

Inhibition of carcinogenesis by polyphenols: evidence from laboratory investigations^{1–4}

Joshua D Lambert, Jungil Hong, Guang-yu Yang, Jie Liao, and Chung S Yang

ABSTRACT

Many plant polyphenolic compounds have been shown to have cancer-preventing activities in laboratory studies. For example, tea and tea preparations have been shown to inhibit tumorigenesis in a variety of animal models of carcinogenesis, involving organ sites such as the skin, lungs, oral cavity, esophagus, stomach, liver, pancreas, small intestine, colon, and prostate. In some of these models, inhibitory activity was demonstrated when tea was administered during the initiation, promotion, or progression stage of carcinogenesis. The cancer-preventing activities of these and other polyphenols, such as curcumin, genistein, and quercetin, are reviewed. In studies *in vitro*, many of these compounds have been shown to affect signal transduction pathways, leading to inhibition of cell growth and transformation, enhanced apoptosis, reduced invasive behavior, and slowed angiogenesis. However, the concentrations used in cell culture studies were much higher than those found *in vivo*. If we propose mechanisms for cancer prevention on the basis of cell line experiments, then these activities must be demonstrated *in vivo*. The bioavailability, ie, tissue and cellular concentrations, of dietary polyphenols is a determining factor in their cancer-preventing activity *in vivo*. For example, compounds such as curcumin are effective when applied topically to the skin or administered orally to affect the colon but are not effective in internal organs such as the lungs. More in-depth studies on bioavailability should facilitate correlation of mechanisms determined *in vitro* with *in vivo* situations, increase our understanding of dose-response relationships, and facilitate extrapolation of results from animal studies to human situations. *Am J Clin Nutr* 2005;81(suppl):284S–91S.

KEY WORDS Tea polyphenols, green tea, curcumin, genistein, quercetin, tumorigenesis

INTRODUCTION

Polyphenolic compounds constitute one of the largest and most ubiquitous group of phytochemicals. They are formed to protect plants from photosynthetic stress, reactive oxygen species, and herbivory. Polyphenolic compounds are an important part of the human diet, with flavonoids and phenolic acids being the most commonly occurring ones in food. Early interest in polyphenols was related to their “antinutritional” effects, ie, decreasing absorption and digestibility of food through their ability to bind proteins and minerals. The astringency of many fruits and beverages is attributable to the precipitation of salivary proteins

by plant polyphenols. Current interest is focusing on the beneficial health effects of dietary polyphenols, because these compounds may have antioxidative, antiinflammatory, and anticarcinogenic activities. The chemical properties, biochemical properties, and bioavailabilities of many polyphenolic compounds have been reviewed (1). This article updates these subjects, with emphasis on the potential cancer-preventing activities of dietary polyphenols and the possible mechanisms involved. We review the data on the tea polyphenols, curcumin, genistein, and quercetin (**Figure 1**). A more extensive discussion of the tea polyphenols is used to illustrate the key issues related to cancer prevention.

TEA AND ITS CANCER-PREVENTING ACTIVITIES

Tea constituents

Tea (*Camellia sinensis*) is a warm-weather evergreen. In the making of green tea, the tea leaves are heated to inactivate the enzymes. Therefore, the constituents are preserved in the dried tea leaves. A typical brewed green tea beverage (eg, 2.5 g tea leaves in 250 mL hot water) contains 240–320 mg of catechins and 20–50 mg of caffeine. The catechins include (–)-epicatechin, (–)-epigallocatechin, (–)-epicatechin-3-gallate, and (–)-epigallocatechin-3-gallate (EGCG), with EGCG being the major catechin in tea (Figure 1). Tea leaves also contain flavonols such as quercetin and myricetin, as well as the nitrogenous compounds caffeine (2–5% by wt) and theobromine (2). During the production of black tea, the tea leaves are withered, crushed, and allowed to undergo an enzyme-mediated oxidation commonly referred to as fermentation. During this process, much of the catechin content of the tea leaves is converted to dimeric theaflavin molecules and polymeric thearubigins. In brewed black tea, catechins, theaflavins, and thearubigins account for 3–10%, 2–6%, and > 20% of the dry weight, respectively (Figure 1). Theaflavins are responsible for the color of and contribute to the taste of black tea.

¹ From the Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, New Jersey.

