Review Article

Cancer prevention by tea and tea polyphenols

Chung S Yang PhD, Jihyeung Ju PhD, Gary Lu PhD, Hang Xiao PhD, Xingpei Hao PhD, Shengmin Sang PhD and Joshua D Lambert PhD

Rutgers, The State University of New Jersey, Department of Chemical Biology, Ernest Mario School of Pharmacy, Piscataway, NJ, USA

The inhibition of tumorigenesis by tea extracts and tea polyphenols has been demonstrated in different animal models, including those for cancer of the skin, lung, oral cavity, esophagus, stomach, small intestine, colon, bladder, liver, pancrease, prostate, and mammary glands. Caffeine is also active in inhibition of tumorigenesis on the skin, lung, and perhaps other organs. In spite of many *in vitro* and *in vivo* studies, the molecular mechanisms for the cancer preventive actions of these compounds are not clearly known. The relationship between tea consumption and cancer risk has not been conclusively demonstrated, and the relationship may become more clear if we consider the effects of specific types of tea, at defined doses, in populations with certain dietary patterns or genetic polymorphisms. Human intervention trials and large prospective studies are needed to further assess the cancer preventive activities of tea constituents.

Key Words: Tea, cancer prevention, polyphenols

INTRODUCTION

Tea, made from the dried leaves of plant *Camellia sinensis*, is a very popular beverage in Asia. Green tea is produced by steaming or pan-frying fresh tea leaves, which inactivates the enzymes and prevents the oxidation of tea constituents. A typical brewed green tea (2 g of tea leaves in 200 mL of hot water) contains 500-700 mg of water extractable materials, of which 30-40% (by dry weight) are catechins. (−)-Epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin, (−)-epicatechin-3-gallate, and (−)-epicatechin are the major tea catechins. Black tea is produced by a process known as fermentation, in which the tea leaves are crushed to promote enzymatic oxidation and subsequent condensation of tea catechins, leading to the formation of oligomeric polyphenols (theaflavins) and polymeric polyphenols (thearubigins). Black tea contains 2-6% theaflavins, >20% thearubigins, and 3-10% catechins in the water-extractable material. Oolong tea is produced by partial fermentation and contains less catechins but contains theasinensins and other polymerized catechin derivatives than green tea. Tea leaves also contain flavonols, such as quercetin and myricetin, as well as nitrogenous compounds, such as caffeine and theobromine. Caffeine accounts for 2-5% of the water-extractable material in green, oolong, and black teas.

The possible cancer preventive activity of tea has received a great deal of attention in recent years. This chapter briefly reviews the available information on the inhibitory effect of tea constituents on tumorigenesis in animal models, possible effects of tea consumption on human cancers, and proposed mechanisms of action of tea constituents. Results from our own laboratory are discussed in more detail to serve as examples of some of the studies in this field of research.

Inhibition of Tumorigenesis in Animal Models

Tea and its constituents have been demonstrated to inhibit tumorigenesis in different organ sites including the lung, oral cavity, esophagus, stomach, small intestine, colon, skin, prostate, mammary glands, liver, pancreas, and bladder. Out of the 132 reported studies, 118 showed an inhibitory effect. Some of the results have been reviewed previously.[1-4] The following is a brief review of some recent studies.

Lung

More than 20 studies on the effect of tea on lung tumorigenesis have been published. In most of these studies, lung tumors were induced by chemicals, such as the tobacco specific and environmental carcinogens, (4-methylnitrosamino)-1-(3-pyridyl)-1-butanon (NNK), and benzo[a] pyrene. Administration of green tea, black tea, EGCG, or theaflavins during the initiation or promotion stages was shown to significantly decrease NNK-induced lung tumorigenesis in rats, mice, and hamsters.[2,5] Treatment with green tea and black tea also inhibited the spontaneous formation of lung tumors in A/J mice.[5] The inhibitory activity of caffeine on lung tumorigenesis was demonstrated in an NNK-induced rat lung tumorigeneisis model, and caffeine may account for most of the inhibitory activity in black tea.

