Protective Effects of Tea on Human Health

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Protective Effects of Tea on Human Health

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Preface

Protective Effects of Tea on Human Health

The ancients have used tea for its health benefits for 5000 years. In China tea was known for its healing powers in 2737 BC. Tea has been likened to a group of medicaments in Ayurveda, the ancient Indian system of medicine, known by the name 'rasayanas' that confer attainment of positive health, resistance to diseases and assured full lifespan of quality living, unlike drugs that cure after disease has struck. Recent clinical data have confirmed that tea has beneficial effects on the senescent brain and prolongs the human lifespan by preventing physiological ageing processes caused by oxidative stress. However, since its introduction in the Western world, tea has become known more as a beverage that relaxes the tired body and mind, brings cheer and is the centrepiece of social gatherings. With the advent of diseases related to modern living, the interest in the health benefits of tea amongst consumers and scientists alike is again increasing.

In earlier times, tea was given to provide relief from allergies, common colds, oedema and gastroenteritis, and was known to improve oral hygiene and intestinal microflora. Detailed research work on health-related aspects of tea is of recent origin and is engaging the attention of scientists in developed countries such as Japan and the USA and in the European Union. To study the health benefits of tea on humans a number of approaches have been adopted. Using epidemiological techniques and comparing tea drinkers with non-drinkers in cohort studies over a long period of time, it was found that tea reduces the risk of several major premature killing diseases, many of which are related to luxurious lifestyles and polluted environments. These include cancer, arteriosclerosis and cardiovascular diseases, neural and obesity problems, diabetes, diseases of the kidneys and liver, pulmonary ailments, flu, SARS and even AIDS. In addition, clinical and laboratory studies have been conducted using animal models, human volunteers and cell studies, as well as other *in vitro* approaches, providing an understanding of the mechanism of action of tea and its constituents against these diseases and their metabolism in human tissues and plasma.

Tea leaves contain a number of bioactive molecules, responsible for health-promoting properties of tea. Flavonoids, the most prominent of which are catechins and their derivative polyphenols, are the most abundant, biologically most reactive molecules and are responsible for most of the health-giving properties of tea. Other bioactive molecules are amino acids like theanine (unique to tea), proteins, caffeine, vitamin C, carbohydrates, polysaccharides and lipids. In the cells of a tealeaf, catechins reside in the cell sap while

oxidative enzymes are located in the cell wall. Not many know that the same green leaf is processed into green, black or oolong tea by controlling biological oxidation, which is wrongly termed 'fermentation' – wrongly because in common parlance the word fermentation connotes microbial production of alcohol, which is forbidden in Islam. For green tea, oxidative enzymes are killed by application of heat by roasting or steaming before processing. For black tea, catechins and oxidative enzymes are mixed by rolling flaccid, withered leaves prior to biological oxidation (misnomer 'fermentation') for almost one hour, followed by processing. Oolong tea is semi 'fermented' i.e. catechins are allowed to oxidize for about 45 min. However, all types of tea contain polyphenols or catechins and their derivatives which impart health benefits to tea. When ingested, tea polyphenols are absorbed in the human system and move to various organs. Often these polyphenols are transformed to different metabolites.

This and the next two paragraphs indicate the mechanism of protective action of tea constituents against specific ailments. Inappropriate diets and smoking generate high levels of reactive oxygen species (ROS) such as peroxides in humans, which are the basic cause of heart diseases. Tea polyphenols have strong scavenging properties that destroy free oxygen radicals, thus lowering the risk of heart ailments. Cancer is the result of uncontrolled proliferation of cells mutated by oxidative stress or carcinogens in food and the environment. Tea polyphenols induce type I and type II enzymes such as glucuronyl transferase which detoxify carcinogens, inhibiting cancer initiation or angiogenesis of cancer tumours. Polyphenols suppress the development of existing neoplasms and even regress tumour growth through death of tumour cells (apoptosis). Skin cancer can be prevented by topical application of catechin-fortified skin cream after exposure to ultraviolet sunrays, rather than used as sunshade before sunbathing, as is required with other sunburn protective lotions.

Catechins, particularly EGCG (epigallocatechin), interact with alpha-amylase in human intestines, to suppress glucose uptake by inhibiting the sodium-dependent glucose transporter SGLTI mechanism, thus preventing diabetes. Kidney diseases are the result of free radical-induced oxidative stress: tea catechins relieve high oxidative stress by inhibiting the production of oxidative uraemic toxins, improve renal blood circulation and are effective in easing the pain caused by renal diseases. Osteoarthritis is also prevented by tea polyphenols through inhibition of the production of catabolic mediators implicated in the progression of arthritis.

Catechins ameliorate beta-amyloid neurotoxicity, which helps in the management of Alzheimer's disease. Dementia, which is the next stage of the disease, is caused by neuronal death: it is prevented by the green tea components theanine and catechin, both of which show a neuron-protective effect in transient ischaemic neuronal death and prevent cerebral stroke. L-Theanine in tea has been reported to generate interferon, which effectively controls flu-like symptoms of the common cold. Viral entities causing influenza and SARS lose their infectivity when tea catechins interact with glycoproteins on the viral surface and render them non-infective. Antiviral and antibacterial properties of tea reduce infections of the respiratory tract and lungs, and prevent pulmonary diseases. Tea also raises the basic metabolic rate so that at equal food intake and exercise levels, body weight is reduced for those who drink 8–10 cups of tea a day. The Japanese are great tea drinkers, which possibly explains why fewer Japanese are obese. Tea polyphenols are also important in oral hygiene. The mutan group of Streptococci bacteria produce GTF enzyme, leading to synthesis of a sticky glucon polymer that forms a plaque, accumulating 300 species of microbes in a matrix, which firmly adheres to tooth surfaces. Black tea polyphenols inhibit cariogenic (causing dental caries) microflora, glucon synthesis, dental plaque formation and enamel eroding acidity, which ultimately lead to periodontal diseases and even loss of teeth.

Based upon the multiple mechanisms of action, it is clear that tea intake is beneficial to human health for preventing various lifestyle ailments. Tea packets sold in sophisticated markets already carry health-related information for catering to the growing interest of consumers. This monograph, conceived in January 2002, envisages meeting the long felt need of the consuming public to be informed of the scientifically supported data on health benefits of tea drinking. In addition, it was meant to inform tea scientists about the status of research in their areas of interest. The book also provides valuable information on the therapeutic value of tea against different diseases, which should appeal to the general public as well as to professionals in various disciplines in the tea industry including those responsible for decision making in the field of public health. This monograph should find an important place in public libraries and scientific institutions.

The editors
26 January, 2006

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1 Tea is a Health-promoting Beverage in Lowering the Risk of Premature Killing Chronic Diseases: a Review

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Abstract
Green and black tea are widely used beverages, second only to water. Tea is an extract of the leaf of the plant *Camellia sinensis*. The major health benefit of tea is that the leaf contains several polyphenols, such as epigallocatechin gallate, and in addition an enzyme, polyphenol oxidase. If upon harvest the leaves are heated, these enzymes are deactivated, and thus, after drying and grinding, the result is green tea. If the leaves are ground and incubated in air at about 40°C, the polyphenol oxidase converts the original polyphenol to a number of other products, such as theaflavin and thearubigins. Upon drying, these are the polyphenols typical of black tea. In most instances, the polyphenols from green and black tea have similar properties in health promotion. Coronary heart disease stems from the oxidation by reactive oxygen species of low-density lipoprotein (LDL) cholesterol. The tea polyphenols are reported to inhibit this reaction. There are data for humans that tea drinkers have a lower risk of heart disease. Similar findings were made with animal models. In the field of cancer causation, we distinguish between genotoxic carcinogens, affecting DNA and genes, and other steps associated with the development of cancer, in part also involving reactive oxygen species. Tea and its polyphenols induce certain enzymes, such as glucuronosyl transferase, that detoxify carcinogens. Thus, tea drinkers have a lower risk of cancer and the mechanisms of these reactions have been explored in animal models and through *in vitro* approaches. Of importance also is that tea decreases the growth of neoplastic cells, but not of normal cells. Tea also enhances apoptosis, a phenomenon of elimination of cancer cells. Tea drinkers also have a healthier intestinal flora, through the inhibition of bacteria that have adverse effects and promotion of the growth of beneficial bacteria. Through the elimination of reactive oxygen species, associated with premature ageing, tea drinkers display good health to an old age.

Keywords
Green tea, black tea, polyphenols, reactive oxygen species, heterocyclic amines, coronary heart disease, cancer, ageing, inhibition, health promotion, disease prevention.

Main Causes of Chronic Diseases

Mainly based on cancer research we have learned to distinguish between agents that modify DNA and generate mutations. Such materials or synthetic chemicals are called mutagenic and genotoxic. On the other hand, there are chemicals or situations that enhance

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the development and growth of lesions initiated through a genotoxic modification of cells. We have learned to distinguish between these two classes of chemicals based on the permanence of their effect and the doses and continuity of exposure needed for their action.

Reactive oxygen species

Living cells require oxygen to generate energy and to develop fully. However, under some circumstances cells generate reactive oxygen species (ROS) in the form of reactive entities such as hydrogen peroxide and especially hazardous oxygen radicals.

Heterocyclic aromatic amines

This class of chemicals was discovered about 25 years ago when Dr Sugimura, at the National Cancer Center Research Institute in Tokyo, wondered about the mechanisms of the browning reaction when cooking, that is, frying or grilling meat and fish. His laboratory showed that the surface of brown meat contained powerful mutagens. At that time, it was thought that mutagens were likely cancer-causing agents, which subsequently were shown to be genotoxic. In part with our collaboration, such cooking-derived mutagens were shown to belong to a new class of carcinogens, the heterocyclic aromatic amines. When they were ingested, this class of chemicals also generated ROS and damaged cells not only through reaction with DNA but also through a cellular toxic reaction, to which the cells responded by attempts to repair, leading to cell regeneration and duplication. Thus, a cell with altered DNA subjected to forced growth would regenerate numerous abnormal cells with abnormal DNA, typical of an early cancer cell.

Tea as a Health-promoting Beverage

Tea is a drink frequently used by people worldwide. It is an extract of the leaves of the plant *Camellia sinensis*. Upon harvest, the best teas are obtained by plucking the top two leaves and the bud of the tea bush. The leaves contain the polyphenol epigallocatechin gallate and an enzyme, polyphenol oxidase. When the leaves are withered (dried) and steamed, the polyphenol oxidase is inactivated and the result is green tea. When the leaves are processed (crushed) and incubated for about 60 min, the polyphenol oxidase converts the polyphenol to other polyphenols, such as theaflavin and thearubigin, typical of black tea. A lesser incubation time, such as about 25–35 min, yields an intermediate product, oolong tea, which is popular in southern China and Taiwan. We have described the history of tea and its use worldwide, including the original discovery of tea in China some 4000 years ago, in the form of green tea, and later of black tea in northern India. Currently, green tea is used mainly in China, Japan and North Africa (Weisburger, 2000).

Tea and heart disease prevention

Epidemiological studies in Europe revealed that drinkers of black tea had a lower incidence of heart disease. The underlying reason rests on the fact the tea polyphenols act as effective antioxidants, inhibit the oxidation of low-density lipoprotein (LDL) cholesterol caused by reactive oxygen species and lead to atherogenesis. It has been shown that the mechanism can be reproduced using copper-catalysed oxidation of LDL cholesterol, inhibited by tea polyphenols. A number of investigations confirmed a lower risk of heart disease as a function of tea intake. A meta-analysis of stroke and coronary heart disease – cardiovascular disease – evaluated the results of many studies and found that heart disease was decreased by 11% with the intake of three cups (about 700 ml) of tea per day. In Japan, it was noted that the relative risk of cardiovascular disease and cancers was significantly lower with ten cups of tea per day. One explanation is that tea also beneficially affects platelet aggregation, a possible cause of heart attacks that are prevented by tea.

Tea and cancer prevention

Mutations of cellular DNA are a key step leading to cancer. Mutational events can be used as markers for environmental genotoxic products that might be possible cancer risks. This approach is effective in research on products that might have antimutagenic and thus likely anticarcinogenic effects. These methods have been applied to study the effect of tea polyphenols from black tea and from green tea. It was found that both types of polyphenols decreased in a dose-related fashion the mutagenicity of different types of carcinogens. In parallel, tea inhibited the formation of cancer of the colon and the mammary gland in rats. Other investigators have found that cancer of the oesophagus is decreased in animal models by tea, just as a lower risk is noted in parts of China of cancer of the oesophagus in people who drink tea. There are more cigarette smokers in Japan than in the USA but the incidence of lung cancer in Japan is lower than in the USA, possibly because there are more tea drinkers in Japan, accounting for this protection. In parallel, mice and rats exposed to the tobacco-specific nitrosamines displayed a lower incidence of lung tumours when the animals were drinking tea. My colleague, Chung, has found that this inhibition by tea was due to lower oxidation of DNA, through the tobacco carcinogen-associated formation of reactive oxygen species, yielding as marker 8-OH-dG.

We described above the formation of powerful mutagens during the frying or grilling of meat, that is heterocyclic aromatic amines. There are epidemiological findings that regular consumers of properly cooked meat have a higher risk of cancer of the colon and breast. These are the target organs in rats, where cancer of the prostate and of the pancreas is also seen. The reason meats generate these kinds of compounds was discovered by a Swedish colleague, Jägerstad, namely meats contain creatinine, which forms the 2-aminomethylimidazo part of the heterocyclic amines. Jägerstad developed an *in vitro* approach to model cooking, namely heating glucose, creatinine and an amino acid, such as glycine or phenylalanine. We have found that addition of black tea or green tea polyphenols to this *in vitro* system prevents the formation of heterocyclic amines. Also, based on that experiment, we have shown that addition of green tea or black tea polyphenols during the frying of ground (minced) meat prevents the formation of mutagenic heterocyclic amines, which seems to be a practical way to cook hamburgers without loss of taste.

Tea induces detoxification enzymes

The administration of 2% solutions of black tea or green tea to rats for 6 weeks modifies the metabolic enzymes in the liver, namely such rats display higher levels of cytochrome P450 1A1, 1A2 and 2B1, but of no other cytochromes. In addition, the Phase II enzyme uridine diphosphate (UDP)-glucuronosyl transferase, which detoxifies many environmental chemicals, was significantly increased (Embola *et al.*, 2001). Heterocyclic amines, described above, are subject to biochemical activation through *N*-hydroxylation, and these *N*-hydroxy compounds are converted to *N*-hydroxy glucuronides. Since green and black tea increased the available UDP-glucuronosyl transferase, it was observed that tea-drinking animals form the detoxified metabolites of heterocyclic amines. Earlier, we reported that decaffeinated tea was less effective than regular tea in carrying out these reactions. Thus caffeine may have a role, most probably together with the tea polyphenols.

Control of growth and apoptosis by tea

Several investigators reported that tea and tea polyphenols decrease the rate of growth of tumour cells through mechanisms involving alterations in gene expression. Tea even inhibited the formation of spontaneous lung tumours in A/J mice, which we reported in 1966 to have a stable incidence. Thus the growth control effect of the polyphenols is remarkable. In addition, tea polyphenols

increase the rate of apoptosis (cell death) of tumour cells and lead to their elimination. Inhibition of angiogenesis may play a role (cell-to-cell communication). This mechanism may hold even during tumour development. A clinical trial with a polyphenol from green tea is under way through the Division of Cancer Prevention at the National Cancer Institute, USA.

Intestinal microflora is improved in tea drinkers

Tea polyphenols are reported to affect microflora, including viruses and bacteria. Enterobacterioaceae in the intestinal tract have mostly unpleasant properties since they generate malodorous chemicals such as skatole and related indoles. The tea polyphenols suppress the growth of these bacteria but have no adverse effects on beneficial bacteria such as lactobacilli. Therefore, regular tea drinkers have a healthier intestinal bacterial flora. Tea polyphenols have antiviral actions, as described in detail in the monograph of Hara (2001) and by Hara in Chapter 3 of this volume.

Effect of tea polyphenols on reactive oxygen species and ageing

Tea and tea polyphenols suppress the reactive oxygen species formed during metabolism of cell systems. It was noted above that the oxidation of LDL cholesterol and of DNA may stem from reactive oxygen. Premature ageing is also a result of cellular reactive oxygen species. It can be concluded that regular intake of five or more cups of tea per day facilitates healthy ageing, which has been demonstrated on cellular systems, in animal models and also through studies of humans where regular tea intake is part of a health-promoting lifestyle, as in Japan and India. In that part of the world, one does find populations at advanced ages in good health.

Summary

Basic biomedical research on the mechanisms of action of tea has uncovered key elements of the health-promoting effects of tea (Table 1.1). When consumed as a warm (not boiling hot) or cold beverage at 1.5–2% concentration at five to ten cups per day – about 1000–1200 ml – it is an

Table 1.1. Mechanisms of action of tea on parameters bearing on improved health.[a]

Action of tea and tea polyphenols	Effects
1. Displays potent antioxidant activity	Lower risk of heart disease; decreased formation of reactive oxygen species and of oxidized nucleotides in DNA and possibly inhibition of carcinogenesis. Improved chance of healthy ageing
2. Selectively induces Phase I and II metabolic enzymes	Increased formation of detoxified metabolites of carcinogens and of chemicals generally
3. Inhibits cell proliferation rates; increases apoptosis	Decreased growth or death of abnormal cells and neoplasm
4. Increases basal metabolism rate	At equal energy intake and exercise, provides basis for control of obesity and excessive body weight
5. Selectively modifies intestinal bacterial flora	Displays some antiviral actions. Undesirable components of the flora replaced by beneficial bacteria, with improved metabolic profiles. Has demonstrated action against influenza virus. Possible role against SARS virus under investigation (2003)

[a]Other vegetables and soy foods may operate through similar mechanisms.
SARS, severe acute respiratory syndrome.

inexpensive, pleasant, tasty means of filling partially the daily required fluid intake, 2–2.5 l per day for adults. When desired or by tradition in some countries, no more than the International Organization for Standardization (ISO) recommended amount of about 2% milk has been shown not to have an adverse affect on the health-promoting properties of tea. Even 5–to 14-year-old children might consume five cups of calorie-free warm or cold decaffeinated tea, instead of high-sugar beverages. After age 14, regular tea may be indicated. Tea is rich in the beneficial polyphenols and similar components that can supplement the recommended five to ten vegetables and fruits per day.

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2 Tea as a *Rasayana*[*]

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Abstract

Rasayanas are a class of natural products that facilitate attainment of positive health, improve resistance to diseases of varied aetiology and promote longevity by slowing the ageing process. They can be food items or drugs and constitute a separate branch of *Ayurveda* distinct from *Kaya Chikitsa* (medicine). Some of their activities can be evaluated in animal models but others require long-term clinical trials or clinico-epidemiological studies on large cohorts, especially in the case of food items. A review of the available clinical and experimental data indicates that tea constituents exhibit most of the properties ascribed to *Rasayanas*. It is recommended that the tea preparations should be promoted as *Rasayanas* rather than as just another beverage or even health food.

Introduction

The health-giving properties of tea have been increasingly recognized during the last two decades. In China, tea was used for its curative value for thousands of years before being introduced as a refreshing beverage. In Japan also, it was introduced for its asoporific properties for Buddhist monks, who also use it for its medicinal values. In India also the health-giving properties of tea are gradually being accepted.

Tea is not a drug. It is a health food, akin to *Rasayanas* known to the ancient Indians. A health food has something positive to offer. It can prevent disease(s). Drugs, on the other hand, are generally taken after a disease process has set in. The drug industry, like other industries, is basically profit-driven. Its profits depend on the disease burden and hence this industry would be much less interested in promoting disease-preventing agents. These would take away their main source of business.

Secondly, the highly competitive drug industry survives on strong patents. These will not be easy to obtain on tea or its constituents. The present review is confined to comparing the health-giving properties of tea with the class of agents known as *Rasayanas* in the ancient Indian medicinal system *Ayurveda.*

What are *Rasayanas*?

It will be necessary to recapitulate briefly the basic tenets and objectives of *Ayurveda*

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to place the activities of *Rasayanas* in a proper perspective. The *Yajurveda* (36/24*) has stated that the aim of *Ayurveda* is not necessarily to ensure a lifespan of 100 years but to enable living 100 years fully and actively. Thus, there is a built-in concept of quality of life. *Rasayana* therapy forms one of the eight main branches of *Ayurveda*, and *Charaka* (Su 30/20) separates *Rasayanas* from medicines (*Kaya Chikitsa*). *Agnipurana* (141/6) also classifies *Rasayanas* as *Sarvarogahari Aushadhi*, thus putting them in a separate class of medicaments.

Rasayana drugs have been defined in Ayurvedic texts in various ways. All the definitions, however, convey the concept of attainment of positive health, increased resistance to diseases and assured longevity. They are often confused with the modern adaptogens. *Rasayanas* have a much broader spectrum of activity, and adaptation to environmental stress is only one of them. *Sushruta* (Su 1/7) says that they re-establish youth, strengthen life and brain power and provide the capability to counteract diseases (Ray *et al.*, 1980). *Sharangdhara* (2/32) offers a more restricted definition, describing them as substances capable of destroying diseases of old age. *Agnipurana* (141/6) improves on this by stating that they cure all illnesses and bestow immortality (Pandey, 1997). *Charaka*, however, provides the most comprehensive definition. The *Charaka Samhita* (CS7,8) says that *Rasayana* drugs lead to fulfilment of life as a whole. They prolong lifespan, ensure a disease-free youthful life with good vigour and control of bodily functions, resounding voice and a glowing complexion. The treatise has classified 34 plants as *Rasayanas*, some of them being *Ayasika* (dietary) *Rasayanas*. The purpose of the present chapter is to evaluate available data on tea and its constituents and to see if tea fulfils the criteria of a dietary or classical *Rasayana*.

Major Activities of *Rasayanas* Amenable to Laboratory Evaluation

It is evident from the above discussion that *Rasayanas* exhibit multiple activities. Hence it is necessary to employ a battery of tests to uncover their activity profile. Three major activities of *Rasayanas* can be evaluated in animal models. These are:

1. Effect on general bodily and mental functions.
2. Altered resistance to diseases caused by stress, toxins, infections, xenobiotics, etc.
3. Effect on ageing and lifespan.

Several parameters have been used to evaluate these activities. These can be grouped under the following six headings:

- Actions on immune system.
- Response to stress.
- Mental activity.
- Selected physiological functions.
- Regeneration following injury.
- Ageing.

It will be beyond the scope of the present chapter to describe the methods used to screen for these activities in laboratory animals. The necessary details can be obtained from publications on screening models (Nodine and Seigler, 1964; Seigler and Moyer, 1967; Turner, 1971; Dhawan and Srimal, 1998).

Comparison of Activities of Tea and *Rasayanas*

Rasayanas re-establish youth, strengthen life and brain function and provide the capability to counteract diseases (*Sushruta*, Su1/7). The Chinese scholar Lu Yu, in a treatise written in AD 780 and entitled *Cha Ching*, states that 'tea tempers the spirits, harmonizes the mind, dispels the lassitude, relieves fatigues, awakens thought, prevents

*The numbers in parentheses after Ayurredic texts refer to the particular verse in the original Sanskrit text containing that information and not the page number. The year of publication in the reference relates to the edited/translated version of the book.

drowsiness, refreshes the body and clears the perspective faculties' (Jhawar, 2000). The similarities are apparent even from these early descriptions. A comparison of their effects in the experimental paradigms mentioned above and some clinical data should enable a better assessment of the similarities.

Response to stress

Rasayanas modulate responses to all types of stress – physical (Ramachandran *et al.* 1990), chemical (Dhawan, 1995), microbiological (Dahanukar *et al.*, 1986) or endogenous, such as cancer (Seena *et al.*, 1993; Dhuley, 1997).

The effect of tea and its constituents has not been systematically investigated on responses to various types of stress. There are reports of many clinical and experimental studies, however, from which data can be extrapolated. These are summarized below under four headings:

1. Physical stress. Reduction of sunburn and DNA damage following ultraviolet exposure of skin following local application of green tea polyphenols has been reported in a study on human volunteers (Elmets *et al.*, 2001).

2. Chemical stress. Effects of tea constituents have been studied on non-specific as well as specific chemotoxins. In the first category, special mention may be made of the prevention of mutagenic effects of carcinogens and pro-carcinogens (Yamada and Tonita, 1994). The effect against specific toxins has been studied in several tissues. Liver is protected against damage by galactosamine, aflatoxins, lipopolysaccharides, etc. (He *et al.*, 2001). The DNA adduct formation by nitropropane is also inhibited (Shukla and Arora, 2002). Renal failure induced by adenine or c-BSA is prevented by green tea polyphenols (Yokozawa *et al.*, 1996a) and a beneficial effect has been reported in patients on renal dialysis (Yokozawa *et al.*, 1996b). Hot-water extract of green tea prevents bone marrow damage by aflatoxin B_1 (Shukla and Arora, 2002). Amelioration of β-amyloid neurotoxicity is seen in cultured hippocampal cells (Choi *et al.*, 2001). Kim *et al.* (2001) have observed selective inhibition of prolyl endopeptidase by (–)-epigallocatechin gallate, (–)-epicatechin gallate and (–)-gallocatechin obtained from green tea. These properties may find application in the management of patients suffering from Alzheimer's disease. Similarly, the protective effect of tea catechins against 6-hydroxydopamine-induced apoptosis in PC-12 cells (Jin *et al.*, 2001) suggests possible utility in patients with Parkinson's disease.

3. Microbiologial stress. Tea extracts have been reported to inhibit the growth of many viruses (including human immunodeficiency virus (HIV)), mycoplasma, fungi, protozoa and Gram-positive as well as Gram-negative bacteria (Miller and Taylor, 2001). They can also suppress the emergence of resistance to antibacterial agents (Pillai *et al.*, 2001) and potentiate the activity of β-lactam antibiotics. Theasinensin A suppresses antibiotic resistance of methicillin-resistant *Staphylococcus aureus* (Hatano *et al.*, 2003). A synergistic effect is observed with some antibacterial agents. This has recently been reported for the effect of leofloxacin against *Escherichia coli* (Isogai *et al.*, 2001). The protection against causative organisms of dental caries is well documented (Banerjee, 1990).

4. Response to endogenous stress. Maximum information is available against several types of cancer (Katiyar and Mukhtar, 1996). A large amount of epidemiological, clinical and experimental data has been accumulated during the last two or three decades. For example, there is significantly lower incidence of lung, digestive tract and skin cancer in communities with a high consumption of tea, particularly green tea (Bushman, 1998). Green tea has been reported to have anti-cancer activity in certain experimental models as well (Oguni *et al.*, 1988). Tea flavonoids can inhibit urethane- and NNK- (a nicotine-derived carcinogen) induced pulmonary neoplasm (Chung, 2001), as well as the induction of apoptosis and have an anti-clastogenic effect in experimental models of leukaemia, gastric carcinoma, etc. (Katsuno *et al.*, 2001). *In vitro* studies have demonstrated the ability of polyphenols to reverse multidrug resistance in cancer cell lines (Zhu *et al.*, 2001) and

synergistic activity with cancer-preventing agents, such as genistein, sulindoc, curcumin, etc., and with anti-tumour agents, such as doxorubicin.

Effect on the immune system

Rasayanas have immunostimulant properties (reviewed by Agarwal and Singh, 1999). Some of them, in addition, exhibit anti-allergic activity (Baruah *et al.*, 1998).

Tea extracts and constituents also share some of these properties. Some pertinent experimental and clinical data are summarized below:

1. Melanin-like pigment from black tea has immunostimulant activity (Sava *et al.*, 2001).
2. Black tea can reverse Ehrlich's ascites carcinoma (EAC)-induced immunosuppression (Bhattacharya *et al.*, 2002).
3. Anti-allergic activity. Tea catechins inhibit passive cutaneous anaphylaxis (PCA) in rat and mouse (Kar *et al.*, 1981) and autoimmune disease in MRL-fas/fas mouse (Sayama and Oguni, 2001). They suppress expression of the high-affinity immunoglobulin E (IgE) receptor Fcε (Fujimura *et al.*, 2001). Green tea polyphenols have a beneficial effect in a guinea pig allergic rhinitis model (Juneja, 2001). Kunishiro *et al.* (2001) have shown facilitation of antigen-specific antibody production through selective augmentation of interleukin-2 (IL-2) generation *in vitro* as well as *in vivo* by tea extracts.
4. Clinical data. Oolong tea has a beneficial effect in patients with atopic dermatitis and allergic rhinitis (Uchara *et al.*, 2001). There is an improvement in the CD4/CD8 ratio with green tea (Tsuboi *et al.*, 2001).

Effect on physiological functions

Rasayanas improve physical performance and stamina (Grover *et al.*, 1995), optimize food utilization (Pushpangadan *et al.*, 1995), are potent antioxidants (Rastogi *et al.*, 1995) and can restore disturbed carbohydrate and lipid metabolism (Khanna *et al.*, 1994).

Tea extracts and constituents share many of these properties. Massive amounts of data are available on some of these activities with products of tea. A brief summary of the major activities is included in the present review under six headings:

1. Work performance. Tea extracts facilitate skeletomotor function by action on L-calcium channels and are known to alleviate post-game fatigue in athletes (Pushpangadan and Latha, 2002). They have been given to horses in Tibet to increase their capacity to work (Emboden, 1979).
2. Food intake and body weight. Green tea ethanolic extract inhibits gastric and pancreatic lipases and stimulates thermogenesis. It exerted a weight-reducing effect in clinical studies in moderately obese patients (Chantre and Lairon, 2002). Intraperitoneal administration of (–)-epigallocatechin-3 gallate produced a reversible 20–30% reduction in body weight in 2–7 days, due to a reduction in food intake (Kao *et al.*, 2000). An anti-obesity effect has been reported in female mice also. More data are required to evaluate this effect.
3. Carbohydrate metabolism. Hypoglycaemic activity has been reported in a variety of experimental models, including normal, streptozotocin, fructose or alloxan diabetic rats and KKA mice (Sugahara *et al.*, 2001). Aldose reductase activity is inhibited in streptozotocin diabetic rats, thereby slowing the progress of nephropathy and cataract (Sakai *et al.*, 2001). The secretion of insulin is not affected in diabetic animals (Sugahara *et al.*, 2001). It did, however, cause a more than 15-fold increase in sensitivity to insulin in an *in vitro* epididymal fat cell assay. The main active ingredient was (–)-epigallocatechin gallate (Anderson and Polansky, 2002). A lowering of blood glucose has also been reported with a polysaccharide from tea that is coordinated with rare earth metals (Wang *et al.*, 2001). Clinically, lowering of blood sugar and haemoglobin A_1C (HbA_1C) (Fukino *et al.*, 2001) and slowing the progress of nephropathy in patients with diabetes mellitus have been reported (Takano *et al.*, 2001).

4. Lipid metabolism. Yang *et al.* (2001) have reported a reduction in raised levels of serum cholesterol and triglycerides, fat storage in the liver and heart and in the weight gain in rats on a high-sucrose diet. Catechins prevent atherosclerosis in hamsters on a high-fat diet (Vinson *et al.*, 2001) and in apoprotein E-deficient mice by inhibiting activity of P-glycoprotein (Miura *et al.*, 2001). Cholesterol biosynthesis is prevented by selective inhibition of squalene peroxides (Abe *et al.*, 2001). Clinical data supporting hypolipidaemic activity of tea constituents include lower levels of serum cholesterol low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), along with a raised level of high-density lipoprotein (HDL), reduction in the atherogenic index (Imai and Nakachi, 1995) and lowered level of the adhesion molecule P-selectin (Hodgson *et al.*, 2001). Green tea extract enhances neutral endopeptidase activity in SK-N-SH cells, thereby preventing the formation of amyloid plaques (Melzig and Jaika, 2003). Oolong tea suppresses oxidation of LDL in a dose-dependent manner (Kurihara *et al.*, 2003).

5. Detoxification mechanisms. Tea polyphenols induce xenobiotic detoxifying enzymes, such as cytochrome P450 $1A_1$, $1A_2$, $2B_1$ and uridine diphosphate (UDP)-glucuronyl transferase (Weisburger, 2002). They can also prevent heavy metal toxicity by chelation (Liao *et al.*, 2001).

6. Antioxidant activity. Tea polyphenols effectively scavenge the reactive oxygen species (ROS) that are the major contributory factor in cellular injury (Yang and Wang, 1993).

Effect on central nervous system (CNS) activity

Rasayanas facilitate learning and consolidation of memory, antagonize CNS effects of stress, are anxiolytic and are capable of interacting with some neurotransmitter mechanisms (Dhawan and Singh, 2002).

Tea constituents improve learning and memory in senescence-accelerated mice, especially older animals (Unno *et al.*, 2001). They can antagonize 6-hydroxydopamine-induced apoptosis in PC-12 neurons (Jin *et al.*, 2001), which is an *in vitro* model of Parkinsonism. The toxicity of nitric oxide (NO) on hippocampal neurons is also antagonized (Nagai *et al.*, 2001). The thearubigin fraction can block the paralytic effect of botulinum (Satoh *et al.*, 2001a) and tetanus (Satoh *et al.*, 2001b) neurotoxins. Theanine protects against ischaemic delayed neuronal death (Kakuda, 2001). The activity of several enzymes linked to monoamine neurotransmitters, such as tyrosinase, COMT and MAO, can also be affected (Siddiqi *et al.*, 2002). (–)-Epicatechin gallate inhibits neurosphere adhesion, cell migration and neurite outgrowth in rat neurospheres (Chen *et al.*, 2003). It might affect neural stem cell survival or differentiation.

Facilitation of tissue regeneration

Rasayanas reduce time for regeneration of damaged tissues and also lead to a better functional recovery after injury due to ischaemia–reperfusion (Singh *et al.*, 2000), toxin (Tandon *et al.*, 1995) or partial resection (Rastogi *et al.*, 1995).

The data for tea constituents are rather limited and further in-depth studies are strongly indicated. Green tea has been reported to facilitate recovery from ischaemia–reperfusion injury of the forebrain in gerbils (Kakuda, 2001) and of the brain and gastric mucosa in rats (Yagi *et al.*, 2001). Similarly, repair of DNA damage by mutagenic agents is facilitated. No data are available on the effect on recovery of organs such as the liver following partial resection.

Anti-ageing effect

Rasayanas improve lifespan, help retain efficient task performance and retard development of biochemical markers of ageing (Pattan, 2003).

A large amount of epidemiological data is available from well-planned surveys in

tea drinkers but there have been very few experimental studies. Sadakata (1995) has reported a lower mortality rate in Japanese women practitioners of the traditional tea ceremony. The incidence of debilitating and killer diseases is also less. Kanis *et al.* (1999) reported protection against hip fracture in a population-based study, and Hegarty *et al.* (2000) found significantly higher bone density in the spine and hip region of 65- to 75-year-old tea drinkers. The Dutch cohort study (Geleijnse *et al.*, 1999) suggests a lower risk of death from coronary artery disease or stroke in tea drinkers. In a Boston study also, the risk of heart attack was assessed to be lower in persons drinking one or more cups of tea daily (Sesso *et al.*, 1999). Bushman (1998) has observed, in a review of cohort studies, a lower incidence of cancer of the oesophagus, stomach, colon and pancreas in green tea drinkers. It is not easy to undertake such studies in tea-drinking countries like India because control cohorts of non-tea-drinking persons are difficult to obtain.

Tea versus Other Health Drinks

Karakaya *et al.* (2001) undertook an interesting study in Turkey. They compared the antioxidant activity of various health drinks with the phenolic content and observed a good correlation ($r^2 = 0.95$). They also found that phenolic contents per serving were higher in liquid than in solid foods. They gave the highest ranking to black tea among the most commonly consumed liquid foods in that country. It was followed in descending order by instant coffee, cola drinks, red wine, carrot juice, apricot nectar, Turkish coffee, grape molasses and white wine. In a more recent study, Parmar *et al.* (2003) have also shown that the antioxidant activity of tea is comparable with that of fruits and vegetables. They have concluded that tea seems to fit the description of an ideal component of a healthy dietary habit. Further such studies are indicated in other countries to compare tea with locally consumed liquid foods.

Does the Colour of Tea Matter?

The majority of tea drinkers (~78%) take black tea as such or with milk (~50%). The polyphenol content of both is similar and largely consists of theaflavins and thearubigins. The next most commonly used tea is green tea (~20%). It is rich in the original polyphenols, such as (–)-epigallocatechin and other catechins. Red (oolong) tea is mainly used in certain parts of China and would account for only ~2% of total consumption. The polyphenol oxidase partially converts catechins to other polyphenols. The use of the least processed form of tea, white tea, would be barely 1%. It contains nine major constituents of green tea, even though their relative percentage may vary. Nishizawa and Van (2001) have made the important observation that all varieties of tea have similar physiological effects even though the composition of polyphenols, etc., may differ.

Epilogue: How Many Cups?

Nothing describes this better than the following English translation of an old Chinese poem by Jhawar (2000):

> One cup does all disorders cure
> With two, your troubles will be fewer
> Thrice, to the bone more vigour gives
> With four, forever you will live
> As young as on your day of birth.

Concluding Remarks

This brief review clearly indicates that tea constituents exhibit, to a significant degree, all the beneficial properties ascribed to *Rasayanas* in the *Ayurveda.* It can be safely predicted that, if tea were available in India when the major Ayurvedic treatises were being compiled, it would have found a place as a *Rasayana.* Its introduction in India during the 18th century was too late for inclusion in Ayurvedic texts. It is suggested that the Indian tea industry should promote tea

as a *Rasayana* rather than just as a refreshing drink or even as health food. It is also necessary that adequate funding should be made available for experimental, clinical and epidemiological studies in a few areas where more information is required.

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3 Prophylactic Functions of Tea Catechins

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Tea catechins constitute the majority of soluble solids in green tea. Catechin fractions of various catechin contents were extracted from green tea and four different individual catechins were purified. Over the last 25 years, we have been investigating the various physiological actions of these catechins. Tea catechins have very potent antioxidative (radical-scavenging) activity and intake of them seems to suppress or retard the process of lifestyle-related, life-threatening diseases such as cancer, hyperlipidaemia, hypertension and hyperglycaemia. Tea catechins also have strong and unique antimicrobial potency that inhibits the proliferation of infectious diseases, many of which plague our daily life. Thus, the importance of green tea drinking and the prophylactic functions of tea catechins should be realized and valued highly, not only in advanced countries where lifestyle- and age-related diseases are increasing, but also in the developing/developed counties where infectious, emerging diseases are threatening the health of people. Many of these functions are described in the book *Green Tea* (Hara, 2001).

Varieties of Catechins

There are four varieties of catechins in green tea, of which (–)-epigallocatechin gallate (EGCG) is dominant and constitutes more than 50% of total catechins. A simplified extraction method of tea catechins (Polyphenons™) is shown in Fig. 3.1, and an example of the breakdown of the individual catechin content is shown in Table 3.1. The structural formulae of catechins are shown in Fig. 3.2. Total catechin content of Polyphenons™ varies from 30% to 90% according to the process.

Antioxidative Action

Lipid peroxidation, induced by boiling lard at 97.8°C with bubbling air, was suppressed by tea catechins, as shown in Fig. 3.3 (Matsuzaki and Hara, 1985). As shown in the figure, 10 p.p.m. of green tea catechin (90% catechin content) was as effective as 200 p.p.m. of α-tocopherol, i.e. 20 times more potent than α-tocopherol. In the same way, green tea catechin was five times as effective as BHA. When catechin was mixed with DPPH radical in ethanol, the notable radical-scavenging potency of catechins was also confirmed, as shown in Fig. 3.4 (Nanjo *et al.*, 1996). The radical-scavenging potency of individual tea catechins corresponds to the preceding data.

Anti-tumour Action

Mice (ddY), fed diets containing 0.5% or 1.0% of catechin powder (green tea catechin)

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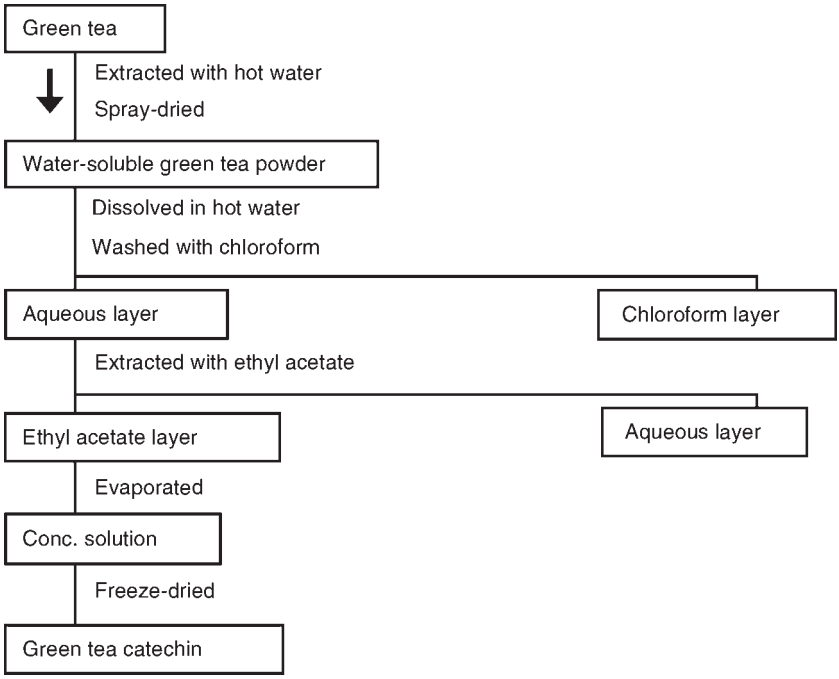

Fig. 3.1. Method of extracting catechin fractions from green tea.

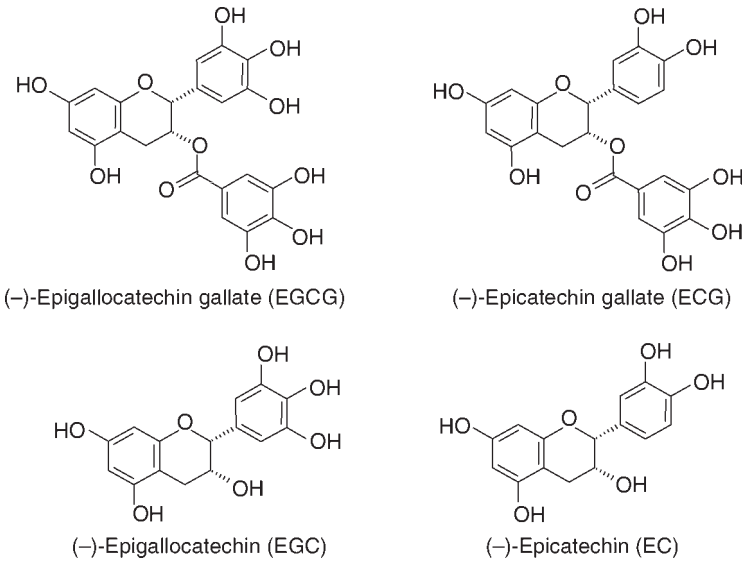

Fig. 3.2. Structural formulae of catechins.

for 9 months, were inoculated subcutaneously with sarcoma 180 cells. After 19 days, tumours grew, as shown in Fig. 3.5. When the tumours were resected and weighed, tea catechin feeding apparently suppressed the growth of tumours, as compared with the control, dose-dependently, as shown in Fig. 3.6 (Hara *et al.*, 1989).

Table 3.1. An example of the breakdown of individual catechin content.

Tea catechins	Polyphenon G	Polyphenon 30	Polyphenon 30S	Polyphenon 60	Polyphenon 60S	Polyphenon 70S	Polyphenon 100	Polyphenon E
(+)-Gallocatechin (+GC)	—	—	—	—	—	—	1.4	0.3
(–)-Epigallocatechin (EGC)	10.0	12.5	11.0	7.5	8.0	18.0	17.6	3.2
(–)-Epicatechin (EC)	1.8	3.5	2.5	8.0	4.5	7.9	5.8	9.6
(–)-Epigallocatechin gallate (EGCG)	13.5	14.5	15.0	29.0	45.5	35.0	53.9	67.4
(–)-Epicatechin gallate (ECG)	2.2	3.5	3.5	9.0	7.0	10.4	12.5	7.7
(–)-Gallocatechin gallate (GCG)	—	—	—	—	—	3.3	—	2.4
Total	27.5	34.0	32.0	63.5	65.0	73.5	91.2	91.6
Caffeine	7.0	7.0	0.5	10.0	0.5	0.5	0.5	0.5

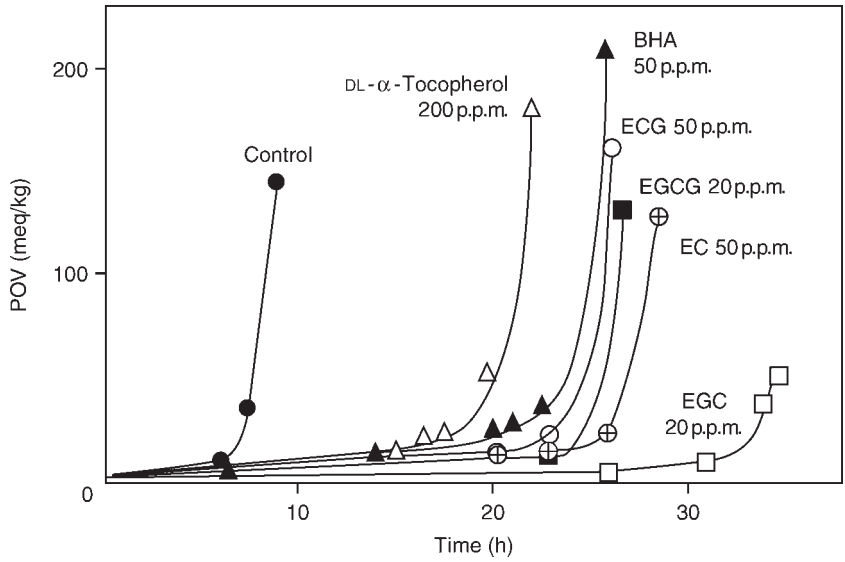

Fig. 3.3. Antioxidative activity of tea catechins on lard (AOM at 97.8).

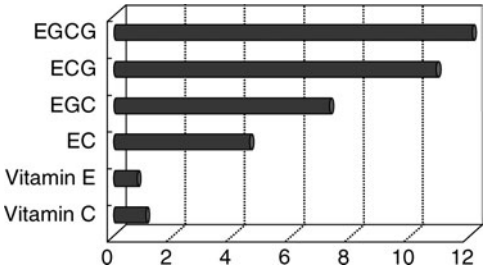

Fig. 3.4. Radical-scavenging potency of catechins (DPPH radicals). Vitamin C: 1.

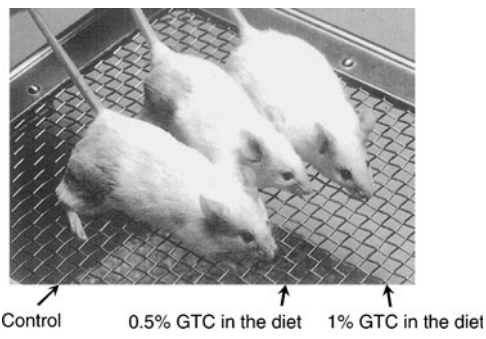

Fig. 3.5. Suppression of tumour growth by feeding green tea catechins (GTC) to ddY mice.

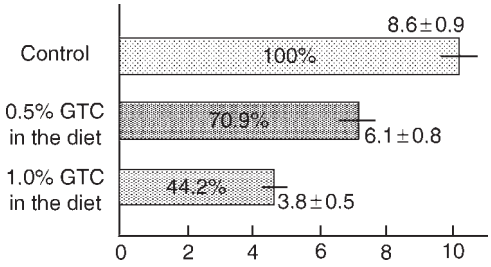

Fig. 3.6. Effect of green tea catechins (GTC) on size of tumours in ddY mice.

Anti-hyperlipidaemic Action

Rats were fed a high-fat (15% lard), high-cholesterol (1%) diet, to which a small amount of cholic acid (0.2%) was added to facilitate lipid absorption. After 4 weeks, all the rats were sacrificed to examine their cholesterol levels. The results in Fig. 3.7 show that the total cholesterol concentration of the test group rose to more than twice the level of that of the control group. Particularly, the low-density lipoprotein (LDL)-cholesterol increased as much as 15 times and the high-density lipoprotein (HDL)-cholesterol decreased to less than half of the control values. But the addition of EGCG (0.5% or 1.0%) to the above test diet moderated

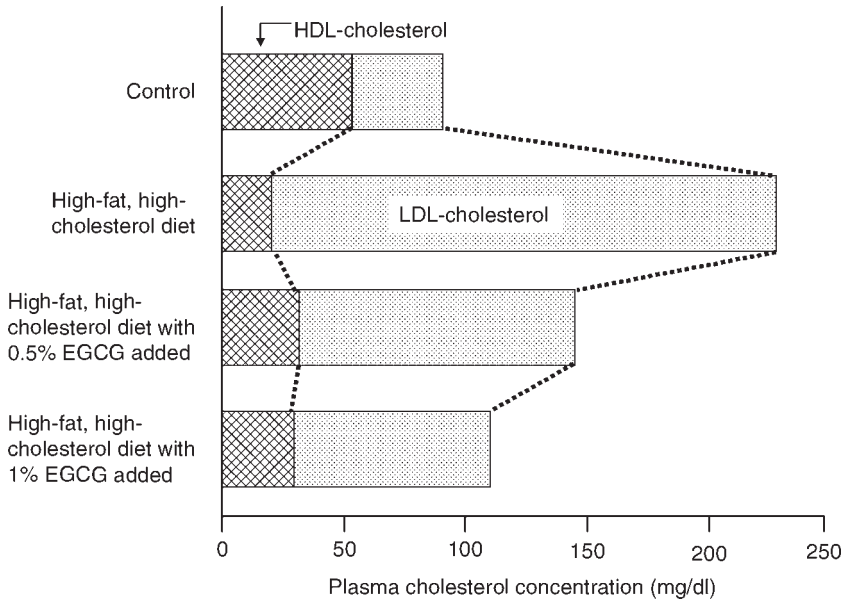

Fig. 3.7. Hypocholesterolaemic effect of EGCG.

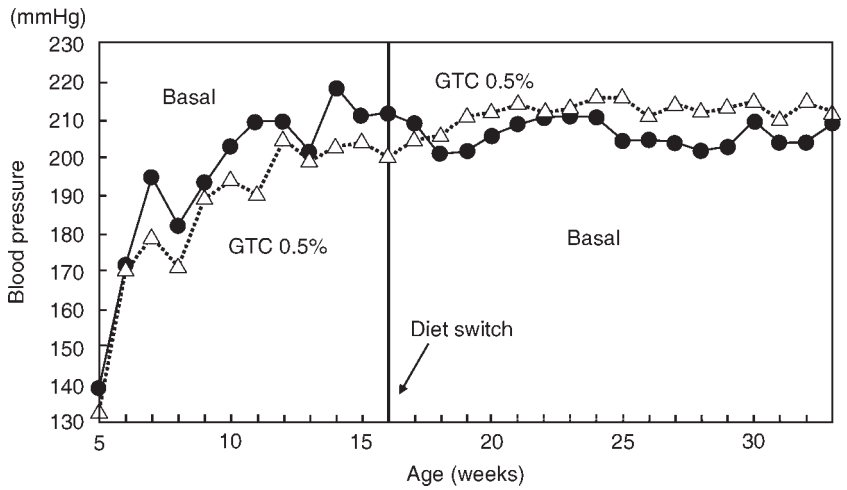

Fig. 3.8. Effect of green tea catechins (GTC) on blood pressure of SHR (spontaneously hypertensive rats).

the increase of LDL-cholesterol and the decrease of HDL-cholesterol dose-dependently (Fukuyo *et al.*, 1986).

Anti-hypertensive Action

Spontaneously hypertensive rats (SHR) were divided into two groups. The control group received a normal diet, while the test group was given 0.5% of green tea catechin (GTC) in the diet from 1 week after weaning. As shown in Fig. 3.8, although the blood pressure in the control group exceeded 200 mmHg at 10 weeks of age, significant suppression was noted in the catechin-fed group. When the diet of both groups was switched at 16 weeks of age, the blood pressures, in time, also changed accordingly (Hara and Tono-oka, 1990).

Anti-hyperglycaemic Action

Wistar rats were divided into two groups. They were starved overnight. Green tea catechin, 80 mg in 1 ml of water, was administered orally to the test group, while the control group was given water (1 ml).

After 30 min, 1.6 g of soluble starch (40% solution/4 ml) was administered orally to rats of both groups and the blood was collected directly afterwards (0 min) and 30 min, 1 h and 2 h after the administration of the starch. As shown in Fig. 3.9, the elevation of the blood glucose level was suppressed in the catechin group as compared with the control group. In the same way, the elevation of the levels of blood insulin and intestinal α-amylase concentration was suppressed (Matsumoto *et al.*, 1993).

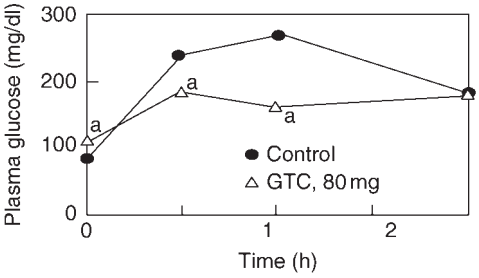

Fig. 3.9. Glucose concentration in blood plasma of rats administered starch. $^{a}P < 0.05$ compared with control

Anti-microbial Action

At much lower concentrations than in a daily brew of green tea, catechins will inhibit the growth of food-borne pathogenic bacteria (Tabel 3.2) (Hara and Ishigami, 1989). In our favour, tea catechins will not inhibit such beneficial bacteria as *Bifidobacterium*/*Lactobacillus* at our drinking concentration. Tea catechins, in particular EGCG, were confirmed to interact with influenza virus and render the virus non-infective to cells. This could be accomplished at a concentration as low as 2 p.p.m. (Nakayama *et al.*, 1993). Electron-microscopic photos of

Table 3.2. Minimum inhibitory concentrations (MIC) of tea catechins against food-borne pathogenic and enteric bacteria.

	MIC (ppm)				
Bacteria	GTC	EC	ECG	EGC	EGCG
Staphylococcus aureus IAM 1011	450	> 800	800	150	250
Vibrio fluvualis JCM 3752	200	800	300	300	200
Vibrio parahaemolyticus IFO 12711	200	800	500	300	200
Vibrio metschnikovii IAM 1039	500	> 1000	> 1000	500	1000
Clostridium perfringens JCM 3816	400	> 1000	400	1000	300
Clostridium botulinium A, B mix	< 10	> 1000	200	300	< 100
Bacillus cereus JCM 2152	600	> 1000	600	> 1000	600
Plesiomonas shigelloides IID No. 3	100	700	100	200	100
Aeromonas sobria JCM 2139	400	> 1000	700	400	300
Lactobacillus brevis subsp. *gravesensis* JCM 1102	> 1000	> 1000	> 1000	> 1000	> 1000
Lactobacillus brevis subsp. *brevis* JCM 1059	> 1000	> 1000	> 1000	> 1000	> 1000
Lactobacillus brevis subsp. *otakiensis* JCM 1183	> 1000	> 1000	> 1000	> 1000	> 1000
Bifidobacterium bifidum JCM 1255	> 1000	> 1000	> 1000	> 1000	> 1000
Bifidobacterium adolescentis JCM 1275	> 1000	> 1000	> 1000	> 1000	> 1000
Bifidobacterium longum JCM 1217	> 1000	> 1000	> 1000	> 1000	> 1000

GTC, green tea catechin; EC, (–)-epicatechin; ECG, (–)-epicatechin gallate; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin gallate.

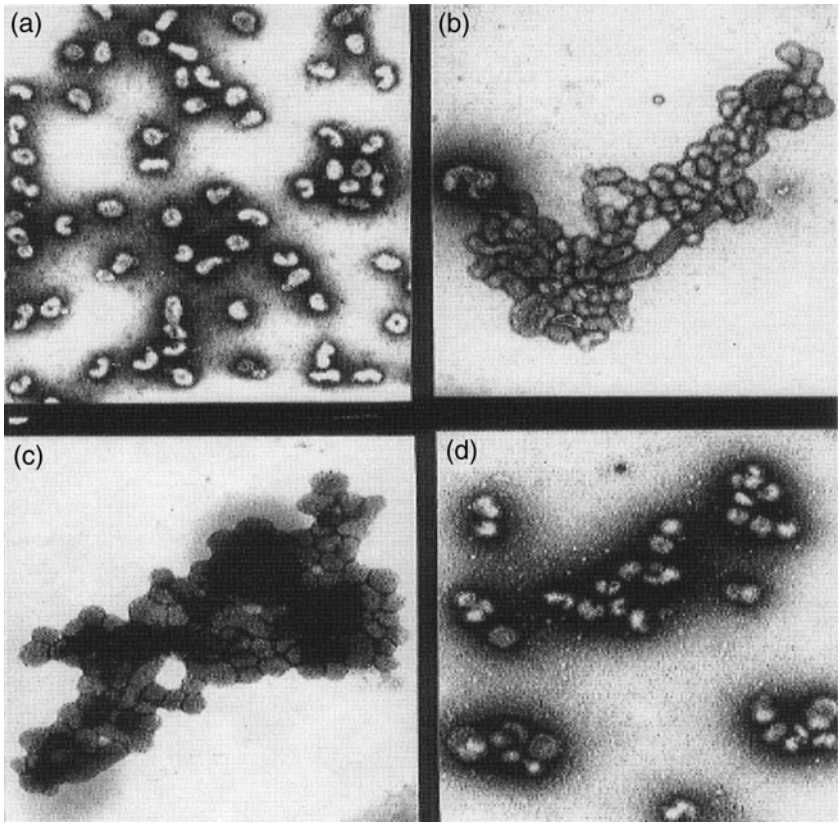

Fig. 3.10. Electron microscopic photographs of agglomerated virus: (a) control, (b) 1 mM EGCg, (c) 1 mM TF3, (d) anti-A virus IgG (6400 HI titre). Virus was negatively stained with 2% sodium phosphotungstate (pH 7.2) for electron microscopy.

agglomerated viruses that have lost infectivity due to a drop of tea catechins/black tea theaflavins are shown in Fig. 3.10. These antimicrobial actions of tea catechins should be fully appreciated and utilized.

Bowel-modulating Action

One hundred mg of tea catechins (160 mg of Polyphenon® 60) were administered to bedridden in-patients at each meal, three times a day for 3 weeks. Their faecal specimens were collected on days 0, 7, 14 and 21 and 1 week after the end of catechin administration (day 28). The analyses of the faeces revealed a significant increase of lactic acid bacteria and a decrease of putrefactive bacteria, as shown in Fig. 3.11. Other faecal parameters also indicated very favourable improvement of intestinal conditions (Goto *et al.*, 1998).

Chemoprevention Trials

In order to prove the chemopreventive efficacy (suppression of the development of carcinogenesis) of tea catechins, and we – Mitsui Norin Co., Ltd. – and the National Cancer Institute, USA, have chosen Polyphenon® E as an active pharmaceutical ingredient, i.e. a composite to be proven as a pharmaceutical. Polyphenon® E is made up of more than 60% EGCG and more than 30% other catechins. Before going into a fully fledged Phase 2 trial against pre-lung cancerous lesions at the British Columbia

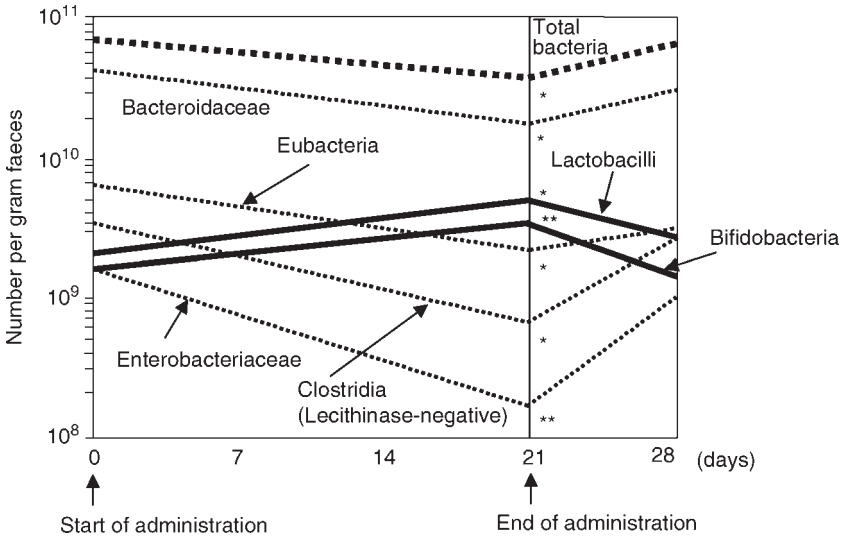

Fig. 3.11. Effect of P-60 administration on faecal flora of 15 human volunteers (300 mg tea catechins/day). The graph is expressed as mean ± SD. *, **, significant difference (* $P < 0.05$, ** $P < 0.01$) from value of day 0 (before administration).

Table 3.3. The change in body weight, blood pressure (BP), cholesterol and triglyceride levels after 1 and 2 months of administering EGCG.

	Baseline ± SD	Month 1	Month 2
Weight	199.3 ± 4.7	197.7 ± 4.5**	195.7 ± 4.5*
Systolic BP (mmHg)	140.6 ± 4.8	130.7 ± 14.9*	125.4 ± 18.6*
Diastolic BP (mmHg)	79.4 ± 1.8	73.9 ± 8.4*	70.7 ± 1.9**
Cholesterol (mmol/l)	5.74 ± 0.41	4.64 ± 0.28*	5.27 ± 0.17
Triglyceride (mmol/l)	1.88 ± 0.30	1.54 ± 0.18**	1.74 ± 0.20

*$P < 0.05$; **$P = 0.01$ for change from baseline.

Cancer Agency, we and Dr Stephen Lam conducted open trials in order to confirm the safety of the intake of Polyphenon® E. To the 20 subjects, heavy smokers of more than 40 pack years, 1300 mg of Polyphenon® E (equivalent to 800 mg EGCG) in capsules was administered twice a day for 2 months. This amount corresponds to almost 20 cups of tea a day. The changes in body weight, blood pressure, cholesterol and triglyceride levels after 1 and 2 months are shown in Table 3.3. The results showed significant reduction in body weight, blood pressure, cholesterol and triglyceride levels. Adverse events consisted of mild heartburn, loose stool, constipation, irritability or fatigue, but these events resolved spontaneously after discontinuation of Polyphenon® E. The recruiting of subjects for the Phase 2 trial of blind, placebo-controlled, randomized systems with Polyphenon® E is under way at the British Cancer Agency (Lam *et al.*, 2004). Clinical chemoprevention trials with Polyphenon® E targeting various other indications, such as oesophagus, bladder, prostate, cervix or colon, are under development of the protocol.

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4 Bioavailabilities of Tea Polyphenols in Humans and Rodents*

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Abstract
Consumption of tea (*Camellia sinensis*) has been suggested to prevent cancer and heart disease. Animal studies have shown that tea and tea constituents inhibit carcinogenesis of the skin, lung, oral cavity, oesophagus, stomach, liver, prostate and other organs. Studies with human cancer cell lines have demonstrated a number of potential cancer prevention mechanisms for tea polyphenols, including protection from or induction of oxidative stress, inhibition of enzymes (mitogen-activated protein (MAP) kinases, cyclin-dependent kinases and topoisomerase I) and inhibition of growth factor-related cell signalling (epidermal growth factor and others). Whereas some studies report effects of epigallocatechin-3-gallate (EGCG) at submicromolar levels, most experiments require concentrations of greater than 10 or 20 μM to demonstrate the effect. In humans, mice and rats, tea polyphenols undergo glucuronidation, sulphation, methylation and ring fission. Recent reports also suggest that EGCG and other catechins may be substrates for active efflux. The peak plasma concentrations of EGCG, epigallocatechin (EGC) and epicatechin (EC) following oral administration of green tea are 0.04–1 μM, 0.3–5 μM and 0.1–2.5 μM, respectively, in humans and rodents. The plasma levels of theaflavins are much lower (~2 nM). The present chapter reviews the literature concerning the biotransformation and bioavailability of tea polyphenols. Such a review should serve as the foundation for future experiments on the bioavailability of tea polyphenols, and as a guide in extrapolating mechanistic data from cell-line studies to animal or human studies.

