

Polyphenols As Inhibitors of Carcinogenesis

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Many polyphenolic compounds have demonstrated anticarcinogenic activities in animal models. These compounds include flavanone, flavonols, isoflavone, and catechins. In this article, tea catechins will be used as an example to illustrate current research in this area. Many laboratory studies have demonstrated the inhibition of tumorigenesis in animal models by different tea preparations. The animal models include tumorigenesis in the mouse lung, rat and mouse esophagi, mouse forestomach, mouse skin, mouse duodenum, rat small intestine, rat and mouse livers, and rat colon. In most of the studies, the inhibitory activity of tea could be demonstrated when tea preparations were given either during or after the carcinogen treatment period. Black tea was also effective, although the activity was weaker than green tea in some experiments. Decaffeinated tea preparations were also active in many model systems. The molecular mechanisms for these broad inhibitory actions are not fully understood. They are most likely related to the biochemical actions of the tea polyphenols, which include antioxidative activities and inhibition of cell proliferation and of tumor promotion-related activities. The effect of tea consumption on human cancers is not clear in spite of numerous investigations. The bioavailability and pharmacokinetics of tea polyphenols are being studied in animals and humans to provide a basis for more quantitative analyses on the effect of tea on carcinogenesis. More mechanistic and dose-response studies will help us to understand the effects of tea consumption on human carcinogenesis. — *Environ Health Perspect* 105(Suppl 4):971–976 (1997)

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Introduction

Frequent consumption of fruits and vegetables has been associated with lower incidence of cancers at different organ sites (1). Several factors may contribute to this association. First, the nutrients in fruits and vegetables, notably vitamin C, vitamin E, folic acid, provitamin A, selenium, and

zinc, are essential for normal cellular functions. A deficiency in these nutrients may enhance the susceptibility of an individual to cancer. Second, some nutrients, such as vitamin C, vitamin E, selenium, and β -carotene, at levels above nutritional needs, may display inhibitory activities against carcinogenesis. A third factor is that nonnutritive constituents, such as polyphenols, organosulfur compounds, and indoles, have anticarcinogenic activities. Finally, fruits and vegetables contribute fiber and bulkiness to the diet. Persons who consume large amounts of fruits and vegetables may eat smaller amounts of meat and other animal products that may contribute to higher cancer incidences in the Western countries.

The involvement of the first two factors is supported by the demonstration that supplementation of a high cancer risk population in Linxian, China, with tablets containing α -tocopherol, β -carotene, and selenium for 63 months significantly lowered the mortality rate of gastric (mainly gastric cardia) cancer (2). The subjects involved in this intervention study were

known to have low micronutrient status (3,4). Supplementation with these antioxidant nutrients apparently produced a protective effect against this cancer. It is not known how much of the beneficial effect is due to the antioxidant functions of these intervention agents. Many plant polyphenolic compounds also possess antioxidative as well as other biological activities. The anticancer activities of some of these compounds have been studied by many investigators. This report discusses the possible anticancer activities of the plant polyphenolic compounds that are widely distributed in our food and beverages. The activities of flavonoids will be discussed in general. Studies on tea polyphenols will be discussed in detail to illustrate the opportunities and difficulties in this area of research.

Effects of Flavonoids on Carcinogen Metabolism and Carcinogenesis

Flavonoids and their glycosides are widely distributed in fruits, vegetables, and nuts. Naturally occurring flavonoids such as flavonols, flavanones, isoflavones, and catechins are structurally related to the parent compound, flavone (2-phenylbenzopyrone) (5). It has been estimated that humans consume approximately 1 g of mixed flavonoids per day (5).

Flavones such as tangeretin and nobiletin, which occur in citrus fruits, affect carcinogen metabolism, including the enhancement of metabolism *in vivo* and either the inhibition or stimulation of metabolism *in vitro* (6). The action *in vitro* appeared to depend on the cytochrome P450 enzymes involved. For example, P4501A2-dependent reactions are inhibited, but P4503A4-dependent reactions are enhanced, by tangeretin (7). The effects of these compounds on carcinogen activation *in vivo* and on carcinogenesis, however, remain to be investigated.