Corresponding Author: Dr. Chung S. Yang, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854-8020, USA
Tel: +732 445 3400 #248; Fax: + 732 445 0687
Email: csyang@rci.rutgers.edu or csyang164@yahoo.com

In our recent study, oral administration of 0.5% Polyphenon E (PPE) or 0.044% caffeine in drinking fluid inhibited the progression of NNK-induced lung ade nomas to adenocarcinomas in A/J mice.[6] PPE is a standardized green tea polyphenol preparation containing 65% EGCG, 25% other catechins, and 0.6% caffeine. Immunohistochemical analysis showed that PPE and caffeine treatment inhibited cell proliferation, enhanced apoptosis, and decreased levels of c-Jun and Erk1/2 phosphorylation in adenocarcinomas. In the normal lung tissues, neither agent had a significant effect on cell proliferation or apoptosis. Previously, we also showed that oral administration of 0.6% green tea as drinking fluid inhibited NNK-induced lung tumor formation, which was associated with enhanced apoptosis and decreased angiogenesis in lung adenomas.[5]

Digestive tract

All 14 studies on the upper digestive tract (oral cavity, esophagus, and stomach) have demonstrated inhibitory activity of different tea preparations.[4] The inhibitory effects of tea and tea polyphenols on intestinal tumorigenesis in mice have also been consistently observed in different laboratories.[4] Administration of EGCG, at doses of 0.08% or 0.16% in drinking fluid, significantly decreased small intestinal tumor formation, but caffeine did not inhibit intestinal tumor formation. In another experiment, small intestinal tumorigenesis was inhibited in a dose-dependent manner by oral administration of 0.02% to 0.32% EGCG. Western blot analysis indicated that oral administration of EGCG resulted in increased levels of E-cadherin as well as decreased levels of nuclear β-catechin, c-Myc, phospho-Akt, and phospho-Erk in the small intestinal tumors.[7] Other studies indicated that treatment with PPE and EGCG decreased intestinal cell proliferation, increased apoptosis, and decreased nuclear β-catenin and phospho-Akt levels in the $Apc^{Min/+}$ mice. In azoxymethane (AOM)-treated mice, oral administration of green tea or EGCG also inhibited aberrant crypt foci (ACF) and tumor formation (unpublished).

The effects of tea preparation on colon tumorigenesis in rats, however, have not been consistent.[4] The lack of a consistent protective effect of polyphenols against colon carcinogenesis is rather surprising because the intestine is considered to be a promising site for chemoprevention with polyphenols that have low systemic bioavailability. EGCG, the major polyphenol in green tea, has only limited systemic bioavailability after oral ingestion. Even the absorbed EGCG is excreted mostly into the intestine through the bile. Therefore, the intestine may actually be exposed to high levels of EGCG after ingestion. Our recent results showed that administration of PPE in the diet to AOM-treated rats effectively inhibited the formation of colon ACF, adenomas, and adenocarcinomas (unpublished).

Skin

Protective activities of tea against skin cancer have been studied extensively in UV-induced or chemically induced tumorigenesis models in mice.[4] The results showed that both tea polyphenols and caffeine, when applied topically to the skin, inhibit skin carcinogenesis. However, when tea polyphenols are administered orally, their low bioavailability in the skin may limit the inhibitory effect; therefore, the contribution of caffeine is more important to the inhibition carcinogenesis. The studies by Conney et al. indicated that caffeine inhibits UVB-induced carcinogenesis in SKH-1 mice by enhancing apoptosis of DNA damaged cells, premalignant cells, and cancer cells, possibly by inhibiting the p53-independent ATR/Chk-1 pathway.[8]

Prostate

Gupta et al.[9] reported that oral infusion of green tea polyphenols significantly inhibited tumor incidence and burden in the prostate as well as the metastases of the tumor to distant sites in an autochthonous transgenic adenocarcinoma of the mouse prostate (TRAMP) model. This inhibition is associated with decreasing insulin-like growth factor (IGF)-1 level and inhibiting the phosphorylation of Akt and Erk 1/2.[10] It is not clear whether tea polyphenols inhibit prostate carcinogenesis by a direct action of tea polyphenols that are bioavailable to prostate or by an indirect action such as by affecting androgen metabolism or by affecting circulating serum IGF-1 levels