Introduction

Tea (*Camellia sinensis* (*Theaceae*)) is second only to water in terms of worldwide popularity as a beverage. Consumption of tea has been associated with many health benefits, including the prevention of cancer and heart disease, but the nature of this association is not clear (Yang *et al.*, 2002).

Green, black and oolong tea are the three major commercial types of tea and differ in how they are produced and in their chemical compositions. Green tea is prepared by pan-frying or steaming withered leaves to heat-inactivate oxidative enzymes; the leaves are then dried. In contrast, black tea is produced by crushing fresh tea leaves and allowing enzyme-mediated oxidation to occur in a process commonly known as fermentation. Green tea is chemically characterized by the presence of large amounts of polyphenolic compounds known as catechins (Fig. 4.1.).

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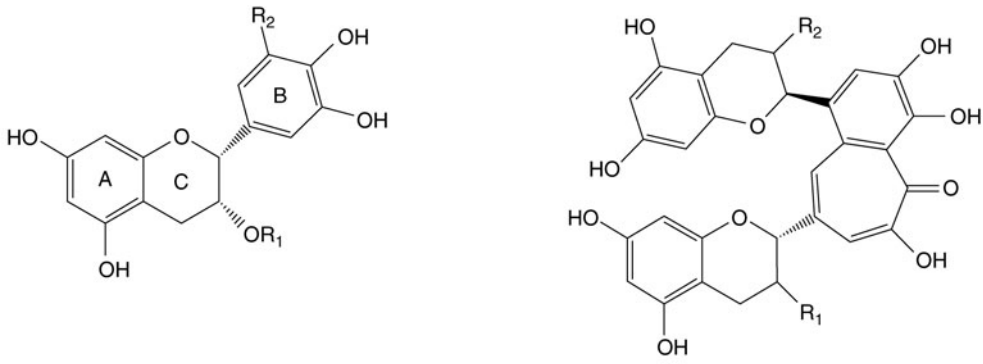

Epicatechin: $R_1 = R_2 = H$

Epigallocatechin: $R_1 = H$; $R_2 = OH$

Epicatechin-3-gallate: R_1 = galloyl; $R_2 = H$

Epigallocatechin-3-gallate: R_1 = galloyl; $R_2 = OH$

Theaflavin: $R_1 = R_2 = OH$

Theaflavin-3-gallate: R_1 = galloyl; $R_2 = OH$

Theaflavin-3-gallate: $R_1 = OH$; R_2 = galloyl

Theaflavin-3,3'-gallate: $R_1 = R_2$ = galloyl

Fig. 4.1. Structure of major tea polyphenols.

A typical cup of brewed green tea contains 30–40% catechins by dry weight, including epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG). Through fermentation, a large percentage of the catechins are converted to oligomeric theaflavins and polymeric thearubigins in black tea. The resulting brewed black tea contains 3–10% catechins, 2–6% theaflavins and 20% thearubigins (Balentine *et al.*, 1997).

Both green and black tea and their constituents have been extensively studied both *in vitro* and in animal models of carcinogenesis (Yang *et al.*, 2002). Whereas these compounds have been shown to be efficacious in a number of models of carcinogenesis, the epidemiological data of cancer prevention remain inconclusive. Numerous mechanisms have been proposed to account for the biological activities of the tea polyphenols. EGCG, the most widely studied catechin, has been shown to inhibit the activity of key enzymes (topoisomerase, matrix metalloproteinases and telomerase), growth factor signalling (epidermal growth factor, vascular endothelial growth factor) and the activity of transcription factors (AP-1, NF-κB) (Cherubini *et al.*, 1999; Ahmad *et al.*, 2001). Typically, the concentrations of EGCG required to observe these effects are in the range of 10–100 μM, which are significantly higher than those observed *in vivo* following consumption of tea. In the present chapter, we discuss our current knowledge regarding the bioavailabilities of tea polyphenols and the inference of these data for potential cancer-preventive activities of tea polyphenols in humans.

Pharmacokinetics

The pharmacokinetics of EGCG and the other catechins have been investigated in rats, mice and humans. Studies of [^{3}H]EGCG in both the rat and the mouse have shown that, following a single intragastric (i.g.) dose, radioactivity is found throughout the body (Suganuma *et al.*, 1998; Kohri *et al.*, 2001). After 24 h, 10% of the initial dose (radioactivity) was in the blood, with about 1% found in the prostate, heart, lung, liver, kidney and other tissues. The major route of elimination was via the faeces. In the rat, 77% of an intravenous (i.v.) dose of [^{3}H]EGCG was eliminated in the bile, while only 2% was eliminated via the urine.

Following i.v. administration of decaffeinated green tea to rats, plasma levels of EGCG, EGC and EC were fitted to a two-compartment model with elimination half-lives of 165, 66 and 67 min, respectively. EGCG levels were highest in the small intestine (43.7 nmol/g) and the kidney (26.2 nmol/g). The absolute bioavailability of EGCG, EGC and EC (free plus conjugated forms of each compound) after i.g. administration of decaffeinated green tea was 0.1, 14 and 31%, respectively (Chen *et al.*, 1997).

In comparison, the absolute bioavailability of EGCG in mice following i.g. administration of EGCG was 26.5%. Concentrations of EGCG in the small intestine and colon were 45 and 7.9 nmol/g following i.g. administration of 75 mg/kg EGCG. The levels in other tissues were less than 0.1 nmol/g. Following i.v. administration of EGCG, levels were highest in the liver (3.6 nmol/g), lung (2.7 nmol/g) and small intestine (2.4 nmol/g). Whereas greater than 50% of plasma EGCG was present as the glucuronide, EGCG was present mainly as the free form in the tissues (Lambert *et al.*, 2003).

Treatment of rats with a green tea polyphenol preparation (0.6% w/v) in the drinking fluid has been shown to result in increased plasma levels over a 14-day period, with levels of EGC and EC being higher than those of EGCG (Kim *et al.*, 2000). Plasma levels then decreased over the subsequent 14 days, suggesting an adaptive effect. EGCG levels were found to be highest in the rat oesophagus, intestine and colon, which have direct contact with tea catechins, whereas EGCG levels were lower in the bladder, kidney, colon, lung and prostate. When the same polyphenol preparation was given to mice, the EGCG levels in the plasma, lung and liver were much higher than in rats. These levels appeared to peak on day 4 and then decreased to less than 20% of the peak values on days 8–10 (Kim *et al.*, 2000).

Several studies of the systemic bioavailability of orally administered green tea and catechins in human volunteers have been conducted (Yang *et al.*, 1998; Chow *et al.*, 2001; Lee *et al.*, 2002). Most recently, we have shown that oral administration of 20 mg green tea solids/kg body weight resulted in C_{max} in the plasma for EGC, EC and EGCG of 223, 124, and 77.9 ng/ml, respectively (Lee *et al.*, 2002). T_{max} was found to range from 1.3 to 1.6 h with $t_{1/2\beta}$ of 3.4, 1.7 and 2 h for EGCG, EGC and EC, respectively. The T_{max} increased with increased dose of catechins (Chow *et al.*, 2001). Plasma EC and EGC were present mainly in the conjugated form, whereas 77% of the EGCG was in the free form (Lee *et al.*, 2002). These findings support earlier work that found that plasma EGC was present as glucuronide (57–71%) and sulphate (23–36%), with only a small free fraction (Yang *et al.*, 1998; Chow *et al.*, 2001). Likewise, plasma EC was largely in the sulphated form (66%), with less glucuronide (33%). EGC, but not EC, was also methylated (4′-*O*-methyl-EGC) in humans. Plasma and urine levels of 4′-*O*-methyl-EGC have been shown to exceed those of EGC by ten- and threefold, respectively (Lee *et al.*, 2002). EGCG has also been shown to undergo methylation. The maximum plasma concentration of 4′,4″-di-*O*- methyl-EGCG was 20% of the maximum concentration of EGCG, but the cumulative excretion of 4′,4″-di-*O*-methyl-EGCG was tenfold higher (140 μg) than that of EGCG (16 μg) over 24 h (Meng *et al.*, 2002). In addition to methylated and conjugated metabolites, the ring-fission metabolites, 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone (M4), 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (M6) and 5-(3′,5′-dihydroxyphenyl)-γ-valerolactone (M6′) have been detected in urine at 8, 4 and 8 μM, respectively, following ingestion of 200 mg EGCG (Li *et al.*, 2000; Meng *et al.*, 2002).

There is one report on the detection of theaflavins in plasma following ingestion of theaflavins. Mulder *et al.* have shown that following ingestion of 700 mg of pure theaflavins, the peak concentrations were 1 ng/ml and 4.2 ng/ml in the plasma and urine, respectively (Mulder *et al.*, 2001).

Whereas the tea polyphenols have a rather low systemic bioavailability, high concentrations have been demonstrated in the oral cavity. We have demonstrated that holding green tea solution (420 mg green tea solids per 60 ml water) in the mouth without swallowing resulted in salivary concentrations of EGCG and EGC of 153 and 327 μM,

respectively (Yang *et al.*, 1999). These concentrations were 400–1000 times greater than those observed in plasma following ingestion of tea. Such locally high levels may support the use of green tea in the prevention of oral cancer and caries. In a more recent study, our laboratory has reported that holding either green tea leaves or black tea extract for 2–5 min resulted in high salivary catechin (2–131 μM) and theaflavin (1–2 μM) concentrations (Lee *et al.*, 2003).

Biotransformation

The catechins are subject to extensive biotransformation, including methylation, glucuronidation, sulphation and ring-fission metabolism (Fig. 4.2). Recent studies on the enzymology of EGC and EGCG methylation have shown that EGC is methylated to form 4′-*O*-methyl-(–)-EGC and EGCG was methylated to form 4″-*O*-methyl-(–)-EGCG and 4′,4″-*O*-dimethyl-(–)-EGCG (Lu *et al.*, 2003b). At low concentrations of EGCG, the dimethylated compound was the major product. Rat liver cytosol showed higher COMT activity towards EGCG and EGC than did human or mouse liver cytosol. Additionally, the K_m and V_{max} values were higher for EGC than for EGCG (e.g. in human liver cytosol, K_m is 4 and 0.16 μM for EGC and EGCG, respectively).

Studies with bile duct-cannulated rats have shown that, after oral administration of 100 mg EGCG, 3.28% of the dose was recovered in the bile as: EGCG (2.65%), 4″-*O*-methyl-EGCG (0.25%), 3″-*O*-methyl-EGCG (0.11%), 4′-*O*-methyl-EGCG (0.11%), 3′-*O*-methyl-EGCG (0.10%), 4′,4″-di-*O*-methyl-EGCG (0.06%) (Okushio *et al.*, 1999). With the exception of 4″-*O*-methyl-EGCG and 4′,4″-di-*O*-methyl-EGCG, which were partially present as the sulphated form, the other metabolites and EGCG were present largely (>58%) as the glucuronidated form, with less sulphate present (<42%).

Treatment of mice with 0.1% or 0.3% green tea as the sole source of drinking fluid resulted in a dose-dependent formation of 4′,4″-di-*O*-methyl-EGCG in the plasma (22–90 nM), urine (13–99 μM) and faeces (24–53 μM) (Meng *et al.*, 2002). This metabolite was also detected in the liver, small intestine and kidney (0.9–11 ng/g).

EGC, but not EC, was also methylated (4′-*O*-methyl-EGC) in humans. Plasma and urine levels of 4′-*O*-methyl-EGC have been shown to exceed those of EGC by ten- and threefold, respectively (Lee *et al.*, 2002). EGCG

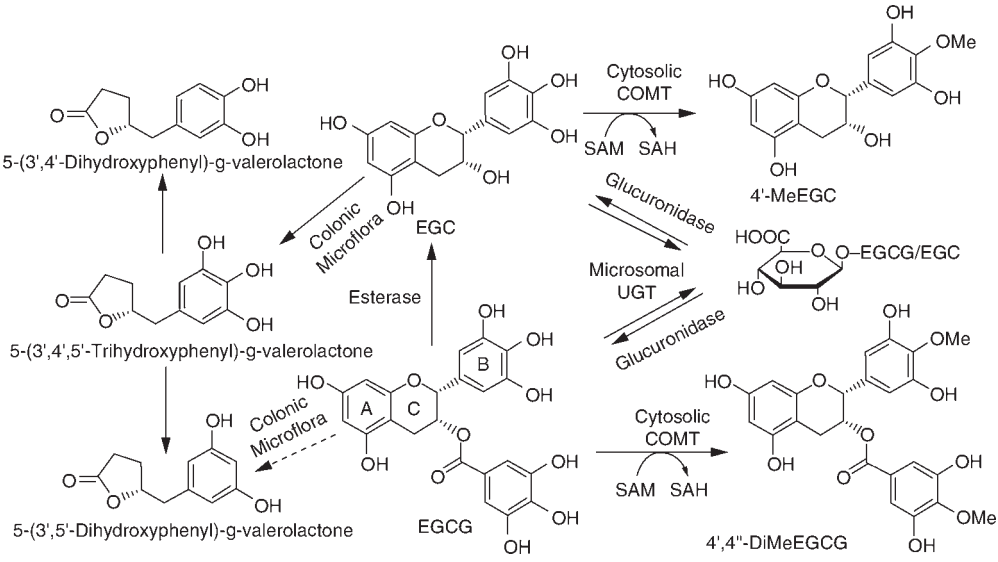

Fig. 4.2. Proposed biotransformation pathways for tea polyphenols.

has also been shown to undergo methylation. The maximum plasma concentration of 4′,4″-di-*O*-methyl-EGCG was 20% that of EGCG, but the cumulative excretion of 4′,4″-di-*O*-methyl-EGCG was tenfold higher (140 μg) than that of EGCG (16 μg) over 24 h (Meng *et al.*, 2002). Other methylated catechins, including 3′- and 4′-*O*-methyl-EGC, have also been reported following oral dosing of rodents and humans with green tea (Yang *et al.*, 2002).

Studies of EGCG and EGC glucuronidation revealed that EGCG-4″-*O*-glucuronide was the major metabolite formed by human, mouse and rat microsomes (Lu *et al.*, 2003a). Mouse small intestinal microsomes had the greatest catalytic efficiency (V_{max}/K_m) for glucuronidation, followed in decreasing order by mouse liver, human liver, rat liver and rat small intestine. Using recombinant human UGT enzymes, it has been determined that UGT1A1, 1A8 and 1A9 have the highest activity towards EGCG, conjugating at the 3″ and 4″ positions, with the intestinal-specific UGT1A8 having the highest catalytic efficiency. EGC-3′-*O*-glucuronide was the major product formed by microsomes from all species. In this case, liver microsomes were shown to have a higher efficiency than intestinal microsomes for glucuronidation.

Vaidyanathan *et al.* have shown that EC undergoes sulphation catalysed by human and rat intestinal and liver cytosol, with the human liver being the most efficient (Vaidyanathan and Walle, 2002). Further studies have revealed that sulphotransferase (SULT)1A1 was largely responsible for this activity in the liver, whereas both SULT1A1 and SULT1A3 were active in the human intestine. The catalytic efficiency for SULT-1A1- and SULT1A3-mediated sulphation of EC was 5834 and 55 μl/min/mg, respectively. Recent work in our laboratory has shown that EGCG was time- and concentration-dependently sulphated by human, mouse and rat liver cytosol (Lu, 2002). The rat had the greatest activity, followed by the mouse and the human. Further studies are required to determine the structure of these sulphated metabolites.

Based on these studies, it appears that mice are more similar to humans than rats in terms of enzymatic ability to conjugate tea catechins. Interestingly, plasma EGCG was present mainly in the free form in humans, but as the glucuronide in mice (Table 4.1) (Lee *et al.*, 2002; Lambert *et al.*, 2003). This indicates that, whereas mice and humans are similar in terms of enzymology, other factors, such as distribution of Phase II enzymes and the role of active transport, may lead to significant species differences. These factors must be considered in choosing the most appropriate animal model to study the potential health benefits of tea constituents.

Anaerobic fermentation of EGC, EC and ECG with human faecal microflora has been shown to result in the production of the ring-fission products M4, M6 and M6′ (Fig. 4.2). Further incubation resulted in

Table 4.1. Comparison of the pharmacokinetic parameters of EGCG in rats, mice and humans.

	Units	Rats[a]	Mice[b]	Human 1[c]	Human 2[d]
Dose	mg/kcal	0.9	0.19	0.07	0.11
T_{max}	min	85.5	89.8	96	126
C_{max}	ng/ml	19.8	128.2	34.7	74.0
$t_{1/2}$	min	85.5	82.8	222.0	120.0
AUC	ng/min/ml	17.4	32.0	12.8	22.0
AUC/dose	N/A	19.3	168.4	189.9	200.0
Form	N/A	Mostly conjugated	Mostly conjugated	Mostly free	Mostly free

[a]Chen *et al.*, 1997.
[b]Lambert *et al.*, 2003.
[c]Lee *et al.*, 2002.
[d]Chow *et al.*, 2001.
AUC = area under the curve.

the formation of lower-molecular-weight phenolic acids (Meselhy *et al.*, 1997). We have found these ring-fission products in human urine and plasma approximately 3 h after oral ingestion of 20 mg/kg decaffeinated green tea (Li *et al.*, 2000). The compounds had a T_{max} of 7.5–13.5 h and reached peak plasma concentrations of 100–200 nM. Following ingestion of 200 mg EGCG, the peak urine concentrations were 8, 4 and 8 μM for M4, M6 and M6′, respectively. M4, M6 and M6′ retain the polyphenolic character of the parent compound, have the addition of a potentially biologically active valerolactone structure and may therefore have biological activities similar to the parent catechins.

In animals, the Phase II metabolism reactions probably compete with one another. The relative concentration of each enzyme and their activities for the tea polyphenols determine the metabolic profile *in vivo*. Since EGCG has a lower k_m for COMT than UGTs, methylation may be favoured at physiological (usually low) concentrations. Indeed, *in vivo*, EGCG is first methylated to form 4″-*O*-methyl-EGCG and then further methylated to form 4′,4″-di-*O*-methyl-EGCG (Meng *et al.*, 2002). At high doses, Lu *et al.* have observed that glucuronidation becomes more prominent, leading to the formation of EGCG-4″-glucuronide in the mouse (Lu *et al.*, 2003b). This compound can be further methylated on the B ring to produce different methylated metabolites. This is consistent with the observation that four monomethylated and two dimethylated compounds are found in mouse urine after hydrolysis with β-glucuronidase and sulphatase, following administration of high doses of EGCG to the mouse (Meng *et al.*, 2002). The methylated compounds were found to have similar peak heights: if conjugation had not preceded methylation, the 4″-*O*-methyl-EGCG peak would have been the predominant monomethylated metabolite.

Whereas no studies have been reported concerning the biotransformation and bioavailability of the theaflavins and the thearubigins, it is possible to make some hypotheses. Based on a calculation of polar surface area and a consideration of Lipinski's Rule of Five, it is predicted that the bioavailability of these molecules will be quite poor (Clark, 1999; Lipinski *et al.*, 2001). In addition, the numerous hydroxyl groups on the theaflavins represent sites for Phase II conjugation. The A ring remains in the theaflavins and may be attacked by the same microorganisms that cleave the catechins, resulting in the formation of M4, M6 and M6′. Much research remains to be done to determine the role of any of these biotransformation pathways in affecting the bioavailability of the theaflavins and thearubigins.

Active Efflux

Active efflux has been shown to limit the bioavailability and cellular accumulation of a number of compounds. The multidrug resistance-associated proteins (MRP or ABCC) are expressed in many tissues: MRP1 is expressed on the basolateral membrane of most cell types, whereas MRP2 is expressed on the apical side of cells in the liver, kidney and intestine (Leslie *et al.*, 2001). Recent studies in our laboratory have shown that treatment of MRP1- and MRP2-overexpressing MDCKII cells with either indomethacin or MK-571 (MRP inhibitors) resulted in three- to tenfold increases in intracellular accumulation of EGCG, 4″-*O*-methyl-EGCG and 4′,4″-di-*O*-methyl-EGCG, as compared with untreated controls (Hong *et al.*, 2003). Treatment of P-glycoprotein (PGP)-overexpressing MDCKII cells with a variety of PGP inhibitors resulted in no significant effect on the intracellular levels of EGCG or its metabolites. Likewise, treatment of HT-29 human colon cancer cells with MRP inhibitors resulted in a twofold increase in cell-associated [^{3}H]EGCG (Hong *et al.*, 2002). Treatment of Caco-2 cells with MK-571 enhanced apical to basolateral movement of EC compared with untreated control cells (Vaidyanathan and Walle, 2001). MK-571 also reduced the efflux of EC-sulphates from the cytosol to the apical wall, suggesting that the EC-sulphates are also substrates for MRP2.

Concluding Remarks

Whereas numerous potential mechanisms have been proposed for the cancer-preventive activity of green tea polyphenols, none has been conclusively demonstrated *in vivo*. Much of the uncertainty stems from the much higher concentrations of tea polyphenols used in cell-line studies compared with those present in the plasma and tissue following normal consumption of tea. A detailed understanding of the levels of tea polyphenols available in the plasma and tissues and a careful consideration of the factors that affect tea polyphenol bioavailability are necessary to understand the biological actions of tea, to develop useful biomarkers for tea consumption and to design effective intervention trials for human diseases.

The currently available literature suggests that for some organ sites, such as the oral cavity, small intestine and colon, the high concentrations (micromolar) used in tissue culture studies are justifiable. For other tissues, the levels achieved following administration of tea and tea components are much lower. In this case, a variety of factors must be considered, including Phase II metabolism and active efflux. The enzymology and, to some extent, the relative importance of glucuronidation and methylation for catechin biotransformation *in vivo* have been established. Significant work remains to characterize fully the enzymology of sulphation and its importance *in vivo*, as well as the importance of the MRP transporters. Likewise, the interplay of Phase II metabolism and active transport *in vivo* and the relative importance of each in affecting tea polyphenol bioavailability remain to be determined.

A proposed model for the interaction of Phase II metabolism and active efflux is shown in Fig. 4.3. In this model, tea polyphenols are absorbed from the intestinal lumen following oral administration. A percentage of these compounds are conjugated. Both the conjugated and free forms are then subject to efflux by MRP2. A remaining fraction of free polyphenols can then be absorbed across the basolateral membrane and into the portal circulation. The conjugated

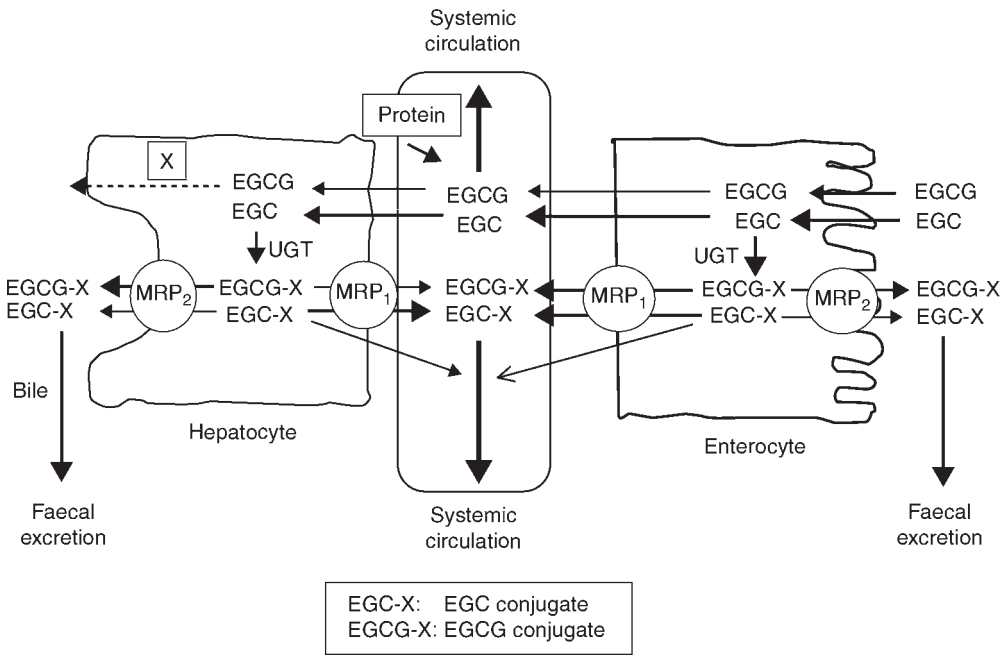

Fig. 4.3. Proposed integration of factors affecting tea polyphenol bioavailability.

polyphenols may be effluxed into the portal circulation by MRP1. These compounds are then filtered by the liver, where they may again be effluxed by MRP2 (into the bile duct) or conjugated and effluxed. A remaining fraction is available for the systemic circulation: a portion of the systemically available tea polyphenols may be metabolized and effluxed into the urine by the kidney. Complete characterization of the absorption, distribution and metabolism of the theaflavins and thearubigins remains to be done.

To summarize, further studies are needed to gain a complete understanding of the factors that affect tea polyphenol bioavailability, particularly with regard to the interplay of these factors *in vivo*. These data, coupled with carefully designed *in vitro* studies and mechanistically oriented *in vivo* studies, will allow greater understanding of the potential health benefits of tea.

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5 Immunomodulatory Activity of Tea

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Abstract
Next to water, tea is the most commonly consumed beverage. The major chemical constituents of tea leaves are polyphenols and among the amino acids L-theanine is present in a high proportion. Tea polyphenols are perhaps the most abundant and efficient antioxidants and are the star players in the immune system, regulating a delicate balance between the immune cell functions by modulating their secretion of specific cytokines. Green tea (–)-epigallocatechin gallate and tea extract have shown immunostimulatory effects in mice, impairing the migration of macrophages/monocytes and neutrophils to the inflammatory lesions by regulating the secretion of interleukin-10 (IL-10), interferon-γ (IFN-γ), tumour necrosis factor α (TNF-α) and IL-12. Tea polyphenols play interesting roles in the development and activation of immune cells, thereby modulating their Th1/Th2 balance. Ethylamine, a degradation product of L-theanine, has shown remarkable effects in priming human Vδ2Vγ2 T cells and further enhancing their memory to abrogate microbial infections. These and other aspects of tea constituents are opening up newer frontiers in their development as therapeutics and nutraceuticals without having any adverse health effects.

Introduction

Several reports in the past have evoked dramatic interest worldwide in the health benefit effects of tea. Next to water, this is the most common beverage consumed in almost all countries of the world. Current scientific research reports have further created an awakening that consumption of tea may possibly prevent many chronic diseases. It has been proved that certain tea types or their constituents, alone or in synergy, may be contributing to such health benefits. A few reviews have been published in the recent past (Balentine, 1997; Balentine *et al*., 1997; Dufresne and Farnworth, 2000) indicating tea's biological activity and health-protective effects. Drinking tea has become associated with lifestyle and living habits of more than 80% of the population, though it is brewed differently to suit one's taste and lifestyle. Its growing importance is evident in almost all rituals and functions. This is a beverage for all occasions and times, next to water.

The major focus of this review is on the immunomodulatory activity of tea, the information being gathered from a few current publications, while some related biological effects will be dealt with briefly. Primarily it is important to know if tea infusion or its isolate can prime or enhance the functions of immune cells. This review is an effort to understand these and some other beneficial effects of tea.

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Types of Tea

It is assumed that tea was used in China more than 5000 years ago as a medicinal herb to purify the body of toxins and diseases by improving resistance. Thereafter, it was exported to European countries and to other parts of the world. Tea comes from the first apical leaves of the plant *Camellia sinensis* (family *Theaceae*) which is grown on hill slopes with a heavy annual rainfall. The plucked apical leaves are processed differently to obtain the three main commercial types of tea, i.e. black, green and oolong tea. Black tea is prepared by biochemical oxidation of crushed leaves, involving multistep enzyme-mediated oxidation of polyphenols. Green tea is readily prepared by pan frying or steaming of withered leaves to inactivate oxidative enzymes, followed by drying. Black tea is most popular, followed by green tea (Hara *et al.*, 1995).

There is another form of tea used in some northern countries called Kombucha tea. This is prepared by fermentation of mostly black and sometimes green tea with sucrose, acetic bacteria and tea fungus. Acetic bacteria and fungus live in symbiosis to inhibit the growth of other undesirable bacteria. The beverage, which has a sour flavour, is filtered through cheesecloth and stored in capped bottles at 4°C (Reiss, 1944; Blanc, 1996). The different methods of tea processing and further brewing may result in altered composition of tea constituents, which may relate to different health effects. Various constituents account for the different aftertaste of the beverages.

Chemical Composition of Tea

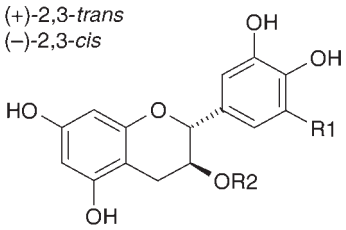
Polyphenols of green tea leaves account for 25–35% of dry weight. Important among them are flavonols of polyphenol types called catechins, such as (–)-epicatechin, (–)-epicatechin gallate, (–)-epigallocatechin gallate (EGCG), (+)-catechin and (+)-gallocatechin. Other simple flavonols and their glycosides found in tea are quercetin, myricetin, kaempferol, etc. (Fig. 5.1) (Hara *et al.*, 1995; Balentine *et al.*, 1997).

In black tea oxidation of polyphenols leads to the formation of catechin oligomers and polymers or their gallate complexes. Common among them are theaflavins, theaflavinic acids, thearubigins and proanthocyanidin. Other common minor constituents are three members of methylxanthine, caffeine, theophylline and theobromine, among others. Taste, colours and the feel in the mouth depend on the interaction between the polyphenols and caffeine. Theanine, a typical tea amino acid, accounts for 50% of the total amino acids found in tea. Kombucha, a Russian wonder-drink, is reportedly less analysed for its chemical profile. However, it contains sucrose fermentation products and a phenolic compound, usnic acid (Dufresne and Farnworth, 2000). Several climatic, ecological and geographical conditions contribute to the chemical signature and quality of tea. Therefore it is likely that health benefits may depend on the source and quality of tea consumed.

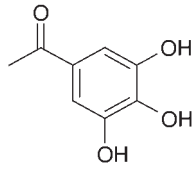
Tea polyphenols were formerly referred to as tannins or tannic acids, which left many with a misguided notion about the adverse effects of tea on the digestive system, as tannins possess the property of hardening animal tissue. However, tannins are not present in tea, though they may appear structurally similar to tea polyphenols called catechins. Unlike tannins, catechins have many health benefit effects and biochemically are very different from tannins.

Tea and its Biological Effects

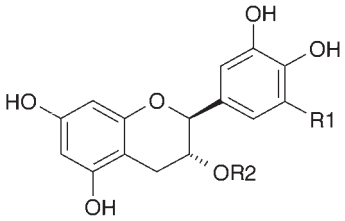
The biological effects of tea ascribed to human health have been gathered from several studies encompassing the influence of total extracts (infusion) or individual major constituents. Current literature indicative of beneficial effects of tea has been accumulated mostly from animal and *in vitro* studies, while not much literature is available from epidemiological studies in humans. An important criterion determining the effect in humans is the bioavailability of tea constituents in the systemic



Catechin



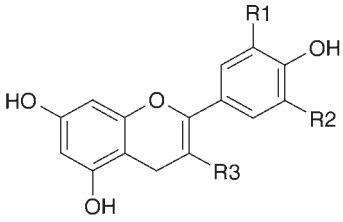
Gallate



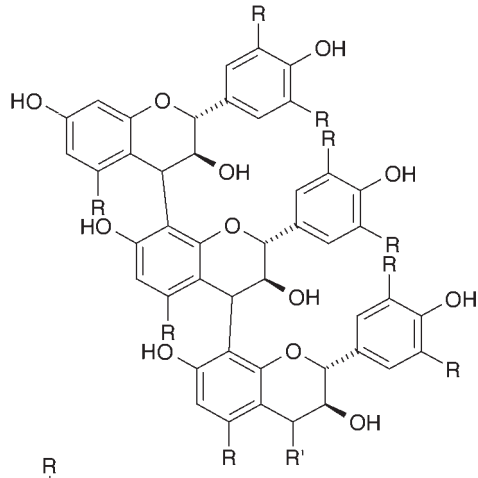
Epicatechin

Catechin, Epicatechin, R1, R2 = H
Gallocatechin, Epigallocatechin, R1 = OH, R2 = H
Catechin & epicatechin gallate, R1 = H, R2 = gallate

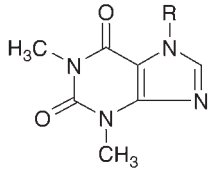
Gallactocatechin gallate & epigallactocatechin gallate, R1 = OH, R2 = gallate



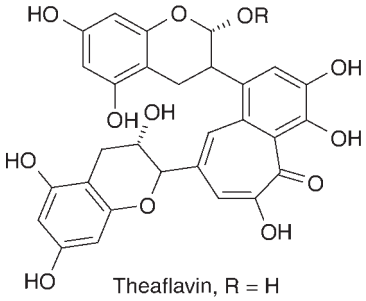
Quericitin, R1 = OH, R2 = H
Kaempferol, R1 = H, R2 = H
Myrecitin, R1 = OH, R2 = OH
R3 = OH or D-glycoside



Proanthocyanidins
R = H or OH
R' = additional units



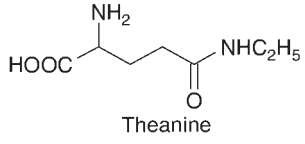
Caffeine, R = CH_3
Theophylline, R = H



Theaflavin, R = H
Theaflavin gallate, R = gallate

$C_2H_5NH_2$

Ethylamine



Theanine

Fig. 5.1. Major constituents of tea.

circulation and their influence on drug transporter proteins. Daily consumption of tea may also have an important influence on the activation of immune cells to save the body from noxious agents, particularly infections and opportunistic organisms. There appears to be a strong relationship between antioxidant, anti-inflammatory and anti-cancer activity and the immune functions of the body.

Anti-cancer and Chemopreventive Activity of Tea

The notion that consumption of tea is associated with reduced risks of developing certain forms of cancer has gained strength with increasing efforts in understanding the role of tea polyphenols in the critical events in cancer cells. This is evident from some recent studies. The green tea polyphenol EGCG inhibits signalling by Her-2/neu, which promotes cell proliferation, survival and transformed phenotype, as evidenced by inhibition of nuclear factor (NF)-κB activity and PI-3 and AKT kinases in overexpressed breast cancer cell lines (Pianetti *et al.*, 2002). This overexpression is associated with increased metastatic potential and resistance to chemopreventive agents, and often poor survival. Inhibition of NF-κB activation, considered important for tumour promotion, has been observed with EGCG and theaflavin in a mouse cancer cell line (Nomura *et al.*, 2000).

Further tea catechins were found to inhibit vascular endothelial growth factor (VEGF)-induced angiogenesis *in vitro* through impairment of VEGFR-2-mediated cascade proteins and cell substratum cum cell–cell interaction (Tang *et al.*, 2003). Another mechanism of EGCG-induced suppression of proliferation of vascular cells is through inhibition of protein tyrosine kinase activity reducing c-jun mRNA expression and inhibiting c-*Jun* N-terminal kinase 1 (JNK1) activation (Lu *et al.*, 1998). Dufresne and Farnworth (2000) gave an earlier account of the influence of tea constituents on cancer and gene mutation. Results of most of these studies on tea have come from experiments with *in vitro* systems or animals. Epidemiological studies on humans, however, are lacking except a report indicating its chemopreventive effects on oral mucosal precancerous lesions (Li *et al.*, 1999).

Antioxidant and Anti-inflammatory Activity of Tea

The antioxidant activity of black tea preparations is higher than that of most reported dietary agents on a daily basis. Strong correlation was observed between the antioxidant activities and the sum of all polyphenols quantified by high-performance liquid chromatography (HPLC). Thearubigins in the gastric environment are cleaved to more potent antioxidant theaflavins (Rechner *et al.*, 2002). Because of the potent antioxidant properties of green tea polyphenols, they appear to have anticancer and anti-inflammatory effects. Tumour necrosis factor α (TNF-α) plays a pivotal role in inflammation, while its bystander NF-κB, an oxidative stress-sensitive nuclear transcription factor, controls the expression of many genes, including that of TNF-α. Tea polyphenols and EGCG down-regulated the expression of this gene in the lipopolysaccharide (LPS)-induced inflammatory condition in BALBc mice and macrophage cell line RAW 264.7 (Yang *et al.*, 1998). The antioxidant activity is due to the ability of tea polyphenols to affect the enzymes of the antioxidant system (Erba *et al.*, 2003). Antioxidants play an important role in oxidative stress-induced degenerative diseases. EGCG inhibits proliferation of quiescent hepatic stellate cells when activated by oxidative stress during liver hepatitis (Chen and Zhang, 2003). Activation of these cells is accompanied by sequential expression of cytokine receptors – platelet-derived growth factor βR – during hepatic fibrosis. EGCG blocks the differentiation of these cells by impairing the activation of transcription factors required for platelet growth factor receptor transcription. These and other

earlier reports demonstrate the antioxidant effects of tea polyphenols, which are efficient free radical and singlet oxygen scavengers (Vinson and Dabbagh, 1998), which may relate to their effects on humans *in vivo* (Serafini *et al.*, 1996). Further, considerable epidemiological and experimental evidence shows beneficial effects of tea, particularly of green tea, in reducing the risk of heart diseases and cancer, most probably due to the antioxidant effect of polyphenols (Vinson, 2000). Thus the tea polyphenols have a strong potential to be used as therapeutic agents against various acute and chronic diseases.

Tea and the Immune System

Awareness about the potential health benefit effects of tea has been in the limelight for the past few years. Not many studies have been conducted regarding the effects on immune functions in primates. An earlier common consensus was that tea in general possesses positive health benefits, which includes the immune system, based on the reported anti-cancer and antioxidant effect of tea constituents. Since a strong immune system is required for healthy sustenance, it is possible that regular tea consumption makes it possible to restore and maintain a functional immune balance to encounter the challenge of microbial infections. Manifestations of several diseases appear to originate from the gastrointestinal tract (GIT); it is possible that regular tea consumption boosts the innate immunity and protects the intestinal epithelium from the harmful effects of several agents entering our body system.

There appears to be a strong relationship between immune suppression and tumour initiation and promotion (Kripke, 1990; Donawho and Kripke, 1991). It has been indicated that ultraviolet (UV)-induced immunosuppression in human is a risk factor for skin cancer development. Tea polyphenols appear to exert prophylactic effects.

Immune regulation of body homeostasis is highly intricate and perhaps least understood; it requires a delicate balance of several immune cell functions with release of cytokines and co-stimulatory molecules. Various approaches to help the immune system can either boost a depressed immune system or mediate the immune cell responses particularly of T-helper cells. Hyperstimulated immunity can lead to allergies or autoimmune diseases, while suppressed immune responses can harbour infectious diseases.

Constituents of tea or tea extract have shown several beneficial effects and tea is thus considered a panacea or elixir of life. Immune functions appear to be influenced in a similar way in mouse and human. Some researchers have made concerted efforts in understanding the influence of tea on immune functions in mice. Katiyar *et al.* (1999) provided some interesting early leads when they prevented UVB-induced immunosuppression in mice by the green tea polyphenol (–)-epigallocatechin-3-gallate (EGCG). In this study EGCG was applied topically on the shaved depilated skin of pathogen-free female C3H HEN mice before a single low dose of UV light irradiation (UVB, 290–330 nm). Biochemical and immunohistochemical studies were performed 48 h after the exposure. EGCG prevented UVB-induced inhibition of the contact hypersensitivity response and tolerance induction to the contact sensitizer 2,4-dintrofluorobenzene. UV irradiation as such can induce immunosuppression to a contact sensitizer primarily by influencing the type or function of cells presenting the antigen stimulus, particularly the Langerhans cells of skin (Toews *et al.*, 1980). One potential mechanism for UV-induced inhibition of contact hypersensitivity involves the release of immunomodulatory cytokines by epidermal cells (Schwarz *et al.*, 1986). A primary role of the cytokine interleukin (IL-10) produced by keratinocytes has been suggested in UV-induced immunosuppression. Topical application of EGCG before UV exposure of mouse skin reduced UV-induced production of IL-10 in skin as well as in draining lymph nodes, because EGCG reduced the number of activated CD11b+ monocytes/macrophages and neutrophils

infiltrating into skin inflammatory lesions considered responsible for creating a UV-induced immunosuppressive state. This indicated that EGCG is capable of impairing the migration of these immune cells into the inflammatory lesions of skin; such cells are CD11b+ monocytes/macrophages and neutrophils (Katiyar *et al.*, 1999), which play a role in UV-induced immunosuppression and inflammation.

CD11b is a surface marker for activated macrophages and neutrophils. The role of IL-10 was earlier suggested in inflammation and immunity (Lalani *et al.*, 1997) and it may thus play an important role in the pathogenesis of several diseases. The levels of IL-10 and IL-12 appeared inversely related to each other. IL-12 is considered to be a mediator and adjuvant for the induction of contact sensitivity and its level was increased in draining lymph nodes (DLN) of EGCG-treated mice. These authors concluded that the green tea polyphenol EGCG protects against UV-induced immunosuppression and tolerance induction by blocking UV-induced infiltration of CD11b+ cells, reducing IL-10 in the skin and markedly increasing IL-12 production in the DLN. IL-12 produced by B cells and macrophages is important in regulating the growth of Th1 cells by stimulating the production of interferon-γ (IFN-γ). EGCG thus appears to regulate the delicate balance between Th1-type and Th2-type cytokines and is capable of reversing the UV-induced inflammatory process. Similar observations were made using total green tea polyphenol (GTP) instead of EGCG on human and mouse skin, where GTP offered remarkable protection against UV-induced DNA damage and immune suppression (Katiyar *et al.*, 1995, 2001). The authors suggested that GTPs are photoprotective and boost the immune system functions against the UVB light-induced skin disorders associated with immune suppression and DNA damage. GTPs may thus prove very useful against human skin photodamage and the onset of skin photocarcinogenesis. EGCG, the major catechin in green tea, therefore appeared most effective in affording protection. There are hardly any reports investigating the comparative influence of various tea constituents.

The results of other studies derived from *in vitro* experiments or animals have shown that high-molecular-weight tea polyphenolic constituents possess antioxidant, anti-inflammatory and chemopreventive properties. There is insufficient literature available regarding their influence in humans. However, an interesting study has recently appeared focusing on perhaps the smallest organic molecule present in tea, which acts as an antigen to boost the antibacterial response in human blood cells *in vivo* and *in vitro* (Kamath *et al.*, 2003). This non-peptide antigen, ethylamine (alkylamine), is the metabolic product of L-theanine, an amino acid which accounts for about 50% of the dry weight of total amino acids in tea. Ethylamine (EA) is found in brewed tea as an intact molecule and also in its precursor form, L-theanine, which is degraded in the body into L-glutamate and ethylamine. Other Gram-positive bacteria, parasites, fungi, tumour cells and some edible plant products also share this non-peptide alkylamine antigen in tea (Hartmann, 1967; Ibe, 1991; Lichtenberger *et al.*, 1991).

The studies conducted by Bukowski and his group (Kamath *et al.*, 2003) indicated that drinking tea, which contains L-theanine, primed peripheral blood γδ T cells, which mediated a memory response upon rechallenging to ethylamine or bacteria, as evidenced by pronounced secretion of IFN-γ by these cells. IFN-γ is produced by T lymphocytes and natural killer cells, whose principal function is to activate macrophages in both the innate immune responses and the adaptive cell-mediated immune responses. Priming of γδ T cells with non-peptide antigen EA provides resistance to bacterial species, irrespective of their secretion of EA. The γδ T cells are unique in that they co-express Vγ2 and Vδ2 T-cell receptor (TCR) subunits. These cells are found only in primates and account for 2–5% of peripheral blood T cells in humans. The cells are functionally uniform and their TCR subunits recognize a family of naturally occurring non-peptide antigens with conserved molecular patterns,

such as alkylamine and organophosphates (Tanaka *et al.*, 1995; Bukowski *et al.*, 1999). These antigenic alkylamines are secreted in millimolar concentration in bacterial supernatants and are also found in certain edible plants.

Several experimental studies have indicated the antimicrobial roles of γδ T cells against bacterial, parasitic and viral infections in mice (Mombaerts *et al.*, 1993; Sciammas *et al.*, 1997). Several-fold expansion of γδ T cells has been reported in human PBMCs after infection (Kersten *et al.*, 1996) and resistance to infection is primarily due to secretion of large quantities of IFN-γ by these cells (Wang *et al.*, 2001). The presence and expansion of γδ T cells are therefore necessary for survival, which is quantitated by assay of IFN-γ or TNF-α secretion and CD3+ cells. Given the large numbers of these memory Vγ2 Vδ2 T cells in adult humans and their expansion during microbial infection, recognition of alkylamine antigens offers the immune system a response of the magnitude of major superantigens for αß T cells, and the γδ T cells thus bridge a gap between innate and adaptive immunity (Bukowski *et al.*, 1999), because γδ T cells as such do not recognize peptide complexes bound to polymorphic major histocompatibility complex (MHC) molecules and are activated by parasite-infected cells by recognizing conserved parasite-induced or parasite-derived antigens in an MHC-unrestricted manner.

Further, it was found that priming of PBMCs with isobutylamine or EA antigen resulted in expansion of γδ T cells, which secreted IFN-γ and TNF-α. These EA-primed cells developed the armoury of responsiveness when challenged with EA or lipopolysaccharide (LPS), or heat-killed bacteria which do not otherwise secrete EA at all. This means that, once the cells are primed with EA, they are activated to recruit monocytes and macrophages, which can abrogate both Gram-negative and Gram-positive bacteria. The results indicated that EA antigen is a powerful tool in offering resistance to infection, which does not depend on γδ T cell TCR-mediated recognition once the cells are primed with EA (Kamath *et al.*, 2003). Thus the antigen priming of γδ T cells *in vitro* results in their IFN-γ production in response to non-peptide antigen, whole bacteria and LPS. This has great relevance to tea drinkers, where priming of γδ T cells by EA in tea can put up a challenge to the onslaught of infection. This health benefit effect is not found in coffee drinkers as coffee is unable to exert an effect on γδ T-cell functions. Again, IFN-γ production is IL-12-dependent in PBMCs primed with alkylamine. This is because of the fact that IL-12, in the presence of IL-2, stimulates human γδ T cells to expand in number and during bacterial infection IL-12 produced by infected monocytes provides a strong stimulus for IFN-γ production by γδ T cells (Skeen and Ziegler, 1995).

Studies conducted on 11 non-tea-drinking healthy volunteers who were asked to drink 600 ml per day of brewed black tea containing 2.2 mM L-theanine for 2 or 4 weeks were compared with another ten healthy non-tea and non-coffee drinkers who also were asked to drink five to six cups of instant coffee per day (Kamath *et al.*, 2003). It should be pointed out that coffee does not contain L-theanine. PBMCs were taken before and 1–4 weeks after drinking the beverages to determine the IFN-γ titres. Drinking tea enhanced the secretion of IFN-γ by PBMCs by twofold compared with before tea drinking (Kamath *et al.*, 2003). Ingestion of tea containing EA caused PBMCs to produce IFN-γ when PBMCs from tea drinkers were challenged *in vitro* with heat-killed bacteria. These studies again show that ingestion of tea or *in vitro* treatment of γδ T cells with EA results in a markedly enhanced innate immune response to bacteria that is γδ T cell-dependent. These studies in human volunteers provide the first evidence that EA in tea provides classic immunological memory in γδ T cells, as well as these cells expressing an innate immune response. Once primed by tea EA, these cells readily take up the challenge to counter pathogens and opportunistic organisms that possess more potent antigens, such as prenyl pyrophosphate antigens from *Mycobacterium tuberculosis* and LPS from

Gram-negative bacteria. Several tumours share non-peptide antigen with bacteria (Gober *et al.*, 2003). Therefore, dietary tea intake-primed γδ T cells may provide immunosurveillance against tumours and other clinical or subclinical microbial infections (Kamath *et al.*, 2003). Such effects, however, were not investigated with other tea constituents.

Some earlier studies reported that antimicrobial activities of tea are dependent upon the net positive or negative charges of the cell surface membrane. Tea extract/catechins have remarkable antimicrobial activities (Hamilton-Miller, 1995). They hypothesized that those antimicrobial activities of tea extract/catechins could be due to the fact that the negatively charged EGCG binds strongly to the positively charged lipid bilayer membrane of Gram-positive bacteria. Therefore, one of the mechanisms of the low susceptibility of Gram-negative bacteria to catechins may be attributed to the presence of negatively charged LPS in the membrane of Gram-negative bacteria (Ikigai *et al.*, 1993). However, polyphenol complexation with positively charged groups may invite criticism when applied to the *in vivo* situation, where such an interaction will depend upon the systemic bioavailability of catechins and their complexation with other body proteins other than those of bacteria. As discussed earlier (Kamath *et al.*, 2003), the priming of immune T cells and recall of memory cells for LPS and non-peptide alkylamine appear a very convincing mechanism to abrogate several bacterial infections by tea drinking. Other investigators (Ikigai *et al.*, 1993), who had earlier proposed antimicrobiological activities of tea catechins based on cell surface charge interactions, have come up recently with interesting complementary studies showing that tea polyphenols, besides such interactions, might offer resistance through enhancement of immune functions (Hisano *et al.*, 2003). These investigators studied the inhibitory effect of green tea extract and EGCG on staphylococcal enterotoxin B (SEB) lethality in mice with potential relevance to atopic dermatitis (AD), an inflammatory condition of skin contributed by staphylococcal superantigens (SsAgs). Tea catechins were found to inhibit the lethal toxicity of SEB and SEB-induced production of TNF-α, IFN-γ and IL-4 following its intraperitoneal (i.p.) administration to D-galactosamine-sensitized BALB/c mice. The expression of TNF-α, IFN-γ, IL-2, IL-10 and IL-12 mRNAs was significantly increased in PBMCs of healthy volunteers when incubated with SEB for 24 h. The catechin treatment significantly inhibited the SEB-induced immunological response in terms of measurements of the above-mentioned cytokines. In these studies, however, the authors could not quantitate the CD4+ T cell population, while the pattern of cytokines induced by SEB appeared to be related to Th1 and Th2 subsets of the T cell population. Catechins, though, may complex with positive charges of proteins, and they apparently inhibit bacterial superantigen-induced skin inflammation through activation of immune functions. The anti-allergic effects of the tea constituents quercetin, kaempferol and myricetin have also been indicated in terms of inhibition of hyaluronidase activity and histamine release by mast cells (Toyoda *et al.*, 1997).

Hepatic injury or hepatitis is another important area where the aetiology appears to share some important components of the immune system and where tea extracts have shown a strong hepatoprotective effect. For instance, the involvement of TNF-α and its secretion in liver injury or hepatitis (Gantner *et al.*, 1995) have been observed in hepatitis caused by viruses, alcohol and autoimmune diseases. TNF-α is synthesized and released by macrophages, including Kupffer cells, the resident macrophages in liver. Stimulation of the macrophages by LPS plays a critical role in LPS + D-galactosamine (GalN)-induced acute liver injury and hepatitis (Bradham *et al.*, 1998). LPS also induces the increased formation of various circulating cytokines, of which TNF-α, IL-1, IL-6 and IL-8 are mainly produced by macrophages while IL-2, IL-4, IL-10 and IFN-γ are produced mainly by T lymphocytes. Green tea extract significantly

suppressed LPS-induced liver injury in GalN-sensitized rats (He *et al.*, 2001); the caffeine-rich fraction of tea extract exhibited the strongest effect. Dietary green tea extract suppressed all the above circulating cytokines except TNF-α, whereas the caffeine-rich fraction of dietary coffee significantly suppressed all the above cytokines. TNF-α-induced apoptosis in GalN-sensitized rat injury appeared to be the mechanism underlying the basis of hepatitis, which was impaired in green tea-fed rats. Tea extract or caffeine increased IL-10 in LPS + GalN-induced liver injury. This is a type of immunosuppressive cytokine produced by Th2 CD4+ cells and macrophages. IL-10 inhibits production of Th1 CD4+ T cells and macrophage function and may thus impair the transmigration of primed neutrophils in liver sinusoids during LPS-induced liver injury.

Concluding Remarks

Total tea extracts or some selective constituents, such as catechins and ethylamine, have shown remarkable health benefit effects in terms of pharmacological actions to boost the immune functions of the body. Antioxidants are star players that permit the immune system to work at its peak. This has been observed with vitamin E and C in terms of elevation of IL-10 and decrease in TNF-α and restoration of T and B cells in clinical cases of common variable immunodeficiency. Tea polyphenols exert anti-inflammatory, antioxidant and chemopreventive effects by inhibiting a vast array of enzymes, signalling pathways involved in cell proliferation, TNF-α secretion, inhibiting the pathway involved in the activation of NF-κB, etc. EGCG and ethylamine in tea are capable of boosting the immune functions of the body and regulating the Th1 and Th2 balance by modulating the secretion of specific cytokines. Tea constituents, therefore, have a strong potential to develop into therapeutic agents against various acute and chronic degenerative diseases. Most of the studies so far have been conducted with selected constituents. There is a need to isolate each constituent and to determine its pharmacological activities vis-à-vis plasma concentration, including immunomodulatory functions *in vitro* and in mice. Not only this, but it is equally important to determine the bioavailability of each constituent and its influence on the bioavailability of other drugs and to investigate the effect on drug efflux and metabolism. Since tea does not appear to have any adverse effects, the use of reverse pharmacology might be very useful in the development of potent therapeutics for various acute and chronic diseases.

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6 Antigenotoxic Activity of Tea

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Introduction

The practice of disease prevention is the most effective means for improving human health. Therefore, different approaches have been actively used, e.g. sanitation, vaccination and lifestyle modifications. With increased awareness of our environment it becomes clear that environmental mutagens and carcinogens (e.g. automobile exhaust, food-borne carcinogens, cigarette smoke, radiation, etc.) can cause a variety of genetic disorders, including cancer. Two general studies have been suggested to cope with this problem: (i) reducing the exposure of an individual to known mutagens as much as possible; and (ii) taking advantage of the inhibitors of mutagenesis with the final purpose of their eventual application as antigenotoxic agents. Since exposure to environmental mutagens is often unavoidable, the latter field has been widely explored with several components of the diet. The advent of natural products as chemotherapeutic agents against human ill-health effects attributable to mutations is the most valid approach due to their relative low cost and non-toxic effects.

Various short-term assays that are used for monitoring environmental mutagens may also be deployed for the detection of antigenotoxic/antimutagenic substances. Inhibitors of mutagenesis assessed by the use of the *Salmonella typhimurium* reverse mutation assay (Ames test) detect antigenotoxic potential in the microbial test system. Besides this, prevention of chromosomal aberrations, micronucleus and sister chromatid exchange (SCE) induction contributes to the evaluation of reduction in genotoxic risk associated with exposure to physical and chemical agents and predicting anticarcinogenic agents. Identification of chemotherapeutic agents through the use of the dominant lethal mutation assay determines its utility in detecting germ cell antimutagens. Hence the above techniques, coupled with antimutagenicity testing, could provide greater insight into the chemoprevention of specific types of DNA damage and be helpful in screening natural antimutagenic and anticarcinogenic agents for chemopreventive activity.

Tea is the most popular and widely consumed beverage, possessing many health-beneficial effects. The pharmacological medicinal properties of tea, including

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antioxidant, antipyretic, anti-inflammatory and anticarcinogenic effects, are well documented in the literature. Of the approximately 2.5 mt of dried tea manufactured annually, only 20% is green tea and less than 2% is oolong tea and the rest, 78%, is black tea, which contains more than 500 chemical constituents (Graham, 1992). These include flavonols, flavandiols, flavonoids and phenolic acids, carbohydrates, minerals and proteins, etc. Most of the polyphenols present in green and black teas are flavonols, tannins commonly called catechins. Some of the major catechins present in tea are (–)-epigallocatechin gallate (EGCG), (–)-epicatechin-3-gallate (ECG), (–)-epicatechin (EC), (–)-epigallocatechin (EGC), theaflavins (TF) and thearubigins (TR) (Table 6.1). TFs are astringent compounds, which contribute importantly to the colour and taste of the black tea beverage. During black tea manufacture some of the catechin mass is converted to a less well-defined group of compounds known as thearubigins. The individual polyphenolic compositions of black and green tea are given in Table 6.2.

Epidemiological and experimental studies displayed the beneficial effects of tea and its polyphenols and utility as a chemopreventive agent. The antimutagenic and anticarcinogenic activities of both green tea and black tea, along with their polyphenolic constituents, have been

Table 6.1. Composition of green and black tea extracts and polyphenols.

Polyphenolic constituents	Green tea extract (%)	Green tea polyphenols (%)	Black tea extract (%)	Black tea polyphenols (%)
Epicatechin	2.5	7.8	0.85	5.6
Epigallocatechin	10.3	4.7	0.97	1.4
Epicatechin gallate	2.3	13.2	1.7	10.6
Epigallocatechin gallate	11.2	40.7	2.9	15.3
Theaflavin	0.00	0.00	0.51	3.2
Theaflavin 3-gallate	0.00	0.00	0.46	2.8
Theaflavin 3′-gallate	0.00	0.00	0.30	1.3
Theaflavin digallate	0.00	0.00	0.22	2.1
Gallic acid	0.00	0.00	0.15	5.2
Caffeine	5.6	0.76	5.0	0.76

Table 6.2. Catechin constituents of green and black tea.

Catechins	Green tea (μg/ml)	Black tea (μg/ml)
(+)-Catechin (C)	21	20
(+)-Gallocatechin (GC)		
(–)-Epicatechin (EC)	98	37
(–)-Epicatechin-3-gallate (ECG)	90	73
(–)-Epigallocatechin (EGC)	411	42
(–)-Epigallocatechin-3-gallate (EGCG)	444	128
Theaflavonoids	0	64
Theaflavin (TF)	0	22
Theaflavin gallate A (TFA)	0	20
Theaflavin gallate B (TFB)	0	13
Theaflavin digallate (TFDG)	0	9
Thearubigins	0	23

characterized in microbial and mammalian somatic and germinal test systems (Katiyar and Mukhtar, 1997).

Epidemiological Studies

Epidemiological studies have provided sufficient evidence suggesting the beneficial role of tea drinking in human health. Drinking of both varieties of tea has been shown to inhibit the genotoxic potential of various mutagens and carcinogens. The chemopreventive role of green tea among cigarette smokers has been demonstrated by the reduction observed in SCE frequency in mutagen-stimulated peripheral blood lymphocytes (Shim *et al.*, 1995). The protective effect of tea has been suggested mainly due to the ability of tea polyphenols to inhibit the endogenous formation of nitroso compounds, which are considered to be major causative factors in gastric cancer (Yang and Wang, 1993).

Antimutagenic activity of tea and its polyphenols in microbial systems

Salmonella typhimurium *reverse mutation assay*

The use of the *S. typhimurium* reverse mutation assay in genetic toxicology is now firmly established for fundamental studies in both mutagenesis and carcinogenesis (Flamond *et al.*, 2000; Kappers *et al.*, 2000). The *S. typhimurium* reverse mutation assay (Ames test) is a widely used method to assess the mutagenic potential of chemicals that can cause base-pair and frame-shift mutations in the genome of this organism (Maron and Ames, 1983). At the same time, this useful tool is also being employed to evaluate the antimutagenic potential of various synthetic or natural products (Heddle *et al.*, 1999).

The antimutagenic activity of tea extracts and polyphenols, including ECG and EGCG, against various mutagens and carcinogens has been demonstrated using microbial systems. EGCG and ECG were inhibitory against the mutagenicity of *N*-methyl-*N*′nitro-*N*-nitrosoguanidine (MNNG) in *S. typhimurium* TA98 and TA100 with and without rat liver S9 mix. (Okuda *et al.*, 1984). EGCG also has a strong inhibitory effect on the mutagenicity of benzo[a]pyrene diol epoxide in TA100 strain without S9 mix (Okuda *et al.*, 1984). Theaflavins, gallate esters and catechins inhibited mutagenicity of 2-amino-1-methyl-6-phenylimidazo-[4,5-b] pyridine (PhIP) in *Salmonella typhimurium* TA98 (Apostolides *et al.*, 1997). The gallate esters of the catechins EC, EGC, EGC and EGCG, the theaflavonoids, TF, TFMG and theaflavin digallate (TFDG), and glucose had a low 50% inhibitory concentration (IC_{50}) in the 80–250 μM range against mutagenicity of 10 μM PhIP. The non-polyphenolic fraction of green tea suppressed 3-amino-1, 4-dimethyl-5H-pyrido[4,3-b]indol (Trp-P-1)- or mitomycin C (MMC)-induced umu C gene expression in *S. typhimurium* TA1535/psk 1002 in the presence or absence of S9 metabolizing enzyme mixture (Okai and Okai, 1997). Standard black and green tea extracts have been known to inhibit mutagenicity caused by PhIP in the *S. typhimurium* reverse mutation assay, with TA98 containing the S9 fraction from the liver of rats induced with alpha-naphthoflavone and phenobarbitone (Apostolides and Weisburger, 1995). The inhibition of the mutagenicity of PhIP by gallated catechins and theaflavins has been found to be due to inhibition of the conversion of the procarcinogen PhIP to the proximate carcinogen N-OH-PhIP (Hayatsu *et al.*, 1992). Antimutagenic activity of green and black tea extracts was also observed towards the food mutagen MeIQx in a direct plate assay with *S. typhimurium* in an *in vitro* gastrointestinal model (Krul *et al.*, 2001). In addition, tea exhibited antimutagenic potential against the mutagenicity of heterocyclic amines in *S. typhimurium* (Stravic *et al.*, 1996). The antigenotoxic effects of specific tea polyphenols, Polyphenon 60 and Polyphenon 100 from green tea and Polyphenon B from black tea, were tested against a battery of genotoxic carcinogens in *S. typhimurium* TA98, TA100 and TA1535, the tester strains (Hara, 1994; Weisburger *et al.*, 1996). The results indicated that tea polyphenols sharply decreased the mutagenicity of a number of aryl- and

heterocyclic amines of aflatoxin B1 (AFB1), BaP, 1,2-dibromoethane (DBE) and, more selectively, of 2-nitropropane, all when an induced rat liver S9 fraction was included. Antimutagenic and antioxidative effects of theaflavins from black tea were reported using *S. typhimurium* TA104 (Shiraki *et al.*, 1994). All theaflavins tested, TF, TFA, TFB and TFDG, suppressed mutagenicity induced by H_2O_2 and inhibited peroxidation of rabbit erythrocyte ghosts induced by *t*-butyl hydroperoxide.

Evaluation of the antimutagenic potential of aqueous black tea extract (ATE) in the Ames test, using TA98 and TA100 tester strains, revealed that the addition of 500 µl of 1, 2 and 4% ATE to the BaP- and CP-treated plates resulted in a dose-dependent inhibition in the number of his^+ revertant colonies. Similarly, supplementation of BTP at the concentration of 100, 200 and 400 µg/plate also led to a significant inhibition in BaP- and CP-induced his^+ revertant colonies. The antimutagenic activity profile of BTP was found to be higher than that of ATE, which may be attributed to the higher amount of polyphenolic ingredients (Taneja *et al.*, 2003).

In other microbial systems

Antimutagenic profiles of both black and green tea and their polyphenolic constituents are well documented in the literature. A homogenate of Japanese green tea gave high bioantimutagenic activity against spontaneous mutations resulting from altered DNA polymerase III in strain NIG1125 of *Bacillus subtilis met his mut-1* (Kada *et al.*, 1985). Green tea and black tea decreased the mutagenic activity of MNNG in *Escherichia coli* WP2 (Jain *et al.*, 1989). Pre-incubation together of the MNNG and tea extracts, before exposure of the cells, also reduced the mutagenic activity of MNNG. EGCG reduced spontaneous mutations in strain NIG1125 of *B. subtilis* (*met his mut-1*), due to the inhibition of a function of error-prone DNA replication involving an altered DNA polymerase III (Kada *et al.*, 1985). ECG, EGC and EGCG reduced ultraviolet C (UVC) (254 nm)-induced mutations in *E. coli* B/r WP2 by altering the fidelity of DNA replication (Shimoi *et al.*, 1986). The antimutagenicity of catechins was identified against UV-induced mutations in *E. coli* B/r WP2 (Shimoi *et al.*, 1986). Tea extracts inhibited the mutagenicity of 1-methyl-1,2,3,4-tetrahydrodiol-β-carboline-3-carboxylic acid on treatment with nitrite in the presence of ethanol (Higashimoto *et al.*, 2000).

