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INHIBITION OF CARCINOGENESIS BY TEA*

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■ **Abstract** Tea has received a great deal of attention because tea polyphenols are strong antioxidants, and tea preparations have inhibitory activity against tumorigenesis. The bioavailability and biotransformation of tea polyphenols, however, are key factors limiting these activities in vivo. The inhibition of tumorigenesis by green or black tea preparations has been demonstrated in animal models on different organ sites such as skin, lung, oral cavity, esophagus, forestomach, stomach, small intestine, colon, pancreas, and mammary gland. Epidemiological studies, however, have not yielded clear conclusions concerning the protective effects of tea consumption against cancer formation in humans. The discrepancy between the results from humans and animal models could be due to 1) the much higher doses of tea used in animals in comparison to human consumption, 2) the differences in causative factors between the cancers in humans and animals, and 3) confounding factors limiting the power of epidemiological studies to detect an effect. It is possible that tea may be only effective against specific types of cancer caused by certain etiological factors. Many mechanisms have been proposed for the inhibition of carcinogenesis by tea, including the modulation of signal transduction pathways that leads to the inhibition of cell proliferation and transformation, induction of apoptosis of preneoplastic and neoplastic cells, as well as inhibition of tumor invasion and angiogenesis. These mechanisms need to be evaluated and verified in animal models or humans in order to gain more understanding on the effect of tea consumption on human cancer.

*ABBREVIATIONS: EGCG, (−)-epigallocatechin gallate; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EC, (−)-epicatechin; TF, theaflavins; DGT, decaffeinated green tea; DBT, decaffeinated black tea; GTPF, green tea polyphenol fraction; DMBA, 7,12-dimethylbenz[a]anthracene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; NNK, (4-methylnitrosamino)-1-(3-pyridyl)-1-butanone; NDEA, *N*-nitrosodiethylamine; BP, benzo[a]pyrene; NDMA, *N*-nitrosodimethylamine; NMBzA, *N*-nitrosomethylbenzylamine; BTP, black tea polyphenols; AOM, azoxymethane; DMH,1,2-dimethylhydrazine; PhIP, 2-amino-1-methyl-6-phenyl imidazo [4,5-p] pyridine; BOP, *N*-nitroso-bis(2-oxopropyl)amine; TFdiG, theaflavin digallate.

INTRODUCTION

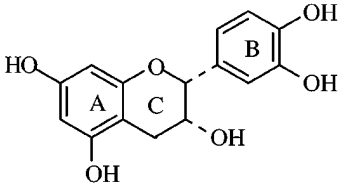
Originated in China and Southeast Asia, tea has been cultivated and consumed for thousands of years. Historically, tea has been lauded for various beneficial health effects. Now, tea is grown in more than 30 countries and is the most widely consumed beverage next to water. The biological activities of tea are beginning to be understood. The possible cancer-preventive potential of tea has received much attention in recent years because of the demonstrated anticancer activities of tea preparations in laboratory studies (1–4). Epidemiological studies in different countries, however, have not generated consistent results concerning the cancer-preventive effect of tea in humans. This chapter reviews the antioxidative properties of tea polyphenols, their bioavailability and biotransformation, the inhibition of tumorigenesis in animal models, epidemiological studies on tea and cancer, and the possible mechanisms of action of tea constituents. The purpose of this review is to provide a better understanding of the effects of tea consumption on cancer. Because of space limitation, many of the earlier publications are not cited, and instead review articles are given as references.

Tea Chemistry

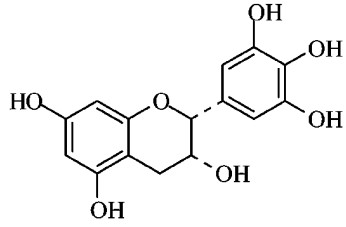
Tea is produced from the leaves of the plant *Camellia sinensis*. Tea composition varies with climate, season, horticultural practices, variety, and the age of the leaves (5). Black tea manufacture involves crushing the tea leaves to promote enzymatic oxidation and subsequent condensation of tea polyphenols in a process known as fermentation, which leads to the formation of theaflavins and thearubigins. To make green tea, fresh tea leaves are steamed or pan-fried, which inactivates the enzymes and prevents the oxidation of tea polyphenols. About 78% of the tea production worldwide is black tea, whereas green tea, mainly consumed in China and Japan, constitutes about 20%. Oolong tea is partially fermented and constitutes about 2% of tea production.

A typical tea beverage, with 2.5 g tea leaves in 250 ml hot water for a 3-min brew, usually contains 620 mg to 880 mg of water-extractable solids (6). Tea polyphenols, known as catechins, usually account for 30% to 42% of the dry weight of the solids in brewed green tea (6). Catechins are characterized by di- or tri-hydroxyl group substitution of the B ring and the meta-5,7-dihydroxy substitution of the A ring. The structures of the four major catechins, (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC) are shown in Figure 1. EGCG is the major catechin in tea and may account for 50% to 80% of the total catechin in tea. Catechin, gallocatechin, epigallocatechin digallates, epicatechin digallate, 3-*O*-methyl EC and EGC, catechin gallate, and

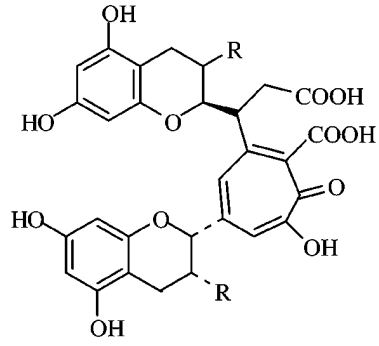
Figure 1 Structures of tea polyphenols. Thearubigins are poorly characterized; the structure shown is one of the proposed structures.



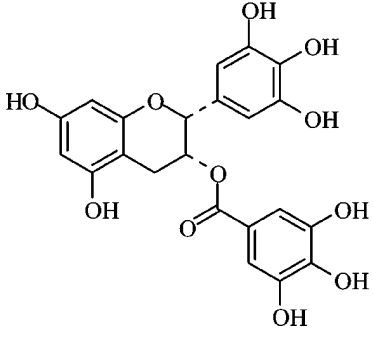
(-)-Epicatechin



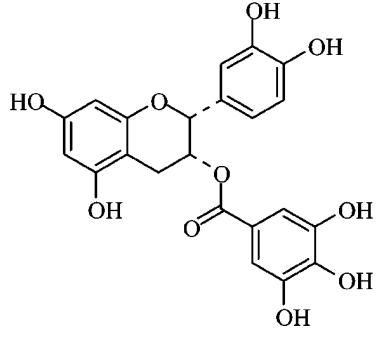
(-)-Epigallocatehcin



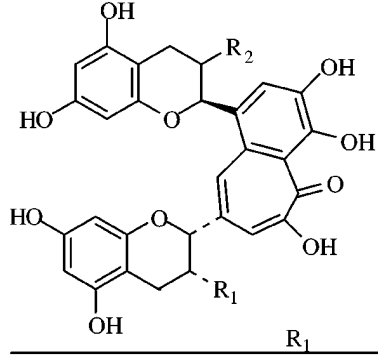
Thearubigins
(R = Gallate or other groups)



(-)-Epigallocatechin-3-gallate



(-)-Epicatechin-3-gallate



	R_1	R_2
Theaflavin	OH	OH
Theaflavin-3-gallate	Gallate	OH
Theaflavin-3'-gallate	OH	Gallate
Theaflavin-3,3'-digallate	Gallate	Gallate

gallocatechin gallate are present in smaller quantities. 3′-*O*-methyl-EGCG has been isolated from oolong tea as a minor component. Flavonols, including quercetin, kaempferol, myricitin, and their glycosides, are also present in tea.

