Chemoprevention of Colon Carcinogenesis by Dietary Curcumin, a Naturally Occurring Plant Phenolic Compound

Chinthalapally V. Rao, Abraham Rivenson, Barbara Simi, and Bandaru S. Reddy

ABSTRACT

Human epidemiological and laboratory animal model studies have suggested that nonsteroidal antiinflammatory drugs reduce the risk of development of colon cancer and that the inhibition of colon carcinogenesis is mediated through the alteration in cyclooxygenase metabolism of arachidonic acid. Curcumin, which is a naturally occurring compound, is present in turmeric, possesses both antiinflammatory and antioxidant properties, and has been tested for its chemopreventive properties in skin and forestomach carcinogenesis. The present study was designed to investigate the chemopreventive action of dietary curcumin on azoxymethane-induced colon carcinogenesis and also the modulating effect of this agent on the colonic mucosal and tumor phospholipase A2, phospholipase C'yl, lipoxygenase, and cyclooxygenase activities in male F344 rats. At 5 weeks of age, groups of animals were fed the control (modified AIN-76A) diet or a diet containing 2000 ppm of curcumin. At 7 weeks of age, all animals, except those in the vehicle (normal saline)-treated groups, were given two weekly s.c. injections of azoxymethane at a dose rate of 15 mg/kg body weight. All groups were continued on their respective dietary regimen until the termination of the experiment at 52 weeks after the carcinogen treatment. Colonic tumors were evaluated histopathologically. Colonic mucosa and tumors were analyzed for phospholipase A2, phospholipase C'yl, ex vivo prostaglandin (PG) E2, cyclooxygenase, and lipoxygenase activities. The results indicate that dietary administration of curcumin significantly inhibited incidence of colon adenocarcinomas (P < 0.004) and the multiplicity of invasive (P < 0.015), noninvasive (P < 0.01), and total (invasive plus noninvasive) adenocarcinomas (P < 0.001). Dietary curcumin also significantly suppressed the colon tumor volume by >57% compared to the control diet. Animals fed the curcumin diet showed decreased activities of colonic mucosal and tumor phospholipase A2 (50%) and phospholipase C'yl (40%) and levels of PG E2 (>38%). The formation of prostaglandins such as PG E2, PG F2α, PG D2, 6-keto PG E1, and thromboxane B2 through the cyclooxygenase system and production of 8(S)-, 8(S)-, 12(S)-, and 15(S)-hydroxyeicosatetraenoic acids via the lipoxygenase pathway from arachidonic acid were reduced in colonic mucosa and tumors of animals fed the curcumin diet as compared to control diet. Although the precise mechanism by which curcumin inhibits colon tumorigenesis remains to be elucidated, it is likely that the chemopreventive action, at least in part, may be related to the modulation of arachidonic acid metabolism.

INTRODUCTION

Large bowel cancer is the leading neoplastic disease that strikes men and women in high frequency in Western countries including North America (1, 2). Although several epidemiological and experimental studies suggest a relationship between colon cancer risk and dietary factors, its etiology is multifactorial and complex in that it may arise from the combined action of environmental factors such as low levels of an assortment of, as yet unidentified, genotoxic agents, dietary factors, and endogenous formation of tumorigenic substances (1–4). Prevention strategies for cancer control involving complete reduction or elimination of human exposure to these environmental factors may not always be possible; however, as an alternative approach, the agents for alleviating the carcinogenic effect of several of these substances have been identified and tested for their chemopreventive action (5). Wattenberg (5), Boone et al. (6), and Kellogg et al. (7) reviewed the results of many chemoprevention studies in laboratory animal models and in a human setting.

Although the use of medicinal plants or their active principles in the prevention and/or treatment of chronic diseases is based on the experience of traditional systems of medicine from different ethnic societies, their use in modern medicine suffers from lack of scientific evidence. Only very few medicinal plants have attracted the interest of scientists, and one such plant is Curcuma longa Linn. The powdered rhizome of this plant, turmeric, has been extensively used for imparting color and flavor to foods and also for the treatment of variety of inflammatory conditions and other diseases (8, 9). Curcumin (diferuloylmethane), a phenolic compound (Fig. 1) that has been identified as the major pigment in turmeric, possesses both antiinflammatory (10–12) and antioxidant properties (13, 14).

Previous studies have shown that topical application of curcumin inhibits TPA3-induced epidermal DNA synthesis, tumor promotion in mouse skin, and edema of mouse ears (15, 16). Topical application of curcumin also inhibited B(α)-induced DNA adducts and skin tumors as well as DMBA-induced skin tumors (17). In other studies, dietary administration of 2% turmeric in the diet inhibited the DMBA-induced skin, B(α)P-induced forestomach, and AOM-induced small and large intestinal tumors in mice (18, 19). In most of these studies, curcumin purity was less than or about 85%. Earlier studies from our laboratory had shown that 2000 ppm curcumin (>98% pure) in the diet significantly suppressed the AOM-induced colon aberrant crypt foci formation, which are early preneoplastic lesions, in male F344 rats (20).