² Presented at the 1st International Conference on Polyphenols and Health, held in Vichy, France, November 18–21, 2004.

³ Supported by NIH grant CA88961.

⁴ Address reprint requests and correspondence to CS Yang, Susan Lehman Cullman Laboratory for Cancer Research, Ernest Mario School of Pharmacy, Rutgers, State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854. E-mail: csyang@rci.rutgers.edu.

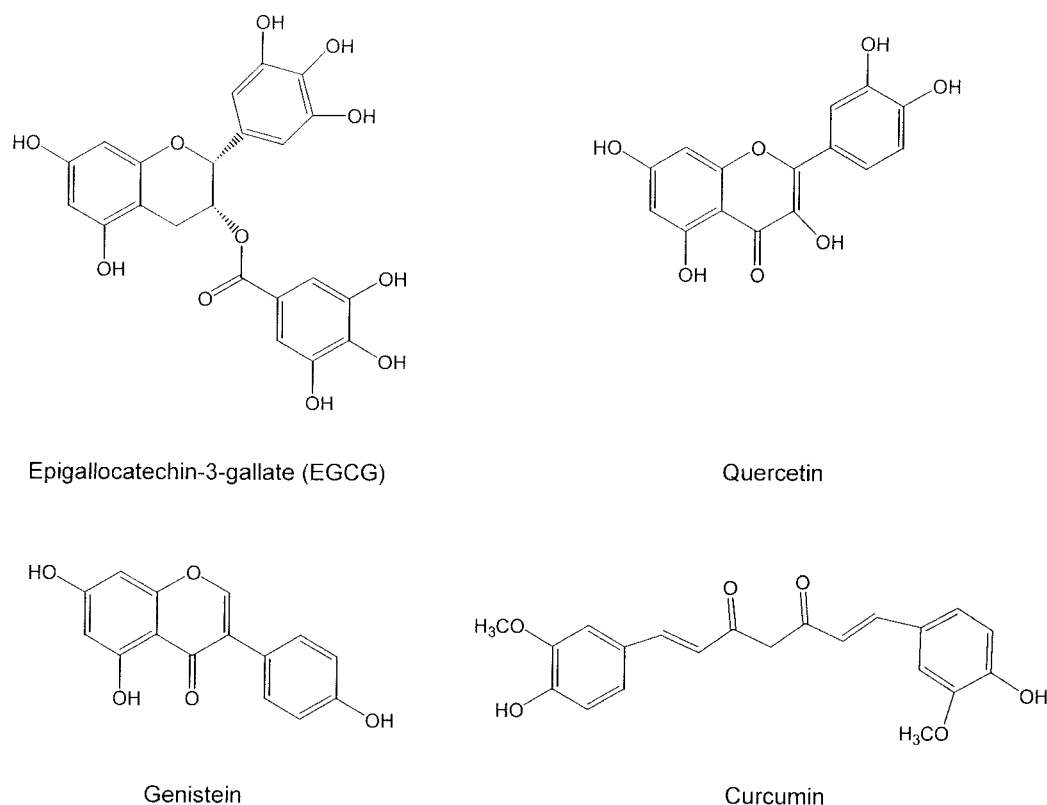


FIGURE 1. Structures of some potentially cancer-preventing dietary polyphenols.

Inhibition of carcinogenesis in animal models by tea and its constituents

Cancer-preventing activity has been demonstrated for green tea, black tea, and their constituents in many animal models of tumorigenesis, including the skin, lung, oral cavity, esophagus, stomach, liver, pancreas, bladder, small intestine, colon, and prostate gland (3–5). **Table 1** summarizes the results of some of those studies.

In most studies, different preparations of tea or tea constituents were administered in drinking water; these included freshly brewed green or black tea (eg, 5–20 g of leaves brewed in 1 L of water, referred to as 0.5–2% tea); green or black tea solids (dehydrated tea extracts), usually reconstituted with distilled water

