Some Perspectives on Dietary Inhibition of Carcinogenesis: Studies with Curcumin and Tea (44173)

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Abstract. **Topical application of curcumin inhibits chemically induced carcinogenesis on mouse skin, and oral administration of curcumin inhibits chemically induced oral, forestomach, duodenal, and colon carcinogenesis. Curcumin and other inhibitors of cyclooxygenase and lipoxygenase are thought to inhibit carcinogenesis by preventing the formation of arachidonic acid metabolites. In contrast to our expectation of a tumorigenic effect of arachidonic acid, we found that treatment of 7,12-dimethylbenz[*a*]anthracene-initiated mouse skin with very high doses of arachidonic acid twice daily, 5 days a week for 26 weeks, failed to result in tumors.**

We considered the possibility that some of the cancer chemopreventive effects of curcumin may be related to an effect of this compound on cellular differentiation, and we investigated the effect of curcumin on differentiation in the human promyelocytic HL-60 leukemia cell model system. Although curcumin alone had little or no effect on cellular differentiation, when it was combined with all-*trans* retinoic acid or 1α,25-dihydroxyvitamin D_3 a synergistic effect was observed. It is possible that many dietary chemicals in fruits, vegetables, and other edible plants can prevent cancer by synergizing with endogenously produced stimulators of differentiation such as all-*trans* retinoic acid, 1α,25-dihydroxyvitamin D_3, and butyrate. More research is needed to test this hypothesis.

Administration of green or black tea inhibits carcinogenesis in several animal models, and tumor growth is also inhibited. Several examples were presented of chemopreventive agents that inhibit carcinogenesis in one animal model but enhance carcinogenesis in a different animal model. Greater efforts should be made to understand mechanisms of cancer chemoprevention and to determine whether a potential chemopreventive agent is useful in many experimental settings or whether it is useful in only a limited number of experimental settings. [P.S.E.B.M. 1997, Vol 216]

Studies in the 1950s by Richardson *et al.* (1) and Miller *et al.* (2) demonstrated that administration of 3-methylcholanthrene or certain other polycyclic aromatic hydrocarbons inhibited hepatocarcinogenesis by the aminoazo dye 3′-methyl-4-dimethylaminoazobenzene. These studies, which were an attempt to enhance tumorigenesis by the simultaneous administration of two carcinogens, were among the first to demonstrate cancer chemoprevention. Additional research demonstrated the mechanism of inhibition by showing that 3-methylcholanthrene and other protective polycyclic aromatic hydrocarbons induced the synthesis of liver enzymes that detoxified aminoazo dyes (3). The strong inhibitory effect of polycyclic aromatic hydrocarbons on azo dye carcinogenesis illustrated the concept that administration of a potentially toxic substance can be a lifesaving cancer chemopreventive regimen. These results indicated that the toxicity of a chemopreventive substance that is permissible depends on the degree of cancer risk and the potency of the protective agent used to prevent cancer. Clearly, it is desirable to identify and to utilize compounds that are safer than 3-methylcholanthrene, and many studies have been done with less-toxic modulators of carcinogen metabolism that inhibit the carcinogenic

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response. Some compounds that inhibit carcinogenesis by enhancing metabolic inactivation when given together with a carcinogen will stimulate the carcinogenic response by functioning as a tumor promoter when given chronically after the carcinogen (4). These observations illustrate the concept that cancer chemopreventive agents may protect animals under one set of circumstances and have the opposite effect under different circumstances. This concept will be discussed again later.

Epidemiological studies suggest that about one-third of human cancer in the United States is related to dietary factors (5, 6), and that people who eat large amounts of fruit and green-yellow vegetables have a lower risk of many kinds of cancer (7–9). These observations in human populations point out the importance of enhancing our understanding of dietary substances that can modulate carcinogenesis. The pioneering research of Wattenberg and his colleagues that demonstrated cancer chemopreventive activity for a large number of dietary and synthetic chemicals (reviewed in Refs. 10 and 11) and the work by Sporn and his colleagues on the chemopreventive effects of retinoids (reviewed in Ref. 12) fostered the field of cancer chemoprevention. In recent years, there has been an increased emphasis on dietary modulators of carcinogenesis, and work in this field is being actively pursued in many laboratories. Our early research on diet and cancer focused on dietary inhibitors of nitrosamine formation (13–16), dietary inhibitors and activators of carcinogen metabolism (17), dietary antagonists of ultimate carcinogens (18–20), and effects of dietary modulation on *in vivo* xenobiotic metabolism by Phase I and Phase II reactions in humans (reviewed in Ref. 21). The later studies showed a stimulatory effect of increasing the ratio of protein to carbohydrate in the diet or of eating cabbage and brussels sprouts or charcoal broiled beef on xenobiotic metabolism in humans. In these studies, it was also observed that dietary changes altered the profile of estradiol and testosterone metabolism. Recent studies in our laboratory have focused on inhibition of carcinogenesis by curcumin, rosemary, and tea. The present manuscript describes some of our studies on the effects of curcumin and tea on carcinogenesis in mice. We also describe the lack of tumor-promoting activity for arachidonic acid and discuss whether or not arachidonic acid and its metabolites play a role in carcinogenesis.

Inhibitory Effect of Curcumin and Some of Its Analogs on Tumor Promotion by TPA

Curcumin is the major yellow pigment in turmeric, curry, and mustard, and it is obtained from the rhizome of the plant *Curcuma longa* Linn. The ground dried rhizome of this plant (turmeric) has been used for centuries for the treatment of inflammatory diseases (22, 23), and curcumin is currently in wide use as a spice, food preservative, and yellow coloring agent for foods, drugs, and cosmetics.