The biosynthesis of PGs through the COX system and of hydroxy fatty acids via LOX pathway from AA has been well documented. These metabolites of AA exert a variety of biological activities. Several studies have shown that COX metabolites, particularly PGs of the type-2 series modulate cell proliferation, tumor growth, and immune responses (21, 22), whereas LOX metabolites can influence various biological responses including chemotactic responses, hormone secretion, ion transport, tumor cell adhesion, stimulation of tumor cell spreading, and regulation of tumor cell metastatic potential (23–25). The generation of AA for biosynthesis of COX and LOX metabolites involves degradation of phosphatidylchinositol via a sequence of reactions regulated by PLC (22, 26). The second pathway in the generation of AA involves a direct action of a PLA2 on a phospholipid (22, 27). There are several forms of PLC and PLCl, among them PLCcyl, a membrane-bound PIP2 specific form, and cytotoxic PLA2, implicated in regulation of eicosanoid biosynthesis and cell proliferation (27, 28). In several studies, increased levels of PLA2 and PLCcyl activities were observed in human colon and breast tumors of animals fed the curcumin diet as compared to control diet. Animals fed the curcumin diet showed decreased activities of colonic mucosal and tumor phospholipase A2 (50%) and phospholipase C'yl (40%) and levels of PG E2 (>38%). The formation of prostaglandins such as PG E2, PG F2α, PG D2, 6-keto PG E1, and thromboxane B2 through the cyclooxygenase system and production of 8(S)-, 8(S)-, 12(S)-, and 15(S)-hydroxyeicosatetraenoic acids via the lipoxygenase pathway from arachidonic acid were reduced in colonic mucosa and tumors of animals fed the curcumin diet as compared to control diet. Although the precise mechanism by which curcumin inhibits colon tumorigenesis remains to be elucidated, it is likely that the chemopreventive action, at least in part, may be related to the modulation of arachidonic acid metabolism.
tumors and melanomas compared to normal tissues (29–31). In this regard, it is noteworthy that the COX metabolite, PGE2, which significantly affects tumor growth and immune responses, has been found at high levels in experimental animal tumors as well as in human tumors (21, 32, 33). We and others have shown that COX inhibitors such as aspirin, indomethacin, piroxicam, sulindac, and ibuprofen suppress colon carcinogenesis in laboratory animal models (21, 33–35). Thus, the possibility exists that the changes in the activities of PLA2 and PLCγ, which are involved in signal transduction and AA release, as well as COX and LOX pathways of AA metabolism produced by exogenous agents, may alter tumorigenesis.

It was, therefore, of interest to study the efficacy of a low dietary level of pure curcumin as a chemopreventive agent in an established colon cancer model. In the present study, we report the chemopreventive efficacy of dietary curcumin on AOM-induced colon tumorigenesis in male F344 rats. In addition, the effects of dietary curcumin on colonic mucosal and tumor PLA2, PLCγ, COX, and LOX activities were analyzed to understand the possible modulating role of this agent in colon tumorigenesis.

MATERIALS AND METHODS

Materials

AOM (CAS: 25843-45-2) was purchased from Ash Stevens (Detroit, MI). [14C]-AA and PAPC were purchased from NEN-Dupont (Boston, MA). PIP2 and 12-C-PGE2, RIA kit were obtained from Amersham (Arlington Heights, IL). AA, 5(S)-, 8(S)-, 9(S)-, 11(S)-, 12(S)-, and 15(S)-HETE as well as PGE2, PGF2α, 6-Keto PGF2α, PGF2β, and TxB2 were purchased from Cayman Chemical Company (Ann Arbor, MI); curcumin (>98% of diferuloylmethane) was purchased from Janssen Chemicals (Beerse, Belgium). The normal phase HPLC (4.6 x 250 mm) silica columns were obtained from Alltech Associates, Inc. (Deerfield, IL), and the reverse phase HPLC (3.9 x 300 mm) μBondpak C18 column was purchased from Waters Associates (Milford, MA). Precoated Silica G plastic TLC plates were purchased from Fisher Scientific Co. (Springfield, NJ).

Animals and Diets

Weanling male F344 rats were purchased from Charles River Breeding Laboratories (Kingston, NY). All ingredients of the semipurified diet were obtained from Dyets, Inc. (Bethlehem, PA) and were stored at 4°C prior to the preparation of diets. Male F344 rats received at weaning were quarantined for 10 days and had access to modified AIN-76A control diet (36). Following quarantine, all the animals were randomly distributed by weight into various groups and transferred to an animal holding room. They were housed in plastic cages with filter tops (three per cage) under controlled conditions of a 12-h light/12-h dark cycle, 50% humidity, and 21°C temperature. The experimental diet was prepared by adding curcumin to the control diet at the expense of dextrose. The incorporation of curcumin into the control diet was done with V-blender after curcumin was premixed with a small quantity of diet in a food mixer to ensure its uniform distribution. All control and experimental diets were prepared weekly in our laboratory and stored in a cold room. Animals had access to food and water at all times, and food cups were replenished with fresh diet three times weekly.