In addition to the above polyphenols, black tea contains thearubigins and theaflavins (TF), which account for 15% to 20% and 2% to 6%, respectively, of the dry weight of black tea solids. Thearubigins, which have higher molecular weights, are poorly characterized chemically. TF are characterized by the benzotropolone ring structure and bright red-orange color, and contribute to the unique taste of black tea. The structures of the four major theaflavins and thearubigins are shown in Figure 1. Theasinensins, which are dimeric gallocatechins linked by C-C bonds, are present in green tea leaves, and additional amounts are produced during preparation of black and oolong tea. Other related compounds in this class include isotheaflavins, neotheaflavins, theaflavic acids and epitheaflavic acids, theafulvins, and theacitrins.

Tea leaves contain about 2% to 5% caffeine and much smaller quantities of theobromine and theophyline. The amount of caffeine in tea beverage is determined by the leaf size, the brewing time, and the temperature. Tea contains around 17% nitrogenous materials as protein (∼6%) as well as amino and nucleic acids (∼8%). Among the 19 amino acids found in tea, theanine (γ-*N*-ethyl glutamine) (∼3%) is unique to tea. Potassium, calcium, magnesium, and aluminum are the predominant minerals found in the ash (10% to 15%) of the water-soluble solids of tea. Tea beverage is a significant source of fluoride at ∼1 mg/serving.

ANTIOXIDATIVE PROPERTIES OF TEA POLYPHENOLS

The potential health benefits associated with tea consumption have been partially attributed to the antioxidative property of tea polyphenols, and this topic has previously been reviewed (7, 8). Tea preparations have been shown to trap reactive oxygen species, such as superoxide radical, singlet oxygen, hydroxyl radical, peroxyl radical, nitric oxide, nitrogen dioxide, and peroxynitrite. The radical quenching ability of green tea is usually higher than that of black tea. Among tea catechins, EGCG is most effective in reacting with most reactive oxygen species. The chemical structures contributing to effective antioxidant activity of catechins include the vicinal dihydroxy or trihydroxy structure, which can chelate metal ions and prevent the generation of free radicals. This structure also allows electron delocalization, conferring high reactivity to quench free radicals. Under certain conditions, however, catechins may undergo autooxidation and behave like prooxidants. During the reactions of tea polyphenols with free radicals, several oxidation products are formed. Reactions of EGCG and other catechins with peroxyl radicals lead to the formation of anthocyanin-like compounds (9), as well as seven-membered B ring anhydride dimers and ring-fission compounds (10, 11). The B ring appears to be the principal site of antioxidant reactions.

Green and black tea can inhibit the oxidation of lipoproteins induced by Cu^{2+} in vitro (12, 13). Pretreatment of macrophages or endothelial cells with green and

black tea polyphenols reduced cell-mediated low-density lipoprotein oxidation (14). The protective activities of tea and tea polyphenols against the oxidation of lipoproteins have been proposed to contribute to the prevention of atherosclerosis and other cardiovascular diseases. Although a reduction of cardiovascular diseases has been associated with tea consumption in some studies, other studies have not demonstrated such a beneficial effect. Even the protective effect against lipoprotein oxidation by tea has not been convincingly demonstrated in vivo (reviewed in 15).

BIOAVAILABILITY AND PHARMACOKINETICS

A good comprehension of the bioavailability of the active components of tea is essential for understanding the biological activities and the mechanisms of actions of tea in vivo. Early studies on the bioavailability of tea polyphenols have been reviewed (16, 17).

Absorption and Biotransformation of Tea Catechins

Glucuronidation, sulfation, and methylation are the major biotransformation reactions for tea catechins. In rats, the highest activity of UDP-glucuronosyltransferase was found in the intestinal mucosa, the highest activity of phenol sulfotransferase occurred in the liver, and that of catechol-*O*-methyl transferase was found in the liver and kidney (18, 19). Methylated catechins including 3′- and 4′-*O*-methyl-EC, 4′-*O*-methyl-EGC, 4′′-*O*-methyl-ECG, and 4′′-*O*-methyl-EGCG have been observed in vivo and in incubations with rat liver homogenates (20, 21). *O*-Methylation of catechins also occurred in incubations with human placenta homogenates; EC and EGC were better substrates than ECG and EGCG (22). After oral administration of EGCG to rats, the biliary metabolites were 3′-, 4′-, 3′′-, and 4′′-*O*-methyl-EGCG, as well as 4′,4′′-di-*O*-methyl-EGCG, almost all of which were in the conjugated forms (23). Following i.v. dosing of [4-^{3}H]EGCG, the major metabolite in the bile was 4′,4′′-di-*O*-methyl-EGCG (24). Perfusion of isolated rat jejunum with (+)-catechin and EC resulted in glucuronidation (∼45%), *O*-methylation (∼30%), and *O*-methyl-glucuronidation (∼20%) during transfer across the enterocytes to the serosal side. In the ileum, however, the majority of the catechins appeared on the serosal side unmetabolized and the percentage of flavanols transferred was about five times higher than that in the jejunum (25).

Following tea ingestion, 4′-*O*-methyl-EGC (mostly in the glucuronidated or sulfated form) was a major metabolite, reaching its peak level within the first 2 h in human plasma at a concentration 4 to 6 times higher than those of EGC (26). The half-lives of EGC and 4′-*O*-methyl-EGC in the blood were 1.0 h and 4.4 h, respectively. The amount of 4′-*O*-methyl-EGC excreted in urine was about three times higher than that of EGC, and 88% 4′-*O*-methyl-EGC was excreted in urine within 8 h.

Tea catechins are also degraded in the intestine by microflora. Several microbial metabolites, including 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid,

3-methoxy-4-hydroxy-hippuric acid, and 3-methoxy-4-hydroxybenzoic acid, were observed in human urine samples (27). 5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone and 5-(3′,4,′,5′-trihydroxyphenyl)-γ-valerolactone were identified in human urine as the ring fusion products of EGC and EC, respectively (28). Both metabolites (mainly in the conjugated form) were detected in the urine and plasma in amounts several fold higher than their respective precursors in some individuals. After green tea administration, the major conjugates that appeared in human, mouse, and rat urine samples were identified by liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) as monoglucuronides and monosulfates of EGC, EC, *O*-methyl-EGC, *O*-methyl-EC, (−)-5-(3′,4′, 5′-trihydroxyphenyl)-γ-valerolactone, and (−)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone. In comparison to rats, the urinary metabolite profiles of tea catechins in mice resemble more closely those in humans (29).

To study the effects of chronic consumption of green tea, rats and mice were given 0.6% green tea polyphenols as the drinking fluid (30). The EGC and EC levels in rat plasma increased over time, reaching peak values (3 times the Day 1 values) on Day 14, and then declined gradually reaching Day 1 values on Day 28. The plasma concentrations of EGCG were much lower than those of EGC or EC, even though EGCG accounted for 78% of the catechins in the drinking fluid. High levels of EGC and EC (but not EGCG) were found in the urine, whereas high levels of EGCG were found in the feces. The amounts of catechins in different tissues reflected the absorption and excretion pattern. Administration of 0.6% green tea polyphenols i.p. resulted in much higher EGCG levels than i.g. administration, which suggests poor intestinal absorption of EGCG by rats. The plasma levels of EGCG in mice were much higher than those in rats. A similar "increase-and-then-decrease" pattern was observed in the catechin levels in mouse plasma, liver, and lung, except that the decrease started four days after the tea treatment. The results demonstrated species difference in the bioavailability of EGCG and adaptive responses owing to consumption of tea by rodents.

The intestinal absorption of tea polyphenols is not well understood. The absorption of catechins is likely to be affected by the conjugation reactions and the efflux pumps in the intestinal mucosa; however, this topic needs further investigation. Information on the bioavailability and biotransformation of black tea polyphenols is scarce. Attempts to detect theaflavins and thearubigins in the blood or urine of animals have not been successful. Therefore, the biological effects of these compounds could be due to their microbial metabolites, which become bioavailable through enterohepatic circulation. When ingested in large quantities, these black tea polyphenols could affect the absorption of fat and other nutrients.

