Cancer Preventive Activities of Tea Polyphenols

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ABSTRACT

The cancer preventive activities of tea (*Camellia Sinensis* Theaceae) have been investigated extensively. Green tea polyphenols have been shown to inhibit tumorigenesis in different animal models, including those for cancers of the lung, oral cavity, esophagus, stomach, small intestine, colon, bladder, liver, pancreas, skin, prostate and mammary glands. Enhancement of apoptosis, suppression of cell proliferation, and inhibition of angiogenesis have been shown to be associated with the inhibition of carcinogenesis by tea polyphenols in animals. Many studies in cell lines have demonstrated the modulation of signal transduction and metabolic pathways by (-)-epigallocatechin-3-gallate (EGCG), the most abundant and active polyphenol in green tea. The cancer preventive activity of green tea in humans, however, has not been conclusively demonstrated in epidemiological studies. The relationship between tea consumption and cancer risk may become more clear if we could better quantify the tea consumption, adjust for confounding factors and consider genetic polymorphisms of the population. Ongoing human studies on the prevention of prostate, oral, lung, colon, and breast cancers by tea polyphenols are expected to yield more information on this important topic.

Key words: Tea, Polyphenols, EGCG, Cancer prevention

INTRODUCTION

Tea, made from the leaves of the plant *Cadamellia sinensis*, is a popular beverage worldwide. The relationship between tea consumption and cancer has been extensively studied and reviewed[1-3]. The cancer preventive activities of green tea and its constituents have been demonstrated in many animal models. Numerous cell line studies have been carried out in an attempt to understand the mechanisms of the anti-cancer actions of tea constituents. Most of the studies have been conducted with green tea and green tea polyphenols, especially the most abundant and biologically active (-)-epigallocatechin-3-gallate (EGCG). The structure of EGCG is shown in Figure 1. This article will discuss the inhibitory activities of tea and tea catechins against tumorigenesis in animal studies, the possible mechanisms involved, and possible implications in humans.

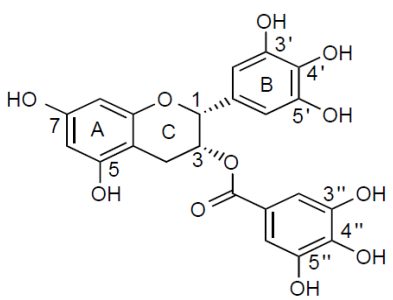

Figure 1. Structure of (-)-Epigallocatechin-3-Gallate (EGCG).

INHIBITION OF TUMORIGENESIS IN ANIMAL MODELS OF THE ORAL DIGESTIVE TRACT

The epithelial cells in the digestive tract have the advantage (over internal organs) of having direct contact with the catechins that are ingested orally. Inhibitory effects of tea against tumorigenesis in the oral cavity, esophagus, stomach, small intestine, and colon have been shown in more than 30 studies[2,3]. For example, in our studies on oral cancers, tea preparations were shown to inhibit 7,12-dimethylbenz[a]anthracene-induced carcinogenesis in the hamster cheek pouch and *N*-nitrosomethylbenzyl-amine-induced esophageal carcinogenesis in rats. EGCG also inhibited tumorigenesis in rat stomach and forestomach induced by *N*-methyl-*N*′-nitro-*N*- nitrosoguanidine. Black tea polyphenols (polyphenon B), given at 0.05% in the diet, also effectively inhibited forestomach tumor formation; this inhibition was associated with increased apoptosis as well as reduced cell proliferation, infiltration and angiogenesis[2,3].

The inhibitory effects of tea and tea polyphenols against intestinal tumorigenesis have been consistently observed in mouse models in different laboratories[2,3]. For example, we showed that administration of EGCG at 0.02 - 0.32% in drinking water dose-dependently inhibited spontaneous small intestinal tumorigenesis in *Apc*$^{Min/+}$ mice, while caffeine did not show an inhibitory effect[4]. The inhibition was associated with increased levels

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of E-cadherin on the plasma membrane, as well as decreased levels of nuclear β-catenin, c-Myc, phospho-Akt, and phospho-Erk in the tumors[4]. Administration of green tea extracts (0.6% in drinking fluid) also inhibited the formation of azoxymethane (AOM)-induced aberrant crypt foci (ACF) in CF-1 mice on a high-fat diet[5]. Recently, Shimizu *et al.*[6] demonstrated the inhibition of AOM-induced ACF formation in male C57BL/KsJ-*db/db* mice by EGCG (0.01 and 0.1% in drinking water) through suppressing the activities of the insulin-like growth factor (IGF)/IGF-1R axis. The elevated levels of IGF-1R, phospho-IGF-1R, phospho-GSK-3*β* and β-catenin in the colonic mucosa were decreased by treatment with EGCG; also decreased were the plasma levels of IGF-1, insulin, triglyceride, cholesterol, and leptin[6].