Antimutagenic activity of tea and its polyphenols in mammalian *in vivo* cytogenetic assays (somatic cell assays)

Cytogenetic tests are well identified for evaluation of mutagenic damage caused by environmental toxicants to the chromosomes (Preston *et al.*, 1987). *In vivo* bone marrow tests, which include metaphase chromosome analysis, the micronucleus assay and SCE assay are used to identify clastogenic compounds, that is, those which are capable of inducing structural damage to chromosomes. Chromosomal aberrations indicate the clastogenic effect induced by mutagens, cytogenetically observed as gaps, breaks, exchanges and multiple aberrations in metaphase-arrested cells. The micronucleus test is a method devised primarily for screening of mutagenic chemicals for chromosome-breaking effects. Micronuclei are the acentric chromosome fragments that lag behind at the anaphase stage of cell division on exposure to genotoxicants, whereas SCEs represent reciprocal exchanges in the DNA between two sister chromatids of duplicating chromosomes and their reunion at apparently homologous loci, which are induced on exposure to genotoxicants**.** All three tests are widely used for screening purposes and are regarded to be of particular importance by many regulatory authorities. These tests are employed in the whole animal, so obvious deficiencies in artificial metabolic activation systems used in *in vitro* systems are minimized.

The applicability of *in vivo* cytogenetic assays in the determination of the antigenotoxic potential of dietary compounds is

well documented (Bronzetti *et al.*, 1996). Studies conducted with green tea polyphenolic ingredients have shown that it possesses potential in inhibiting the genotoxicity of polycyclic aromatic hydrocarbons, nitrosoamines, etc. in a mammalian test system (Kuroda and Hara, 1999). Tea and its polyphenols inhibited MMC-induced micronuclei induction in V79 cells (Liu *et al.*, 1998). Moreover, oral administration of 0.1% green tea reduced BaP-induced micronuclei in the peripheral blood of mice (Sasaki *et al.*, 1993). Tea polyphenols, EGCG and TFG sharply reduced the mutagenicity of IQ and PhIP, and induced DNA repair in rat hepatocytes (Weisburger *et al.*, 1996). ECG and EGCG had inhibitory effects against 6-thioguanine (6TG)-resistant mutations induced by 4-nitroquindine 1-oxide (4NQO) in cultured Chinese hamster V79 cells (Kuroda, 1996). The antimutagenic activity of the catechins was found only when the cells were post-treated with catechins during the mutation expression time after treatment with 4NQO, and was not found by simultaneous treatment with 4NQO and catechins (Kuroda, 1996). This suggests that the catechins may act intracellularly as bioantimutagenic blocking agents or suppressive agents. In addition, extracts of green tea effectively suppressed AFB1-induced chromosome aberrations in bone marrow cells in rats (Ito *et al.*, 1989). In another report, green tea and black tea retarded 4(methylnitrosamino)-1-(3-pyridyl)-1-butanone bioactivation and DNA methylation in A/J mice (Shi *et al.*, 1994). Crude tea extracts decreased the mutagenic activity of MCCG *in vitro* and in the intragastric tract of rats (Jain *et al.*, 1989). Black and green tea imparted protection against 2-amino-3-methylimidazo-[4,5-f]quinoline-induced DNA adducts and colonic aberrant crypts in the F344 rat (Xu *et al.*, 1996). Anticlastogenic effects of black tea and its two active polyphenols, theaflavins and thearubigins, were identified towards CP and DMBA in an SCE and chromosomal aberration assay *in vivo* in Swiss albino mice (Gupta *et al.*, 2001). A protective effect of green tea has been observed against BaP-induced mutations in the liver of Big Blue mice (Jiang *et al.*, 2001). A chemopreventive effect of green tea has been attributed against cigarette smoke-induced mutations (SCE) in humans (Lee *et al.*, 1997). Tea exhibited anticlastogenic activity against environmental tobacco smoke in the SCE assay (Zhau *et al.*, 2000). Green tea antioxidant strongly inhibited the increase in SCE and micronuclei induced by fried fish extract and its component MeIQ in V79 or IAR20 cells (Liu, 1998). Besides this, in another study green tea exhibited chemopreventive effects on SCE induction among cigarette smokers (Shim *et al.*, 1995). Tea tannin components inhibited the induction of SCEs and chromosome aberrations in mutagen-treated cultured mammalian cells (Imanshi *et al.*, 1991). Hot-water extracts of green tea effectively suppressed AFB1-induced chromosome aberrations in bone marrow cells in rats that were given green tea extract 24 h before they were injected with AFB1 (Ito *et al.*, 1989). The suppressive effect of green tea extracts on AFB1-induced chromosome aberrations was directly related to the dose of green tea extract when given in the range between 0.1 and 2 g/kg. Catechins have been found to inhibit tobacco-specific 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK)-induced DNA strand breaks in rat hepatocytes (Liu and Castonguay, 1991). Green tea suppressed NNK-induced levels 8-OH-guanosine levels in lung DNA (Xu *et al.*, 1992). Administration of 2% green tea as sole source of drinking solution for 2 weeks reduced 2-nitropropane-induced levels of 8-OH-deoxyguanosine adducts in liver nuclear DNA. Reductions in levels of DNA adducts were also found in rats given extracts of 2% green tea or 1% black tea for 8 weeks before oral administration of IQ (Xu *et al.*, 1996). The amount of IQ and other promutagens decreased in both urine and faeces. Binding of AFB1 to hepatic nuclear DNA was inhibited in rats given 0.5% instant green tea for 2 or 4 weeks before a single injection of AFB1 (Quin *et al.*, 1997). The oral administration of 0.2% green tea or 0.1% black tea for 28 days decreased the extent of micronuclei formation in the peripheral blood of

mice subsequently treated with BaP and 3-methylcholanthrene (Sasaki *et al.*, 1993). It was also found that green tea given by stomach perfusion had distinct inhibitory effects on micronuclei in the colon crypt cells of C57BL mice (Zao *et al.*, 1992).

ATE at concentrations of 1, 2 and 4%, when given as a sole source of drinking solution for 2 weeks, resulted in inhibition of chromosomal aberrations, micronuclei and SCEs induced by BaP or CP (Tables 6.3 and 6.4). The anticytotoxic potential of ATE was also evident, as the status of the mitotic index, which declined in the BaP/CP-alone-treated group, was found to be increased. Similarly, the oral dosage of 40, 80 and 160 mg/kg body weight (bw) of BTP for five consecutive days prior to BaP/CP injection led to more significant inhibition in chromosomal aberrations, micronuclei induction and SCEs. Different kinds of chromosomal damage, including gaps, breaks, exchanges and multiple aberrations, induced by BaP or CP were found to be inhibited. Furthermore, BaP- and CP-induced cytotoxicity was also found to be protected by different doses of BTP. Hence the study reveals that administration of black tea has potential in suppressing chromosomal aberrations, micronuclei induction, SCEs and cytotoxicity produced by mutagens *in vivo* (Shukla and Taneja, 2002).

Antimutagenic potential of black tea and its constituents in mammalian germ cells using a dominant lethal mutation assay

The dominant lethal assay analyses the meiotic mutagenic damage induced by genotoxicants, which results in the death of the embryo. A positive result in the dominant lethal test (DLT) provides evidence for damage transmitted via the gametes. DLT is widely used to assess the mutagenic potential of chemicals in whole animals and to detect agents that produce chromosomal aberrations in sperm and thereby affect the viability of progeny (Ashby and Clapp, 1995).

Clastogenicity induced by genotoxicants, leading to non-disjunction in chromosomes in the meiotic cycle resulting in a chromosomal-deficient embryo that dies *in utero*, is the cause of dominant lethality (Green *et al.*, 1987; Ashby and Clapp, 1995). However, dietary agents have been found to inhibit the clastogenetic damage induced by many xenobiotics in germ cells (Ferguson, 1994; Waters *et al.*, 1998). The antimutagenic properties of tea and its polyphenols are well documented in mammalian somatic cell assays (Kuroda and Hara, 1999). However, no study on the effect of tea on germ cells has been conducted.

The work carried out in our laboratory demonstrates the antimutagenic potential of ATE and BTP against BaP- and CP-induced dominant lethal mutations in male Swiss albino mice (Shukla and Taneja, 2001). In different sets of experiments, BaP and CP were injected through the intraperitoneal (i.p.) route at the doses of 100 mg/kg bw and 60 mg/kg bw, respectively. Soon after BaP/CP injection the animals were mated with untreated virgin females for 3 weeks of mating intervals. The females were analysed for living and dead implants. During 3 weeks of mating, BaP/CP administration resulted in a decrease in the number of living implants and an increase in dead implants. However, pre-treatment of 1, 2 and 4% ATE as sole source of drinking solution for 2 weeks prior to BaP or CP injection resulted in dose-dependent inhibition of dominant lethality (Tables 6.5 and 6.6). The numbers of living implants were found to be increased by ATE. The induction of post-implantation losses by BaP/CP was found to be protected by ATE. Similarly, an oral dosage of 40, 80 and 160 mg/kg bw of BTP for five consecutive days also led to more significant inhibition of dominant lethal mutations induced by either BaP or CP. The status of decline in the number of living implants by BaP/CP treatment was found to be protected by BTP. Furthermore, significant inhibition of BaP/CP-induced post-implantation losses was also notified by treatment of BTP. Hence the study reveals that black tea has a potential in inhibiting meiotic mutagenic damage induced by germ cell mutagens.

Table 6.3. Antimutagenic effect of ATE and BTP on BaP-induced chromosomal aberrations in Swiss albino mice bone marrow cells.

Treatment groups	Mitotic index (mean ± SE)	% Chromosomal aberrations with (mean ± SE)				Incidence of aberrant cells (%) (mean ± SE)	Suppression (%)
		Breaks	Fragments	Exchanges	Multiple damage		
Untreated	4.67 ± 0.47	0.77 ± 0.17	0.71 ± 0.020	0.02 ± 0.01	0.97 ± 0.26	2.47 ± 0.61	—
BaP	3.01 ± 0.38[a]	3.77 ± 0.89	4.53 ± 1.07	0.98 ± 0.12	2.50 ± 1.01	11.78 ± 1.82[a]	—
1% ATE + BaP	3.69 ± 0.36	3.53 ± 0.63	4.22 ± 0.96	0.81 ± 0.19	2.16 ± 1.07	9.51 ± 1.17	19.2
2% ATE + BaP	4.01 ± 0.37[b]	2.94 ± 0.59	4.10 ± 0.89	0.65 ± 0.18	2.02 ± 0.89	8.52 ± 1.03	27.6
4% ATE + BaP	4.36 ± 0.39[b]	2.19 ± 0.48	2.17 ± 0.67	0.51 ± 0.10	1.86 ± 0.78	6.10 ± 0.81[b]	48.2
BTP (40) + BaP	3.89 ± 0.38[a]	2.28 ± 0.39	3.13 ± 0.67	0.65 ± 0.09	1.76 ± 0.61	7.93 ± 0.92	32.7
BTP (80) + BaP	4.09 ± 0.36	2.23 ± 0.63	2.72 ± 0.46	0.37 ± 0.09	1.26 ± 0.57	6.90 ± 0.80[b]	41.4
BTP (160) + BaP	4.58 ± 0.37[b]	1.64 ± 0.19	2.10 ± 0.59	0.22 ± 0.09	1.02 ± 0.29	4.43 ± 0.61[b]	62.4

[a]Significant difference from Gr. I, $P < 0.05$.
[b]Significant difference from Gr. II, $P < 0.05$.
SE, standard error.

Table 6.4. Antimutagenic effect of ATE and BTP on CP-induced chromosomal aberrations in Swiss albino mice bone marrow cells.

Treatment groups	Mitotic index (mean ± SE)	% Chromosomal aberrations with (mean ± SE)				Incidence of aberrant cells (%) (mean ± SE)	Suppression (%)
		Breaks	Fragments	Exchanges	Multiple damage		
Untreated	4.67 ± 0.47	0.77 ± 0.17	0.71 ± 0.020	0.02 ± 0.01	0.97 ± 0.26	2.54 ± 0.66	—
CP	2.89 ± 0.38[a]	7.58 ± 0.89	8.63 ± 1.07	0.96 ± 0.12	3.76 ± 1.01	19.86 ± 2.02[a]	—
1% ATE + CP	3.19 ± 0.36	7.03 ± 0.63	7.51 ± 0.96	0.87 ± 0.19	2.16 ± 1.07	16.98 ± 1.57	14.5
2% ATE + CP	3.68 ± 0.37[b]	6.14 ± 0.59	6.51 ± 0.89	0.67 ± 0.10	2.02 ± 0.89	15.35 ± 1.17	22.7
4% ATE + CP	4.01 ± 0.39[b]	5.68 ± 0.58	5.78 ± 0.67	0.51 ± 0.30	1.86 ± 0.78	12.10 ± 1.06[b]	38.6
BTP (40) + CP	3.86 ± 0.47	6.77 ± 0.17	5.71 ± 0.50	0.69 ± 0.01	1.97 ± 0.26	13.36 ± 1.42	24.4
BTP (80) + CP	4.18 ± 0.38	6.18 ± 0.89	4.51 ± 0.67	0.53 ± 0.32	1.66 ± 1.01	12.17 ± 1.27[b]	38.7
BTP (160) + CP	4.48 ± 0.36	4.53 ± 0.63	3.21 ± 0.96	0.38 ± 0.29	1.16 ± 1.07	9.55 ± 0.97[b]	51.9

[a]Significant difference from Gr. I, $P < 0.05$.
[b]Significant difference from Gr. II, $P < 0.05$.
SE, standard error.

Table 6.5. Antimutagenic effect of ATE and BTP on BaP-induced dominant lethal mutation (DLM) in Swiss albino mice.

Treatment groups	Mating weeks	Living implants/ female (mean ± SE)	Dead implants/ female (mean ± SE)	DLM rate (%)	Suppression (%)
Untreated	1	7.86 ± 0.32	0.32 ± 0.06	—	—
	2	7.84 ± 0.28	0.30 ± 0.09	—	—
	3	7.85 ± 0.30	0.28 ± 0.09	—	—
BaP	1	5.45 ± 0.36[a]	2.87 ± 0.78[a]	31.7	—
	2	6.17 ± 0.28[a]	1.89 ± 0.62[a]	21.2	—
	3	6.51 ± 0.30	0.92 ± 0.23[a]	18.7	—
1% ATE + BaP	1	5.94 ± 0.38	2.16 ± 0.72	24.4	21.1
	2	6.46 ± 0.28	1.50 ± 0.48	17.5	18.7
	3	6.96 ± 0.36	0.66 ± 0.28	11.3	36.4
2% ATE + BaP	1	6.30 ± 0.28	1.84 ± 0.60	19.8	37.6
	2	6.71 ± 0.36	1.09 ± 0.42	14.3	32.8
	3	7.13 ± 0.28[b]	0.52 ± 0.20[b]	9.1	51.3
4% ATE + BaP	1	6.84 ± 0.35	1.26 ± 0.38[b]	12.9	59.3
	2	7.04 ± 0.36	0.89 ± 0.17[b]	10.1	52.2
	3	7.37 ± 0.28	0.41 ± 0.09[b]	6.1	68
BTP (40) + BaP	1	6.15 ± 0.28[b]	1.77 ± 0.72	22.3	29.6
	2	6.61 ± 0.28	1.10 ± 0.48	15.6	30.9
	3	7.04 ± 0.30[b]	0.70 ± 0.18[b]	10.3	47.4
BTP (80) + BaP	1	6.55 ± 0.28	1.55 ± 0.60	16.6	45.8
	2	7.03 ± 0.30[b]	0.85 ± 0.42	10.4	46.9
	3	7.27 ± 0.30	0.60 ± 0.15[b]	7.3	62.7
BTP (160) + BaP	1	6.86 ± 0.28[b]	1.10 ± 0.58	10.3	66.2
	2	7.10 ± 0.35[b]	0.70 ± 0.21[b]	9.4	58.4
	3	7.38 ± 0.35	0.45 ± 0.09[b]	5.5	72

[a]Significant difference from Gr. I, $P < 0.05$.
[b]Significant difference from Gr. II, $P < 0.05$.
SE, standard error.

Mechanism of Antigenotoxicity of Tea

The mechanisms proposed for the antimutagenic effect of tea involve interaction between the reactive genotoxic species of the various promutagens and nucleophilic tea component(s) present in tea (Kuroda and Hara, 1999). Tea ingredients have the property of mopping up mutagenic reactive free-radical intermediates generated by genotoxicants by various metabolic pathways (Johnson and Loo, 2000; Maliakal *et al.*, 2001). The second mechanism involves inhibition of the cytochrome P-450-dependent bioactivation of the promutagens (Wang *et al.*, 1988). Black tea exhibits the potential to inhibit *in vitro* cytochrome P-450-dependent metabolic activation of mutagens and displays antioxidative activity (Grinberg *et al.*, 1997). Tea ingredients were found to inhibit aryl hydrocarbon hydroxylase (AHH) activity in liver microsomes (Wang *et al.*, 1988). The inhibition of cytochrome P-450 activity may be due, at least partly, to impairment of the electron flow from nicotinamide adenine dinucleotide phosphate (NADPH) to the cytochrome (Wang *et al.*, 1988). Tea catechins have been shown to inhibit cytochrome P-450-mediated activation of carcinogens, including polycylic aromatic hydrocarbons (PAHs), AFB1 and nitrosoamines (Wang *et al.*, 1988; Yang

Table 6.6. Antimutagenic effect of ATE and BTP on CP-induced dominant lethal mutation (DLM) in Swiss albino mice.

Treatment groups	Mating weeks	Living implants/ female (mean ± SE)	Dead implants/ female (mean ± SE)	DLM rate (%)	Suppression (%)
Untreated	1	7.86 ± 0.28	0.32 ± 0.06	—	—
	2	7.84 ± 0.36	0.30 ± 0.09	—	—
	3	7.85 ± 0.35	0.28 ± 0.09	—	—
CP	1	5.69 ± 0.28[a]	2.03 ± 0.78[a]	27.5	—
	2	4.98 ± 0.36[a]	3.04 ± 0.62[a]	36.4	—
	3	6.32 ± 0.28	1.43 ± 0.23[a]	19.4	—
1% ATE + CP	1	5.97 ± 0.28	1.77 ± 0.72	24	12.6
	2	5.48 ± 0.28	2.49 ± 0.48	30.1	17.2
	3	6.75 ± 0.30	1.04 ± 0.28[b]	13.9	28.6
2% ATE + CP	1	6.16 ± 0.28	1.55 ± 0.60	21.6	21.2
	2	5.77 ± 0.30	2.22 ± 0.42	26.4	27.6
	3	7.00 ± 0.30	0.79 ± 0.20[b]	10.8	44.2
4% ATE + CP	1	6.46 ± 0.28	1.25 ± 0.38	17.7	35.6
	2	6.06 ± 0.35[b]	1.89 ± 0.41[b]	22.6	37.8
	3	7.08 ± 0.35	0.68 ± 0.09[b]	9.8	49.6
BTP (40) + CP	1	6.09 ± 0.30	1.51 ± 0.72	22.4	18.2
	2	5.84 ± 0.30	2.34 ± 0.48	25.4	21.6
	3	6.80 ± 0.36	0.87 ± 0.28[b]	13.3	31.4
BTP (80) + CP	1	6.38 ± 0.36	1.24 ± 0.60	18.8	31.6
	2	6.02 ± 0.36	1.90 ± 0.42	23.1	36.4
	3	6.03 ± 0.28[b]	0.54 ± 0.20[b]	10.0	48.2
BTP (160) + CP	1	6.53 ± 0.28	1.12 ± 0.58	16.9	38.2
	2	6.20 ± 0.30[b]	1.71 ± 0.21[b]	20.8	42.6
	3	7.19 ± 0.36	0.40 ± 0.09[b]	8.4	56.7

[a]Significant difference from Gr. I, $P < 0.05$.
[b]Significant difference from Gr. II, $P < 0.05$.

et al., 2000). EGCG, the major catechin present in both varieties of tea, has been found to modulate the activity of cytochrome P-450 IA1, IA2, IA3 and NADPH reductase, thereby reducing conversion of promutagens to ultimate mutagens (Wang *et al.*, 1988). Both Phase I and Phase II enzymes were induced in rats, using black or green tea infusions (Katiyar and Mukhtar, 1997; Kuroda and Hara, 1999). Besides this, tea catechins EGCG, EC, EGC and ECG have also been found to enhance the Phase II enzymatic pathways, resulting in detoxification of carcinogens (Kuroda and Hara, 1999; Maliakal *et al.*, 2001). For theaflavins, polyphenolic ingredients of black tea, it has been shown that inhibition of DNA single-strand cleavage and mutagenicity induced by hydrogen peroxide in *S. tyhphimurium* is due to *in vitro* antioxidant activity (Shiraki *et al.*, 1994). It has also been found to possess antimutagenic effects towards peroxy radical damage (Shiraki *et al.*, 1994). Catechins are competitive inhibitors of the NADPH-cytochrome c reductase enzyme (Hasaniya *et al.*, 1997; Katiyar and Mukhtar, 1997).

Induction of antioxidant enzymes against oxidative stress plays an important role in inhibiting clastogenicity of genotoxicants. Tea polyphenols are strong scavengers against superoxide, hydrogen peroxide, hydroxy radicals and nitric oxide produced by various chemicals (Katiyar and Mukhtar, 1997). Black tea has been identified to act as a powerful chemopreventer of reactive oxygen and nitrogen species (Sarkar and Bhaduri,

2001). EGCG also exhibited protective effects against oxidative damage to cellular DNA (Johnson and Loo, 2000). EGCG is known to interact with DNA polymerase III and influence the excision repair system, favouring repair of genetic damage (Kada *et al.*, 1985).

Conclusion

The levels at which tea has been consumed worldwide aroused interest in the possibility of its use in cancer chemoprevention and other related genetic disorders. The possible beneficial health effects of tea consumption have been suggested by epidemiological studies and supported by laboratory research. Considerable work has been carried out on both varieties of tea as infusions and their principal constituents. An overview of the findings on antigenotoxicity indicates that both varieties of tea have significant antimutagenic and anticlastogenic properties. Green tea was found to have no mutational toxicity but was able to inhibit mutations at concentration levels equivalent to daily human consumption. In the case of black tea, reports on thearubigins show that it has significant antimutagenic and anticlastogenic effects, as in the case of theaflavins. The knowledge gained at tissue levels and biological activities of tea polyphenols would be useful in planning future epidemiological studies and human cancer prevention trials. Therefore it may be concluded that both varieties of tea have potential to prevent cancer and other genetic disorders, but more in-depth studies are needed to study their mechanism of action.

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7 Methodological Issues in Population Studies of Tea and Disease Prevention

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Introduction

The protective role of tea and its constituents in chronic disease prevention in humans is under continuous debate. Methodological pitfalls may explain the inconsistencies within and between different study designs. The inconsistent epidemiological findings may be attributed to confounding variables, such as individual differences in tea preparation and consumption patterns, variability associated with tea production, variability in the bioavailability of the active green tea constituents, concomitant use of tobacco and alcohol and individual differences in lifestyle. We need to understand the advantages and limitations of the different study approaches. Observational studies have the advantage of studying the population at large, with ultimate disease as the study end point. However, these studies are limited by the difficulty in estimating the intake of individual compounds by questionnaires and the lack of biological markers of relevant exposure. Controlled experimental short-term studies in humans rely on biological markers of disease as intermediate end points. The relatively low sensitivity and specificity of these markers may complicate extrapolation of results. In the case of long-term and large-scale human intervention studies with disease end points, issues such as time, dose and duration of intervention, compliance and choice of the study population influence the interpretation of results.

Increasing recognition of the potential importance of tea polyphenols in the aetiology of various diseases has highlighted the need for methods to measure their consumption that are sufficiently simple to be used in large epidemiological studies and whose reproducibility and accuracy have been quantified.

Assessment of Tea Consumption

The concentration of flavonoids in tea is likely to vary with preparation technique. Inconsistencies between epidemiological studies may arise from the lack of information on methods of preparation. Tea leaves are primarily manufactured as green, black or oolong, with black tea representing approximately 80% of the tea consumed. Catechins and theaflavins are the two major groups of tea flavonoids. Green tea is the non-oxidized/non-fermented product of the leaves and contains several polyphenolic

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components, such as epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate (EGCG). EGCG is the major green tea polyphenol (>40% dry weight) (Graham, 1992). In the manufacture of black tea, the polyphenols in tea leaves undergo polyphenol oxidase-catalysed oxidative polymerization, which leads to the formation of bisflavonols, theaflavins, thearubigins and other oligomers in a process commonly known as 'tea fermentation' (Graham, 1992). The major components of black tea (the fermented product) are theaflavins (1–3% dry weight) and thearubigins (10–40% dry weight) (NCI, 1996).

In human populations, the amount of tea polyphenols ingested is determined not only by the frequency and amount of tea intake but also by the strength of the tea consumed. Catechins from green tea and black tea were rapidly absorbed and addition of milk did not impair the bioavailability of tea catechins (van het Hof *et al.*, 1998). Moreover, recently published data showed that addition of milk to black or green tea did not affect its antioxidant activity (Leenen *et al.*, 2000).

Variability in tea preparation

Since dry tea leaves are not consumed directly, brewing conditions may influence the final antioxidant capacity in the tea as consumed. Tea composition, including catechins, varies with climate, season, horticultural practices, variety and the age of the leaf (Graham, 1992). Moreover, methods of beverage preparation vary greatly. The traditional tea beverage is prepared by steeping tea leaves or tea bags in water at 90–100°C in teapots or cups. Additional hot water may be added to residual leaf in teapots to produce a weaker beverage.

Forty samples, representing the most typical preparation techniques of hot, iced and sun tea in Arizona (USA), were analysed by high-performance liquid chromatography (HPLC) for total flavonoids, catechins, theaflavins, thearubigins, caffeine and gallic acid. Our database shows that tea composition, including catechins, varies with tea-leaf concentration, brewing time and beverage temperature (Hakim *et al.*, 2000). In black tea, the total phenolic concentration as well as the antioxidant activity increased with the brewing time (Liebert *et al.*, 1999; Hakim *et al.*, 2000). One cup of black tea, brewed using one tea bag (regular strength), has approximately three times the amount of total flavonoids and six times the amount of catechins as the same volume prepared by two successive brews of loose tea leaf (diluted).

Iced tea was first introduced in 1904 at the St Louis World Fair during a spell of hot weather (Weisburger, 1997). The custom of drinking iced tea remains largely American. In the USA nearly 75% of tea is consumed cold, and the practice is spreading elsewhere (Weisburger, 1997). It is difficult to state a definitive composition for iced tea beverage, as methods of beverage preparation vary greatly. This beverage is prepared by either cooling traditionally brewed tea or by prolonged (6–15 h) steeping of tea in the sun (sun tea). Moreover, the wide variability in preparation techniques of iced tea was translated into corresponding variability in tea composition in the resulting beverages. Iced tea is likely to be consumed more diluted than hot tea. Iced tea is prepared in larger amounts, using fewer tea bags (6–10 bags/gallon = 0.86–1.44 g/240 ml), while regular hot tea is usually prepared by extracting one tea bag per cup (2.26 g/240 ml) of hot water. The wide variability in preparation techniques of iced tea is translated into corresponding variability in flavonoid concentrations (202–847 μg/ml) in the resulting tea infusions (Hakim *et al.*, 2001).

The demand for convenience led to the creation of instant tea, iced tea mixes and ready-to-drink iced tea in cans, bottles and plastic containers. Canned and bottled tea drinks are becoming popular worldwide (Komatsu *et al.*, 1991). Although total catechins in green tea, oolong tea and black tea have been reported, information on the catechin composition of canned and bottled tea drinks is limited (Ho and Zhu, 1999). Chen and co-workers (Chen *et al.*, 2001) have analysed 14 brands of canned and bottled tea drinks and 11 brands of green, black

and oolong teas which are mostly consumed in Hong Kong. They found that green tea catechin content was very low in canned and bottled tea when compared with that of tea traditionally prepared in a porcelain cup or teapot. Thus, one will ingest 400–500 mg green tea catechins if one cup of tea drink is brewed traditionally. In contrast, one will ingest only 3–60 mg green tea catechins when one canned or bottled tea drink (250 ml) is consumed. In Arizona, our group analysed 40 black tea samples (Hakim *et al.*, 2000). Our results show that tea concentration, brewing time and beverage temperature also have major influences on flavonoid concentrations. The highest concentrations of flavonoids (μg/ml) were found in brewed hot tea (range: 541–692) while the lowest concentrations were for instant tea preparations (range: 90–100). We found that one glass of brewed black iced tea has approximately five times the amount of total flavonoids available in a similar glass of instant iced tea. Moreover, instant iced tea contains negligible amounts of catechins. These differences should be accounted for in the epidemiological studies evaluating the effect of tea on health.

Levels of tea consumption

Although there is wide variability in levels of tea consumption around the world, it is believed that tea consumption is second only to water, with an average consumption of 120 ml/day per capita (Katiyar and Mukhtar, 1996; Ahmed *et al.*, 1998). Furthermore, not only does tea consumption vary from country to country, but also there is enormous variation in any given population. This ranges from no tea at all to as many as 20 or more cups per day. For large-scale epidemiological studies, food frequency questionnaires are often the method used to obtain dietary exposure data. The amount of tea estimated from the usual number of cups consumed is frequently then used as the marker of tea and/or polyphenol consumption. However, this may be an inadequate measure of intake since tea drinking practices vary considerably among and between populations. Depending on the population studied, a cup of tea could be 80 ml, as in Saudi Arabia, 100 ml, as in Japan, 135 ml, as in Italy, 150 ml, as in England, or 240 ml (8 oz mug), as in the USA (Hakim *et al.*, 2000, 2003a; Ferraroni *et al.*, 2004). In the case of iced tea the amount is larger (360 ml); however, in almost all cases, it will be diluted.

Assessment of Exposure in Epidemiological Studies

Food frequency questionnaires

Studies of the potential relationship between tea consumption and chronic disease typically use food frequency questionnaires (FFQs) to estimate tea intake. The amount of tea estimated from the usual number of cups consumed is frequently then used as the marker of tea and/or polyphenol consumption. Short-term recall and diet record methods are generally expensive, often unrepresentative of the usual intake and inappropriate for assessment of past diet. The FFQ always refers to the year preceding the interview and ideally it should contain questions on the average monthly, weekly and/or daily consumption of foods and beverages and on the type of tea consumed. However, some of the FFQs contain only questions related to weekly consumption (Ferraroni *et al.*, 2004). Decaffeinated tea was sometimes excluded or grouped with herbal tea, while other FFQs grouped tea and coffee together. Many of the FFQs did not distinguish between green and black tea or between hot (brewed), iced or instant tea. All of these inconsistencies in collecting tea consumption data have resulted in inconsistencies in the results obtained from epidemiological studies.

Lack of specific information on the type of tea consumed (e.g. black or green), amount and duration of tea intake and method of tea preparation (e.g. hot or iced, strong or weak) has limited all studies. Therefore, we developed a detailed tea questionnaire (TQ) after a series of focus groups identified usual tea

drinking preparations and behaviours. This questionnaire asked about average tea intake over the past year, as well as lifetime consumption pattern and any changes made. Detailed information was sought for the past year's tea intake during winter and summer by type of tea consumed (black, green or herbal and hot or iced). Information was also sought for use of regular or decaffeinated tea products and the usual brewing strength (weak, medium or strong). Usual or typical recipes for tea preparation were obtained, e.g. number of tea bags/cup and brewing time. This questionnaire was then evaluated for short- (1 week) and long-term (6 months) reliability (Hakim *et al.*, 2001).

In future epidemiological studies, it is important to collect more specific information on the qualitative and quantitative aspects of tea consumption. The use of specific questions focusing on tea preparation and the availability of quantitative estimates of tea flavonoids should enhance future epidemiological studies of the relationship between tea and chronic disease prevention.

Selection of the control group

Most of the epidemiological studies that examined the association between tea consumption and health outcomes were not originally designed for that. Therefore, the selection and criteria of a control group vary considerably among studies. While the best approach is to use non-tea drinkers as a control group, most of the studies (due to limitation of the FFQ used and data collected) included black tea drinkers in their control group when they studied the association with green tea (Suzuki *et al.*, 2004) and vice versa. Similarly, many studies based their results on comparing subjects in the highest and lowest categories of green tea consumption (Suzuki *et al.*, 2004). Therefore, it is not surprising to see inconsistent results when studying the association between tea consumption and cancer (Hoshiyama *et al.*, 2004; Jian *et al.*, 2004; Suzuki *et al.*, 2004).

Randomized Trials of High Tea Intake

Randomized controlled trials are commonly regarded as the definitive study design for proving causality. In controlled trials, the random assignment of subjects to the intervention eliminates the problems of dietary recall and controls the effects of both known and unknown confounding factors. Relationships between tea and cancer risk, previously postulated only on the basis of observation, have now been studied in a number of completed randomized controlled trials; many others are still ongoing. The health benefits of green tea consumption, by means of extract or beverage, have been investigated in human studies, but intervention studies report controversial results, probably due to differences in the considered population (i.e. dietary habit and lifestyle) and/or in experimental protocols (i.e. dose and length of treatment) (Serafini *et al.*, 1996; van het Hof *et al.*, 1997; Pietta *et al.*, 1998; Klaunig *et al.*, 1999; Young *et al.*, 2002; Hakim *et al.*, 2003b, 2004).

Dose selection

Tea beverage

It is reported that the lowest effective dose of 0.016 mmol EGCG/kg body weight (bw)/day in animal models is comparable with the consumption of four cups of green tea or 17.7 μmol/kg bw/day of EGCG by a 70 kg man (NCI, 1996). Following this recommendation, we completed a 4-month randomized tea intervention trial among smokers. Our results revealed a 30% significant decrease in DNA damage, as measured by urinary 8-hydroxydeoxy guanosine (8-OHdG), among smokers in the green tea group (Hakim *et al.*, 2003b). Erba *et al.* (2005) investigated the effect of the addition of two cups of green tea (GT), containing approximately 250 mg of total catechins, to a controlled diet in a group of healthy volunteers with respect to a group following the same controlled diet but not consuming GT. Their study suggests that, in healthy subjects, a moderate intake

of GT ameliorates antioxidant defences in plasma and protects lymphocytes from DNA oxidative damage.

Tea extracts

We completed a series of Phase I clinical trials with green tea extracts to determine bioavailability, safety and maximum tolerable dose. We performed a Phase I pharmacokinetic study to determine the systemic availability of green tea catechins after single oral dose administration of EGCG and Polyphenon E (decaffeinated green tea catechin mixture). Twenty healthy volunteers were randomly crossed over to receive the two catechin formulations at one of the dose levels (200, 400, 600 and 800 mg based on EGCG content). We found that oral administration of EGCG and Polyphenon E at the same dose level (based on EGCG content) resulted in similar plasma EGCG levels. From the economic standpoint in chemoprevention, these results are encouraging because it would be less expensive to produce the Polyphenon E formulation than the pure EGCG formulation. Throughout the study, we recorded all side effects experienced by our study subjects. Both tea polyphenol formulations administered as a single oral dose over the dose range studied were well tolerated by the study participants (Chow *et al.*, 2003). Moreover, oral bioavailability of tea catechins appeared to increase following 4 weeks of tea catechin intervention at high daily bolus doses. Four weeks of daily oral intake of green tea polyphenols appeared to be safe and well tolerated at 800 mg EGCG/day (Chow *et al.*, 2003).

Dosing condition

We have recently shown that greater oral bioavailability of free catechins is achieved by taking the Polyphenon E capsules on an empty stomach. Polyphenon E up to a dose that contains 800 mg epigallocatechin gallate is well tolerated when taken under the fasting condition (Chow *et al.*, 2005). This dosing condition is also expected to optimize the biological effects of tea catechins in clinical trials.

Conclusion

In summary, tea polyphenol intake may vary not only because the actual amount of consumption differs, but also because the concentrations of tea polyphenols differ by type of tea preparation. Estimation of total amount of tea intake in epidemiological studies should include information on the type, preparation and strength of the consumed tea. More focused questionnaires may be needed to assess tea preparation techniques. Use of specific questions focusing on tea preparation and availability of quantitative estimates of tea flavonoids should enhance epidemiological studies of the relationship between tea consumption and disease risk. In addition, an integrated approach combining designs and implementing new techniques to identify biomarkers may clarify the role of tea in health promotion and disease prevention.

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8 Protective Effects of Tea against Cardiovascular Diseases

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Abstract
Tea beverages derived from infusions of *Camellia sinensis* are consumed daily by millions of people globally. A body of epidemiological data, albeit derived primarily from studies carried out in Europe and the USA, indicates that tea consumption may reduce the risk of cardiovascular events, notably mycocardial infarction and stroke. Tea beverages, both black and green, contain significant amounts of flavonoids – catechins, flavonols, theaflavins and thearubigins – which have the potential to influence free-radical-related and other pathophysiological processes *in vivo*. The effects of tea beverages on antioxidant and vascular function, in relation to mechanisms that may reduce the risk of cardiovascular disease, are discussed.

Keywords
Tea, flavonoid, antioxidant, cardiovascular disease, endothelial function, flow-mediated vasodilation.

Introduction

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in all regions of the world, in both developed and developing countries. Cardiovascular mortality accounts for approximately 15–55% of all deaths, varying from, for example, 17% in South Africa to 55% in the Russian Federation. According to World Health Organization (WHO) estimates, 16.6 million people die of CVD each year (World Health Report, 2002). The pathogenesis of cardiovascular disease is thought to involve a major oxidative component, primarily in terms of lipid oxidation (Lusis, 2000), and it has been suggested that dietary antioxidants, e.g. flavonoids from tea, could offer protective benefits. Tea has been considered to be a 'functional food' in China for centuries due to one of the legends surrounding the origins of tea as a beverage. While boiling some water for a drink to allay stomach pains after overindulgence at dinner, the Chinese emperor Shen-Nung, who lived around 2700 BC, failed to notice that some leaves from a nearby shrub had dropped into his boiling vessel. He drank the resulting brew and found that it not only tasted delicious but it also caused the spontaneous disappearance of his stomach pains, thereby demonstrating for the first time the beneficial health effects of tea. Nowadays green and black tea are beverages prepared by

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infusing the dried leaves of *Camellia sinensis* in hot water to achieve a desirable taste profile. Both green and black tea originate from the leaves of *C. sinensis* and are characterized by a high content of antioxidant polyphenols called flavonoids, a typical UK brew of black tea providing approximately 150 mg flavonoids per cup (Lakenbrink *et al.*, 2000).

Tea Consumption and Cardiovascular Disease: Epidemiology

As CVD is preceded by a process that develops over many years, it is difficult to investigate directly whether the dietary intake of certain foods or beverages (e.g. tea) can prevent CVD. To show an effect through a clinical intervention trial would take many years and many participants. Most research in the area of tea intake and CVD therefore relies on observational epidemiological research, and a significant number of trials have now been performed.

The data from published trials that have investigated the relationship between tea consumption and CVD have been evaluated in a meta-analysis (Peters *et al.*, 2001). This meta-analysis included three outcome categories (myocardial infarction, stroke and a broader category of coronary heart disease (CHD), which had been examined in at least three studies. Table 8.1 gives an overview of all studies included in the meta-analysis, together with additional cohort studies that were found in the literature. There were large variations between studies in the definition of the size of a cup of tea (from 80 to 250 ml tea per cup) and large differences in the classification of tea consumption categories. For each study Peters *et al.* standardized the risk estimates for measuring the effects per three cups of tea per day (i.e. 3×237 ml). This made it possible to compare all study outcomes.

Most studies included in the analysis suggest a decrease in the rate of CVD outcomes with increasing tea consumption (the risk decreased between 1 and 75% per three cups/day for CHD or myocardial infarction and between 26 and 66% for stroke). Five studies (four based on CHD or myocardial infarction outcomes and one study that looked at stroke incidence) indicated an increased risk with increasing tea consumption (respective increased relative risks were 4–126% and 51%).

It appeared that the study-specific effect estimates for stroke and CHD were too heterogeneous to be summarized in the meta-analysis. Much of the heterogeneity among the results could be explained by the geographical region in which the studies were conducted. With increasing tea consumption, the risk for CHD increased in the UK and for stroke in Australia, whereas the risk decreased in other regions, particularly in continental Europe. Only the relative risk estimates for myocardial infarction appeared relatively homogeneous. The incidence rate of myocardial infarction was estimated to decrease by 11% with an increase in tea consumption of three cups per day (95% confidence interval: 0.79, 1.01). Possible explanations for the discrepancy in results from the UK and Australia versus other regions are twofold. It has been suggested that the addition of milk to tea, a common practice in the UK and Australia, affects flavonoid bioavailability (Haslam, 1989; Serafini *et al.*, 1996), thus explaining the lack of a protective effect in these studies. However, more recent research has shown that milk proteins do not impede the absorption of catechins from the gut (Hollman *et al.*, 1999; van het Hof *et al.*, 1999), although no definitive conclusions can be drawn as there are currently no data available on the effect of milk on the absorption of other tea flavonoids, such as theaflavins and thearubigins. Another explanation for the UK findings could be residual confounding. In contrast to tea consumption in most other countries (Schwarz *et al.*, 1994), tea consumption in the UK is positively associated with a less healthy lifestyle (e.g. smoking and fat intake) and with lower social class (Hertog *et al.*, 1997; Woodward and Tunstall-Pedoe, 1999). Residual confounding by inaccurately measured or unmeasured confounders has been suggested as a likely explanation for

Table 8.1. Overview of observational studies investigating the effect of tea or flavonoids on cardiovascular disease included in the meta-analysis (from Peters *et al*., 2001).

Study	Country	Outcome	RR for 3 cups/day	95% CI	Follow-up (years)	All subjects (no.)	Cases (no.)	% of population consuming ≥ *x* cups of tea per day
Cohort studies								
Hirvonen *et al*., 2000	Finland	Stroke	0.69	0.35, 1.36	6	26,415	736	17.7 ≥ 0.7
Yochum *et al*., 1999	USA	CHD	0.90	0.64, 1.26	10	34,492	438	25.0 ≥ 0.7
		Stroke	0.73	0.38, 1.41	10	34,492	131	
Woodward and Tunstall-Pedoe, 1999	UK	CHD	2.26	1.10, 4.64	8	11,567	206	66.6 ≥ 1.3
Hertog *et al*., 1997	UK	CHD	1.48	1.03, 2.12	14	1,900	131	85.8 ≥ 1.3
Rimm *et al*., 1996	USA	CHD	1.47	0.95, 2.28	6	44,303	279	91.2 ≥ 2
Keli *et al*., 1996	Netherlands	Stroke	0.34	0.17, 0.69	15	552	42	75.7 ≥ 1.4
Hertog *et al*., 1993	Netherlands	CHD	0.29	0.11, 0.74	5	805	43	66.7 ≥ 1.1
Klatsky *et al*., 1993	USA	CHD	0.95	0.80, 1.14	8	12,893	539	19.4 ≥ 1
		MI	0.91	0.74, 1.11		12,893	433	
		Stroke	0.84	0.64, 1.10		12,893	275	
Stensvold *et al*., 1992	Norway	CHD	0.25	0.12, 0.50	12	20,089	159	25.9 ≥ 1
Sato *et al*., 1989 (green tea)	Japan	Stroke	0.68	0.56, 0.82	4	14,360	174	81.9 ≥ 1
Case–control studies								
Sesso *et al*., 1999	USA	MI	0.31	0.09, 1.02		680	340	32.0 ≥ 1
Thrift *et al*., 1996	Australia	Stroke	1.51	0.89, 2.56		662	331	67.1 ≥ 1
Gramenzi *et al*., 1990	Italy	MI	0.29	0.10, 0.81		936	287	23.3 ≥ 1
Rosenberg *et al*., 1988	USA	MI	1.04	0.66, 1.66		351	146	39.0 ≥ 1
Rosenberg *et al*., 1980	USA	MI	0.96	0.76, 1.20		1,423	472	40.9 ≥ 1
Jick *et al*., 1973	USA	MI	0.91	0.63, 1.33		12,759	440	1.9 ≥ 5
BCDSP, 1972	USA	MI	0.81	0.58, 1.13		1,380	276	60.8 ≥ 1

RR, relative risk; CI, confidence interval; CAD, coronary artery disease; CHD, coronary heart disease; MI, myocardial infarction; BCDSP, Boston Collaborative Drug Surveillance Program.

the reported increased risk of CHD in the UK study (Hertog *et al.*, 1997).

The meta-analysis also identified the potential for publication bias. It appeared that smaller studies producing results inconsistent with the hypothesis of a preventive effect of tea for myocardial infarction may have had a low probability of being published which may have resulted in an artificially favourable effect of tea on CVD risk reduction. However, these smaller studies may not have had sufficient power to identify relationships with tea consumption, so, while the results of the meta-analysis do indeed need cautious interpretation, the general picture that has emerged from observational studies is that tea consumption may be of benefit in reducing the incidence of CVD.

Human Trials: Tea and Plasma Antioxidant Potential

Tea flavonoids have been identified as powerful antioxidants in a range of chemical and biological *in vitro* assay systems (Wiseman *et al.*, 1997). There is considerable interest in the potential of tea to exert functional antioxidant effects in the human body, as the pathophysiology of a number of major human degenerative diseases, including CVD, is believed to have an oxidative stress component (Harrison *et al.*, 2003). A number of human intervention trials have reported on the effect of black or green tea consumption on the plasma antioxidant potential, a parameter that although by itself does not imply antioxidant functionality *in vivo* indicates the potential to do so. A variety of methods are currently in use to determine plasma antioxidant potential, namely the FRAP, or ferric iron-reducing ability of plasma, assay (Benzie *et al.*, 1999), the TRAP, or total radical-trapping ability of plasma, assay (Ghiselli *et al.*, 1995), the TEAC, or trolox equivalent antioxidant capacity, assay (Miller *et al.*, 1993) and the ORAC, or oxygen radical- absorbing capacity assay (Cao *et al.*, 1995). The human studies that have applied these assays to investigate the effects of tea on plasma antioxidant potential are discussed below.

In a crossover study with 24 healthy volunteers, each subject received different tea treatments on separate days in a randomized order, with 1 week washout between treatments (Leenen *et al.*, 2000). The treatments consisted of a 300 ml single dose of black or green tea or an equal volume of hot water. Each 300 ml of tea was prepared with 2 g of lyophilized tea solids, equivalent to three normal cups. Ingestion of dissolved green or black tea solids significantly increased the plasma FRAP value by 2–3%, the effect becoming significant at 30 min post tea ingestion. Addition of milk to green or black tea had no effect on the plasma FRAP response. This study was repeated in a further 24 volunteers and a single dose ingestion of green and black tea again resulted after 30 min in a significant increase in plasma antioxidant activity (Fig. 8.1) and total catechins in plasma (Fig. 8.2), both relative to consumption of water (unpublished data). As anticipated from the higher catechin concentration in green tea, the rise in plasma total catechins was significantly higher following consumption of green tea as compared with black tea (P <0.001). Due to the large difference in plasma catechin levels between green and black tea, a similar difference in the FRAP values after green and black tea consumption could be anticipated if the increase relative to water

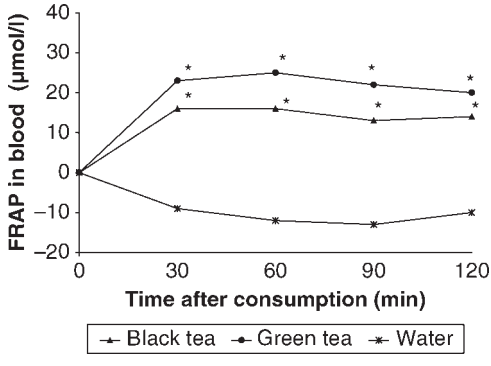

Fig. 8.1. Changes in plasma FRAP activity (µmol/l) after a single dose of black tea, green tea and water. *, P < 0.001: green tea versus water; *, P < 0.001: black tea versus water.

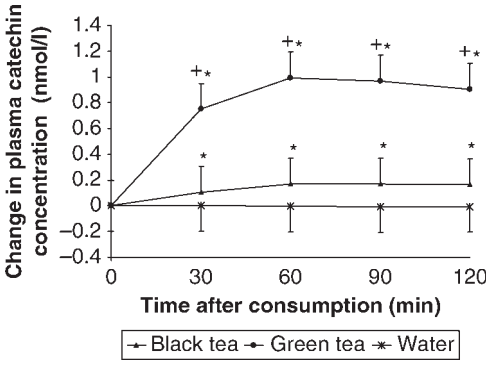

Fig. 8.2. Changes in total catechin concentration in plasma (nmol/l SEM) after a single dose of water, black tea and green tea. *, $P < 0.001$: green tea and black tea versus water; *, $P < 0.001$: green tea versus black tea.

was determined solely by catechin content. However, the much smaller difference found in these two studies suggests that flavonoids from black tea other than catechins are taken up and contribute to increased antioxidant potential. Peak levels in FRAP were reached 60 min after consumption of black or green tea. Plasma uric acid concentration (a major contributor to the total FRAP value) decreased over time but this response was apparent in all treatments. Contributions of uric acid to plasma FRAP activity were evaluated by analysis of covariance and, after adjustment for the contribution of uric acid, the effects of tea consumption on FRAP were still highly significant.

Similar findings to the above have been reported in other human studies. In a study with three groups of five volunteers taking water, black tea or green tea, respectively, Serafini *et al.* demonstrated a significant increase in TRAP values in the tea groups between 30 and 60 min after a single consumption of 300 ml of either green or black tea (Serafini *et al.*, 1996). The radical-scavenging capacity returned to its initial level after 80 min and there was no significant difference between green and black tea consumption. In contrast to data reported by Leenen *et al.* (2000), Serafini *et al.* (1996) observed that the addition of milk to tea completely abolished the increase in plasma antioxidant potential.

In a small crossover study with ten healthy subjects, green tea consumption resulted in a 4% increase in plasma FRAP values 40 min after ingestion as compared with water (Benzie *et al.*, 1999). FRAP values returned to baseline levels after 120 min. A significant increase in FRAP value was also found in urine after green tea consumption. In this study, tea was prepared from 20 g dry tea leaves, about eight times more than commonly used per cup of tea.

In a parallel intervention study by van het Hof *et al.*, healthy subjects consumed six cups of green tea, black tea or water per day for 4 weeks (van het Hof *et al.*, 1997). The total antioxidant capacity of plasma was measured with the TEAC assay. A small but significant increase in the total antioxidant capacity of plasma could be observed after 4 weeks of green tea but not black tea consumption as compared with water. Princen *et al.* (1998) analysed plasma levels of antioxidant vitamins E and C, β-carotene and uric acid to determine whether tea polyphenols have a sparing or regenerating effect on these antioxidants. In this placebo-controlled parallel study, 64 smokers were randomized into four groups and consumed either green tea, black tea, green tea polyphenol isolate or water for 4 weeks. No significant differences were found in endogenous plasma antioxidant levels between tea and control groups, indicating that tea flavonoids do not have a sparing effect on these antioxidants.

Serum antioxidant activity was measured by Maxwell *et al.* (1996) in ten healthy subjects after ingestion of black tea but no significant effects were observed. Data from this study were based on an enhanced chemiluminescence assay, which depends on enzymatic activity, therefore differing from the more commonly applied methods, such as FRAP and TRAP. Also, the study did not include a control treatment. The chemiluminescence assay has been applied in one other tea study (McAnlis *et al.*, 1998), in which also no change in the antioxidant capacity of plasma was found. In contrast to the results observed in these two studies, a strong increase in plasma antioxidant activity was found in a randomized crossover

study with black tea (Langley-Evans, 2000). The increase was measured using the FRAP assay 3 h after the first cup of tea. The antioxidant potential was further increased at 5 h after the first intake. A more moderate increase in plasma antioxidant capacity (measured as TEAC) was found in ten young healthy subjects who received green tea on three occasions each separated by 1 week, with the amount of tea increasing stepwise from 150 ml to 300 and 450 ml (Sung *et al.*, 2000). In the first week, a non-significant increase compared with baseline values was found. After doubling and tripling the amount of green tea, the increase became progressively significant, thereby indicating a positive dose–response relationship. Apart from taking baseline blood samples, there was no control treatment in this study.

The plasma TRAP value was assessed in a randomized crossover study with black tea, green tea, water or water with caffeine treatments (Hodgson *et al.*, 2000). A small but non-significant increase in TRAP was found at 60 min post-consumption in both black and green tea groups as compared with caffeine in water (*P* for trend 0.09). Finally, a double-blind, placebo-controlled crossover trial in 60 coronary artery disease subjects was performed by Duffy and colleagues to determine the effect of tea consumption on antioxidant status and endothelial function (Duffy *et al.*, 2001). ORAC and FRAP were determined before and after beverage consumption and values obtained in both assays tended to increase following 4 weeks' black tea consumption (*P* for trend 0.09).

In conclusion, a small but consistent increase in antioxidant capacity of plasma after tea ingestion has been demonstrated in a number of independent studies using a variety of methods, indicating that tea flavonoids enter the bloodstream and have an antioxidant function (Table 8.2).

Tea and Vascular Function

It is now becoming widely accepted that endothelial dysfunction is likely to play a role in the early development of atherosclerosis (Quyyumi, 2003). In accordance, all major CVD risk factors, including high blood cholesterol, diabetes, hypertension, ageing, smoking and hyperhomocysteinaemia, have been shown to be accompanied

Table 8.2. Effect of tea consumption on plasma antioxidant potential.

			Plasma antioxidant capacity (%)	
Study	Assay	Time post-consumption	Green tea	Black tea
Serafini, 1996	TRAP	30–50 min	+ 34	+ 29
Maxwell and Thorpe, 1996	ECL	60, 120 and 180 min	—	No effect
van het Hof, 1997	TEAC	4 weeks	+ 3	No effect
McAnlis, 1998	ECL	0–180 min and 4 weeks	—	No effect
Benzie, 1999	FRAP	40 min	+ 4	—
Leenen, 2000	FRAP	30–120 min	+ 3	+ 2
Unpublished, data	FRAP	30–120 min	+ 2.4	+ 1.5
Serafini, 2000	TRAP	30 min GT, 50 min BT	+ 40	+ 52
Sung, 2000	TEAC	120 min	+ 13	—
Langley-Evans, 2000	FRAP	180–300 min	—	+ 76
Hodgson, 2000	TRAP	60 min	+ 4 (ns)*	+ 3 (ns)*
Duffy, 2001	FRAP/ORAC	4 weeks	+ 11 (ns)*	+ 11 (ns)*

**P* for trend = 0.09.

TRAP, total radical-trapping ability of plasma; ECL, enhanced chemiluminescence; TEAC, trolox equivalent antioxidant capacity; FRAP, ferric iron-reducing ability of plasma; ORAC, oxygen radical-absorbing capacity; GT, green tea; BT, black tea; ns, not significant.

by endothelial dysfunction long before morphological alterations can be observed in blood vessels. Data have now accumulated that suggest a predictive value of endothelial (dys)function for the development of future CVD (Schroeder *et al.*, 1999; Teragawa *et al.*, 2001), atherosclerotic disease progression and cardiovascular events (Schachinger *et al.*, 2000).

The endothelium is the inner lining of all blood vessels. It functions as a selectively permeable barrier between blood and tissues. The normal endothelium plays a key role in vascular homeostasis by controlling the vascular tone, growth and interaction of the vessel wall with platelets and leucocytes. Endothelial cells in regions of arterial branching or curvature, where flow is disturbed, show increased permeability to macromolecules such as low-density lipoprotein (LDL) and these sites are preferential for atherosclerotic lesion formation. The accumulation and subsequent oxidation of LDL within the subendothelial matrix trigger endothelial cells to produce a number of pro-inflammatory molecules, including adhesion molecules and growth factors (Lusis, 2000). The adhesion molecules (vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM)) mediate the adhesion of leucocytes to the endothelium. This step is critical in the initiation and progression of atherosclerosis. The rolling and adhesion of the leucocytes to the vessel wall are mediated by E- and P-selectin. Oxidized LDL can also inhibit the endothelial production of nitric oxide (NO), a chemical mediator with multiple anti-atherogenic properties, including vasorelaxation. The assessment of flow- mediated dilation (FMD) of the brachial artery is now being widely used as a simple and non-invasive method of determining changes in NO-regulated vascular tone and thus of endothelial function. Flow-mediated dilation is the widening response of the artery to increased blood flow, which occurs after the release of an inflatable cuff. The FMD technique correlates well with more invasive tests of endothelial function (Anderson *et al.*, 1995) and the FMD response is impaired in subjects with risk factors for CVD and clinical disease. Supplementation with antioxidants such as vitamin C and E has been found to improve endothelial dysfunction (Plotnick *et al.*, 1997; Gokce *et al.*, 1999; Borovnicar *et al.*, 2000). Improvements in endothelial dysfunction may also be mediated through antioxidant polyphenols in black tea and this has now been investigated in a number of intervention trials.

Human Trials: Tea and Vascular Function

Four clinical trials have studied the effects of tea on vascular function and/or vascular inflammation. Two studies investigated the effect of black tea on the FMD response (Duffy *et al.*, 2001; Hodgson *et al.*, 2002) and found significant beneficial effects. Duffy *et al.* investigated both the long- and short-term effects of tea on FMD outcome in subjects with existing CVD. Tea consumption, both after an acute dose and after 4 weeks' consumption, improved the FMD response to levels observed in disease-free subjects. Hodgson *et al.* demonstrated benefits of 4 weeks' tea consumption on FMD in subjects with mild cholesterolaemia or triglyceridaemia. De Maat *et al.* (2000) investigated vascular inflammation markers in response to black and green tea consumption and Hodgson *et al.* (2001) studied the effect of black tea on cell adhesion molecules. No effects of tea on vascular markers were observed in either study. The amounts of tea ingested in the studies were not uniformly described. They ranged from 4.4–10 g/day tea leaf to 3 g/day tea solids and 500–1250 ml tea/day. One can assume that in each study at least 500 ml tea was consumed.

The effect of tea on endothelial function, measured as the FMD response, may be based on improvement of NO metabolism. NO released from the endothelium acts on the underlying smooth-muscle cells to regulate blood-vessel diameter and blood flow. It has been reported that flavonoids accumulate in vascular tissue between the

endothelium and the vascular smooth-muscle cells (Neumann *et al.*, 1992). Endothelial dysfunction may involve a component of oxidative stress with enhanced production of superoxide free radicals, which could potentially react with NO to form peroxynitrite. Bioavailability of NO (and therefore the potential of the blood vessel to vasodilate) could therefore be limited in the presence of oxidative stress. The beneficial effect of black tea on the FMD response observed in subjects with expected oxidative stress (CVD patients and hyperlipidaemics) could be due to scavenging of the superoxide free radical by tea flavonoids and preservation of NO availability in the endothelium.

Other antioxidants, such as vitamin C, have been shown to be similarly effective in restoring the FMD response (Plotnick *et al.*, 1997; Gokce *et al.*, 1999). This activity was shown to be due to increased endothelial NO synthase activity. Since a similar effect was observed for tetrahydrobiopterin (BH_4) reductase, a mechanism was proposed that involves the reduction of dihydrobiopterin to BH_4 (Huang *et al.*, 2000). Only the completely reduced tetrahydro form of biopterin supports NO synthase coupling of nicotinamide adenine dinucleotide phosphate (NADPH) oxidation to NO production. Because NO synthesis is associated with BH_4 oxidation, vitamin C and possibly tea flavonoids may function as antioxidants by keeping BH_4 in a reduced state.

In addition to effects on endothelium-dependent vasodilation, an effect on endothelium-independent vasodilation suggests an effect of tea on smooth-muscle cells. Zhang *et al.* (2000) have shown that both endothelial cell and smooth-muscle cell function may be impaired in patients with CHD. In the FMD study, in which coronary artery disease patients were investigated (Duffy *et al.*, 2001), no effect on endothelium-independent vasodilation was detected, whereas in the non-symptomatic population of Hodgson *et al.* (2002) an effect of long-term tea consumption on endothelium-independent vasodilation was present.

In conclusion, evidence is accumulating indicating that both short- and long-term consumption of black tea (in relatively normal doses) improves flow-mediated vasodilation and thus vascular function. This has been shown in apparently healthy subjects as well as those with coronary artery disease. More well-controlled studies are now needed to confirm the effects observed in these two studies and to elucidate the mechanism of action.

Future Prospects

A body of observational epidemiological data suggests that tea may be of benefit in reducing rates of CVD in regular users. This suggestion of benefit needs to be confirmed by intervention trials that indicate potential mechanisms. Studies in humans show that consumption of tea beverage acutely enhances the antioxidant capacity of plasma, which could reduce the potential for arterial lipid peroxidation. The latter, however, still remains to be proven. Endothelial dysfunction is known to be associated with a range of risk factors for CVD, and emerging data suggest that tea may improve vascular function in both apparently healthy subjects and those with CVD. This effect of tea may be mediated via an antioxidant mechanism in the blood-vessel wall, but again more data are required. Tea beverage remains an important candidate for dietary prevention of CVD.

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9 Potential Targets of Tea Polyphenols in Cancer Prevention

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Abstract
Cancer is perhaps the most progressive and devastating disease posing a threat of mortality to the entire world, despite significant advances in medical technology for diagnosis and treatment, because selective killing of tumour cells without harming the normal cells of the same tumour bearer still remains as complex as the disease itself. The mechanisms of carcinogenesis involve multiple stages of biochemical and molecular alterations in target cells. To achieve full malignancy, cells must acquire certain transformation characteristics, including: (i) self-sufficiency in growth signalling and limitless replicative potential; (ii) unresponsiveness to antiproliferative signals; (iii) evading apoptosis; (iv) inducing and sustaining angiogenesis; and (v) acquiring the ability to invade and metastatize. This sequence of events presents many opportunities for intervention, with the aim of preventing, slowing down or reversing the transformation process. However, mainstream therapies (relying upon surgery, chemotherapy and radiation) may initially appear successful in the treatment of cancer, but they have their own specific shortcomings. In fact, during the regimen of cancer chemotherapy, various popular and effective drugs in use these days exert concurrent toxic manifestations, including oxidative stress, liver damage and immunosuppression in the tumour bearer. Thus, from the point of view of successful cancer therapy, selective targeting and low toxicity for normal host tissues are fundamental requisites for proposed chemopreventive agents. Recently, the concept of cancer prevention using naturally occurring substances that could be included in the diet is under investigation as a practical approach towards reducing cancer incidence and the mortality and morbidity associated with this disease. Tea, which is the most popularly consumed beverage aside from water, has been particularly associated with decreased risk of cancer in humans. A wealth of research suggests numerous mechanisms of action to explain these observations. Various studies have provided evidence that polyphenols are the strongest biologically active agents in tea, which, in addition to their antioxidative properties, also affect the molecular mechanisms involved in angiogenesis, metastasis and regulation of cell death. This chapter will discuss the effects and the biological activities of tea and its polyphenols in relation to these mechanisms, each of which plays a crucial role in the development of cancer in humans.

Introduction

Carcinogenesis – an overview

Cancer is one of the leading causes of death in industrialized nations. After a quarter century of rapid advances, cancer research has generated a rich and complex body of knowledge, revealing cancer to be a disease consisting of a multistep process involving genetic alterations that drive the progressive transformation of normal cells into highly

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malignant types. The paths that cells take on their way to becoming malignant are highly variable. Further, mutations in certain oncogenes and tumour suppressor genes can occur early in some tumour progression pathways and late in others. As a consequence, the acquisition of biological capabilities such as resistance to apoptosis, sustained angiogenesis as well as metastasis, and unlimited replicative potential can appear at different times during these various progressions. Moreover, cancer development depends upon changes in the heterotypic interactions between incipient tumour cells and their normal neighbours. Once formed, virtually all types of cancers, including their metastatic outgrowths, continue to harbour complex mixtures of several cell types that collaborate to create malignant growth (Hanahan and Weinberg, 2002). The process of carcinogenesis involves the stepwise accumulation of genetic changes, ultimately leading to malignancy (DiGiovanni, 1992). There are three main steps:

1. Initiation. In this stage irreversible modification of DNA occurs, perhaps resulting in one or more mutations.
2. Promotion. This step is much slower and leads to the specific expansion of initiated cells. These changes are believed to result from epigenetic mechanisms.
3. Progression. The process of tumour progression is characterized by a high level of genetic instability that leads to a number of chromosomal alterations.