TABLE 1

Tea and cancer prevention: results of studies with animal models

Site	No. of positive studies (refs)	No. of negative studies (refs)
Prostate	4 (6–9)	
Breast	3 (10–12)	3 (5)
Colon	9 (5, 13–16)	3 (5, 17)
Intestine	2 (5, 18)	1 (19)
Liver	7 (5)	1 (5)
Stomach	6 (5)	
Esophagus	4 (5)	
Oral cavity	2 (5, 20)	
Lung	9 (5)	1 (5)
Skin	15 (5, 21)	
Pancreas	2 (5)	
Bladder	3 (5, 22, 23)	

at concentrations of 0.4% or 0.6%; decaffeinated green or black tea solids prepared as described above; or green tea or black tea polyphenol preparations (which are enriched in tea polyphenols but also may contain some caffeine). The tea preparations were administered either during or after the carcinogen treatment period or during the entire experimental period. Tea preparations were found to be effective in inhibiting 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis in A/J mice with all 3 of the dosing schedules listed above (5). Inhibition of tumor multiplicity (number of tumors per mouse) was observed in most of the studies, although inhibition of tumor incidence (number of animals with tumors) was reported in some publications. In the NNK-induced lung tumorigenesis model, administration of a black tea preparation to adenoma-bearing mice significantly inhibited tumor cell proliferation and the progression of adenoma to carcinoma (24). Inhibition of tumor invasion and metastasis in transplanted and spontaneous metastasis models by orally administered green tea infusion or EGCG was also reported (25, 26).

Other experiments did not demonstrate inhibition of lung tumorigenesis by tea preparations. For example, Witschi et al (27) reported that green tea extract did not reduce lung tumor multiplicity in male A/J mice treated with a single dose of NNK or in a cigarette smoke-induced lung tumorigenesis model.

Whereas most of the studies were conducted with chemical- or ultraviolet (UV) light-induced tumorigenesis models, tea has also been shown to inhibit spontaneous tumorigenesis. For example, administration of 1% or 2% freshly brewed green or black tea significantly inhibited the spontaneous development of lung adenoma and rhabdomyosarcoma in A/J mice (28). In that

experiment, as well as in some other experiments, the tea-treated groups had lower body weight and body fat than did the control group, although food consumption was not significantly different between the groups. For example, with 1% green tea in the aforementioned experiment, the body weights and retroperitoneal fat pad weights were 14% and 35% lower, respectively, than those for the control group (28). It remains unclear whether these differences in body weight and fat are related to the mechanisms of cancer prevention or are simply coincidental.

With the TRAMP mouse model of prostate carcinogenesis, it was recently demonstrated that oral consumption of 0.1% green tea polyphenols decreased tumor incidence by 65%. In contrast to water-treated animals, which demonstrated a high rate of distal metastasis (25–95%), tea-treated mice showed no distal metastasis. Biochemical and histologic analyses showed a significant decrease in proliferating cell nuclear antigen and a 10-fold increase in tumor cell apoptosis (6). Inhibition of matrix metalloproteinase (MMP)-2 and MMP-9, as well as vascular endothelial growth factor (VEGF), was also observed (29).

The bioavailability of tea constituents is apparently a key factor determining the effectiveness of tea in inhibiting tumor formation. In this respect, the oral cavity and digestive tract, which have direct contact with orally administered tea, may represent good targets for chemoprevention. In the 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced oral carcinogenesis hamster model, treatment with 0.6% green tea, as the sole source of drinking fluid, reduced the number of visible tumors by 35% and reduced tumor volume by 57%. In addition, immunohistochemical analyses showed that tea increased the apoptotic index of the tumors while decreasing the proliferation index and microvessel density (20). Tea preparations were also shown to inhibit esophageal, forestomach, and intestinal cancer (5). Inhibition of colon cancer was observed by some investigators but not others (5).

Purified tea constituents have also been reported to inhibit tumorigenesis. For example, EGCG inhibited lung tumorigenesis in A/J mice induced by NNK and cisplatin (30, 31). Theaflavins (a mixture of theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, and theaflavin-3,3'-digallate [TFdiG]) reduced NNK-induced lung tumor multiplicity and volume in A/J mice (32). These findings are interesting, given the extremely poor bioavailability of theaflavins, and may suggest that the theaflavins are metabolized to a more-bioavailable active metabolite. In NNK-treated F344 rats, the inhibitory activity of black tea could be fully accounted for by the caffeine content of the tea (33). Caffeine has also been shown to play an important role in the inhibition of skin carcinogenesis. For example, Huang et al (34) reported that, whereas orally administered green tea and black tea were effective in reducing the incidence and multiplicity of UVB light-induced skin tumors, orally administered decaffeinated teas were much less effective. The addition of caffeine restored the activity of the decaffeinated teas. A recent study showed that topical application of caffeine or EGCG to SKH-1 hairless mice that had been pretreated twice weekly for 20 wk with UVB light decreased the multiplicity of skin tumors by 44–72% or 55–66%, respectively. In addition, both compounds were shown to increase the apoptotic index of the tumors by 56–92%, as measured with immunohistochemical assays for caspase-3-positive cells (21). The inhibition of skin tumorigenesis by caffeine or tea was shown to be closely correlated with the reduction of body fat (24).