The purity and stability of curcumin was analyzed by HPLC. Curcumin was extracted from the diet with 2 volumes of acetonitrile:acetic acid (95:5). The organic layer was dried and redissolved in a mobile phase containing 0.1 M sodium phosphate (pH 4) and acetonitrile and injected into HPLC. Curcumin was separated on a Waters C18 column with gradient elution and was monitored at a 380-nm wavelength in a Waters 990 photodiode array detector. The results indicate that >96% of curcumin could be accounted for in feed samples stored in a cold room for 14 days.

Experimental Procedure

The experiment was design to determine the efficacy of 2000 ppm curcumin on AOM-induced colon carcinogenesis. Although curcumin administered in the diet to rats at 100,000 ppm had no significant toxic effect (8), we have decided to use low dietary levels of curcumin, based on our previous study in which 2000 ppm in the diet significantly inhibited colonic aberrant crypt foci development (20). As indicated in Fig. 2, beginning at 5 weeks of age, groups of animals were fed the control diet or the experimental diet containing 2000 ppm curcumin. At 7 weeks of age, groups of animals intended for carcinogen treatment received AOM s.c., once weekly for 2 weeks at a dose rate of 15 mg/kg body weight. Animals intended for vehicle treatment were administered an equal volume of normal saline. Animals were maintained on control or experimental diets until the termination of the experiment. Body weights were recorded every 2 weeks for the first 10 weeks and then every 4 weeks. Animals were monitored daily for general health. The experiment was terminated 52 weeks after the second AOM treatment, at which time all animals were sacrificed by CO2 euthanasia. After laparotomy, the entire stomach, small intestine, and large intestine were resected and opened longitudinally, and the contents were flushed with normal saline. Using a dissection microscope, colon and small intestinal tumors were noted grossly for their location, number, and size. For each tumor, the length (L), width (W), and depth (D) were measured with calipers. Estimates of tumor volume (V) were made using the formula $V = L \times W \times D / \pi/6$ (37). All other organs, including the kidney

![Fig. 2. Experimental design for evaluation of chemopreventive efficacy by curcumin. Curcumin was fed 2 weeks prior to exposure to AOM, during treatment, and until the termination experiment. AOM was given to the animals by s.c. at the beginning of 7 and 8 weeks of age, 15 mg/kg body weight.](image-url)
and liver, were also grossly examined under the dissection microscope for any abnormalities. Colon tumors with a diameter of more than 0.4 cm were cut into two halves; one portion of the tumor was used for analyses of PLA2, PLCγ1, COX and LOX metabolites, and the other one-half was used for histopathological examination of tumor types. Mucosa that was free of tumors in AOM-treated animals and from saline-treated animals was scraped from each colon for biochemical analysis according to our previously described method (37). Colon mucosa and portions of tumors intended for biochemical determinations were quickly frozen in liquid nitrogen and stored at −80°C.

For histopathological evaluation, tumors were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed by routine procedures with hematoxylin and eosin staining. The stained sections were examined histologically for tumor types according to the classification of Pozharisski (38) with minor modifications. Most of the colon tumors in this experiment were adenocarcinomas, either invasive or noninvasive. The invasive adenocarcinomas were mostly signet ring mucinous type, invading muscularis mucosa deep into the intestinal wall and beyond. The noninvasive adenocarcinomas were those growing outward towards the intestinal lumen and not invading the muscularis mucosa. They were usually well differentiated adenocarcinomas.

Biochemical Analysis

Samples of colonic mucosa and tumors for PLA2 and PLCγ1 assay were homogenized in 1:3 (w/v) volumes of homogenizing buffer containing 30 mM Tris-HCl (pH 7.4), 140 mM NaCl, 5 mM KCl, 20 μM EDTA, 10 μg/ml leupeptin, 50 μg/ml trypsin inhibitor, and 1 mM phenylmethanesulfonyl fluoride, and the homogenates were centrifuged at 100,000 × g at 4°C for 1 h. The resulting supernatant fraction was used for cytosolic PLA2 activity, and the pellet fraction was redissolved in 30 mM HEPES-NaOH buffer (pH 7.2) containing 0.2% Triton X-100 and used for the analysis of membrane-bound PLCγ1 activity.