Mammary

There are a total of 13 studies on the effect of tea on mammary tumorigenesis; 8 of the studies showed inhibitory effects but 5 studies did not. The reason for the discrepancy is not clear. One possible factor that contributes to the lack of an inhibitory effect is the suspected low bioavailability of tea polyphenols in the mammary tissues. The observed inhibitory effect of tea on mammary tumorigenesis may be due to an indirect action of tea, such as affecting lipid absorption and estrogen metabolism.[4]

Liver

There are a total of 8 studies on the effect of tea on liver tumorigenesis, and 7 of the studies showed significant inhibitory activities of tea on formation of hepatic tumors or preneoplastic foci. For example, oral administration of black and green tea was shown to decrease the incidence of NNK-induced liver tumors in rats and the multiplicity of diethylnitrosamine-induced liver tumors in mice.[4]

Bladder and pancreas

There are 4 studies that showed inhibitory activity of tea on bladder cancer. For example, oral administration of green tea or green tea polyphenols during the promotion or entire experimental period inhibited N-butyl-N-(4-hydroxybutyl)-nitrosamine-induced urinary bladder tumors in rats. There are 3 studies that showed inhibitory activity of tea on nitrosamine-induced pancreatic cancer and related ductal lesions in hamsters.[4]

MECHANISTIC STUDIES ON THE ACTIVITIES OF EGCG IN CELL LINES

Many studies on the mechanisms of action of EGCG and other catechins have been conducted in cell lines, and this topic has been reviewed previously.[2,4,10,11] The proposed mechanisms include the inhibition of MAP kinases and PI3K/AKT pathways, NFκB- and AP-1-mediated transcription, growth factor-mediated signaling, aberrant ara-

chidonic acid metabolism, proteinase activities, and other activities. The end result of these effects may be the inhibition of tumor cell growth or induction of apoptosis. Some of these activities have been demonstrated to be associated with the inhibition of carcinogenesis in animal models. In most cases, however, the concentrations of EGCG required to observe these biological effects *in vitro* exceed the concentrations achievable in plasma and tissues by 10- to 100-fold, and questions remain concerning the relevance of these *in vitro* observations to the mechanisms of the cancer-preventive activities *in vivo*.[4,11]

In general, if an effect can be observed *in vitro* at concentrations lower or similar to those observed *in vivo*, then the event may occur *in vivo*. However, there are big differences between the effective concentrations determined with pure enzymes and those in cell lines or tissues, possibly due to the non-specific binding of EGCG to many proteins and the limited amount of EGCG that can enter the cells. When a small amount of pure enzyme is used in an enzymatic assay, inhibition may be observed with nanomolar concentrations of EGCG, but it may take much higher concentrations of EGCG to inhibit the activity in cell lines or tissues. This point is illustrated in the inhibition of 20 s proteasome chymotryptic activities by EGCG; i.e. the IC_{50} observed in a cell-free system was 0.1–0.2 μM, but it was 1–40 μM in tumor cell lines. EGCG was reported to bind to the 67-kDa laminin receptor with a K_d of 0.04 μM, to vimentin with a K_d of 3.3 nM, and interact with Bcl-2 with a K_i of 0.33 μM, but it required much higher concentrations of EGCG to cause growth inhibition and induce apoptosis.[4] In all these studies, there were experiments demonstrating the biological relevance of the effects in their specific experimental systems. The general applicability of these mechanisms for cancer prevention is still not known.

Another concern in the use of redox-sensitive compounds in a cell culture system is the oxidation and stability of the compound. When added to the cell culture medium, EGCG is oxidized to produce superoxide radicals and H_2O_2. We have demonstrated that, depending on the cell lines and culture conditions, the EGCG-induced apoptosis can be completely or partially blocked by the addition of catalase in the culture medium, suggesting that the apoptosis is mediated by H_2O_2. Autooxidation of EGCG generated reactive species which may inactivate the epidermal growth factor receptor in cells in culture.[4] It is not clear whether the EGCG autooxidation-induced effects occur inside animal tissues, because these tissues are endowed with anti-oxidative enzymes and are usually under lower oxygen partial pressure than the cell culture medium.