Pharmacokinetics of Tea Polyphenols

A detailed phamacokinetic study regarding the absorption, distribution, and elimination of tea polyphenols was conducted in rats (31). Following i.v. administration of decaffeinated green tea (DGT), the plasma concentration-time curves ($t_{1/2\beta}$)

of EGCG, EGC, and EC could be fitted into a two-compartment model. The β-elimination half-life of EGCG (212 min) was higher than that of EGC (45 min) or EC (41 min). The clearances were 2.0, 7.0, and 13.9 ml • min/kg, and the distribution volumes were 0.15, 0.21, and 0.36 L/kg for EGCG, EGC, and EC, respectively. When pure EGCG was given i.v., a shorter $t_{1/2\beta}$ (135 min) was observed, which suggests that other components in DGT may affect the plasma concentration and elimination of EGCG. Following i.v. administration of DGT, the highest tissue level of EGCG was in the intestine, whereas the highest levels of EGC and EC were observed in the kidney. After i.g. administration of DGT, 14% of EGC and 31% of EC appeared in the plasma, but only 0.1% of EGCG was bioavailable. A similar study found EGCG levels in the blood and tissues corresponding to between 0.0003% and 0.45% of the ingested EGCG (32). Both studies showed the poor bioavailability of EGCG in rats.

Following i.v. administration of [4-^{3}H]EGCG to bile-duct–cannulated rats, about 77% of the radioactivity was excreted in the bile within 48 h, whereas only 2.0% was recovered in the urine (24). After i.g. administration of [^{3}H]EGCG to mice, radioactivity was detected in the digestive tract, liver, lung, pancreas, mammary gland, and skin, as well as in other organs (brain, kidney, uterus and ovary, and testes) (33). Within 24 h, about 6.5% of total administered radioactivity was excreted in the urine and 35% in the feces. About 0.03% to 0.59% of administered [^{3}H]EGCG and five metabolites were excreted into the urine. It is interesting that a second equal dose of [^{3}H]EGCG 6 h later increased the radioactivity levels 4 to 6 fold in the blood, brain, liver, pancreas, bladder, and bone.

The pharmacokinetics of tea polyphenols in humans have been studied by several groups. In one of our studies, 18 volunteers were given a beverage containing DGT solids (1.5, 3.0, or 4.5 g) after overnight fasting (34). The plasma concentrations of EGCG, EGC, and EC reached peak levels between 1.5 and 2.5 h and declined to undetectable levels after 24 h. When the dose of green tea was increased from 1.5 to 3.0 g, the maximum plasma concentrations and the areas under the curve of EGCG, EGC, and EC increased 2.5- to 5-fold, but a further increase in dose to 4.5 g of green tea did not significantly alter these parameters. The half-lives of the terminal elimination phase of EGCG, EGC, and EC ranged from 3 to 5 h. The total amounts of EGC and EC excreted through the urine appeared to increase with increasing dose, but a dose-response relationship was not established. No EGCG was detected in the urine. Over 90% of the total EGC and EC was excreted between 0 to 8 h, and after 24 h, their levels were negligible. The bioavailability of EGCG appeared to be lower than that of EGC. In another study, EGCG and Polyphenon E (decaffeinated green tea catechin mixture) at dose levels of 200, 400, 600, and 800 mg EGCG were given to 20 subjects (35). The mean areas under the plasma concentration-time curve of unchanged EGCG were 22.5, 35.4, 101.9, and 167.1 min • μg/mL at the 200-, 400-, 600-, and 800-mg dose levels, respectively. In plasma, EGCG was mainly present in the free form, whereas the plasma and urine EGC and EC were mostly in the form of glucuronide/sulfate conjugates. The plasma level of EGCG elevated markedly with increase in the

dose from 400 to 600 mg, possibly owing to saturable presystemic elimination of orally administered green tea polyphenols.

More studies in this area are needed in order to develop blood and urine levels of catechins and metabolites as exposure markers of tea consumption, as well as to develop appropriate dosage regimens for intervention studies with tea.

INHIBITION OF TUMORIGENESIS IN ANIMAL MODELS

Many studies have demonstrated the inhibitory action of tea against tumorigenesis in animal models on different organ sites such as skin, lung, oral cavity, esophagus, forestomach, stomach, small intestine, colon, liver, pancreas, and mammary gland. Some of the previously reviewed and recent results are summarized in Table 1. The effectiveness of tea in different organs appears to be dependent on the amount of tea constituents that can reach the target tissues.

Protection Against Skin Tumorigenesis

Rodent skin tumor model has been utilized extensively to study the cancer chemopreventive potential of tea. Inhibition of tumorigenesis at the initiation, promotion, and progression stages by different preparations of tea has been shown in more than 20 studies (Table 1). Earlier studies have demonstrated that topical application of tea preparations or EGCG to the mouse skin inhibited 7,12-dimethylbenz[a]anthracene (DMBA) and UV-induced skin carcinogenesis (36, 37). In a study by Katiyar et al. (38), topical application of a green tea polyphenol fraction (GTPF) to the skin of DMBA-initiated mice, prior to that of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) or mezerein, resulted in protection against skin tumor promotion as judged by the decrease in tumor incidence (by 32% to 60%), multiplicity (by 49% to 63%) and tumor volume/mouse (by 73% to 90%). GTPF also protected against the malignant conversion of papillomas to squamous cell carcinomas (SCC). The antiinitiation and antipromotion effects of black tea polyphenols (BTP) were demonstrated in a two-stage mouse skin carcinogenesis model in which BTP was applied topically, either twice a week for three weeks prior to initiation with a dose of DMBA or prior to each treatment with the promoter TPA (39). Record et al. (40) reported that in mice receiving simulated solar radiation, the group consuming tea (with 10% whole milk) had 30% fewer papillomas, 50% fewer tumors, and 55% smaller lesions than the group consuming water.

The effects of orally administered tea, decaffeinated tea, and caffeine (in drinking fluid) on skin tumor development and growth in mice were studied systematically by Conney et al. (41–44). In one study, SKH-1 female mice were treated with UVB light twice weekly for 22 weeks, were allowed to develop tumors during the following 13 weeks, and then treated with black tea for 11 weeks (41). The tea treatment markedly decreased the number and volume of nonmalignant and malignant tumors (by 54% to 84%). In CD1 mice with established papillomas (initiated with

DMBA and promoted with TPA), treatment with black tea for 11 to 15 weeks inhibited the growth of papillomas (by 35% to 48%), but decaffeinated black tea gave inconsistent results. In another study, oral administration of green or black tea inhibited UVB-induced complete carcinogenesis, but decaffeinated green or black tea was much less effective (42). Oral administration of caffeine alone had a substantial inhibitory effect on UVB-induced carcinogenesis, and adding caffeine to the decaffeinated teas restored the inhibitory effects.

The effects of oral administration of tea, decaffeinated tea, and caffeine on the formation and growth of tumors in high-risk SKH-1 mice were also investigated (43). Treatment of mice with UVB light twice a week for 22 to 23 weeks resulted in tumor-free animals with a high risk of developing tumors in the absence of further UVB treatment. Oral administration of green tea or black tea for 18 to 23 weeks to these high-risk mice inhibited the formation and decreased the size of nonmalignant tumors and malignant SCC. The decaffeinated teas were inactive or less effective inhibitors of tumor formation, but they decreased the tumor size. Adding caffeine back to the decaffeinated teas restored the inhibitory activity. Oral administration of the caffeine alone also decreased the number and size of nonmalignant and malignant tumors. The inhibition of UVB-induced mouse skin tumor by orally administered tea or caffeine is suggested to be due to, at least partly, the ability of caffeine to decrease body fat, especially the fat in the dermis layer. This conclusion was based on the observation that tea or caffeine administration significantly decreased the size of the parametrical fat pads and thickness of the dermal fat layer, which was correlated with inhibition of tumorigenesis (44).