PLA2 Activity. Cytosolic PLA2 activity was measured by the method of Leslie (39) with some modifications using [14C]PAPC (40–60 mCi/mmol) as substrate. PLA2 activity of cytosolic proteins was carried out in a total volume of a 100-μl reaction mixture containing 50 mM sodium HEPES (pH 7.3), 0.8 mM CaCl2, 0.02% Triton X-100, and 20–30 μg of cytosolic protein. The reaction was initiated by adding 40 μM of PAPC (10 μCi/μmol, adjusted with cold substrate). The reaction mixture was incubated at 37°C in a shaking water bath for 30 min. The reaction was terminated by the addition of 300 μl chloroform:methanol (3:2, v/v). An additional 200 μl chloroform was added to each sample and mixed thoroughly. The samples were then centrifuged, and the chloroform layer was separated and evaporated to dryness under N2. Five μg AA was added to dried extract and redissolved in chloroform. An aliquot of chloroform extract was then subjected to TLC on precoated plastic TLC plates (Silica G). The TLC plates were developed with a solvent system containing chloroform:methanol:acetic acid:water (90:12:2:1, v/v/v/v) and were exposed in an iodide chamber for 5 min for visualization of AA. The area of each [14C]AA metabolite was determined with a Bioscan system 200 image-scanning counter (Bioscan Inc., Washington, DC) equipped with a β-detector. Protein content was determined by the Bio-Rad method. Results are expressed as pmol [14C]AA released/mg protein/min.

PLCγ1 Activity. Membrane bound PLCγ1 activity was measured by the method of Blesadale et al. (40) with some modifications using [3H]PIP2 (1–5 Ci/mmol) as a substrate. PLCγ1 activity of membrane proteins (100–200 μg) was carried out in a total volume of 250 μl reaction mixture containing 30 mM HEPES-NaOH buffer (pH 7.2), 5 mM DTT, 4 mM CaCl2, 2 mM EGTA, 0.9 mM MgSO4, and 50 μM [3H]PIP2 (50 μCi/mmol). The reaction was initiated by adding substrate to the mixture and incubating at 37°C for 20 min in a shaking water bath. The reaction was terminated by the addition of 0.2 ml of chloroform:methanol (1:2, v/v), followed by 0.3 ml of 1 M HCl. The incubation mixture was mixed and centrifuged to yield two phases. An aliquot of 0.3 ml of aqueous layer containing [3H]inositol 1,4,5-trisphosphate was transferred to a scintillation vial containing 10 ml of scintillation cocktail, and radioactivity was counted in a Beckman model LD6800 scintillation counter. The activity is expressed as pmol of [3H]inositol 1,4,5-trisphosphate formed from [3H]PIP2/mg protein/15 min.

PGE2 Production

Colonic mucosa and tumors intended for PGE2 analysis were homogenized with a Polytron in 0.1 M Tris-HCl buffer (pH 7.4) containing 20 mM EDTA. Ex vivo production of PGE2 was determined in colonic mucosal and tumor homogenates (0.3 ml) by incubating them at 37°C for 0 basal level and 15 min (ex vivo PGE2 production). All the incubations were terminated by adding 5.6 μl indomethacin. Then 0.5 ml ice-cold methanol was added to each incubation mixture and mixed vigorously. After acidifying the samples to pH 3.5 with 1% formic acid, the aqueous layer was extracted with an equal volume of chloroform. Following centrifugation at 2000 × g for 20 min, the organic phase was removed and evaporated to dryness under N2. The dried extract was resuspended in the assay buffer for RIA of PGE2. Extraction of samples typically results in <100% recovery of PGE2. Consequently, recovery estimates were made by adding 3000 dpm of tritiated PGE2 to the sample prior to processing. Corrections for the proportion of analytically recovered PGE2 was made by measuring the tracer in an aliquot after extraction. RIA was done under equilibrium conditions according to standard methodology (41). The results are expressed as ng PGE2/mg protein.

LOX and COX Activities

Colonic mucosa and tumors from individual animals were homogenized in 1:3 (w/v) volumes of 100 mM Tris-HCl buffer (pH 7.2) using a Polytron tissue homogenizer. The samples were then centrifuged at 9000 × g for 4°C for 10 min. The supernatant fraction was centrifuged at 100,000 × g for 1 h. The resulting supernatant was used for LOX activity. Microsomal pellets were resuspended in 50 mM potassium phosphate buffer (pH 7.4) for assay of COX activity.

Cyclooxygenase Activity. The COX activity of colonic mucosa and tumors was measured by previously published methods (42, 43). Briefly, 150 μl reaction mixture containing 12 μM [14C]AA (420,000 dpm), 1 mM epinephrine, 1 mM glutathione in 50 mM phosphate buffer, and 25–35 μg of mucosal or tumor microsomal protein was incubated at 37°C for 15 min. The reaction was then terminated by the addition of 40 μl of 0.2 M HCl. The COX metabolites of AA were extracted three times with 0.5 ml of ethyl acetate. The combined extracts were evaporated to dryness under N2 and redissolved in chloroform and subjected to TLC using Silica G. The TLC plates were developed in a solvent system containing chloroform:methanol:acetic acid:water (100:15:1:25:1, v/v/v/v) and were exposed in an iodide chamber for 5 min for visualization of the standards. The metabolites of [14C]AA corresponding to PGE2, PGF2α, PGD2, 6-keto PGF1α, and TXB2 were detected by their comigration (RF values) with authentic standards. The area of each metabolite was determined with a Bioscan System 200 image-scanning counter equipped with a β-detector.