Several studies indicated that compounds that possess antioxidant or anti-inflammatory activity inhibit TPA-induced tumor promotion in mouse skin. Since curcumin was reported to possess both antioxidant and anti-inflammatory activity (24–29), we studied the effect of topical applications of curcumin on TPA-induced tumor promotion on mouse skin, and we also studied the effect of curcumin as a potential inhibitor of tumor initiation by polycyclic aromatic hydrocarbons. Studies in our laboratory showed that topical application of curcumin inhibited benzo[*a*]pyrene– and 7,12-dimethylbenz[*a*]anthracene (DMBA)–induced tumor initiation and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)–induced tumor promotion on mouse skin (30, 31). TPA– or arachidonic acid–induced inflammation (mouse ear edema), hydrogen peroxide formation, ornithine decarboxylase activity, ornithine decarboxylase mRNA level, DNA synthesis, and hyperplasia in mouse skin were also inhibited (30, 32–34).

We examined the potential inhibitory effects of topical applications of several structural analogs of curcumin on TPA-induced tumor promotion on mouse skin. The structures of the compounds tested and the results obtained are shown in Figure 1. Among the compounds tested, demethoxycurcumin (~17% of commercial grade curcumin) and bisdemethoxycurcumin (~3% of commercial grade curcumin) are naturally occurring substances present in the rhizome of *Curcuma longa* Linn and in turmeric. Caffeic acid phenethyl ester (CAPE) is a constituent of the propolis of honeybee hives, and caffeic acid, ferulic acid, and chlorogenic acid are widespread constituents of fruits and vegetables. Chlorogenic acid accounts for 6%–8% of the dry weight of the coffee bean (35). The results of our studies indicate that curcumin, demethoxycurcumin, and caffeic acid phenethyl ester are highly active inhibitors of TPA-induced tumor promotion on mouse skin (Fig 1; 30, 34, 36, 37). Bisdemethoxycurcumin, tetrahydrocurcumin, caffeic acid, ferulic acid, and chlorogenic acid were less active than curcumin (30, 36), and bisdemethylcurcumin (a dicatechol synthesized recently by Dr. Toshihiko Osawa at Nagoya University [38] was inactive (Fig. 1). Commercial grade curcumin had the same inhibitory effect as pure curcumin on TPA-induced tumor promotion (36).

Examination of antioxidant activities for several of the curcumin derivatives that were tested as potential inhibitors of tumor promotion indicated that the antioxidant activities of these compounds when evaluated *in vitro* did not parallel their activities as inhibitors of TPA-induced tumor promotion *in vivo*. The *in vitro* studies indicated that curcumin, demethoxycurcumin, and bisdemethoxycurcumin were equally potent inhibitors of iron-stimulated lipid peroxidation in rat brain homogenate and rat liver microsomes (39). In other studies, the relative activities of curcumin derivatives as *in vitro* inhibitors of lipid peroxidation were tetrahydrocurcumin > curcumin > demethoxycurcumin > bisdemethoxycurcumin (40–42). In addition, bisdemethylcurcumin (a dicatechol) was found to have potent antioxidant activity *in vitro* (Osawa T, unpublished results). Although