Based on the above discussions, we summarize our understanding of the mechanisms of cancer prevention by EGCG as follows: 1) multiple mechanisms are likely to be involved in different experimental systems; 2) some of the proposed mechanisms based on studies in cancer cell lines may not be relevant to cancer prevention; 3) mechanisms of cancer prevention need to be demonstrated in relevant models or human tissues; and 4) many of the observed effects are probably secondary events or down-stream events and it is important to identify the direct targets of EGCG action.

TEA CONSUMPTION AND HUMAN CANCER

The relationship between tea consumption and human cancer risk has been reviewed in many articles. In 1991, the Working Group of the International Agency for Research on Cancer (IARC) reviewed the effects of tea on cancers of different sites and concluded that "there is inadequate evidence for the carcinogenicity in human and experimental animals of tea drinking".[12] In 1993, we reviewed more than 100 published papers and summarized that, while some studies showed a negative association between tea consumption and cancer risk, others showed no association or positive association.[1] We recently reviewed approximately 200 epidemiological studies regarding the association between tea consumption and human cancer risks of different organ sites.[4] The inhibitory effect was more frequently observed in case-control studies (than cohort studies) in Asia where green tea is consumed. However, there are more reports that did not show a cancer preventive effect than those that show a preventive effect of tea consumption. Early case-control studies on the stomach cancer preventive activities of tea encouraged many other studies on this topic, but the results from subsequent cohort studies did not confirm such an effect.[13] Because of the difficulties in accurately assessing the quantity of tea consumption, the possible different etiological factors for cancer in different populations, and the metabolic differences in the handling tea constituents among individuals, a cancer preventive effect may only be observed when tea consumption is better quantified or in certain sub-populations, but not in the general population. For example, the cancer preventive effect of green tea against gastric and colon cancers was observed when urinary metabolites of tea catechins, such as epigallocatechin and 4'-*O*-methylepigallocatechin, were used as indicators of tea consumption. Green tea consumption was associated with the prevention of esophageal cancer only in women or nonsmokers, lung cancer only in nonsmoking women, and breast cancer only among women with low activity allele of catechol-*O*-methyltransferase or high-activity angiotensin-converting enzyme.[4]

Intervention studies are a more effective way to determine whether tea preparation reduces the risk for certain types of cancer. For example, a recent double-blind study by Bettuzzi *et al*.[14] followed 200 individuals with high-grade prostate intraepithelial neoplasia (PIN) that were given either 600 mg of green tea catechins daily or placebo (100 individuals in each group) for 12 months. Only 3% of the patients in the catechin treatment group developed prostate cancer, whereas the rate of cancer development in the placebo group was 30%. No side or adverse effect was associated with the treatment. These results are very exciting, and their impact would be tremendous if the results could be reproduced in similar trials with a larger number of subjects.

CONCLUDING REMARKS

Although animal studies have demonstrated the broad cancer preventive activities of tea constituents in different

organs, results from human studies are not consistent. This discrepancy may be related to the higher doses of tea preparation used in animal studies in comparison to those consumed by humans. Unlike studies done on free living humans, animal studies are generally optimized for the detection of an experimental effect. The results of epidemiological studies are affected by the lack of accuracy in assessing tea consumption, the different etiological factors for cancer in different populations, the individual differences in genetic polymorphism, the lifestyles associated with tea consumption in different cultures, and other confounding factors. More clear-cut results may be obtained if the quantity of tea consumption could be measured more accurately; the etiological factors and the status of smoking and the drinking of alcoholic beverage are better known; and relevant genetic polymorphism are considered. Large cohort studies on this topic are needed. Even though the results from epidemiological studies on tea and cancer prevention are not conclusive, tea constituents could still be used for cancer prevention (or treatment of precancers) at selected organ sites if such activity can be demonstrated in human intervention trials. More studies of this type, as well as of oral and colon cancer prevention, with well defined preparations of tea constituents in high risk populations are needed.

AUTHOR DISCLOSURES

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