Flavonols, such as quercetin and its glycoside, rutin, are prevalent in fruits, vegetables, and cereal grains. Rutin is hydrolyzed by intestinal bacteria to quercetin in humans. Quercetin is a potent inhibitor of P450 reactions including the activation of aflatoxin B₁ by P4503A4 and the activation of benzo[*a*]pyrene (B[*a*]P) by P4501A1 (6). In addition, quercetin has been suggested to inhibit lipoxygenase and cyclooxygenase activities (8). Quercetin inhibits

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Abbreviations used: B[*a*]P, benzo[*a*]pyrene; DBT, decaffeinated black tea; DGT, decaffeinated green tea; EC, (–)-epicatechin; ECG, (–)-epicatechin-3-gallate; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin gallate; NDEA, *N*-nitrosodiethylamine; NMBzA, *N*-nitrosomethylbenzylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

chemically induced tumorigenesis in the skin, mammary gland, and colon (9).

Among the flavanones, naringenin has received much attention. It is an effective inhibitor of the P4503A4-catalyzed oxidation of calcium-blocker drugs such as nifedipine and felodipine (10). This inhibitory effect has been shown *in vivo* by the ingestion of grapefruit juice (11), which is rich in naringin, the glycoside of naringenin. Another flavanone, hesperetin, and a flavone, apigenin, which are present in grapefruit juice, had similar inhibitory activities (10). Naringenin also inhibits the activation of aflatoxin B₁ and B[a]P (10,12). At low doses, flavanones induced glutathione S-transferase and glucuronidation activities (13). Because of their activities in inhibiting carcinogen activation and inducing phase II enzymes, flavones are expected to inhibit tumorigenesis induced by the above carcinogens; however, this remains to be demonstrated.

Isoflavones and their glucosides are present in high concentrations in soy products; daidzein and genistein have received the most attention. These compounds are known to be inhibitors of carcinogenesis, agonists and antagonists of estrogen, inhibitors of protein tyrosine kinase, inhibitors of angiogenesis, inducers of apoptosis, and to have other biological functions (14).

Tea Polyphenols and Inhibition of Carcinogenesis in Animal Models

The chemistry of tea has been reviewed previously (15,16). Although it remains to be substantiated further, the major anticancer components in tea are believed to be the tea polyphenols, also known as catechins. In green tea, (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epicatechin (EC) are the major polyphenols. In the manufacture of black tea, most of these compounds are oxidized and polymerized to form theaflavins, which give the reddish color of black tea, and thearubigins, which are not well characterized chemically. The structures of some of the major tea polyphenols are shown in Figure 1.

Tea extracts and tea polyphenols inhibit tumorigenesis in a variety of animal models in different organ sites. Some recent studies from our laboratory and others are discussed herein.

Lung Tumorigenesis

Green tea infusion (e.g., 1.25 g of tea leaves brewed in 100-ml boiling water) as the sole source of drinking fluid to A/J mice significantly decreased *N*-nitrosodiethylamine (NDEA)-induced lung tumor incidence (by 36 to 44%) and tumor multiplicity (by 44 to 60%) (17). Decaffeinated green tea (DGT) or decaffeinated black tea (DBT) extracts (e.g., 0.6-g dehydrated tea extract reconstituted in 100-ml distilled water) also inhibited lung tumorigenesis caused by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (17). The tumor multiplicity was reduced by 65 to 85%, and tumor incidence was inhibited to a lesser extent, which showed 14 to 30% inhibition in some of the experiments. In both

models, tea preparations were effective when administered to mice either during or after the carcinogen treatment period. To gain mechanistic information, mice were given DGT in different time periods before or after the NNK treatment (18). Treatment with DGT starting 2 weeks before and continuing until 1 week after the NNK injection was more effective in reducing tumor multiplicity (56%) than treatment starting 2 days before NNK injection (31%). When tea was given to the mice immediately after the NNK treatment for a period of 1 week, tumor multiplicity was also reduced (20%). Tumor multiplicity was inhibited (54%) even when DGT administration was started at 5 weeks after NNK treatment.