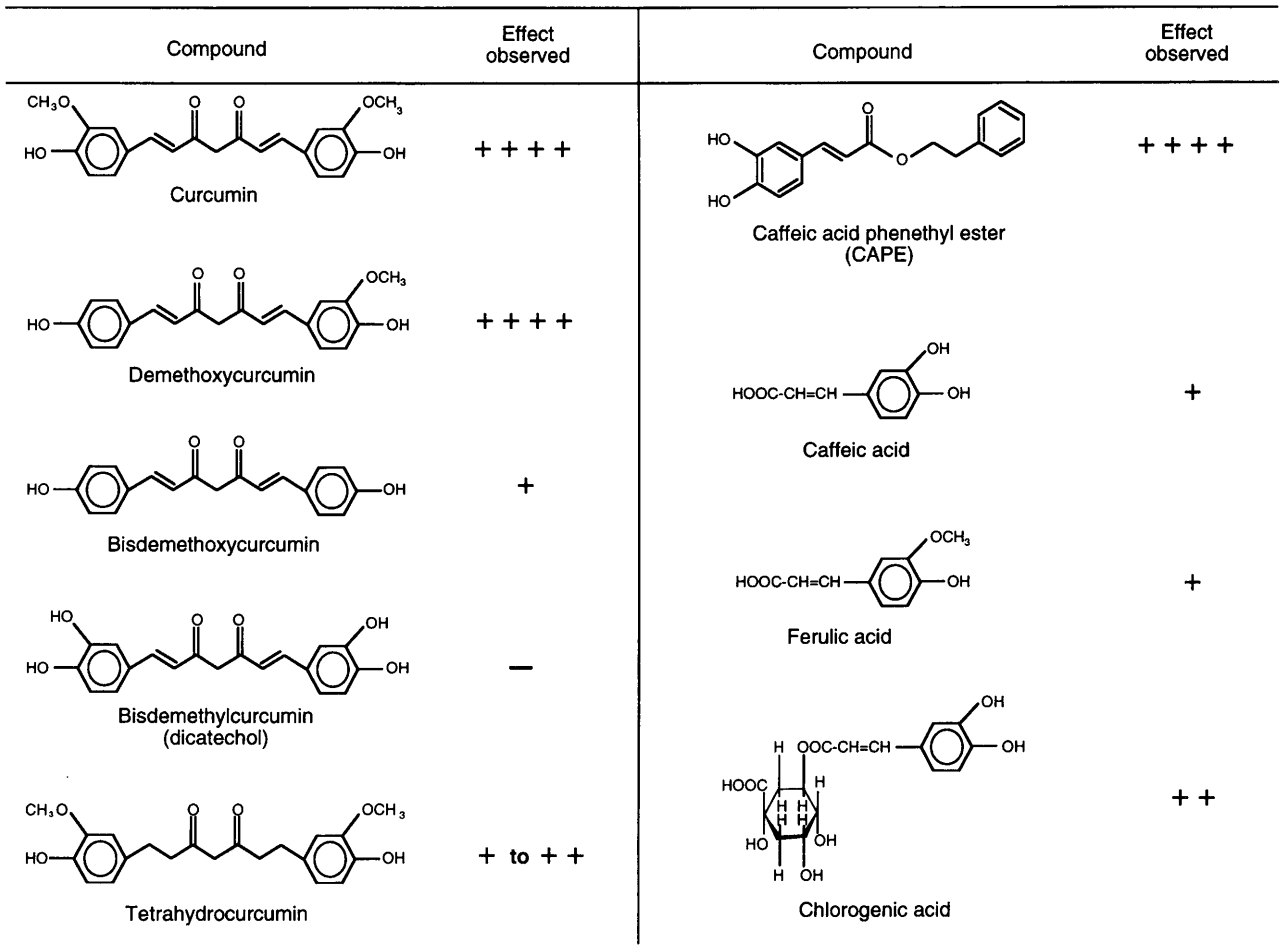

Figure 1. Structures and inhibitory effects of curcumin and some analogs on tumor promotion by TPA. (Taken from data described in the text and from Refs. 30, 34, 36, and 37).

the topical application of 100 nmol of curcumin together with 5 nmol of TPA twice a week for 18 weeks to the backs of CD-1 mice previously initiated with 200 nmol of DMBA inhibited the number of TPA-induced tumors per mouse by 58%, application of 100 nmol of bisdemethylcurcumin together with TPA did not inhibit tumorigenesis (unpublished observations). The results of the studies described above indicate that the *in vitro* antioxidant activities of several curcumin derivatives do not predict their activities as inhibitors of TPA-induced tumor promotion. Although *in vitro* studies indicated that bisdemethylcurcumin and tetrahydrocurcumin were more potent antioxidants than curcumin, it was found that tetrahydrocurcumin was less active than curcumin as an inhibitor of TPA-induced tumor promotion *in vivo,* and bisdemethylcurcumin was inactive (Fig. 1).

Inhibitory Effect of Dietary Curcumin on Chemically Induced Oral, Forestomach, Duodenal, and Colon Carcinogenesis

The results of recent studies indicate that administration of curcumin in the diet suppresses chemically induced oral, forestomach, duodenal, and colon carcinogenesis (43–45). In our studies (44), feeding 0.5% or 2.0% curcumin in the diet to CF-1 mice inhibited the number of azoxymethane-induced colon adenomas per mouse by 49% and 55%, respectively, and the number of colon adenocarcinomas per mouse was inhibited by 57% and 100%, respectively (Table I). Feeding curcumin also had a marked inhibitory effect on the size of adenomas and adenocarcinomas (Table I). A strong inhibitory effect of curcumin on colon carcinogenesis was observed when this compound was fed either during the initiation or postinitiation phase of azoxymethane-induced colon carcinogenesis (Table II). The results of our studies and those of others indicating an inhibitory effect of curcumin on oral, skin, and colon carcinogenesis suggest that curcumin may be a useful cancer chemopreventive agent in patients with oral leukoplakia or in patients with colon polyps who have a high risk of colon cancer.

Inhibitory Effect of Curcumin on Arachidonic Acid Metabolism

Several inhibitors of arachidonic acid metabolism inhibit TPA-induced inflammation and tumor promotion (46–48) and it is thought that arachidonic acid metabolites are important for TPA-induced inflammation and tumor

Table I. Inhibitory Effect of Dietary Curcumin Administration during Initiation and Postinitiation on Azoxymethane-Induced Colon Carcinogenesis in Mice

Dietary inhibitor	% mice with adenomas	Adenomas per mouse	Volume per adenoma (mm^3)	% mice with adenocarcinomas	Adenocarcinomas per mouse	Volume per carcinoma
Control diet	93	4.9 ± 0.6	7.0 ± 1.1	40	0.72 ± 0.15	22 ± 6
0.5% curcumin	69	2.5 ± 0.5	4.0 ± 0.1	22	0.31 ± 0.11	7 ± 3
2.0% curcumin	68	2.2 ± 0.5	2.7 ± 0.6	0	0	0

Note. CF-1 female mice were injected sc with azoxymethane (AOM; 10 mg/kg body wt) once weekly for 6 weeks. The mice were fed AIN 76A diet or curcumin in AIN 76A diet for 2 weeks before the first AOM administration and until the end of the experiment. The mice were killed 27 weeks after the last dose of AOM, and colon tumors were counted. Data are expressed as the mean ± SEM from 34–56 mice/group. (Taken from Ref. 44.)

Table II. Inhibitory Effect of Dietary Curcumin Administration either during Initiation or Postinitiation on Azoxymethane-Induced Colon Tumorigenesis in Mice

Treatment	Adenomas per mouse	% mice with adenomas	Carcinomas per mouse	% mice with carcinomas
Control diet	4.91 ± 0.56	93	0.72 ± 0.15	40
2.0% curcumin given during and after the initiation period	2.15 ± 0.48	68	0	0
2.0% curcumin given during the initiation period	1.78 ± 0.30	61	0.11 ± 0.06	9
2.0% curcumin given after the initiation period	3.97 ± 0.54	85	0.24 ± 0.08	24

Note. Female CF-1 mice were given sc injections of azoxymethane (AOM; 10 mg/kg body wt) once weekly for 6 weeks. Curcumin in AIN 76A diet was given during the initiation period (2 weeks before, during, and for 1 week after AOM), during the postinitiation period (1 week after the last dose of AOM until the end of the experiment), or during the initiation and postinitiation periods. The mice were sacrificed 27 weeks after the last dose of AOM. Each value was obtained from 34–56 mice. (Taken from Ref. 44.)