In rat models, the effects of tea preparations on colon tumorigenesis have not been consistent[2,3]. This inconsistency in colon carcinogenesis is rather surprising, because the intestine is considered to be a promising site for chemoprevention with polyphenols that have low systemic bioavailability[7]. Orally ingested EGCG has only limited systemic bioavailability, with most of it passing through the colon; and the absorbed EGCG is excreted mostly through the bile into the intestine. Our recent animal study showed that, after injection with AOM, treatment of rats with Polyphenon E (PPE, a standardized green tea polyphenol preparation containing 65% EGCG, 25% other catechins, and 0.6% caffeine), 0.12 or 0.24% in the diet for 8 weeks, dose-dependently decreased the total number of ACF per rat. The inhibitory activity was associated with decreased levels of nuclear β-catenin and cyclin D1, and increased levels of retinoid X receptor *α* (RXRα), in the ACF with high-grade dysplasia[8]. After treatment with 0.24% PPE for 34 weeks, the incidence of adenocarcinoma decreased from 57 to 23%, and the multiplicity of adenocarcinoma and adenoma decreased by 80 and 45%, respectively (Yang, C. S. *et al.,* unpublished). Loss of expression of RXRα was observed in colonic dysplastic ACF, adenomas, and adenocarcinoma, but the RXRα expression was (partially) retained in PPE treated rats in these lesions.

INHIBITION OF LUNG TUMORIGENESIS IN RODENT MODELS

The inhibitory effects of tea preparations against lung tumorigenesis have been demonstrated in at least 20 studies[2,3]. Most studies used (4-methylnitrosamino)-1-(3- pyridyl)-1-butanone (NNK) or benzo[a]pyrene as the carcinogen. Oral administration of extracts or solutions of green tea, black tea, EGCG, (-)epigallocatechin (EGC) or theaflavins (from black tea) significantly decreased lung tumorigenesis in rats, mice, or hamsters[2,3]. Treatment of A/J mice with extracts of green or black tea for 60 weeks also inhibited the spontaneous formation of lung tumors and rhabdomyosarcomas. In addition, oral administration of green tea infusion reduced the number of lung colonies of mouse Lewis lung carcinoma cells in a metastasis model.

Chung and coworkers demonstrated that caffeine effectively inhibited NNK-induced lung tumorigenesis in rats, and that the inhibitory effect of 2% black tea (containing 680 ppm caffeine) against lung tumorigenesis due to caffeine[9]. This conclusion is different from the experiments with A/J mice, which demonstrated the inhibition of lung tumorigenesis by decaffeinated green and black tea preparations[10]. A possible interpretation of this difference is that the systemic bioavailability of tea polyphenols in mice is much higher than in rats[7]. In our recent study, administration of 0.5% PPE or 0.044% caffeine in the drinking water, to tumor-bearing A/J mice (induced by a single dose of NNK 20 weeks earlier) for 32 weeks, inhibited the progression of lung adenomas to adenocarcinomas[11]. Imunohistochemical analysis showed that PPE and caffeine treatment inhibited cell proliferation in adenocarcinomas, enhanced apoptosis, and decreased levels of c-Jun and phospho-Erk1/2. In the normal lung tissues, neither agent had a significant effect on cell proliferation or apoptosis, suggesting that the effect is selective against tumor cells. These results demonstrate the broad inhibitory activity of tea preparations in the inhibition of lung neoplasia at different stages of carcinogenesis.

In our effort in looking for agents that can generate synergistic inhibitory actions, together with tea polyphenols, we recently demonstrated the synergistic inhibitory action of a combination of PPE and the cholesterol-lowering agent, atorvastatin (trade name Lipitor), against NNK-induced lung carcinogenesis in A/J mice[12]. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. The synergistic action of this combination was also demonstrated in human lung cancer H1299 and H460 cells. In both the cell lines and the mouse lung tumors, downregulation of the anti-apoptotic proteins MCL1 and BCL-X_L and induction of apoptosis were associated with the synergistic inhibitory action[12]. The possible synergistic actions between atorvastatin and tea in humans warrant future investigation.