All these studies demonstrated the protective effect of tea on skin carcinogenesis. However, a critical analysis of the results raises the question whether this effect is due to tea polyphenols or due to the caffeine present in tea. When applied topically to the skin, both tea polyphenols and caffeine can inhibit skin tumor formation and growth. When tea polyphenols are administered orally, their bioavailability in the skin is a factor affecting the outcome of the experiment, and the relative contribution of caffeine becomes more important.

Inhibition of Lung Tumorigenesis

Inhibition of lung tumorigenesis by green and black tea preparations has been reported in 14 publications (Table 1). This includes lung tumorigenesis induced by (4-methylnitrosamino)-1-(3-pyridyl)-1-butanon (NNK), *N*-nitrosodiethylamine (NDEA), benzo[a]pyrene (BP), and cisplatin, as well as those that develop spontaneously. In the NNK-induced lung tumorigenesis model with A/J mice, inhibitory activities of green and black tea preparations at the initiation and promotion stages have been demonstrated (4). Brewed black tea and theaflavins have been shown to protect against NNK-induced pulmonary hyperproliferation and tumorigenesis in mice initiated with NNK (45, 46). In mice that already developed lung adenomas (16 weeks after the NNK dose), oral administration of brewed black tea inhibited the progression of adenoma to adenocarcinoma (45).

TABLE 1 Inhibition of carcinogenesis in animal models by tea

Target organ	Species	Carcinogen/promoter[a]	Tea preparation (mode of treatment) used during [tumorigenesis stage]	Study outcome	References[b]
Skin	Mouse	DMBA/TPA	BTP, GTPF, or EGCG (topical) [initiation or promotion]	↓ Tumor incidence or multiplicity	1[98, 100], 3[83] 39
		DMBA/TPA	BT (p.o.) [promotion] DBT (p.o.) [promotion]	↓ Tumor growth Inconclusive	41
		DMBA/TPA, UVB	GTPF or GT (topical or p.o.) [promotion or entire course]	↓ Tumor incidence and/or multiplicity ↓ Progression of papillomas to SCC	1[96, 100, 102] 3[86, 90], 38
		DMBA/teleocidin or okadaic acid	EGCG (topical) [promotion]	↓ Tumor growth	36
		DMBA/TPA, BPO or TPA/4-NQO or spontaneous	GTPF (topical) [progression]	↓ Tumor progression Less effectiveness in spontaneous malignant conversion	3[92]
		BPDE or BP/TPA, 3-MC	GTPF (topical) [initiation or promotion or entire course]	↓ Tumor incidence or multiplicity	1[97, 98, 100]
		UVA + UVB	BT, GT, BT + milk (p.o.) [entire course]	↓ Tumor multiplicity	40
		UVB	GT, BT, or caffeine (p.o.) DGT, DBT (p.o.) [entire course or progression]	↓ Tumorigenesis Had less or inconsistent effect	41, 42
		UVB	GTPF (p.o.) [entire course]	↓ Tumor incidence & multiplicity	1[96]
		UVB	EGCG (topical) or EGCG (p.o.) [entire course]	↓ Tumor incidence or no effect, respectively	37
		UVB	GT, BT, or caffeine (p.o.) [postinitiation] DGT (p.o.) [postinitiation]	↓ Papilloma formation & size No effect	43
		UVB/TPA	GT (p.o.) [initiation & promotion]	↓ Tumor incidence & multiplicity	1[102]

Lung	Mouse	NDEA (i.p.)	DGT or DBT (p.o.) [entire course]	↓ Tumor multiplicity	62
		NDEA or BP (i.g.)	GT or GTPF (p.o.) [initiation or entire course]	↓ Tumor incidence & multiplicity	1[107,109], 3[99]
		NNK (i.p.)	DGT or DBT (p.o.)	↓ Tumor incidence & multiplicity	1[107,108]
		NNK (i.g.)	GT, EGCG or caffeine (p.o.) [initiation or postinitiation]		2[3]
		NNK (i.p.)	BT (p.o.) [postinitiation/ progression]	↓ Progression of adenoma to adenocarcinoma	45
		NNK (i.p.)	TF (p.o.) [postinitiation]	↓ Tumor incidence & multiplicity	46
		MNNG	GT (p.o.) [initiation]	↓ Tumor multiplicity	2[31]
		Tobacco smoke	Green tea [postinitiation]	No effect	52
		Spontaneous	GT or BT infusion (p.o.) [entire course]	↓ Tumor incidence, multiplicity & volume	47
		Cisplatin	EGCG (p.o.) [entire course]	↓ Tumor incidence & multiplicity	51
	Rat	Asbestos + BP	GT (p.o.) [entire course]	↓ Tumor incidence	50
		NNK	BT or caffeine (p.o.) [entire course]	↓ Tumor incidence & multiplicity	48
Oral cavity	Rat	DMBA (topical)	GT, mixed tea (p.o.) [entire course]	↓ Tumor incidence & multiplicity	53
Esophagus	Rat	NMBzA or NMBzA precursors (i.g.)	GT, BT, oolong (p.o.) [entire course]	↓ Tumor incidence & multiplicity	1[110,111]
		NMBzA (i.g.)	GT, DGT, DBT (p.o.) [initiation/postinitiation]	↓ Tumor incidence & multiplicity	2[4]
		NMBzA (s.c.)	EGCG, TF, GTPF or BTP [entire course]	↓ Tumor multiplicity (only results with TF were statistically significant)	54
Fore-stomach	Mouse	Precursors of NMBzA or NSACS (i.g.)	GT (p.o.) [entire course]	↓ Tumorigenesis	1[112], 2[21]
		NDEA or BP (i.g.)	GT (p.o.) [entire course]	↓ Tumor incidence & multiplicity	1[109]
		NDEA or BP (i.g.)	GT, GTPF (i.g./p.o.) [entire course]	↓ Tumor incidence & multiplicity	1[107], 3[99]
	Rat	NDEA (i.g.)	GT (p.o.) [entire course]	↓ Tumor incidence & multiplicity	1[2]

(*Continued*)

TABLE 1 (*Continued*)

Target organ	Species	Carcinogen/promoter[a]	Tea preparation (mode of treatment) used during [tumorigenesis stage]	Study outcome	References[b]
Stomach	Rat	MNNG (p.o.)	GT, DGT (p.o.) [entire course] EGCG (p.o.) [entire course]	↓ Tumor incidence and/or multiplicity	2[25] 55
Small intestine	Mouse	ENNG (i.g.)	EGCG [postinitiation]	↓ Tumor promotion	1[114]
		ENNG	GT, EGCG (p.o.) [entire course]	↓ Tumor incidence and/or multiplicity	56
	Min mouse		GT (p.o.) [from 6 weeks]	↓ Tumor multiplicity	57
	Rat	Multiple carcinogens	GTPF (p.o.) [initiation]	↓ Initiation and postinitiation	1[115]
Colon	Rat	AOM (s.c.)	GTPF (p.o.) [postinitiation]	↓ Tumor incidence and multiplicity	1[116]
		AOM (s.c.)	GT, EGCG (p.o.)	↓ Tumor multiplicity	56
		AOM (s.c.)	EGCG (p.o.) [postinitiation]	No effect	59
		AOM (s.c.)	Tea or tea + milk (p.o.) [postinitiation/entire course]	No effect	60
		AOM (s.c.)	BT (p.o.) [postinitiation] GT (p.o.) [postinitiation]	↓ Tumor incidence & multiplicity No effect	58
		MNU (p.r)	GTE (p.o.) [entire course]	↓ Tumor incidence	3[112]
	Mouse	DMH (s.c.)	GTPF or EGCG (i.g.) [entire course]	↓ Tumor incidence	1[29]
Liver	Rat	NDEA (i.p.) or Aflatoxin	GT (p.o.) [entire course]	↓ Hepatocarcinogenesis	1[118], 3[113]
		NDEA (i.p.)	GTPF, BT or oolong tea (p.o.) [initiation or promotion or entire course]	↓ GST positive hepatic foci	63
		NDEA (i.p.)	Epicatechin complex (p.o.) [initiation]	↓ Initiation of hepatocarcinogenesis	3[115]
		NDEA (i.p.)	DGT or DBT (p.o.) [entire course]	↓ Tumor multiplicity	62

