI	body mass index
Bq	becquerel
C	(-)-catechin
CG	(-)-catechin gallate
CGMP	current good manufacturing practice
CHD	coronary heart disease
CLL	chronic lymphocytic leukemia
COX	cyclooxygenase
CPT	carnitine palmitoyl transferase
CRC	colorectal cancer
CT	computed tomography
DIT	diet-induced thermogenesis
DM	diabetes mellitus
DMF	drug master file
EC	(-)-epicatechin
ECG	(-)-epicatechin gallate
ECG3"Me	(-)-epicatechin-3-*O*-(3-*O*-methyl) gallate
eEF	eukaryotic elongation factor
EGC	(-)-epigallocatechin
EGCG	(-)-epigallocatechin gallate
EGCG3"Me	(-)-epigallocatechin-3-*O*-(3-*O*-methyl) gallate
EGCG4"Me	(-)-epigallocatechin-3-*O*-(4-*O*-methyl) gallate
EGFR	epidermal growth factor receptor
eNOS	endothelial nitric oxide synthase
ERK	extracellular signal-regulated kinase
FAS	fatty acid synthase
FDA	Food and Drug Administration
FOSHU	food for special health users
GA	gallic acid
GalN	D-galactosamine
GC	(-)-gallocatechin
GCG	gallocatechin gallate
GLUT	glucose transporter
GPDA	glycerol-3-phosphate dehydrogenase
GT	germline transcript
GTC	green tea catechin
GTE	green tea extract
GTP	green tea powder
Hb	hemoglobin
HBV	hepatitis B virus
HCV	hepatitis C virus
HDL-C	high density lipoprotein cholesterol
HGPIN	high-grade prostatic intraepithelial neoplasia

HI	hemagglutination inhibition
HIF	hypoxia-inducible factor
HSP	heat shock protein
I/R	ischemia-reperfusion
ICAM	intracellular adhesion molecule
IFN	interferon
IgE	immunoglobulin E
IGF	insulin-like growth factor
IGF-1R	insulin-like growth factor-1 receptor
IL	interleukin
IRB	institutional review board
LDL	low density lipoprotein
LDL-C	low density lipoprotein cholesterol
LPS	lipopolysaccharide
MCAO	middle cerebral artery occlusion
MCP	monocyte chemoattractant protein
MDCK	Madin-Darby canine kidney
MM	multiple myeloma
MMP	matrix metalloproteinase
MRLC	myosin regulatory light chain
M-SHRSP	malignant stroke-prone spontaneously hypertensive rat
MYPT	myosin phosphatase target subunit
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NCI	National Cancer Institute
NEFA	non-esterified fatty acid
NO	nitric oxide
Nrf2	nuclear factor erythroid 2 related factor
OR	odds ratio
ORAC	oxygen radical absorbance capacity
PBMC	peripheral blood mononuclear cell
PDE	phosphodiesterase
PET	polyethylene terephthalate
PGN	peptidoglycan
PI3K	phosphatidylinositol 3-kinase
PK	pharmacokinetic
PKC	protein kinase C
PMN	polymorphonuclear neutrophil
PP	protein phosphatase
RAR	retinoic acid receptor
RBC	red blood cells
RCT	randomized controlled trial
RFU	relative fluorescence unit
ROS	reactive oxygen species
RSV	respiratory syncytial virus
RTK	receptor tyrosine kinase
SAM	senescence-accelerated mouse
SCD	stearoyl-CoA desaturase
SFA	subcutaneous fat area
sGC	soluble guanylate cyclase
SOD	superoxide dismutase
Sv	sievert
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TFA	total fat area
TG	triacylglycerol/triacylglyceride
TLR	Toll-like receptor
TNF	tumor necrosis factor
Tollip	Toll-interacting protein
TRAMP	transgenic adenocarcinoma of the mouse prostate
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VFA	visceral fat area
VLDL	very low density lipoprotein
WBC	white blood cells

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