Cancers are caused by the progressive growth of the progeny of a single transformed cell. In the pre-vascular phase, the tumour is rarely larger than 2 to 3 mm^3 and may contain a million or more cells. Up to this size, tumour cells can obtain the necessary oxygen and nutrient supplies required for growth and survival by simple passive diffusion. Beyond this size, solid tumours depend on angiogenesis for growth and metastasis in a hostile environment. All these make the cancer extremely complex, and any successful therapy will thus depend on complete knowledge of each and every mechanism of carcinogenesis. Thus, there has been increasing emphasis on research to develop an understanding of cancer, as well as the action of modulating factors, as the basis for prevention and therapy.

Why cancer therapy fails

Curing cancer requires that all the malignant cells be removed or destroyed without killing the patient. An attractive way to achieve this would be to use an agent that would discriminate between the cells of the tumour and their normal cell counterparts and be selectively lethal to the malignant phenotypes. However, for the past 30 years, cancer therapies have experienced tremendous setbacks because of an associated toxic response, resulting in significant numbers of treatment-induced deaths rather than disease-induced fatalities. Mainstream medicine (relying upon surgery, chemotherapy and radiation) may initially appear successful in the treatment of cancer, but has specific shortcomings. In fact, during the regimen of cancer chemotherapy, cancer itself and various popular and effective drugs in use these days exert concurrent toxic manifestations, including oxidative stress (Bhimani *et al.*, 1993; Gopalakrishna and Jaken, 2000), liver damage (Ray and Das, 1998; Wolff *et al.*, 1998; Gopalakrishna and Jaken, 2000; Kong *et al.*, 2000) and immunosuppression (Ferraro *et al.*, 2000; Molto *et al.*, 2003; Sosin and Handa, 2003; Finke *et al.*, 2004; Rayman *et al.*, 2004) in the tumour bearer. Furthermore, failure of clinical management of this disseminated disease is also due to the lack of detailed information on the molecular mechanisms of carcinogenesis, for which reason targeted inhibition at a molecular level has not been effective to date. These facts may well explain why, in spite of the availability of different modalities of cancer therapy and the existence of various anti-cancer drugs, successful cancer therapy is still the Cinderella of investigation. Limited available options for the treatment of cancer and increasing incidence of the disease have, therefore, spurred the search for novel preventive approaches for the management of this disease.

Probable solution – if any

The discussion presented above clearly indicates that, from the point of view of successful cancer therapy: (i) selective targeting; and (ii) low toxicity for normal host tissues (Fig. 9.1) are fundamental requisites for proposed chemopreventive agents.

1. Selective targeting, in turn, demands clear understanding of the mechanisms of carcinogenesis and how these can be exploited when designing new therapeutic agents. Dysregulated processes involved in tumorigenesis, such as regulation of cell cycle progression, angiogenesis, metastasis and apoptosis, provide rational targets for novel therapies. Thus, improved molecular characterization and greater understanding of tumorigenesis are considered to enable more effective treatment.
2. On the other hand, targeting one or more of the above-mentioned processes by the use of dietary agents, which are non-toxic to normal cells, could be one such strategy that may block the neoplastic inception or delay disease progression without generating toxicity in the normal systems of the host.

Prevention/Inhibition of Carcinogenesis by Dietary Constituents

Cancer is a dynamic process that involves many complex factors, which may explain why a 'magic bullet' cure for cancer has not been found. Death rates are still rising for many types of cancers, which possibly contributes to the increased interest in chemoprevention as an alternative approach to the control of cancer. This strategy for cancer control is based on the presumption that, because cancer develops through a multistep process, each step may be a prospective target for reversing or suppressing the process. Thus, the design and development of chemopreventive agents that act on specific and/or multiple molecular and cellular targets are gaining support as a rational approach to control cancer. Recently, considerable attention has been focused on identifying naturally occurring chemopreventive substances capable of inhibiting, retarding or reversing the process of multistage carcinogenesis. Wide arrays of phenolic substances, particularly flavonoids, those present in dietary and medicinal plants, have been reported to possess substantial anticarcinogenic and antimutagenic effects (Lin and Liang, 2000). These flavonoids are naturally occurring, low-molecular-weight, polyphenolic compounds, widely distributed in fruits, vegetables and beverages. People who eat diets rich in fruits and vegetables have lower incidences of diseases such as cancer. Numerous experimental studies have examined the role of specific flavonoids in disease prevention. For example, increased flavonoid intake was associated with decreased risk of carcinogenesis (Hertog *et al.*, 1993). The majority of these naturally occurring phenolic compounds retain antioxidative and anti-inflammatory properties, which appear to contribute to their chemopreventive activity (Hertog *et al.*, 1993). Because of their safety and the fact that they are not perceived as medicine, food-derived products have high potential for development as chemopreventive and therapeutic agents that may find widespread and long-term use. Moreover, *in vitro* studies are now being conducted to identify the molecular targets within cancer cells that are modulated by these dietary constituents (Manson, 2003). Tea is one dietary substance that has diverse biomodulatory activities and therefore may be a good candidate as a safe potential anti-cancer agent.

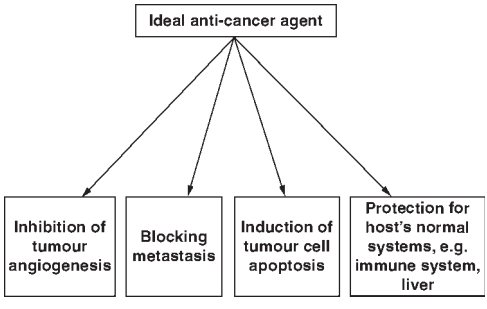

Fig. 9.1. Required properties of any potent anti-cancer agent.

Tea – can it be a candidate?

It is recognized that, next to water, tea is the most ancient and widely consumed beverage in the world. The inhibition of carcinogenesis by tea has been demonstrated in cancers of skin, lung, oesophagus, stomach, liver, duodenum and small intestine, pancreas, colon, bladder, prostate and mammary gland (Dreosti *et al.*, 1997; Katiyar and Mukhtar, 1997; Yang *et al.*, 1998). Green tea has been shown to possess cancer chemopreventive effects in a wide range of target tumours. Furthermore, the Japanese and Chinese populations, who regularly consume tea, have one of the lowest incidences of prostate cancer (Gupta *et al.*, 1999). Multiple biological effects of flavonoids have been described, among them anti-inflammatory, anti-allergic, anti-haemorrhagic, antimutagenic, anti-neoplastic and hepatopreventive activities. The flavonoids in green tea have been studied intensely in many laboratories. The major fraction of these flavonoids is the group of catechins in green tea. The chemopreventive effects of green tea against tumorigenesis and tumour growth have been attributed to the biochemical and pharmacological activities of its polyphenolic constituents, most notably epigallocatechin gallate (EGCG) (Gupta *et al.*, 2000). Epidemiological studies also suggest a protective effect of green tea consumption on some cancer types in humans (Bushman, 1998). Black tea has also been shown to inhibit tumorigenesis in animal model systems, including skin, lung, colon, oesophagus, mammary gland and ascites carcinoma (Lu *et al.*, 2000; Bhattacharyya *et al.*, 2003a; Mandal *et al.*, 2004), and to block neoplastic transformation in a mouse epidermal cell line (Liang *et al.*, 1999). However, besides some scattered reports and supporting epidemological evidence on the protective role of black tea, the detailed molecular mechanisms underlying its anti-tumour effect are still unclear and inconclusive, although the worldwide production and consumption of black tea far exceeds that of green tea (Bickers and Athar, 2000). This chapter describes the role of tea in regulating cancer cell angiogenesis, metastasis and apoptosis selectively without harming the normal cells of the tumour bearer.

Tea and its constituents – a historical view

From its legendary discovery in the year 2737 BC by the emperor Sheng Nung, when leaves fell into a pot of boiling water, to the universally enjoyed beverage it is today, tea has had a significant role in human history. It has various health-friendly properties in addition to its taste as a drink. Moreover, various reports from different parts of the world have demonstrated its antitumour, anti-cancer and immunomodulatory properties. Tea has been used as a daily beverage and crude medicine in China and India for several thousand years. This beverage is primarily manufactured as green, black or oolong tea, according to the degree of fermentation involved. Most Japanese and Chinese people in northern China prefer green tea, whereas Indians, Americans and Europeans prefer black tea. Oolong and paochong tea are favoured in Taiwan and parts of China. The composition of tea varies with the species, season, age of the leaf (plucking position), climate and horticultural practices. Non-fermented green tea contains several groups of polyphenols, which account for up to 40% of the dry leaf weight. Flavonols are the major polyphenolic fraction of green tea and include catechins (Yang *et al.*, 2000), e.g. (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), (–)-epicatechin (EC), (+)-gallocatechin (GC) and catechin (C) (Fig. 9.2) (Graham, 1992). In the manufacture of black tea, the 'fermentation' process causes green tea catechins to oxidize and form oligomeric flavonols, including theaflavins, thearubigin and other oligomers. Theaflavins are a mixture of theaflavin (TF-1), theaflavin-3-gallate (TF-2a), theaflavin-3′-gallate (TF-2 b) and theaflavin-3,3′-digallate (TF-3) (Fig. 9.2). However, thearubigins are the most abundant phenolic fraction in black tea. They vary greatly in molecular weight and have structures that are not yet well characterized. Partially fermented oolong or paochong tea contains both green tea catechins,

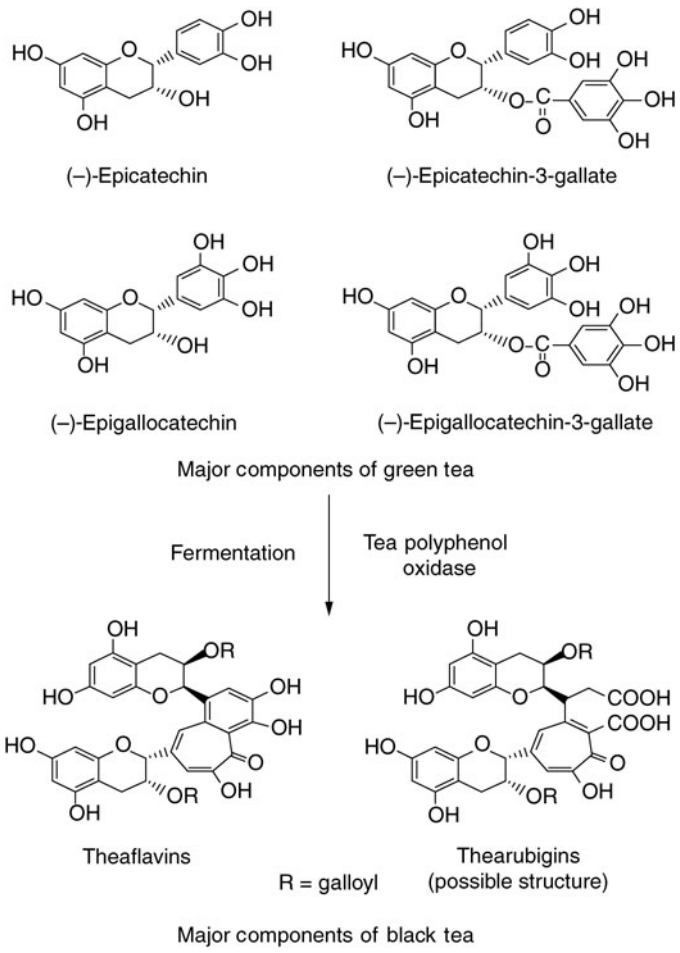

Fig. 9.2. Major components of green tea and black tea.

black tea theaflavins and, possibly, thearubigins. Some components in oolong or paochong tea, such as proanthocyanidins, are less well characterized and they may be important in disease prevention.

Hypothesis

We hypothesize that tea may act as an effective anti-cancer agent by: (i) regulating tumour angiogenesis, metastasis and apoptosis; and (ii) protecting the intrinsic defence machinery of the tumour-bearing host. Some recent studies in our laboratory and by others which support this hypothesis are discussed below.

Effect of Tea and Tea Polyphenols in Inhibiting Tumour Angiogenesis

Tumour angiogenesis and angiogenic factors – an overview

Tumour angiogenesis is the proliferation of a network of blood vessels that penetrates into cancerous growths, supplying nutrients and oxygen and removing waste products (Gupta and Qin, 2003). In the pre-vascular phase, the tumour is rarely larger than 2 to 3 mm^3 and may contain a million or more cells. Up to this size, tumour cells can obtain the necessary oxygen and nutrient supplies required for growth and survival by simple passive diffusion. Tumour angiogenesis actually starts

with tumour cells inducing or releasing several angiogenic and anti-angiogenic factors that send signals to surrounding normal host tissue. This signalling activates certain genes in the host tissue, which, in turn, make proteins to encourage the growth of new blood vessels.

Among the most commonly found angiogenic growth factors are vascular endothelial growth factor (VEGF) (Doanes *et al.*, 1999; Ferrara, 2001; Gupta and Qin, 2003), basic fibroblast growth factor (bFGF) (Mandriota and Pepper, 1997; Gupta and Qin, 2003), angiopoietin-1 (Lobov *et al.*, 2002), transforming growth factor-β (Pepper *et al.*, 1990), interleukin-8 (IL-8) and matrix metalloproteinease-2 (MmP-2) (Kitadai *et al.*, 1999), etc. On the other hand, tumour suppressor protein, p53 (Kieser *et al.*, 1994), IL-4 (Volpert *et al.*, 1998), IL-12 (Duda *et al.*, 2000), angiopoietin-2 (Kim *et al.*, 2000; Lobov *et al.*, 2002), endostatin and angiostatin (Hajitou *et al.*, 2002), thrombospondin (Jimenez *et al.*, 2000), etc., are the commonly found anti-angiogenic factors. As one or more of the positive regulators of angiogenesis are up-regulated and, simultaneously, certain negative regulators of angiogenesis are down-regulated, tumours become angiogenic. Hence, anti-angiogenic therapy can be realized through the regulation of cm 'angiogenic switch' by interfering with different mechanisms. The potential to block tumour growth by inhibition of the angiogenic process represents an intriguing approach to the treatment of solid tumours. In fact, the high proliferation rate in the tumour deprived of proper vascularization would be balanced by cell death due to lack of diffusion of nutrients and oxygen. The main targets of an increasing number of trials approved to test the tolerance and therapeutic efficacy of anti-angiogenic agents are, therefore, MMPs, angiogenic growth factors and their receptors.

Advantages of anti-angiogenic therapy

Anti-angiogenic therapy of cancer represents a highly effective strategy for destroying tumours because the fundamental requirement of tumour growth is dependent on a blood supply. Unlike standard chemotherapy, which targets tumour cells and other normal proliferating cells of the host, angiogenesis inhibitors target dividing endothelial cells that have been recruited into the tumour bed. Moreover, angiogenesis inhibitors do not attack cancer cells directly. Instead, they affect the blood-vessel cells serving the tumours and these cells do not go through rapid genetic changes or develop drug pumps. Therefore, unlike many chemotherapeutic drugs, anti-angiogenic drugs may work for much longer periods of time, control tumour growth independently of growth fraction or tumour cell heterogeneity or even tumour cell type, and do not induce acquired drug resistance. Moreover, inhibition of angiogenesis also blocks tumour metastasis, thereby making anti-angiogenic therapy more important as far as cancer regression is concerned.

Tea and angiogenesis

The concept of cancer prevention by use of naturally occurring substances that could be included in the diet is under investigation as a practical approach towards reducing cancer incidence and therefore the mortality and morbidity associated with this disease. Tea, which is the most popularly consumed beverage aside from water, has been particularly associated with decreased risk of various proliferative diseases such as cancer in humans (Demeule *et al.*, 2002). Recent observations have raised the possibility that green tea catechins, in addition to their antioxidative properties, also affect the molecular mechanisms involved in angiogenesis (Demeule *et al.*, 2002). According to Pfeffer *et al.* (2003), green tea flavonoids possess anti-angiogenic activities that could account – at least in part – for the tumour prevention effects observed with these compounds. These drugs appear to target common mechanisms of tumour angiogenesis that may permit identification of critical targets for anti-angiogenic therapy and anti-angiogenic chemoprevention (Pfeffer *et al.*, 2003). The major green tea polyphenol,

EGCG, has also been found to inhibit activation of the epidermal growth factor receptor, thereby inhibiting VEGF production by inhibiting both the constitutive activation of Stat3 and nuclear factor (NF)-kappa B, but not extracellular-signal-regulated kinase (ERK) or Akt (Masuda *et al.*, 2002). Green tea extract (GTE) and its individual catechin components were reported to suppress breast cancer xenograft size and decrease the tumour vessel density *in vivo* (Sartippour *et al.*, 2001). Sartippour *et al.* (2002) have observed that GTE and EGCG inhibit VEGF transcription and VEGF promoter activity in breast cancer cells. Their studies further revealed that GTE decreased c-fos and c-jun RNA transcripts, suggesting that activation protein (AP)-1-responsive regions present in the human VEGF promoter may be involved in the inhibitory effect of GTE. Furthermore, GTE suppressed the expression of protein kinase C, another VEGF transcription modulator, in breast cancer cells (Sartippour *et al.*, 2002). Inhibition of MMP-2 and MMP-9 by EGCG, causing angioprevention, has also been reported (Garbisa *et al.*, 2001; Tosetti *et al.*, 2002). Modulation of insulin-like growth factor-I-induced signalling by inhibition of protein expression of phosphatidylinositol 3′-kinase, phosphorylated forms of Akt and ERK 1/2 is being suggested as a prime pathway for green tea polyphenol-mediated inhibition of prostate cancer, which limits the progression of cancer through inhibition of angiogenesis and metastasis (Adhami *et al.*, 2004). Black tea polyphenols have been found to act synergistically with genistein to inhibit MMP-9 and VEGF and thereby to arrest angiogenesis (Ravindranath *et al.*, 2004). The above discussion clearly indicates the angiopreventing effect of tea and its polyphenols as a whole.

Role of Tea and its Polyphenolic Components in Blocking Metastasis

What is metastasis?

Metastases are responsible for most cancer deaths. Despite dramatic advances in cancer therapy, the presence of metastases implies a significantly shortened survival and reduced quality of remaining life. Metastasis is a complex series of events in which metastatic cells migrate beyond tissue compartment boundaries and spread into different organs. To form clinically evident metastases, the main cause of death in cancer patients, tumour cells must complete a highly complex series of steps called the metastatic cascade, including local invasiveness, intravasation, circulation, adhesion and extravasation, survival, proliferation and angiogenesis (Gassmann *et al.*, 2004). Since failure of any one of these steps results in metastatic failure, understanding the metastatic cascade may guide us to new therapeutic concepts.

The expression of a considerable number of genes has been shown to affect the metastatic capacity of any tumour. However, it seems probable that many of these effects were indirect and attention has therefore focused on changes specific to tumour cells in the expression of cell surface adhesion molecules and of proteolytic enzymes that might be involved in degradation of the basement membrane. The major families of such gene products are cadherins (Guilford, 1999), integrins (Guo and Giancotti, 2004), MMPs (Chakraborti *et al.*, 2003), tissue inhibitors of metalloproteinases (Lambert *et al.*, 2004), putative metastasis-suppressor gene products (Stark *et al.*, 2005), etc.

Therapeutic targets for antimetastatic therapy

Aside from prevention of cancer altogether or significant improvements in early detection for most cancers, effective novel therapeutic strategies targeting metastasis should provide the greatest clinical benefit. Metastasis research has shown that many of the initial steps in metastasis are completed with a high degree of efficiency and may have occurred by the time of clinical diagnosis. Therefore, targeting the later stages of metastasis may offer a more promising therapeutic approach for the development of antimetastatic therapies. Appropriate clinical strategies include targeting dormant solitary

cells, active pre-angiogenic metastases or vascularized metastases. Since metastasis depends largely on tumour angiogenesis, angiopreventive agents might also have antimetastatic potency.

Tea and metastasis

As discussed above, tea and its polyphenols have anti-angiogenic effects in many cancer models. EGCG, the main flavonol present in green tea, exerts inhibition of membrane type 1 MMP (MT1-MMP), which restrains activation of MMP-2 (Yamakawa *et al.*, 2004), which plays a crucial role in the degradation of the extracellular matrix instrumental to invasion. Theaflavin and theaflavin digallate, which are components of black tea, inhibited invasion by mouse Lewis lung carcinoma LL2-Lu3 cells by inhibiting MMPs, as did EGCG (Sazuka *et al.*, 1999). EGCG also inhibits all activities of prostate-specific antigen, which is able to cleave extracellular matrix glycoproteins, thereby affecting cell migration and metastasis (Pezzato *et al.*, 2004). In the cell migration assay, a mixture of EGCG and ascorbic acid significantly suppressed the migration of SMMC-7721 cells by 65.9%, while EGCG and/or ascorbic acid did so by 28.9% and 18.7%, respectively, possibly with a mechanism associated with the scavenging of reactive oxygen species (Wei *et al.*, 2003). Inhibition of the expression of genes related to metastasis, such as MMP-2 and MMP-9, has been reported in prostate cancer of TRAMP mice (Adhami *et al.*, 2003). According to Oneda *et al.* (2003), green tea polyphenols also inhibit human MMP-7. In human fibrosarcoma HT1080 cells, suppression of MMP-2 and MMP-9 activities was consistent with the decreased levels of MMP-2 and MMP-9 mRNAs and suppression of MMP-9 promoter activity (Maeda-Yamamoto *et al.*, 2003). EGCG also inhibited the phosphorylation of ERK 1/2 as well as p38 mitogen-activated protein kinase (MAPK) activity, which are necessary for MMP-9 up-regulation (Maeda-Yamamoto *et al.*, 2003). In animal experiments, EGCG alone reduced lung metastases in mice bearing B16-F3m melanomas (Taniguchi *et al.*, 1992). Further mechanistic studies indicated that EGCG significantly inhibited the tyrosine phosphorylation of focal adhesion kinase (FAK) and the activity of MMP-9 in B16-F3 melanoma cells (Taniguchi *et al.*, 1992). All these reports support the canditure of tea and its polyphenols as antimetastatic agents.

Induction of Apoptosis in Tumour Cells by Tea and Tea Polyphenols

Apoptosis – can it be a target for cancer therapy?

While in multicellular organisms all cells inexorably die, there are several different ways provided for the realization of cell death. One of them, apoptosis, represents a universal energy-dependent and tightly regulated physiological process of cell death in both normal and pathological tissues. The execution of apoptosis appears to be uniformly mediated through a cascade of reactions. It is acknowledged that deregulated apoptosis contributes to malignant progression in the genesis of cancer. Aberrant proliferation and modulated apoptosis, leading to impaired cellular homeostasis, represent crucial early events in the multistep carcinogenic process. Thus, to increase apoptosis in cancer cells may result in successful therapy of cancer. Apoptosis is also the main response of cells to many chemotherapeutic agents. However, since many of the antitumour agents that induce tumour cell apoptosis are also toxic to normal cells of the host, a search is still ongoing for a nontoxic anti-cancer agent from phytochemicals.

Tea and cancer cell apoptosis

It has been reported that tea polyphenols inhibited the growth of a human lung cancer cell line, PC-9 cells, with G2/M arrest (Fujiki *et al.*, 1998). EGCG can inhibit cervical cancer cell growth in a human papillomavirus HPV-16-associated cervical cancer cell

line, CaSki cells, through the induction of apoptosis and cell cycle arrest, as well as regulation of gene expression *in vitro* (Ahn *et al.*, 2003). Cells were arrested at the G1 phase, suggesting that cell cycle arrests might precede apoptosis in these cell lines upon EGCG treatment (Ahn *et al.*, 2003). The anti-tumour effects of the green tea compound EGCG have been studied in detail in head and neck squamous cell carcinoma (HNSCC) cells (Masuda *et al.*, 2001). Treatment with EGCG increased the proportion of cells in the G1 phase of the cell cycle and induced apoptosis. In cells treated with EGCG, there was a decrease in the cyclin D1 protein, an increase in the p21(Cip1) and p27(Kip1) proteins and a reduction in the hyperphosphorylated form of pRB, changes that may account for the arrest in G1. EGCG also caused a decrease in the Bcl-2 and Bcl-XL proteins, an increase in the Bax protein and activation of caspase 9, suggesting that EGCG induces apoptosis via a mitochondrial pathway (Masuda *et al.*, 2001). In one study, it was shown that treatment of A431 (human epidermoid carcinoma) cells with green tea polyphenols and its components, EGCG, EGC and ECG, resulted in the formation of internucleosomal DNA fragments, characteristic of apoptosis (Ahmad *et al.*, 1997). Treatment with EGCG also resulted in apoptosis in human keratinocyte carcinoma, human prostate carcinoma cells and mouse lymphoma cells (Ahmad *et al.*, 1997). The DNA cell cycle analysis showed that, in A431 cells, EGCG treatment resulted in arrest in the G0–G1 phase of the cell cycle and a dose-dependent apoptosis (Ahmad *et al.*, 1997). It is now well recognized that whether a cell becomes committed to apoptosis partly depends upon the balance between proteins that mediate cell death, e.g. p53, p21, Bax, and proteins that promote cell viability, e.g. Bcl-2 (Oltvai *et al.*, 1993). A report from our laboratory has already established the relationship between p53 status, p21 induction, Bcl-2/Bax ratio, cell cycle deregulation and apoptosis in black tea-treated tumour cells (Bhattacharyya *et al.*, 2003). The results imply an apoptosis-enhancing capability of black tea in Ehrlich's ascites carcinoma (EAC) cells that was effected by modulating the tumour cell cycle as well as the balance between pro- and anti-apoptotic factors. The anticarcinogenic effect of black tea on diethylnitrosoamine-induced pulmonary tumours, dimethylbenzanthracene-induced solid tumours and transplantable tumours in Swiss albino mice has been documented (Javed and Shukla, 2000; Shukla and Taneja, 2002). Black tea polyphenols have been shown to exert an antitumorigenic effect in a mouse skin model of carcinogenesis by altering both genetic and epigenetic pathways (Javed *et al.*, 1998).

Protection of Host's Defence Machinery from Tumour Insult by Tea

Amelioration of tumour-induced immunosuppression

The tumour microenvironment influences the functional potential of immune cells. Escape from immune surveillance pre-figures the rapid progression of cancers (Chouaib *et al.*, 1997). Various immune escape mechanisms in cancer have been proposed (Pawelec *et al.*, 1997). Certain cancer cells may secrete immunosuppressive factors to modify the host immune responses (Molto *et al.*, 2003; Finke *et al.*, 2004; Rayman *et al.*, 2004). In addition anaemia is a common complication of malignancies. Cancer-related anaemia may occur as a direct effect of the neoplasm, it may be due to products of the cancer or it may develop as a result of the cancer treatment itself. Tea has been found to have a protective function for the immune system in cancer (Zvetkova *et al.*, 2001), although the underlying mechanism is still unclear. We have already reported that an anti-cancer dose of black tea significantly protects immune cells from tumour-induced depletion (Bhattacharyya *et al.*, 2004; Mandal *et al.*, 2004). Moreover, green tea and its major components have been found to ameliorate immune dysfunction in mice bearing Lewis lung carcinoma and treated with the carcinogen NNK (Zhu *et al.*, 1999). In C57/BL6J mice bearing Lewis lung cancer,

green tea showed protective effects on adverse changes in immune parameters, e.g. the weight of the thymus and its index declined, the proportion of the positive CD4 subgroup of T lymphocytes and the ratio of CD4+ to CD8+ reduced, the baseline chemiluminescence decreased in peripheral white blood cells and the number of immunoglobulin-M formation cells decreased (Zhu *et al.*, 1997). According to Yan *et al.* (1992), GTE stimulates the proliferation of T-lymphocyte and natural killer cell activity in BALB/c mice bearing EAC, HAC and S-180 tumours. All this information leads us to conclude that, by potentiating the host's immune system, tea helps in regressing tumours.

Modulation of metabolizing/detoxifying enzymes of tumour bearers by tea

It is now well accepted that, with the development of cancer, the detoxifying system of the host becomes jeopardized. According to Han and Jia (2003), chemopreventive effects of tea pigments may be partly due to the activation of detoxifying enzymes such as QR and GST. EGCG has been found to exert protective effects against tumours of the lung, the gastrointestinal tract and the liver by inhibiting the metabolic activation of carcinogens and to induce detoxifying enzymes in humans at the same time (Bertram and Bartsch, 2002). Moreover, in the case of cancer chemotherapy, various popular and effective drugs exert concurrent toxic manifestations, including oxidative stress and liver damage, in the tumour bearer. Most pro-carcinogens require metabolic activation by metabolite enzymes, such as Phase I and II enzymes, in order to convert to electrophiles before they can exert carcinogenic effects (Conney, 1982). As a result, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase are elevated in serum (Wolff *et al.*, 1998). Green tea polyphenols have been found to inhibit rat liver microsomal monooxygenase activities, including Phase I enzymes of aryl hydrocarbon hydroxylase, 7-ethoxyresorufin *O*-deethylase, and 7-ethoxycoumarin *O*-deethylase (Wang *et al.*, 1998). Another report indicated that treatment of rats with green and black tea for 4 or 6 weeks caused significant induction of cytochrome P450 enzymes, such as CYP1A2, CYP1A1, CYP2B and CYP4A1 (Sohn *et al.*, 1994). Tea has been shown to effectively inhibit tumorigenesis induced by various carcinogens in the skin, lung, fore-stomach and oesophagus by modulating the detoxification systems of the host (Conney, 1982). Our findings also indicate that an anti-cancer dose of black tea ameliorates cancer-induced liver toxicity and haemotoxicity (unpublished data), thereby indicating that it can be a good candidate as a safe anti-cancer agent. Activation of the host detoxification system by black tea has also been confirmed by our work (Bhattacharyya *et al.*, 2003a; Mandal *et al.*, 2004).

All this information leads us to the conclusion that tea and its polyphenols can protect the intrinsic defence machinery of the host from tumour insult.

Tea: a Multiedged Sword

Based on the above discussion, it can be concluded that tea and its polyphenols can regress cancer. The above review also leads us to conclude that this popular beverage can regress tumours directly by inhibiting tumour angiogenesis, blocking metastasis and inducing apoptosis in cancer cells. Moreover, unlike many other anti-cancer agents/drugs, tea potentiates the suppressed defence machinery of the tumour bearer and in that way it indirectly helps in defending against cancer (Fig. 9.3). Thus, tea, previously considered only as a popular beverage, can now emerge as a 'multiedged sword' against the deadly disease of cancer.

Prospects for the Future

The above review leads us to conclude that tea may directly act as an anti-cancer agent by killing tumour cells or it may act as 'rescue drink' that strengthens the defence

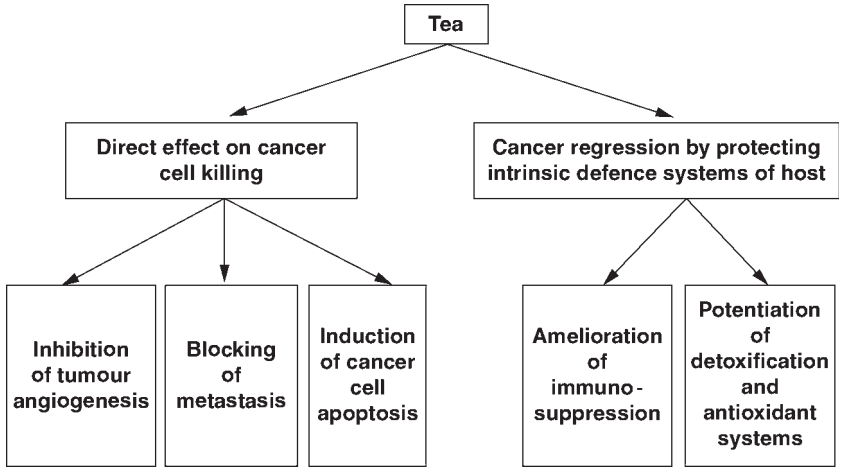

Fig. 9.3. Tea-induced cancer prevention by regulating tumour angiogenesis, metastasis and cancer cell apoptosis as well as by protecting the intrinsic defence machinery of the tumour bearer.

machinery of the host, which is otherwise suppressed due to developing cancer as well as due to the anti-cancer drugs used, and ultimately prevent cancer. This knowledge of tea and its polyphenols thus adds a new dimension to our understanding of the use of dietary constituents either during therapy of cancer patients or as a preventive measure, not only in cancer patients but also in high-risk individuals who work in an environment containing carcinogenic/toxic chemicals.

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10 The Beverage Tea in Chemoprevention of Prostate Cancer

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Abstract
In recent years, many laboratories around the world have reported several health-beneficial, especially cancer-preventive, effects of tea consumption in animal models and human population studies. We reasoned that prostate cancer represents an ideal candidate disease for chemoprevention because even a modest delay achieved through intervention through drugs or diet could have a significant impact on the outcome of this disease. Based on the epidemiological studies and recent data, amassed from various laboratories around the world, there is convincing evidence that polyphenolic antioxidants present in the beverage tea, such as epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC) and epicatechin-3-gallate (ECG), may have the potential to lower the risk of prostate cancer in the human population. Our recent study has demonstrated that green tea polyphenols, when given to transgenic adenocarcinoma of the mouse prostate (TRAMP), a transgenic mouse model that mimics progressive forms of human prostate cancer, exerts remarkable preventive effects against prostate cancer development. The results from a recent human study conducted in China have demonstrated that the prostate cancer risk declined with increasing frequency, duration and quantity of green tea consumption. This chapter addresses the issue of possible use of tea beverage, especially green tea, for the chemoprevention of prostate cancer.

Introduction

Because of increased life expectancy and changes in dietary habits and lifestyle behaviours, cancer of the prostate gland has become a significant health concern for the male population, especially in the Western world. Almost 6 years ago, this laboratory established a programme to examine whether tea consumption has preventive effects on prostate cancer. According to a projection by the American Cancer Society, one out of six males has a lifetime probability of developing prostate cancer. The incidence of prostate cancer increases rapidly with advancing age and, because of increasing life expectancy and better diagnosis of the disease, its rate of incidence is expected to increase. The incidence of progressive prostate cancer is 15-fold higher in US men than it is in men from Asian countries, and studies of the incidence and mortality of prostate cancer showed that Asian men who adopt a Western lifestyle show increased incidence of progressive disease (Saleem *et al.*, 2003).

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Prostate cancer is a complex process and multiple genetic and epigenetic factors have been implicated in the oncogenesis of prostate cancer. The limitations in the clinical management of prostate cancer are derived not only from the fact that no single gene or molecule has served as a reliable marker of prostate cancer but also from the reality that, as yet, an effective therapeutic regimen is lacking. It is therefore necessary to intensify our efforts for a better understanding of prostate cancer and for the development of novel approaches for its prevention and treatment.

An effective approach to reducing the incidence of prostate cancer is chemoprevention, which involves the use of natural or synthetic agents to suppress, block or reverse the process of carcinogenesis. Consistent with this notion, several single natural agents, particularly dietary agents, are under study for their assessment as chemopreventive agents against prostate cancer. Among dietary products the beverage tea has received much attention by researchers and marketers around the world, due to its wide-ranging pharmacological properties. The beverage tea, derived from *Camellia sinensis*, was shown to have antimutagenic and cancer-chemopreventive effects in animal tumour models (Wang *et al.*, 1989; Saleem *et al.*, 2003).

Almost 6 years ago, we initiated a programme to assess whether tea consumption could afford chemopreventive effects against prostate cancer development. This chapter summarizes the laboratory and clinical trial and epidemiological observations for the use of tea beverage or its constituent polyphenols for prostate cancer chemoprevention.

Chemoprevention and Prostate Cancer

Chemoprevention of cancers differs from cancer treatment in that the goal of this approach is to lower the rate of cancer incidence; hence one way of considering chemoprevention is preventive maintenance of the body by use of natural agents (Mukhtar and Ahmad, 1999). The recurrence of the metastatic form of the disease is often responsible for the low survival rate in prostate cancer patients and chemoprevention seems to be a promising approach against this disease because therapy and surgery have not been fully effective. Since prostate cancer is commonly diagnosed in men over the age of 50, it is an ideal candidate disease for chemoprevention because a delay by 5 years through chemoprevention would decrease the incidence and mortality from this disease. The chemoprevention of prostate cancer by the intervention of polyphenolic compounds found in various foods and beverages consumed by humans seems to be an attractive possibility.

A Brief Introduction to Tea

Next to water, tea is the most popular drink around the world; it is recorded as a healthful beverage in ancient scriptures of the East and is currently grown and cultivated in at least 30 countries around the world. It is estimated that the per capita worldwide consumption of tea beverage is approximately 120 ml per day. The three most popular tea types are black tea (78%), green tea (20%) and oolong tea (2%). All three types of tea preparations originate from the same plant source (*C. sinensis*), but have different processing methods.

Tea and its constituents

Tea leaves are a rich source of polyphenolic compounds, such as catechins, caffeine and theanine, which impart flavour and taste to tea beverages (Table 10.1). The catechins that are found commonly in green tea are (–)-epicatechin-3-gallate (ECG), epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG) and (–)-epicatechin (EC) (Fig. 10.1). EGCG is the most abundant polyphenol among all catechins found in green tea and has received by far the most attention. A brewed cup of green tea contains up to 300 mg of EGCG. Black tea is a rich source of thearubigins and theaflavins. Caffeine content varies in green and black tea,

Table 10.1. Chemical composition of polyphenolic constituents of tea.

Constituents	Amount (mg/l)	
	Green tea	Black tea
Epigallocatechin gallate	129.0	16.0
Epicatechin gallate	1.0	29.0
Gallocatechin gallate	42.0	4.0
Epicatechin	8.0	12.0
Epigallocatechin	177.0	11.0
Gallocatechin	207.0	86.0
Catechin	4.0	38.0
Catechin gallate	84.0	6.0
Caffeine	233.0	177.0
Theaflavins	0	8.0
Gallated theaflavins	0	10.0
Thearubigens	0	840.0

i.e. caffeine content in green tea ranges from 3 to 6% and black tea contains 2–4% of dry weight of caffeine. Besides EGCG and theaflavins, phenolic acids, mainly caffeic, quinic and gallic acids, are found in tea. Catechins and other polyphenols, such as quercetin, myricitin and kaempferol, constitute one-third of the dry weight of tea.

Biological properties of tea and its constituents

Tea polyphenols are widely recognized for their antioxidant activity, and the long-term consumption of green tea has been reported to influence the incidence of obesity, diabetes and cardiovascular diseases. Based on a number of studies in laboratory animals in various target-organ bioassay protocols,

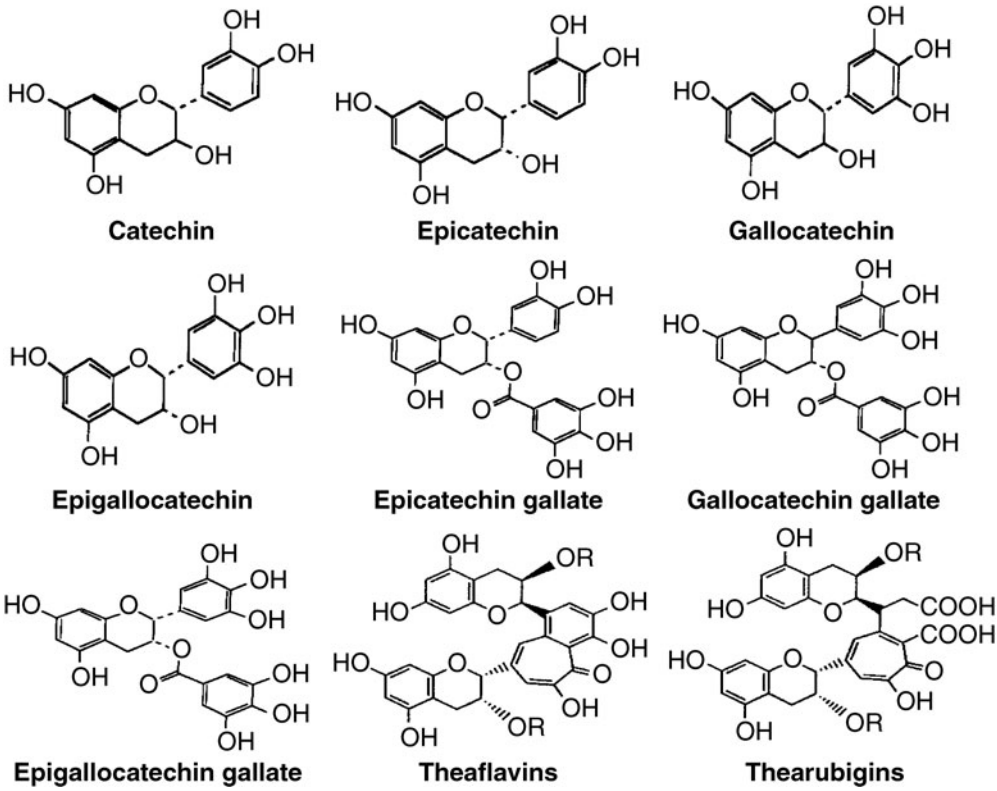

Fig. 10.1. Structures of polyphenolic constituents found in green and black tea.

conducted in many laboratories around the world, there is convincing evidence that the polyphenolic compounds present in tea are capable of affording protection against cancer initiation and its subsequent development (Ahmad *et al.*, 1998; Yang *et al.*, 1999). Feeding tea to various animal model systems has been demonstrated to inhibit lung, skin, oesophagus, stomach, liver, duodenum, small intestine and pancreatic tumour formation (Katiyar *et al.*, 1996). The growth of a variety of mouse and human cancer cell types in culture systems is inhibited by both green tea extract and ECGC, without affecting normal cells. Our laboratory showed for the first time that green tea may protect against cancer by causing cell cycle arrest and inducing apoptosis in various cell lines, such as HaCaT and L5178Y, and this was followed by many laboratories who reached similar conclusions with other cell types (Ahmad *et al.*, 1997). The activities of transcription factors activation protein-1 (AP-1) and nuclear factor-κB (NF-κB) and synthesis of nitric oxide have been shown to be inhibited by tea polyphenols. Tea catechins and theaflavins have been reported to inhibit cell transformation and cell growth, and these activities have been attributed to the inhibition of several steps of signal transduction pathways relevant to cancer development (Ahmad *et al.*, 2002). Based on wide-ranging information, clinical trials in human cancer patients with green tea are being conducted or planned.

Tea and Prostate Cancer: Experimental Studies

The effect of green tea on prostate cancer was first assessed by Liao *et al.* who showed that intraperitoneal (i.p.) administration of EGCG rapidly reduced the size of human prostate tumour growth in nude mice (Liao *et al.*, 1995). This group further suggested that there might be a possible relationship between the higher consumption of green tea and the lower incidence of prostate cancer in some Asian countries. Our laboratory has a long-term ongoing research programme on green tea and cancer chemoprevention, and we started systematically to evaluate the effect of green tea consumption on prostate carcinogenesis. At first, we showed that ornithine decarboxylase (ODC), a rate-controlling enzyme in the polyamine biosynthesis pathway, is overexpressed in prostate cancer and prostate fluid in humans (Gupta *et al.*, 1999). High testosterone levels are known to mediate the induction of ODC activity, and exposure of prostate cancer cells to EGCG and an infusion of green tea given to Cpb:WU rats caused a down-regulation of ODC activity in prostate cancer cells as well as in rats. We then reasoned that these preclinical studies should be carried out in a model system that develops prostate cancer in a similar fashion to the human disease (Gupta *et al.*, 1999). Transgenic adenocarcinoma of the mouse prostate (TRAMP) is one such model for prostate cancer, in which progressive forms of the disease develop in a manner similar to human disease. We provided convincing evidence that oral infusion of green tea polyphenols (equivalent to six cups of human consumption of green tea) to TRAMP inhibits prostate carcinogenesis (Gupta *et al.*, 2001). Tea polyphenols have been shown to inhibit the *in vitro* formation of the heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo-[4,5-b]-pyridine (PhIP), a suspected human prostate carcinogen. The development of prostate cancer is known to depend upon a variety of factors as it progresses from small latent carcinoma to a large metastatic lesion; hence it will be important to establish the stage of prostate carcinogenesis which is most responsive to green tea.

Tea polyphenols and androgen

Androgens have been reported to play a key role in the pathogenesis of prostate cancer, and androgen ablation therapy is commonly suggested for men with this nonorgan-confined disease. EGCG has been reported to decrease the circulating testosterone level by 70% in male Sprague–Dawley rats; however, the mechanism through which tea polyphenols decrease the androgen level

remained unknown at that time. Since populations with impaired androgen metabolism, such as congenital 5α-reductase deficiency (a key enzyme that converts testosterone to 5α-dihydrotestosterone), do not develop prostate cancer, researchers showed that tea polyphenols might obstruct the androgen synthesis pathway. It is suggested that the inhibition of 5α-reductase prevents prostate cancer in rats, and various 5α-reductase inhibitors, such as finasteride, are approved by the US Food and Drug Administration for the treatment of benign prostatic hyperplasia in humans (Saleem *et al.*, 2003). The green tea constituents EGCG and ECG (5–200 μM) have been reported to inhibit the activity of type 1 rat 5α-reductase.

Alterations in the function of the androgen receptor (AR) in prostate cancer cells have been shown to promote a growth advantage, since AR is expressed in all stages of prostate cancer. Hence, it has been of great interest to seek more effective means of minimizing or eliminating the function of the AR in order to achieve preventive and/or therapeutic treatments for prostate neoplastic disease. Extensive studies have shown that tea polyphenols down-regulate the expression of the AR in LNCaP prostate cancer cells (Gupta *et al.*, 2000). The basal activity of the AR promoter has been shown to be determined by an Sp1. Since Sp1 is involved in the expression of many critical genes, the decrease in this protein by tea polyphenols could somewhat decrease the growth rates of prostatic cells. EGCG has been reported to decrease the Sp1 DNA-binding activity significantly. Although prostate cancer is known to undergo a transition from an early androgen-sensitive form of cancer to a late androgen-insensitive cancer, our laboratory has shown that EGCG is capable of inhibiting the prostate cancer cell growth, irrespective of the androgen association, in a dose- and time-dependent manner. A recent study has shown that testosterone administration induced oxidative stress in rat prostate; however, administration of aqueous tea extract afforded significant protection against androgen-induced oxidative injury that may result in the development of prostate cancer (Siddiqui *et al.*, 2005). However, additional work is required to substantiate that tea polyphenols could affect androgen synthesis or its receptor in a manner that could lead to protection against prostate cancer development.

Tea polyphenols and prostate-specific antigen

Prostate-specific antigen (PSA), a glycoprotein secreted by the prostate gland, is the most clinically used marker for the detection of prostate cancer. Elevated levels of PSA have been linked with the occurrence of benign prostatic hyperplasia, the incidence of which increases with age, similarly to prostatic cancer. It has been argued that serum PSA levels can be decreased by the agents that lower serum testosterone levels, such as leuteinizing hormone-releasing hormone agonists, antagonists, anti-androgens and 5α-reductase inhibitors. It has been shown that green tea polyphenols decrease PSA levels in human prostate cancer cells (LNCaP) in a dose-dependent manner in a culture medium (Saleem *et al.*, 2003, and references therein).

Tea polyphenols and polyamine synthesis

Prostate tissue has been reported to be rich in polyamines and polyamine-metabolizing enzymes. Polyamine synthesis has been reported to increase during various types of cancers, including prostate cancer, and the activity of ODC, a key regulatory enzyme for polyamine synthesis, has been reported to be elevated in such cases. Therefore, ODC has been used as a biomarker for chemopreventive studies for a long time (76). In prostate cancer cells, testosterone has been reported to lead to an induction of ODC activity and ODC mRNA expression. Earlier, our laboratory showed that green tea polyphenols significantly reverse the induction of ODC activity as well ODC mRNA expression in LNCaP cells. Testosterone caused a twofold increase in ODC activity in the ventral prostate of mice, while prior oral infusion of 0.2% (w/v) green tea

polyphenol in drinking water resulted in 40% inhibition in this induction (Gupta *et al.*, 1999).

Tea polyphenols and gene expression

Several genes that regulate growth, cell signalling, differentiation, cell death, cell division and cell migration are either over-expressed or suppressed during cancer development and play an important role in the pathogenesis of cancer. Tea polyphenols have been shown to modulate the function of various genes at various levels (Wang *et al.*, 2002). Recently we reported that EGCG down-regulates the expression of nine genes, comprising six kinases and three phosphatases, in human prostate LNCaP cancer cells (Wang *et al.*, 2002). These genes include adenosine kinase, protein kinase C (PKC) and type I β cGMP-dependent protein kinase and are reported to play a role in cell proliferation. PKC is involved in diverse cellular functions, including cell differentiation, growth control, tumour promotion and cell death, and is a regulator of cell cycle events during G1 progression and G2/M transition. We have shown that EGCG inhibits the expression of the PKC-α gene, adenosine kinase and type I β cGMP-dependent protein kinase in LNCaP cells and hence is able to block the intracellular cyclic-nucleotide signalling cascade.

Tumour suppressor genes and the genes producing anti-growth factors are lost during cancer development, including prostate cancer, and hence are considered important biomarkers for cancer chemoprevention. EGCG has been shown to induce the expression of tumour suppressor gene SHP-1 and 16 other genes, including pyrroline-5-carboxylate and prostatic acid phosphatase. Prostatic acid phosphatase and pyrroline-5-carboxylate inhibit growth by deactivation of erbB-2 and p38 mitogen-activated protein (MAP) kinases and survival of cancer cells, respectively. The most frequently mutated gene found in human malignancies, including prostate cancer, is the p53 tumour suppressor gene. Generally, no correlation between p53 mutation and early-stage prostate cancer has been noticed, but p53 mutations are shown to be associated with a small group (10–20%) of advanced prostate cancer patients with a high Gleason score and distant-site metastasis. Recently, we reported that EGCG up-regulated p53 in LNCaP cells (with wild-type p53) but not in DU145 cells (with mutant p53). The stabilization of p53 is a critical step during the induction of apoptosis. Our laboratory has shown that EGCG-induced apoptosis in LNCaP cells occurs through the stabilization of p53 by phosphorylation on critical serine residues and $p14^{ARF}$-mediated down-regulation of MDM2 protein (Hastak *et al.*, 2003).

Tea polyphenols and essential and trace elements

Hypoxia-inducible factor 1 (HIF-1) is an alpha–beta heterodimeric transcription factor and contributes to hypoxia-mediated tumour angiogenesis. Recent studies have shown that concomitant treatment of prosate cancer cells with EGCG and ferrous ions abolished the increase in HIF-1-mediated transcription, suggesting that EGCG may act as a ferrous ion chelator. Green tea is an effective chemopreventive agent for human prostate cancer adenoma. EGCG inhibited the growth of prostate cancer cells and induced apoptosis. Cu^{2+} is a trace element necessary to our health. Many studies have proved that bioactivity of EGCG is altered in the presence of Cu^{2+}. Studies have shown that the addition of EGCG and Cu^{2+} to the growth medium decreased the relative viability of androgen-sensitive and -insensitive human prostate cancer cells. These studies suggested that EGCG-induced apoptosis and growth inhibition of prostate cancer cells are mediated through the generation of free radicals in the presence of elements.

Tea polyphenols and programmed cell death (apoptosis)

Since a balance between cell growth and cell death is maintained in other normal

cells including normal prostate, this balance is lost in favour of cell growth in prostate cancer. It is suggested that correction of this imbalance could lead to the prevention and even ablation of prostatic cancer. As human prostate cancer is present as a heterogeneous mixture of androgen-dependent and androgen-independent cells, surgery and chemotherapy have failed to address this problem. Therefore, one potential strategy to eradicate this mixture of cells is to modulate the apoptotic machinery by chemopreventive agents. Studies from this and other laboratories have shown that EGCG results in the apoptosis of both androgen-insensitive DU145 and androgen-sensitive LNCaP cells (Gupta *et al.*, 2000). We have reported that EGCG-induced apoptosis in human prostate carcinoma LNCaP cells is mediated via modulation of two related pathways: (i) stabilization of p53 and down-regulation of MDM2 protein; and (ii) negative regulation of NK-κB activity, thereby decreasing the expression of the pro-apoptotic protein Bcl-2 (Hastak *et al.*, 2003).

Tea polyphenols and angiogenesis in prostate cancer

The phenomenon that makes cancer a deadly and life-threatening disease is the movement of cancer cells from their original sites to invade surrounding tissues. Invasion of cancer cells in new sites is subsequently followed by new blood-vessel formation (angiogenesis) and is a major event of later stages of carcinogenesis. Matrix metalloproteases (MMPs) and some hydrolases have been reported to be over-expressed during the invasion of cancer cells and angiogenesis. Once tumours become aggressive and metastasize to other organs, even systemic chemotherapy may be in vain, and an interesting report from Jankun *et al.* (1997) demonstrated that EGCG inhibits urokinase, implicated in tumour invasion. The activities of MMP2 and MMP9, the most frequently expressed proteases during metastasis and angiogenesis, were found to be inhibited by EGCG. Oral feeding of tea polyphenol to TRAMP mice (which develop prostate cancer spontaneously) inhibited metastasis and angiogenesis, and this was found to be due to inhibition of vascular endothelial growth factor, a marker of angiogenesis, and also due to suppression of the activities of MMP2 and MMP9 (Cao and Cao, 1999; Adhami *et al.*, 2003). Recently, it was reported that tea polyphenols slow progression of LNCaP human prostate tumours in SCID mice, partly by inhibiting the formation of new blood vessels.

Tea polyphenols and insulin growth factor in prostate cancer

Recent studies have demonstrated that elevated circulating levels of insulin-like growth factor (IGF-I) are associated with increased risk of several common cancers, including those of the breast, prostate, lung and colorectum. The level of IGF-binding protein (IGFBP-3), a major IGF-I-binding protein in serum that, in most situations, suppresses the mitogenic action of IGF-I, has been reported to be inversely associated with the risk of these cancers. The increased levels of IGF-I with concomitant decreased levels of IGFBP-3 in serum are excellent biomarkers of prostate cancer progression in humans. Therefore, it is argued that the identification of agents that inhibit the IGF-I signalling pathway could lead to the development of highly successful prevention strategies for prostate cancer. IGF-I has been linked with the initiation and progression of prostate cancer in TRAMP mice and we observed that oral infusion of green tea polyphenols significantly lowered the IGF-I levels and restored the deficient levels of IGFBP-3 levels in TRAMP mice (Adhami *et al.*, 2004). On the basis of these results, it could be argued that prostate regression induced by green tea polyphenols may be related to alterations in availability of IGF-I as a result of increased production of IGFBP-3. The mechanism that leads to the modulation of IGF-1/IGFBP-3 by green tea polyphenol needs further extensive research.

Tea polyphenols and proteasome activity in prostate cancer

The ubiquitin–proteasome complex plays an important role in the specific degradation of cellular proteins and tumour cell survival during tumour progression of many cancer types. The cell cycle and cell death regulators, such as p53, pRB, p21/CIP1, p27/KIP1, IκBα and Bax, have been identified as targets of the ubiquitin–proteasome-mediated degradation pathway. Recent studies have shown that the ubiquitin–proteasome system is involved in the regulation of AR protein in prostate cancer cells. Tea polyphenols, such as EGCG, potently and specifically inhibit the chymotrypsin-like activity of the proteasome *in vitro* (50% inhibitory concentration (IC_{50}) = 86–194 nm) and *in vivo* (1–10 μm) at the concentrations found in the serum of green tea drinkers. The inhibition of the proteasome activity by EGCG in several tumour and transformed cell lines results in the accumulation of two natural proteasome substrates, p27(Kip1) and IκBα, an inhibitor of transcription factor NF-κB, followed by growth arrest in the G1 phase of the cell cycle. This study proposes that the proteasome complex acts as a molecular target of tea polyphenols and that inhibition of the proteasome activity by tea polyphenols may contribute to the cancer-preventive effect of tea. Treatment of prostate cancer LNCaP cells with either of the green tea polyphenol (GTP) analogues (+/–EGCG and gallocatechin gallate (+/–GCG)) accumulated p27 and IκBα proteins, associated with an increased G1 population. The treatment with (+)-EGCG accumulated the pro-apoptotic Bax protein and induced apoptosis in LnCaP cells and the synthetic GTPs significantly inhibited colony formation by LNCaP cancer cells.

Tea polyphenols and the cell cycle in prostate cancer

A controlled cell-cycle progression is also an important physiological event that is regarded to be essential for normal tissue homeostasis, and most cancer types, including prostate cancer, possess defects in one or more cell-cycle checkpoints. Normal cell-cycle progression depends on the cell's efficiency to translate extracellular signals, such as mitogenic stimuli, and intact extracellular matrices in order to replicate DNA efficiently and divide. Such signals are received by cyclin-dependent kinases (cdks) and are pushed through the cell cycle. Cyclins are cdk-binding partners that are needed for kinase activity, and their protein levels are intimately linked to the cell-cycle stage. The cyclin amplification, cdk or substrate mutation as well as the inactivation of inhibitors, such as WAF1/p21, INK4a/p16 and INK4c/p18, may lead to abnormal cdk activity. The amplification of positive growth signals and mutation of checkpoints cause a selective growth advantage of cancer cells, and the loss of cell-cycle checkpoints may result in drug resistance, invasion and metastasis. Therefore, inhibition of the cell cycle has been appreciated as a potential target for the management of cancer, including prostate cancer. Tea polyphenols are reported to arrest the cell division of cancer cells and increase the expression of cdk inhibitors. We have demonstrated that EGCG exhibited a dose-dependent arrest of cells in the G0/G1 phase of the cell cycle in prostate cancer cells, hence slowing down the growth of prostate cancer cells. Recent studies from our laboratory have shown that EGCG treatment of human prostate carcinoma cells LNCaP and DU-145 caused a significant dose- and time-dependent: (i) up-regulation of the protein expression of WAF1/p21, INK4a/p16 and INK4c/p18; (ii) down-modulation of the protein expression of cyclin D1, cyclin E, cdk2, cdk4 and cdk6, but not of cyclin D2; and (iii) increase in the binding of cyclin E to cdk2. These events lead to a blockade of the G1 to S transition, causing a G0/G1 phase arrest of the cell cycle (Gupta *et al.*, 2003). However, further investigation is needed to study the effect of EGCG on the interlinking between the different components of the cki–cyclin–cdk network.

Epidemiological Studies

To date, no extensive case–control study has been conducted to assess the effect of consumption of green tea on human prostate cancer. All published reports seeking an association between tea consumption and the risk of prostate cancer considered undefined tea preparations, mostly black tea (Table 10.2). There are two epidemiological studies that have shown that people who regularly drink tea have a lower incidence of prostate cancer (Saleem *et al.*, 2003). In a prospective cohort study employing 7833 men with Japanese ancestry living in Hawaii, a weak but significant negative association between black tea intake (more than one cup per day) and prostate cancer incidence ($P = 0.02$) was observed. The case–control studies conducted in Canada showed a decrease in prostate cancer risk with the intake of tea. Other epidemiological studies conducted in Italy, Utah and Canada did not find any difference of risk for prostate cancer between tea drinkers and non-drinkers. Since most of these studies include populations that were predominantly black tea drinkers, these studies lacked appropriate controls for comparison in categorization of tea consumption, the type of tea consumed and the ethnicity of the subjects, which weakens the overall impact of the study. Therefore, epidemiological investigations looking for an association between green tea and prostate cancer should be undertaken to establish the validity of cell culture and animal data for human prostate cancer patients.

Green Tea and Clinical Trials

Recently, a Phase II clinical trial exploring the anti-neoplastic effects of green tea in patients with metastatic androgen-independent prostate cancer was conducted (Jatoi *et al.*, 2003). This study comprised patients who were instructed to take 6 g of green tea/day orally in six divided doses, and patients were monitored monthly for response and toxicity. The results of this study revealed that only one patient within this 42-patient cohort manifested a 50% decline in PSA values from baseline and this decrease was not sustained beyond 2 months. Green tea was tolerated well for the most part; however, a notable percentage of patients did experience toxicity such as insomnia, fatigue, etc., presumably from the caffeine present in the tea. A recent clinical trial conducted at the Ottawa Hospital Regional Cancer Centre, Canada, on hormone-refractory prostate cancer patients who were prescribed green

Table 10.2. Summary of published epidemiological reports showing an association between tea and prostate cancer.

Location	Sample size	Tea intake	OR/RR
Hawaii, USA	149 cases, 7833 subjects	2–4 cups/week >1 cup/day	RR = 0.4 RR = 0.6 ($P = 0.02$)
Canada	335 cases, 344 controls	0–500 g/day	OR = 0.89 (95% CI = 0.69–1.16) ($P = 0.05$)
	99 cases, 124 controls	>500 g/day	OR = 0.7 (95% CI = 0.5–0.99) ($P = 0.05$)
Italy	107 cases, 6147 controls	≥1 cup/day	RR = 0.9
Utah, USA	362 cases, 685 controls	>5 cups/week	RR = 0.9
Canada	145 cases, 3400 subjects	500 ml/day	RR = 1.02 (95% CI = 0.62–0.65)

OR, odd ratio; RR, relative risk ratio; CI, confidence interval; *P*, *P* value for trend.

tea extract capsules at a dose level of 250 mg twice daily, revealed that green tea, as an alternative complementary therapy, had minimal clinical activity against disease. It should be noted that these studies were conducted in patients with metastatic androgen-independent prostate cancer and therefore, in principle, are not representative of the chemopreventive effects of green tea. Further, in this trial, the median time of the study was only 1 month, whereas previous preclinical data suggest that green tea requires prolonged exposure to exert its anti-tumour activity. Moreover, this trial was conducted in patients with androgen-independent prostate carcinoma only and it is possible that green tea may exert anti-neoplastic affects in patients with hormone-sensitive prostate carcinoma. In view of the drawbacks of this trial, the negative findings of this study do not nullify the results of previous epidemiological studies that suggest that green tea may confer an anti-tumour effect in a relatively healthy population. For a perfect prospective study, a population with a high risk for prostate cancer development should be considered and the length of time of green tea drinking by the subjects should be taken into consideration.

Future Perspectives

Based on the information gained to date in mice or in human prostate cancer cell culture systems, tea or its constituents have been reported to cause cell-cycle dysfunction, induce apoptosis and inhibit or induce certain enzymatic pathways that play a role in the cancer development process. Although tea polyphenols have been demonstrated to act as a potent chemopreventive agent against prostate cancer, there are still many gaps in our existing knowledge before a recommendation could be made for tea polyphenols as a preventive or therapeutic agent for humans. Certain factors, such as bioavailability, tissue level of tea constituents that can be achieved, tea type, drinking habits, race and association with other dietary factors, should be taken into consideration before recommending the beverage tea as a therapeutic agent against prostate cancer. Therefore, extensive laboratory research and well-planned epidemiological studies are needed to obtain conclusive evidence. The increase or decrease of PSA and the ratio of IGF-I to IGFBP-3 in relation to tea consumption and levels of tea polyphenols in urine samples may be used as potential predictors in prostate cancer chemoprevention. Such studies could possibly answer the question of how much tea should be consumed by humans for prostate cancer chemoprevention. Prostate cancer is a disease with many aetiological factors and involves several mechanisms in its progression, and the modulation of a single mechanism alone by tea polyphenols may not completely stop the progression of prostate cancer. Although tea polyphenols have been reported to modulate various molecular targets involved in the progression of prostate cancer, as discussed elsewhere in this review, there is no information available that links the various molecular pathways involved in the progression of prostate cancer to each other. Currently, the combinational studies of cancer chemopreventive agents have become an area of research interest and it is suggested that various chemopreventive agents in combination may produce synergistic or additive effects. Although tea exhibits broad chemopreventive efficacy, from inhibition of carcinogen formation to suppression of prostate cancer progression, the uniqueness of its action lies in its specificity of killing cultured cancer cells without affecting the growth of normal cells. An indepth study in animal models and humans is warranted to establish the chemopreventive potential of tea polyphenols against prostate cancer, after integrating mechanistic studies *in vitro* and *in vivo* and paying major attention to the bioavailability of tea constituents and its metabolites. The experimental and epidemiological information collected to date is encouraging and should form the basis for a further examination of the utility of tea beverage in chemoprevention of human prostate cancer.