Mechanistic studies in vitro

Antioxidant/prooxidant activity

Numerous potential mechanisms have been proposed for the cancer-preventing activity of tea and tea constituents, on the basis of studies with cancer cell lines. One problem encountered in most studies is that the concentrations of tea compounds used usually exceed those found in animal or human plasma or tissues after tea consumption.

Tea polyphenols have been shown to have strong antioxidant activity in vitro, but such activity was associated with inhibition of tumorigenesis in only a few cases (30, 35). The importance of this potential mechanism in vivo remains unclear. In contrast, some studies suggested that the cell-killing activity of these compounds, at least in vitro, might be related to their prooxidant activity. For example, we showed that EGCG-induced apoptosis, but not growth inhibition, in H661 lung cancer cells and Ras-transformed human bronchial cells was completely or partially blocked with the inclusion of catalase in the medium (36, 37). When EGCG was added to cell culture systems (eg, HT29 cells in McCoy's 5A medium), production of H₂O₂ was observed (38). Preincubation of cells with EGCG (before the addition of a growth factor) was shown to block the epidermal growth factor (EGF)- and platelet-derived growth factor-induced signaling systems (39). Our recent results with KYSE510 esophageal squamous cells indicated that inhibition of the EGF signaling system was associated with degradation or inactivation of the EGF receptor, and the effect could be abolished with the inclusion of superoxide dismutase (Z Hou and CS Yang, unpublished results, 2004). The addition of superoxide dismutase also stabilized EGCG, suggesting that the effect was not attributable to competition between EGCG and EGF for the EGF receptor or inhibition of the activated receptor tyrosine kinase activity by EGCG. These observations suggest a role for EGCG prooxidation in some of the observed activities of EGCG in vitro. It is unclear whether such reactions occur in vivo.

Specific protein kinases

Enhanced activity of the transcription factors activator protein 1 (AP-1) and nuclear factor κ B (NF- κ B) is key in carcinogenesis. EGCG and other tea polyphenols have been shown to inhibit the activation of AP-1 and NF- κ B. Although antioxidative mechanisms have been implicated in this activity, the results can be better explained by the direct inhibition of specific protein kinases by these tea polyphenols.

Treatment of 30.7b Ras 12, Ras-transformed, mouse epidermal cells with 20 μ mol/L EGCG and theaflavins was shown to decrease amounts of phosphorylated Erk1/2 and Mek1/2 (40), as well as the association between Raf-1 (an upstream protein kinase) and Mek1. TFdiG and EGCG were also shown to directly inhibit the kinase activity of Erk1/2 by competing with Elk-1 for access to the active site (40). Similar results were observed after treatment of Ras-transformed human bronchial cells with EGCG and TFdiG (37).

EGCG and TFdiG were shown to inhibit the activity inhibitor κ B (I κ B) kinase in tumor necrosis factor- α -stimulated IEC-6 intestinal epithelial cells and lipopolysaccharide-stimulated RAW 264.7 murine macrophages (41, 42). EGCG and theaflavins were also reported to inhibit cdk4 and cdk2, leading to the hyperphosphorylation of retinoblastoma protein, which is expected to result in G₀/G₁ phase arrest (43).

Enhancement of apoptosis

EGCG was reported to induce apoptosis of cultured cells (5). As discussed previously, the H_2O_2 generated in the cell culture system through autooxidation of EGCG could account for a large proportion of the reported activity. A recent study suggested that certain green and black tea polyphenols bind to the antiapoptotic proteins Bcl-2 and Bcl-xL and thus may induce apoptosis (44). The study used a combination of nuclear magnetic resonance binding assays, fluorescence polarization assays, and computational docking studies. The results, with very low (nanomolar) concentrations of EGCG, were very impressive but must be confirmed in whole cells. To date, apoptosis has been observed only with much higher doses of EGCG (20–100 $\mu\text{mol/L}$) (5).

