Chemoprevention of Colon Carcinogenesis by Phenylethyl-3-methylcaffeate

Chinthalapally V. Rao, Dhimant Desai, Abraham Rivenson, Barbara Simi, Shantu Amin, and Bandaru S. Reddy


ABSTRACT

Previous studies from this laboratory have established that caffeic acid esters present in propolis, a natural resin produced by honey bees, are potent inhibitors of human colon adenocarcinoma cell growth, carcinogen-induced biochemical changes, and preneoplastic lesions in the rat colon. The present study was designed to investigate the chemopreventive action of dietary phenylethyl-3-methylcaffeate (PEMC) on azoxymethane-induced colon carcinogenesis and to examine the modulating effect of PEMC on phosphatidylinositol-specific phospholipase C (PI-PLC), phospholipase A₁, lipooxygenase (LOX), and cyclooxygenase activities in the colonic mucosa and tumor tissues in male F344 rats. At 5 weeks of age, groups of rats were fed the control (modified AIN-76A) diet, or a diet containing 750 ppm of PEMC. At 7 weeks of age, all animals except those in the vehicle (normal saline)-treated groups were given 2 weekly s.c. injections of azoxymethane at a dose rate of 15 mg/kg body weight/week. All groups were maintained on their respective dietary regimen until the termination of the experiment 52 weeks after the carcinogen treatment.

Colonie tumors were evaluated histopathologically. Both colonic mucosa and tumors were analyzed for PI-PLC, phospholipase A₁, cyclooxygenase, and LOX activities. The results indicate that dietary administration of PEMC significantly inhibited the incidence and multiplicity of invasive, noninvasive, and total (invasive plus noninvasive) adenocarcinomas of the colon (P < 0.05–0.004). Dietary PEMC also suppressed the colon tumor volume by 43% compared to the control diet. Animals fed the PEMC diet showed significantly decreased activities of colonic mucosal and tumor PI-PLC (about 50%), but PEMC diet had no effect on phospholipase A₁. The production of 5(S)-, 8(S)-, 12(S)-, and 15(S)-hydroxyeicosatetraenoic acids via the LOX pathway from arachidonic acid was reduced in colonie tumors by 15–30%. The precise mechanism by which PEMC inhibits colon tumorigenesis remains to be elucidated. It is likely that the chemopreventive action may be related, at least in part, to the modulation of PI-PLC-dependent signal transduction and LOX-mediated arachidonic acid metabolism.

INTRODUCTION

Large bowel cancer is one of the leading neoplastic diseases in both men and women in Western countries, including North America (1, 2). Although several epidemiological and experimental studies suggest a relationship between colon cancer risk and dietary factors, the etiology of colon cancer is multifactorial and complex in that it may arise from the combined action of low level environmental (many of which as yet unidentified) and genotoxic agents, and dietary and host factors (1–4). Prevention strategies for cancer control involving reduction or elimination of human exposure to environmental risk factors may not always be possible; however, agents that can alleviate or diminish the carcinogenic effect of several environmental agents have been identified and tested for their chemopreventive efficacy (5).

Wattenberg (5), Boone et al. (6), and Kelloff et al. (7) reviewed the results of many chemoprevention studies in laboratory animal models, as well as in the human setting.

The use of naturally derived products or their active principles in the prevention and/or treatment of chronic diseases is based on the experience of traditional systems of medicine practiced in various ethnic societies, and on epidemiological observations of the relationship of dietary practices and disease patterns. Isolation, identification, and testing of active substances not only provide naturally occurring novel agents as inhibitors of cancer development but also offer unique opportunities to study the mechanisms of carcinogenesis. Propolis from honey bees contains various chemical constituents that exhibit a broad spectrum of activities including antibacterial, antifungal, cytostatic, and anti-inflammatory properties (8–10). Griebel and Pashinskii (11) have shown that honey possesses moderate antitumor and pronounced antimitotic effects in tumors in five different strains of rats and mice. Caffeic acid (3,4-dihydroxycinnamic acid) and its esters, which are present in propolis at levels of 20–25% (12), are agents suspected of having a broad spectrum of biological activities including tumor suppression. Because of this potential we and others have synthesized MC, PEC, PEMC, PEDMC, and related compounds that are present in propolis, and we have examined their antimutagenic and antitumorigenic activities (13, 14). Our bioassays confirmed that these agents are antimutagenic in Salmonella typhimurium strains TA98 and TA100 and that they inhibited colon adenocarcinoma HT-29 and HCT-116 cell growth (14). Further, these agents inhibited the AOM-induced colonic mucosal ornithine decarboxylase, tyrosine protein kinase and LOX activities, and colonic abrant crypt foci formation in F344 rats (15). Among the caffeic acid esters evaluated, PEMC was found to be most effective in inhibiting AOM-induced aberrant crypt foci formation (15).