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11 Anti-diabetic Effects of Tea and its Constituents

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Abstract
Diabetes is a disorder of carbohydrate metabolism where the sugar level increases in the blood beyond the normal level. In diabetic patients, reduced antioxidant defences are observed and they suffer from an increased risk of free-radical-mediated disease, such as coronary heart disease. Epidemiological evidence has suggested that antioxidant dietary flavonoids may protect against diabetes but a biological effect has yet to be demonstrated directly in humans. Chemical analysis has indicated that tea is a source of many flavonoids. These tea flavonoids are reported to have a glucose-lowering effect in animals. The exact mechanisms of the antidiabetic effects of tea are not clear, although several hypotheses have been proposed. Tea components mimic the insulin by increasing tyrosine phosphorylation of the insulin receptor, insulin receptor substrate, phosphoinositide 3-kinase, mitogen-activated protein kinase and p^{70s6k} activity. They also regulate genes that encode gluconeogenic enzymes and protein tyrosine phosphorylation and reduce phosphoenolpyruvate carboxykinase gene expression in a phosphoinositide 3-kinase-dependent manner. Free-radical-mediated diseases are also known to be reduced by tea components due to their antioxidant properties. Thus tea can be considered as anti-diabetic without any toxicity in animals. Tea is therefore worthy of serious consideration for further investigation in respect of the prevention and treatment of diabetes.

Introduction and Global Status of the Ailment

Diabetes, or 'sugar diabetes' as it is most commonly referred to, is a disease of disturbed carbohydrate metabolism, where the sugar level increases in the blood beyond normal levels. Diabetes is a disease characterized by the insufficient secretion or improper functioning of insulin. Insulin regulates the amount of blood sugar in our tissues. Improper absorption of blood sugar leads to excess concentrations that must be released through urine. If this continues for long periods of time, it can lead to a number of more serious illnesses. Diabetes can be classified as type 1 – insulin-dependent diabetes mellitus (IDDM); and type 2 – non-insulin-dependent diabetes mellitus (NIDDM). Type 1 diabetes (juvenile-onset diabetes) results from a lack of insulin,

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and usually develops before age 30. Type 2 diabetes (adult-onset diabetes) results when the body cannot process insulin correctly.

Both type 1 and 2 diabetic patients exhibit abnormal antioxidant status, autoxidation of glucose and excess glycosylated proteins. Oxidative stress in diabetes leads to tissue damage, with lipid peroxidation, inactivation of proteins and protein glycation as intermediate mechanisms (Wolffe *et al.*, 1991) resulting in complications including retinopathy, nephropathy and coronary heart disease (Oberley, 1988; Jennings *et al.*, 1991; Lyons, 1991; Valezquez *et al.*, 1991). Dietary antioxidant compounds, including ascorbic acid and tocopherol, offer some protection against these complications through their roles as inhibitors of glycation and free-radical scavengers (Davie *et al.*, 1992; Sinclair *et al.*, 1992).

Despite the introduction of hypoglycaemic agents from natural and synthetic sources, diabetes and its complications continue to be a major problem in the world. According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the American Diabetes Association (2004) diabetes was the seventh leading cause of death in 1996 and the sixth leading cause of death in 1997 from the disease. It was found that diabetes affects an estimated 16 million people in the USA (90–95% have type 2 diabetes – 10.3 million have been diagnosed, but 5.4 million are unaware that they have the disease). Those affected comprise 8.1 million women, 7.5 million men, 123,000 children under age 20 and 6.3 million adults over 65.

In India, the number of cases of diabetes was found to be 31.7 million in 2002 and the estimated number of diabetes cases in India for 2030 is predicted to be 79.4 million (estimated by the American Diabetes Association, 2004). In view of the above, in the present report, the beneficial health effect of tea and its constituents is described.

Types and Components of Tea

There are three types of tea: green, oolong and black. They are produced from a single plant species, but they are distinguished by the processing technique. Oolong tea is partially fermented during processing, whereas green tea is not fermented and black tea is fully fermented (Hosoda *et al.*, 2002). Green tea, a beverage commonly consumed in Asian countries, is a significant source of a type of flavonoids called catechins, which are monomeric polyphenols. The green tea catechins include (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin, (–)epicatechin gallate and (–)epicatechin (Guo *et al.*, 1999). In contrast to green tea, black tea contains a lower monomeric polyphenol content (Yang *et al.*, 1993; Katiyar and Mukhtar, 1996). During the manufacture of black tea, the green tea catechins undergo oxidation of polyphenol oxidase to form the complex condensation products theaflavins (TF) and thearubigins (TR), by a process commonly known as fermentation. TF are a mixture of TF and TF-gallates. However, TR are more complex oligomeric flavonols; they vary greatly in molecular weight, ranging from 1 to 40 kDa (Lee *et al.*, 2000). The contents of TF and TR vary with the species of tea and the process of fermentation. Nevertheless, TR are the most abundant phenolic fractions in black tea. Black tea contains ~1–4% TF, 10–20% TR and catechins (Yang *et al.*, 1993; Lee *et al.*, 2000). Oolong tea contains epigallocatechin, caffeine and epigallocatechin gallate as main components (Hosoda *et al.*, 2003).

Historical References to Tea for Disease Prevention

Initial work on tea suggested that it possesses human health-promoting effects. Diseases for which tea drinkers appear to have a lower risk range from simple bacterial and viral infections to chronic debilitating diseases, including cancer, coronary heart disease, stroke and osteoporosis (Siddiqui *et al.*, 2004). Inhibition of carcinogenesis by tea and tea polyphenols has been demonstrated in many animal models, including those for cancer of the skin, lung, oral cavity, oesophagus, small intestine, stomach, colon, liver,

pancreas, bladder and prostate. *In vitro* cell culture studies show that tea polyphenols potently induce apoptotic cell death and cell-cycle arrest in tumour cells but not in their normal cell counterparts. Green tea polyphenols affect several signal transduction pathways, including growth factor-mediated, the mitogen-activated protein kinase (MAPK)-dependent and ubiquitin–proteasome degradation pathways. Epidemiological studies have suggested that the consumption of green tea lowers the risk of cancer (Chen *et al.*, 2004).

Maron (2004) reveals the numerous plausible mechanisms by which tea polyphenols may confer cardiovascular protection: they inhibit low-density lipoprotein oxidation, reduce thrombosis, improve endothelial function and reduce inflammation. Kawai *et al.* (2003) showed that *in vitro* EGCG, the main component of tea, can block the binding of human immunodeficiency virus (HIV) envelope glycoprotein to human CD4 molecules on human T cells. The CD4 molecule acts as a binding target for HIV vesicles and plays an important role in the aggressive infection process. Other researchers had previously reported that this catechin, as well as a further two polyphenolic catechins from green tea, inhibited HIV reverse transcriptase activity, like other antiviral agents (Nakane *et al.*, 1989; Chan *et al.*, 1994). Several other scientists have also investigated the possible therapeutic application of EGCG in the fight against acquired immune deficiency syndrome (AIDS) (Fassina *et al.*, 2002; Weber, 2003).

Tea and Diabetes

Several lines of evidence, including human epidemiological data and clinical intervention data, indicate that plants containing flavonoids are used to treat diabetes in Indian medicine, and the green tea flavonoid EGCG is reported to have glucose-lowering effects in animals (Waltner-Llaw *et al.*, 2002; Tsuneki *et al.*, 2004). Green tea-derived products are mainly extracts of green tea in liquid and powdered form varying in the proportion of polyphenols (45–90%) and caffeine content (0.4–10%). The polyphenolic fraction of green tea has been reported to have multiple pharmacological actions (Mukhtar *et al.*, 1992; Sano *et al.* 1995). According to the study by Kobayashi *et al.* (2000), epigallocatechin gallate was found to inhibit intestinal glucose uptake by sodium-dependent glucose transporter SGLT1, indicating increase of glucose in controlling blood sugar. Streptozotocin-diabetic rats showed increased sensitivity to platelet aggregation and thrombosis, and this abnormality could be improved by dietary catechin of green tea (Choi *et al.*, 1998; Yang *et al.*, 1999).

In a report, injection of EGCG into lean and obese Zucker rats significantly lowered blood glucose and insulin levels, and green tea extract increased glucose metabolism in adipocytes (Broadhurst, 2000; Kao *et al.*, 2000). The amelioration of insulin resistance by green tea is associated with the increased expression level of glucose transporter IV in a fructose-fed rat (Wu *et al.*, 2004). The study presented by Gomes *et al.* (1995) also reveals that, like green tea, black tea also possesses anti-diabetic activity. Black tea and green tea extracts were found to possess both a preventive and a curative effect on streptozotocin-induced diabetes in rats. While green tea was more effective as a preventive, black tea was more effective as a curative. An antihyperglycaemic effect of oolong tea has also been reported by Hosoda *et al.* (2003). Based on these findings, the anti-diabetic effects of tea and its constituents have drawn considerable attention.

Experimental Studies

Role of polyphenolic fraction of green tea in reducing the alloxan-induced diabetes in *in vivo* studies

Alloxan produces oxygen radicals in the body, which cause pancreatic injury (Halliwell and Gutteridge, 1985), which is responsible for the increased blood sugar

seen. Sabu *et al*. (2002) reported that an aqueous solution of green tea polyphenols (GTP) was found to reduce the serum glucose level in alloxan-diabetic rats significantly at a dose level of 100 mg/kg body weight (bw). Continued daily administration (15 days) of the extract at 50 and 100 mg/kg bw produced 29 and 44% reduction, respectively, in the elevated serum glucose level produced by alloxan administration. Elevated hepatic and renal enzymes produced by alloxan were found to be reduced by GTP. The serum lipid peroxidation (LP) level, which was increased by alloxan, was reduced significantly by the administration of 100 mg/kg bw of GTP. Decreased liver glycogen, after alloxan administration, showed a significant increase after GTP treatment. GTP treatment showed an increased antioxidant potential, as seen from improvements in superoxide dismutase and glutathione levels. However, catalase, LP and glutathione peroxidase levels were unchanged. These results indicate that alloxan-induced diabetes and subsequent elevation of blood sugar were reversed by simultaneous administration of green tea polyphenols.

Insulinomimetic activity of EGCG in hepatoma cells

The study of Waltner-Law *et al*. (2002) shows that EGCG decreases glucose production in hepatoma cells. The production of glucose in response to insulin or EGCG was examined in H4IIE rat hepatoma cells incubated in a medium containing pyruvate and lactate as substrates for gluconeogenesis. The cells were treated with a combination of 500 nM dexamethasone and 0.1 mM 8(-4-chlorophenylthio)-cAMP (Dex/cAMP), in the presence or absence of insulin or EGCG, for 5 h to maximize glucose production capacity. Cells were incubated for an additional 3 h with Dex/cAMP, with or without insulin or EGCG, in glucose production buffer. At the end of this incubation, 0.5 ml of medium was taken to measure the glucose concentration in the culture medium, using a glucose assay kit. Insulin, at physiological concentrations of 10 nM, and 25 μM EGCG were comparable in repressing glucose production to basal levels. However, higher concentrations of EGCG had no further glucose-lowering effect.

Insulinomimic activity of (–)epicatechin in erythrocyte membrane

Another study, conducted by Rizvi *et al*. (2001), also shows the *in vitro* effect of (–)epicatechin and/or insulin tested in normal and type 2 diabetic patients to test the efficacy of epicatechin to mimic insulin in its effect on erythrocyte membrane acetylcholine esterase. They found that the acetylcholinesterase activity was significantly lower in type 2 diabetic patients than in normal controls and *in vitro* insulin treatment restored this activity to normal levels. Epicatechin (1 mmol/l) also caused an elevation in acetylcholine esterase activity in diabetic erythrocytes, an effect that was similar to the effect of insulin.

Mechanism

Mechanism of action of EGCG in comparison with insulin

Like insulin, EGCG increases tyrosine phosphorylation of the insulin receptor and insulin receptor substrate 1 (IRS-1), and it reduces phosphoenolpyruvate carboxykinase gene expression in a phosphoinositide 3-kinase-dependent manner. EGCG also mimics insulin by increasing phosphoinositide 3-kinase, mitogen-activated protein kinase and p^{70s6k} activity. Furthermore, EGCG regulates genes that encode gluconeogenic enzymes and protein tyrosine phosphorylation by modulating the redox state of the cell (Waltner-Law *et al*., 2002). One possible mechanism for the observed actions of EGCG in hepatoma cells is the inhibition of protein tyrosine phosphatases (PTPs), which contain an oxidizable cysteine in their active site (Denu *et al*., 1998; Robinson *et al*., 1999). It is possible that EGCG causes

oxidation of this cysteine residue in redox-sensitive phosphatases, and *N*-acetylcysteine (NAC) and superoxide dismutase (SOD) reverse this effect. Several PTPs, including PTP-1 B and leucocyte antigen-related phosphatase, dephosphorylate the insulin receptor and IRS-1, making these phosphatases candidates for modification by reactive oxygen species (ROS) produced in response to EGCG (Mooney *et al.*, 1997; Goldstein *et al.*, 2000; Salmeen *et al.*, 2000). The disruption of the PTP-1 B gene leads to decreased blood glucose level and increased insulin sensitivity. EGCG differs from insulin, however, in that it affects several insulin-activated kinases in slower kinetics.

Mechanism of action of EGCG as antioxidant

Diabetic patients have reduced antioxidant defences and suffer from increased risk of free-radical-mediated diseases. There is growing awareness that free-radical processes may be of particular importance in the microvascular and macrovascular complications of diabetes (Gazis *et al.*, 1997). Oxidation of membrane proteins and lipids is a self-perpetuating process that can damage the cellular membrane integrity and ultimately result in cell dysfunction (Mead, 1991). Decreased lipid peroxidation and improved antioxidant status may be one mechanism by which dietary treatments contribute to the prevention of diabetic complications (Leinonen *et al.*, 1997). It has been suggested by others (Katiyar and Mukhtar, 1997) that EGCG can interact with peroxy radicals and inhibit lipid peroxidation. The exact mechanism of EGCG is not known but it is proved to be a very effective inhibitor of formation of thiobarbituric acid-reactive substances (TBARS). EGCG also protects erythrocyte membrane-bound ATPase (Ca^{2+} pumps ATPase and CaM/Ca^{2+} ATPase activity) against tertiary butyl hydroperoxide (BHP)-induced damage (Saffari and Sadezadeh, 2004). The protective effect of EGCG is either due to scavenging peroxides before attacking membranes and/or due to blocking the oxidation of membrane lipids. Because of the presence of the catechol structure, most tea polyphenols are strong metal ion chelators. They can bind and thus decrease the level of free cellular ferric and ferrous ions, which are required for the generation of reactive oxygen radicals (Wang *et al.*, 1989).

Human Studies

To determine the efficacy of tea and its constituents for lowering plasma glucose in type 2 diabetic patients, a study has been done in Taiwan by Hosoda *et al.* (2003). The study was done on 20 free-living subjects who had type 2 diabetes and took hyperglycaemic drugs. Total caffeine, which is one of the most important components of tea, and tea polyphenol consumption for subjects consuming 1500 ml of the tea were 352.7 and 149.0 mg, respectively, for 30 days. It was found that caffeine and tea polyphenols significantly decreased plasma glucose (from an initial concentration of 229 ± 53.9 to 162.2 ± 29.7 mg/dl) and fructosamine concentration decreased significantly (from an initial concentration of 409.9 ± 96.1 to 323.3 ± 56.4 μmol/l) with the tea treatment. This result clearly supports the concept that tea and its components are effective in lowering plasma glucose levels.

Future Prospects

Diabetes is widely recognized as one of the leading causes of death and disability worldwide. The available epidemiological information does not indicate that tea consumption has a statistically significant causative effect on diabetes. Therefore, studies on the absorption, distribution and metabolism of key components of tea in animals and humans are of great importance. There is a need to collect more specific information on the qualitative and quantitative aspects of tea consumption and its beneficial effect on diabetes. Thus, researchers should continue to look for the cause or causes of diabetes and ways to prevent and cure the disease. It has been shown that

EGCG has an insulin-like effect, but further experiments should be directed to determine the action of EGCG, which will lead to the identification of molecular targets for the generation of therapeutic agents useful in the treatment of diabetes.

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12 Green Tea Catechins against Oxidative Stress of Renal Disease

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Abstract
Renal disease, widely associated with various diseases, is affected by free-radical-induced oxidative stress. Green tea catechins, known for their powerful antioxidant and active free-radical scavenger activities, were examined against the oxidative stress of renal disease and related complications in rat models and humans. The catechins strongly inhibited the oxidative stress of free radicals and decreased the cell injury in a renal epithelial cell line. The catechins relieved the high oxidation stress condition and renal hypertension by inhibiting the production of oxidative uraemic toxins and improving the renal blood circulating state, respectively. The application of catechins improved renal function by inhibiting mesangial cell proliferation. The catechins were found to be effective in easing the pains connected with renal disease. The antioxidant properties of catechins against oxidative stress and related complications of renal disease have established the activity of green tea in regulating renal function.

Keywords
Antioxidant, glomerular function, glomerular sclerosis, renal hypertension, uraemia.

Introduction

Renal disease is a growing menace to the world population. In the USA, the number of patients with end-stage renal diseases has been rising at the rate of 8% annually for the last 10 years. Nearly 20 million people have been found to have an impaired kidney function, based on serum creatinine and urinary albumin values. Renal disease is associated with various diseases, such as diabetes (36%), followed by hypertension (24%) and glomerulonephritis (14%). Almost 3% of the world population suffers from diabetes mellitus. The USA (6.2%), Japan (5.3%) and India (3.1%) have large percentages of diabetic patients in their population. World statistics invariably suggest that renal disease is yet another lifestyle-related disease of modern society.

Functional disorders of the kidneys may lead to the accumulation of waste metabolites, such as urea and guanidine compounds, which can cause a state of oxidative stress in renal disease. The oxidative stress, generally recognized as 'uraemia', is mediated by free-radical species. Also, a decrease in antioxidative enzyme activities, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px), and an

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increase in the amount of hydroxyl radical (OH) in an animal model of renal disease suggest the weakening of the free-radical scavenging system in renal disease (Yokozawa *et al.*, 1999). Therefore, it is believed that substances that have strong free-radical scavenging activities may ameliorate the oxidative stress conditions in renal disease (Rehan *et al.*, 1984; Diamond *et al.*, 1986; Shah and Walker, 1988; Yokozawa *et al.*, 1993a).

Green tea catechins have been recognized as powerful antioxidants and active scavengers of free radicals (Xie *et al.*, 1993; Chen and Ho, 1995; Serafini *et al.*, 1996; Koketsu, 1997; Chu and Juneja, 1998; Hara, 2001; Nakagawa *et al.*, 2002), and are found to be effective in the prevention of various free-radical-induced and -mediated diseases such as cancer, cardiovascular diseases and arthritis. Our laboratory, in collaboration with Dr Yokozawa's group (Toyama Medical and Pharmaceutical University, Toyama, Japan), has exclusively examined the antioxidative and physiological functions of green tea catechins against renal disease, using animal models and humans. A refined form of green tea catechins, Sunphenon® (Taiyo Kagaku Co., Ltd, Japan), was used in all our studies.

Prevention of Oxidative Stress

Oxidative stress is linked to tissue damage and the development of several chronic diseases, including renal disease. Renal disease is known to occur as a result of reperfusion after a certain period of blood flow blockage (Yokozowa *et al.*, 1997a). In the case of ischaemia–reperfusion, the generation of superoxide (O_2^-) in renal proximal tubule cells increases, leading to lipid peroxidation and cell and tissue injury (Paller *et al.*, 1984). In addition, recent studies have indicated that nitric oxide (NO) is also produced in proximal tubules as a result of ischaemia–reperfusion (Yu *et al.*, 1994). Excess NO produced during ischaemia–reperfusion is considered to act as a toxic radical and to cause renal dysfunction like O_2^- (Paller *et al.*, 1984; Yu *et al.*, 1994). NO and O_2^- cause ischaemic renal injury individually and they work together to bring about further damage. The degree of damage increases several-fold when the two radicals react, forming peroxynitrite ($ONOO^-$), which can lead to a series of toxic reactions with biomolecules such as proteins, lipids and nucleic acids (Moncada *et al.*, 1991; Radi *et al.*, 1991a, b; Yermilov *et al.*, 1995). Further, the succession of renal disease is then associated with the accumulation of highly oxidative uraemic toxins. Hence, the symptoms of uraemia and high oxidative stress conditions are common phenomena in renal disease patients (Fillet *et al.*, 1981; Giardini *et al.*, 1984; Kuroda *et al.*, 1985; Flament *et al.*, 1986). The uraemic toxins are mainly produced by the involvement of hydroxyl radicals (Ienaga *et al.*, 1991; Nakamura *et al.*, 1991; Yokozawa *et al.*, 1991a, b, 1992, 1997b, c). The free-radical species-mediated oxidative stresses are therefore the main factors in the occurrence and progression of renal disease (Yokozawa *et al.*, 1996, 1997a, 1998, 2000).

The effect of green tea extract on oxidative stress damage in renal disease was assessed using a porcine kidney-derived cultured epithelial cell line, LLC-PK_1 (Yokozawa *et al.*, 1997, 1999). This cell line had the nature of a proximal uriniferous tubule, known to be severely injured in ischaemic acute renal disease. When LLC-PK_1 cells were cultured under hypoxic conditions (<2% oxygen) before reoxygenation was applied (95% air, 5% CO_2), the leakage of lactate dehydrogenase (LDH) into the medium increased. This phenomenon was inhibited by dimethyl sulphoxide, a free-radical scavenger, suggesting the involvement of free radicals. Similarly, cisplatin, an anti-tumour drug, was known to induce acute renal failure by decreasing the radical scavenger SOD. The induction of oxidative stress by hypoxia/reoxygenation and cisplatin in LLC-PK_1 cells was inhibited dose-dependently by Sunphenon (Fig. 12.1). In another study, a hydrophilic azo compound, namely 2,2′-azobis-(2-amidinopropane) dihydrochloride (AAPH), was used to generate peroxyl radicals in the LLC-PK_1 line (Yokozawa *et al.*, 2000).

AAPH is known to generate peroxyl radicals via interaction with carbon-centred radicals and molecular oxygen, eventually causing the oxidation of lipid and protein in biomolecules. Green tea polyphenols significantly decreased the AAPH-induced lipid peroxidation in the cell line and dramatically increased cell viability (Fig. 12.2). Green tea catechins also showed direct scavenging of NO and O_2^- radicals (Fig. 12.3) (Nakagawa and Yokozawa, 2002). These studies have confirmed the beneficial effects of green tea catechins in relieving oxidative stress in renal disease.

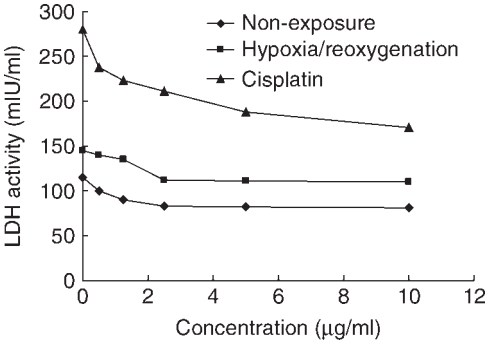

Fig. 12.1. Effect of green tea catechins on lactate dehydrogenase (LDH) leakage from LLC-PK_1 cells exposed to hypoxia/reoxygenation or cisplatin (from Yokozawa *et al.*, 1997, 1999).

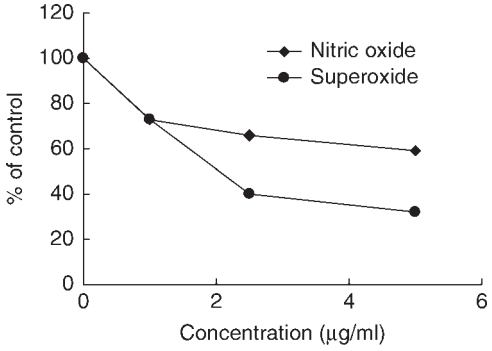

Fig. 12.3. Effect of green tea extract on the inhibition of NO and O_2^- (from Nakagawa and Yokozawa, 2002).

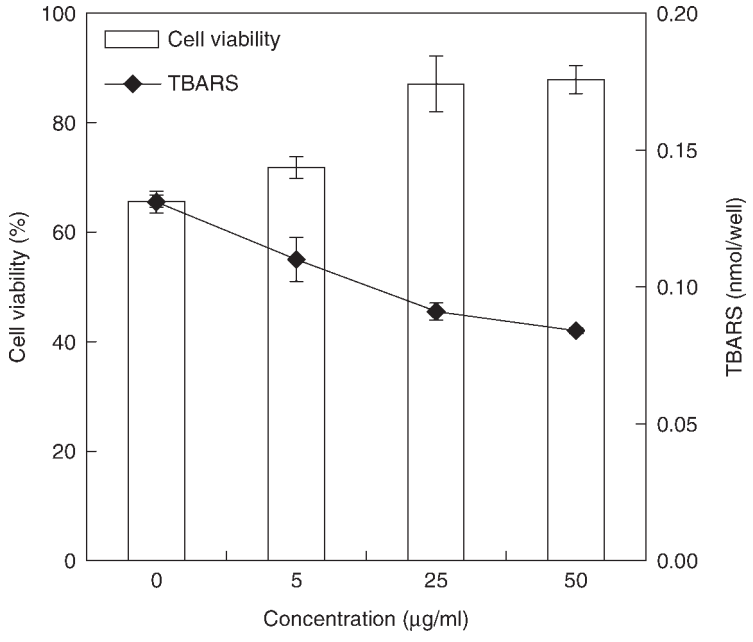

Fig. 12.2. Effect of green tea catechins on thiobarbituric acid-reactive substances (TBARS) and cell viability of LLC-PK_1 cells treated with AAPH (from Yokozawa *et al.*, 1997, 1999).

The suppression of oxidative stress and the free-radical scavenging activity of green tea are mainly due to its composition of low-molecular catechins, namely epigallocatechin gallate (EGCG), epigallocatechin (EGC), gallocatechin gallate (GCG), epicatechin gallate (ECG), epicatechin (EC), gallocatechin (GC) and catechin (C). These catechins are largely known as catechins and structurally they belong to the flavon-3-ol group. Chemically they are highly reactive, with properties of metal chelation, oxidative radical scavenging, nitrosation inhibition, etc. Using a lipid peroxidation assay, Hirose *et al.* (1990, 1991) proposed the following chemical reactivity of catechins in radical scavenging action. The catechins liberate a hydrogen radical from the hydroxyl groups of 3′ and 4′ positions in the B ring, and the hydrogen radical joins with other free radicals (e.g. lipid peroxide) to become stabilized. On the other hand, the catechin itself changes to a phenoxy radical; then the transfer of the radical electron occurs by contribution of the resonance structure of the benzene ring, and the carbons at the 3′ and 4′ positions form a double bond with a remaining oxygen atom to form a ketone structure. The bond between C-3′ and C-4′ of the B ring is oxidatively cleaved, leaving one radical electron each on both of the carbons. The radical electron of C-3′ forms a lactone ring with the alcoholic hydroxyl group of the 3 position of the C ring in the same way that hydroxyl carboxylic acid forms an intramolecular ester. Another radical electron of C-4′ captures the hydroxyl radical existing in the reaction system and becomes stable by the formation of carboxylic acid. By this reaction process, a polyphenol can scavenge four radicals per mole, and thus the above reaction may explain the mechanism of the antioxidative action of catechins.

Inhibition of Uraemia

At the inception of chronic renal failure, the concentration of uraemic toxins increases, leading to the state of uraemia and a high oxidation stress condition (Giovannetti *et al.*, 1973; Sakanaka and Kim, 1997). The toxins currently known include methylguanidine, guanidinosuccinic acid, dimethylamine, myoinositol and β_2-microglobulin. Among these toxins, methylguanidine is the pertinent toxin to induce the uraemic condition (Giovannetti *et al.*, 1968). Examination of urine specimens from chronic renal disease patients revealed that methylguanidine is produced from creatinine via creatol (5-hydroxycreatinine) by the OH radical (Ienaga *et al.*, 1991; Nakamura *et al.*, 1991; Yokozawa *et al.*, 1991a, b). Green tea catechins known for their active free-radical scavenging activity inhibit the production of methylguanidine and thus alleviate chronic renal failure both in animals (Yokozawa *et al.*, 1992, 1994, 1996a, 1997b, c; Sakanaka and Kim, 1997) and in humans (Yokozawa *et al.*, 1996b; Sakanaka and Kim, 1997).

In rats, Yokozawa *et al.* (1992, 1993a) examined the effect of green tea catechins on adenine-induced renal disease. They examined urinary methylguanidine excretion as an index of scavenging reaction. The rats were administered with different doses of green tea catechins (as Sunphenon®) or only EGCG orally for 14 days after adenine administration for 20 days. A dose-dependent decrease in methylguanidine excretion was observed (Fig. 12.4), whereas a dose of 0.5 mg/day of green tea polyphenol or EGCG

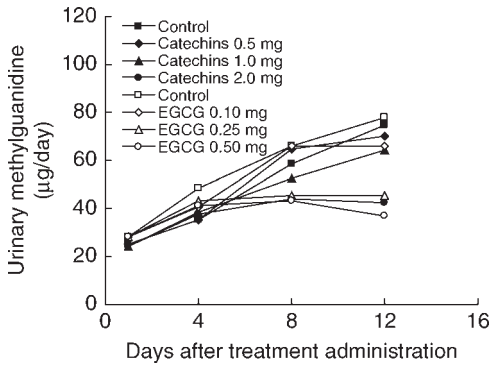

Fig. 12.4. Effect of different doses of green tea catechins (Sunphenon®) or EGCG (open symbols) on the urinary methylguanidine excretion in adenine-induced renal disease rats (from Yokozawa *et al.,* 1992).

did not show any appreciable inhibition in the production of methylguanidine. However, an increase in the dose to 1.0 or 2.0 mg/day strongly inhibited the production of methylguanidine. Methylguanidine production was about 40% lower compared with the control group with the administration of 2 mg green tea catechins at 12 days (32nd day) after adenine-induced chronic renal failure, while a similar decrease (40%) or much greater inhibition (about 50%) was noticed with the administration of exclusively EGCG at the rate of 0.25 and 0.5 mg/day, respectively. These results suggested that EGCG, the powerful antioxidant component of green tea catechins, has a strong inhibitory effect on methylguanidine production and may thereby induce recovery from oxidative stress.

The clinical efficacy of green tea catechins in suppression of creatinine and methylguanidine production was observed in 50 dialysis patients (Yokozawa *et al.*, 1996b). The patients were administered 200 mg green tea catechins twice a day in the form of either jelly or capsules consecutively for 6 months. The blood samples were collected just before dialysis each month and the creatinine and methylguanidine levels were analysed. The creatinine level was significantly lowered after 3 months of the administration, showing an almost 8% decrease in 5–6 months (Table 12.1). A decrease in methylguanidine preceded the decrease of creatinine, reaching a significantly low level within a month. In 5 months the level of methylguanidine was 20% lower than the initial level. These results suggested that green tea catechins influenced the radicals involved in the production of methylguanidine from creatinine, which was clearly evident in the ratio methylguanidine/creatinine. Concurrently a significant decrease in β_2-microglobulin was also observed within 1 month of green tea administration in patients with high oxidative stress (Table 12.1). Aggressive removal of β_2-microglobulin is desirable to prevent the complications of prolonged dialysis, including amyloidosis. It was noteworthy that the administration of green tea catechins relieved 35 to 100% of the pain in the shoulder, knee, hips, cubitus, coxa and fingers of dialysis patients (Table 12. 2). These results suggest that green tea catechins are very effective in the suppression of oxidative stress and post-dialysis arthralgia in dialysis patients.

Inhibitory Effect on Proliferation of Mesangial Cells

Glomerulorenal disease and diabetic nephropathy are recognized as the main functional disorders underlying the development of renal disease. Histological studies

Table 12.1. Changes in creatinine (Cr), methylguanidine (MG), MG/Cr ratio and β_2-microglobulin in serum during the application of green tea catechins (Sunphenon®) in dialysis patients (from Yokozawa *et al.*, 1996b).

Duration of treatment (months)	Creatinine (Cr) (mg/dl)	Methylguanidine (MG) (μg/dl)	MG/Cr ($\times 10^{-3}$)	β_2-Microglobulin (mg/dl)
0	13.51	56.43	4.12	39.0
1	13.33	53.65*	3.99	35.0***
2	13.28	51.92**	3.86*	37.5
3	12.81***	48.66***	3.78**	36.4**
4	12.65***	49.12***	3.87*	36.1***
5	12.37***	45.06***	3.62**	35.7**
6	12.43***	48.41***	3.85*	35.4**

Significantly different from the pre-treatment value: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

have characterized these disorders with deteriorated glomerular filtration by excessive proliferation of mesangial cells. Nearly 70 to 80% of chronic renal disease patients have a background of either glomerulorenal disease or diabetic nephropathy disorders. These disorders, which are associated with deteriorated glomerular filtration, are characterized histologically by excessive proliferation of mesangial cells. Grond *et al.* (1985) suggested that mesangial cells affect the haemodynamics of glomerular capillaries via vascular contraction, and relaxation thereby regulates glomerular filtration. Apparently this suggests that the proliferation of mesangial cells may interfere with the function of glomerular filtration (Kashgarian and Sterzel, 1992).

Yokozawa *et al.* (1993b) have examined the effect of green tea catechins, using Sunphenon®, on the proliferation of mesangial cells. They determined the proliferation in terms of [^{3}H]thymidine uptake in cultured mouse mesangial cells. Mesangial cells were isolated from mouse renal glomeruli and cultured in a medium with D-valine MEM containing 20% fetal calf serum (FCS) for 10 days. For the measurement of [^{3}H]thymidine uptake, the cultured mesangial cells were seeded in a 96-well microtitre plate at a density of 10^4 cells/well in 0.2 ml D-valine MEM containing 20% FCS and incubated for 48 h with or without Sunphenon® (6.25 to 200 μg/ml). Twelve hours prior to the end of the incubation period, the cultures were pulsed with 1 μCi of [^{3}H]thymidine. At the end of the incubation period, the cells were harvested and the radioactivity was measured. Simultaneously the effect of individual catechins (EGCG and ECG) on mesangial cell proliferation was also examined by a similar method.

A concentration-dependent decrease in the uptake of [^{3}H]thymidine was observed with catechins in mesangial cells (Fig. 12.5). A dose of 25 μg/ml of catechins EGCG or ECG completely inhibited the uptake. The EGCG exerted inhibitory effects even at relatively low concentrations. Since EGCG is the strongest antioxidant of all the green tea catechins, the inhibitory effects could be attributed to this major component present in Sunphenon®. These results suggest that green tea catechins inhibit the proliferation of mesangial cells and could improve the function of glomerular filtration. This was noticed when an oral administration of ECG induced an increase in glomerular filtration in rats (Oura and Yokozawa, 1990).

Table 12.2. Effect of green tea catechins (Sunphenon®) on arthralgia in dialysis patients. Values in parentheses indicate the percentage of total patients (from Yokozawa *et al.*, 1996b).

	Number of patients with arthralgia			
	Disappearance	Relief	No change	Total
Shoulder	5 (31)	1 (6)	10 (63)	16
Knee	4 (31)	1 (8)	8 (62)	13
Finger	3 (23)	4 (31)	6 (46)	13
Coxa	5 (50)	1 (10)	4 (40)	10
Hip	5 (83)	1 (17)	0 (0)	6
Cubitus	1 (100)	0 (0)	0 (0)	1

Inhibition of Renal Hypertension

Besides relieving oxidative stress and improving glomerular filtration during renal disease, green tea catechins have also been

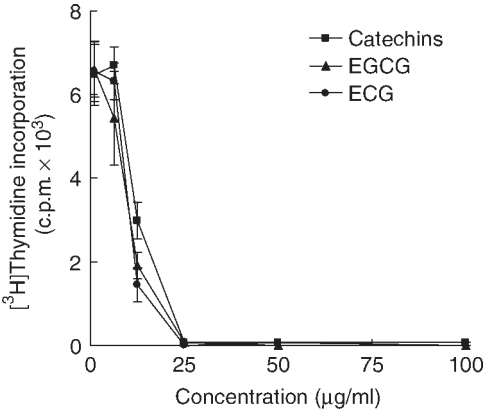

Fig. 12.5. Effect of green tea catechins (Sunphenon®) and their components on the proliferation of mesangial cells (from Yokozawa *et al.*, 1993b).

found to be effective in controlling renal hypertension by manipulating the blood pressure in kidneys. The kinin–kallikrein system seems to be involved in the mechanism of blood pressure regulation by its mutual action with other vasoactive systems, such as the renin–angiotensin– aldosterone, sympathetic nerve vasopression and prostaglandin systems, and by its direct action on the cardiovascular system and the mechanism for water and sodium (Abe *et al.*, 1978; Levinsky, 1979; Abe, 1981). Decreased production by the kinin–kallikrein system in patients with essential hypertension has been reported, suggesting its involvement in the aetiology of this condition (Margolius *et al.*, 1974; Zineer *et al.*, 1976). On the other hand, prostaglandin E_2 (PGE_2) is thought to be involved not only with maintaining the blood flow but also with sodium metabolism, and it has been suggested to act as a modulator of the haemodynamic changes associated with hypertension (Coleman *et al.*, 1975).

Yokozawa *et al.* (1994) examined the changes in blood pressure, kallikrein and PGE_2 with the administration of green tea catechins in adenine-induced renal failure rats. After the induction of renal failure, the rats were administered green tea catechins (as Sunphenon®) at a dose of 2 or 4 mg/kg bw for 24 consecutive days. The catechins were dissolved in water and given to the rats as drinking water. A 24 h urine sample was collected and analysed for kallikrein and PGE_2. Simultaneously, systolic, mean and diastolic blood pressures were determined by a tail-pulse pickup method. The administration of green tea catechins significantly reduced the systolic, mean and diastolic blood pressure (Fig. 12.6). On the other hand, green tea polyphenol administration significantly increased the urinary kallikrein level and the excretion of PGE_2 and sodium (Table 12.3). These data suggest that green tea catechins may ameliorate the development of hypertension by improving the renal circulating state. Stokes and Kokko (1977) have found in a study on isolated perfused tubules that PGE_2 caused direct tubular inhibition of sodium reabsorption. Ruilope *et al.* (1982) have demonstrated the protective role of renal PGE_2 in the maintenance of hypertension. Thus, it appears that the antihypertensive effect of green tea catechins results from their direct action in the kidney.

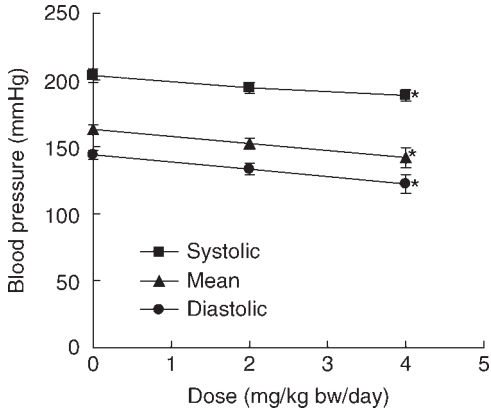

Fig. 12.6. Effect of green tea catechins (Sunphenon®) on systolic, mean and diastolic blood pressure in adenine-induced renal disease rats. Significantly different from the control value: $*P < 0.05$ (from Yokozawa *et al.*, 1994).

Table 12.3. Effect of green tea catechins (Sunphenon®) on the urinary excretion of PGE_2, kallikrein and sodium (from Yokozawa *et al.*, 1994).

	Dose of green tea catechins (mg/kg bw/day)		
	0	2	4
PGE_2 (ng/day)	8.00	14.87***	20.07***
Kallikrein (mU/day)	14.87	21.44**	31.81***
Na (mM/day)	1.50	1.72	1.96*

Significantly different from the control value: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Inhibition of Renal Tissue Lesions

To analyse the effect of green tea polyphenol on renal tissue lesions, Yokozawa *et al.* (1996a) examined the mesangial proliferation and glomerular sclerosis index in nephrectomized rats. An experimental

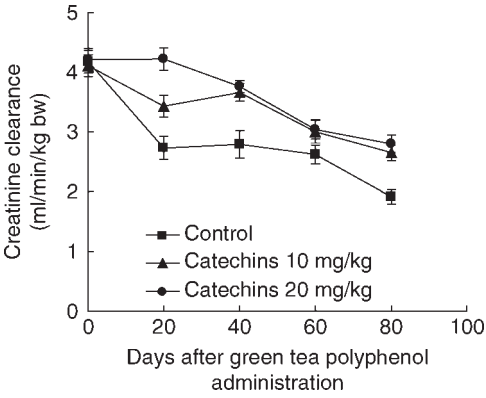

Fig. 12.7. Effect of green tea catechins (Sunphenon®) on creatinine clearance in nephrectomized rats (from Yokozawa *et al.*, 1996a).

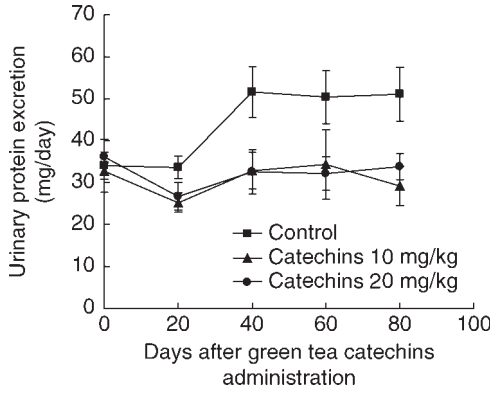

Fig. 12.8. Effect of green tea catechins (Sunphenon®) on urinary protein excretion in nephrectomized rats (from Yokozawa *et al.*, 1996a).

model to induce non-inflammatory renal disease was established by the excision of part of a kidney. It was earlier pointed out that, following subtotal nephrectomy, growth factors may simultaneously induce glomerular hypertrophy and mesangial proliferation, the former leading to a disorder in the glomerular basement membrane or epithelial cells, resulting in protein leakage, and the latter leading to glomerular sclerosis. In the study, the urinary creatinine clearance, protein excretion and oxidative activities were measured with the administration of 10 or 20 mg/kg body weight green tea polyphenol (as Sunphenon®) for 80 consecutive days.

Nephrectomized rats receiving oral green tea polyphenol exhibited milder lesions. The decrease in creatinine clearance was also significantly reversed after administration of green tea catechins (Fig. 12.7). These results suggested that green tea catechins inhibited mesangial cell proliferation to retain the function of the glomeruli, thereby inhibiting the progression of glomerular sclerosis. The study also showed that green tea catechins suppressed the leakage of urinary protein (Fig. 12.8), suggesting that the green tea catechins also delayed the progression of glomerular hypertrophy.

Harris *et al.* (1988) and Schrier *et al.* (1988) have suggested that free radicals are involved in various ways in the occurrence and progression of renal disease. The renal disease model produced by partial resection of the renal parenchyma, as used in this study, results in swelling of the remaining kidney tissue, which might cause increased oxygen consumption and enhanced ATP synthesis, where active free radicals could be involved. In such a scenario, measurements of antioxidative enzymes, such as SOD, catalase and GSH-Px activities, may suggest the level of free-radical-scavenging activity in the system. In the current study, the activities of SOD and catalase were significantly higher in rats given green tea polyphenol after nephrectomy (Table 12.4).

Table 12.4. Effect of green tea catechins (Sunphenon®) on the activities of reactive oxygen species-scavenging enzymes in rats after excision of three-quarters of their kidney volume (from Yokozawa *et al.*, 1996a).

	Dose of Sunphenon (mg/kg bw/day)		
	0	10	20
SOD (U/mg protein)	8.75	10.68**	11.66***
Catalase (U/mg protein)	142.7	213.2***	224.4***
GSH-Px (U/mg protein)	69.63	71.91	76.97*

Significantly different from the control value:
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Since these rats showed low activity of GSH-Px (an enzyme that is present in the mitochondrial matrix and eliminates H_2O_2, like the enzyme catalase), it was speculated that the site of action of green tea polyphenol is the peroxisome. These results suggest that green tea catechins maintain the enzyme activities that are related to free-radical scavenger action, thereby inhibiting renal tissue lesions.

Conclusions

Green tea, a simple refreshing beverage, has been believed to have therapeutic uses for many centuries. Recently enormous research findings have confirmed the therapeutic functions of green tea extract in preventing a wide range of diseases, in particular the diseases concerned with modern life (Weisburger and Chung, 2002) such as cancer (Yang and Wang, 1993; Katiyar and Mukthar, 1996), cardiovascular diseases (Ross, 1993) and allergy (Matsuo *et al.*, 2000). Nevertheless, green tea extract has been noted for a number of physiological functions, such as antibacterial (Juneja *et al.*, 2000), antiviral (Ebina, 1991), anticariogenic (Ahn *et al.*, 1991), antimutagenic (Nakagawa *et al.*, 2002), anti-atherogenic (Luo *et al.*, 1997), anticarcinogenic (Yang and Wang, 1993; Katiyar and Mukthar, 1996; Weisburger, 1997) and so on. Therefore, if listed, the functions of green tea in the prevention of various diseases would be endless. Scientists have recognized that this wide range of physiological functions in green tea is due to the low-molecular-weight catechins, which are especially abundant in green tea. They have also recognized that the efficacy of green tea catechins over others was related to their powerful antioxidant and free-radical scavenging activities.

Our studies particularly examined the effect of green tea catechins on renal disease. We examined the effect of refined green tea catechins (Sunphenon®, Taiyo Kagaku Co. Ltd, Japan) on the oxidative stress of renal disease and related complications in both animal and human models. The studies showed that green tea catechins can strongly inhibit free-radical-induced oxidative stress and can alleviate many complications related to renal disease. Green tea catechins were found to be effective in: (i) the inhibition of mesangial cell proliferation, improving glomerular function; (ii) the inhibition of the production of methylguanidine, a prominent uraemic toxin that causes uraemia, relieving oxidative stress in dialysis patients; (iii) the regulation of blood pressure, suppressing hypertension; and (iv) scavenging the free radicals, preventing renal tissue lesions and relieving pain from renal disease complications. These studies not only suggested the therapeutic use of green tea catechins in renal disease but also extended their physiological functions as 'antinephropathic' activity.

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13 Hepatoprotective Properties of Tea

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Introduction

The liver has the unique function of processing chemicals and drugs that enter the bloodstream. Many of these chemicals are difficult for the kidneys to excrete out of the body. The liver helps by removing these chemicals from the bloodstream and changing them into products that can be readily removed through the bile or urine. In this process, unstable toxic products are sometimes produced which can attack and injure the liver. Symptoms of chemical injury to the liver can resemble any form of acute or chronic liver disease. Acute liver injury can resemble viral hepatitis or blockage of the bile ducts. In other cases, a patient with fever, abdominal pain and jaundice may have a form of chemical injury that can be confused with conditions such as stones blocking the bile ducts that may require other surgery. Chemicals can also cause chronic liver disease and cirrhosis. Usually, chronic liver disease develops only after long-term use of the drug. Excessive exposure to certain drugs and chemicals may cause tumours of the liver. Treatment options for common liver diseases, such as cirrhosis, fatty liver and chronic hepatitis, are problematic. The effectiveness of treatments such as interferon, colchicine, penicillamine and corticosteroids is inconsistent at best and the incidence of side effects is profound. All too often, the treatment is worse than the disease. Conservative physicians often counsel watchful waiting for many of their patients, waiting in fact for the time when the disease has progressed to the point that warrants the use of heroic measures. Physicians and patients are in need of effective therapeutic agents with a low incidence of side effects.

In recent years, many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat diseases of the liver. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and modes of action of these plants, as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies. Traditional beverages are not necessarily major sources of nutrients, except for tea, which contains relatively large amounts of vitamin C. However, a number of studies have shown that traditional beverages, as represented by teas, are beneficial to human health (Trevisanato and Kim, 2000), suggesting that

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these beverages might warrant investigation from the viewpoint of nutrition. To date, a number of studies have shown that tea or its constituents possess a variety of effects, including antioxidation (Matsuzaki and Hara, 1985), antimutation (Kada *et al.*, 1985), anticarcinogenesis (Fujiki *et al.*, 1996), antibiotic action (Toda *et al.*, 1989), antihypercholesterolaemia (Muramatsu *et al.*, 1986), antihypertension (Hara and Tonooka, 1990), antihyperglycaemia (Shimizu *et al.*, 1988) and anti-inflammatory action (Sagesaka *et al.*, 1996). In addition, it has been shown in various studies that tea can demonstrate protective effects against liver injury.

Tea Consumption, Composition and Chemistry

According to the *Encyclopaedia Britannica*, China introduced tea to the world. The word tea comes from a Chinese ideogram pronounced 'tay' in the Amoy dialect and came into English with that pronunciation, changing to its present form in the 18th century. Tea is made from the young leaves and leaf buds of the tea plant, *Camellia sinensis* – a species of evergreen shrub of the *Theaceae* family. Ancient legends refer to a beverage made from an infusion of dried tea leaves, which was introduced by Emperor Shen Nung in about 2737 BC. From that royal beginning, tea drinking gradually spread around the world and now it is the most commonly used non-alcoholic beverage. The China tea plant – brought to Japan in about AD 800 – was regarded as a medicine for 500 years, until green tea was developed and became a popular Japanese beverage.

Consumption

The tea plant is native to South-East Asia and is grown in about 30 countries, but it is consumed worldwide, although at greatly varying levels. It is the most widely consumed beverage, aside from water, with a per capita worldwide consumption of approximately 0.12 l per year. Nowadays consumption of tea is just as important as the consumption of drinking water. On average, about 2.5 Mt of tea are produced worldwide. The UK is the largest per capita consumer of tea, averaging about 3.5 to 4.0 cups per day.

Composition

The composition of tea leaf varies with climate, season, horticultural practices, variety of the plant and age of the leaf, i.e. the position of the leaf on the harvested shoot. There are three main types of tea – green, black and oolong tea. These come from the same plant, but have been treated differently: green tea is heated soon after picking and is not subjected to further processing; black tea, on the other hand, is dried and then exposed to the air before it is heated. Consequently, green and black tea differ noticeably in appearance, taste and chemical composition. Green tea is more common in Asia, while black tea is the tea of choice for most people in Western countries.

In Table 13.1, the principal polyphenolic components present in typical green and black tea beverages are shown, but variation may be considerable. Oolong tea composition in general falls between that of green and black tea.

Table 13.1. Polyphenolic composition of green and black tea (% w/w) (from Graham, 1992).

Constituents	Green tea	Black tea
Catechins	30–42	3–10
Flavonols	5–10	6–8
Other flavonoids	2–4	—
Theagallin	2–3	—
Gallic acid	0.5	—
Quinic acid	2.0	—
Theanine	4–6	—
Methylxanthines	7–9	8–11
Theaflavins	—	3–6
Thearubigins	—	12–18

Chemistry

Steaming or drying fresh tea leaves at elevated temperatures makes commercial green tea. Its chemical composition is similar to that of fresh tea leaves. Green tea contains polyphenols, which include flavonols, flavandiols, flavonoids and phenolic acids; these compounds may account for up to 30% of the dry weight (Balentine, 1997). Most of the green tea polyphenols are flavonols, commonly known as catechins. Some major green tea catechins are (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), (–)-epicatechin (EC), (+)-gallocatechin and (+)-catechin (Graham, 1992). Caffeine, theobromine and theophylline, the principal alkaloids, account for about 4% of the dry weight. In addition, there are phenolic acids, such as gallic acids, and characteristic amino acids, such as theanine. A cup (200 ml) of green tea (Gun Powder, Hangzhou, China) contains about 142 mg EGCG, 65 mg EGC, 28 mg ECG, 17 mg EC and 76 mg caffeine.

In the manufacture of black tea, the monomeric flavon-3-ols undergo polyphenol oxidase-dependent oxidative polymerization, leading to the formation of bisflavonols, theaflavins, thearubigins and other oligomers in a process commonly known as 'oxidation'. Theaflavins (about 1–2% of the total dry matter of black tea), including theaflavin, theaflavin-3-*O*-gallate theaflavin-3′-*O*-gallate and theaflavin-3,3′-*O*-digallate, possess benzotropolone rings with dihydroxy or trihydroxy substitution systems, which give the characteristic colour and taste of black tea. About 10–20% of the dry weight of black tea is due to thearubigins, which are even more extensively oxidized and polymerized, have a wide range of molecular weights and are less well characterized. Oolong tea, a partially oxidized tea, contains monomeric catechins, theaflavins and thearubigins. Some characteristic components, such as epigallocatechin esters, theasinensins, dimeric catechins and dimeric proanthocyanidins, are also found in oolong tea. The flavonols are easily oxidized to the corresponding *O*-quinones. These flavonols and quinones can function as either hydrogen acceptors or hydrogen donors. In addition, tea polyphenols effectively interact with reactive oxygen species. In the flavonol structure, the 5- and 7-dihydroxy groups and 1-oxygen make the carbons at positions six and eight strongly nucleophilic. During enzyme oxidation or non-enzyme oxidation, including auto-oxidation or coupled oxidation, tea flavonols may undergo oxidative condensation via either C–O or C–C bond formation in oxidative polymerization reactions. Tea polyphenols also have high complexation affinity for metals, alkaloids and biological macromolecules, such as lipids, carbohydrates, proteins and nucleic acids.

Many describing certain tea constituents have used the term 'tannins'. In industrial and botanic literature, tannins are characterized as plant materials that give a blue colour with ferric salts and produce leather from hides. Thus, tannins are a group of chemicals usually with large molecular weights and diverse structures. Monomeric flavonols, the major components in green tea, are precursors of condensed tannins. It would be more appropriate to use the term 'tea polyphenols' or 'tea flavonols' because they are quite distinct from commercial tannins and tannic acid.

Pharmacokinetics

The pharmacokinetics of EGCG and the other catechins have been investigated in rats, mice and humans. Studies of [^{3}H]EGCG in both the rat and mouse have shown that, following a single intragastric (i.g.) dose, radioactivity is found throughout the body (Suganuma *et al.*, 1998). After 24 h, 10% of the initial dose (radioactivity) was in the blood, with about 1% found in the prostate, heart, lung, liver, kidney and other tissues. The major route of elimination was via the faeces. Following intravenous (i.v.) administration of decaffeinated green tea to rats, plasma levels of EGCG, EGC and EC were fitted to a twocompartment model with elimination half-lives of 165, 66 and 67 min, respectively. Following i.v.. administration of EGCG, levels were highest in the liver

(3.6 nmol/g), lung (2.7 nmol/g) and small intestine (2.4 nmol/g). Whereas greater than 50% of plasma EGCG was present as the glucuronide, EGCG was present mainly as the free form in the tissues (Lambert *et al.*, 2003).

Protection against Liver Tumorigenesis – Laboratoy Studies

Administration of 5% green tea leaf in diet given to rats from 10 days prior to treatment with the carcinogenic compound aflatoxin B1 (AFB1) until 3 days after treatment resulted in a significant inhibition of AFB1-induced γ-glutamyl transpeptidase-positive foci in rat liver (Chen *et al.*, 1987). In another study, it was also shown that 2.5% green tea leaf in the diet given to rats produced significant inhibition of diethylnitrosamine (DEN)-induced hepatocarcinogenesis (Li, 1991). Mao showed the inhibitory effect of epicatechin complex on DEN-induced liver pre-cancerous lesions, variant-cell foci and nodule formation in rats (Mao, 1993). Epicatechin complex treatment resulted in a marked decrease in the number of N-ras-overexpressing lesions. In another study, it was shown that the administration of decaffeinated black tea extract by oral gavage to male Swiss mice resulted in a decrease of tobacco-induced liver tumours (Nagabhushan *et al.*, 1991).

In another study (Qin *et al.*, 1997), in a Fischer rat model, the effect of green tea (given through diet) was examined on the initiation of AFB1-induced hepatocarcinogenesis, as assessed by hepatic AFB1–DNA binding *in vivo*, by AFB1 metabolism *in vitro* and by the appearance of AFB1-induced glutathione *S*-transferase placental form (GST-P)-positive hepatocytes detected by immunohistochemical methods. Green tea feeding did not affect the microsome-mediated formation of non-toxic hydroxylated metabolites of AFB1. Hepatic nuclear AFB1–DNA binding *in vivo* was significantly inhibited by green tea treatment. The authors concluded that green tea inhibits initiation of AFB1-induced hepatocarcinogenesis in the rat by modulation of AFB1 metabolism, thereby inhibiting AFB1–DNA binding and AFB1-induced GST-P-postive hepatocytes.

Inhibitory effects of individual tea catechins ((–)-epicatechin, (–)-epigallocatechin, (–)-epicatechin gallate, (–)-epigallocatechin gallate), black tea extract and oolong tea extract on hepatocarcinogenesis were investigated. Male F344 rats received a single dose of diethylnitrosamine (200 mg/kg, i.p.), and thereafter phenobarbital (0.05%) was administered in the drinking water for a period of 6 weeks. Tea catechins, black tea extract or oolong tea extract was given during the entire experimental period, during only the initiation period or during only the promotion period. All four tea catechins, black tea extract and oolong tea extract (0.05 or 0.1%) significantly decreased the number and area of pre-neoplastic GST-P positive foci in the liver. These results suggest that tea catechins, black tea extract and oolong tea extract have a chemopreventive action against hepatocarcinogenesis (Matsumoto *et al.*, 1996).

2-Nitropropane, used as an industrial solvent and found in cigarette smoke (Hoffmann and Rathkamp, 1972), is strongly hepatotoxic (Zitting *et al.*, 1981). It is also reported to be a strong hepatocarcinogen in male rats (Lewis *et al.*, 1981). Hasegawa *et al.* showed that green tea can effectively block oxidative DNA damage to the liver, as well as hepatotoxicity in rats treated with 2-nitropropane (Hasegawa *et al.*, 1995).

Epidemiological Studies

A population-based study was conducted in Taixing, Jiangsu province, comprising 206 cases and 415 population controls. Results showed that green tea might have protective effects against liver cancer (Mu *et al.*, 2003). In Shizuoka prefecture of Japan, a negative association between green tea consumption and liver cancer was observed (Oguni *et al.*, 1992), while no relationship was observed in three other studies (Stocks, 1970; Heilbrun *et al.*, 1986; La Vecchia *et al.*, 1992). Tea has

been investigated as a protective agent against cancer and cardiovascular disease. One study investigated the association between consumption of green tea and various serum markers in a Japanese population. Peripheral blood samples of men aged over 40 years were subjected to biochemical assays and a survey of habits and of daily consumption of green tea was conducted. Increased consumption of green tea was associated with decreased serum concentrations of total cholesterol and triglyceride, an increase in the proportion of high-density lipoprotein cholesterol and a decreased proportion of low- and very-low-density lipoprotein cholesterols, producing a decreased atherogenic index. A decreased concentration of hepatological markers in serum was also related to increased consumption of green tea. The authors conclude that green tea might act protectively against liver disorders and cardiovascular disease (Imai and Nakachi, 1995).

Protection against Ischaemia–Reperfusion Injury

Ischaemia–reperfusion injury to the liver occurs in trauma and haemorrhagic shock and after hepatic surgery, including tumour resection and transplantation. Reactive oxygen species produced on reperfusion play a critical role in the injury caused by ischaemia–reperfusion (Parks *et al.*, 1982; McCord, 1987; Farber *et al.*, 1990). Accumulation of purine derivatives due to ATP degradation and reduction of mitochondrial ubiquinone produces superoxide radicals on reoxygenation (Boveris and Chance, 1973; Parks *et al.*, 1982; McCord, 1987; Jaeschke and Mitchell, 1989). In addition, macrophages and neutrophils in previously ischaemic tissue are activated and produce oxygen radicals via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Bellavite, 1988). Indeed, superoxide production by Kupffer cells and neutrophil accumulation increase over fivefold after hepatic ischaemia–reperfusion (Jaeschke, 1992). Moreover, gadolinium chloride, a drug that selectively destroys and/or inactivates Kupffer cells, minimizes liver injury after ischaemia–reoxygenation (Bremer, 1994). Reactive radical species not only directly damage cell membranes, DNA and protein, they also trigger formation of toxic cytokines and increase adhesion molecules, leading to an inflammatory response, tissue damage and multiple organ failure (Farber *et al.*, 1990; Abello, 1994; Hensley *et al.*, 2000). Green tea extract (GTE) and one of its major polyphenolic components, epicatechin, significantly reduced liver injury after ischaemia–reperfusion. Protection by GTE and epicatechin was associated with decreased free-radical formation. In addition, GTE prevented nuclear factor-κ B (NF-κB) activation and pro-inflammatory cytokine formation. Consistent with these observations, a previous report showed that green tea polyphenols blunted endotoxin-induced NF-κB activation and tumour necrosis factor (TNF) production (Yang *et al.*, 1998). Based on the present data, we cannot rule out the possibility that GTE inhibits NF-κB activation by a non-antioxidant mechanism. However, a variety of structurally diverse antioxidants and antioxidative enzymes have been shown previously to inhibit NF-κB-mediated cytokine production stimulated by endotoxin, consistent with pro-oxidant stimulation of NF-κB activation and cytokine synthesis (Anderson *et al.*, 1994; Pinkus *et al.*, 1996; Yang *et al.*, 1998; Wolin, 2000; Han *et al.*, 2001, Lakshminarayanan *et al.*, 2001; Torrie *et al.*, 2001). Therefore, GTE probably also inhibits NF-κB activation by scavenging free radicals.

Suppression of Lipopolysaccharide (LPS)-induced Liver Injury

Tumour necrosis factor is synthesized and released by macrophages, including Kupffer cells, the resident macrophages in the liver, in response to stimulation by LPS and plays a critical role in LPS + D-galactosamine-induced acute liver injury or hepatitis (Bradham *et al.*, 1998). Yang *et al.* (1998) reported that tea catechins could inhibit

LPS-induced TNF-α production in both mouse peritoneal macrophages and mice *in vivo*. LPS is a component of the outer membrane of Gram-negative bacteria, which have been used frequently in combination with D-galactosamine (GalN) to induce liver injury in rodents. These results suggest that tea catechins may be effective in suppressing liver injury induced by LPS, because LPS is known to cause liver injury through enhanced TNF-α production (Tiegs *et al.*, 1989).

Protection against Liver Cirrhosis and Liver Fibrosis

Using the carbon tetrachloride liver cirrhosis rat model, the protective effect of GTE on liver cirrhosis was studied. Male SD rats were randomly divided into three groups: normal group, GTE group and cirrhosis group. The GTE group and the cirrhosis group were injected subcutanuously twice per week over 9 weeks with 40% CCl_4. In the second and ninth week, the rats were sacrificed to measure malondialdehyde (MDA) and hydroxyproline concentrations and transforming growth factor (TGF)-β_1 mRNA expression in liver tissue, as well as to conduct a histological examination of various organs. Compared with the cirrhosis group, the MDA and the hydroxyproline concentrations in the GTE group were significantly reduced ($P < 0.05$). Liver necrosis and cirrhosis were extenuated in the GTE group by means of a histological examination. The expression of the TGF-β_1 mRNA was reduced significantly in the GTE group. Dietary supplementation of GTE can protect against CCl_4-induced liver damage and cirrhosis in rats (Xiao *et al.*, 2004).

Accumulation of hydrophobic bile acids during cholestasis leads to generation of oxygen free radicals in the liver. Accordingly, this study investigated whether polyphenols from green tea, which are potent free-radical scavengers, decrease hepatic injury caused by experimental cholestasis. Rats were fed a standard chow or a diet containing 0.1% polyphenolic extracts from *C. sinensis* starting 3 days before bile-duct ligation. After bile-duct ligation, serum alanine transaminase increased to 760 U/l after 1 day in rats fed a control diet. Focal necrosis and bile-duct proliferation were also observed after 1–2 days, and fibrosis developed 2–3 weeks after bile-duct ligation. Additionally, procollagen-α1(I) mRNA increased 30-fold 3 weeks after bile-duct ligation, accompanied by increased expression of α smooth-muscle actin and TGF-β, and the accumulation of 4-hydroxynenonal, an end product of lipid peroxidation. Polyphenol feeding blocked or blunted all of these bile-duct ligation-dependent changes by 45–73% (Zhong *et al.*, 2003). Another study was designed to observe the effect of tea polyphenols on hepatic fibrosis in rats with alcoholic liver disease and to explore the related mechanisms. Hepatic fibrosis was less severe in the rats of the alcohol groups given tea polyphenols than in the group given only alcohol. Tea polyphenols increased the serum antioxidant capacity and decreased the endotoxin level (Li *et al.*, 2004).