		NNK (s.c.)	BT or caffeine (p.o.) [entire course]	↓ Tumor incidence	48
		Glu-P-1 or HCA	GTPF (p.o.)	↓ GST positive hepatic foci	64, 65
	Mouse	Tobacco	DBT (p.o.)	↓ Tumorigenesis	3[116]
Pancreas	Hamster	BOP (s.c.)	GTPF or (p.o.) [postinitiation]	↓ Tumor initiation	1[119], 67
		BOP	GTPF (p.o.) [progression]	↓ Hyperplasia and total duct lesion	68
Urinary bladder	Rat	BBN (p.o.)	GT, BT & oolong (p.o.) [postinitiation]	↓ Formation of bladder tumor	66
Mammary gland	Rat	DMBA (i.g.)	GTPF (p.o.) [postinitiation]	No effect on tumor incidence & multiplicity, but ↓ tumor size	70
		DMBA (i.p., i.g.)	GTPF (p.o.) [postinitiation]	↓ Tumor incidence or multiplicity	71, 2[27]
		DMBA (i.g.)	Crude EGCG preparation (p.o.) [progression]	No effect	72
		DMBA (i.g.)	Tea extract (p.o.) [entire course]	↓ Tumor incidence & ↓ tumor burden only in the high fat diet group	73
		DMBA/IQ	BT (p.o.) with or without milk [entire course]	↓ Tumor incidence & multiplicity.	69
	Rat & mouse	DMBA	Catechins	↓ Tumor incidence.	61
	Mouse	Spontaneous	GTPF (p.o.) [postinitiation]	↓ Tumorigenesis	2[34]

GT, green tea; BT, black tea; TF, theaflavins; 4-NQO, 4-nitroquinoline-*N*-Oxide; BPDE, benzo(a)pyrene diolepoxide; BPO, benzoal peroxide; 3-MC, 3-methylcholanthrene; NM-BzA, *N*-nitrosomethylbenzylamine; NSACS, Nitrosarcosine; Glu-P-1, 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole; BBN, *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; ENNG, *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine; HCA, heterocyclic amine; MNU, *N*-methyl *N*-nitrosurea; EGCG, (−)-epigallocatechin gallate; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EC, (−)-epicatechin; DGT, decaffeinated green tea; DBT, decaffeinated black tea; GTPF, green tea polyphenol fraction; DMBA, 7,12-dimethylbenz[a]anthracene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; NNK, (4-methylnitrosamino)-1-(3-pyridyl)-1-butanone; NDEA, *N*-nitrosodiethylamine; BP, benzo[a]pyrene; NDMA, *N*-nitrosodimethylamine; BTP, black tea polyphenols; AOM, azoxymethane; DMH,1,2-dimethylhydrazine; PhIP, 2-amino-1-methyl-6-phenyl imidazo [4,5-p] pyridine; BOP, *N*-nitroso-bis(2-oxopropyl)amine; TFdiG, theaflavin digallate.

[a]For skin carcinogenesis all carcinogens/promoters were applied topically.

[b]The superscript denotes the reference cited in that particular review article.

Black tea and green tea infusions have also been shown to inhibit the spontaneous formation of lung tumors and rhabdomyosarcomas in A/J mice (47). Treatment with black tea or green tea for 60 weeks significantly reduced lung tumor incidence, multiplicity, and volume. The incidence and multiplicity of rhabdomyosarcomas were also reduced significantly by 2% green or black tea. The body weights and particularly the body fat weights (as measured by the retroperitoneal fat pad weight) of the mice in the tea-treated groups were significantly lower than in the control group. The mice in all groups consumed about the same amount of diet. The body weight reduction effect could be attributed to 1) decreased absorption of fat and proteins, and 2) increased energy expenditure due to tea administration. The contribution of caffeine in increasing energy expenditure and body fat reduction could be a factor in the inhibition of carcinogenesis.

Chung et al. (48) examined the effect of black tea and caffeine on lung tumorigenesis in F344 rats induced by NNK in a two-year bioassay. After 20 weeks of NNK administration, the animals were given either 2% black tea infusions or caffeine (680 ppm, corresponding to those in tea infusions) for 22 weeks. Black tea caused a significant reduction of the total lung tumor (adenomas, adenocarcinomas, and adenosquamous carcinomas) incidence (from 47% to 19%). Caffeine treatment also caused a remarkable reduction of the lung tumor incidence (from 47% to 10%). It appears that caffeine could account for all the inhibitory activity of black tea. In a previous study with A/J mice, however, pure EGCG was shown to be slightly more effective than caffeine in inhibiting lung tumorigenesis when both compounds were used at concentrations corresponding to those present in 2% green tea (49).

The above results clearly demonstrated the inhibition of lung tumorigenesis by different tea preparations. It appears that both tea polyphenols and caffeine contribute to the inhibitory activity. In rats, which have poor bioavailability for EGCG (and perhaps ECG and theaflavins), the contribution of caffeine could proportionally be higher.

Inhibition of Tumorigenesis in Gastrointestinal Tract

The chemopreventive effect of tea against tumors in different parts of the gastrointestinal tract including mouth, esophagus, small intestine, and colon has been shown in 21 out of 23 studies (Table 1). Li et al. (53) demonstrated that oral administration of 1.5% green tea or other tea preparations as drinking fluid, starting two weeks before initiation with DMBA and proceeding until the end of the experiment, significantly reduced the incidence of oral dysplasia and carcinoma in Syrian golden hamsters. The treatment also reduced the frequency of micronucleated cells, the proliferation index, and the level of epidermal growth factor (EGF) receptor expression in oral mucosal cells. Recent studies in our laboratory also showed that a solution of 0.6% green tea solids, when given as drinking fluid to Syrian golden hamsters during the postinitiation stage inhibited DMBA-induced

oral carcinogenesis in terms of the number and volume of visible tumors and the number of SCC (H. Li, X. Chen, Y. Josephson, M-T Huang, & C.S. Yang, unpublished results). The inhibition was much more pronounced when curcumin was also applied to the cheek pouch of the hamster.

The inhibition of *N*-nitrosomethylbenzylamine (NMBzA)-induced esophageal carcinogenesis in rats by different tea preparations has been demonstrated in four studies (Table 1). Morse et al. (54) compared the effects of BTP, GTPF, TF, and EGCG by administering them to rats in the drinking water at concentrations of 360 and 1200 ppm starting two weeks before the administration of NMBzA. By 25 weeks, there were trends of dose-related inhibition of esophageal tumor multiplicity by BTP, GTPF, TF, and EGCG; however, only the high dose TF group was significantly different from the control.

Tea preparations have also been shown to inhibit tumorigenesis in the mouse forestomach induced by NDEA or precursors of NMBzA or *N*-nitrososarcosine (Table 1). The inhibitory effect of EGCG on *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG)-induced glandular stomach carcinogenesis in rats and on *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine (ENNG)-induced duodenal carcinogenesis in mice has been reported (55, 56). Green tea extract administration also inhibited intestinal tumor formation in the Apc mutant *Min* mice, and the effect was enhanced synergistically when combined with sulindac (57).