LOX Activity. Colon mucosal and tumor LOX activity was determined by the modification method of Huang et al. (16) and Rao et al. (43). In brief, this method involved HPLC measurement of [13C]labeled 5(S)-, 8(S)-, 9(S)-, 11(S)-, 12(S)-, and 15(S)-HETEs that were formed from the [14C]AA. The reaction mixture (200 μl) containing 100 mM Tris-HCl (pH 7.2) and 2 mM CaCl2 [14C]AA (6 nmol, 480,000 dpm) and cytosol fraction (300–500 μg protein) was incubated for 15 min at 37°C. The reaction was terminated by the addition of 12 μl of 0.2 M HCl, and the metabolites of [14C]AA were extracted with 0.6 ml of ethyl acetate three times. The HETEs were analyzed by normal phase HPLC as described previously (20, 28).

Statistical Analysis

Body weights, tumor incidence, tumor multiplicity, tumor volume and biochemical parameters were compared between the animals fed the control and curcumin diets. Tumor incidence, which is expressed as the percentage of animal with tumors, was analyzed statistically by χ2 test. Tumor multiplicity, expressed as mean number of tumors/animal, was analyzed by the unpaired t test accounting for unequal variance. Differences in body weights, tumor volume, and biochemical parameters between the groups were analyzed statistically by the Student t test and analysis of variance. Differences were considered statistically significant at P < 0.05.
RESULTS

General Observations. The body weights of animals treated with vehicle or AOM and fed the control and curcumin diets were comparable throughout the study (Table 1). In vehicle-treated animals, the chronic feeding of curcumin did not produce any gross changes in the liver, kidney, stomach, intestine, or lungs or any kind of histopathological changes in the liver or intestine attributable to toxicity.

Tumor Incidence. Table 2 summarizes the AOM-induced colon tumor incidence (percentage of animals with tumors) and multiplicity (number of tumors/animal). There was no evidence of tumors in vehicle-treated animals fed the control or curcumin diet. The results indicate that administration of curcumin significantly inhibited the incidences of noninvasive (P < 0.015) and total (invasive plus noninvasive) adenocarcinomas (P < 0.004). Although dietary curcumin inhibited invasive adenocarcinoma incidence to 47%, the differences did not reach a statistical significance (P = 0.054). With regard to tumor multiplicity, dietary curcumin significantly inhibited invasive (P < 0.015) and noninvasive (P < 0.01) adenocarcinomas of the colon. In addition, AOM-induced colon tumor volume was reduced to 57% in animals fed curcumin as compared to those fed the control diet (Table 3).

Biochemical Studies. The activities of PLA2 and PLCγ1 analyzed in colonic mucosa and tumors are summarized in Figs. 3 and 4. AOM administration elevated the activities of colonic mucosal PLA2 and PLCγ1, irrespective of dietary regimen. Interestingly, there was a 3-4-fold increase in the activities of PLA2 and PLCγ1 in colon tumors when compared to surrounding colonic mucosa. Long-term feeding of curcumin resulted in a significant suppression PLA2 and PLCγ1 activities in colonic mucosa and in tumors.

Table 3 summarizes the effect of dietary curcumin on basal levels and ex vivo production of PGE2 in the colonic mucosa and tumors of animals treated with AOM or vehicle.

As summarized in Table 5, the results of COX metabolites in colonic mucosa demonstrate that AOM treatment had minimal effect on the production of PGs and TxB2. Markedly increased levels (3-5-fold) of PGs and TxB2 were observed in the colonic tumors of animals fed the control diet when compared to colonic mucosa of animals fed control diet. Animals fed the curcumin diet showed significantly lower levels of PGs (25-50%) in the colonic mucosa and tumors compared to those fed the control diet. In contrast, administration of curcumin had no measurable effect on colonic mucosal and tumor TxB2.

The effect of dietary curcumin on colonic mucosal and tumor LOX metabolites is shown in Table 6. AOM administration had no significant effect on the production of HETEs in the colonic mucosa compared to those treated with vehicle and fed similar diets. The levels of HETEs were significantly higher in colonic tumors compared to colonic mucosa. Feeding of curcumin diet significantly suppressed the colonic mucosal and tumor 5(S)-, 8(S)-, 12(S)-, and 15(S)-HETEs formation to more than 49, 44, 47, and 27%, respectively.

DISCUSSION

The major aim of this investigation, which is a part of a large-scale investigation to identify the compounds present in fruits and vegetables for their potential chemopreventive properties, was to elucidate the colon tumor-inhibitory role of curcumin, a naturally occurring antiinflammatory agent. Previous studies have demonstrated that topical application and dietary administration of curcumin inhibited DMBA-induced skin and B(α)P-induced forestomach tumors in mice, respectively (17). A recent study in mice which showed that dietary administration of 5,000-20,000 ppm curcumin reduced the incidence of intestinal tumors (19), as well as another study from our laboratory that demonstrated an inhibitory effect of dietary curcumin on colonic aberrant crypt foci (20), provided an impetus to investigate the effect of this agent in a well-established colon cancer model. To our knowledge, this is the first report to demonstrate chemopreventive activity of curcumin in the F344 rat model of colon cancer.