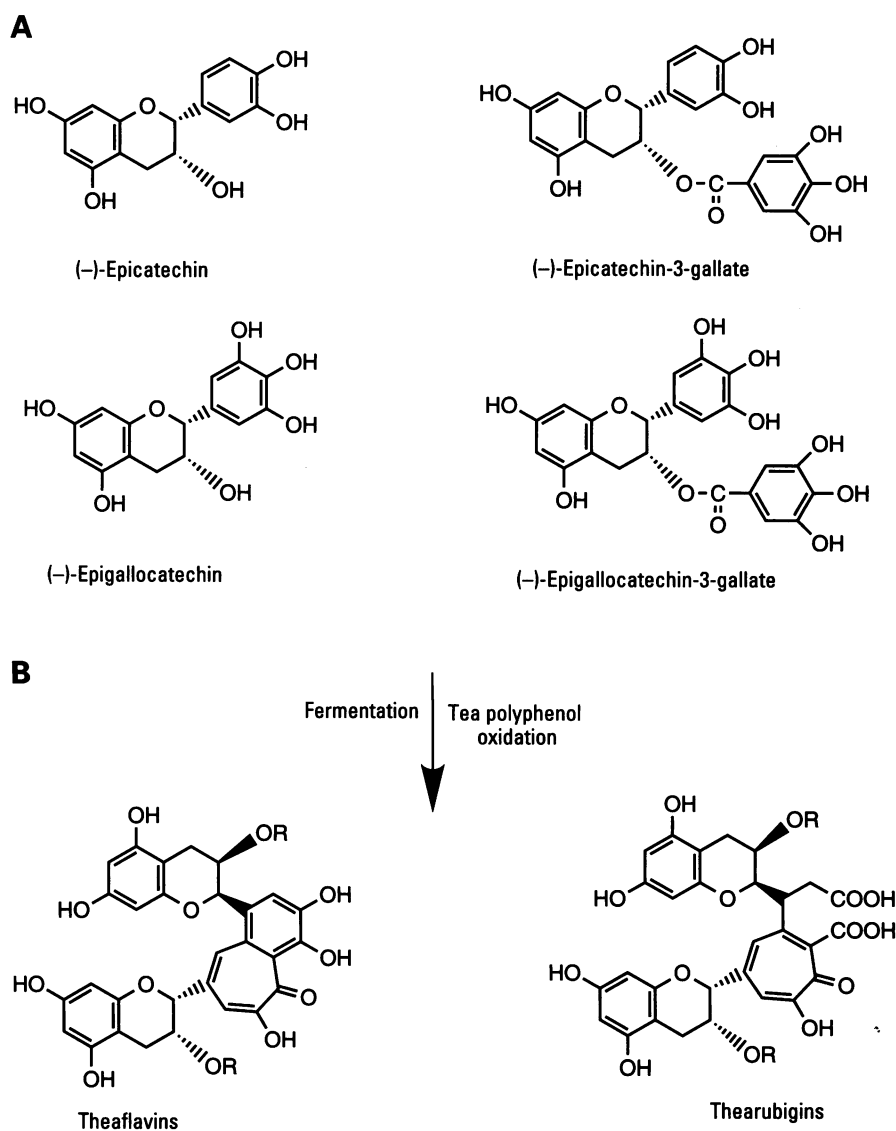


Figure 1. Structures of major tea polyphenols in (A) green tea and (B) black tea. R, galloyl.

Similar inhibitory activity was also observed by other investigators in lung tumorigenesis induced by NDEA, B[a]P, and NNK (17,19,20).

Esophageal Carcinogenesis

When 0.6% DGT or DBT extracts were given to male Sprague-Dawley rats as the drinking fluid during the *N*-nitrosomethylbenzylamine (NMBzA) treatment period, esophageal tumor incidence and multiplicity were reduced by approximately 70% (21). When the tea preparations were given after the NMBzA treatment period, the esophageal papilloma incidence and multiplicity were reduced by approximately 50%. The volume per tumor was also reduced in rats that received DBT after the carcinogen treatment period. In a second experiment, the rats received 0.9% regular green tea or DGT after the NMBzA treatment period; the tumor multiplicity was decreased by more than 55% at 16 weeks after the first dose of NMBzA. The volume per tumor was reduced by approximately 60% in the rats receiving 0.9% regular green tea. Histological analysis indicated that both the incidence and multiplicity of esophageal carcinoma were decreased by treatment with either regular green tea or DGT (21).