Inhibition of Lipid Peroxidation

Tea provides protection against liver damage by lowering lipid peroxidation. Rats were permitted free access to solubilized extract of green tea. Bioactive ingredients of green tea extract caused an increase in the activity of glutathione peroxidase and glutathione reductase and in the content of reduced glutathione, as well as a marked decrease in lipid hydroperoxides (LOOH), 4-hydroxynonenal (4-HNE) and MDA. The concentration of vitamin A increased by about 40%. Minor changes in the measured parameters were observed in the blood serum. Glutathione (GSH) content increased slightly, whereas the index of the total antioxidant status increased significantly. In contrast, the lipid peroxidation products, particularly MDA, were significantly diminished. In the central nervous tissue, the activity of superoxide dismutase and glutathione peroxidase decreased, while the activity of glutathione reductase and catalase

increased after drinking green tea. Moreover, the level of LOOH, 4-HNE and MDA significantly decreased. The use of green tea extract appeared to be beneficial to rats in reducing lipid peroxidation products. These results support and substantiate traditional consumption of green tea as a protection against lipid peroxidation in the liver, blood serum and central nervous tissue (Skrzydlewska *et al.*, 2002).

The antioxidative activity of theaflavins (TFs) and thearubigin (TR) purified from the infusion of black tea leaves was examined using the tert-butyl hydroperoxide-induced lipid peroxidation of rat liver homogenates. The concentrations that produced 50% inhibition of lipid peroxidation (IC_{50}) by TF, theaflavin monogallate-A (TFM-A) and TR were 4.88×10^{-4}, 4.09×10^{-4} and 4.95×10^{-4}% (w/v), respectively. The antioxidative activity of these compounds was higher than that of glutathione, L(+)-ascorbic acid, DL-alpha-tocopherol, butylated hydroxytoluene, butyl hydroxyanisole, etc., but was lower than the activity of (–)-epicatechin gallate, (–)-epigallocatechin and (–)-epigallocatechin gallate. As for the IC_{50} in molarity, the antioxidative activity of TFM-A was the second highest among all the samples used in this study. The antioxidative activity of lyophilized tea infusions was compared. The activity of black tea was about as potent as that of green tea. These results suggest that the black tea infusion, containing TFs and TR, could inhibit lipid peroxidation in biological conditions in the same way as the green tea infusion, containing epicatechins (Yoshino *et al.*, 1994).

Mechanism of Action

Modulation of metabolizing/detoxification enzymes

Sugiyama *et al.* (2004) showed that theanine, a specific glutamate derivative in green tea, decreased doxorubicin (DOX)-induced adverse reactions, such as the induction of the lipid peroxide level and the reduction of glutathione peroxidase activity, in normal tissues. In order to clarify how theanine attenuates the adverse reactions of DOX, we have focused on the effects of theanine on glutamate and GSH levels in normal tissues. The administration of theanine to mice increased the glutamate concentration in the liver and heart, and not in tumours. *In vitro* examinations indicated that theanine was metabolized to glutamate mainly in the liver. Moreover, theanine inhibited GSH reduction induced by DOX in the liver and heart. Therefore, these results suggested that theanine attenuated the DOX-induced adverse reactions involved in oxidative damage due to the increase in glutamate and the recovery of GSH levels in normal tissues. The mechanism of action of tea as an effective chemopreventive agent for toxic chemicals and especially carcinogens. Uridine diphosphate-glucuronosyltransferase (UDP-GT) activities towards *p*-nitrophenol were markedly increased (51.8% or 1.5-fold) in rats that consumed tea compared with the control animals on water. Induction of UDP-GT activity by tea may involve the UDP-GT1 (UGT1A) gene complex of the UDP-GT multigene family. Therefore, a major mechanism of tea as a chemopreventive agent is induction of the microsomal detoxification enzyme, UDP-GT (Embola *et al.*, 2002).

Antioxidative effects

Increasing evidence indicates the role of oxidative stress in liver injury, cirrhosis development and carcinogenesis (Yamamoto *et al.*, 1998; Yamamoto and Yamashita, 1999; Stal and Olsson, 2000). Tea polyphenols act as antioxidants *in vitro* by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. They may also function indirectly as antioxidants through: (i) inhibition of the redox-sensitive transcription factors, NF-κ-B and activator protein-1; (ii) inhibition of 'pro-oxidant' enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclo-oxygenases and xanthine oxidase; and (iii) induction of Phase II and antioxidant enzymes, such as

glutathione *S*-transferases and superoxide dismutases. The fact that catechins are rapidly and extensively metabolized emphasizes the importance of demonstrating their antioxidant activity *in vivo*. Animal studies offer a unique opportunity to assess the contribution of the antioxidant properties of tea and tea polyphenols to the physiological effects of tea administration in different models of oxidative stress. Most promising are the consistent findings in animal models of skin, lung, colon, liver and pancreatic cancer that tea and tea polyphenol administration inhibits carcinogen-induced increases in the oxidized DNA base, 8-hydroxy-2′-deoxyguanosine.

Free-radical scavengers

Harmful free radicals, such as superoxide anion (a reactive oxygen species (ROS)), are produced during aerobic respiration in all tissues because of only partial reduction of some oxygen molecules in mitochondria: this is due to one-electron reduction of each atom of oxygen, instead of four-electron reduction per molecule of oxygen to form water. Similarly, in liver and many other tissues, such as lung and brain, an electron transfer chain from NADPH to water occurs (with insertion of one oxygen atom into xenobiotic substrates) that uses cytochrome P450 as the electron acceptor. Here, futile recycling of electrons, in the absence of substrate, produces the superoxide anion O_2^-. ROS and reactive sulphur species (RSS) may act in unison to damage biomolecules. (Wiseman, 2004). Liver ischaemia–reperfusion causes free-radical production and green tea polyphenols are effective free-radical scavengers. GTE and one of its major polyphenolic components, epicatechin, significantly reduced liver injury after ischaemia–reperfusion. Protection by GTE and epicatechin was associated with decreased free-radical formation (Zhong *et al.*, 2003).

Conclusions

The liver is the largest organ in the body and it has many vital tasks to perform. For example, it gets rid of or neutralizes toxins (such as poisons, germs and bacteria) in the blood and controls infection. The liver also produces proteins that regulate blood clotting, and bile, which helps absorb fats and fat-soluble vitamins. When the liver does not work normally, many complications can develop. Scarring of the liver, once it occurs, cannot be repaired. But, because cirrhosis progresses very slowly, early treatment can prevent further damage. In view of the available experimental data on the protection of liver damage by tea polyphenols, an intervention study on humans, including a dose–response effect, could be of great importance. Similarly, the data collected on the prevention of liver cancer in animal experimental models by tea polyphenols provide convincing evidence for a chemoprevention trial in high-risk human populations. Since only limited data are available on the bioavailability of tea polyphenols following tea consumption by the human population, studies on the absorption, distribution and metabolism of green tea and black tea polyphenols in animals and humans are of great importance. After careful evaluation of additional studies, it may be possible to recommend consumption of tea polyphenols by humans.

Because research findings in laboratory animals clearly point to a hepatoprotective role of tea and its constituents and chemoprevention of liver tumorigenesis, the well-defined and naturally occurring polyphenols found in tea should be evaluated in clinical interventions in human trials so that tea polyphenols can be utilized for preventing damage in the liver.

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14 Preventive Effects of Tea against Obesity

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Introduction

Obesity, like acquired immune deficiency syndrome (AIDS) and severe acute respiratory syndrome (SARS), is one of the most serious diseases in humans. The level of obesity is usually checked by using body mass index (BMI) (weight in kilograms divided by height in metres, squared). Generally, a BMI of 25 or more (>30 in the USA) is considered obese, and a high BMI brings a high risk of many diseases, such as heart disease, diabetes, cancer, hypertension, etc. The incidence of obesity is very high in advanced nations, including the USA, Europe and Japan: one out of every three to five adults is obese in these countries. As a result, the fiscal burden of medical expenses is increasing due to the diseases that are caused by obesity. Moreover, obesity is a serious problem not only in adults but also in children. Therefore, the prevention and medical treatment of obesity are tackled as a most important subject on a national level.

On the other hand, for many years, it has been known that tea has anti-obesity effects, and the effects were described in an old Chinese medical book, *Ben Cao Shi Yi*, published around AC 740. Recently, many interesting results concerning anti-obesity actions of various kinds of tea, such as green tea, oolong tea, black tea and Pu-Erh tea, and some of their components have been clarified. Moreover, the research into the anti-obesity action of green tea and oolong tea is advanced not only in animal experiments but also in clinical tests.

Thus, in this chapter, the details of the anti-obesity effects of several kinds of tea and their components, mainly the effects of green tea, are explained, divided into experimental and clinical research.

Experimental Studies of Anti-obesity Effects of Tea and its Components

Green tea and its components

Green tea

Green tea is one of the most popular beverages consumed worldwide. Epidemiological studies have suggested that green tea drinking reduces blood cholesterol in Japanese people (Kono *et al.*, 1996). Therefore, green tea is considered to have suppressive effects on lipid metabolism *in vivo*. Moreover, green tea catechins have a hypocholesterolaemic effect (Muramatsu *et al.*, 1986). On the other hand, Sano *et al.* (1986) reported

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that drinking water extracts of green tea had no effect on the body weight, weight of intraperitoneal adipose tissue (IPAT) and lipid metabolism in rats. However, it was reported that increase of body weight in mice was suppressed by the administration of food containing green tea powder (Sayama *et al.*, 1996). Thus, to clarify the anti-obesity effect of green tea powder, the effects of green tea on the weight of the body and several organs, food intake, lipid and leptin levels in mice were investigated.

Green tea powder (GTP) was mixed with a commercial powder diet for mice at concentrations of 1, 2 and 4% and administered to 4-week-old female ICR mice for 16 weeks. During the feeding period, individual mice were assessed monthly for body weight and food intake. After feeding, liver, kidneys, spleen, adrenals, brain, pituitary and IPAT from individual mice were excised and weighed. In addition, serum was collected, and the levels of lipids and leptin were analysed. Moreover, lipid levels in the liver were also determined.

Figure 14.1 shows the body weight increase during the feeding period of GTP. The body weight increase in mice was significantly suppressed by the 2 and 4% GTP diets from 1 or 2 months to the end of the feeding, but not by the 1% GTP diet. Finally, the body weight gain in the 2 and 4% GTP groups significantly decreased by 64% and 55%, respectively, compared with the control.

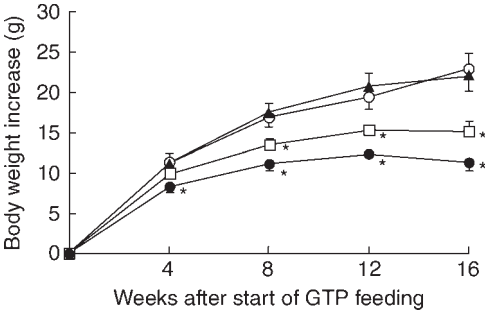

Fig. 14.1. Effects of green tea powder (GTP) on the body weight increase in mice. ○, Control; ▲, diet containing 1% GTP; □, diet containing 2% GTP; ●, diet containing 4% GTP. The means and standard errors (SE) for ten mice are plotted. *Significant difference at $P < 0.05$ compared with the control.

The weights of the body, kidneys, adrenals, liver, spleen, brain, pituitary and IPAT after the feeding are shown in Table 14.1. It was noted that the IPAT weights were remarkably decreased in the mice fed the diets containing 2 and 4% green tea powder: by 35% and 13%, respectively. Other organ weights in the GTP-fed mice were almost the same as those in the controls. These results clearly demonstrate that the suppression of body weight increase in GTP-fed mice resulted mainly from reduced fat accumulation.

On the other hand, significantly lower food intake than the control was seen in the group receiving the 4% GTP diet but not in the group receiving the 2% GTP diet. These results notably indicate that the mice fed the 2% GTP diet had a decrease in both the body and the IPAT weight, without reduction of food intake. Moreover, as shown in Table 14.2, fatty metabolism in the mice was analysed after 2 and 4% GTP feeding. The serum levels of triglyceride (TG) and non-esterified fatty acids (NEFA) were significantly lower than those of the control. Levels of TG and total cholesterol (TC) in the liver were also decreased by GTP feeding. GTP clearly suppressed the body weight increase and fat accumulation in mice when added to the diet at 2% and higher concentrations. These suppressive effects were suggested to be due to the inhibitory effect of GTP on lipid metabolism. The TG levels in serum and liver were remarkably lowered by GTP. GTP contains caffeine and green tea catechins. Caffeine reduced the concentration of plasma TG in rats (Naismith *et al.*, 1969), and green tea catechins inhibited TG accumulation in adipocytes by suppression of lipid synthesis (Watanabe *et al.*, 1998). Thus, the reduction of the TG level by green tea ingestion might be ascribed to caffeine and green tea catechins. On the other hand, caffeine raised the plasma levels of TC, phospholipids (PL) and NEFA in rats (Naismith *et al.*, 1969). In contrast, green tea catechins reduced plasma TC level (Fukuyo *et al.*, 1986) and this effect was partly

Table 14.1. Effects of the feeding of green tea powder (GTP) on the weights of organs and intraperitoneal adipose tissues (IPAT) in mice.

	Dietary groups			
	Control	1% GTP	2% GTP	4% GTP
Ovaries	24.1 ± 2.2[a]	28.3 ± 1.8	27.9 ± 2.5	20.7 ± 2.3
Adrenals	14.1 ± 0.4	15.6 ± 0.8	13.6 ± 0.9	11.0 ± 0.3[b]
Kidneys	516.1 ± 15.3	544.1 ± 22.3	556.0 ± 29.2	534.6 ± 50.4
Liver	1633.8 ± 58.9	1840.8 ± 117.7	2076.3 ± 213.2	1743.5 ± 222.4
Spleen	175.7 ± 27.7	165.6 ± 16.2	146.6 ± 14.9	164.3 ± 13.5
Brain	476.7 ± 34.4	473.6 ± 9.3	470.5 ± 7.7	446.0 ± 14.1
Pituitary	3.2 ± 0.2	3.3 ± 0.2	3.7 ± 0.2	2.9 ± 0.2
IPAT	3508.1 ± 600.1	2968.2 ± 588.3	1230.0 ± 178.4[b]	463.7 ± 52.8[b]

[a]Values are mean ± standard error (SE) for ten mice.
[b]A significant difference at $P < 0.05$ compared with the control.

Table 14.2. Effects of the feeding of green tea power (GTP) on the levels of lipids in serum and liver and serum leptin in mice.

	Dietary groups		
	Control	2% GTP	4% GTP
Serum lipids			
Triglyceride (g/l)	1.87 ± 0.15[a]	133 ± 0.15[b]	1.11 ± 0.12[b]
Phospholipids (g/l)	1.56 ± 0.12	1.70 ± 0.11	1.49 ± 0.12
Total cholesterol (g/l)	1.16 ± 0.12	1.26 ± 0.09	1.16 ± 0.08
Non-esterified fatty acids (mEq/l)	2.15 ± 0.21	1.51 ± 0.11[b]	1.78 ± 0.13
Liver lipids (μmol/g liver)			
Triglyceride	26.13 ± 1.48	23.01 ± 2.04	13.68 ± 1.35[b]
Phospholipids	34.76 ± 0.71	34.40 ± 1.29	34.23 ± 0.67
Total cholesterol	23.58 ± 0.72	21.95 ± 1.03	20.54 ± 1.00[b]
Serum leptin (ng/ml)	10.14 ± 1.95	3.11 ± 1.05[b]	3.13 ± 0.96[b]

[a]Values are mean ± standard error (SE) for ten mice.
[b]A significant difference at $P < 0.05$ compared with the control.

caused by suppression of cholesterol absorption from the intestine (Ikeda *et al.*, 1992). Moreover, green tea catechins exerted inhibitory activity on acetyl CoA carboxylase, the first key enzyme of fatty acid biosynthesis (Watanabe *et al.*, 1998), indicating that lipid synthesis in the liver may be suppressed by green tea catechins. Thus, it is likely that the reduction of NEFA levels in the serum and TC levels in the liver resulted from the inhibitory activity of green tea catechins and that the comparable levels of PL in serum and liver and of TC in serum between GTP and control groups might be a result of the balance between the enhancing effect of caffeine and the suppressive effect of catechins. Furthermore, levels of leptin, a protein produced by fat cells and identified as playing a role in regulating body fat, had a suppressive effect on food intake in the mice analysed. If a reduction of body fat is achieved through dietary limitation, leptin levels would decline, and consequently food intake would increase

following the reduction. Leptin levels in the mice fed GTP diets were significantly lower than in the control. However, the food intake of the mice did not increase, notwithstanding the reduced fat accumulation. Leptin is an anti-obesity protein *in vivo* and the body weights of mice were reduced by leptin treatment (Halaas *et al.*, 1995). Thus, it is plausible that the leptin synthesis stimulated *in vivo* by green tea might lead to the suppression of body weight increase and fatty accumulation. However, the leptin level was decreased by GTP feeding. Leptin is produced by adipose tissues and its level is closely correlated with the weight of adipose tissues (Maffei *et al.*, 1995). Therefore, the decreased leptin level due to GTP ingestion may be attributable to the decrease of adipose tissues by GTP. It is reported that hyperphagia and obesity in C57BL 6J *ob/ob* mice, which have an abnormal leptin gene, were improved by treatment with leptin (Halaas *et al.*, 1995). Therefore, reduction of the leptin level may induce hyperphagia and obesity. However, the food intake was not affected by the 2% GTP diet, and the 4% GTP diet rather exhibited an anorexic effect. Therefore, these results indicate that GTP suppressed the activation of eating behaviour following reduction of the leptin level. Racotta *et al.* (1994) reported that caffeine has an anorexic effect. Thus, the suppression of food intake by green tea might be brought about by caffeine contained in GTP, and the suppressed body weight increase and fatty accumulation by 4% GTP may be partly explained by the anorexic effect of caffeine.

Green tea components

The previous section demonstrated that green tea has an anti-obesity action. However, it has never been clearly determined which component of green tea is responsible for the anti-obesity effects of green tea. Among many green tea components, catechins are the main components of green tea and are linked to many physiological functions, such as anti-cancer, suppression of lipid absorption, etc. Caffeine is a main constituent in the anti-obesity action of oolong tea and promotes thermogenesis *in vivo*. Theanine is a main amino acid and a component peculiar to green tea. Thus, an investigation was carried out as to the effects of these three major components of green tea – caffeine, catechins and theanine – on the weights of the body and several organs, food intake and lipid levels in mice. From the results, at least caffeine and theanine appear to be responsible for the suppressive effects of GTP on body weight increase and fat accumulation. Moreover, it was demonstrated that catechins acted synergistically with caffeine in manifestation of anti-obesity activities.

Nine kinds of diets containing GTP and different combinations of GTP components were used in these experiments. GTP was mixed with a commercial powder diet for mice at a concentration of 2%. Catechins, caffeine and theanine were mixed with diets at concentrations of 0.3% catechins, 0.05% caffeine and 0.03% theanine, which correspond, respectively, to their concentration in a 2% green tea powder diet. Different diets included these components singly and in combination. Just as in the methods for testing the anti-obesity effects of GTP, each of the diets was administered to mice for 16 weeks. During the feeding period, body weight increase and food intake were measured. After feeding, the weights of several organs and IPAT were measured, and lipid levels in serum and liver were analysed.

Figure 14.2 shows the body weight increase during the feeding period of single

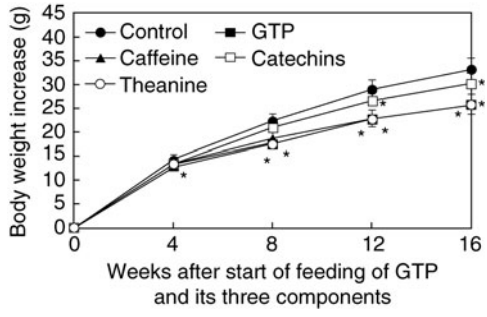

Fig. 14.2 Effects of green tea components on the body weight increase in mice. The means and standard errors (SE) for ten mice are plotted. *Significant difference at $P < 0.05$ compared with the control.

administration of GTP and GTP components. The body weight increase was significantly suppressed by the GTP, caffeine and theanine diets compared with the control, but not by the catechins diet.

Figure 14.3 shows the results of administering combinations of GTP components. Diets containing caffeine + catechins, caffeine + theanine and caffeine + catechins + theanine suppressed the weight increase, but the catechins + theanine diet did not. Particularly, among the diets with GTP components in combination, a caffeine + catechins diet was most effective to the body weight increase in mice. The results for the weights of the body, several organs and IPAT after the feeding are shown in Tables 14.3 and 14.4.

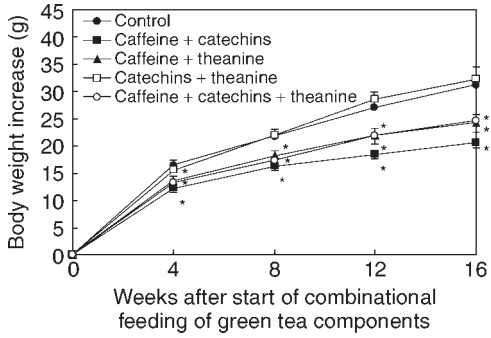

Fig. 14.3. Effects of combinations of green tea components on the body weight increase in mice. The means and standard errors (SE) for ten mice are plotted. *Significant difference at $P < 0.05$ compared with the control.

Table 14.3. Effects of green tea powder (GTP) and its components on weights of organs and intraperitoneal adipose tissues (IPAT) in mice.

	Control	GTP	Caffeine	Catechins	Theanine
Liver (g)	1.62 ± 0.06[a]	1.52 ± 0.07	1.71 ± 0.09	1.62 ± 0.06	1.51 ± 0.06
Kidneys (mg)	491.9 ± 13.9	491.6 ± 10.0	526.8 ± 15.7	499.8 ± 13.0	503.1 ± 15.2
Spleen (mg)	161.8 ± 8.4	159.0 ± 8.6	162.0 ± 8.8	132.0 ± 6.1[b]	142.0 ± 10.4
Brain (mg)	538.5 ± 8.1	516.8 ± 5.3	531.1 ± 6.3	524.0 ± 6.0	520.9 ± 14.9
Adrenals (mg)	11.89 ± 1.57	12.57 ± 0.49	14.81 ± 0.73[b]	12.75 ± 0.75	11.56 ± 0.79
Pituitary (mg)	3.04 ± 0.13	3.10 ± 0.10	2.98 ± 0.22	2.83 ± 0.24	2.71 ± 0.17
IPAT (g)	6.29 ± 0.83	3.16 ± 0.39[b]	3.47 ± 0.74[b]	6.01 ± 0.67	3.84 ± 0.53[b]

[a]Values are means ± standard errors (SE) for ten mice.
[b]A significant difference at $P < 0.05$ compared with the control.

Table 14.4. Combinational effects of green tea components on weight of organs and intraperitoneal adipose tissues (IPAT) in mice.

	Control	Caffeine + catechins	Caffeine + theanine	Catechins + theanine	Caffeine + catechins + theanine
Liver (g)	1.71 ± 0.05[a]	1.59 ± 0.08	1.75 ± 0.05	1.68 ± 0.05	1.72 ± 0.10
Kidneys (mg)	549.1 ± 16.5	479.2 ± 10.9[b]	550.4 ± 12.1	540.0 ± 23.4	535.4 ± 18.8
Spleen (mg)	184.5 ± 26.0	157.0 ± 13.7	176.5 ± 12.8	156.5 ± 10.0	163.5 ± 6.5
Brain (mg)	530.8 ± 4.2	530.5 ± 3.4	538.9 ± 7.0	536.6 ± 5.9	543.0 ± 7.1
Adrenals (mg)	13.79 ± 1.06	13.60 ± 0.42	12.71 ± 0.27	12.54 ± 0.54	12.42 ± 0.54
Pituitary (mg)	3.13 ± 0.14	3.09 ± 0.15	3.39 ± 0.15	3.18 ± 0.09	2.93 ± 0.11
IPAT (g)	5.12 ± 0.62	1.19 ± 0.26[b]	2.44 ± 0.49[b]	5.96 ± 0.74	2.75 ± 0.66[b]

[a]Values are means ± standard errors (SE) for ten mice.
[b]A significant difference at $P < 0.05$ compared with the control.

The IPAT weights in the mice were remarkably decreased by all of the diets except for the catechins diet and the catechins + theanine diet. Like the results of the GTP diet, the results for body and IPAT weights seen after the feeding of GTP components suggest an adequate correlation. These results clarified that the suppression of body weight increase in GTP-fed mice mainly results from caffeine and theanine. Moreover, it was shown that catechins promoted the suppression of body weight increase and fat accumulation by caffeine. However, none of the diets with GTP components affected the food intake in mice.

As shown in Tables 14.5 and 14.6, lipid metabolism was also analysed in the mice fed GTP components. Table 14.5 shows the results from a single administration of GTP and GTP components. Caffeine had no effects on lipid levels in serum and liver, but a tendency of total cholesterol to increase in serum was seen in the caffeine-fed mice. On the other hand, serum levels of TG and NEFA were significantly lower in the mice fed GTP, catechins and theanine diets.

Table 14.5. Effects of green tea powder (GTP) and its components on lipid levels in serum and liver in mice.

	Control	GTP	Caffeine	Catechins	Theanine
Serum lipids					
TC (g/l)	1.23 ± 0.11[a]	1.21 ± 0.11	1.41 ± 0.11	1.14 ± 0.09	1.09 ± 0.08
TG (g/l)	1.70 ± 0.17	1.21 ± 0.11[b]	1.58 ± 0.11	1.25 ± 0.05[b]	1.18 ± 0.11[b]
PL (g/l)	1.54 ± 0.04	1.67 ± 0.08	1.67 ± 0.12	1.45 ± 0.09	1.53 ± 0.10
NEFA (mEq/l)	1.86 ± 0.08	1.41 ± 0.09[b]	1.82 ± 0.10	1.31 ± 0.10[b]	1.36 ± 0.10[b]
Liver lipids (μmol/g liver weight)					
TC	24.61 ± 0.98	23.00 ± 0.94	25.06 ± 0.66	23.23 ± 0.40	24.64 ± 0.76
TG	30.66 ± 0.96	28.59 ± 1.48	30.25 ± 1.25	26.76 ± 1.42[b]	29.00 ± 1.51
PL	26.91 ± 1.27	25.89 ± 0.78	26.39 ± 1.21	26.38 ± 1.14	25.80 ± 0.83

TC, total cholesterols; TG, triglycerides; PL, phospholipids; NEFA, non-esterified fatty acids.
[a]Values are means ± standard errors (SE) for ten mice.
[b]A significant difference at $P < 0.05$ compared with the control.

Table 14.6. Combinational effects of green tea components on lipid levels in serum and liver in mice.

	Control	Caffeine + catechins	Caffeine + theanine	Catechins + theanine	Caffeine + catechins + theanine
Serum lipids					
TC (g/l)	0.91 ± 0.07[a]	0.85 ± 0.06	0.96 ± 0.08	0.91 ± 0.05	0.87 ± 0.04
TG (g/l)	1.16 ± 0.11	0.99 ± 0.07	1.10 ± 0.09	1.10 ± 0.11	0.95 ± 0.10
PL (g/l)	1.44 ± 0.09	1.54 ± 0.08	1.55 ± 0.10	1.59 ± 0.06	1.45 ± 0.07
NEFA (mEq/l)	2.00 ± 0.12	1.58 ± 0.08[b]	1.58 ± 0.05[b]	1.70 ± 0.08	1.33 ± 0.07[b]
Liver lipids (μmol/g liver weight)					
TC	35.66 ± 1.64	32.93 ± 1.54	32.28 ± 1.60	32.37 ± 1.60	31.81 ± 1.87
TG	16.40 ± 1.02	14.47 ± 0.62	14.86 ± 0.64	13.22 ± 0.65[b]	14.00 ± 0.75
PL	39.83 ± 1.32	38.67 ± 0.77	38.90 ± 1.42	39.39 ± 1.17	37.53 ± 0.78

TC, total cholesterols; TG, triglycerides; PL, phospholipids; NEFA, non-esterified fatty acids.
[a]Values are means ± standard errors (SE) for ten mice.
[b]A significant difference at $P < 0.05$ compared with the control.

Levels of TG in the liver were significantly decreased by the feeding of catechins. Therefore, it was considered that reduction of lipid levels by GTP might be caused by the catechins and theanine in GTP. Moreover, for the administration of combinations of GTP components, NEFA in serum was reduced by all of the diets, and TG in the liver was reduced by the catechins and theanine diet, and a tendency of TG to decrease in serum was shown in mice fed the caffeine + catechins diet and the caffeine + catechins + theanine diet (Table 14.6). It has been reported that caffeine ingestion elevated the metabolic rate and fat oxidation *in vivo* through lipolysis in fat cells and the release of catecholamines (Robertson *et al.*, 1978; Jung *et al.*, 1981; Arciero *et al.*, 1995). Moreover, caffeine enhanced noradrenaline- or adrenaline-induced lipolysis in fat cells (Dulloo *et al.*, 1992; Han *et al.*, 1999). We also obtained results that supported the anti-obesity activities of caffeine. Thus, it was considered that the anti-obesity effect of caffeine in GTP was due to enhancement of thermogenesis and fat metabolism. The diet containing 0.3% catechins did not influence fat accumulation and body weight increase in mice. However, it was reported that catechins significantly inhibited TG accumulation and synthesis in 3T3-L1 cells (Watanabe *et al.*, 1998). Murase *et al.* (2002) clarified that a diet containing tea catechins at 0.2% showed anti-obesity action in mice at 27 weeks of feeding although the body weight was significantly lower in the mice fed a 0.5% tea catechins diet at 12 weeks of feeding. Moreover, epigallocatechin gallate (EGCG), one of the tea catechins, significantly reduced or prevented body weight gain, with reduction of food intake in lean and obese rats when it was intraperitoneally injected at a daily dose of 81 to 92 mg/kg for 7 days (Kao *et al.*, 2000). These reports indicated that catechins have an anti-obesity potential. We also showed that the levels of TG and NEFA in the serum and TG level in the liver were decreased by catechins and previously reported that GTP had an anorexic effect and was more effective in reduction of the body weight increase and fat accumulation when added to the diet at 4% than at 2% concentration (Sayama *et al.*, 2000). Thus, catechins might exhibit an anti-obesity action at a higher dose. Although catechins did not show anti-obesity effects, a combination of caffeine and catechins induced stronger suppression of the body weight increase and fat accumulation than caffeine alone. The suppressive effect of caffeine and catechins in combination was strongest in all experimental groups and almost equal to the effect achieved by GTP addition. Dulloo *et al.* (1999) reported that thermogenesis and fat oxidation were synergistically enhanced by catechins, and that both green tea components might be used in the management of obesity. Therefore, it might be possible to prevent obesity by continuous and long-term administration of caffeine and catechins. On the other hand, theanine also had anti-obesity action. It was reported that theanine could pass through the blood– brain barrier and induced an increase in dopamine release (Kobayashi *et al.*, 1998) and reduction of serotonin concentration in the brain (Matsumoto *et al.*, 1993; Yokogoshi *et al.*, 1995, 1998). Therefore, the anti-obesity effect of theanine might be caused by changes of neurotransmitters in the brain. However, theanine did not augment the anti-obesity action of caffeine and/or catechins. Thus, it seems that the mechanism of anti-obesity effects of theanine are very complicated.

Addition of GTP to the diet decreased serum TG and NEFA levels in mice. TG and NEFA levels in serum were also significantly reduced by administration of catechins or theanine. It was reported that EGCG decreased the serum TG level in rats (Kao *et al.*, 2000). Thus, the reduction of serum lipid levels by green tea might be caused by the catechins and theanine contained in green tea.

Green tea catechins

In the previous section, it was shown that anti-obesity action did not occur by administration of diet containing 0.3% green tea catechins. However, Murase *et al.*

reported that the anti-obesity action of catechins was exerted by higher-concentration and longer-term administration (Murase *et al.*, 2002). Briefly, the weights of the body and visceral adipose tissues in mice fed a high-calorie diet containing 30% lipids and 13% sucrose for 11 months were significantly increased compared with those in the control mice, which were maintained on the standard diet containing 5% lipids (Figs 14.4 and 14.5).

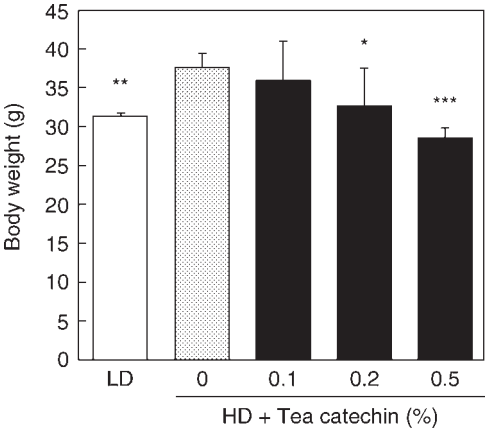

Fig. 14.4. Effects of green tea catechins on body weight in mice. Values are means ± standard deviations (SD), $n = 5$. LD, standard diet containing 5% lipids; HD, high-fat, high-sucrose diet containing 30% lipids and 13% sucrose. Significant difference compared with HD (tea catechin: 0 mg) group ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

However, in mice fed a high-calorie diet with catechins at 0.2 and 0.5%, the body weight increase and fat accumulation were significantly suppressed compared with those in the mice fed a high-calorie diet without catechins. Previous research showed that catechins affect the reduction of energy intake (Kao *et al.*, 2000) and suppression of absorption of lipids and carbohydrates (Muramatsu *et al.*, 1986; Matsumoto *et al.*, 1993) in mice or rats. Therefore, these effects of catechins might give rise to the anti-obesity action. Usually, the weights of the body and fat are reduced when energy expenditure exceeds energy intake. Suppression of fat accumulation in mice after administration of catechins may be due to increased energy expenditure, not decreased energy intake, as: (i) the suppression was observed with the same energy intake (Kao *et al.*, 2000; Murase *et al.*, 2002); and (ii) it has been confirmed that the inhibitory action of lipid and carbohydrate absorption under the same concentration of catechins was very weak (Meguro *et al.*, 2001). Moreover, an investigation was carried out as to the effect on lipid

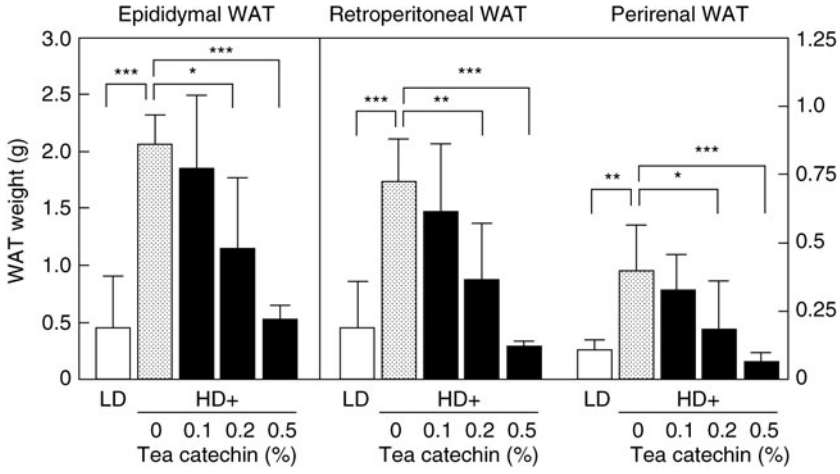

Fig. 14.5. Effects of green tea catechins on visceral fat weight (epididymal, retroperitoneal, perirenal WAT) in mice. Values are means ± standard deviations (SD), $n = 5$. LD, standard diet containing 5% lipids; HD, high-fat, high-sucrose diet containing 30% lipids and 13% sucrose; WAT, white adipose tissue. Significant difference compared

metabolism after treatment with tea catechin for 1 month, at which time suppression of body weight gain was not shown, by measuring the activity of β-oxidation in the major tissues. It was found that the activity was increased in the liver (Fig. 14.6).

Expression of the mRNA of a peroxisome β-oxidation-related enzyme, acyl-coenzyme A (CoA) oxidase (ACO), and a mitochondrial β-oxidation-related enzyme, medium-chain acyl-CoA dehydrogenase (MCAD), was also increased in the liver (Fig. 14.7). It was reported that tea catechin induced an increase in energy expenditure (Osaki *et al.*, 2001), and tea catechin induced acceleration of oxidative decomposition of fatty acids by using stable isotope-labelled lipids (Onizawa *et al.*, 2001). These reports strongly suggest that suppression of fatty accumulation in mice fed catechins may be due to the acceleration of energy expenditure through a tea catechin-induced increase in β-oxidation activity in the liver.

Other teas

Oolong tea

As well as green tea, oolong tea is also traditionally known to have anti-obesity and hypolipidaemic effects. Recently, Han *et al.* showed that oolong tea has an anti-obesity action in mice fed a high-fat diet (Han *et al.*, 1999). A high-fat diet with oolong tea powder was orally administered to mice for 10 weeks. As a result, food consumption was not significantly different between the high-fat diet group and high-fat diet + oolong tea group. However, increase of the body weight and lipid levels in livers in mice fed a high-fat diet was suppressed by oolong tea. Moreover, activities of noradrenaline-induced lipolysis of water extract from oolong tea and some fractions isolated from the extract were analysed by using isolated fat cells and a cell-free system, consisting of lipid droplets and hormone-sensitive lipase (HSL). A water extract of oolong tea enhanced noradrenaline-induced lipolysis, and the active substance was identified as caffeine. Caffeine enhanced noradrenaline-induced lipolysis in fat cells without a concomitant increase in HSL activity. Moreover, the hormone-induced lipolysis in a cell-free system consisting of lipid droplets and HSL was also accelerated, but not in the cell-free system with sonicated lipid droplets and HSL. Pancreatic lipase activity was inhibited by oolong tea extract. The same results in which oolong tea and its caffeine have an anti-obesity action are also proved in clinical tests. On the other hand, it was

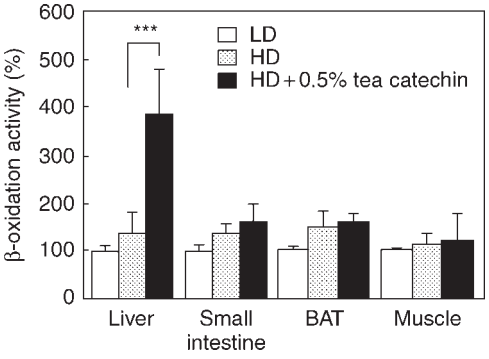

Fig. 14.6. Effects of green tea catechins on β-oxidation activity in the liver, small intestine, BAT and skeletal muscle at 1 month. Values are means ± standard deviations (SD), $n = 5$. LD, standard diet containing 5% lipids; HD, high-fat, high-sucrose diet containing 30% lipids and 13% sucrose; BT, brown adipose tissue. Significant difference compared with HD (tea catechin: 0 mg) group ($^{***}P < 0.001$).

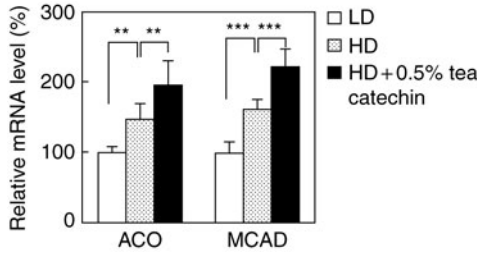

Fig. 14.7. Effects of green tea catechins on ACO, MCAD and FAS mRNA expression in the liver at 1 month (*1). Values are means ± standard deviations (SD), $n = 5$. LD, standard diet containing 5% lipids; HD, high-fat, high-sucrose diet containing 30% lipids and 13% sucrose. ACO, acyl-CoA oxidase; MCAD, medium-chain acyl-CoA dehydrogenase, FAS, fatty acid synthase. Significant difference compared with HD (tea catechin: 0 mg) group ($^{**}P < 0.01$, $^{***}P < 0.001$).

reported that not only body weight gain and the levels of plasma TG and cholesterol but also food intake in rats was suppressed by oral administration of drinking water with 1% ethanol-soluble extract of oolong tea. Caffeine has not only an anti-obesity action but also an anorexic effect (Racotta *et al.*, 1994). Therefore, it was suggested that the anti-obesity action of oolong tea was mainly caused by caffeine. Moreover, oolong tea suppressed an increase in the plasma TG level by restraint stress and the suppression did not depend on caffeine (Rumpler *et al.*, 2001). Therefore, constituents other than caffeine in oolong tea may also be related to the anti-obesity action.

Pu-Erh tea

It was reported that Pu-Erh tea, a Chinese tea produced mainly in the Yunnan district in China, also has an anti-obesity action (Sano *et al.*, 1986). Two kinds of Pu-Erh tea preserved for 2 and for 20 years were used in this research. Eight-week-old female rats were given boiled water extracts of Pu-Erh teas and administered a 1% cholesterol diet for 8 and 16 weeks *ad libitum*. After the treatments, the body, several organs and adipose tissues were weighed. As a result, the weights of adipose tissues in rats treated with Pu-Erh tea extracts were significantly lower than those in controls, but not the body weights. Plasma cholesterol and TG levels in the rats were also suppressed by Pu-Erh tea treatments. Moreover, the activity of adrenalin-induced lipolysis in adipose tissue was low and lipoprotein lipase activity was higher in the rats. Pu-Erh tea contains very little catechin as a result of long-term fermentation. Therefore, it was considered that suppression of fat accumulation by Pu-Erh tea was caused by caffeine or other peculiar components of Pu-Erh tea.

Black tea

It was reported that black tea has suppressive effects on plasma lipid levels in rats (Matsumoto *et al.*, 1998). A diet with 15% lard and 1% cholesterol was supplemented with 1% black tea polyphenols extracted and condensed from black tea. When this 1% black tea diet was administered to rats, the lipid levels in the plasma were reduced and the faecal excretion of total lipids and cholesterol were increased compared with those in rats fed the lard–cholesterol diet. Moreover, Yang *et al.* (2001) showed that black tea suppressed not only the lipid levels but also body weight gain in hyperlipidaemic rats fed a high-sucrose diet. The rats were fed a sucrose-rich diet and a drink with black tea extracts (1% w/v). As a result, black tea extracts significantly decreased body weight gains and food efficiency in the rats. Moreover, the hypertriglyceridaemia and hypercholesterolaemia induced by the sucrose-rich diet were normalized by black tea.

Studies of Anti-obesity Effects of Tea and its Components in Humans

Green tea

Obesity and green tea

Obesity, an excess amount of body fat, is a major risk factor for various lifestyle-related diseases, such as hyperlipidaemia, diabetes, hypertension and arteriosclerosis (Rimm *et al.*, 1995; Matsuzawa *et al.*, 1995; Zimmet *et al.*, 2001). Many epidemiological studies have clearly demonstrated that the incidence of arteriosclerotic diseases and mortality is increased in obese people compared with those with normal body weight (Lee *et al.*, 1993; Manson *et al.*, 1995; Rimm *et al.*, 1995; Seidell and Verschuren, 1996; Tsugane *et al.*, 2002). Thus, obesity is a major worldwide health problem (World Health Organization, 1998; Flegal *et al.*, 2002).

Green tea contains a variety of physiologically active substances, such as caffeine, saponins, vitamins and theanine, and their possible effects have been reported. Tea catechin is also a physiologically active component of green tea, for which antibacterial, antiviral, anti-cancer, anti-hypertension, antidiabetic, anti-allergic and anti-inflammatory effects and an ability to improve lipid metabolism have been reported (Hara, 2001). Among the green tea components, only tea

catechins have been verified to have anti-obesity effects in humans.

Body fat-decreasing effect

Hase *et al.* (2001), in a study involving human subjects, demonstrated a body fat-decreasing effect of tea catechins. Twenty-three adult males (mean age, 38.7 years; mean BMI, 24.6) were divided into two groups, and one group ingested one bottle per day of a beverage containing 483.0 mg of tea catechins (catechin group), while the other group ingested a beverage containing 180.0 mg of tea catechins (control group), for 12 weeks. The amount of body fat (evaluated based on the abdominal fat area in computerized tomography (CT) images) was significantly decreased in the catechin group, whereas no decrease was observed in the control group. In subjects with a BMI ≥25.0, the body fat in the catechin group showed a significantly greater decrease compared with those in the control group, but this was not significant in subjects with a BMI <25.0.

Otsuka *et al.* (2003) performed almost the same study using 40 adult females (mean age, 35.8 years; mean BMI, 22.4). In this study, body fat was significantly decreased in the catechin group (a beverage containing 562.0 mg of tea catechins, one bottle per day) compared with that in the control group (a beverage containing 33.0 mg of tea catechins, one bottle per day), and the decreases were higher in subjects with a high initial amount of body fat and subjects with a high BMI before ingestion of the test beverage. In females with a BMI ≥22.0, tea catechins significantly decreased body fat compared with the initial value and the control group, whereas the decreases were not significant in females with a BMI <22.0 compared with the initial value or the control group. Based on these findings, it can be concluded that tea catechins decrease body fat only in subjects with a high BMI, suggesting that the risk of unnecessary reduction of body fat is low in humans with a low BMI.

Nagao *et al.* (2001) have also investigated the relationship between the amount of tea catechins ingested and the body fat-decreasing effect. Twenty-seven adult males (mean age, 36.0 years; mean BMI, 25.1) were divided into three groups, who drank beverages containing 101.5, 555.4 and 901.9 mg of tea catechins, respectively, one bottle per day. A comparison with the tea catechins 101.5 mg group indicated that body fat was significantly decreased in the other two groups, but no significant difference was observed between the 555.4 mg and 901.9 mg tea catechins groups.

The above three studies were randomized, double-blind, placebo-controlled studies, the most reliable study method among intervention studies investigating the efficacy of materials.

Based on the results described above, Tsuchida *et al.* (2002) performed a randomized, double-blind, placebo-controlled study in 43 adult males with a BMI of 24–30 (mean age, 42.1 years old; mean BMI, 26.5) and 37 post-menopausal females (mean age, 54.8 years; mean BMI, 25.9) in an extensive investigation. After a 2-week observation period, the subjects ingested a control beverage (126.5 mg of tea catechins/340 ml) or a catechin beverage (587.5 mg tea catechins/340 ml), one bottle per day for 12 weeks, and the parameters of obesity or the amount of body fat, BMI, body fat weight, as measured by a biological impedance method, and abdominal fat area in CT images were measured. Blood chemistry, a blood test, urinalysis and an enquiry into their medical history were also performed. BMI was lower by 0.49 (Fig. 14.8A) and the amount of body fat was lower by 1.37 kg (Fig. 14.8B) in the catechin group compared with those in the control group, and the differences were significant. The difference in BMI corresponded to 1.25 kg of body weight in the subjects in this study.

The abdominal fat area showed a similar tendency, and the differences in visceral fat areas, subcutaneous fat area and the total of visceral and subcutaneous fat areas between the control and catechin groups were 9.0, 15.5 and 24.5 cm^2, respectively, showing significant decreases for the catechin group (Fig. 14.9). Since the caffeine contents in the control and catechin beverages were similar (control group, 81.3 mg;

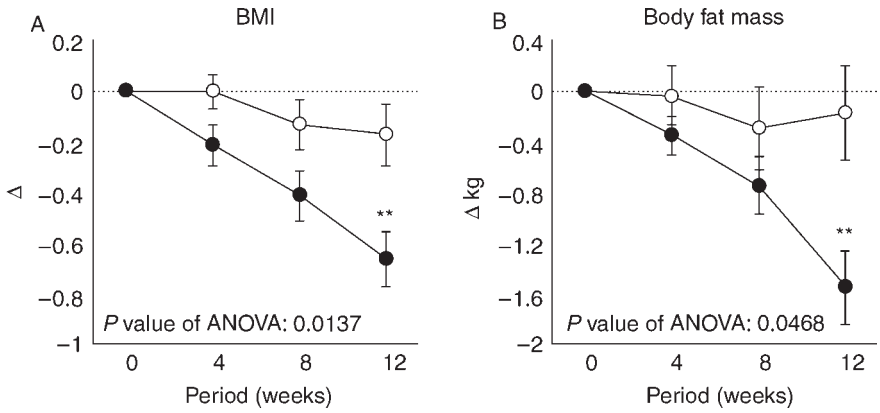

Fig. 14.8. Effects of tea catechins on BMI and body fat mass (15). A, BMI (body mass Index), B, body fat mass. Values (mean ± standard error of mean (SEM)) are expressed as changes (○, control group ($n = 41$); ●, tea catechin group ($n = 39$)). Significant difference compared with the control group by *t*-test (unpaired, $^{**}P < 0.01$). *P* values represented the results of comparison between the control and the tea catechins group by two-factor factorial analysis of variance (ANOVA).

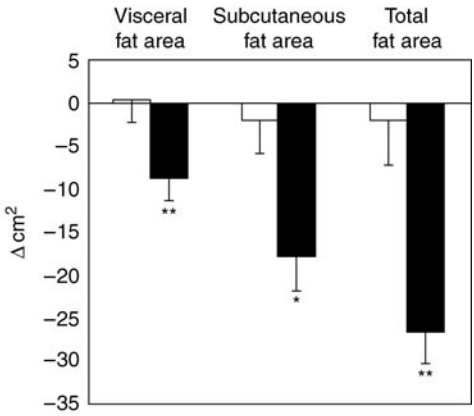

Fig. 14.9. Effects of tea catechins on abdominal fat area in CT images (15). Values (mean ± standard error of mean (SEM)) are expressed as changes (white bars, control group ($n = 41$); black bars, tea catechin group ($n = 39$)). Significant difference compared with the control group by *t*-test ($^{*}P < 0.05$, $^{**}P < 0.01$).

catechin group, 83.0 mg), the above findings can be attributed to the difference in the amount of tea catechins ingested. The energy and lipid intakes were essentially the same in the two groups throughout the study period.

In this study, changes after completion of ingestion of the beverages were also observed for 12 weeks. Exceeding the initial values of BMI and the amount of body fat before ingestion of the test beverage, the so-called rebound phenomenon, was not observed, and the decrease in abdominal fat area by tea catechins was maintained relatively well after the completion of ingestion. There were no problematic findings in blood chemistry, blood tests, urinalysis or the results of an enquiry into medical history throughout the study period.

Findings reported by Chantre and Lairon (2002) also suggested a body fat-decreasing effect of tea catechins, although the study was monadic. Seven males and 63 females, a total of 70 subjects (mean age, 44.7 years; mean BMI, 28.9), ingested 375 mg/day of tea catechins powder packed in four capsules, divided over two times per day, for 12 weeks. Although no statistical discussion was reported, body weight and waist circumference were decreased with time.

Furthermore, Hase *et al.* (2003) recently performed a study of 140 adult males (mean age, 38.4 years; mean BMI, 26.7) and 100 adult females (mean age, 46.3 years; mean BMI, 26.8), a total of 240 subjects. Unlike the study reported by Tsuchida *et al.* (2002), the female subjects were not limited to those who were post-menopausal, and were selected from a wide age group.

The 240 subjects were divided into two groups, the control (96.3 mg tea catechins/340 ml) and catechin (582.8 mg tea catechins/340 ml) groups, and the subjects ingested the test beverage for 12 weeks. The differences between the two groups after 12 weeks were 0.59 in BMI (1.59 kg in body weight) and 2.48 cm in waist circumference and 6.4, 9.7 and 16.1 cm^2 in visceral fat area, subcutaneous fat area and total of visceral and subcutaneous fat areas, respectively, in the abdominal fat area in CT images (Table 14.7). All parameters were significantly decreased in the catechin group. These effects were similar in the male and female groups in a subclass analysis. The caffeine contents in the catechin and control beverages were the same (control group, 75.0 mg; catechin group, 72.3 mg), and there were no differences in energy and lipid intake throughout the study period.

Combining the studies performed so far, human clinical studies were performed in a total of 480 subjects in the above six studies, and indicate that the ingestion of about 100 mg tea catechins per day does not decrease body fat, while the ingestion of about 400 mg/day slightly decreases body fat, and the ingestion of more than 500–600 mg/day significantly decreases body fat.

Acceleration of energy expenditure

As described above, a reduction in body fat by the ingestion of 500–600 mg/day of tea catechins was observed in the absence of any difference in energy intake between the groups during the study period in all studies. What is the action mechanism that leads to decreases in body fat? Body fat decreases when energy expenditure exceeds energy intake, and conditions that cause such an energy balance may be a decreased energy intake and/or an increased energy expenditure.

Some previous studies suggest that the ingestion of tea catechins decreases energy intake. Regarding the possible inhibition of lipid absorption, Juhel *et al.* (2000) revealed that tea catechins inhibited gastric and pancreatic lipase activity in an *in vitro* study, and Muramatsu *et al.* (1986) showed increases in faecal lipid excretion in rats. Regarding the inhibition of carbohydrate absorption, Matsumoto *et al.* (1993) proposed that tea catechins decrease the absorption of carbohydrates by inhibition of intestinal α-amylase and sucrase. Kao *et al.* (2000) reported that an intraperitoneal administration of tea catechins decreased food intake in rats. However, none of these effects have been investigated in humans.

Dulloo *et al.* (1999) reported a tea catechins-induced increase in energy expenditure in humans. In their study, ten males randomly ingested three types of samples containing a placebo, 150 mg caffeine or 150 mg caffeine + 375 mg tea catechins in a crossover design, a total of three times, and the energy expenditure per 24 h and the effects on the respiratory quotient were observed using a respiratory chamber. Caffeine did not increase the energy expenditure compared with the placebo, but caffeine +

Table 14.7. Effects of tea catechin on weight, BMI, waist circumference and abdominal fat area in CT images: changes from baseline at 12 weeks.

	Control group ($n = 117$)	Catechin group ($n = 123$)
Weight (kg)	−0.09 ± 0.16	−1.68 ± 0.14***
BMI	−0.04 ± 0.06	−0.63 ± 0.05***
Waist circumference (cm)	0.00 ± 0.23	−2.48 ± 0.20***
Total fat area (cm^2)	0.13 ± 3.01	−15.97 ± 4.20**
Visceral fat area (cm^2)	−3.87 ± 2.30	−10.28 ± 2.10*
Subcutaneous fat area (cm^2)	4.00 ± 2.30	−5.70 ± 3.47*

Values are means ± standard errors of the means (SEM).
Significantly different from control subjects at 12 weeks, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

tea catechins significantly increased the energy expenditure compared with the placebo and caffeine (Table 14.8). These findings verify that tea catechins are essential for increasing energy expenditure. The difference in energy expenditure after ingestion of the placebo and caffeine + catechin was 329.0 kJ (78.6 kcal) per 24 h.

Caffeine did not decrease the respiratory quotient compared with the placebo, but caffeine + tea catechins significantly decreased the respiratory quotient compared with the placebo and caffeine, suggesting that the increased energy expenditure was derived from lipids (Table 14.9).

Nagao *et al.* (2002) recently investigated the relationship between the amount of ingested tea catechins and increases in energy expenditure. Ten adult males randomly ingested beverages containing 0, 167, 537 and 900 mg of tea catechins, a total of four times in a crossover design, and energy expenditure and respiratory quotient were measured for 5 h after ingestion by expiratory gas analysis, using an indirect calorimeter. While no difference was observed between the energy expenditures after the ingestion of 0 and 167 mg of tea catechins, significant increases were observed after the ingestion of 537 mg and 900 mg of tea catechins, compared with the ingestion of 0 and 167 mg. Similar results were obtained with regard to the lipid-derived energy expenditure calculated from the energy expenditure and respiratory quotient. The caffeine content was about 80 mg

Table 14.8. Effects of tea catechin on energy expenditure (EE) for a 24 h period.

	Placebo	Caffeine	Caffeine + catechin	P^a
Total 24 h EE	9538 ± 521	9599 ± 518	9867 ± 448[b, c]	<0.01

Values are means ± standard errors of the means (SEM), $n = 10$.
[a]For differences across treatments (analysis of variance (ANOVA)).
[b]Significantly different from the placebo, $P < 0.05$ (*post hoc* pairwise comparison with Tukey test).
[c]Significantly different from caffeine, $P < 0.05$ (*post hoc* pairwise comparison with Tukey test).

Table 14.9. Effects of tea catechin on substrate oxidation during 24 h.

	Placebo	Caffeine	Caffeine + catechin	P^a
Protein				
(g)	65.6 ± 3.1	66.9 ± 4.7	68.3 ± 3.5	NS
(% of 24 h EE)	13.2 ± 1.0	13.4 ± 1.0	13.3 ± 1.0	NS
Carbohydrate				
(g)	336 ± 16.0	324 ± 16.0	258 ± 17.0[b]	< 0.001
(% of 24 h EE)	55.1 ± 2.4	52.7 ± 2.1	45.2 ± 2.7[c]	< 0.001
Fat				
(g)	76.2 ± 10.6	81.9 ± 8.7	103.0 ± 13.0[c]	< 0.001
(% of 24 h EE)	31.6 ± 3.1	33.8 ± 2.4	41.5 ± 3.1[c]	< 0.001

EE, energy expenditure; NS, not significant.
Values are means ± standard errors of the means (SEM), $n = 10$.
[a]For differences across treatments (analysis of variance (ANOVA)).
[b]Significantly different from the placebo, $P < 0.05$ (*post hoc* pairwise comparison with Tukey test).
[c]Significantly different from the placebo and caffeine, $P < 0.05$ (*post hoc* pairwise comparison with Tukey test).

in all test beverages, which was lower than the content (150 mg) at which Dulloo *et al.* (1999) reported no increase in energy expenditure, suggesting that tea catechins are essential for increasing energy expenditure. The amount of tea catechins required to increase energy expenditure significantly in this study was consistent with that for decreasing body fat.

Regarding the body fat-decreasing mechanism of tea catechins, inhibition of the activities of various gastrointestinal digestive enzymes may decrease the absorption of lipids and carbohydrates, thus decreasing the energy derived from the diet. However, it is obvious that an increase in energy expenditure is closely involved in the body fat-decreasing mechanism of tea catechins, based on the following reasons: (i) tea catechins are readily absorbed compared with other flavonoid compounds (Dulloo *et al.*, 1999); (ii) some studies in humans indicate that the ingestion of tea catechins increases energy expenditure, mainly lipid-derived energy; (iii) as described in the previous section, tea catechins increase β-oxidation activity and mRNA expression of β-oxidation-related enzymes in the liver (Murase *et al.*, 2002), energy expenditure (Osaki *et al.*, 2001) and oxidative decomposition of fatty acids (Onizawa *et al.*, 2001) in experimental animal models.

The prevention of obesity by drinking tea has been known from experience for a long period, and is described in *Ben Cao Shi Yi*, written more than 1000 years ago in China. It is interesting to note that the preventive effect of green tea with regard to obesity, based on experience, was scientifically verified to be the body fat-decreasing effect of tea catechins after more than 1000 years. People have loved drinking green tea for a long time, and the components of green tea are quite varied. The daily ingestion of the physiologically active components of green tea, tea catechins in particular, would be expected to contribute to the prevention of various lifestyle-related diseases, such as hyperlipidaemia, diabetes and hypertension, by decreasing body fat, and to reduce the incidence of arteriosclerotic diseases.

Oolong tea

Regarding the body fat-decreasing effect of oolong tea, Chen *et al.* (1998), in a study performed in 102 Chinese subjects (male, 42; female, 60), reported that the ingestion of oolong tea (2 g/tea bag × 4 times/day) for 6 weeks decreased body weight, waist circumference and abdominal subcutaneous fat, but this study lacked a control group. No double-blind study in humans with a control group such as those performed for oolong tea has been reported.

On the other hand, Rumpler *et al.* (2001) investigated the acceleration of energy expenditure by the ingestion of oolong tea in humans. They randomly assigned 12 males to four types of beverages, water, low-concentration oolong tea (1.5 g tea/300 ml, extracted for 20 min, 1500 ml/day), high-concentration oolong tea (3 g tea/300 ml, extracted for 20 min, 1500 ml/day) and the caffeine equivalent of the content in the high-concentration oolong tea (270 mg/day). The four treatments were represented in each of four blocks according to a 4 × 4 Latin square design. Effects of the beverages on energy expenditure and respiratory quotient were observed over a 24 h period, using a respiratory chamber.

Compared with the water group, energy expenditure was significantly increased in the high-concentration oolong tea group (2.9% increase) and the caffeine group (3.4% increase). Oxidation of lipids was also significantly increased by 12% after ingestion of the high-concentration oolong tea compared with the ingestion of water. Based on these findings, Rumpler *et al.* proposed that the caffeine contributes to the effect of oolong tea.

Komatsu *et al.* (2003) measured energy expenditure 2 h after drinking oolong tea (15 g tea/300 ml, extracted for 5 min), green tea (5 g powder/300 ml) and the same volume of water (300 ml) in 11 non-obese Japanese females with a mean BMI of 21.2, using a gas monitor. Since oolong tea led to an increase in energy expenditure, although the caffeine and tea catechins were low compared with those in green tea, Komatsu *et al.* suggested that polymerized

polyphenols contribute to the effect of oolong tea. As Komatsu *et al.* described in their report, the measurement time was not long enough to comprehensively discuss the function of oolong tea, including the amount of lipid oxidation, and further investigation is expected.

Future Prospects

Previous research clearly demonstrated that many kinds of tea and its components have anti-obesity action and implied that tea is a dietary food. The interest in prevention of obesity is very high in advanced nations. In Japan, a new special green tea drink has recently been launched. The green tea drink has anti-obesity action and has been approved as a 'Food for Special Health Use' by the Ministry of Health in Japan, and the sales of the drink are increasing explosively. More extensive studies are in progress to clarify the mechanism of anti-obesity action by tea and its components. Therefore, in the near future, it is expected that more effective anti-obesity foods, supplements or medicines using tea and tea components will be developed and obesity will be prevented by them.

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15 Protective Effects of Tea against Lung/Pulmonary Ailments

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Abstract
Lung/pulmonary ailments, diseases of the respiratory system, are often classified with the airway, pulmonary vascular and parenchymal lung diseases, based on the anatomical structures. Among the lung/pulmonary ailments, experimental and clinical studies related to tea are mainly focused on lung cancer and respiratory tract infections. The experimental studies show the consistent anti-cancer effects of tea extracts, but epidemiological evidence regarding the association between the consumption of tea and lung cancer is conflicting. For the respiratory tract infections, the experimental studies have revealed that tea extracts show antiviral and antibacterial activities, and their antibacterial activities are bactericidal as well as synergistic effects with antibiotics. Clinically, the effect of gargling with tea on the prevention of influenza and the effects of tea catechin inhalation on methicillin-resistant *Staphylococcus aureus* (MRSA) have been reported. There have been a few limited reports on the clinical effects up to now; therefore, further studies should be performed for the evaluation of the chemopreventive effects of tea against lung/pulmonary ailments.

Keywords
Lung cancer, respiratory tract infection, influenza virus, methicillin-resistant *Staphylococcus aureus* (MRSA), catechin.

Introduction

Profile and global status of the ailments

The major function served by the respiratory system is the exchange of gas between the atmosphere and the inner body circulation. The respiratory system consists of three major anatomical structures: the tracheobronchial tree, the pulmonary circulation and the alveolar bed. Diseases of the respiratory system are often classified according to these three elements: (i) airway diseases, those that affect primarily the trachea and bronchi; (ii) pulmonary vascular diseases, affecting pulmonary circulation; and (iii) parenchymal lung disease, affecting primarily the alveolar bed and its supporting stroma (Table 15.1). The representative airway diseases are upper and lower respiratory infections, such as bronchitis, pneumonia, chronic obstructive pulmonary disease (COPD), including emphysema and bronchial asthma. Also the representative pulmonary vascular diseases are pulmonary embolism or primary pulmonary hypertension, and the parenchymal lung disease includes pulmonary fibrosis. On the other

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Table 15.1. Diseases of the respiratory system classified according to the anatomical elements.

Diseases classified according to the anatomical elements	Representative diseases
Airway diseases	Bronchitis, pneumonia, COPD (e.g. emphysema), bronchial asthma
Pulmonary vascular diseases	Pulmonary embolism, pulmonary hypertension
Parenchymal lung diseases	Pulmonary fibrosis

COPD, chronic obstructive pulmonary disease.

hand, lung cancer is usually progressive and affects all these elements even when the origin is parenchyma.