Several studies have demonstrated chemopreventive effects of different types of tea extracts against colon tumorigenesis in rats or mice induced by azoxymethane (AOM), 1,2-dimethylhydrazine (DMH), 2-amino-3-methylimidazo-4,5-f]quinoline (IQ), and 2-amino-1-methyl-6-phenylimidazo [4,5-p] pyridine (PhIP) (Table 1). The protective effect of black tea extract on AOM-induced intestinal carcinogenesis in F344 rats has recently been shown by Caderni et al. (58). However, lack of a protective effect was reported by Weisburger et al. in the AOM-induced colon cancer model in rats (59, 60).

Protection Against Hepatocarcinogenesis

A total of nine studies have been reviewed in this area (Table 1). A significant decrease in the incidence of NNK-induced liver tumors (from 34% to 12%) in rats receiving 2% black tea was reported by Chung et al. (48). Caffeine, at a concentration corresponding to that in 2% black tea, also displayed a similar inhibitory effect. Green and black tea administration also led to a significant reduction in mouse liver tumor multiplicity induced by NDEA (62).

Inhibitory effects of individual tea catechins, black tea extract, and oolong tea extract (0.05 or 0.1%) on hepatic preneoplastic glutathione S-transferase (GST)-positive foci formation were demonstrated in rats treated with a single dose of NDEA and then phenobarbital (0.05%) in the drinking water for a period of six weeks (63). In a medium term bioassay, GTPF inhibited the development of heterocyclic amine-, NDMA- and NDEA-induced preneoplastic GST-positive hepatic foci (64, 65).

Protection Against Pancreatic and Bladder Carcinogenesis

There are three studies on the effects of tea on pancreatic carcinogenesis (Table 1). In a carcinogenesis model in which Syrian golden hamsters were treated with *N*-nitroso-bis(2-oxopropyl)amine (BOP) and then put on a protein-deficient diet consisting of DL-ethionine and L-methionine for tumor promotion, dietary supplementation with tea polyphenols during the promotion stage reduced pancreatic tumorigenesis. In a study by Hiura et al. (67), the incidence and multiplicity of pancreatic tumors in hamsters decreased when green tea extract was administered after the initiation of pancreatic tumorigenesis by BOP. They also observed the inhibitory effect of green tea extract on the growth of transplanted pancreatic cancer. Tea polyphenols in the drinking fluid at 500 and 5000 ppm decreased the numbers of hyperplasia and total ductal lesions initiated by BOP in hamsters (68). A combination of low dose tea polyphenol (50 ppm) and β-carotene also inhibited the lesion development. Inhibition of *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine-induced urinary bladder tumors in rats by green tea has been reported, when administered either as an infusion in the drinking fluid or in a solid form in the diet (66).

Inhibition of Mammary Gland Tumorigenesis

The results from a total of eight studies concerning the effect of tea on mammary gland tumorigenesis are mixed (Table 1). Weisburger et al. (69) showed a decrease in the tumor multiplicity and tumor volume of DMBA-induced mammary tumors in rats by oral administration of tea commenced at the preinitiation stage. Hirose et al. (70) reported that administration of GTPF to rats after DMBA treatment decreased the mammary tumor size, but had no effect on tumor incidence and multiplicity. In a study by Tanaka et al. (71), in the DMBA-induced mammary tumorigenesis model, inhibition of tumor multiplicity was observed with rats on 1% and 0.01% tea catechins, but strangely not with rats on 0.1% tea catechins.

No protective effect was observed by Hirose et al. (72) when tea catechins were given at the late promotion or progression stage of mammary carcinogenesis. In this study, after the initiation of tumors with DMBA in rats, tea polyphenol preparations containing 0.29% and 0.4% EGCG were given to rats as drinking fluid. The incidence, multiplicity, and size of mammary tumors were not significantly influenced by the treatment. Two other unpublished studies also showed a lack of inhibition by tea (M-T. Huang, R. Karmali, personal communication). Similarly, Rogers et al. (73) showed no significant effect of black tea administered during the promotion stage of DMBA-induced mammary tumorigenesis in rats on an AIN-76 diet. It is interesting that in rats on a high fat diet, a reduction of the tumor number and size by black tea was observed.

Because of the low bioavailability of tea polyphenols, especially in the mammary tissues, the observed inhibitory effect of tea on mammary tumorigenesis is likely an indirect action; for example, by affecting body fat or estrogen metabolism.

Inhibition of Multi-Organ Tumorigenesis

In a multiorgan carcinogenesis model, F344 male rats were treated with NDEA, *N*-methylnitrosourea, DMH, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, and 2,2′-dihydroxy-di-n-propylnitrosamine for a period of 4 weeks (74). The numbers of small intestinal tumors (adenomas and carcinomas) per rat were significantly reduced in the groups treated with 1% or 0.1% tea catechins during the entire period of the experiment. On the other hand, the numbers of hepatic GST-positive foci were significantly increased by tea catechins. Schut & Yao (75) demonstrated the potential of green and black tea to inhibit PhIP-induced mammary, intestine, colon, and liver tumorigenesis in F344 female rats by assaying the inhibition of PhIP-DNA adduct formation at the corresponding target sites.

Inhibitory Action Against Transplantable Tumors

Hara et al. (76) reported that EGCG and crude catechin preparations (administered i.p., s.c., or p.o.) produced inhibitory effects against the growth of transplanted tumors (mouse sarcoma, mouse Ehrlich solid tumors, and rat solid tumors). Taniguchi et al. (77) demonstrated the inhibition of lung metastasis in mice transplanted with B16 melanoma cells by 0.05% EGCG administered orally. Oral administration of green tea infusion reduced the metastasis of Lewis lung carcinoma cells in mice (78). Zhu et al. (79) reported the inhibition of the growth of transplanted Lewis lung carcinoma along with improvement in immune function in mice receiving green tea in drinking water. Liao et al. (80) reported the inhibition of the growth of inoculated human prostate cancer cells (PC-3 and LNCaP 104-R) and mammary cancer cells (MCF-7) in nude mice by EGCG. Nevertheless, EGC, EC, and ECG were not effective against PC-3 cells.

EPIDEMIOLOGICAL STUDIES ON TEA AND CANCER

Since our initial review on this topic in 1993 (1), many other reviews have been published (3, 15, 81–83). The association of tea consumption with lower risk for certain types of cancer has been demonstrated in some studies, but not in others.

Since the ingested tea is in direct contact with the digestive organs, these organs are more likely to be protected than other organs. Of seven case-control studies on the relationship between green tea consumption and gastric cancer risk, two studies in Japan and two studies in China found a significant inverse association, one study each in Japan and China found a nonsignificant inverse association, and one study in Taiwan found a nonsignificant positive association (reviewed in 84). In a recent population-based prospective cohort study in Miyagi, Japan, however, no association between green tea consumption and gastric cancer risk was observed (84). The result was from a follow-up of 199,748 person-years from 1984 to 1992 involving 419 cases of gastric cancer. Analysis was conducted after adjustment for sex, age, smoking status, alcohol consumption, and other factors. Nevertheless,

smoking could still be a confounding factor; the men in the highest category of tea consumption ($\geq$5 cups/day) also had the largest percentage of heavy smokers and the lowest percentage of nonsmokers.