Our results (21) confirmed the observations by Han and Xu (22), who demonstrated the inhibitory activity of three brands of green tea, one brand of oolong tea, and one brand of black tea against esophageal tumorigenesis in Wistar rats. In their study, tea and the carcinogen NMBzA were administered during the entire experimental period. Our results (21) demonstrated that tea administration was effective when given during either the initiation or postinitiation stage, and that DGT is also effective. Green tea extracts have also been shown to inhibit esophageal tumorigenesis in mice induced by precursors of *N*-nitrososarcosine (23).

Gastric Carcinogenesis

In the previously described NDEA-induced carcinogenesis model with A/J mice, forestomach tumorigenesis was also significantly inhibited by green tea infusion (17). In this model, hyperplasia, papillomas, carcinoma *in situ*, and squamous cell carcinomas in the forestomach were observed. Tea treatment inhibited the tumor multiplicity (up to 63% inhibition) more effectively than tumor incidence (up to 26% inhibition). The tumor volume was lower in the mice in the tea treatment group than in the control group (17).

Similar inhibitory activity by green tea preparations has also been observed in mouse forestomach tumors induced by NDEA or B[a]P (19,24) and by precursors of nitrosamines (23,25). Inhibition of gastric cancer induced by *N*-methyl-*N*-nitro-*N*-nitrosoguanidine in rats by green tea extract (26) and EGCG (27) has also been reported. In the latter study, EGCG (0.05% in water) administration to the rats in the drinking fluid for 15 weeks after a 28-week treatment with the carcinogen caused a 50% reduction in glandular tumor incidence (27).

Other Laboratory Studies

More than 20 laboratories in several countries have reported the inhibitory action of tea against tumorigenesis in animal models on different organ sites, such as skin, esophagus, forestomach, stomach, duodenum and small intestine, colon, lung, liver, pancreas, and mammary gland. Among these, skin tumorigenesis has been studied most extensively. Some of this work has been reviewed previously (16,28,29). Skin tumorigenesis caused by ultraviolet light, chemicals, and tumor promoters was inhibited by different tea preparations and tea polyphenols (30,31). When mice bearing chemically induced or ultraviolet light-induced skin papillomas were given different tea preparation or EGCG, partial tumor regression or marked inhibition of tumor growth was observed (32).

Tea Consumption and Human Cancer

A 1991 International Agency for Research on Cancer monograph (33) concluded, "There is inadequate evidence for the carcinogenicity in humans and experimental animals of tea drinking." In the review on "Tea and Cancer" in 1993, we concluded that no clear-cut conclusion could be drawn concerning the protective effects of tea against human cancers (16). The conclusion still held true after inclusion of the 17 new case-control studies (28,34-50) published after our review. A summary of case-control studies on the relationship between tea consumption and human cancers is shown in Table 1.

Tea Consumption and Esophageal Cancer

As reviewed previously (16), early literature suggested that excessive consumption of tea was a causative factor for esophageal cancer. Several case-control studies, however, showed that there was no association

Table 1. Recent case-control studies on the association between tea consumption and human cancers.^a

Organs	Number of reports		
	Negative	Positive	No relationship
Bladder and urinary tract	—	—	17
Breast	—	—	6
Colon and rectum	4	1	6
Esophagus	1	7 ^b	6
Kidney	—	1	5
Liver	—	—	1
Lung	—	1	1
Nasopharynx	—	—	3
Oral and tongue	1	—	1
Pancreas	2	1	8
Prostate	—	—	1
Stomach	4	1	8

^aStudies since 1992. ^bSix studies were with hot tea.