Among the respiratory diseases, respiratory tract infections are very common in primary care, and a large number of patients visit doctors every year. Upper respiratory tract infections are usually self-limited and have a good clinical course. On the other hand, lower respiratory tract infections, e.g. severe pneumonia, are sometimes life-threatening and require intensive care with hospitalization. There are many pathogens that cause respiratory tract infections, such as bacteria, fungi or viruses. Among these pathogens, methicillin-resistant *Staphylococcus aureus* (MRSA) is a very serious pathogen, because it is resistant to many antibiotics and offers a serious social problem of hospital-acquired infections in some susceptible patients, especially in the elderly.

Lung cancer is one of the leading causes of cancer-related death worldwide. Treatment of the disease has incrementally advanced, but the improvement of the survival rates is slight. Cessation of smoking is therefore emphasized for the prevention of the disease. Recently, chemoprevention has introduced a new treatment option for early intervention in lung carcinogenesis, and the use of molecular targeted therapeutic agents constitutes a novel strategy for lung cancer prevention.

Introduction to tea in relation to the disease

Tea components have various possibilities for chemopreventive effects on lung/pulmonary ailments, such as antioxidative, anticancer, antiviral, antibacterial, antiallergic and anti-inflammatory effects (Mukhtar and Ahmad, 2000; Wang, 2000; Cooper *et al.*, 2005). On the basis of experimental results, clinical studies have investigated the chemopreventive effects of tea in the pulmonary system, mainly focused on the upper and lower respiratory infections, lung cancer or COPD.

Historical reference to tea in prevention/treatment

It has been said since the old days that tea prevents the common cold. Gargling with tea has been recommended for the prophylaxis of flu in elementary schools in certain districts in Japan. Tea nebulization therapy has also been tried as a folk medicine against respiratory tract infection in certain areas of Japan; the therapy is called 'chanebu', abbreviations of tea nebulization therapy ('cha' means tea in Japanese).

Epidemiological References

Among the diseases of the pulmonary system, epidemiological studies are mainly focused on lung cancer. Though experimental studies show the consistent anti-cancer effects of tea extracts, epidemiological evidence regarding the association between the consumption of tea and lung cancer is conflicting.

Hirvonen *et al.* (2001) studied the associations between the intake of flavonols and flavones, including tea, and the risk of cancer in a cohort study, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, in Finland. The study consisted of

27,110 male smokers, and the intake of flavonols and flavones was reported to be inversely associated with the risk of lung cancer. Mendilaharsu *et al.* (1998) investigated the effect of drinking tea on the risk of lung cancer in male cigarette smokers in a case–control study in Uruguay and found that black tea consumption decreased the risk.

In Asian countries, Zhong *et al.* (2001) studied the association between the consumption of green tea and the risk of lung cancer in a population-based case–control study in Shanghai. They found that the consumption of green tea was associated with a reduced risk of lung cancer among nonsmoking women, and the risks decreased with the increase of consumption. Imai *et al.* (1997) and Nakachi *et al.* (2000) studied the significance of drinking green tea for the prevention of cancer from a prospective cohort study on a total of 8552 general residents in Saitama, Japan. They revealed a negative association between green tea consumption and cancer incidence, especially among females drinking more than ten cups a day, and found a significant delay in cancer onset associated with increased consumption of green tea. Ohno *et al.* (1995) also conducted a case–control study conducted in Okinawa, and found that the greater the intake of Okinawa tea (a partially fermented tea), the smaller the risk, particularly in women, and the risk reduction was reported mainly in squamous cell lung carcinoma.

On the contrary, there are some epidemiological studies that find against the association between the consumption of tea and lung cancer. Zheng *et al.* (1996) studied tea consumption and cancer incidence in a prospective cohort study of post-menopausal women and reported no association of tea drinking found with lung cancer. Kohlmeier *et al.* (1997) reviewed the epidemiological literature and could not find a consistent protective effect of tea drinking on the total risk of cancer and the site-specific studies revealed more complex from the limited studies. Arts *et al.* (2001) studied the association between intake of catechins and incidence of epithelial cancers, including lung cancer, from a prospective cohort study, and reported that catechins derived from tea were not significantly inversely associated with the incidence of lung cancer. Nagano *et al.* (2001) examined the association between green tea consumption and cancer incidence in a prospective study in Japan, and found that green tea consumption was not related to the incidence of cancers.

Except for lung cancer, there are few epidemiological studies found in the literature concerning the association between the consumption of tea and pulmonary ailments. In COPD, results of epidemiological studies relating individual dietary factors are inconsistent. Tabak *et al.* (2001) studied intake of catechins, flavonols and flavones in relation to pulmonary function and COPD symptoms in 13,651 adults from three Dutch cities (the MORGEN Study). They found a beneficial effect of a high intake of catechins and solid fruits against COPD, but not with tea consumption. Also there have been few reports on the association of tea consumption and preventive effects with regard to bronchial asthma.

Mechanism of Action and Experimental Work

Mechanism of action

Tea polyphenols are known to have antioxidative, anti-cancer, antiviral, antibacterial, anti-allergic and anti-inflammatory effects in lung/pulmonary ailments (Mukhtar and Ahmad, 2000; Wang, 2000; Cooper *et al.*, 2005). Green tea blocks the cancer growth in its initiation and promotion, and also prevents metastasis, with the suppression of the development of new blood vessels required for tumour growth, by blocking angiogenesis (Cao *et al.*, 2002). For bacterial infections, tea catechins, the major components of tea flavonoids, show important physiological activities as bactericides and with synergistic effects towards antibiotics. For viral infections such as influenza, tea catechins are reported to inhibit infectivity and proliferation by blocking adsorption, haemagglutination, virus

assembly or the maturation cleavages. These mechanisms are precisely explained in the following experimental work.

Experimental work

Among lung/pulmonary ailments, experimental work is mainly focused on respiratory tract infections and lung cancer.

Respiratory tract infections

Tea components are known to have antiviral and antibacterial effects on the pathogens of respiratory tract infections. Those viral pathogens reported are influenza, parainfluenza, adenovirus and respiratory syncytial virus (RSV) (Nakayama *et al.*, 1993, 1994; Wyde *et al.*, 1993; Sidwell *et al.*, 1994; Imanishi *et al.*, 2002; Weber *et al.*, 2003). The bacterial pathogens reported are *S. aureus*, *Mycoplasma pneumoniae*, *Bordetella pertussis* and *Legionella pneumophila* (Toda *et al.*, 1991; Chosa *et al.*, 1992; Horiuchi *et al.*, 1992; Ikigai *et al.*, 1993; Kono *et al.*, 1994; Takahashi *et al.*, 1995; Yam *et al.*, 1998; Hu *et al.*, 2001, 2002a, b; Matsunaga *et al.*, 2001, 2002; Shiota *et al.*, 1999; Zhao *et al.*, 2001, 2003).

Green tea extract (GTE) is reported to inhibit the growth of influenza virus by preventing its adsorption and haemagglutination. Nakayama *et al.* (1993) revealed that epigallocatechin gallate (EGCG) and theaflavin digallate (TF-3) inhibit the infectivity of both influenza A and B virus in Madin–Darby canine kidney (MDCK) cells *in vitro*. They also found that black tea extract inhibits the infectivity of influenza virus to mice at the beverage concentration (2% w/w) (Nakayama *et al.*, 1994). Imanishi *et al.* (2002) found an additional inhibitory effect of tea extract on the acidification of intracellular compartments such as endosomes and lysosomes, suppressing the growth of influenza A and B viruses in MDCK cells.

With regard to RSV and parainfluenza type 3 (PIV3) viruses, Wyde *et al.* (1993) reported an antiviral activity of SP-303, a natural plant polyphenolic polymer, administered to cotton rats (*Sigmoden hispidus*). They also attempted to administer SP-303 by small-particle aerosol to influenza A/HK virus-infected mice and RSV-infected cotton rats and found that it was effective (Sidwell *et al.*, 1994). Weber *et al.* (2003) studied the effects of green tea catechins on adenovirus infection in cell culture. They reported the anti-adenoviral activity of EGCG through mechanisms both outside and inside the cell, with the suppression of virus assembly and maturation cleavages carried out by the viral protease adenain.

For MRSA, tea catechin extracts show both antibacterial activity and induction of synergistic effects with antibiotics against MRSA (Toda *et al.*, 1991; Kono *et al.*, 1994; Takahashi *et al.*, 1995; Yam *et al.*, 1998, 1995; Shiota *et al.*, 1999; Hu *et al.*, 2001, 2002a, b; Zhao *et al.*, 2001, 2003). Toda *et al.* (1991) revealed the bactericidal activities of EGCG against MRSA. Tea catechin extracts can reverse both methicillin resistance in MRSA and, to some extent, penicillin resistance in β-lactamase-producing *S. aureus*. These phenomena are explained by the prevention of penicillin-binding protein (PBP) 2 synthesis and the inhibition of secretion of β-lactamase (Yam *et al.*, 1998). Takahashi *et al.* (1995) reported that oxacillin (MPIPC) shows antibacterial activity against MRSA in the presence of catechin below the minimum inhibitory concentration (MIC). Zhao *et al.* (2001) also studied the mechanism of synergy between EGCG and β-lactams against MRSA. MICs of EGCG against methicillin-susceptible and methicillin-resistant *S. aureus* (MSSA and MRSA) were 100 μg/ml or less. Less than 25 μg/ml of EGCG obviously reversed the high-level resistance of MRSA to β-lactams, such as benzylpenicillin, oxacillin, methicillin, ampicillin and cephalexin. For non-β-lactam antibiotics, Hu *et al.* (2002a) reported that combinations of carbapenems and epigallocatechin gallate showed potent synergy against MRSA. They also studied additive, indifferent and antagonistic effects in combinations of epigallocatechin gallate with 12 non-β-lactam antibiotics against MRSA (Hu *et al.*, 2002b). The combinations of EGCG with such antibiotics, the inhibitors of either protein or nucleic

acid synthesis, such as tetracycline, minocycline, chloramphenicol, streptomycin, gentamicin, kanamycin, erythromycin, rifampicin and ofloxacin, showed additive or indifferent effects, whereas EGCG was reported to have an antagonistic tendency against glycopeptide antibiotics, such as vancomycin, teicoplanin and polymyxin B. The common property of these antibiotics is the peptide backbone structure, suggesting a direct binding of EGCG to the antibiotics.

For *M. pneumoniae* infection, Chosa *et al*. (1992) reported that EGCG purified from green tea and TF-3 from black tea showed marked bactericidal activities. Horiuchi *et al*. (1992) also reported that green and black tea showed marked bactericidal activity against *M. pneumoniae* at the beverage concentrations.

For *L. pneumophila* infection, Matsunaga *et al*. (2001) revealed that EGCG selectively alters the immune responses of macrophages to *L. pneumophila* and leads to an enhanced anti-*L. pneumophila* activity of macrophages mediated by enhanced production of both tumour necrosis factor (TNF)-α and interferon (IFN)-γ. They also reported that cigarette smoke condensate selectively alters the immune responses of macrophages to *L. pneumophila* infection and leads to an enhancement of bacterial replication in alveolar macrophages, and EGCG induced the recovery of anti-*L. pneumophila* activity impaired by nicotine (Matsunaga *et al*., 2002).

Lung cancer

Menon *et al*. (1995) studied the effects of several polyphenolic compounds on the inhibition of lung metastasis induced by B16F10 melanoma cells in mice. Oral administration of polyphenols, such as curcumin and catechin, at concentrations of 200 nmol/kg body weight was found to inhibit lung metastasis, as seen by the reduction in the number of lung tumour nodules (80%), rutin (71.2%) and epicatechin (61%). Taniguchi *et al*. (1992) also reported an inhibitory effect of EGCG on lung metastasis with mouse B16 melanoma cell lines. Sazuka *et al*. (1995) showed inhibitory effects of green tea infusion on *in vitro* invasion and *in vivo* metastasis of mouse lung carcinoma cells. They also investigated theaflavin, theaflavin digallate and EGCG inhibiting tumour cell invasion by inhibiting type IV collagenases of the mouse Lewis lung carcinoma LL2-Lu3 cells (Sazuka *et al*., 1997). Fujimoto *et al*. (2002) recently reported that EGCG or epicatechin-3-gallate (ECG) showed dose-dependent growth inhibition of human lung cancer cell lines A549 and PC-9.

Suganuma *et al*. (2000) studied the mechanisms of cancer prevention by tea polyphenols based on the inhibition of TNF-α expression with TNF-α-deficient mice. TNF-α is an endogenous tumour promoter and they reported that EGCG dose-dependently inhibited activation protein 1 (AP-1) and nuclear factor (NF)-κB activation in BALB/3T3 cells treated with okadaic acid, resulting in inhibition of TNF-α gene expression. They also found synergistic effects of EGCG with epicatechin, sulindac or tamoxifen on cancer-preventive activity in the human lung cancer cell line PC-9 (Suganuma *et al*., 1999).

EGCG is shown to be a potent natural inhibitor of leucocyte elastase, which may be used to reduce elastase-mediated progression to emphysema and tumour invasion. Sartor *et al*. (2002) reported that EGCG inhibited leucocyte elastase, potentially hindering inflammation, emphysema and tumour invasion.

Human Trials, Impact of Tea and Future Prospects

Human trials and the impact of tea

Though the experimental studies have revealed that tea extracts show antiviral and antibacterial activity, there have been only a few limited reports on the clinical effects up to now (Saito *et al*., 1994; Iwata *et al*., 1997; Yamada *et al*., 2001, 2003, 2006).

Iwata *et al*. (1997) studied the preventive effect of gargling with black tea on influenza infection. The black tea group gargled with 0.5% (w/v) black tea extract

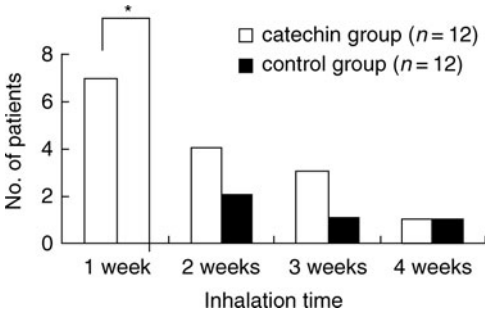

Fig. 15.1. Tea catechin inhalation effects on methicillin-resistant *Staphylococcus aureus*. After a week of inhalation, the number of patients with a decrease or disappearance of MRSA in their sputum was significantly higher in the catechin group, compared with that in the control group (*$P < 0.05$) (from Yamada *et al.*, 2003).

twice daily for 5 months, whereas the control group followed their normal daily routine. They found the HI titres of influenza viruses were raised fourfold or more in 49% of the control group, but in only 35% of the black tea group; this was significant between the two groups.

Yamada *et al.* (2001) investigated tea catechin inhalation effects on MRSA in elderly patients, the MRSA being detected in their sputum. Patients received an inhalation of two different concentrations of tea catechins (2.2 mg/ml and 3.7 mg/ml, half of them being composed of EGCG) three times daily with a hand nebulizer for 4 weeks. They showed that the catechin inhalation seemed to be temporally effective for MRSA elimination in a dose-dependent manner. They also studied the tea catechin inhalation effects on MRSA compared with that in the control group with the inhalation of saline/bromhexine alone (Yamada *et al.*, 2003). After a week's inhalation, the number of patients with a decrease or disappearance of MRSA in their sputum was significantly higher in the catechin group compared with that in the control group (Fig. 15.1). The results were confirmed by a randomized clinical study (Yamada *et al.*, 2006). Moreover, the number of patients discharged during the study increased significantly and the days of hospital stay were significantly decreased in the catechin group compared with those in the control group (Yamada *et al.*, 2003). No serious adverse events occurred in the study, and tea catechins have been reported to be well tolerated except for tea-induced asthma (Shirai *et al.*, 1994; Yamane *et al.*, 1996). Therefore tea catechin inhalation might be a possibility as an add-on treatment combined with the standard therapy for the control of MRSA.

Future prospects

In spite of the experimental studies showing anti-cancer, antiviral and antibacterial activities of tea polyphenols, there has been little clinical evidence reported up to now on the preventive effects on lung/pulmonary ailments. Further studies are recommended in future for the clinical evaluation of the chemopreventive effects of tea on lung/pulmonary ailments.

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16 Antibacterial and Antiviral Effects of Tea – from Influenza to SARS

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Abstract
The effects of green tea and its extracts on the lowering of the cholesterol level, prevention of cancer and some other physiological support have been widely studied and found evident. However, its effects on infections are less known and less studied. This chapter reviews the antimicrobial effects of green tea: from its direct *in vitro* suppressive influence on microbacteria and viruses to its *in vivo* indirect modulating interference with the immunological responses of the human body, in either an upgrading or down-regulatory direction.

A clinical trial using green tea extracts in the prevention of influenza is described in detail to illustrate the exploration of the practical value of tea in prophylaxis against viral infections.

Keywords
Green tea, antimicrobial, antiviral effects.

Introduction

The value of tea as a means of boosting normal physiological activities has long been known. Experiments and tests were designed in the laboratory to evaluate and eventually confirm the observations. It would be difficult, if at all possible, to achieve objective confirmation in the human *in vivo* situation because tea is consumed as a casual drink or food supplement, which takes lengthy periods to produce measurable changes. Unlike modern pharmaceuticals, herbal preparations do not produce immediate target results. Instead, herbal preparations modulate physiological functions, regulate disharmony and promote long-term well-being (Isogai *et al.*, 2001).

Scientists therefore resort to laboratory tests to identify the biological influences of tea extracts on various physiological and pathological conditions. A number of biological influences of tea have been worked out, using *in vitro* and *in vivo* models. The following are well-established areas of interest already studied:

- Tea consumption, survival and longevity.
- Tea influence on vitality.
- Tea influence on immunological responses.
- Tea influence on cancer growth.
- Tea influence on cardiovascular states.
- Tea influence on hepatic functions.
- Tea influence on infections.

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It has long been assumed that tea drinking is beneficial for the prevention of infections, and with tea drinking the individual enjoys better health.

This chapter attempts to review the laboratory studies available in the literature and to explore how tea drinking could protect the human body from different types of infection. Although proper clinical trials are scanty for the exploration of the relationship between tea drinking and infections, recommendations could be made on the need and required design, based on the limited data available.

Laboratory Tests Exploring the Antimicrobial Activities of Tea

Laboratory tests investigating the antimicrobial activities of tea have been microbacteria- or virus-targeted. Since viral infections are often areas of treatment insufficiency, interest has naturally grown in recent years. Laboratory tests were designed to look at the direct effects of tea on microbial activities, or indirectly to explore the series of cellular activities in *in vitro* cell culture environments or specific physiological changes in the animal *in vivo* situation.

Antibacterial properties

If tea drinkers developed fewer infections or were more resistant to infections, where did the mechanism lie? Was it direct antibacterial or antiviral effects of the tea or was it due to the immunological boosting effects helping to build up the natural resistance of the human body? The assumption has always been the latter (Hara, 2004). However, when green tea extract, taken together with antibiotics, was found to produce synergistic effects on infection, it must be logical to consider that other, more complicated mechanisms might be responsible. Indeed, Isogai *et al.* (2001) found that, when a green tea extract was used together with levofloxacin against enterohaemorrhagic *Escherichia coli* O157 infection in a gnotobiotic mouse model, the animal's ability to resist the infection was significantly stronger, although complete elimination of the infection did not occur. The overall survival rate was increased and the animal's organs were protected from damage.

There has been a steady rise in the incidence of nosocomial infections due to methicillin-resistant *Staphylococcus aureus* (MRSA); staphylococci show a strong tendency to accumulate antibiotic resistance genes and the majority of MRSA isolates are now resistant to a range of antibiotics. Taylor *et al.* (2004) examined the therapeutic potential of agents that did not directly kill the target bacterial population but modified them to produce a 'less fit' phenotype with reduced capacity to survive at the site of infection. Galloyl catechins from tea reduced the minimum inhibitory concentration (MIC) of MRSA strains from full resistance to below the antibiotic break point, raising the possibility that such molecules could be used in combination with inexpensive β-lactam antibiotics to treat MRSA infections. Epicatechin gallate and stable semi-synthetic derivatives appeared to reduce the efficiency by intercalation into the cytoplasmic membrane, an event that also disrupts the secretion of virulence determinants that enable the pathogen to cause serious infections.

Shiota *et al.* (1999) found that epicatechin gallate, a constituent of an extract of tea leaves (green tea), markedly lowered the MIC of oxacillin and other β-lactams, but not of other antibacterial agents tested, in strains of MRSA. The antibacterial action of epicatechin gallate plus oxacillin was a bactericidal one.

Yam *et al.* (1998) found that extracts of tea (*Camellia sinensis*) could reverse methicillin resistance in MRSA and also, to some extent, penicillin resistance in β-lactamase-producing *S. aureus*. These phenomena were explained by prevention of PBP2′ synthesis and inhibition of secretion of β-lactamase, respectively. Synergy between β-lactams and tea extracts was demonstrated by disc diffusion,

chequerboard titration and growth curves. Partition chromatography of an extract of green tea on Sephadex LH-20 yielded several fractions, one of which contained a virtually pure compound that showed the above-mentioned activities at concentrations above about 2 mg/l. The observed activities were novel and distinct from the previously reported direct antibacterial activity of tea extracts. Prevention of PBP2′ synthesis offered an interesting possible new approach for the treatment of infections caused by MRSA.

There appeared to be a genuine potential for green tea extracts to be used to facilitate the control of drug-resistant strains of bacteria.

Pillai *et al.* (2001) found that a number of antimutagenic agents, e.g. green tea catechins, and other antioxidants, etc., were able to suppress the emergence of resistance. In many cases, these agents were capable of exerting these effects at doses which by themselves produced no visible effects on growth. In a number of cases, antimutagenic substances capable of preventing resistance emergence were present in normal foodstuffs. These effects were exerted against the resistance to tetracyclines, fluoroquinolones, macrolides, β-lactams, aminoglycosides and the like.

Not only had observations been made on the direct effects of tea extracts on bacterial control in *in vitro* and *in vivo* experiments, but dental experts were keen to study the influence of tea extracts on dental plaque bacteria when used as a local agent. Wu *et al.* (2004) reported the effects of *in vivo* rinsing with tea extracts on human plaque regrowth/metabolism, composition of cariogenic microflora and pH/fluoride of saliva and plaque. They also studied the effects of tea extract and its components on *in vitro* growth and viability of cariogenic streptococci. Short-term frequent tea rinses inhibited subsequent regrowth and glycolysis of human supragingival plaque bacteria compared with the water rinse group. Tea polyphenols, including catechins, inhibited *in vitro* growth of selected oral pathogens associated with dental caries, periodontal disease and halitosis.

Antiviral properties

Viral infections were as much a headache as drug-resistant bacterial infections, since agents developed for their control had not been as effective as those against bacteria, in spite of repeated efforts by scientists. This major clinical disappointment happened for lethal conditions, such as acquired immune deficiency syndrome (AIDS), and in less debilitating common viral attacks, such as influenza. The frequent occurrences of the latter infection and its variances affecting birds and subsequently spreading to humans, giving high mortalities, initiated genuine interest and efforts to look for a possible treatment alternative.

Imanishi *et al.* (2002) found that green tea extract inhibited the growth of influenza virus by preventing its adsorption. The additional inhibitory effects on the acidification of intracellular compartments, such as endosomes and lysosomes (referred to as ELS), inhibited the growth of influenza A and B viruses in Madin–Darby canine kidney (MDCK) cells. The vital fluorescence microscopic study showed that the extract inhibited acidification of ELS in a concentration-dependent manner. Moreover, the growth of influenza A and B viruses was equally inhibited when the cells were treated with the extract as early as 5 to 15 min after infection, depending on the virus strains. The fact that (–)-epigallocatechin (EGC), one of the major catechin molecules in the extract, exerted inhibitory effects on the acidification of ELS and virus growth in a manner similar to that of the extract strongly suggested that EGC was one of the active components in the extract.

Nakayama *et al.* (1993) found that (–)-epigallocatechin gallate (EGCG) and theaflavin digallate (1–10 μM) inhibited the infectivity of both influenza A virus and influenza B virus in MDCK cells *in vitro*. Study by electron microscopy revealed that EGCG and theaflavin digallate (1 mM) agglutinated influenza viruses as well as did antibody, and that they prevented the viruses from adsorbing to MDCK cells. They both more weakly inhibited adsorption of

the viruses to MDCK cells. They (1–16 μM) also inhibited haemagglutination by influenza viruses. These findings suggested that tea polyphenols bind to the haemagglutinin of influenza virus, inhibit its adsorption to MDCK cells and thus block its infectivity.

The same group of scientists (Nakayama *et al.*, 1990, 1994) tried to determine whether black tea extract inhibited the infectivity of influenza virus to mice. When mice were inoculated intranasally (i.n.) with 10(5.3) plaque-forming units (p.f.u.) of influenza viruses (10(1.3) median lethal dose (LD_{50}), their body weight decreased and all died within 10 days, whereas, when mice were inoculated i.n. with the mixture of influenza viruses and 2% (w/w) black tea extract, 5 min after mixing, all mice showed normal body-weight increase and survived. Neutralizing antibody to influenza virus was not detected in nine of ten surviving mice. The results indicate that black tea extract at beverage concentration (2% w/w) inhibits almost completely the infectivity of influenza virus to mice and that *in vivo* reversion of the tea-inactivated influenza virus did not occur.

The antiviral properties of tea extracts had been tested on other viruses. Chang *et al.* (2003) found that EGCG, abundant in green tea, was a potent antimicrobial and anti-tumour compound. He used immunoblot, flow crystametry, microarray and indirect immunofluorescence analyses to show that, at concentrations exceeding 50 μM, EGCG inhibited the expression of Epstein–Barr virus (EBV) lytic proteins, including Rta, Zta and EA-D, but did not affect the expression of EBNA-1. Moreover, DNA microarray and transient transfection analyses demonstrated that EGCG blocked the EBV lytic cycle by inhibiting the transcription of immediate–early genes, thus inhibiting the initiation of the EBV lytic cascade.

Cheng *et al.* (2002) found that prodelphinidin B-2 3-*O*-gallate, a proanthocyanidin gallate isolated from green tea leaf, when investigated for its anti-herpes simplex virus (HSV) type 2 properties *in vitro*, exhibited antiviral activity with 50% inhibitory concentration (IC_{50}) of 5.0 ± 1.0 μM and 1.6 ± 0.3 pM for XTT and plaque reduction assays (PRA), respectively. A cytotoxicity assay showed that prodelphinidin B-2 3′-*O*-gallate possessed a cytotoxic effect towards Vero cells at a concentration higher that its IC_{50}. The 50% cytotoxic concentration for cell growth (CC_{50}) was 33.3 ± 3.7 μM. Thus, the selectivity index (SI) (ratio of IC_{50} to CC_{50}) for the XTT assay and PRA was 6.7 and 20.8, respectively. Prodelphinidin B-2 3′-*O*-gallate significantly reduced viral infectivity at concentrations of 10 μM or more. The result of time-of-addition studies suggested that prodelphinidin B-2 3′-*O*-gallate affected the late stage of HSV-2 infection. In addition, it was also shown to inhibit the virus from attaching to and penetrating into the cell. Thus, prodelphinidin B-2 3′-*O*-gallate was concluded to possess antiviral activity with a mechanism of inhibiting viral attachment and penetration and disturbing the late stage of viral infection.

Mukoyama *et al.* (1991) found that EGCG from green tea and theaflavin digallate from black tea inhibited infections of cultured rhesus monkey kidney MA 104 cells with rotaviruses and enteroviruses. Antiviral effects were maximally induced when directly added to the virus, and pre- and post-treatment of the cells produced much weaker antiviral activity. Antiviral activity of the extracts therefore seems to be attributable to interference with virus adsorption.

Green tea catechins have been reported to inhibit proteases involved in cancer metastasis and infection by influenza virus and human immunodeficiency virus (HIV). To date there are no effective anti-adenoviral therapies. Consequently, Weber *et al.* (2003) studied the effects of green tea catechins, and particularly the predominant component, EGCG, on adenovirus infection and the viral protease adenain in cell culture. Adding EGCG (100 μM) to the medium of infected cells reduced virus yield by two orders of magnitude, giving an IC_{50} of 25 μM and a therapeutic index of 22 in Hep2 cells. The agent was most effective when added to the cells during the transition from the early to the late phase of viral

infection, suggesting that EGCG inhibits one or more late steps in virus infection. One of these steps appeared to be virus assembly, because the titre of infectious virus and the production of physical particles were much more affected than the synthesis of virus proteins. Another step might be the maturation cleavages carried out by adenine. Of the four catechins tested on adenine, EGCG was the most inhibitory, with an IC_{50} of 109 μM, compared with an IC_{50} of 714 μM for PCMB, a standard cysteine protease inhibitor. EGCG and different green teas inactivated purified adenovirions with an IC_{50} of 250 and 245–3095, respectively. Weber *et al.* concluded that the anti-adenoviral activity of EGCG manifests itself through several mechanisms, both outside and inside the cell, but at effective drug concentrations well above that reported in the serum of green tea drinkers.

To determine the effects of EGCG on HIV infection, peripheral blood lymphocytes were incubated with either LAI/IIIB or Bal HIV strains and increasing concentrations of EGCG. Fassina *et al.* (2002) found that EGCG strongly inhibited the replication of both virus strains as determined by reverse transcriptase and p24 assays on the cell supernatants.

The long-term efficacy of new combination drug therapies for HIV infection might be limited by the tendency of transferred HIV to mutate to drug-resistant forms. This argued for the use of safe antimutagenic measures as adjuvants to such therapies. Certain nutrients and food factors – notably selenium, green tea polyphenols and cruciferous phytochemicals – can suppress cancer initiation and mutagenesis in animal and cell culture models; epidemiological studies suggested that ambient variations in consumption of these food factors could have an important impact on human cancer rates. Low-fat diets might reduce DNA base damage in human leucocytes, whereas increased body iron stores were likely to increase mutation rates. Thus, McCarty (1997) reported that ample but safe intakes of selenium, green tea polyphenols and cruciferous vegetables, in the context of a diet low in fat and assimilable iron, could be expected to prolong the efficacy of drug therapy in subjects infected with HIV.

Catechin derivatives, including (–)-epicatechin gallate (ECG), EGCG, EGC and green tea extract (GTE), were found by Tao (1992) to inhibit the activities of cloned human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT), duck hepatitis B virus replication complexes reverse transcriptase (DHBV RCs RT), herpes simplex virus 1 DNA polymerase (HSV-1 DNAP) and cow thymus DNA polymerase alpha (CT DNAP alpha). EGCG and ECG were shown to be very potent inhibitors of HIV-1 RT. According to the IC_{50} values for HIV-1 RT, these compounds can be ordered as EGCG 0.0066 μmol/l > ECG 0.084 μmol/l > GTE 0.1 μg/ml > EGC 7.2 μmol/l. DHBV RCs RT was the least sensitive to these compounds. Kinetic study showed that EGCG exerts a mixed inhibition in respect of external template inducer poly(rA)·oligo(dT)[12–18] and a non-competitive inhibition in respect of substrate dTTP for HIV-1 RT. Bovine serum albumin significantly reduced the inhibitory effects of catechin analogues and GTE on HIV-1 RT. In tissue culture, GTE inhibited the cytopathic effect of Coxsackie B3 virus, but did not inhibit the cytopathic effects of HSV-1, HSV-2, influenza A or influenza B viruses.

Immunological properties

Although so much expectation and so many studies have been directed towards the direct control of microbial infections, it might be easier and more logical to base one's efforts on the exploration of the indirect effects of tea and tea extracts on the internal regulation of the human body towards the achievement of counteracting infections. If the immunological state of the individual could be modulated to suit the situation, whereby microbial invasion could be better checked, the control or prevention of infection would be achieved. Studies on immunological changes after tea or tea extract consumption have been plentiful.

Human γδ T cells mediate innate immunity to microbes via T-cell receptor-dependent recognition of unprocessed antigens with conserved molecular patterns. These non-peptide alkylamine antigens are shared by tumour cells, bacteria, parasites and fungi but also by edible plant products, such as tea, apples, mushrooms and wine. Here we show that priming of γδ T cells with alkylamine antigens *in vitro* results in a memory response to these antigens. Such priming also results in a non-memory response to whole bacteria and to lipopolysaccharide, characterized by interleukin (IL)-12-dependent secretion of interferon (IFN)-γ by γδ T cells and by γδ T-cell proliferation. Drinking tea, which contains L-theanine, a precursor of the non-peptide antigen ethylamine, primed peripheral blood γδ T cells to mediate a memory response on re-exposure to ethylamine and to secrete IFN-γ in response to bacteria. This unique combination of innate immune response and immunological memory shows that γδ T cells can function as a bridge between innate and acquired immunity. In addition, these data provide an explanation for the health benefits of tea. Kamath *et al.* (2003) proved these effects in his studies.

The immunomodulatory property of *Crinum latifolium* (L.) extracts was investigated and compared with those of black and green tea. Human peripheral mononuclear cells were cultured in the presence of tea extracts with or without mitogens or IFN-γ. The effect of plant extracts on cultured cells was assayed by neopterin production, a sensitive marker reflecting the activation of cell-medicted immunity. The experiments showed that extracts of *C. latifolium* slightly enhance neopterin production in unstimulated peripheral mononuclear cells, whereas an effective reduction of neopterin formation in cells stimulated with concanavalin A (Con A), phytohaemagglutinin (PHA) or IFN-γ was observed (Zvetkova *et al.*, 2001). Green and black tea extracts displayed similar immunomodulatory properties in our *in vitro* system, whereas *C. latifolium* extracts seemed to be more effective in reducing neopterin formation in stimulated cells.

If tea extracts can prolong the efficacy of HIV drug therapy, they might work through an immuno-enhancing channel (McCarty, 1997).

As it had been shown that the sustained virological response (SVR) rate was at an estimated 10% level of the national average with interferon-α2b and ribavirin combination (IFN/Rib) therapy in chronic hepatitis C patients with a very high genotype 1 hepatitis C virus (HCV) load (> 850 KIU/ml), Yoichi *et al.* (2004) conducted a clinical study to improve SVR by adding green tea powder, which has an antioxidant and immunomodulator effect, to standard IFN/Rib therapy for 6 months. Nine patients were recruited in this study. All patients completed the therapy. No significant adverse effects were noted. Of the nine patients, six completed the observation period of 6 months, and the efficacy of our new protocol was estimated. SVR was achieved in three patients (50%). For the remaining three patients, the observation is still ongoing at the time of this report. Further studies will be continued to determine the full efficacy of triple combination therapy, including green tea, in the treatment of HCV infection.

The effects of polyphenols in Pu'er tea on prostaglandin E_2 (PGE_2) production, cell growth rates and toxicology of RAW 264.7 macrophage were explored. Six kinds of Pu'er tea available in Taiwan markets were used as materials. The results showed that the epicatechin (EC) content in grade A Pu'er Tou tea was the highest (>5.13 mg/g) while the Yunnan Pu'er Zhuan tea contained the highest amount of EGC (>59.10 mg/g). The cell survival rates in RAW 264.7 macrophage with Yunnan Pu'er Zhuan tea and Meng-Hai tea were elevated as from 17 to 61% and no cytotoxicity was found. The grade A Pu'er tea extracted for 20 min showed the best biological immune and anti-inflammatory function for lowering PGE_2 production in macrophages among all the treatments, possibly due to the high contents of EC, EGC and EGCG (Chiang *et al.*, 2004).

Excessive inflammation is a manifestation of overactive immunological responses, which could be harmful to the human body.

Allergy is another manifestation of an excessive immunological response. Tea has been studied for its anti-inflammation and anti-allergy effects.

The flavonol EGCG was shown to be a potent natural inhibitor of leucocyte elastase that might be used to reduce elastase-mediated progression to emphysema and tumour invasion. This phytofactor, abundant in green tea, exerted a dose-dependent, non-competitive inhibition of leucocyte elastase at a non-cytotoxic concentration and was effective in neutrophil culture. This inhibition showed an IC_{50} of 0.4 μM, 30 times higher than the α1-protease inhibitor but lower than other known natural and synthetic elastase inhibitors. The flavonol inhibited leucocyte elastase at concentrations 50, 150 and 2500 times lower than that effective on gelatinases (matrix metalloproteinase (MMP)-2 and MMP-9), thrombin and cathepsin G, respectively, and also blocked elastase-mediated activation of MMP-9 (Sartor *et al.*, 2002).

Junko *et al.* (2004) found inhibitory effects of black tea extracts on fMLP-induced aggregating responses in a rabbit polymorphonuclear leucocyte (PMN)–platelet system. To elucidate whether platelet-activating factor production in PMNs and/or PAF-stimulated platelet activation were inhibited, the effects of tea polyphenols were investigated on the enzyme activity of acetyl-coenzyme A (CoA):lyso-PAF acetyltransferase (EC2.3.1.67), PAF biosynthesis in A23187-activated rabbit PMNs and rabbit platelet aggregation. By comparing the inhibitory effects of 31 galloyl esters and gallic acid, the structure–inhibitory activity relationship was characterized. Theaflavin and its galloyl esters, dimeric flavon-3-ols with a galloyl group at C-3 and pentagalloylglucose were found to be potent inhibitors of acetyltransferase (IC_{50} = 25–50 μM) and PAF biosynthesis, as were ECG (IC_{50} = 72 μM) and EGCG (IC_{50} = 2 μM). In addition, theaflavin and its galloyl esters (IC_{50} = 23–72 μM) and isoprenyl gallates (IC_{50} = 6.2–3.6 μM) were found to be potent inhibitors of PAF- and TPA-induced rabbit platelet aggregation, but not A23187-induced aggregation. Theaflavin and its galloyl esters in black tea extract and isoprenyl gallates were potent inhibitors of PAF synthesis and platelet aggregation, and these activities may be relevant to the claimed therapeutic effects of tea extracts.

In a systematic screening of commonly used herbs to study *in vitro* immunomodulating effects on mouse lymphocytes, ginger and tea were found consistently immunosuppressive, while dong quai, milk thistle and St John's wort were consistently immunostimulatory *in vitro* (Chumpon and Smita, 2002).

Clinical Studies on the Efficacy of Tea against Viral Infections

While laboratory studies have been plentiful for the influence of tea and tea extracts on the direct control or prevention of infections, properly designed clinical trials on the efficacy of tea against viral infections are scanty, if available.

The author has the experience of conducting a properly designed clinical trial to look at the efficacy of a herbal formula or green tea for the prevention of influenza (Leung, 2004).

The study

During the annual seasonal outbreak of influenza in Hong Kong in the spring of 2004, elderly people in homes and community centres in Hong Kong were given a random choice of either a herbal or tea preparation for 2 months for the prevention of the expected endemic. These elderly clients were randomly divided into two groups: Group A received the standard herbal preparation consisting of 12 herbs and Group B was given a green tea drink prepared in standard sachets. The clinical responses of the two groups were measured during and after consumption. The parameters included quality of life, occurrence of fever and some objective blood tests investigating possible changes in the immunological state. Adverse effects were another target of concern. In total, 877 clients were recruited. Of these,

112 had their blood checked for immunological changes after taking the herbal preparation or tea extract.

The primary objective of this study was to investigate the effectiveness of the two interventions, i.e. herbal formula and tea. They acted as an alternative to treatment by influenza vaccination for prevention of influenza and flu-like symptoms among the elderly during the expected influenza outbreak season from January to May 2004. The secondary objectives were to compare the differences of the two interventions with regard to immune function and quality of life. The third objective was to establish the safety of the herbal and tea preparations.

Materials and methods

Two randomized double-blind controlled trials were conducted for two groups of elderly people. The first study was for those aged 65 or above and living in homes for the elderly in Hong Kong (Study I). Another study was on those of the same age who were living in the community (Study II). All subjects had to comply with the inclusion and exclusion criteria and were invited to give informed consent before the study started.

Inclusion criteria

1. Those aged 65 years or older.
2. Those who had been vaccinated against influenza in the last 6 months.
3. Those who had not received vaccination against influenza in the past 6 months.

Exclusion criteria

1. Those who took medications that affect immune functioning, e.g. steroid and most anti-cancer medications.
2. Those who were regularly taking other forms of Chinese medicine.
3. Those who had cognitive dysfunctions.
4. Those with severe morbidity and seen as obviously not suitable for the study.

Randomization

The eligible recruited participants were randomized into two treatment groups by equal opportunity. One group would take the herbal formula and other would take the green tea preparation. The assignment was blind to all the staff and the participants to avoid bias. The study preparations were made in powder form with a similar colour, and the packaging and labelling were identical.

Treatment schedule

There were 8 weeks' treatment with a 4-week follow-up period. All participants were being served for a total of 3 months after recruitment into the study. During the treatment period, participants took the assigned preparations three times per week (Monday, Wednesday and Friday), with one sachet (4 g) each time.

Study design

STUDY I: RANDOMIZED CONTROLLED TRIAL IN HOMES FOR THE ELDERLY. Study I was a double-blind randomized controlled trial that was conducted in eight homes for the elderly in the northern district of Hong Kong. All of them had been vaccinated for influenza on or before November 2003. There were 377 enrolled and 241 of them were recruited at 12 health seminars. All of them fulfilled the inclusion and exclusion criteria. They were randomly assigned into the herbal group or the green tea group by equal chance.

STUDY II: RANDOMIZED CONTROLLED TRIAL IN DAY CARE CENTRES FOR THE ELDERLY. Study II was a double-blind randomized controlled trial that was conducted in ten day care centres for the elderly in the northern district of Hong Kong. Some of them had received influenza vaccination and some of them had not. There were 1106 enrolled and 636 of them were recruited at 27 health seminars. Of the enrolled group, 400 were vaccinated and 236 of them were not vaccinated. All of them fulfilled the inclusion and exclusion criteria. Within each group, clients were randomly assigned into two treatment groups by equal chance.

Outcome measurements

During the 12 weeks of study, the number of episodes of influenza and influenza-like

illness was recorded. Influenza was defined as those suffering from typical symptoms and nasal and pharyngeal swabs subsequently proved to be positive for influenza virus. Influenza-like illness was defined by a fever (37.5°C) plus any one of the following symptoms: sore throat, general myalgia, cough or runny nose. Research nurses visited those with influenza-like illness and collected a nasopharyngeal swab specimen for virus check. These specimens were sent to the Prince of Wales Hospital for laboratory tests to detect viral pathogens.

Quality of life (QoL) data as measured by the SF-36 (Vitality and Mental Health Subscales) were used as the secondary end points. QoL was measured at the beginning of the trial treatment and every 4 weeks during the trial. Compliance data were obtained by telephone checks on the consumption of herbal formula or green tea preparation.

The immunomodulatory effects of the herbal formula or green tea preparation were assessed for those willing to supply blood samples, before and 8 weeks after taking the preparations (Table 16.1).

Results

During the trial period, the expected influenza endemic was not encountered. Not a single case of influenza was proven after throat and nasal swabs on the suspected individuals presenting with fever, runny nose and/or sore throat. Seventy-two incidents of flu-like cases were found. Only 9.7% of those who took either tea or Chinese medicine developed flu-like symptoms compared with 18.3% of those who did not take these (P = 0.001). The QoL for those who took either of the preparations improved in both mental and vitality parameters. The difference between the tea group and the Chinese medicine group was statistically insignificant. For the study of immunomodulation, 112 clients completed blood tests. Parameters included T lymphocytes, T suppressor cells, T helper cells, natural killer lymphocytes, B lymphocytes and T helper/suppressor ratio. Both T lymphocytes and T helper cells were increased in the tea group and Chinese medicine group. Other parameters did not show significant changes. The differences between the tea and Chinese medicine groups did not reach statistical significance (Table 16.2).

Clients taking either tea or the Chinese medicine preparation did not experience serious adverse effects apart from occasional giddiness and abdominal discomfort.

Conclusions

Both the tea and Chinese medicine preparations were found to have beneficial effects on elderly people, who apparently developed fewer influenza-like symptoms during the taking of the preparations. The biological action of the two preparations should be quite different because we found 0.06% epigallocatechin and 0.06% epicatechin in our tea preparation and 0% for both chemicals in our Chinese medicine preparation. The objective data on the immunomodulating effects showing mild degrees of up-regulation might explain the better well-being of those who took the preparations. Future studies might be able to catch the influenza endemic so that more direct proof as to the efficacy of tea or Chinese medicine

Table 16.1. Number of blood samples taken.

	Homes for the elderly		Centres for the elderly			
	Vaccinated group		Vaccinated group		Non-vaccinated group	
Herbal	22	(17)	31	(22)	17	(13)
Green tea	21	(17)	28	(22)	22	(21)
Total	43	(34)	59	(44)	39	(34)

Numbers in parentheses are those that completed all blood tests.

Table 16.2. Immunomodulation difference between BFT* and tea.

	BFT (%)	Tea (%)
T lymphocytes	0.40 (improved)	0.57 (improved)
T suppressor cells	0.21 (not improved)	0.48 (not improved)
T helper cells	0.05 (improved)	0.41 (improved)
NK lymphocytes	0.35 (improved)	0.45 (not improved)
B lymphocytes	0.68 (not improved)	0.16 (not improved)
T helper/suppressor ratio	0.04 (not improved)	0.04 (not improved)

*BFT Bu Fei is the herbal drink used for prevention of influenza

could be acquired when used as prophylactic agents against viral infections affecting the respiratory tract.

Clinical studies relied on the abundance of relevant patients for the study. Our influenza prevention trial using either Chinese medicine or tea preparation had been a failure. Normally during the months of February to April in Hong Kong, we expected an endemic outbreak of influenza. This had happened in the 4 years preceding the study. For some reason, this time, when we needed the cases, the endemic did not come. Indeed, all our suspected cases were eventually proven to be just cases of common cold, not influenza, as all the nasal swabs taken for the viral study turned out to be negative for influenza. We are therefore planning a similar but more specific study in 2005, during the autumn when again, as in the past, high frequencies of the outbreak will be expected.

Prevention of Influenza

Broadly speaking, vaccination is effective against influenza. Vaccination coverage can be increased by administering vaccine to persons during hospitalization or routine health-care visits before the influenza season, making special visits to physicians' offices or clinics. However, the effectiveness of influenza vaccine depends primarily on the age and immunocompetence of the recipient and the degree of similarity between the viruses in the vaccine and those in circulation. The majority of vaccinated children and young adults develop high post-vaccination haemagglutination in the antibody titres (La Montagne and Noble, 1983; Neuzil and Dupart, 2001). Older persons and persons with certain chronic diseases might develop lower post-vaccination antibody titres than healthy young adults and thus can remain susceptible to influenza-related upper respiratory tract infection (McElhancy and Beattie, 1990; Blumberg and Albano, 1996). A randomized trial among non-institutionalized persons aged >60 years reported a vaccine efficacy of 58% against influenza respiratory illness, but indicated that efficacy might be lower among those aged >70 years (Govaert and Thijs, 1994).

Among elderly persons living outside nursing homes or similar chronic-care facilities, influenza vaccine was 30–70% effective in preventing hospitalization for pneumonia and influenza (Mullooly and Bennett, 1994; Nichol and Wuorenma, 1998). Among elderly persons residing in nursing homes, influenza vaccine is most effective in preventing severe illness, secondary complications and deaths. Among this population, the vaccine can be 50–60% effective in preventing hospitalization or pneumonia and 80% effective in preventing death, although the effectiveness in preventing influenza illness often ranges from 30% to 40% (Patriarca and Weber, 1985; Arden and Patriarca, 1986).

Annual vaccination is recommended for health-care workers. None the less, vaccination coverage of only 34% and 36% among health-care workers was reported in the 1997 and 2001 surveys, respectively (unpublished NHIS data; NIP, CDC, 2003).

Vaccination of health-care workers has been associated with reduced work absenteeism and fewer deaths among nursing home patients (Potter and Stott, 1997; Wilde and McMellan, 1999; Carman and Elder, 2000).

In view of the absence of perfect results of vaccination, other means of preventing influenza infection should be explored.

An influenza outbreak could bring disaster. In the USA, approximately 36,000 deaths/year resulted from the infection during 1990–1999. Influenza viruses also cause pandemics, during which rates of illness and death from influenza-related complications can increase dramatically worldwide. Influenza viruses cause disease among all age groups. Rates of infection are highest among children, but rates of serious illness and death are highest among persons aged >65 years or persons of any age who have medical conditions that place them at increased risk for complications of influenza.

Influenza vaccination is the primary method for preventing influenza and its severe complications. In the report from the Advisory Committee on Immunization Practices (ACIP), the primary target groups recommended for annual vaccination are: (i) groups that are at increased risk for influenza-related complications (e.g. persons aged >65 years and persons of any age with certain chronic medical conditions); (ii) the group aged 50–64 years, because this group has an elevated prevalence of certain chronic medical conditions; and (iii) persons who live with or care for persons at high risk (e.g. health-care workers and household contacts who have frequent contact with persons at high risk and who can transmit influenza to persons at high risk). Vaccination is associated with reductions in influenza-related respiratory illness, physicians visits among all age groups, hospitalization and death among persons at high risk, otitis media among children and work absenteeism among adults. Although influenza vaccination levels increased substantially during the 1990s, further improvements in vaccine coverage levels are needed, chiefly among persons aged <65 years who are at increased risk for influenza-related complications.

Influenza A and B are the two types of influenza viruses that cause epidemic human disease. Influenza A viruses are further categorized into subtypes on the basis of two surface antigens: haemagglutinin (H) and neuraminidase (N). Influenza B viruses are not categorized into subtypes (Colacino and Laver, 1997; Jai and Escarpe, 1998).

A person's immunity to the surface antigens, including haemagglutinin, reduces the likelihood of infection and severity of disease if infection occurs. Antibody against one influenza virus type or subtype has limited or no protection against another. Furthermore, antibody to one antigenic variant of influenza might not protect against a new antigenic variant of the same type or subtype. Frequent development of antigenic variants through antigenic drift is the virological basis for seasonal epidemics and the reason for the usual incorporation of more than one new strain in each year's influenza vaccine. Vaccination against influenza, therefore, could face particular difficulties because of the changing antigenic variants.

Outlook

We have been attempting to unearth the evidence that tea and tea extracts could have direct and indirect antimicrobial effects in the human body. Laboratory data are apparently quite adequate to offer a positive outlook. Clinical trials, however, are difficult to conduct and the parameters of assessment are not clear-cut.

In 2001, Tomita *et al.* (2004) found that green tea administration significantly suppressed DNA methylation of the mouse oestrogen receptor gene. Recently EGCG was shown to inhibit DNA methyltransferase, suppress DNA methylation and re-express RNA and protein of four genes in various human cancer lines. DNA methylation of the human collagen type 1 gene (Colia l and Colia 2) promoter region was also significantly suppressed *in vivo* by the administration of green tea. These antioxidant effects did have significant implications on issues

of ageing and disease control. In infection, when we look at the agent responsible or the host under challenge, whenever DNA methylations are involved, there might be room to develop an active programme towards the further development of the utilization of tea against infections.

While different types of tea were found to have different chemical components (Oi, *et al.*, 2004), and different chemical components might have different antimicrobial activities (Shin *et al.*, 2004), it was indicated that more refinement in the research on tea would bring more exciting information, not only on infection control, but on the prolongation of longevity and vitality in general (Nakachi *et al.*, 2003). Before specific means of viral infection control, e.g. more reliable vaccinations, become reality, exploration of the use of tea and other herbal preparations for the early control of the infection, which would be equivalent to the prevention of the disease, would be rewarding.

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17 Green Tea and the Prevention of Arthritis

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Abstract
All over the world there is a growing interest in using botanicals as part of a complementary strategy to manage or prevent the onset of arthritis and other chronic diseases. Amongst the agents of plant origin that are under rigorous evaluation, green tea has gained attention due to its established anti-inflammatory and antioxidant properties. In this chapter, we have attempted to summarize critically the socio-economic impact of arthritis, with the focus on osteoarthritis (OA), limitations of the current treatment modalities and the emerging role of green tea in OA and, where appropriate, for rheumatoid arthritis (RA), treatment/prevention with reference to its historical importance and use, present research status and its mechanism of action. A survey of the published studies also suggests that consumption of green tea may be beneficial in inhibiting OA progression, as polyphenols present in green tea inhibit the production of catabolic mediators implicated in the progression of both RA and OA, both *in vitro* and in animal models. Thus it is safe to conclude that consumption of green tea may have beneficial health effects, including prevention of OA and inhibiting its progression in affected individuals.

Introduction

Osteoarthritis (OA) is the most common form of synovial joint disorder, characterized by progressive erosion of articular cartilage and loss of bone with concomitant loss of joint function (van den Berg, 2000; Malemud *et al.*, 2003). On the other hand, rheumatoid arthritis (RA) is an autoimmune disease with systemic manifestations and has clear associations with the specific genetic composition of susceptible individuals. Both RA and OA are debilitating conditions, with different profiles and unknown aetiology, but sustained chronic production of common mediators of cartilage destruction is an important characteristic of both diseases (Haqqi *et al.*, 2000). Arthritis is present in every population and affects more women than men (3 : 1). The severity of the disease varies from person to person, but the consonant clinical signs include pain, ranging from minor pain with discomfort to severe inflammation, and reduced range of motion with joint destruction and deformity (Yelin and Callahan, 1995). Until recently, OA and RA were thought to be a progressive and non-reversible disorders, resulting ultimately in joint destruction, but with a better understanding of their pathogenesis and with the

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development of new 'non-conventional' approaches, the spontaneous arrest or reversal of the disease may occur (Haqqi *et al.*, 1999; McAlindon, 2000). The high prevalence of arthritis (both OA and RA) in every population, with its associated loss of joint function, requires expensive and long-term therapies and poses a significant socio-economic burden and health challenge globally. An estimated 43 million Americans and many more around the world suffer from arthritis and other rheumatic conditions (Lawrence *et al.*, 1998), with the number expected to reach approximately 60 million by the year 2020 (Center for Disease Control and Prevention, 1998).

Socio-economic Impact

The economic, social and psychological impact associated with rheumatic diseases, including arthritis, is enormous (Yelin *et al.*, 1995). Although some of the effects can be translated into economic terms, such as lost wages and medical care costs, many more, such as pain, reductions in housekeeping activities and the victim's inability to enjoy leisure activities, cannot be easily determined. Arthritis clearly has an adverse impact on ambulation and results in significant reduction in social activities and job performance, which eventually leads to higher levels of psychological distress in affected individuals than that expected in the general population (Yelin and Callahan, 1995). Risk factors associated with arthritis include non-modifiable and modifiable factors, as well as some demographic factors (Falson *et al.*, 1991; Callahan and Rao, 1996; Falson and Zhang, 1998). Non-modifiable risk factors are female sex, older age and genetic predisposition. Modifiable risk factors are obesity, joint injuries, infections and certain occupations with repetitive knee bending (Yelin and Callahan, 2001).

Role of Pro-inflammatory Cytokines in the Pathophysiology of OA and RA

Cytokines are low-molecular-weight proteins that mediate communication between cells in a redundant, overlapping and synergistic manner, and include colony-stimulating factors, growth factors, interleukins and interferons. However, the cytokine network is largely self-regulating, and pathophysiological consequences usually result from the unregulated action or inappropriate production of pro-inflammatory cytokines.

The pro-inflammatory cytokines interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) have been shown to play an important role as mediators of inflammation and tissue destruction in both RA and OA (Arend and Dayer, 1995; Dinarello, 1996; Goldring, 2000; Dayer, 2004). Their importance in RA pathogenesis is evident from the observation that therapeutic agents that inhibit IL-1β and TNF-α also ameliorate the disease (Arend and Dayer, 1995).

TNF-α exhibits many biological properties that are relevant to the pathogenesis of RA and OA. Along with IL-1, TNF-α induces the production of collagenase and prostaglandin E_2 (PGE_2) in synovial fibroblasts and chondrocytes (Goldring, 2000; Dayer, 2004). TNF-α has also been shown to inflict oxidative tissue injury in the affected joint via enhanced production of reactive oxygen/nitrogen species (ROS/RNS), such as singlet oxygen (O^-), peroxynitrite ($OONO^-$) and nitric oxide (NO) (den Broeder *et al.*, 2003).

IL-1β, primarily produced by activated synovial fibroblasts and chondrocytes, on binding to its receptor IL-1R1, induces systemic and local detrimental effects in acute and chronic diseases, including OA (Goldring, 2000; Dayer, 2004). In chondrocytes and synovial fibroblasts, IL-1β has been shown to enhance the expression of adhesion molecules on endothelial cells and induce chemotaxis of neutrophils, monocytes and lymphocytes into the synovium (Dinarello, 2002). IL-1β induces the production of NO and PGE_2 in OA chondrocytes via enhanced activity and expression of their respective synthesizing enzymes, inducible nitric oxide synthase (iNOS) and cyclo-oxygenase-2 (COX-2) (Ahmed *et al.*, 2002). IL-1β induces systematic degradation of articular cartilage in arthritis by activating matrix metalloproteinases (MMPs), especially collagenases (MMP-1, 3, 8 and 13), a group of enzymes

involved in effectively chewing the cartilage matrix (Poole *et al.*, 2003).

Botanicals as Complementary Therapies for the Treatment of Arthritis

With the increasing socio-economic burden in pursuing expensive, and yet ineffective, current treatment modalities based on synthetic drugs, there has been a global resurgence for a better understanding of disease pathogenesis and the use of herbal medicines and dietary supplements for the alleviation of chronic diseases (Soeken *et al.*, 2003). It is noteworthy that there has been a remarkable increase in the use of botanicals and other dietary foods not only in developing countries but also in the USA and European countries for better and healthier living and as complementary and alternative therapies for many conditions, including RA, OA and cancer (Borchers *et al.*, 2000).

Recent advances in our understanding of the pathogenesis of inflammatory and progressive degenerative joint diseases have markedly influenced the design of interventional pharmacological approaches to block or inhibit disease progression. Conventional treatment methods for arthritis are limited to the widespread use of non-steroidal anti-inflammatory drugs (NSAIDs) and, more recently, COX-2 inhibitors, as well as of glucocorticoids (Brune, 2004). However, the disease-modifying effects of these current treatments are very limited and only provide temporary relief from joint pain, stiffness or swelling. More recently, the use of biologicals has been introduced for treating both OA and RA, but a significant portion of patients do not respond to these treatments either (Moreland *et al.*, 1999). Due to these limitations, an increasing number of people in the USA, as many as 42%, and many more around the world are adopting complementary and alternative medicine (CAM) approaches to help meet their personal health problems (Soeken and Miller, 2003). Another epidemiological study, based on reports and visits to primary care physicians, showed that there has been an increase in the use of herbal remedies (Engel and Straus, 2002). Arthritis is one of the foremost diseases for which patients seek a CAM option (Kessler *et al.*, 2001). Because there is a paucity of evidence in respect of modifying/inhibiting the progression of joint dysfunction and its associated adverse effects, the American College of Rheumatology recommends the careful use of dietary supplements and herbal medicines during early stages of treatment to limit the degree of joint destruction. Although the use of botanicals for the treatment of RA or OA is not universally accepted, the evaluation of traditional medicine in the light of modern science has revealed the efficacy of some of these herbs and dietary constituents in certain disease conditions (Huxtable, 1992).

Green Tea

Historical perspective

Tea (*Camellia sinensis*) is one of the most popular beverages consumed around the world. An estimated 2.5 Mt of dried teas are manufactured annually. Of that amount, ~20% is green tea, which is prepared by chopping and rolling the leaves and quickly drying them in heat/steam to inactivate polyphenol oxidase, and ~78% is black tea, which is prepared by the oxidation of leaves by polyphenol oxidase (Zuo *et al.*, 2002). Oolong tea, which constitutes ~2% of total tea production, is prepared by partial oxidation of tea leaves before drying (Zuo *et al.*, 2002). By tradition, oriental, North African and Middle East populations favour green tea, whereas black tea is the most popular drink in the Indian subcontinent, Europe and North America, with oolong tea being popular in China and Taiwan (Wu and Wei, 2002).

Composition of green tea

Green tea has high concentrations of polyphenols, such as flavonols, flavonediols, phenolic acids, catechins and caffeine as

well as minerals and trace elements (Cabrera *et al.*, 2003). Catechins, the major constituents of green tea polyphenols, including epigallocatechin-3-gallate (EGCG), epicatechin (EC), epicatechin-3-gallate (ECG) and epigallocatechin (EGC), account for ~30% of its water-soluble dry weight. The majority of the reported pharmacological effects of green tea are mediated by EGCG, which constitutes 70% of the total catechin content in green tea (Zuo *et al.*, 2002). During the fermentation of black tea, a large portion of the catechins is converted to theaflavins and thearubigins, which account for the strong taste and dark brown colour of black tea (Wu and Wei, 2002). The remaining catechins account for 3–10% of solids in brewed black tea (see also Carbera *et al.*, 2003).

Green Tea and the Treatment of Chronic Diseases

Green tea has been reported to possess antimicrobial, immune-stimulating, anticarcinogenic and anti-inflammatory properties (Katiyar and Mukhtar, 1996; Katiyar *et al.*, 1999; Ahmad *et al.*, 2001). The established pharmacological properties of green tea are attributed to its high content of polyphenols, mainly EGCG, which mimics most of the biological effects of green tea (Ahmad *et al.*, 2001). Green tea has also been shown to possess antioxidant activity against a broad range of oxidants and anti-inflammatory activity against pro-inflammatory mediators of chronic diseases, both *in vitro* and in animal models (Katiyar and Mukhtar, 1997). In this regard, EGCG has been shown to inhibit superoxide- and NO-induced oxidative damage in several animal models (Mukhtar and Ahmad, 2000).

TNF-α has been shown to play a role in regulating the inflammatory response in numerous degenerative and inflammatory disorders, including arthritis (Surh *et al.*, 2001). EGCG has been demonstrated to modulate catabolic pathways induced by TNF-α in several models of inflammation by inhibiting nuclear factor-κB (NF-κB), an oxidant-sensitive transcription factor that regulates the expression of various genes, including the pro-inflammatory cytokines (Lin *et al.*, 1999). Green tea extracts have also been shown to attenuate neuro-inflammatory effects of 6-hydroxydopamine by inhibiting NF-κB activation and cell death in neuronal cultures (Levites *et al.*, 2002). In another study, prophylactic feeding of green tea (50 mg/100 ml drinking water) to 6-month-old rats for 22 weeks showed a marked reduction in age-associated glycation and oxidative modification of proteins that are implicated in ageing, diabetes, coronary heart disease and atherosclerosis (Song *et al.*, 2002).

Green Tea and Arthritis

The potential disease-modifying effect of green tea on inflammatory arthritis came to light with a study in which it was shown that collagen type II-induced arthritis (CIA) in mice was ameliorated by prophylactic administration of green tea polyphenols (GTP) in drinking water (Haqqi *et al.*, 1999). In three independent experiments, mice given GTP in water exhibited a significantly reduced incidence of arthritis (33% to 50%) as compared with mice not given GTP in water (84% to 100%). The arthritis index was found to be significantly lower in GTP-fed animals when compared with non-GTP-fed animals. Protein analysis showed a marked reduction in the expression of inflammatory mediators, such as COX-2, interferon-γ (IFN-γ) and TNF-α, in arthritic joints of GTP-fed mice. Histological and immunohistochemical analysis of the arthritic joints in GTP-fed mice demonstrated only marginal joint infiltration by IFN-γ- and TNF-α-producing cells, as opposed to massive cellular infiltration and fully developed pannus in arthritic joints of non-GTP-fed mice. The neutral endopeptidase activity was found to be approximately sevenfold higher in arthritic joints of non-GTP-fed mice in comparison with non-arthritic joints of unimmunized mice, whereas it was only twofold higher in the arthritic joints of

GTP-fed mice. Additionally, total immunoglobulins (Ig) and type II collagen-specific IgG levels were found to be lower in serum and arthritic joints of GTP-fed mice. The results of reduction in biochemical markers were supported by the marked reduction of inflammation in the synovium and milder cellular infiltrates, coupled with reduced cartilage or bone erosion, in the green tea-fed mice.