INHIBITION OF PROSTATE TUMORIGENESIS IN MOUSE MODELS

Mukhtar *et al.* demonstrated that administration of a green tea polyphenol infusion (0.1% in drinking water) to transgenic adenocarcinoma of the mouse prostate (TRAMP) mice for 24 weeks markedly inhibited prostate cancer development and distant site metastases[13,14]. The inhibition was associated with increased apoptosis, decreased cell proliferation, decreased IGF-1 level, and restored IGF binding protein 3 (IGF-BP3) in both serum and the dorso-lateral prostate[13,14]. This modulation of IGF-1 and IGF-BP3 levels was associated with reduced

levels of phosphotidylinositol 3-kinase (PI3K) as well as phosphorylated forms of Akt, ERK1 and ERK2. The green tea polyphenol treatment also significantly decreased levels of angiogenic and metastatic markers[14]. These results suggest that the inhibition of the IGF-1 signaling pathway, such as vascular endothelial growth factor A (VEGFA) and matrix metalloproteinases, contribute to the cancer prevention activity of green tea polyphenols. Caporali *et al.*[15] reported similar inhibitory activity of orally administered green tea catechins on prostate tumor formation in the TRAMP model. The IGF-1 signaling pathway appears to be a key target for the inhibition; it is not clear whether tea polyphenols inhibit IGF-1 pathway by a direct action of tea polyphenols that are present in the prostate or by indirect or systemic actions.

INHIBITION OF MAMMARY TUMORIGENESIS

There are at least 10 studies on possible inhibitory effects of tea against mammary tumorigenesis, but the results were inconsistent[2,3]. For example, in one study, tea catechin administration in the diet only reduced the volume of mammary tumors[16]. In a second study, green tea was found to increase the latency to first mammary tumor, but did not affect the tumor multiplicity[16]. In our study, even at a high dose of 1,000 mg/kg b.w./day, *i.g.*, EGCG only slightly decreased mammary tumor incidence and multiplicity (statistically nonsignificant) in rats treated with *N*-nitrosomethylurea[17]. The lack of robust inhibition against mammary tumorigenesis is likely to be due to 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. For example, Rogers *et al.*[18] showed no significant inhibitory effect of black tea administered during the promotion stage of 7,12-dimethylbenz[*a*]-anthracene-induced mammary tumorigenesis in rats maintained on AIN76 diet. However, in rats on a high-fat diet, black tea was found to reduce the tumor number and size. The results suggest that black tea may affect fat absorption and metabolism, which subsequently influence estrogen metabolism and mammary tumorigenesis.

INHIBITION OF CARCINOGENESIS IN OTHER ORGAN SITES

Green tea has shown efficacy against rat bladder cancer induced by N-(4-hydroxybutyl)-*N*-butyl-nitrosame (OH-BBN)[19,20]. In our study, PPE was administered (100 or 250 mg/kg b.w./day, intragestrically) to rats at 126 days of age (1 week after the final dose of OH-BBN of a total of 16 doses in 8 weeks). Palpable urinary bladder tumor incidence was reduced from 59% (control) to 40% (by 100 mg/kg) or 18% (by 250 mg/kg)[17].

Catechins are not the only cancer preventive constituents in tea; caffeine has also been shown to inhibit lung and skin carcinogenesis in mouse models[9,11,21]. The mechanism of action of caffeine in the inhibition of skin tumorigenesis has been thoroughly studied and discussed by Conney *et al.*[21]

POSSIBLE MECHANISMS FOR THE CANCER PREVENTIVE ACTION OF TEA POLYPHENOLS

Many mechanisms have been proposed for the cancer prevention by tea constituents, and this subject has been reviewed recently[3]. It is uncertain whether all these proposed mechanisms are relevant for cancer prevention *in vivo*. Apparently, mechanisms derived from cancer prevention studies in animal models should be more relevant. These include the induction of apoptosis in different animal models, inhibition of the activation of c-Jun and Erk1/2 by phosphorylation in lung tumorigenesis model, suppression of phospho-Akt and nuclear β-catenin levels in colon cancer models, inhibition of the IGF/IGF-1R axis in colon and prostate cancer models, and suppression of VEGF-dependent angiogenesis in lung and prostate cancer models. It is still unclear whether these molecules are direct targets for EGCG or downstream events of the primary action. In theory, *in vitro* experiments could provide more information on the detailed mechanisms. It is reasonable to assume that the high affinity binding proteins reported in the literature[3] could serve as initial targets, but this point remains to be substantiated in animal models.

CONCLUDING REMARKS

In contrast to the strong evidence for the cancer preventive activity of tea constituents in animal models, results from epidemiological studies have not been consistent concerning the cancer preventive effect of tea consumption in humans. The difference between the results from animal and human studies is likely to be due to the lower quantities of human tea consumption as compared to the doses used in animal studies as well as the many confounding factors in epidemiological studies, which reduce the power for detecting a cancer preventive effect in a population. Whereas in animal studies, the doses of tea preparations and the experimental conditions are set to maximize the opportunity to detect a cancer prevention effect. From the limited human studies that are available, action of tea constituents in reducing oxidative stress and enhancing the elimination of carcinogens may be important.

With the strong evidence provided by laboratory studies for the cancer preventive activities of tea con-

stituents, even though the results from epidemiological studies are not conclusive, tea preparations can still be used for the prevention of certain types of human cancer. Results from laboratory studies will help us to design the optimal conditions for cancer prevention trials and prospective studies as well as for interpreting results from epidemiological studies.

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