Metabolites of AA exert a variety of biological activities. Several studies have shown that COX metabolites (particularly PGs derived from ω-6 fatty acids) modulate cell proliferation, tumor growth, and immune responses (16, 17), whereas LOX metabolites can influence various biological responses including chemotaxis, hormone secretion, ion transport, stimulation of tumor cell adhesion, tumor cell spreading, and regulation of the metastatic potential of tumor cells (18–20). The generation of AA for biosynthesis of COX and LOX metabolites involves degradation of phosphatidylcholine via a sequence of reactions regulated by PLC (17, 21). The second pathway in the generation of AA involves direct action of a PLA₂ on a phospholipid (17, 22). Several forms of PLC and PLA₂, among them PI-PLC, membrane-bound a PIP₂-specific, and cytosolic PLA₂, have been implicated in the regulation of eicosanoid biosynthesis and cell proliferation (22, 23). Increased levels of PLA₂ and PI-PLC activities have been observed in human colon and breast tumors and in melanomas compared to normal tissues (24–26). It is also noteworthy that the COX metabolite, PGE₂, which significantly affects tumor growth

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2 To whom requests for reprints should be addressed, at Division of Nutritional Carcinogenesis, American Health Foundation, Valhalla, NY 10595.
and immune responses, has been found at high levels in tumors in laboratory animals, as well as in neoplasms in humans (16, 27, 28). We and others have shown that inhibitors of COX such as aspirin, indomethacin, piroxicam, sulindac, and ibuprofen suppress colon carcinogenesis in laboratory animal models (16, 28–30). Thus, it is possible that changes in the activities of PLA₂ and PI-PLC that are involved in signal transduction and AA release, as well as COX and LOX pathways of AA metabolism produced by exogenous agents, may alter the tumorigenesis.

It was therefore of interest to evaluate the chemopreventive efficacy of PEMC (Fig. 1) in an established colon cancer model. PEMC, also known as phenethyl ferulate, is closely related to the naturally occurring turmeric compound curcumin, or diferuloylmethane. In the present study, we report on the chemopreventive efficacy of dietary administration of PEMC on AOM-induced colon tumorigenesis in male F344 rats. In addition, the effects of dietary PEMC on PLA₂, PI-PLC, COX, and LOX activities in colonic mucosal and tumor tissues were analyzed to better understand the 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 bought from DuPont New England Nuclear (Boston, MA). PIP₃ was obtained from Amersham (Arlington Heights, IL). AA, 5(S)-HETE, 8(S)-HETE, 9(S)-HETE, 11(S)-HETE, 12(S)-HETE 15(S)-HETE, PGE₂, PGF₁₂, 6-Keto PGF₁₂, PGE₂, AND TxB₂ were procured from the Cayman Chemical Co. (Ann Arbor, MI). PEMC was prepared from 4-hydroxy-3-methoxycinnamic acid by a procedure similar to one reported earlier (14). Chromatography on silica gel with hexane:ethyl acetate (1:1) as an eluent gave PEMC (71%), melting point 57–59°C, and a 1H nuclear magnetic resonance (CDCl₃) with following characteristics: δ 3.02 (t, 2H, COOCH₂, J = 7.05 Hz), 3.93 (s, 3H, OCH₃), 4.43 (t, 2H, CH₂Ph, J = 7.06 Hz), 6.28 (d, 1H, CH, J = 15.90 Hz), 6.92 (d, 1H, aromatic, J = 8.18 Hz), 7.00–7.09 (m, 2H, aromatic), 7.21–7.35 (m, 5H, aromatic), 7.61 (d, 1H, CH, J = 15.92 Hz); 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) µ Bondpak C₁₈ column was purchased from Waters Associates (Milford, MA). Precast Silica G plastic TLC plates were bought 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 replenished with fresh diet three times weekly. Animals were fed PEMC 2 weeks prior to exposure to AOM, during treatment, and until the termination of experiment. AOM (15 mg/kg body weight/week) was given to the animals s.c. at beginning of 7 and 8 weeks of age. Wk, week.