Table 1 Effect of dietary curcumin on body weight gain in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of animals</th>
<th>0</th>
<th>4</th>
<th>9</th>
<th>14</th>
<th>22</th>
<th>32</th>
<th>42</th>
<th>52</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOM-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>36</td>
<td>73 ± 5</td>
<td>190 ± 12</td>
<td>260 ± 16</td>
<td>317 ± 22</td>
<td>369 ± 22</td>
<td>418 ± 27</td>
<td>437 ± 27</td>
<td>448 ± 32</td>
</tr>
<tr>
<td>2000 ppm curcumin diet</td>
<td>30</td>
<td>73 ± 4</td>
<td>194 ± 12</td>
<td>265 ± 15</td>
<td>322 ± 21</td>
<td>376 ± 20</td>
<td>426 ± 24</td>
<td>444 ± 26</td>
<td>456 ± 27</td>
</tr>
<tr>
<td>Saline-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>12</td>
<td>73 ± 5</td>
<td>200 ± 9</td>
<td>268 ± 12</td>
<td>321 ± 15</td>
<td>378 ± 19</td>
<td>434 ± 24</td>
<td>457 ± 27</td>
<td>469 ± 26</td>
</tr>
<tr>
<td>2000 ppm curcumin diet</td>
<td>12</td>
<td>73 ± 5</td>
<td>198 ± 8</td>
<td>269 ± 10</td>
<td>324 ± 13</td>
<td>380 ± 17</td>
<td>435 ± 22</td>
<td>460 ± 22</td>
<td>472 ± 25</td>
</tr>
</tbody>
</table>

* At 5 weeks of age, groups of animals were fed control or 2000 ppm curcumin diet. This period is denoted as 0 weeks.

Table 2 Effect of dietary curcumin on AOM-induced colon adenocarcinoma incidence in male F344 rats

<table>
<thead>
<tr>
<th>Experimental diets</th>
<th>Invasive (%)</th>
<th>Noninvasive (%)</th>
<th>Total (%)</th>
<th>Tumor multiplicity (Adenocarcinomas/animal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOM-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>38</td>
<td>67</td>
<td>81</td>
<td>0.53 ± 0.7</td>
</tr>
<tr>
<td>2000 ppm Curcumin</td>
<td>20</td>
<td>37*</td>
<td>47*</td>
<td>0.20 ± 0.4*</td>
</tr>
<tr>
<td>Saline-treated</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Significantly different from control diet group by [Chi]² test, * P < 0.01; ** P < 0.004.

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with no gross changes in the liver, kidney, stomach, intestine, and lungs. It is also noteworthy that, when compared to other naturally occurring colon cancer chemopreventive agents, such as dithiole thiones and diallyl disulfide, to cite a few, curcumin produced a better or equally potent colon tumor-inhibitory action (44, 45). Interestingly, curcumin fed even at 5–10% in the diet had no measurable toxic effects in the animals (46) as compared to substituted dithiole thiones, such as oltipraz, anethole trithione, and diallyl disulfide, which produced significant toxicity even at 0.05% in the diet (44, 45).

Although the precise mechanism by which curcumin inhibits the AOM-induced colon tumorigenesis has not been established, it would appear that one possible action involves antiinflammatory activity by curcumin. Previously, Srimal and Dhawan (11) showed that either oral or i.p. administration of curcumin effectively inhibited carrageenin-induced acute inflammation in mice and rats. In addition, several studies have also demonstrated the antiinflammatory action of curcumin on laboratory rodents by inducing the inflammation with carrageenin and kaolin (reviewed in Ref. 8). Several previous studies

**Table 3** Effect of dietary curcumin on AOM-induced colon tumor size and volume in male F344 rats

<table>
<thead>
<tr>
<th>Tumor size and volume</th>
<th>Control diet</th>
<th>2000 ppm curcumin diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-0.5 cm</td>
<td>26 (48.8)</td>
<td>14 (70)</td>
</tr>
<tr>
<td>0.5-1.0 cm</td>
<td>18 (33.3)</td>
<td>6 (30)</td>
</tr>
<tr>
<td>&gt;1.0 cm</td>
<td>10 (18.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor volume (mm³)</td>
<td>133 ± 240²</td>
<td>56 ± 68 (58)²</td>
</tr>
</tbody>
</table>