promotion in mouse skin. Early studies by Flynn *et al.* showed an inhibitory effect of curcumin on 5-lipoxygenase activity in human neutrophils and on cyclooxygenase activity in bovine seminal vesicles (49). Because of the possibility that curcumin might inhibit carcinogenesis in mouse skin by inhibiting arachidonic acid metabolism, we evaluated the effect of curcumin on the metabolism and action of arachidonic acid in the epidermis. We found that topical application of curcumin inhibited arachidonic acid-induced inflammation on mouse ears (30, 50). Studies on the metabolism of arachidonic acid by mouse epidermis revealed that curcumin (3 μM) inhibited the metabolism of arachidonic acid to 5- and 8-HETE by 40% (lipoxygenase pathway), and the metabolism of arachidonic acid to PGE_2, $PGF_{2\alpha}$, and PGD_2 (cyclooxygenase pathway) was inhibited 42%, 24%, and 34%, respectively (50) (Table III). Curcumin (10 μM) inhibited the epidermal metabolism of arachidonic acid to 5- and 8-HETE by 60% and 51%, respectively, and the metabolism of arachidonic acid to PGE_2, $PGF_{2\alpha}$, and PGD_2 was inhibited 70%, 64%, and 73%, respectively (50) (Table III). In another study, dietary administration of curcumin (2000 ppm) to rats inhibited azoxymethane-induced colon carcinogenesis and decreased colonic mucosal phospholipase A_α, phospholipase Cδ1, and PGE_2 levels (45). In this study, dietary curcumin also decreased enzyme activity in colonic mucosa for the formation of PGE_2, $PGF_{2\alpha}$, PGD_2, and 6-keto $PGF_{1\alpha}$ *via* the cyclooxygenase system, and production of 5(S)-, 8(S)-, 12(S)-, and 15(S)-HETE *via* the lipoxygenase pathway of arachidonic acid metabolism was also inhibited.

Lack of Tumor-Promoting Activity of Arachidonic Acid in DMBA-Initiated Mouse Skin

Indirect evidence suggests that arachidonic acid metabolites that are formed *via* the cyclooxygenase and lipoxygenase pathways may be active participants in the carcinogenic process. Some of these arachidonic acid metabolites enhance DNA synthesis or cell proliferation (51, 52), many tumors contain or synthesize high levels of arachidonic acid metabolites (53–56), and many inhibitors of cyclooxygenase and lipoxygenase are inhibitors of chemically induced carcinogenesis in animals (46–48, 53, 54, 57). Epidemiological studies indicate that certain cyclooxygenase inhibitors such as aspirin decrease the risk of colon cancer in humans, and sulindac inhibits the development of colon polyps in high-risk individuals with familial adenomatous polyposis (FAP) (58–61). The role of cyclooxygenase in the inhibition of tumor formation by sulindac, however, has recently been questioned (62–65). Sulindac inhibits colon carcinogenesis in rodent models (66, 67), and this inhibition was thought to result from metabolism to sulindac sulfide, which is a potent anti-inflammatory agent and an inhibitor of cyclooxygenase activity. Sulindac sulfone (another metabolite of sulindac) lacks anti-inflammatory activity and does not inhibit cyclooxygenase activity. Even though sulindac sulfone does not inhibit cyclooxygenase activity, it exerts an inhibitory effect on chemically induced mammary and colon carcinogenesis in rodents (63, 64), and it stimulates apoptosis in cultured HT-29 human colon carcinoma cells (65). These results indicate that sulindac and its derivatives may exert their anticancer effects by mechanisms independent of cyclooxygenase inhibition.

Table III. Inhibitory Effect of Curcumin on Epidermal Lipoxygenase and Cyclooxygenase Activity

Curcumin (μ*M*)	Inhibition of metabolite formation (%)				
	Lipoxygenase inhibition		Cyclooxygenase inhibition		
	5-HETE	8-HETE	PGE_2	$PGF_{2\alpha}$	PGD_2
1	ND	ND	32	2	22
3	40 ± 14	40 ± 16	42	24	34
10	60 ± 6	51 ± 6	70	64	73
30	66 ± 7	77 ± 9	86	81	54
100	83 ± 3	85 ± 6	99	85	94

Note. Arachidonic acid was incubated with enzyme preparations from mouse epidermis. ND, not determined. (Taken from Ref. 50.)

Proof of the involvement of arachidonic acid metabolites in the carcinogenic process requires the direct demonstration of carcinogenic activity for arachidonic acid and/or its metabolites. We recently evaluated the possible tumor-promoting effect of direct application of arachidonic acid to DMBA-initiated mouse skin. In initial studies, we found that topical application of 1 mg of arachidonic acid or 0.1 nmol of TPA twice daily for 8–12 days to the ears of mice increased the number of epidermal layers, the epidermal thickness, leukocyte infiltration, and intracellular edema (Table IV). In an additional study, we initiated the right ear of mice with 30 nmol DMBA. One week later, the same ear was treated with topical applications of 1 mg of arachidonic acid once a day (5 days a week) or with 1 mg of arachidonic acid twice a day (5 days a week) for 26 weeks. Control mice were treated with acetone vehicle twice a day (5 days a week) or with 0.5 nmol of TPA twice a week for 26 weeks. Although treatment of DMBA-initiated mice with TPA for 26 weeks resulted in 87% of the animals with tumors (2.9 tumors/mouse), no tumors were observed in DMBA-initiated mice treated with arachidonic acid (Table V). Histology studies with these animals revealed that topical application of 1 mg of arachidonic acid twice a day (5 days a week) for 26 weeks caused a mild to moderate increase in the number of epidermal layers, the epidermal thickness, intracellular edema, and the keratin content of the epidermis (Table IV). Topical application of TPA twice a week for 26 weeks had a more marked effect on the morphology of the skin than twice daily applications of arachidonic acid for 26 weeks (Table IV). Although other studies indicate that mouse epidermis metabolizes arachidonic acid to prostaglandins (50, 68) and 5-, 8-, 12-, and 15-HETEs (50, 69–71), our study indicates that arachidonic acid is inactive as a tumor promoter when high doses are applied topically for 26 weeks (Table V). Similar results were observed by

Table IV. Arachidonic Acid– and TPA-Induced Morphological Changes in the Ears of Mice