Inhibition of angiogenesis

Cao and Cao (45) demonstrated inhibition of endothelial growth and angiogenesis in the chorioallantoic membrane assay with EGCG (20 $\mu\text{mol/L}$). Those authors also showed that oral administration of 1.25% green tea to mice inhibited corneal neovascularization stimulated by VEGF (45). EGCG was also shown to inhibit the expression of VEGF by head and neck squamous cell, breast, and colon carcinoma cells (13, 46, 47).

Inhibition of other enzymes

EGCG was shown to selectively inhibit the activity of topoisomerase I, but not topoisomerase II, in human colon cancer cell lines at concentrations that effectively inhibit cell growth (48). Inhibition of MMP-2 and MMP-9 by EGCG was also demonstrated at relatively low doses (49). Others showed that EGCG similarly inhibited the activity and expression of membrane type 1 MMP, a protein responsible for the activation of MMPs (50). Because of the physiologically achievable concentrations used, these activities represent an attractive mechanism for the observed antiangiogenic activity of EGCG and green tea *in vivo* (5).

EGCG has been reported to inhibit the chymotryptic activity of 20S proteasome in leukemic, breast cancer, and prostate cancer cell lines (51). This inhibition causes accumulation of p27^{Kip1} and $\text{I}\kappa\text{B}$, which results in G₀/G₁ phase cell cycle arrest and inhibition of NF- κB activity, respectively. The 50% inhibitory concentration for EGCG-mediated inhibition of proteasomes in cells (1–10 $\mu\text{mol/L}$) is 10-fold higher than the 50% inhibitory concentration in a cell-free system (86–194 nmol/L). This is likely attributable to the nonspecific binding of EGCG to other cellular components. Such nonspecific binding may lead to a higher dose requirement *in vivo*.

CURCUMIN

Topical application of 10 mmol curcumin to the cheek pouch of Syrian golden hamsters was shown to reduce the number of visible DMBA-induced oral tumors by 39.6% and the tumor volume by 61.3% (20). Administration of 4% curcumin in the diet of azoxymethane-treated mice during the initiation period reduced the number of colon tumors by 66%. If treatment was started after initiation, then tumor number was reduced by 25% (52). In APC^{min} mice given 0.2% or 0.5% curcumin in the diet, intestinal adenoma multiplicity was reduced by 39–40% and hematocrit amounts, which were reduced in untreated mice, were partially restored (53). Topical application of curcumin has also been shown to reduce tumor incidence and multiplicity

in both the benzo[*a*]pyrene/12-*O*-tetradecanoyl phorbol-13-acetate (TPA) and DMBA/TPA models of skin carcinogenesis in mice (34, 54).

As with the tea polyphenols, the poor bioavailability of curcumin limits its cancer-preventing activity. Curcumin was found to be ineffective in preventing NNK-induced lung tumorigenesis in A/J mice and liver and kidney tumorigenesis in Long-Evans Cinnamon rats (55, 56). All of these tissues require systemic bioavailability for cancer prevention.

Studies with cell lines have shown that curcumin possesses both antioxidant and prooxidant activities. Curcumin-induced, Akt-mediated apoptosis of Caki renal cells was partially inhibited by cotreatment with *N*-acetylcysteine, which suggests the involvement of oxidative stress (57). Cyclooxygenase-2 and inducible nitric oxide synthase have also been reported as potential targets for curcumin (58, 59). Treatment of lipopolysaccharide-treated mice with curcumin (92 $\mu\text{g/kg}$, administered intragastrically) reduced hepatic inducible nitric oxide synthase mRNA expression by 50–70% (60).

Curcumin has also been shown to inhibit activation of the transcription factors AP-1 and NF- κB in human leukemia cell lines (61, 62). Ablation by curcumin of AP-1 and NF- κB activation has been demonstrated in TPA-treated mouse skin. Chun et al (63) reported that topical curcumin application to mouse skin inhibited TPA-mediated activation of Erk and p38 mitogen-activated protein kinase and subsequent activation of NF- κB .

It is unclear from the currently available data what the primary target (or targets) of curcumin is. Many but not all of the animal models studied involve inflammatory mediators such as TPA or lipopolysaccharide, which indicates that curcumin may act primarily as an antiinflammatory agent. Additional studies with *in vivo* biomarkers are needed.