between drinking tea at normal temperature and esophageal cancer; ingestion of very hot tea, however, was associated with a 2- to 3-fold increase in risk for esophageal cancer. The high temperature of hot tea, rather than the chemicals in the tea, was suggested to be an important etiological factor in human esophageal cancer (33). A recent small case-control study conducted in northern China (38) reported that the high temperatures of the meals and drinks had a strong association with esophageal cancer risk. After adjustment for the temperature, however, tea consumption was still a risk factor. On the other hand, a recent report by Gao et al. (36) indicated that, in a case-control study on 902 patients and 1552 controls in Shanghai, frequent consumption of green tea was associated with a lower incidence of esophageal cancer. After adjustment for age and other possible confounding factors, a protective effect of green tea drinking on esophageal cancer was observed among women: odds ratio, 0.50; 95% confidence interval, 0.30 to 0.83; this risk decreased ($p \leq 0.01$) as the quantity of tea consumption increased. Among men, the protective effect was not statistically significant. Among nonsmokers and drinkers of nonalcoholic beverages, however, statistically significant decreases in risk were observed among tea drinkers for both men (69 cases and 192 controls) and women (194 cases and 564 controls).

Tea Consumption and Stomach Cancer

Early correlative and cohort studies indicated that tea consumption was either negatively or positively associated with stomach cancer (51-53). Many case-control studies on stomach cancer and tea consumption

conducted in different areas, however, indicated that there was no significant association between tea consumption and cancer of the stomach (16,29). Some more recent case-control studies suggested that tea consumption reduced the risk of stomach cancer in Kyushu (Japan) (54), Shanghai (China) (55), northwestern Turkey (47), and central Sweden (42). For example, in Kyushu (139 cases and 2574 controls), individuals who consumed green tea more frequently or in larger quantities (10 or more cups a day) tended to have a lower risk for gastric cancer (54). In a population-based case-control study (669 cases and 880 controls) in central Sweden, tea (mostly black tea) consumption was associated with a lower incidence of gastric cancer (42).

Different etiological factors and molecular mechanisms may be involved in varying types of cancers in different geographic areas. Therefore, it is not surprising that a clear-cut conclusion on the relationship between tea consumption and human cancer has not been found. Many confounding factors, such as cigarette smoking, alcohol drinking, and the temperature of tea, have been adjusted in many newer studies, and these results tend to be more informative. Another key factor that needs to be incorporated in future studies is the quantity and type of tea consumed.

Possible Active Tea Components and Their Tissue Levels

One factor to be considered in correlating laboratory observations to epidemiological studies is the quantities of tea used in animal studies in comparison to those consumed by humans. To conduct quantitative analyses, we developed a method for the quantification of plasma and tissue levels of tea polyphenols (56). Plasma EGCG, EGC, and EC exist in free and conjugated (glucuronide and sulfate) forms. The plasma tea polyphenol levels in rats and mice in our anticarcinogenesis experiments were comparable to the peak levels in humans after consuming two or three cups of tea (29). The peak plasma polyphenol levels were observed at 1.5 to 2 hr after ingestion, and plasma EGCG, EGC, and EC had half-lives of 3 to 5 hr (C Yang et al., unpublished results).

In a preliminary experiment, after administration of regular green tea in drinking fluid to rats, substantial amounts (220 and 46 ng/g wet weight, respectively) and esophagus (850 and 280 ng/g, respectively). The EGCG was detected in the esophagus

(410 ng/g) but not in the lung. The EGCG, EGC, and EC levels in the small intestine and intestinal contents were rather high (1.5–5.5 mg/g) due to the unabsorbed and biliary excreted glucuronides of polyphenols in the intestine. High EGC and EC levels were also observed in the colon tissues (1.8 and 0.3 mg/g, respectively). Due to possible glucuronidase and esterase activities in the colon, most of the EGC and EC were found in the free form, and EGCG was found at lower levels. EGCG has been usually considered the active anticarcinogenic component in tea because it is the most abundant polyphenol in tea. Indeed, inhibition of lung and colon carcinogenesis by EGCG has been reported (20,57,58). Our results on tissue levels suggest that EGCG is converted to EGC, and thus EGC rather than EGCG may be the main active compound involved in both models. EGC and EGCG had similar potency (IC_{50} of 30–50 μ M) in inhibiting the growth of colon cancer cell line HT-29 (G Yang, J Liao, F Kim, C. Yang, unpublished results). These results demonstrate the importance of active components, especially when a mixture of compounds is used.