Experiment	Treatment	No. of mice	No. of epidermal layers	Epidermal thickness (μm)	Leukocyte infiltration	Intracellular edema	Keratin
1	Acetone (20 μl) twice daily for 8–12 days	6	1.40 ± 0.09	15.8 ± 0.7	0	0	0
	AA (1 mg) once daily for 8–12 days	6	2.43 ± 0.09[a]	20.5 ± 0.8[a]	0 to +	0 to +	0
	AA (1 mg) twice daily for 8–12 days	6	6.03 ± 0.14[a]	31.0 ± 1.3[a]	+ to ++	+	0
	TPA (0.1 nmol) twice daily for 8 days	3	7.00 ± 0.20[a]	43.7 ± 2.1[a]	+ to ++	+ to ++	0
2	Acetone (20 μl) twice daily for 26 weeks	9	1.36 ± 0.07	15.2 ± 0.6	0	0	0
	AA (1 mg) once daily for 26 weeks	9	2.24 ± 0.10[a]	24.1 ± 0.9[a]	0 to +	0	0
	AA (1 mg) twice daily for 26 weeks	9	2.84 ± 0.11[a]	29.1 ± 0.9[a]	+	0 to +	0 to +
	TPA (0.5 nmol) twice weekly for 26 weeks	9	6.60 ± 0.19[a]	92.1 ± 3.8[a]	++	+	++

Note. In Experiment 1, female CD-1 mice (6–8 weeks old) were treated topically on an ear with acetone (20 μl twice daily for 8–12 days), arachidonic acid (AA; 1 mg/20 μl acetone once daily for 8 or 12 days), arachidonic acid (1 mg/20 μl acetone twice daily for 8 or 12 days), or 12-*O*-tetradecanoylphorbol-13-acetate (TPA; 0.1 nmol/20 μl acetone twice daily for 8 days). The animals were sacrificed at 24 hr after the last application. Since the data obtained after 8 days of treatment were not different from that obtained after 12 days of treatment (3 mice/time point), the two sets of data were combined. In Experiment 2, female CD-1 mice (6–8 weeks old) were treated topically on an ear with acetone (20 μl twice daily, 5 days a week for 26 weeks), AA (1 mg/20 μl acetone once or twice daily, 5 days a week for 26 weeks), or TPA (0.5 nmol/20 μl acetone twice a week for 26 weeks). Some biological effects of TPA or AA are indicated by 0 (no effect), + (mild effect), or ++ (moderate effect). Each value represents the mean ± SEM.

[a] Statistically different from the acetone-treated control group ($P < 0.01$) as determined by the Student's *t* test.

Table V. Lack of Tumor-Promoting Activity of Arachidonic Acid on Mouse Skin

Treatment	% mice with tumors	Tumors per mouse
Acetone	0	0
Arachidonic Acid (1 mg once daily)	0	0
Arachidonic Acid (1 mg twice daily)	0	0
TPA (0.5 nmol twice weekly)	87	2.9 ± 2.3

Note. Female CD-1 mice (6–8 weeks old; 30/group) were initiated with 30 nmol of DMBA in 20 μl of acetone on the right ear. One week later the mice were treated topically on the right ear with 20 μl of acetone or with 1 mg of arachidonic acid in 20 μl of acetone once or twice a day (5 days/week) for 26 weeks. Additional DMBA-initiated animals (positive controls) were treated with 0.5 nmol of TPA twice a week for 26 weeks.

Fischer and her associates, who used lower dose levels of arachidonic acid and certain prostaglandins. These investigators found that topical application of arachidonic acid (100 μg), PGE_1 (20 μg), PGE_2 (20 μg), or $PGF_{2\alpha}$ (20 μg) twice a week to the backs of DMBA-initiated SENCAR mice for 15 weeks did not result in any tumors (72).

In conclusion, our present study indicating the lack of tumor-promoting activity for arachidonic acid on mouse skin, coupled with earlier studies demonstrating epidermal metabolism of arachidonic acid *via* the cyclooxygenase and lipoxygenase pathways by mouse epidermis, suggests several possibilities: (i) insufficient amounts of putative tumorigenic arachidonic acid metabolites were formed in the epidermis by cyclooxygenase and/or lipoxygenase to exert a tumorigenic effect under the conditions of our expeiment; (ii) high levels of cyclooxygenase and/or lipoxygenase metabolites were formed from arachidonic acid, and they are necessary but not sufficient for tumor formation; or (iii) the metabolic products of cyclooxygenase and lipoxygenase are not tumorigenic. If the last explanation is correct, why do inhibitors of cyclooxygenase and lipoxygenase inhibit tumorigenesis? One possible explanation could be that inhibition of cyclooxygenase and lipoxygenase activities results in higher tissue levels of arachidonic acid, which may inhibit carcinogenesis *per se* or cause more metabolism of arachidonic acid *via* the alternative cytochrome P-450 pathway to products that may inhibit the carcinogenic process. In support of a possible inhibitory effect of arachidonic acid or its metabolites on the carcinogenic process, topical application of arachidonic acid inhibits TPA-induced tumor promotion in the skin of DMBA-initiated SENCAR mice (72). In addition, recent studies by Dr. Floyd Chilton at the Bowman Gray School of Medicine have shown that arachidonic acid or acyl transferase inhibitors that increase levels of cellular arachidonic acid stimulates apoptosis in certain cell-culture systems (personal communication), which suggests that inhibitors of cyclooxygenase and lipoxygenase may inhibit carcinogenesis by increasing the levels of arachidonic acid, which can then function as a stimulator of apoptosis. The possibility that arachidonic acid or some of its noncyclooxygenase or nonlipoxygenase metabolites can inhibit carcinogenesis has not received serious consideration and is worthy of further investigation. This concept is consistent with the observed inhibitory effect of cyclooxygenase and lipoxygenase inhibitors on carcinogenesis.