* a Number of tumors having particular size.
* b Percentage number of tumors having particular size.
* c Mean ± SD.
* d Percentage tumor suppression.
Table 4 Effect of dietary curcumin on AOM-induced colonic mucosal and tumor PGE2 levels in male F344 rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Saline-treated</th>
<th>AOM-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal level</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Mucosa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>0.24 ± 0.08bc</td>
<td>0.93 ± 0.31</td>
</tr>
<tr>
<td>2000 ppm Curcumin</td>
<td>0.13 ± 0.04d</td>
<td>0.44 ± 0.2d</td>
</tr>
<tr>
<td>Tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>4.74 ± 1.2</td>
<td>11.7 ± 2.6</td>
</tr>
<tr>
<td>2000 ppm Curcumin</td>
<td>2.93 ± 0.1d</td>
<td>7.2 ± 1.4d</td>
</tr>
</tbody>
</table>

a Samples were incubated at 37°C for 15 min for PGE2 synthetic activity.  

b ng of PGE2/mg protein.  
c Mean ± SD (n = 6).  
d Significantly different from control diet fed groups by the Student t test, P < 0.05.

have established that AA metabolites may play a modulatory role in several immunological and inflammatory diseases (22). The results of our present study demonstrated that the levels and production of PGs in colon tumors were higher than in colonic mucosa, suggesting an increased synthesis of COX metabolites in tumors. In addition, the role of COX metabolites, particularly PGE2, in colon tumor promotion has been established (21, 34, 35). Earlier studies from our laboratory and others have shown that PG inhibitors, such as piroxicam, indomethacin, sulindac, and aspirin, inhibit colon tumorigenesis in rodents (21, 32). LOX metabolites such as 12(S)-HETE promote tumor cell adhesion, stimulate tumor cell spreading, and augment the tumor cell metastatic potential (23–25). Also, a positive correlation was observed between the levels of 8(S)-HETE and degree of inflammation, hyperproliferation, clastogenicity, and tumor development induced by TPA (47). In addition, the activities of 5(S)- and 15(S)-HETEs, which are potent modulators of inflammation suppressed by lipooxygenase inhibitors, indicate a mediating role of HETEs in tumor promotion (42, 48). Thus, the inhibition of colon adenocarcinomas by curcumin was consistent with the reduction in COX and LOX metabolites in the colonic mucosa and tumors, suggesting that the chemopreventive action of curcumin might be mediated through the inhibition of production of COX and LOX metabolites.

In the present experiment, we studied the colonic mucosal and tumor levels of PLA2 and PLCy1, which are dominant pathways for the AA release (Fig. 5). Also, PLCy1 is responsible for diacylglycerol formation and protein kinase C-dependent signal transduction and cell proliferation (49). One of the pathways leading to generation of AA involves a direct action of PLA2 on a phospholipid that could include 1,2-diacyl- or 1-O-alkyl-2-acyl-phosphatidylinositol, phosphatidylethanolamine, or phosphatidylcholine. The second pathway mediated by PLCy1 involves the degradation of phosphatidylinositol 4,5-bisphosphate via a sequence of reactions beginning with PLCy1, followed by diglyceride lipase and monoglyceride lipase (27). Our results demonstrated that dietary curcumin significantly inhibited the PLA2 and

Table 5 Effect of dietary curcumin on colonic mucosal and tumor COX activity in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>PGE2</th>
<th>PGF2α</th>
<th>PGD2</th>
<th>6-keto PGF1α</th>
<th>TxB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline-treated</td>
<td>245 ± 32d</td>
<td>280 ± 28</td>
<td>156 ± 31</td>
<td>325 ± 34</td>
<td>278 ± 40</td>
</tr>
<tr>
<td>2000 ppm curcumin</td>
<td>148 ± 21b (39)c</td>
<td>211 ± 27d (25)</td>
<td>114 ± 24b (27)</td>
<td>212 ± 31b (35)</td>
<td>265 ± 38b (5)</td>
</tr>
<tr>
<td>AOM-treated</td>
<td>328 ± 34</td>
<td>355 ± 43</td>
<td>242 ± 33</td>
<td>378 ± 41</td>
<td>258 ± 32</td>
</tr>
<tr>
<td></td>
<td>184 ± 23c (44)</td>
<td>236 ± 41c (34)</td>
<td>156 ± 17d (30)</td>
<td>284 ± 35 (25)</td>
<td>264 ± 36</td>
</tr>
<tr>
<td>Tumors</td>
<td>1578 ± 116d</td>
<td>937 ± 108d</td>
<td>549 ± 77d</td>
<td>1193 ± 157d</td>
<td>972 ± 111d</td>
</tr>
<tr>
<td>2000 ppm curcumin</td>
<td>789 ± 88c (50)</td>
<td>543 ± 84c (42)</td>
<td>375 ± 59 (32)</td>
<td>820 ± 73 (31)</td>
<td>788 ± 93 (19)</td>
</tr>
</tbody>
</table>

a Mean ± SD (n = 6—8).  
b Significantly different from the respective control groups by the Student t test, P < 0.05.  
c Values in the parentheses are the percentage of inhibition from their respective control groups.  
d Significantly different from colonic mucosal values by the Student t test, P < 0.0001.