A series of studies by ourselves further probed the mechanisms of action of EGCG on chondrocytes to determine how EGCG might prevent, inhibit or reverse the pathological events associated with OA in affected joints. In this regard, it is known that IL-1β induces the production of several inflammatory mediators of cartilage degradation, such as NO, PGE_2 and MMPs, and suppresses the biosynthesis of type II collagen and aggrecan and the proliferation of chondrocytes (Abramson and Amin, 2002). In chondrocytes, NO has been shown to inhibit collagen and proteoglycan synthesis, increase susceptibility to injury by other ROS produced and induce apoptosis (Blanco *et al.*, 1995; Kraan and Berg, 2000). In addition, increased PGE_2 production induces inflammation by increasing local blood flow and enhancing the effects of other catabolic mediators, such as bradykinins and NO (Nedelec *et al.*, 2001). In a recent study, we showed that EGCG inhibited the IL-1β-induced expression and activity of iNOS and COX-2, resulting in reduction in NO and PGE_2 production by human OA chondrocytes (Ahmed *et al.*, 2002). In this study it was shown that pre-treatment of human OA chondrocytes with EGCG resulted in a dose-dependent inhibition of the production of NO and PGE_2 by 48% and 24%, respectively, and correlated with the inhibition of iNOS and COX-2 activities. Additionally, the IL-1β-induced expression of iNOS and COX-2 was also markedly inhibited in human OA chondrocytes pre-treated with EGCG. Parallel to these findings, it was observed that EGCG also inhibited the IL-1β-induced lactate dehydrogenase (LDH) release in OA chondrocytes, indicating a chondroprotective effect. Overall, this study suggested that EGCG affords protection against IL-1β-induced production of catabolic mediators NO and PGE_2 in human chondrocytes by regulating the expression and catalytic activity of their respective enzymes.

Several studies have shown that the expression of iNOS is dependent on the activation of the ubiquitously expressed transcription factor NF-κB (Ghosh *et al.*, 1998; Li and Verma, 2002). NF-κB remains inactive in cytoplasm when bound to its endogenous inhibitor (IκBα) in normal physiological situations (Li and Verma, 2002). However, IL-1β and TNF-α have been shown to activate NF-κB by proteosomal degradation of IκBα and a consequent translocation of NF-κB to the nucleus, where it binds to the promoter region of the iNOS gene to enhance mRNA and protein synthesis. In further exploring this phenomenon, we showed that EGCG is a potent inhibitor of IL-1β-induced iNOS mRNA and protein expression in human OA chondrocytes and that it exerts this effect by inhibiting the activation and translocation of NF-κB to the nucleus by suppressing the degradation of IκBα in the cytoplasm (Singh *et al.*, 2002). A critical observation of this study was that the inhibition of NO production by EGCG appeared to be structure-specific, as other compounds, namely EGC or sodium gallate alone or in combination, were not effective in inhibiting IL-1β-induced NO production in human OA chondrocytes.

The activation of mitogen-activated protein kinases (MAPK) is a critical event in the activation of the pro-inflammatory cytokine-induced signalling cascade in synoviocytes and chondrocytes, which leads to the production of several mediators of cartilage damage in an arthritic joint (Johnson and Lapadat, 2002). IL-1β is known to activate the MAPK family, including the extracellular signal-regulated kinase (ERKp44/p42), c-*Jun N*-terminal kinase (JNK) and p38-MAPK in human chondrocytes (Liacini *et al.*, 2002). The most relevant MAPKs for the expression of IL-1β-induced catabolic mediators in OA are the p38-MAPK and JNK, as these are involved in the activation of transcription factor activation protein-1 (AP-1) (Eder, 1997; Vincenti and Brinckerhoff, 2001).

Transcription factor AP-1 plays a central role in the regulation of gene expression for several MMPs, which degrade proteoglycan and type II collagen in cartilage (Vincenti and Brinckerhoff, 2001). In another study evaluating the effect of EGCG on IL-1β-induced MAPKs in human chondrocytes, we showed that EGCG inhibited principally the IL-1β-induced phosphorylation of the JNK p46 isoform, accumulation of phospho-c-Jun and DNA binding activity of AP-1 in human OA chondrocytes. Further, immune complex kinase assays revealed that EGCG preferentially inhibited JNK activity and not p38-MAPK or ERK1/2 activity in chondrocytes.

Collagenases (MMP-1, 3, 8 and 13) are a large group of enzymes that play a crucial role in tissue remodelling in normal physiological conditions, as well as in the destruction of cartilage and bone in an arthritic joint, due to their ability to degrade a wide variety of extracellular matrix (ECM) components (Brinckerhoff and Matrisian, 2002). Among the MMPs, MMP-1 and 13 are critical, as they are found elevated in arthritic joints (Mitchell *et al.*, 1996). MMP-13 can cleave type II collagen, the major component of cartilage matrix, more efficiently than MMP-1 (Mitchell *et al.*, 1996). Studies have documented the fact that, in arthritic joints, degradation of type II collagen is excessive, due to increased activity of MMPs (Billinghurst *et al.*, 2000). Other studies have shown that excessive activity of MMP-13 can produce the type of pathology seen in arthritic joints (Neuhold *et al.*, 2001).

In OA chondrocytes, activation of the JNK pathway and the transcription factor Runx-2 is required for the activation of MMP-13 promoter activity (Mengshol *et al.*, 2001). Physical interaction between transcription factors AP-1 and Runx-2 is a necessary step for the optimum activation of MMP-13 promoter. An important role of the JNK pathway in the pathogenesis of arthritis is also evident from studies showing that inhibitors of JNK prevent arthritis in animal models (Han *et al.*, 2001). Our recent studies showed that pre-treatment of human OA chondrocytes with EGCG (20–100 μM) significantly inhibited the expression and activities of MMP-1 and MMP-13 in human chondrocytes (Ahmed *et al.*, 2004). These results also demonstrated for the first time that, of the two MMPs, EGCG preferentially inhibited MMP-13 at the transcriptional level. A similar differential dose-dependent inhibition of NF-κB and AP-1 was also found in these studies. Using regression analysis the 50% inhibitory concentration (IC_{50}) values for the inhibition by EGCG were found to be 27 μM and 16.5 μM for MMP-1 and 13, respectively, both of which are within a physiologically achievable range.

The results of this study were further independently confirmed by other investigators who showed that different catechins from green tea inhibited the degradation of human cartilage proteoglycan and type II collagen (Adcocks *et al.*, 2002) and selectively inhibited ADAMTS-1, 4 and 5 *in vitro* (Vankemmelbeke *et al.*, 2003). The study also showed that the concentration needed for the total inhibition of these members of the ADAMTS group was approximately two orders of magnitude lower than what is needed partially to inhibit collagenase or ADAM-10 activity. EGCG and ECG were the most effective catechins in inhibiting cytokine-induced cartilage degradation, and none of the catechins studied was found to produce any adverse/toxic effect in cultured cartilage explants.

Mechanism of Action

Chondrocytes are the only cell type present in the cartilage and they play a key role not only in joint degradation but also in the attempted repair of the damaged cartilage in OA joints. It is likely that multiple factors, such as injury, inflammatory cytokines, oxidative stress, loading, etc., act on chondrocytes *in vivo* to activate unique signalling pathways that lead to gene expression profiles that are associated with differential rates of disease progression at the level of the cartilage. At the cellular level, recent studies suggest that the observed arthritis-inhibitory effects of green tea are manifested through the regulation of signalling pathways and

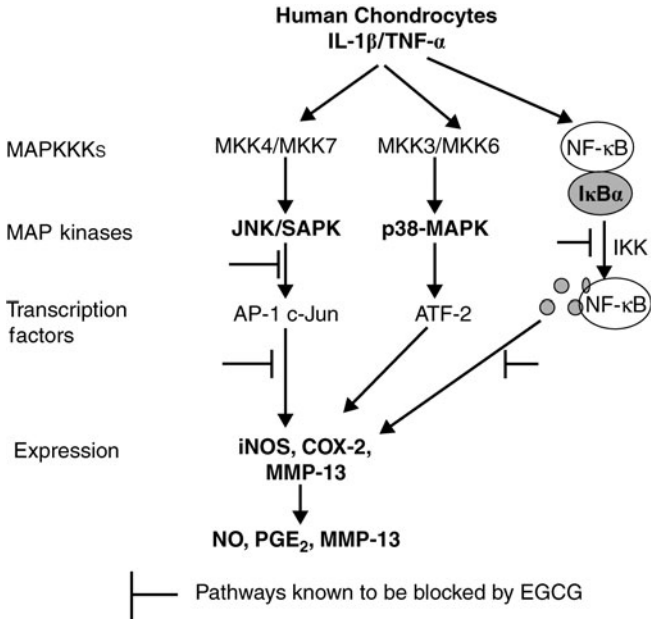

Fig. 17.1. Activation of signal transduction pathways and transcription factors by pro-inflammatory cytokines and their modulation by green tea polyphenols in OA chondrocytes *in vitro*.

transcription factors known to be critical for the induction and expression of crucial inflammatory and catabolic mediators in arthritic joints. Some of these pathways experimentally shown to be affected by green tea polyphenols, at least *in vitro*, are depicted in Fig. 17.1.

Conclusions

These studies, combined with growing evidence that green tea and its constituents provide chondroprotection, warrant evaluation in human subjects. With the availability of green tea globally, a standardized method of preparation, cost-effectiveness and proven safety, with no reported adverse effects, regular consumption of green tea combined with a healthy lifestyle may prove beneficial in preventing arthritis. Additionally, in combination with current treatment modalities, consumption of green tea may also aid in alleviating the discomfort, pain and progression of arthritis.

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18 Chemoprevention Effect of Tea against Neuronal Death–Dementia

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Abstract
It has been thought that the number of Alzheimer's disease patients is higher in Western nations, whereas patients with vascular dementia-type diseases are more common in Japan. An epidemiological study in Japan has shown that the incidence of stroke was significantly lower in people who consume more than five cups of green tea per day. The green tea chemical component theanine showed a neuroprotective effect in transient ischaemic neuronal death. Tea catechins are acknowledged to have radical scavenging actions and preventive effects against arteriosclerosis. Thus, the neuroprotective effects of tea catechins may be inferred, and the preventive effects of green tea consumption on dementia are presently the focus of considerable attention.

Profile and Global Status of the Ailment

Dementia is a progressive, irreversible decline in mental function caused by Alzheimer's disease, interruption of cerebral blood flow by cerebral stroke, Creutzfeldt–Jakob disease, and so on. Alzheimer's disease, which has a high incidence in Western populations, is thought to be the cause of senile spot formation by the extracellular accumulation of β-amyloid protein and neurofibrillary tangle by intracellular accumulation of excessive phosphorylated tau protein (Aisen, 1997; Cummings *et al.*, 1998). Some hold that excess ingestion of fat causes Alzheimer's disease. The severity of dementia is thought to involve mainly the number of cerebral neurons, caused by the toxicity of amyloid protein, injury by active oxygen and an abnormal calcium metabolism. For this reason, the use of calcium antagonists and so on has been considered. Also, vitamin E and ginkgo leaf extract have been tried as radical-scavenging agents.

According to the Japanese Ministry of Health, Labour and Welfare statistics for 1997, cerebral stroke was the third highest cause of mortality after cancer and heart disease, accounting for 15.7% of all deaths, or about 138,000 people. Of these strokes, 63% were caused by cerebral infarction, 23% by cerebral haemorrhage and 10% by subarachnoid haemorrhage. Thus, the majority of strokes in Japan are cerebral infarctions. At present, the percentage of dementia patients over 65 years old in Japan is approximately 7%, but it is thought to be growing, together with the increase in the elderly population. Also, there are

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various opinions about the increasing number of Alzheimer's patients.

In the present chapter, the neuroprotective effects of theanine and catechins on transient ischaemic neuronal death are described, and the possibility of prevention of dementia by green tea consumption is discussed.

Tea in Relation to Disease

An epidemiological study showed that the incidence of stroke was significantly lower in people who consume more than five cups of green tea per day (Sato *et al.*, 1989). We have paid special attention to the r-glutamylethylamide (theanine) contained in green tea, which is a compound similar to the excitatory neurotransmitter glutamic acid, and we investigated the neuroprotective effect of theanine against transient forebrain ischaemic neuronal death (Kakuda *et al.*, 2000). Moreover, the suppressive effects of tea catechins on lipid peroxidation and the inhibitory effects on arteriosclerosis have also been reported (Miura *et al.*, 1994; Nakagawa *et al.*, 1999). Studies have also been published on the prevention of neuronal death in the hippocampal CA1 region by transient ischaemia by oral administration of tea (–)-catechin and suppression of amyloid peptide toxicity by tea catechin (Inanami *et al.*, 1998). Based on these findings, the neuroprotective effects of theanine and catechins contained in tea have received considerable attention.

Historical References to Tea for Disease Prevention

Chemical components

Tea is broadly classified, according to the production method, as unfermented tea (green tea), half-fermented tea (oolong tea) and fully fermented tea (black tea). The green tea produced in Japan is unfermented tea. The most common production method is steaming, although a small amount of tea in certain districts is produced by panning. Steaming or panning deactivates the oxidase in tea leaves, so that the tea retains its brilliant green colour and has the name 'green tea'. The major chemical components of green tea are shown in Table 18.1 (Goto *et al.*, 1996). Theanine and catechins are thought to be the chemical components that confer neuroprotective effects.

Neuroprotective effect of theanine

Neuroprotective effects of theanine on glutamate toxicity from *in vitro* testing using rat cortical neurons have been reported (Nozawa *et al.*, 1998), as well as in *in vivo* studies with a transient ischaemic model using gerbils (Kakuda *et al.*, 2000b), and in neuronal death in the cortex region using a

Table 18.1. Contents of major chemical component (%) dry weights in green tea (from Goto *et al.*, 1996).

Type of tea	Grade	Total catechins	Caffeine	Vitamin C	Theanine
Gyokuro	High	12.02	4.03	0.17	2.35
	Low	13.69	3.53	0.21	1.52
Matcha	High	9.37	3.87	0.09	2.43
	Low	12.63	3.53	0.15	1.37
Sencha	High	15.06	3.20	0.41	1.55
	Low	16.21	2.98	0.25	0.60
Bancha	—	13.45	2.05	0.23	0.46

middle cerebral artery occlusion rat model (Kakuda *et al.*, 2000a).

Neuroprotective effects of catechins

Arteriosclerosis (atherosclerosis and arteriolosclerosis) is a main cause of ischaemic cerebrovascular disease, and prevention of cerebral arteriosclerosis is important in suppressing neuronal death. The relationship between active oxygen species and one of the mechanisms for the development of arteriosclerosis has recently received attention. Low-density lipoprotein (LDL) undergoes oxidative changes due to active oxygen species, and this oxidatively altered LDL is ingested by macrophages to form foam cells. Inhibiting the formation of oxidatively altered LDL is thus important in terms of preventing arteriosclerosis.

On the other hand, green tea contains catechins, such as (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG) and (–)-epicatechin (EC), at levels of 10–15%. The oxidative alterations of LDL were shown to be prevented by tea catechins (Miura *et al.*, 1994), and oral administration of EGCG in humans reportedly causes a decrease in peroxidative phospholipids and an increase in free catechins in the serum (Nakagawa *et al.*, 1999). The increase in stroke incidence was reported to be lowered and survival time increased by long-term oral administration of EGCG to stroke-prone spontaneously hypertensive rats (SHRSP) (Uchida *et al.*, 1995). The reasons for this are the radical scavenger effects and the preventive effect on lipid peroxidation. Daily ingestion of tea as an antioxidant has also been reported to prevent stroke (Sato *et al.*, 1989).

Mechanism of Action

Ischaemia and neuronal death

Severe cerebral infarction with no post-ischaemic reperfusion may result in neuronal death. In cases of slight ischaemia with reperfusion, on the other hand, neuronal damage may be minor. However, some brain regions are reported to be particularly vulnerable to ischaemia (Diemer *et al.*, 1981; Pulsinelli *et al.*, 1982), and ischaemia of between 5 and 10 min can lead to neuronal death about 2 days later (Kirino, 1982). This is thought to be due to the elevation of the extracellular concentrations of the excitatory neurotransmitter glutamic acid with transient cerebral ischaemia, which induces neuronal damage by its excitotoxicity (Olney, 1990; Benveniste, 1991). The relationship between ischaemic neuronal death and cerebral vascular dementia has attracted the attention of researchers, and minimizing neuronal damage after reperfusion is considered vital in cases of transient ischaemia.

Glutamate toxicity and neuronal death

Glutamic acid plays an important role in memory and learning by producing synaptic plasticity, known as long-term potentiation (LTP) or long-term depression (LTD). Neurons contain between 1 and 10 mM glutamic acid, which is released from the cell by transmission of an impulse. This glutamic acid contributes to signal transduction by stimulating glutamate receptors and opening ion channels. Afterwards, the glutamic acid is taken into neurons or glial cells by glutamate transporters, so that the concentration in the extracellular space is maintained at between 1 and 2 μM (Benveniste *et al.*, 1984; Peghini *et al.*, 1997). The glutamic acid taken into glial cells is transformed into glutamine by the action of glutamine transporters and transferred into neurons, where this glutamine is transformed again to glutamic acid. However, when the intracellular energy source ATP is depleted by injury, such as ischaemia, depolarization of the neuronal membrane leads to excessive release of glutamic acid and inability to reabsorb this glutamic acid by the glutamate transporter, resulting in an excessive concentration of glutamic acid in the extracellular space. This excessive glutamic acid binds to *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors in the postsynaptic membrane, increasing cell permeability to Ca^{2+}

(Nicholls and Attwell, 1990; Gorter *et al.*, 1997; Pellegrini-Giampietro *et al.*, 1997). A high concentration of free Ca^{2+} is thought to cause neuronal death by abnormal activation of various enzymes in the cell or by increasing the amount of superoxide radicals (Kitagawa *et al.*, 1990; Lafon-Cazal *et al.*, 1993). A correlation has been shown between the intracellular concentration of Ca^{2+} and neuronal death (Ogura *et al.*, 1988). This theoretical relationship between glutamic acid/ calcium ions and cell death is now widely accepted.

Mechanism of theanine neuroprotection

Since theanine is a natural glutamate analogue, we paid special attention to the action of theanine on glutamate receptors as one of the mechanisms of neuroprotection (Fig. 18.1) (Kakuda, 2002). The binding activity of theanine on glutamate receptor subtypes α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainate and NMDA was evaluated, using radiolabelled ligands [^{3}H]AMPA and [^{3}H]kainite and the NMDA glycine antagonist [^{3}H]MDL105,519 (Kakuda *et al.*, 2002). Given the demonstrated ability of theanine to inhibit the binding of radiolabelled ligand to glutamate receptors, it was suggested that theanine acts on glutamate receptors as an antagonist. However, the 50% inhibitory concentration (IC_{50}) value of theanine was about 80- to 30,000-fold less than that of glutamic acid, and therefore the binding activity of theanine proved to be very weak. The inhibitory effect of theanine on glutamate subtypes AMPA and kainite receptor was shown to be one order higher than that of the NMDA receptor.

Although the IC_{50} value of theanine on the AMPA receptor was 80-fold lower than that of glutamic acid, the binding concentration was 10^{-5} M, which is thought to be pharmacologically effective (Kakuda *et al.*, 2002). Sheardown *et al.* (1990) reported that the death of CA1 pyramidal neurons was reduced with the administration of AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(*F*)quinoxaline (NBQX) before and after ischaemia, and suggested that AMPA receptors play a functional role in neuronal death induced through NMDA receptors. Nellgård and Wieloch (1992)

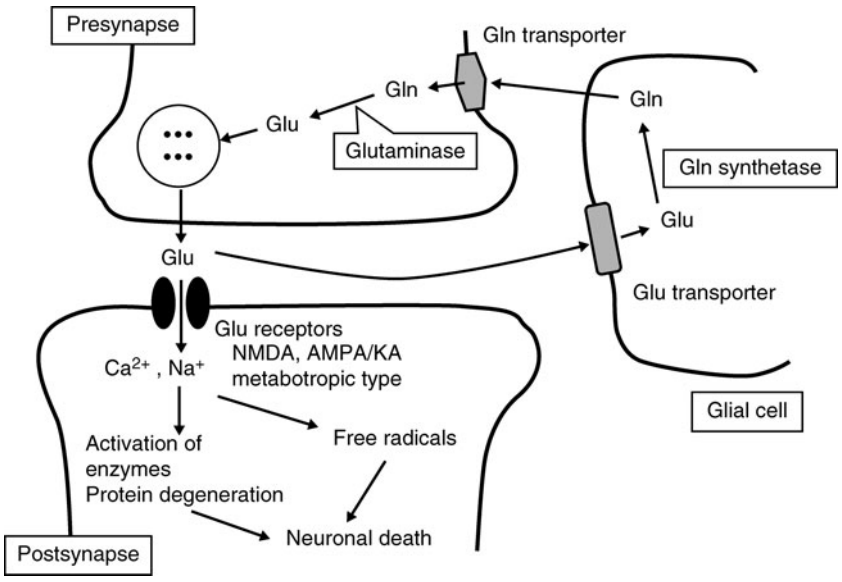

Fig. 18.1. A schematic depiction of the glutamate receptor, glutamine (Gln)/glutamate (Glu) cycle and neuronal death (from Kakuda, 2002).

reported that neuronal death was prevented by administration of AMPA receptor antagonist even after ischaemia, and thus theanine might also prevent neuronal death when administered after ischaemia. However, more detailed studies on this are needed.

The IC_{50} value of theanine on kainate receptors was also about 80-fold lower than that of glutamic acid (Kakuda *et al.*, 2002). While the activity was very low, the binding concentration was 10^{-5} M, which is thought to be pharmacologically effective. Hippocampal CA3 pyramidal neurons showed the highest uptake of radiolabelled kainate in the brain (Cotman *et al.*, 1987), and were killed by exposure to kainate (Ben-Ari, 1985). In our previous study, neuronal death in the hippocampal CA3 region was seen with intraperitoneal injection of kainate at 8 mg/kg, but this neuronal death was prevented by the administration of theanine into the ventricle before the intraperitoneal injection of kainate (Kakuda *et al.*, 2001). These findings suggest that theanine also binds to kainite receptors as an antagonist and that theanine plays a role in neuroprotection.

The hippocampal CA1 region, the neurons of which are vulnerable to ischaemia, contains many NMDA receptors (Monaghan *et al.*, 1983; Cotman *et al.*, 1987). The IC_{50} value of theanine on NMDA receptors was about 30,000-fold less than that of glutamic acid, and one order less than that of AMPA or kainate receptors. Thus, the binding capacity of theanine to NMDA receptors was very low, and the binding concentration was 10^{-4} M (Kakuda *et al.*, 2002). Maruyama and Takeda (1994) suggested that, even though the binding capacity of theanine to glutamate receptors is low, theanine may act as a competitive antagonist to glutamate receptors. Yokogoshi *et al.* (1998) reported that the administration of theanine into brain striata increased dopamine release markedly, but that this release was inhibited by pre-treatment with D-2-amino-5-phosphonopentanoate (D-APV). These findings suggest that, while the binding activity of theanine on NMDA receptors is very weak, theanine acts on glutamate receptors as an antagonist.

It is known that neuroprotective agents such as MK801 act by lowering the brain temperature (Busto *et al.*, 1987; Buchan and Pulsinelli, 1990). However, the results of one of our previous studies, in which the brain temperature was maintained at 37.5 ± 0.2°C, suggest that this is not the case with the neuroprotective effect of theanine (Kakuda *et al.*, 2000).

The above results suggest that one of the mechanisms of the neuroprotective effects of theanine against neuronal death from transient ischaemia is related to the fact that theanine binds to glutamate receptor subtypes such as AMPA, kainate and NMDA receptors as an antagonist. However, it has been shown that the binding capacity of theanine to glutamate receptors is much lower than that of glutamic acid, which suggests that the antagonistic effect of theanine may be very weak. Therefore, other mechanisms for the neuroprotective action of theanine in transient ischaemia are thought to exist. The glutamic acid discharged from the presynapse acts on glutamate receptors in the postsynaptic membrane (Fonnum, 1984). The Ca^{2+} channel then opens, and Ca^{2+} flows into the cells. The extracellular glutamic acid is then taken back up through glutamate transporters in glial cells and the synaptic membrane (Bridges *et al.*, 1987; Amara and Kuhar, 1993). Glutamine is then synthesized by glutamine synthetase from the glutamic acid taken into the glial cells (Martinez-Hernandez *et al.*, 1977; Norenberg, 1979). Glutamine is taken into the presynapse through glutamine transporters. This glutamine is converted to glutamic acid by glutaminase and stored again in synaptic vesicles through a glutamic acid–glutamine cycle (Amara and Kuhar, 1993). We earlier confirmed the effects of theanine on glutamine transporters.

Theanine metabolism

Kitaoka *et al.* (1996) reported that theanine, similar to glutamic acid, was absorbed into the blood from the intestinal tract through co-transport with Na^{+}. Unno *et al.* (1999) reported that the serum theanine concentration

reached a peak 0.5 to 2 h after oral administration of theanine 200 mg/kg in rats, and Yokogoshi *et al.* (1998) showed that orally administered theanine entered the brain through the blood–brain barrier. Thus, it is clear that orally administered theanine is absorbed into the blood from the intestinal tract, and then a very small amount of theanine in blood flows into the brain through the blood–brain barrier.

Radical-scavenging action of tea catechins

Active oxygen species generated in the body include the superoxide anion radical ($\cdot O_2^-$), hydroxyl radical ($\cdot OH$) and hyperperoxy radical ($\cdot OOH$). Of these active oxygen species, $\cdot O_2^-$ is not as active alone, but $\cdot OH$ and $\cdot OOH$ are generated by $\cdot O_2^-$ to trigger the generation of $\cdot OH$ and $\cdot OOH$. Therefore the scavenging of $\cdot O_2^-$ is important. Unno *et al.* (2000) reported that EGCG, which accounts for about 50% of tea catechins, EGC, (–)-gallocatechin gallate (GCG) and (–)-gallocatechin (GC) exhibited strong scavenging activity on $\cdot O_2^-$.

Effect of tea catechins on neuroprotection

A small percentage of the catechins consumed in tea are absorbed into the blood vessels from the intestinal tract (Unno *et al.*, 1996; Nakagawa and Miyazawa, 1997), and trace amounts of tea catechins pass through the blood–brain barrier (Nakagawa and Miyazawa, 1997; Suganuma *et al.*, 1998). Moreover, it has been reported that neuronal death following transient ischaemia was inhibited by oral administration of (–)-catechin (Inanami *et al.*, 1998), and that the green tea polyphenol EGCG has protective effects against hippocampal neuronal damage after transient global ischaemia in gerbils (Lee and Kim, 2000); also β-amyloid peptide toxicity was prevented by tea catechin in an *in vitro* test. These results suggest that the neuroprotective effect of tea catechins is due to prevention of lipid peroxidation and a radical-scavenging effect.

Experimental Work

Inhibitory effects of theanine on glutamate toxicity in *in vitro* studies

Nozawa *et al.* (1998) exposed cultured rat cortical neurons to between 25 and 800 μM of glutamic acid and found that 50% of the cells died from exposure to 50 μM glutamic acid. The death of cultured rat cortical neurons induced by glutamic acid was suppressed by simultaneous exposure to 500 μM theanine (Nozawa *et al.*, 1995), suggesting its neuroprotective effect on glutamate toxicity. However, neuronal death was observed at higher concentrations.

Neuroprotective effect of theanine in gerbils

The neuroprotective effect of theanine on post-ischaemic neuronal death in field CA1 of the gerbil hippocampus was examined using a transient ischaemia model (Fig. 18.2) (Kakuda *et al.*, 2000b). Transient forebrain ischaemia was induced by bilateral occlusion of the common carotid arteries for 3 min. The studies were done with the following three experimental groups: sham-operated animals administered 500 μM theanine 30 min before the sham operation; control animals administered saline 30 min before ischaemia; and theanine-treated animals administered theanine concentrations of 50 μM, 125 μM and 500 μM 30 min before ischaemia. The theanine solutions were administered with a glass micropipette through a lateral ventricle. Transient forebrain ischaemia was induced as described above. Then, after a survival period of 7 days, the animals were deeply anaesthetized with pentobarbital and perfused transcardially. Their brains were removed and embedded in paraffin. Three coronal sections with a thickness of 4 μm, taken from a level of 2.0 mm posterior to the bregma, were made using a microtome and stained with cresyl-violet. The number of intact CA1 neurons in the hippocampus in an ocular grid (rectangle, 500 μm wide and 100 μm high) was assessed.

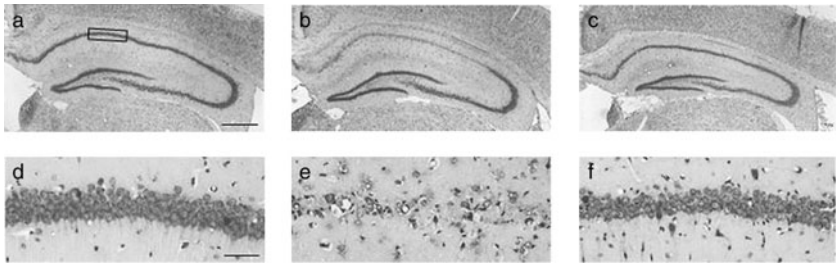

Fig. 18.2. Photomicrographs of sections of hippocampus after survival for 7 days following administration of theanine in animals 30 min before sham operation (a, d), in the control group administered saline 30 min before 3 min forebrain ischaemia (b, e) and in the theanine-treated group administered 1 µl of 500 µM theanine 30 min before ischaemia (c, f). Lower photomicrographs show a higher magnification of the CA1 sector. Bars: 500 µm in (a), 50 µm in (d). Neuronal damage was not observed in the sham-operated group (a, d). Widespread neuronal destruction was observed in the CA1 sector, whereas almost all neurons in the CA3 sector and dentate gyrus were preserved (b, e). Ischaemic neuronal destruction in the CA1 sector is prevented by theanine administration (c, f) (from Kakuda, *et al*., 2000b).

Most CA1 neurons degenerated or disappeared in the control group. In contrast, these neurons were largely preserved in the 500 µM theanine pre-treated group. Ischaemic neuronal death in field CA1 of the hippocampus was suppressed in the theanine-pre-treated groups in a dose-dependent manner, with approximately 60% and 90% survival with 125 µM and 500 µM theanine, respectively. Thus, a neuroprotective effect of theanine was also shown against neuronal death in the CA1 region by transient brain ischaemia in an *in vivo* study.

Human Trials

At present, with the cooperation of a patient with dementia, we are testing the safety and effects on a human of using green tea containing theanine, catechin and so on. The results will be published in the near future. From the standpoint of preventing arteriosclerosis, we have reported catechin's cholesterol-lowering effect on a human for 12 weeks (Nozawa *et al.*, 2002). Its safety was examined using 197.4 mg of tea catechins in a 250 ml beverage consumed daily. The results showed that the cholesterol level was significantly lowered 8 weeks after commencing intake and was significantly lower than the placebo group after 12 weeks. These results clearly showed the cholesterol-lowering effect of catechins.

Future Prospects

Though research and development of compounds with a chemopreventive effect against neuronal death–dementia have been promoted around the world, specific medicines have not yet been developed with few side effects and clear effectiveness. Vitamin E and vitamin C are also used as radical scavengers (Majewska and Bell, 1990; Packer, 1991).

On the other hand, the present study revealed that the antagonistic effects of theanine on glutamate receptors were very weak compared with those of glutamic acid. Tea catechins are very effective in preventing arteriosclerosis through their antioxidant effect and scavenging of active oxygen species. High-quality green tea powder also contains natural vitamin E and vitamin C. There is no doubt as to their safety, since most Japanese consume green tea almost daily. Therefore it is important to confirm the mechanisms and clinical applications of the neuroprotective effects of green tea components, such as theanine and catechins, in order to protect brain health in our ageing society.

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19 Chemopreventive Action of Tea against Senescence/Ageing

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Abstract
Senescence is an ageing process occurring after maturity, with physiological dysfunctions and failing homeostasis. Although its causative mechanisms are complex and not fully understood, oxidative stress is thought to be involved in senescence/ageing as an important factor. It also remains to be clarified whether tea as a beverage has a chemopreventive effect on senescence. Some data have shown that tea has beneficial effects on the senescent brain in aged mice and prolongs the human lifespan by preventing premature death. Sufficient daily intake of tea might be an effective means to suppress senescence.

Keywords
Senescence, ageing, oxidative stress, longevity, learning, memory, brain atrophy.

Senescence/Ageing

Senescence is an ageing process towards death after maturity and is characterized by progressive and irreversible dysfunction of various physiological systems and failures of homeostasis. Physiological senescence with advancing age is not a disease but it causes impairments such as cognitive dysfunctions and lowered motor skills in all elderly people. Moreover, ageing is an important risk factor of some diseases such as cancer, hypertension, diabetes and dementia. The ageing process accompanied by these diseases, morbid senescence, is frequently appended to physiological senescence and affects the lifespan and quality of life of elderly people. That is, the characteristic phenomena followed by senescence are summarized as physiological dysfunctions, shortened longevity and age-related diseases.

It is thought that the process of ageing is roughly programmed because the maximal longevity and the number of maximal cell divisions are fixed in each organism (Hayflick, 1965). However, significant differences in senescent progression among individuals indicate involvement of other factors in the ageing process. It has been known that oxidized cellular macromolecules, such as DNA, lipids and proteins, accumulate in aged organisms, and these products appear to be harmful to physiological functions and defence systems. In fact, resistance to oxidative stress allows nematodes and *Drosophila* to have a prolonged lifespan (Jazwinski, 1996; Ishii *et al.*, 1998; Lin *et al.*, 1998). Aerobic organisms consume a large amount of oxygen and generate abundant free radicals as normal products of cellular metabolism. Under normal conditions, the antioxidant defence system acts against increased

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reactive oxygen species (ROS) or peroxide production. However, if imbalance between the production of ROS and the cellular defensive ability occurs, it gives rise to oxidative stress to critical cellular components (Finkel and Holbrook, 2000). In aged organisms, the defensive response may decline or become defective in dealing with oxidative damage. In addition to the oxidative damage, some other alterations, such as instability of damaged DNA, epigenetic modifications leading to altered gene expression patterns, shortened telomeres, and glycation of long-lived proteins, are also thought to be closely associated with the ageing process.

Introduction to the Significance of Tea in Relation to Senescence/Ageing

To prevent senescence, caloric restriction (CR) has been known to extend lifespans in a wide range of animal species and decrease the oxidative damage of macromolecules (Sohal and Weindruck, 1996). In fact, the stress response was increased in the aged rat brain. However, CR suppressed the increased stress response significantly (Unno *et al.*, 2000). Recently it has been found that CR variously alters the gene expression and profile affects protein turnover and carbon metabolism (Lee *et al.*, 1999; Lin *et al.*, 2002). Although more studies are needed to clarify the mechanisms of the anti-ageing effect of CR and suppression of oxidative stress, accumulation of oxidative damage is thought to contribute to senescence.

As another method to suppress oxidative damage, administration of antioxidants has been expected to protect the organism from oxidative damage and to have beneficial effects on age-associated diseases and longevity (Meydani, 2001). Antioxidants present in foods and beverages, such as vitamins A, C and E, carotenoids, flavonoids and polyphenols, play an important role in preventing cellular oxidative damage (Esposito *et al.*, 2002). Tea polyphenols exhibit a potent antioxidative effect; therefore, the chemopreventive action of tea on senescence will be an important subject in the field of research on senescence.

Historical References

Tea is the beverage that has the longest history. The anti-senescence effect of tea was first recorded in China (around the year 500). In Japan, Eisai, a Zen priest, wrote about the life-prolonging effect of green tea in his book *Kissa-yojouki* in the year 1211. In those days, tea was used as a medicine. In Europe, tea was imported from Japan and China in the 17th century. Dirx, a famous Dutch doctor, wrote about the effect of tea on longevity in his book (1641). Tea, i.e. green tea in Japan and black tea in England, became a popular beverage in the 18th century; however, scientific investigations on the effect of tea and its mechanisms of action only started in the last century. In particular, studies on the effect of tea on senescence/ageing have only begun in recent years. These studies deal with a very important subject, because elderly people have been increasing in number in many countries and their demand for healthy ageing has become higher than ever before.

Epidemiological References

The characteristic phenomena accompanying senescence are physiological dysfunctions, shortened longevity and age-related diseases. Thus, the efficacy of tea against senescence/ageing centres on whether these phenomena are improved by tea consumption.

There are many epidemiological studies of the effect of tea consumption on age-related diseases such as cancer, cardiovascular and neurodegenerative diseases (Higdon and Frei, 2003). As the effect of tea consumption on these diseases has been described in detail in the preceding chapters in this book, I would like to focus on the effect of tea consumption on longevity and physiological dysfunctions in this chapter. Many epidemiological investigations have indicated improvement of morbidity and mortality by tea consumption, though longevity might be altered through the preventive effect on age-related diseases.

Nakachi *et al.* (2003) observed the effect of daily consumption of tea on people

over the age of 40 for 13 years. The results showed that lifespan was significantly longer in men and women who consumed over ten cups of green tea every day than that of those who drank fewer than three cups. The mean age of death among men or women who consumed more than ten cups a day was 4.3 and 3.8 years higher, respectively, than those who had fewer than three cups a day. The association between the increased consumption of green tea and the higher age of death was clearly observed in the age of death below 79 among both men and women. They suggested that daily consumption of green tea in sufficient amounts helped to prolong life by preventing premature death.

The quality of elderly people's life might be improved by daily consumption of tea or other antioxidants, though the action of tea on longevity might be slightly less effective than caloric restriction, which has been confirmed to prolong the lifespan of various experimental animals. Many epidemiological studies are needed to clarify the effect of tea consumption on longevity. Moreover, it is necessary to investigate the effect of tea consumption on the cognitive function and motor skills of elderly people.

Mechanism of Action

The mechanism of action of tea against senescence/ageing is little known, because the mechanism and cause of senescence/ageing have not been clarified. However, the accumulation of oxidative damage in critical macromolecules is thought to be an important factor for senescence. The antioxidative activity of tea, especially that of chronic consumption of tea, might afford useful information about the mechanism of action of tea on senescence, because senescence is a long-term process and ROS are constantly produced.

It has been known that vitamin C (ascorbate) scavenges ROS and vitamin E (α-tocopherol) inhibits lipid peroxidation. Tea polyphenols, mainly (–)-epigallocatechin gallate in green and black tea, have more potent antioxidative and radical-scavenging activities on a molar basis than vitamin C or E *in vitro* (Guo *et al.*, 1999; Rice-Evans, 1999; Valcic *et al.*, 2000; Hu and Kitts, 2001; Nakagawa *et al.*, 2002; Hagerman *et al.*, 2003). Green tea catechins are rapidly absorbed and distributed mainly into the mucous membrane of the small intestine, the liver and plasma, and only slightly into the brain (Nakagawa and Miyazawa, 1997). Green tea catechins are excreted into the urine with a half-life of less than 2 h in accordance with the short-term effects on the plasma antioxidant capacity (Young *et al.*, 2002). However, in humans, continuous intake of tea catechins has been reported to bring about an elevation of antioxidant activity in plasma (Kimura *et al.*, 2002) and a decrease in the oxidative damage to DNA, blood proteins and plasma lipids (Young *et al.*, 2002). It has been observed that antioxidative enzymes are induced by the intake of green tea catechins (Khan *et al.*, 1992). These results suggest that long-term tea consumption may effectively suppress oxidative stress. Actually, the level of 8-oxodeoxyguanosine, a marker of oxidative damage of DNA, was significantly suppressed in the liver and kidney of aged mice that had been given green tea catechins in drinking water for a long period (Unno *et al.*, 2004).

Experimental Work

The effect of green tea catechins on cognitive function and brain atrophy

The effect of tea consumption on physiological dysfunctions, especially cognitive dysfunctions occurring as brain senescence, was investigated (Unno *et al.*, 2004). Oxidative damage has long been considered to be a cause of age-associated brain dysfunctions because the brain is believed to be particularly vulnerable to oxidative stress (Forster *et al.*, 1996; Floyd, 1999). To investigate the complex mechanism of senescence, it is necessary to use a suitable animal model. A murine model of accelerated senescence (SAM), developed by Takeda *et al.* (1981), was used for the investigation of the effect

of the intake of green tea catechins on learning and memory functions. SAM consists of two strains: one prone to accelerated senescence (SAMP) and the other resistant to accelerated senescence (SAMR). SAMP10, a substrain of SAMP, is characterized as a model of inherited brain atrophy with cognitive dysfunctions (Shimada *et al.*, 1992). In this study, SAMP10 mice were supplied with water containing 0.02% green tea catechins (Polyphenon 70S, Tokyo Food Techno Co., Ltd) from the age of 1 month. Polyphenon 70S contains about 70% green tea catechins and 0% caffeine. The green tea catechins consist of 31.7% (–)-epigallocatechin gallate, 15.7% (–)-epigallocatechin, 10.0% (–)-epicatechin gallate, 8.5% (–)-epicatechin, 4.5% (–)-gallocatechin gallate and 1.0% (–)-catechin gallate. Each mouse consumed 5–15 ml of catechin water (0.02%) a day. Therefore, the mean dose of green tea catechins was calculated to be about 35 mg/day/kg. The dose corresponded to an intake of over ten cups of tea for humans.

To investigate the effect of tea consumption on learning, a step-through passive avoidance task was carried out according to the method of Yagi *et al.* (1988), using the test system of MST-01S (Muromachi Kikai Co., Ltd). When the mouse entered the dark chamber, the door was closed and an electric foot-shock was delivered at 0.5 mA for 1 s. Acquisition of the avoidance response was judged successful if the mouse remained in the light chamber for 300 s. The trial was repeated until the mouse satisfied the acquisition criterion within five trials. The time spent in the light chamber was reduced from 300 s. That was calculated as a time needed for learning. The time of each trial was summed and expressed as 'learning time'.

The learning time was monitored with 5-, 8- and 11-month-old SAMP10 and SAMR1 mice. The learning time in control SAMP10 mice was significantly longer in 11-month-old SAMP10 mice than in younger SAMP10 mice and aged SAMR1 mice. However, the elongated time for learning was significantly improved in 11-month-old SAMP10 mice when given green tea catechin water (Fig. 19.1).

Then, the effect of tea consumption on memory was investigated. To confirm the avoidance response, the mice underwent the passive avoidance test on the day after the acquisition test described above. One month later, we examined these mice to see whether they could remain in the light chamber for 300 s. The number of mice that were not able to remain in the light chamber was compared between the 6- and 12-month-old SAMP10 mice. The failure ratio was clearly suppressed in the 12-month-old SAMP10 mice given green tea catechins

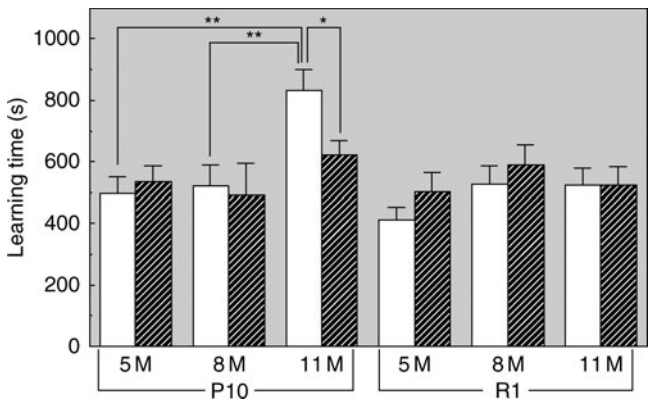

Fig. 19.1. Effect of green tea catechins on learning in senescent SAM mice. The time was measured in mice that had taken green tea catechins (hatched column) and in control mice (open column). Each value represents a mean ± standard error of the mean (SEM) (n = 10–24). An asterisk represents a significant difference (*P < 0.05, **P < 0.01). M, months of age.

Table 19.1. Effect of intake of green tea catechins on the memory of SAMP10 mice.

	Failure ratio (mice entering dark chamber/tested mice)	
Age (months)	Control	GT catechin
6	0.142 (2/14)	0.158 (3/19)
12	0.400 (12/30)	0.152* (5/33)

*A significant difference between the control and 12-month-old mice $P < 0.05$, Fisher's exact and χ^2 tests).

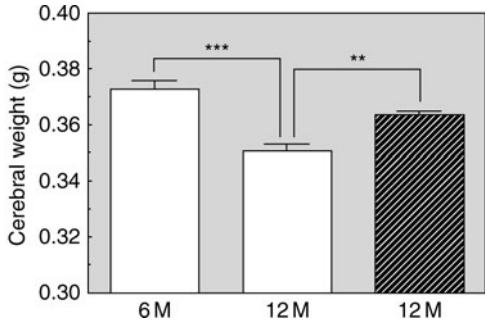

Fig. 19.2. Effect of green tea catechins on cerebral atrophy in senescent SAMP10 mice. Cerebral weights were compared among 6- and 12-month-old control SAMP10 (open column) mice and 12-month-old mice given catechin water (hatched column). Each value represents a mean ± standard error of the mean (SEM) ($n = 22–32$). An asterisk represents a significant difference ($^{**}P < 0.001$, $^{***}P < 0.0001$). M, months of age.

compared with the control mice (Table 19.1). That is, the memory function was maintained in the aged SAMP10 mice given tea catechin water. Besides the step-through test, the effect of tea consumption on working memory was investigated in aged SAMP10 mice using a Y-maze test that was based on the searching behaviour of mice. The result also showed that the intake of green tea catechins improved the lowered cognitive function of aged SAMP10 mice (data not shown).

With advancing age, brain atrophy was observed in SAMP10 mice (Shimada *et al.*, 1992, 1993, 1994). The cerebral weight was significantly lower in 12-month-old control SAMP10 mice than in 6-month-old ones. However, atrophy was clearly suppressed in 12-month-old SAMP10 mice that had been given catechin water (Fig. 19.2).

These results indicate that brain senescence, such as the dysfunction of learning/memory and brain atrophy, was significantly suppressed by long-term intake of green tea catechins. Although distribution of the tea catechins into the brain is thought to be very low (Nakagawa and Miyazawa, 1997), daily consumption of tea might be helpful for vitamins E and C to act cooperatively to prevent the brain from oxidative damage.

SAMP10 mice began to die from the age of about 6 months and the maximal longevity was about 18 months. Although the mortality of SAMP10 mice was not altered by the consumption of green tea catechins (Table 19.2), SAMP10 mice that had drunk tea catechin water were thought to have

Table 19.2. Effect of intake of green tea catechins on the mortality of SAMP10 mice.

		Mortality (dead mice/tested mice)	
Age (months)		Control	GT catechin
12	Male	0.143 (5/30)	0.163 (7/36)
	Female	0.103 (3/29)	0.056 (2/36)
15	Male	0.333 (12/36)	0.333 (10/30)
	Female	0.111 (3/27)	0.111 (2/18)

healthy ageing, because the mice of about 12 months, which were regarded to be past middle age, exhibited improved brain functions and decreased oxidative damage in the liver and kidney. Using SAMP8 mice, another substrain of SAMP, Kumari *et al.* (1997) reported a significant effect of antioxidants comprising a green tea extract, ascorbate, dunaliella carotene, vitamin E and sunflower seed extract on longevity. There are very few data of the effect of tea on longevity and mortality. More cautious

experiments are needed for evaluation of the effect of tea consumption on these parameters.

Human Trials – Impact of Tea Intake

The population of the elderly over 65 years of age has been rising in many countries. All elderly people desire to be exempt from age-related diseases and die of old age with normal healthy ageing. It is not only an individual's problem but also an important social and economic problem to suppress senescence and maintain the healthy life of elderly people. Tea is the most popular beverage other than water. If tea could partly suppress senescence, it would be an easy and beneficial anti-ageing method for many people.

However, further extensive research is required to clarify tea's action against senescence. Epidemiological investigations against age-related diseases, such as cancer, cardiovascular diseases and dementia, have been reported. For comparison of longevity, these data might supply useful information. Evaluation of the action of tea on physiological dysfunctions, which directly concern the quality of life, would be much more difficult. A lot of factors, such as genetic backgrounds, nutritional and environmental conditions and various kinds of stress, are concerned with the progression of senescence. Even in experimental animals, whose genetic background and living conditions are thought to be uniform, the longevity and the rate of brain atrophy differ among individual animals. In humans, the progression of senescence differs greatly among individuals. The difference creates large fluctuations in the statistical data. Thus, large-scale and detailed investigations will be needed to clarify the effect of tea consumption vis-à-vis cognitive dysfunctions and motor skills.

Future Prospects

Among the various age-related alterations, brain dysfunctions are the most detrimental for the quality of life in elderly people. Brain atrophy is observed physiologically in aged humans. Normal brain ageing is suggested to be associated with subtle morphological and functional alterations. In patients with dementia, such as Alzheimer's disease and Creutzfeldt–Jakob disease, much more severe cerebral atrophy has been observed. In studies to clarify the mechanism of senescence/ageing, important alterations have been found in the brain of SAMP10 mice (Moriguchi *et al.*, 1997; Nishiyama *et al.*, 1997; Saito *et al.*, 1999; Shimada, 1999; Okuma *et al.*, 2000; Numata *et al.*, 2002; Shimada *et al.*, 2002, 2003; Miyazaki *et al.*, 2003). SAMP10 mice, with characteristic age-related brain atrophy, would be a useful tool for the study of senescence-related alterations of brain functions. Interestingly, the intake of green tea catechins exhibited some preventive effect on the brain of SAMP10 mice past middle age. Although the mortality of SAMP10 mice was not altered by the intake of green tea catechins, SAMP10 mice appeared to undergo healthy ageing. Tea catechins and other dietary antioxidants are thought to have a preventive effect on neurodegenerative diseases (Esposite *et al.*, 2002). The study of tea and other antioxidants for use against senescence, which has just started, is expected to lead to the development of preventive and therapeutic means to treat senescence and thus maintain the quality of life in an ageing society.

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20 Tea and Oral Health

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Dental Plaque and Caries

Oral diseases, including dental caries, periodontal disease and tooth loss, affect more persons than any other single disease in the USA. Millions of Americans suffer from these diseases, which are the major cause of lost work or school days next to the common cold. Oral diseases and/or disorders can have a significant impact on a person's overall health (National Institute of Dental Research (NIDR), 1989). Recent research has shown that oral bacteria may contribute to increased risk of heart attacks, strokes and lung disease, and may be associated with premature childbirth in some women (Offenbacher *et al.*, 1996).

Dental caries is a multifactorial infectious disease that depends on diet and nutrition, microbial infection and host response. Dental plaque has been implicated as the most important aetiologic agent in the formation of dental caries (Hamada and Slade, 1980). The mutans group of streptococci (MS), including *Streptococcus mutans* and others found prominently in plaque, have been strongly implicated as one of the aetiological agents of dental caries in both humans and experimental animals. MS produce glucosyltransferase (GTF) enzymes, which hydrolyse sucrose (table sugar), leading to the synthesis of sticky water-insoluble glucan polymers, which facilitate the attachment and accumulation of oral bacteria on to saliva-coated tooth surfaces. As time goes on, co-aggregation among oral bacteria takes place, resulting in a complicated plaque community consisting of more than 300 species of bacteria embedded in a chemical and biological matrix firmly adhering to the tooth surface. These plaque bacteria ferment carbohydrates in the environment and produce acids that demineralize the enamel surface, which causes caries formation (Hamada and Slade, 1980). Dental plaque is also one of the causative factors for periodontal disease, a leading cause of tooth loss and dysfunction among the adult population (Theilade and Theilade, 1976).

To prevent dental/oral diseases, plaque control through daily personal oral hygiene and regular professional care is of utmost importance. While mechanical removal by tooth brushing remains the most effective means for plaque control, antimicrobial compounds have been incorporated into toothpastes and mouth rinses as adjuncts to mechanical plaque control. These chemicals may prevent caries or gingivitis by: (i) reduction of existing plaque; (ii) prevention of new plaque formation; (iii) inhibition of the expression of cariogenic determinants; (iv) selective suppression of cariogenic, bacterial species, especially MS; or (v) inhibition of plaque bacterial metabolism (Marsh, 1993). These agents often produce adverse effects, such as staining or increased calculus (tartar) formation, thus justifying further

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research and development of alternative agents that are safe for the host while retaining efficacy. In recent years, public desire for useful natural anti-plaque products has increased.

Anticariogenic Potential of Tea

Besides being a traditional and popular drink, tea has been shown to have a wide range of beneficial physiological and pharmacological effects, including antioxidative, antimicrobial and hypocholesterolaemic activities (Hamilton-Miller, 1995). Depending upon the manufacturing process, teas are classified into three major types: non-fermented green tea, semi-fermented oolong tea and fermented black tea. The chemical composition of tea is fairly complex, consisting of polyphenols, catechins, caffeine, amino acids, carbohydrates, protein, chlorophyll, volatile compounds, fluoride, minerals and other undefined compounds (Graham, 1992).

The first clue to the cariostatic effects of tea came from studies in the 1940s and 1950s showing fluoride to be the active component (Gershon-Cohen, 1954). Earlier reports suggested that tea consumption led to a reduction in dental caries in humans and experimental animals, and that tannins contributed to the inhibitory effect (Elvin-Lewis and Steelman, 1968; Rosen *et al.*, 1984). Despite the positive animal data supporting the positive relationship between tea and dental caries prevention, relatively little attention has been given to this field of research by Western scientists. Instead, the majority of research on tea and its dental relevance has been performed by Japanese researchers. Green tea extracts, or polyphenols, have been reported to inhibit *in vitro* growth, acid production and water-insoluble glucan synthesis by GTF enzyme of *S. mutans* (Elvin-Lewis and Steelman, 1968; Kashket *et al.*, 1985; Hattori *et al.*, 1990; Otake *et al.*, 1991). Similar findings have been reported for oolong tea (Nakahara *et al.*, 1993; Ooshima *et al.*, 1993). Taiwanese green, black and oolong teas have also been shown to inhibit *in vitro* growth of selected cariogenic and periodontal pathogens (Bhalla *et al.*, 1997). A review on the anticariogenic properties of tea has been presented (Hamilton-Miller, 2001). In an adult human study by Wu *et al.* (2001), rinsing with black tea ten times a day for 7 days resulted in significantly less pronounced pH fall, a lower plaque index ($P < 0.05$) and lower numbers of mutans streptococci and total oral streptococci in plaque but not in saliva. Fluoride concentrations in plaque and saliva increased, reaching a maximum at day 7. Black tea and its polyphenols may benefit human oral health by inhibition of dental plaque, acidity and cariogenic microflora.

Tea Polyphenols and Caries

Tea polyphenols, commonly known as catechins, contribute to the observed antioxidant and antimicrobial properties. These compounds act primarily on and damage bacterial membranes, often leading to cell death. The common catechins are: (–)-epigallocatechin gallate (EGCG), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC), (–)-epicatechin (EC), (+)-gallocatechin and (+)-catechins. Among these, EGCG is considered the most potent antimicrobial polyphenolic compound. The important polyphenols in black tea are bisflavonols, theaflavins (found mainly in black tea), theaflagallins and thearubigins.

Animal studies have shown that specific pathogen-free (SPF) rats infected with *S. mutans* and then fed a cariogenic diet containing green tea polyphenols demonstrated significantly lowered caries scores (Otake *et al.*, 1991). Supplementing drinking water of rats with 0.1% green tea polyphenol along with a cariogenic diet also significantly reduced total fissure caries lesions (Sakanaka *et al.*, 1996). Animal studies using oolong tea gave similar results and it was suggested that active substances may affect bacterial virulence factors other than the GTF enzymes (Ooshima *et al.*, 1998).

Drinking tea has also been associated with lower caries levels in humans. Caries were found to be significantly lower among children who drank a cup of tea immediately after lunch and the tea polyphenols, rather than fluoride, were found to be responsible for the anticariogenic effects (Sakanaka *et al.*, 1989). Another study reported that rinsing with 0.2% Chinese green tea while brushing decreased plaque and the gingival index significantly (You, 1993). Tea drinking has been attributed as one of the factors in the declining prevalence of caries in Tunisia (Abid, 2004). Tea extracts have also been shown to inhibit human salivary amylase and tea consumption may reduce the cariogenic potential of starch-containing foods, such as biscuits and cakes, because tea may reduce the tendency for these foods to serve as slow-release sources of fermentable carbohydrate (Zhang and Kashket, 1998). It is likely that cariogenic challenge in a cariogenic diet may be overcome by the simultaneous presence of green tea in the diet.

So far, a majority of the research on antimicrobial activity and oral health benefits of teas has focused on green tea, while less attention has been directed to fully fermented black tea. Worldwide, 80% of the tea consumed is black tea, which is also a popular drink in Europe and North America. An anticariogenic potential of black tea has been suggested in various *in vitro* studies (Bhalla *et al.*, 1997; Wei *et al.*, 1999; Sarkar *et al.*, 2000). Black tea and its polyphenols inhibited growth, acid production, metabolism and GTF enzyme activity of MS and dental plaque bacteria.

Tea Fluoride and Caries

The incidence and severity of dental caries have decreased in recent years due in part to the use of fluoride (F) (Maitheler, 1984; Wei, 1985). The multifactorial nature of the caries process allows fluoride to interact on several levels. One interaction is directly with the hard tissue – enamel/dentin/root. In this regard, the incorporation of fluoride into the tooth due to the demineralization of soluble mineral and remineralization of less soluble crystals may lead to an acid resistance to further decay. This process occurs at low concentrations of fluoride when present at the reacting sites. Low concentrations may also affect bacterial metabolism via inhibition of the enzyme enolase. Large concentrations of fluoride are needed to affect various aspects of microbial adherence, growth, agglutination and metabolism (Wefel, 1981). These high concentrations may accumulate in plaque over time but are not normally found in water, drinks or food. Professionally applied fluorides, dentifrices and rinses have all been used to provide higher levels of fluoride. Fluoride is currently thought to exert itself more as a topical agent than a systemic one. As such, its ability to prevent demineralization and enhance remineralization is recognized as one of its major mechanisms of action.

Teas are a source of fluoride as well as many other dietary trace elements. The caries-preventive effect of teas was first believed to be due to its fluoride content. More recent studies, however, have pointed out that the polyphenol contents of tea may affect plaque formation and metabolism as well (Yu *et al.*, 1995). The commercial tea plant, *Camellia sinensis*, takes up F from the soil by passive diffusion and concentrates it in the leaves by transpiration (Wei *et al.*, 1989). Due to differing soils, types of tea leaves, infusion times and methods of analysis, a great deal of variation in tea content has been found. Coupled with the various drinking habits among different people, it is very difficult to calculate the contribution of tea to total F intake. This amount is important, however, as a means of predicting caries prevention and as a possible significant contributor to dental fluorosis. The ingestion of too much fluoride during tooth development may lead to dental fluorosis.

A recent animal study showed that rats consuming black tea (prepared from fluoride-free water) over a 2-week period had a significantly lower rate of caries than those consuming non-fluoridated water. Furthermore, the caries scores in the group receiving tea were significantly greater than

those in the group receiving fluoridated water. The authors suggested that black tea consumption attenuates the development of caries in young, caries-prone rats (Touyz and Amsel, 2001).

Infusion of tea leaves results in a variety of fluoride concentrations due to types of tea, infusion times and analytical techniques. Wei *et al.* (1989) found a 15-min infusion to result in a mean F concentration of 1.75 p.p.m. for 15 Chinese teas, 1.24 p.p.m. for 11 Ceylon/Indian teas and a negligible F amount for six herbal teas. The bioavailability of fluoride in tea has been said to be approximately 85%. Most studies on fluoride consumption from tea conclude that the total fluoride intake needs to be assessed prior to supplementation (Kiristy *et al.*, 1996). A review by Kavanagh and Renehan (1998) lists over ten papers that measured the fluoride content of various teas. Several studies have also calculated the total F intake from tea consumption. Jenkins (1991) measured the highest tea consumers he could find and calculated a total of 9 mg that could be consumed. When using the assumptions of 2.5 cups/day, 150 ml per cup and 2.2 p.p.m. F diluted in half with milk for children, an ingested range of 0.1 mg to 1.08 mg was found. Wei *et al.* (1989) estimated a daily F intake at a level of 1.05 mg. These authors also state that 'tea contributes up to one-fifth (0.33 mg) of the optimal daily F intake if an 8-year-old child (27 kg) consumes one cup of tea'. With regard to dental fluorosis, the total amount of fluoride consumed on a daily basis in children undergoing tooth formation is most critical and therefore the early use of high F-containing products needs to be done in moderation. With regard to inhibition of dental caries, the amount of fluoride retained in the mouth after rinsing may be the more important parameter. This fluoride may bind to the enamel and salivary pellicle components, which could lead to local topical effects if the concentration becomes sufficient (Simpson *et al.*, 2001).

The optimal ingestion of fluoride has been stated to be 0.05–0.07 mg F/kg body weight. An acute lethal dose has been calculated as 35–70 mg F/kg body weight. It is very unlikely that enough tea could be consumed at one time to cause a lethal overdose. Dental fluorosis, however, may occur when the water fluoride levels exceed twice the optimal. Because dental fluorosis can only occur during tooth development, the over-ingestion of fluoride will be reflected in those teeth undergoing active mineralization. As shown by Wei *et al.* (1989), teas are unlikely to cause fluorosis by themselves, but they may be significant contributors to the total fluoride intake of children.

Tea and Gingivitis/Periodontal Disease

Green tea catechin has been shown to be bactericidal against *Porphyromonas gingivalis* and *Prevotella* spp. *in vitro*. The combined use of mechanical treatment and the application of green tea catechin using a slow-release local delivery system was found to be effective in improving periodontal status, including the reduction in pocket depth and the suppression of peptidase activities in the gingival crevicular fluid (Hiasawa *et al.*, 2002). Tea catechins containing the galloyl radical (ECG and EGCG) possess the ability to inhibit both eucaryotic and procaryotic cell-derived collagenase, an enzyme that plays an important role in the disruption of the collagen component in the gingival tissues of patients with periodontal disease (Osawa, 1991; Demeule *et al.*, 2000; Maeda-Yamamoto, 2003).

Catechin derivatives have been reported to inhibit certain proteases of *P. gingivalis* and may reduce periodontal breakdown (Okamoto *et al.*, 2004). Green tea catechins EGCG have also been shown to inhibit protein tyrosine phosphatase in *Prevotella intermedia* (Okamoto *et al.*, 2003). EGCG has been reported to inhibit production of toxic metabolites of *P. gingivalis* (Sakanaka and Okada, 2004). Zhu *et al.* (2003) have shown that purified tea polyphenols inhibited *in vitro* growth and H_2S production of *P. gingivalis* and *Fusobacterium nucleatum* associated with human halitosis. Schwartz *et al.* (2005) have studied the molecular and cellular effects of green tea on oral cells of smokers. During the course of green tea

administration, smoking-induced DNA damage was decreased, cell growth was inhibited and the percentage of cells in the S phase was reduced, although accumulation of cells in the G1 phase was observed. DNA content also became more diploid and the presence of biochemical markers for apoptosis increased. Drinking green tea reduced the amount of DNA damaged by inducing cell growth arrest and apoptosis. Although a high amount of tea catechins may be consumed by a tea drinker on a daily basis, it is not known whether effective concentration of tea catechins may be present in the gingival crevicular fluid or the circulating blood. A recent human study investigated the effect of tea polyphenols in the form of chew candies on gingival inflammation over a 4-week period (Krahwinkel and Willershausen, 2000). The approximal plaque index (API) and sulcus bleeding index (SBI) were determined at the end of day 7 and day 28. These authors suggested that tea polyphenols might exert a positive influence on gingival inflammation. However, based on data presented, no statistically significant differences were noted between the test group and the placebo group. Further laboratory and controlled clinical studies are warranted in this respect.

Conclusion

Although there has been a substantial amount of research related to the study of teas and their health benefits, it has been difficult to compare data between laboratories due to the lack of standardization in experimental procedures. Teas used in studies often differed in their types, sources, method of manufacture and procedures for extraction. Analytical data of tea preparations were often not specified or provided, making the comparison of *in vitro* or *in vivo* data difficult among laboratories. Improvement in this aspect and encouragement in designing new multidisciplinary research approaches will strengthen our knowledge concerning this ancient beverage with its many health attributes. At present, the use of tea in clinical application is still a long way from reality, and further controlled clinical trials in humans are warranted. Furthermore, consumption of tea may have added oral health benefits by controlling 'through prevention' the most prevalent infectious disease of humankind, namely caries (Anon., 2001a, b). With the added dental health implication among many other bioregulatory functions, tea can be considered as a functional food for oral health.

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