Synergistic Effects of Curcumin on All-*trans* Retinoic Acid–and 1α,25-Dihydroxyvitamin D_3–Induced Differentiation

We considered the possibility that some of the cancer chemopreventive effects of curcumin may be related to an effect of this compound on cellular differentiation, and we investigated the effect of curcumin on differentiation in the human promyelocytic HL-60 leukemia cell model system. We also investigated the effects of combinations of curcumin together with 1α,25-dihydroxyvitamin D_3 or all-*trans* retinoic acid on differentiation in HL-60 cells. Treatment of HL-60 cells with 10 μ*M* curcumin for 48 hr resulted in small increases in differentiation (100%–200%) as measured by the proportion of cells that reduced nitroblue tetrazolium and expressed Mac–1 (73) (Tables VI and VII). Synergistic induction of differentiation as measured by the above markers was observed when 1–10 μ*M* curcumin was combined with 10–100 n*M* all-*trans* retinoic acid or with 100 n*M* 1α,25-dihydroxyvitamin D_3 (73) (Table VI and VII). Combinations of all-*trans* retinoic acid and curcumin stimulated differentiation predominantly to granulocytes, whereas combinations of 1α,25-dihydroxyvitamin D_3 and curcumin stimulated differentiation predominantly to monocytes (73). Independent studies by Sokoloski and his colleagues have also shown a synergistic effect of curcumin and 1α,25-dihydroxyvitamin D_3 on differentiation in HL-60 cells, and they suggest the importance of NF-κB inhibition for this effect (74).

As indicated earlier, epidemiology studies have pointed

Table VI. Effect of Curcumin and All *trans* Retinoic Acid on the Differentiation of HL-60 Cells as Measured by Nitroblue Tetrazolium Reduction and Mac-1 Expression

Treatment	% NBT-positive cells	% Mac-1–positive cells
Control	3.0	5.6
CUR 1 μ*M*	4.6 (1.6)	8.6 (3.0)
CUR 10 μ*M*	13.9 (10.9)	16.5 (10.9)
RA 10 n*M*	12.9 (9.9)	16.0 (10.4)
RA 100 n*M*	20.6 (17.6)	17.4 (11.8)
RA 10 n*M* + CUR 10 μ*M*	79.4 (76.4)	64.7 (59.1)
RA 100 n*M* + CUR 1 μ*M*	47.1 (44.1)	34.8 (29.2)
RA 100 n*M* + CUR 10 μ*M*	76.3 (73.3)	71.9 (66.3)

Note. HL-60 cells were treated with curcumin (CUR) and all-*trans* retinoic acid (RA) for 48 hr, and nitroblue tetrazolium (NBT)–positive or Mac-1–positive cells were measured. The numbers in parentheses were corrected for background values from control samples. (Taken from Ref. 73.)

Table VII. Effect of Curcumin and 1α,25-Dihydroxyvitamin D_3 on the Differentiation of HL-60 Cells as Measured by Nitroblue Tetrazolium Reduction and Mac-1 Expression

Treatment	% NBT-positive cells	% Mac-1–positive cells
Control	7.8	5.5
CUR 1 μ*M*	12.6 (4.8)	9.8 (4.3)
CUR 10 μ*M*	17.8 (10.0)	16.1 (10.6)
VD_3 100 n*M*	19.1 (11.3)	33.0 (27.5)
VD_3 100 n*M* + CUR 1 μ*M*	34.2 (26.4)	53.9 (48.4)
VD_3 100 n*M* + CUR 10 μ*M*	54.0 (46.2)	74.8 (69.3)

Note. HL-60 cells were treated with curcumin (CUR) and 1α,25-dihydroxyvitamin D_3 (VD_3) for 48 hr, and nitroblue tetrazolium (NBT)–positive or Mac-1–positive cells were measured. The numbers in parentheses were corrected for background values from control samples. (Taken from Ref. 73.)

out that people who eat large amounts of fruit and green-yellow vegetables have a lower risk of many kinds of cancer (7–9). It is possible that many dietary chemicals such as curcuminoids, tocopherols, carotonoids, and other substances in fruit, vegetables, and other edible plants can prevent human cancer in part by synergizing with endogenously produced stimulators of differentiation such as all-*trans* retinoic acid, 1α,25-dihydroxyvitamin D_3, and butyrate. More research is needed to test this hypothesis.

Inhibitory Effects of Tea and Tea Constituents on Carcinogenesis in Experimental Animals

Early studies in our laboratory showed a potent inhibitory effect of tannic acid and hydroxylated flavonoids in tea on the mutagenic activity of a bay-region diol expoxide that is the ultimate carcinogenic metabolite of benzo[*a*]pyrene (19, 20). These observations were followed by *in vivo* studies indicating a potent inhibitory effect of plant phenolics on the carcinogenic action of the tumorigenic bay-region diol epoxide metabolite of benzo[*a*]pyrene in mice (75, 76), but the plant phenolics were only weakly active or inactive when tested for inhibitory effects on the tumorigenic action of benzo[*a*]pyrene (75). Additional studies showed a strong inhibitory effect of green tea, black tea, or (−)-epigallocatechin gallate (the major catechin in green tea) on the mutagenicity of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (77).

Yoshizawa and his colleagues demonstrated that topical application of (−)-epigallocatechin gallate inhibited tumor promotion by teleocidin in DMBA-initiated mouse skin (78). Subsequent studies showed that tea and some of its constituents have a very broad spectrum of cancer chemopreventive activity (reviewed in Refs. 79 and 80). Oral administration of green tea, black tea, (−)-epigallocatechin gallate, or a green tea polyphenol fraction to rodents has been reported to inhibit chemically induced carcinogenesis in many organs including esophagus (81, 82), forestomach (83–85), stomach (86), duodenum/small intestine (87, 88), colon (89, 90), lung (83–85, 91–93), liver (94), and pancreas (95). Orally administered decaffeinated green or black tea has been reported to effectively inhibit chemically induced esophageal, lung, and forestomach carcinogenesis in rodents (82, 84), indicating that caffeine may not play a major role for the inhibitory effects of tea in these chemically induced carcinogenesis models. In addition to these studies, oral administration of (−)-epigallocatechin gallate (the major polyphenol in green tea) inhibits the formation of spontaneous liver tumors in mice (96). It is of interest that a strong inhibitory effect of green tea on NDEA-induced forestomach and lung tumorigenesis was observed when the tea was given either during the initiation or postinitiation period (84).