Table 6 Effect of dietary curcumin on colonic mucosal and tumor COX activity in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>5(S)-HETE</th>
<th>8(S)-HETE</th>
<th>12(S)-HETE</th>
<th>15(S)-HETE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline-treated</td>
<td>178 ± 29a</td>
<td>272 ± 36</td>
<td>242 ± 39</td>
<td>237 ± 39</td>
</tr>
<tr>
<td>2000 ppm curcumin</td>
<td>84 ± 13b (53)b</td>
<td>144 ± 28b (47)</td>
<td>123 ± 14b (49)</td>
<td>173 ± 31 (27)</td>
</tr>
<tr>
<td>AOM-treated</td>
<td>211 ± 33</td>
<td>312 ± 42</td>
<td>266 ± 37</td>
<td>308 ± 52</td>
</tr>
<tr>
<td></td>
<td>93 ± 17c (56)</td>
<td>176 ± 31c (44)</td>
<td>140 ± 18b (47)</td>
<td>189 ± 43c (38)</td>
</tr>
<tr>
<td>Tumors</td>
<td>312 ± 30a</td>
<td>348 ± 44</td>
<td>585 ± 83c</td>
<td>428 ± 69c</td>
</tr>
<tr>
<td>2000 ppm curcumin</td>
<td>158 ± 27c (49)</td>
<td>224 ± 35c (36)</td>
<td>253 ± 56c (57)</td>
<td>212 ± 48c (50)</td>
</tr>
</tbody>
</table>

a Mean ± SD (n = 6).  
b Values in the parentheses are the percentage of inhibition from their respective control groups.  
c Significantly different from their respective controls by the Student t test, P < 0.01.  
d Significantly different from colonic mucosal values by the Student t test, P < 0.0001.
In conclusion, the present study demonstrates that dietary curcumin significantly inhibited AOM-induced colon tumorigenesis in male F344 rats. In addition, curcumin significantly suppressed the colonic mucosal and tumor PLA2, PLCγ1, and COX and LOX activities, which are relevant to colon carcinogenesis. Although the exact mechanisms of chemopreventive action of curcumin remain to be elucidated, it would appear that modulation of AA metabolism by curcumin may play a role in its inhibitory action. Based on our current efficacy study, the lack of toxicity and side effects, as well as its availability in large quantities as natural products that has been used in population groups for several centuries, further studies are warranted to test the colon cancer chemopreventive action of curcumin in human clinical trials. It is reasonable to state that we believe curcumin possesses several advantages over other synthetic nonsteroidal anti-inflammatory drugs such as sulindac, piroxicam, and aspirin that are already in human clinical trials.

ACKNOWLEDGMENTS
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REFERENCES

Fig. 5. PLA2 and PLCγ1 pathways that may be involved in the release of AA from cellular phospholipids for the biosynthesis of COX and LOX metabolites. PC, phosphatidylcholine; Acyl PC, 1-acylphosphatidylcholine; PIP2, phosphatidylinositol 4,5-biphosphate; IP3, inositol 1,4,5-triphosphate; AMG, 2-arachidonyl-monoglyceride; LTs, leukotrienes, LXS, lipoxins.

PLCγ1 activities in the colonic mucosa and tumors. The exact mechanism by which curcumin inhibits these enzyme activities are not clear. It may be possible that curcumin may exert inhibitory activity by directly acting on PLA2 or PLCγ1, or alternatively, by acting on the regulators of PLA2 or PLCγ1, resulting in decreased levels of AA and its metabolite formation. We are not aware of any previous studies in which curcumin was tested on these enzyme activities. Based on these findings, it is reasonable to state that curcumin may modulate not only PLA2 and PLCγ1 to alter endogenous AA available as a substrate for production of COX and LOX metabolites but also COX and LOX pathways. Thus, the results from this study further support the modulatory role of curcumin as a dual inhibitor of AA metabolism.

In addition, it is also possible that inhibition of AOM-induced colon carcinogenesis by curcumin administered during the initiation phase may be mediated through the modulation of AOM metabolism, which is mediated through the cytochrome P450 2E1 system. The metabolic activation of AOM takes place in two steps, i.e., the hydroxylation of AOM to methylazoxymethanol in the liver and the oxidation of methylazoxymethanol to methylazoxymethamidine in the liver and colon (50). Although there is no direct evidence that curcumin modulates the metabolism of AOM, another antiinflammatory drug, aspirin, has been shown to suppress AA-induced decomposition of methylazoxymethanol, a metabolite of AOM, to formaldehyde in colonic mucosal homogenate. In this connection, Huang et al. (51) showed that TPA-induced c-jun/AP-1, an early gene, activation was significantly suppressed by curcumin in fibroblast cells. Further studies on the effect of curcumin on AOM metabolism, arachidonic acid metabolism, and early gene activation could clearly provide the possible mode of action of curcumin in colon carcinogenesis.
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Chemoprevention of Colon Carcinogenesis by Dietary Curcumin, a Naturally Occurring Plant Phenolic Compound

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