In black tea, the level of catechins is about 30% that of green tea, but the inhibitory activity against tumorigenesis of black tea was comparable to that of green tea in several animal models (17,21,31). The effective components in black tea are not clearly understood. Theaflavins and thearubigins contain multiple hydroxyl groups and possess antioxidative activities. The antioxidative and antimutagenic effects of theaflavins from black tea have been reported recently (59). Other non-polyphenolic constituents may also play a role in the antitumorigenic activities of tea. Tumorigenesis studies indicated that decaffeinated tea displayed similar inhibitory activity to regular green tea in some experiments (17,21) but was less effective in other experiments (31), suggesting that caffeine also possesses inhibitory activities in some animal models. Xu et al. (20) reported that oral feeding of caffeine to A/J mice also inhibited NNK-induced lung tumorigenesis.

Possible Mechanisms for the Inhibition of Tumorigenesis

The most noteworthy properties of tea polyphenols and other flavonoids are their antioxidative activities (16). Reactive oxygen species may play important roles in carcinogenesis through damaging DNA,

altering gene expression, or affecting cell growth and differentiation (60,61). The anticarcinogenic activities of tea polyphenols are believed to be closely related to their antioxidative properties. The findings that green tea preparations inhibited 12-*O*-tetradecanoylphorbol-13-acetate-induced hydrogen peroxide formation in mouse epidermis (62) and NNK-induced 8-hydroxydeoxyguanosine formation in mouse lung (20) are consistent with this concept. Inhibition of tumor promotion-related enzymes such as ornithine decarboxylase (62,63), protein kinase C (64), lipoxigenase, and cyclooxygenase (19) by tea preparations has also been reported. Although inhibition of carcinogen activation by tea or green tea polyphenol fractions could be demonstrated *in vitro* and, in certain cases, *in vivo* (65,66), this mechanism was not demonstrated for NNK bioactivation *in vivo* (18,20). Oral administration of tea preparations to animals has been reported to moderately enhance the activities of glutathione peroxidase, catalase, glutathione *S*-transferase, NADPH-quinone oxidoreductase, uridine diphosphate-glucuronosyltransferase, and methoxyresorufin *O*-dealkylase (67–69). The effects of a mild induction of these enzymes on carcinogenesis are not clear. Mechanisms relating to the quenching of activated carcinogens, antiviral activity, and enhancing immune functions have also been suggested, but their relevance to carcinogenesis remains to be determined (16). Inhibition of nitrosation by tea preparations has been demonstrated *in vitro* and in humans (70,71); this may be an important factor in preventing certain cancers, e.g., gastric cancer, if the endogenously formed *N*-nitroso compounds are causative factors. Our recent results (72) suggest that the antiproliferative effect of tea is important for the anticarcinogenic activity. One may speculate that tea polyphenols inhibit growth-related signal transduction pathways.

Conclusion

Although the anticarcinogenic activity of tea and polyphenols has been demonstrated in many animal studies, such activity has not been clearly demonstrated in humans. More epidemiological investigations, especially prospective studies, concerning the effect of polyphenol consumption on human cancer risk are needed. Because the causative factors may be different for different cancers and for the same cancer in different populations, the effects may vary in different situations. Therefore, in future epidemiological studies,

it is important to consider the etiological factors of the specific cancers and to collect specific information on the quantitative aspects of polyphenol consumption. Urinary excretion of metabolites of polyphenols may be used as an exposure biomarker.

Definitive information on the protective effects of polyphenol consumption on cancer will come from intervention studies. Based on the suggestive epidemiological data from Shizuoka, Kyushu (54), Shanghai (55), northwestern Turkey (47),

and central Sweden (42), future intervention studies on gastric and esophageal cancers with tea preparations among high-risk populations should be very worthwhile. Intervention trials with other polyphenols should also be encouraged.

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