The results of earlier studies from our laboratory indicated a strong inhibitory effect of green tea, decaffeinated green tea, black tea, and decaffeinated black tea on UVB-induced skin carcinogenesis in DMBA-initiated mice (97) (Table VIII). In these studies, tea was administered as the sole source of drinking fluid, and the concentration (2–4 mg tea solids/ml) was similar to that commonly present in tea brews ingested by humans. We found that all four tea preparations strongly inhibited UVB-induced carcinogenesis in DMBA-initiated mice, but the decaffeinated teas were somewhat less effective than the regular teas (Table VIII).

In subsequent studies in a different animal model, we found that administration of green tea or black tea inhibited UVB-induced complete carcinogenesis (animals were not initiated with DMBA prior to twice a week UVB adminis-

Table VIII. Inhibitory Effects of Green and Black Tea on UVB-Induced Keratoacanthomas and Carcinomas in DMBA-Initiated SKH-1 Mice

Treatment	Tea solids (mg/ml)	% mice with keratoacanthomas	No. of keratoacanthomas per mouse	% mice with carcinomas	No. of carcinomas per mouse
Water control		97	6.33	33	0.60
Green tea	4.0	43	1.37	7	0.07
Black tea	4.4	35	1.35	4	0.04
Decaf green tea	3.6	77	1.90	17	0.17
Decaf black tea	3.9	67	1.73	17	0.20

Note. Female SKH-1 mice were treated topically with 200 nmol of DMBA. One week later the mice were treated with gradually increasing concentrations of tea leaf extracts as drinking fluid for 6 days and full-strength teas (1.25 g tea leaves/100 ml hot water; ~4 mg tea solids/ml) for an additional 8 days prior to and during treatment with UVB (30 mJ/cm^2) twice weekly for 31 weeks. (Taken from Ref. 97.)

tration), but the teas were less effective than observed previously in the DMBA/UVB model (98). In the UVB complete carcinogenesis experiments, decaffeinated green or black tea (~4 mg tea solids/ml) had little or no inhibitory effect, and high dose levels of decaffeinated green or black tea (~9 mg tea solids/ml) had a stimulatory effect on UVB-induced complete carcinogenesis (unpublished observations). Administration of caffeine in the drinking water was also shown to inhibit UVB-induced complete carcinogenesis (98). During the course of our studies on the effect of green and black tea on UVB-induced carcinogenesis, we observed an inhibitory effect of the teas on tumor size (97, 99). Accordingly, we investigated the effects of green and black tea administration on the growth of papillomas in mice with established papillomas.

Effect of Green Tea on Tumor Growth in Mice with Established Papillomas

In six separate experiments, mice with established skin papillomas were treated continuously with green tea as the sole source of drinking fluid (2–10 mg tea solids/ml) or with ip injections of (−)-epigallocatechin gallate (25–50 mg/kg) three times a week for several weeks. Inhibition of tumor growth was observed in all six experiments (Fig. 2) (100). A similar inhibitory effect of orally administered black tea on the growth of previously established skin papillomas was also observed (101). Recent studies by Liu and his colleagues showed an inhibitory effect of injections of (−)-epigallocatechin gallate on the growth of transplanted malignant human prostate and breast-cancer cells in immunodeficient mice (102).

An important question is whether or not tea drinking inhibits cancer formation in humans. Although several epidemiology studies have been done, the results are not conclusive, and additional research is needed (103, 104).

Inhibitors of Carcinogenesis in One Experimental Model May Stimulate Carcinogenesis in Another Experimental Model

There are several examples of cancer chemopreventive agents that are effective inhibitors in one experimental setting but have the opposite effect in another experimental setting. Administration of phenobarbital together with 2-acetylaminofluorene, 4-dimethylaminoazobenzene, or aflatoxin B_1 in rats inhibits the carcinogenicity of these compounds by enhancing their metabolic detoxification (Reviewed in Ref. 105). However, when phenobarbital is chronically administered, after a short exposure to 2-acetylaminofluorene or another initiator of liver tumors, phenobarbital is a promoting agent that enhances the formation of liver tumors (4).