Historically, tea consumption has been considered a risk factor for esophageal cancer. Nevertheless, of the seven publications reporting a positive association between tea consumption and esophageal cancer, six have been attributed to the hot temperature of tea (reviewed in 2). On the other hand, a population-based study conducted in Yangzhong, China, recently revealed that green tea drinkers had 48% and 51% reduced risks of gastric cancer and chronic gastritis respectively, after adjusting for confounders (85a). This study included 133 gastric cancer cases, 166 chronic gastritis cases, and 433 healthy controls. In a population-based case-control study involving 734 esophageal cancer patients in Shanghai, frequent consumption of green tea was associated with a lower risk for esophageal cancer, especially among nonsmokers and nonalcoholic-drinkers (85). In this study, drinking "burning-hot" fluids, including tea, soups, and other drinks was associated with a five-fold increase in the risk of esophageal cancer.

Studies in Saitama, Japan have shown that women consuming more than 10 cups of tea per day are associated with a lower risk for cancer (all sites combined) (86). On the other hand, in the Netherland Cohort Study on Diet and Cancer, consumption of black tea did not affect the risk for colorectal, stomach, lung, and breast cancers (87).

The above examples illustrate the inconsistent results from epidemiological studies on tea and cancer. The results could be confounded by lifestyle-related factors such as smoking, alcohol consumption, and diet. The different results may also be due to diverse etiological factors involved in different populations. Tea may be effective only against specific types of cancer caused by certain etiological factors.

MECHANISMS OF ANTICANCER ACTIVITIES

Many mechanisms have been proposed for the inhibitory action of tea against carcinogenesis. Much of the information, however, was obtained from studies in cell lines with tea polyphenols (mainly EGCG) levels that may not be achievable in vivo. In this section, we review the biological activities of tea constituents that may contribute to the inhibition of carcinogenesis and assess their possible importance in vivo.

Studies in Cell Lines

The inhibition of cell growth and the induction of apoptosis by tea polyphenols have been demonstrated in cell lines from different origins (reviewed in 88). Many of these studies were conducted in cancer cell lines with EGCG (and other tea polyphenols) at rather high concentrations (50 μM or higher), and the results should be interpreted with caution. Katdare et al. (89) demonstrated that 1 to 2 μM of EGCG caused the inhibition of transformation of preneoplastic human

mammary epithelial cells by BP. Dong et al. (90) showed that 2 to 5 μM of EGCG or TF inhibited TPA- or EGF-induced transformation of mouse epidermal cell line JB6, and the activity was closely related to the inhibition of the transcription factor AP-1 activity. These two studies may be relevant to cancer prevention because of the inhibition of cell transformation and the low effective concentrations observed. Newer studies have confirmed the growth inhibition and apoptosis induction activities of tea polyphenols and attempted to elucidate the underlying mechanisms. Some major activities of tea polyphenols are summarized as follows:

1. Inhibition of MAP-kinases, AP-1, and related activities—Elevated MAP-kinase and AP-1 activities are involved in many disease processes such as inflammation, neoplastic transformation, cancer cell invasion, metastasis, and angiogenesis. Inhibition of MAP-kinase and AP-1 activities by EGCG and other tea polyphenols has been demonstrated in JB6 cells and the corresponding H-ras transformed cell line (30.7b Ras 12) (90, 91). In the JB6 cells, the phosphorylation of c-Jun was inhibited. In the 30.76 Ras 12 cells, EGCG and theaflavin digallate (TFdiG) inhibited the phosphorylation of both c-Jun and Erk1/2, as well as the phosphorylation of MEK1/2 and Elk-1, which are upstream and downstream of Erk1/2, respectively, in the MAP-kinase cascade. In addition, TFdiG promoted the degradation of Raf-1, which is upstream of MEK1, and EGCG decreased the association between Raf-1 and MEK1 (92). These results suggest that EGCG affects different protein kinases directly. However, there may also be inhibitory actions that are cell type–dependent. For example, in the inhibition of UVB-induced c-fos gene expression in human keratinocyte HaCaT cells by EGCG (5 μM), decrease in phospho-p38 (but not in phospho- c-Jun or -Erk) was observed (93).
2. Inhibition of NFκB and related activities—The transcription factor NFκB is involved in inflammation and the survival of cancer cells. The activation of NFκB involves the phosphorylation of IκB, which leads to its degradation and allows the functional p50/p65 complex to be translocated into the nucleus to bind to the κB site and activate transcription. Lin et al. observed that EGCG (10 μM) prevented the degradation of IκB, the activation of NFκB, and the induction of inducible nitric oxide (iNOS) by lipopolysaccharide in macrophages (reviewed in 94). Ahmad et al. (95) also observed that EGCG (20 μM) inhibited TNFα-induced degradation of IκB and activation of NFκB in human epidermoid carcinoma A431 cells; but with normal human epidermal keratinocytes (NHEK), much higher concentrations of EGCG were needed to cause similar effects. Okabe et al. (96) observed the inhibition of TNFα gene expression as well as the inhibition of the tumor promoter okadaic acid–induced AP-1 and NFkB activation in human stomach cancer cell line KATOIII. Since NFκB is known to be antiapoptotic and promote survival in cancer cells, the inhibition of NFκB by tea polyphenols may promote apoptosis.
3. Induction of apoptosis—The induction of apoptosis in different cancer cell lines, including those from skin, lung, stomach, prostate, and blood, by

EGCG and other tea polyphenols has been reported and reviewed (88, 97, 98). In general, the reported effective concentrations for the induction of apoptosis are higher than those required for growth inhibition. In the induction of apoptosis by EGCG (25 μM) in H-ras–transformed human bronchial epithelial cell line 21BES and lung cancer cell line H661, H_2O_2 production was involved and the effect was abolished by the addition of catalase (99, 100). This suggests that prooxidant activity of EGCG could play a role in the induction of apoptosis. In the induction of apoptosis of human chondrosarcoma HTB-94 cells by EGCG (5 μM), induction of caspase-3 was observed (101). In the induction of apoptosis in human histolytic lymphoma U937 cells by theasinensin A (25 μM) (from oolong tea) and other tea polyphenols, a rapid generation of reactive oxygen species, a loss of mitochondrial transmembrane potential, the release of cytochrome c, and activation of caspase-9 and caspase-3 were observed (102).

4. Modulation of cell-cycle regulation—During the blockade of cell-cycle progression at G_1 phase of asynchronous breast cancer MCF-7 cells by EGCG (30 μM), the hypophosphorylation of Rb protein was associated with the loss of cyclin-dependent kinase (cdk)2 and cdk4 activities (103). The p53 levels and the expression of the cdk inhibitors, WAF1/p21 and KIP1/P27, also increased. Ahmad et al. (104) reported that EGCG (20–80 μM) inhibited the cell cycle G_1 arrest, downregulated the protein expression of cdk 4, cdk 6, and cyclin D1, and upregulated the protein expression of WAF1/p21, K1PI/p27, Ink4a/p16, and Ink4c/p18. Similarly, G_0/G_1-phase arrest, increase of p53, and induction of WAF1/p21 by EGCG (40 and 80 μM) were also observed in human prostate carcinoma cells (105). When added to cells in the G_0 and G_1 phase, EGCG (50 μM) induced WAF1/p21, inhibited cyclin D1-associated pRb kinase activity, and impaired Rb phosphorylation in EGF-stimulated MCF10A breast epithelial cells (106).
5. Interference on receptor binding and related activities—Liang et al. (107) observed that EGCG (1–2 μM) blocked EGF-binding to its receptor (EGF-R) and the autophosphorylation of EGF-R in A431 cells. In vitro assays demonstrated the inhibition of protein tyrosine kinase activities of EGF-R, PDGF-R, and FGF-R by EGCG (IC_{50} 1–2 μM). On the other hand, protein kinase C (PKC) and protein kinase A activities were less susceptible to EGCG ($IC_{50} > 20$ μM). Similar and stronger activities were also observed with TFdiG (108). TFdiG (20 μM) also inhibited TPA-induced PKC activity as well as AP-1 activity and c-Jun expression in NIH3T3 cells, but EGCG had weaker inhibitory activity (93). Sachinidis et al. (109) reported the inhibition of the transformation of A172 human glioblastoma cells; the tyrosine phosphorylation of PDGF-receptorβ (PDGFRβ); and phosphatidylinositol 3′-kinase (PI 3′-K) activity by ECGG, ECG, and catechin gallate (50 μM), but not by those without the gallate group. Ren et al. (110) reported the growth inhibition and down regulation of androgen receptor in LNCap prostate cancer

cell lines by EGCG (5–20 μM), and suggested the possible involvement of SP1 transcription factor as a target.