GENISTEIN

Genistein and daidzein (isoflavones derived from soybeans) have been shown to inhibit the development of both hormone-related and non-hormone-related cancers, including mouse models of breast, prostate, and skin cancer. Treatment of TRAMP mice with 100–500 mg genistein/kg diet reduced the incidence of advanced-stage prostate tumors, in a dose-dependent manner (64). A high-isoflavone diet was also shown to inhibit methylnitrosourea-induced prostate tumors in Lobund-Wistar rats (65).

Topically applied genistein was shown to reduce the incidence and multiplicity of skin tumors in the DMBA-initiated and TPA-promoted Sencar mouse model by 20% and 50%, respectively (66). In the UVB light-induced complete carcinogenesis model, topical pretreatment of SKH-1 mice with 10 μmol genistein significantly reduced the formation of H_2O_2 and 8-hydroxy-2'-deoxyguanosine but not pyrimidine dimers in the epidermis (66).

Treatment of mouse mammary tumor virus-neu mice with diet containing 250 mg/kg genistein or daidzein significantly increased the latency for spontaneous breast tumors but did not affect tumor size or multiplicity (67). In contrast, it was reported that treatment of mice in the DMBA-initiated and medroxyprogesterone acetate-promoted model with diet containing 1 g/kg genistein increased progression of mammary adenomas, compared with control animals (68).

The ability of genistein to inhibit colon cancer in animals remains unclear. In carcinogen-treated rats, both positive and

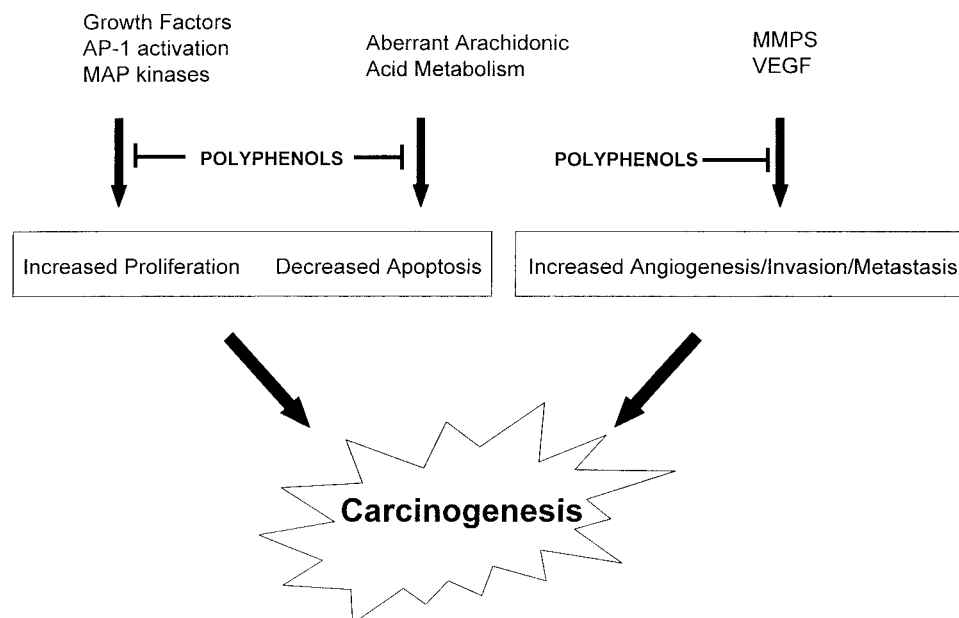


FIGURE 2. Proposed mechanistic scheme for the prevention of cancer by dietary polyphenols.

null effects on the formation of aberrant crypt foci have been reported. No inhibitory effect on intestinal carcinogenesis was observed with APC^{min} mice fed a Western-style (high-fat/low-fiber/low-calcium) diet containing 16–475 mg/kg soy isoflavones (69).