Topical application of all-*trans* retinoic acid or 1α,25-

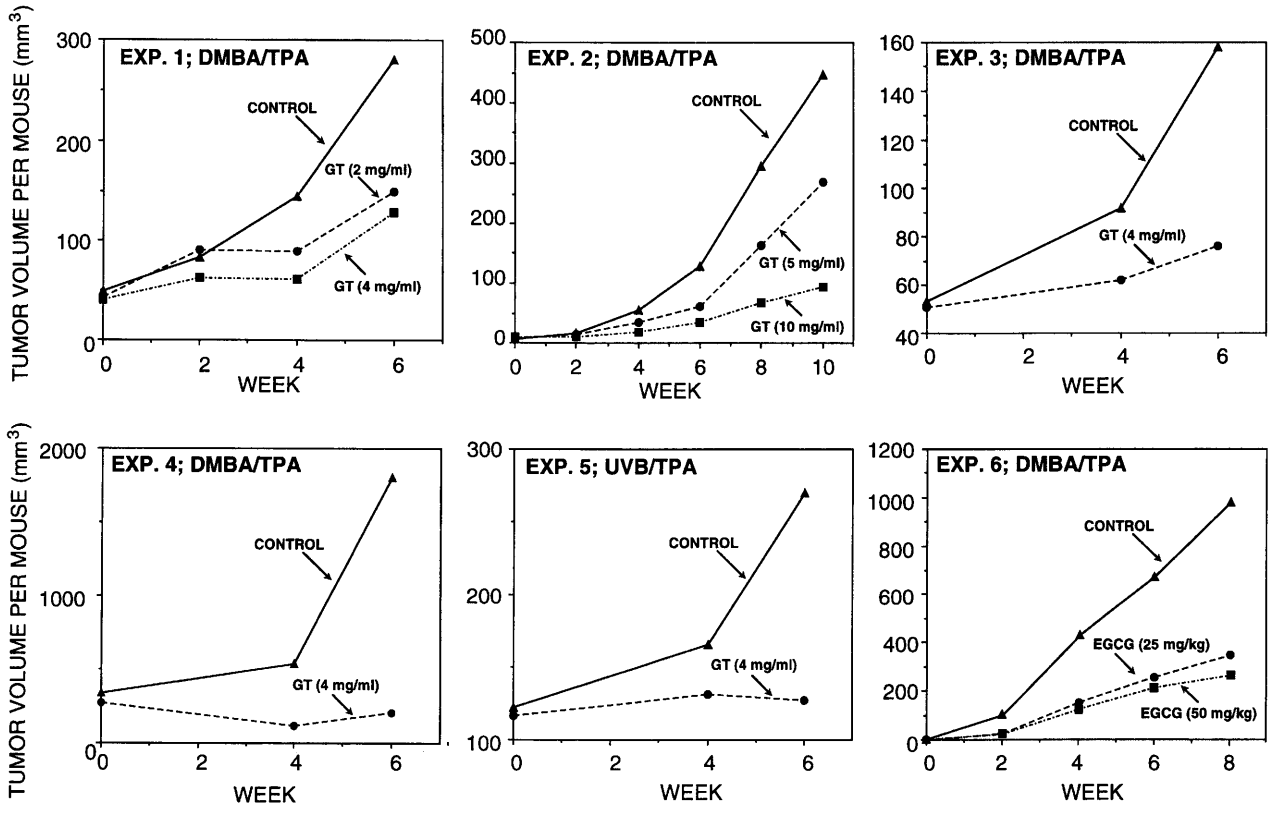

Figure 2. Inhibitory effect of orally administered green tea or intraperitoneally administered (−)-epigallocatechin gallate on the growth of established papillomas in tumor-bearing mice. CD-1 mice were initiated with DMBA or UVB and promoted with TPA. Application of TPA was stopped, and papilloma-bearing animals were treated with green tea (2–10 mg tea solids/ml) as their sole source of drinking fluid (Exps. 1–5) or injected ip with (−)-epigallocatechin gallate (EGCG; 25–50 mg/kg three times a week) (Exp. 6). (Taken from Ref. 100.)

dihydroxyvitamin D_3 strongly inhibits tumor promotion by TPA in mice previously initiated with DMBA (106, 107). However, when all-*trans* retinoic acid or 1α,25-dihydroxyvitamin D_3 is administered topically twice a week together with DMBA using a complete carcinogenesis protocol, a markedly enhanced tumor response was observed (108, 109). The inhibitory effects of all-*trans* retinoic acid and 1α,25-dihydroxyvitamin D_3 on tumor promotion by TPA and the stimulatory effect of these compounds on complete carcinogenesis by DMBA is shown in Table IX. An important unanswered question is whether all-*trans* retinoic acid and 1α,25-dihydroxyvitamin D_3 have a cancer chemopreventive effect in some individuals and an adverse effect in others.

An optimal cancer chemopreventive regimen for heavy smokers may be different from an optimal cancer chemopreventive regimen for people exposed to aflatoxin B_1 or for heavy smokers who have stopped smoking. The recent observation that administration of β-carotene may have increased the carcinogenic risk in smokers (110–112) could be analogous to the stimulatory effect of all-*trans* retinoic acid treatment on complete carcinogenesis by DMBA and may not have occurred in people who had stopped smoking. Greater efforts should be made to understand mechanisms of cancer chemoprevention and to determine whether a potential chemopreventive agent is useful in many experimental settings or whether it is only useful in a limited number of experimental settings. As described above, some compounds may be extremely effective cancer chemopreventive agents in one experimental setting but enhance carcinogenesis in another experimental setting. Accordingly, it may be necessary to tailor the cancer chemopreventive agent to individual subjects with known carcinogen exposures or to individuals at high risk for cancer with mechanistically understood pathways of carcinogenesis so that chemopreventive agents can be better tailored to the individual and selected on a more rational basis.

Table IX. Effect of 1α,25-Dihydroxyvitamin D_3 and All-*trans* Retinoic Acid on Tumor Promotion by TPA or Tumor Formation in Mouse Skin Induced by Chronic Twice Weekly Treatment with 7,12-Dimethylbenz[a]anthracene (DMBA)

Experiment	Treatment	% tumor-bearing animals	Tumors/mouse (mean ± SEM)
1	TPA	92	20.0 ± 2.5
	TPA + VD_3 (0.5 nmol)	63	3.9 ± 1.0
	TPA + RA (2.0 nmol)	33	1.3 ± 0.5
2	TPA	88	8.61 ± 1.09
	TPA + VD_3 (0.5 nmol)	59	3.39 ± 0.91
3	DMBA	63	1.20 ± 0.26
	DMBA + VD_3 (0.5 nmol)	100	5.67 ± 0.76
	DMBA + RA (0.5 nmol)	80	2.57 ± 0.43
	DMBA + RA (25 nmol)	93	8.40 ± 1.13

Note. In Experiment 1, CD-1 mice previously initiated with 200 nmol of DMBA were treated topically with the indicated dose of all-*trans* retinoic acid (RA) or 1α,25-dihydroxyvitamin D_3 (VD_3) together with 5 nmol of TPA twice a week for 15 weeks. In Experiment 2, mice previously initiated with 50 nmol of DMBA were treated with solvent vehicle or VD_3 in vehicle 1 hr before 16 nmol of TPA twice a week for 16 weeks. In Experiment 3, animals were treated with the indicated compounds or solvent vehicle 1 hr prior to treatment with 50 nmol of DMBA twice a week for 16 weeks. Mice treated twice weekly with solvent, VD_3 or all-*trans* retinoic acid in the absence of DMBA did not develop any tumors. (Data from Experiment 1 are from unpublished observations in our laboratory. Data from Experiments 2 and 3 were taken from Refs. 107 and 109.)

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