6. Inhibition of invasiveness and angiogenesis—Sazuka et al. reported that pretreatment of Lewis lung carcinoma LL2-Lu3 cells with green tea infusion resulted in the inhibition of their invasion into matrigel, and this may be related to the antimetastasis activity of tea in vivo (78). EGCG, ECG, and TF (20 μg/ml) strongly suppressed the invasion of human fibrosarcoma HT1080 cell into the monolayer of human umbilical vein matrix endothelial cells/gelatin membrane and inhibited gelatin degradation mediated by metalloproteinases (MMP) 2 and 9 (111). EGCG also inhibited the activities of MMP2 and MMP9 (IC_{50} 0.8–6 μM) as well as ConA-induced pro-MMP-2 activation in glioblastoma cell (112). Zhang et al. showed that EGCG (50 μM) inhibited the invasion of rat ascites hepatoma AH109A cells when cocultured with a rat mesentery-derived mesothelial cell monolayer (113). Cao & Cao (1999) demonstrated the inhibition of endothelial growth and angiogenesis in the chorioallantoic membrane assay by EGCG (20 μM). These authors also showed that oral administration of 1.25% green tea to mice inhibited corneal neovascularization stimulated by vascular endothelial growth factor (VEGF) (114).

Possible Mechanisms for the Inhibition of Carcinogenesis

Tea is likely to inhibit carcinogenesis by multiple mechanisms. Some of them have been reviewed (1–3). Among the proposed mechanisms, some may be relevant to many situations, some may be important only in specific cases, and some others may not be relevant. The antioxidant theory for cancer prevention by tea has been widely mentioned. Nevertheless, this mechanism has not been fully substantiated. Although tea polyphenols are strong antioxidants, their antioxidative effect in vivo is weak, demonstrable in some studies but not in others, which is possibly owing to the low bioavailability of these compounds. The contribution of the antioxidative activity of tea to inhibition of carcinogenesis needs to be further assessed. Another proposed mechanism is that tea polyphenols can inhibit the activation and increase the elimination of carcinogens, and thus inhibit carcinogenesis. The inhibition of carcinogen activation by tea polyphenols has been demonstrated in vitro, but the results from in vivo studies are not as convincing (reviewed in 1). Tea consumption has been shown to moderately induce glutathione *S*-transferase, UDP-glucuronsyltransferase, NADPH-quinone oxidoreductase, and antioxidant enzymes, which may enhance the elimination of carcinogens and reactive oxygen radicals. The contribution of these activities to cancer prevention needs to be quantified.

The inhibition of MAP-kinases and cyclin-dependent kinases as well as the suppression of the activation of transcription factors AP-1 and NFκB by tea can result in cell-cycle arrest, increase in apoptosis, as well as inhibition of cell proliferation, cell transformation, tumor invasion, and angiogenesis. These activities, if they can

be verified in vivo, may have broad applications for cancer prevention. Enhancement of apoptosis by tea treatment is beginning to be demonstrated in animal models. Recently Lu et al. (115) showed that administration of 0.6% green tea solids or corresponding amounts of caffeine (0.044%) as drinking fluid to SKH-1 mice for 2 weeks enhanced UV-induced increase in the number of apoptotic sunburn cells as well as p53-positive cells and WAF1/p21-positive cells in the epidermis. Recent studies from our laboratory indicated that the inhibition of NNK-induced lung tumorigenesis in mice by 0.6% green tea solids (observed at 16 weeks after the NNK dose) was associated with enhanced apoptosis and decreased angiogenesis (J. Liao, G-Y. Yang, E.S. Park, D.N. Seril, C.S. Yang, unpublished results). The apoptotic indices in lung adenomas as determined by morphological analysis and the TUNEL method increased by 37% and 93%, respectively, due to tea treatment. Tea treatment also decreased the blood vessel number (as determined by immunostaining against von Willebrand factor) by 50% on the basis of per adenoma or by 32% based on tumor area. The scores for VEGF staining in the adenomas were also decreased by 32% due to the tea treatment. Additional studies with this approach are needed to further verify these mechanisms for the inhibition of tumorigenesis by tea.

Early studies demonstrated that topical application of GTPF or EGCG to mice inhibited cyclooxygenase and lipoxygenase activities (reviewed in 1). Because aberrant arachidonic acid metabolism is involved in inflammation and tumorigenesis, such inhibitory activities could contribute to the inhibition of carcinogenesis. The inhibition of arachidonic acid metabolism via the cyclooxygenase and lipoxygenase pathways by tea polyphenols has also been demonstrated in human colon mucosa and colon tumors (116). A related observation is that administration of one or two cups of green tea to human volunteers caused a decrease of prostaglandin E_2 levels in rectal biopsies at 4 h and 8 h (117). The possible relevance of this effect to the reduction of colon cancer risk deserves further investigation.

In many studies with orally administered tea, reductions in body weight and body fat of the animals have been observed. Caffeine, which increases thermogenesis, may play a key role in this effect. This effect may contribute to the inhibition of tumorigenesis in a manner partially resembling that caused by caloric restriction. Lu et al. proposed that decreased body fat, especially the dermal fat, plays a key role in the inhibition of skin tumorigenesis (44). One possibility is that the dermal fat, or the body fat store in general, can serve as a source of arachidonic acid or energy for tumor development and growth.

CONCLUDING REMARKS

The inhibitory activity of tea against tumorigenesis has been demonstrated in different organ sites in animal models, but the active constituents and the mechanisms of action are not clearly understood. Many investigators believe EGCG (plus other polyphenols) is the active constituent, but the bioavailability of tea polyphenols is rather low and a large portion of catechins are present in the methylated,

glucuronidated, and sulfated forms. The biological activities of these catechin metabolites need to be characterized to enhance our understanding of the biological effects of tea consumption. Many mechanisms for the action of tea have been proposed based on studies in cell lines with tea polyphenols. These mechanisms need to be evaluated in the context of inhibition of carcinogenesis in vivo and verified in animal models or humans. In evaluating the relevance of the proposed mechanisms, the effective concentration should be a key criterion; i.e., the mechanistically relevant biological activities that can be produced by lower concentrations of tea constituents are likely to be more important. The physiological effects of tea consumption, such as the decrease in body weight or fat store mainly caused by caffeine, may contribute to the inhibition of tumorigenesis in animal models. It remains to be determined whether such effects occur in humans.

Epidemiological studies have not yielded clear conclusions concerning the effect of tea consumption on cancer. The difference in results between human and animal studies on this topic may be due to the following reasons. 1) In animal studies, the doses of tea used are generally higher than those consumed by humans. 2) In animal studies, the experimental conditions are generally optimized for the detection of a protective effect. Some of these conditions, such as the use of a particular carcinogen and at a certain dosage regimen, may not occur in human carcinogenesis. 3) In human studies, many confounding factors may provide false negative or false positive results. In many studies, the amounts of tea consumed by the subjects (based on dietary recalls) may not be accurate.

In order to understand the effect of tea consumption on cancer in humans, additional research on the pharmacokinetics of tea constituents as well as their mechanisms of action is needed. Definitive conclusions concerning the protective effect of tea have to come from well-designed observational epidemiological studies and intervention trials. The development of biomarkers for tea consumption, as well as molecular markers for its biological effects, will contribute to better future studies in this area.

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