Several mechanisms have been proposed for genistein. Genistein and other isoflavones have demonstrated weak estrogenic activity at lower concentrations but are estrogen receptor antagonists at higher concentrations. At 40 $\mu\text{mol/L}$, genistein inhibited proliferation of LNCaP prostate cancer cells and enhanced apoptosis. Treatment of human bladder cancer cells with genistein resulted in inhibition of cdc2 kinase activity and G₂/M phase cell cycle arrest (70). Genistein has been shown to inhibit 20S proteasomal activity in LNCaP and MCF7 cells, resulting in accumulation of p27^{Kip-1}, I κ B, and Bax (71). Genistein has also been shown to inhibit tyrosine kinase and topoisomerase activity (72). The relative importance of each of these mechanisms remains to be determined in vivo.

The bioavailability of genistein appears to be better than that of the green tea polyphenols or curcumin (73, 74). Plasma concentrations of genistein in tumor-bearing nude mice fed a diet containing 1 mg/g genistein were 3.4 $\mu\text{mol/L}$ (75). Among humans, a single oral dose of 460 mg resulted in peak plasma concentrations of 20–25 $\mu\text{mol/L}$ (76). The concentrations achieved in the target tissues must still be accurately determined.

QUERCETIN

Studies with animal models of carcinogenesis have yielded mixed results regarding the cancer-preventing activity of quercetin. For example, it was reported that 2% dietary quercetin inhibited azoxymethane-induced hyperproliferation and focal dysplasia in mice (77). Quercetin also reduced tumor incidence by 76% and tumor multiplicity by 48%. However, no inhibitory effects were observed in APC^{min} mice treated with quercetin (78). Similarly, although quercetin inhibited local, UVB light-induced immunosuppression in SKH-1 hairless mice, it had no

effect on skin tumorigenesis (79). Quercetin did inhibit *N*-nitrosodiethylamine-induced lung tumorigenesis in mice when administered during the initiation phase (80).

Mechanistic studies have shown that treatment of colon cancer cells with quercetin results in reduced expression of Ras protein (81). Inhibition of cell growth in pancreatic and colon cancer cells has been correlated with inhibition of EGF receptor expression and tyrosine kinase activity (82, 83). In HL-60 promyelocytic leukemia cells, it was found that the ability of quercetin to damage DNA was largely attributable to the formation of H₂O₂ (84).

POSSIBLE MECHANISMS FOR INHIBITION OF CARCINOGENESIS

Although many biological actions of polyphenols have been described, the mechanisms responsible for inhibition of carcinogenesis in animals remain unclear. One of the problems with extrapolating results of in vitro studies to animals is that the concentrations of test compounds used in vitro usually exceed those found in plasma or tissues after polyphenol consumption; genistein may be an exception. Even when physiologically achievable concentrations are effective, as has been reported for some enzyme systems (44, 49, 51), caution is still needed in the extrapolation from purified enzymes to animals, as we previously discussed. Therefore, any proposed mechanisms must be verified in animal models or human tissues.

Multiple mechanisms appear to be involved in the inhibition of carcinogenesis by dietary polyphenols (Figure 2). The relative importance of these mechanisms depends on the model system used. Tea polyphenols and curcumin have been shown to inhibit key signal transduction protein kinases, such as mitogen-activated protein kinase, I κ B, and certain cyclin-dependent kinases. This action would inhibit cell growth and transformation, induce apoptosis, and inhibit angiogenesis. As mentioned above, inhibition of cell growth and induction of apoptosis by tea polyphenols, curcumin, genistein, and quercetin have been noted in

one or more of the following tissues: skin, prostate, colon, and lung. Tea consumption and genistein demonstrated antiangiogenic activity in NNK-induced lung adenoma (J Liao and CS Yang, unpublished observations, 2004) and Lewis lung xenograft (85) mouse models, respectively. Inhibition of arachidonic acid metabolism by tea polyphenols and curcumin has been reported (86–90), and such activity may contribute to the inhibition of carcinogenesis. Other mechanisms, such as inhibition of topoisomerase or inhibition of proteasome, remain to be validated *in vivo*.

CONCLUSIONS

Because of the extensive consumption of polyphenols in the diet, the biological activity of these compounds is an important topic of scientific investigation. Given the potential cancer-preventing activities of these compounds, we would expect to observe these activities in human populations. However, epidemiologic studies have not provided sufficient evidence for such conclusions. On the basis of what is known about the bioavailabilities of different dietary polyphenols, it is likely that organ sites that are most accessible to dietary polyphenols experience the protective effects of these compounds. Future studies, especially carefully designed, mechanism-based, animal studies, may facilitate better understanding of the potential health benefits of dietary polyphenols.



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