The study was designed to determine the chemopreventive efficacy of 750 ppm PEMC on colon carcinogenesis. The rationale for the selection of this dose was based on our biochemical and short-term bioassay results in F344 rats (14, 15). The experimental procedure was described previously (27). As indicated in Fig. 2, beginning at 5 weeks of age, groups of rats were fed the control diet or the experimental diet containing 750 ppm of PEMC. Two weeks later, 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, whereas those 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 every 4 weeks there after. All animals were sacrificed by CO₂ euthanasia 52 weeks after the second AOM treatment. After laparotomy, the entire gastrointestinal tract was resected and opened longitudinally, and the contents were flushed with normal saline. With the use of a dissection microscope, colonic and small intestinal tumors were noted grossly for their location, number, and size. For each tumor, the length, width, and depth were measured with calipers. Estimates of tumor volume were made with the use of the formula V = L x W x D x π/6 (32). All other organs, including kidney and liver, were also examined grossly under the dissection microscope for abnormalities. Colon tumors with a diameter of more than 0.4 cm were counted; one portion of the tumor was used for analyses of PLA₂, PI-PLC, and COX and LOX metabolites, the other half for histopathological examination. Mucosa that was free of tumors in AOM-treated animals and from saline-treated animals was scraped from each colon for comparative biochemical analysis according to our previously described method (32). Colonic mucosa and portions of tumors intended for biochemical determinations were quickly frozen in liquid nitrogen and stored at −80°C until analysis.

For histopathological evaluation, tumors were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed with hematoxylin and eosin staining. The stained sections were examined histologically according to the tumor classification of Pozharisski (33) with minor modifications. All 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 toward the intestinal lumen and not invading the muscularis mucosa. They were usually well differentiated adenocarcinomas.
Biochemical Analysis

Samples of colonic mucosa and tumors intended for PLA₂ and PI-PLC 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 phenylmethylsulfonyl fluoride, and the homogenates were centrifuged at 100,000 x 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, which was used for the analysis of membrane-bound PI-PLC activity.

PI-PLC Activity. Membrane-bound PI-PLC activity was measured by the method of Blesa et al. (34) with some modifications using [3H]-PIP₂ (5 Ci/mmol) as a substrate. PI-PLC activity of membrane proteins (100–200 μg) was determined 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/mmole). 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 addition of 0.2 ml of chloroform: methanol (1:2, v/v) and then 0.3 ml of 1 M HCl. The incubation mixture was mixed vigorously and centrifuged to yield two phases. An aliquot of 0.3 ml of the aqueous layer containing [3H]inositol 1,4,5-triphosphate was transferred into a scintillation vial containing 10 ml of scintillation cocktail. Radioactivity was counted in a Beckman Model LD6800 scintillation counter. The activity is expressed as pmol of [3H]inositol 1,4,5-triphosphate formed from [3H]-PIP₂/mg protein/15 min.

PLA₂ Activity. Cytosolic PLA₂ activity was measured by the method of Leslie (35) with some modifications with the use of [14C]-PAPC (40–50 mCi/mmol) as substrate. PLA₂ activity of cytosolic protein was carried out in a total volume of 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 μl PMAPC (10 μCi/μmol, adjusted with cold substrate), and the reaction mixture was incubated at 37°C in a shaking water bath for 30 min. Reaction was terminated by adding 300 μl chloroform:methanol (3:2, v/v). An additional 200 μl of chloroform were 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 of AA were added to the dried extract and redissolved in chloroform. An aliquot of the chloroform extract was then subjected to chromatography 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 exposed in an iodide chamber for 5 min for visualization of the standards. The metabolites of [14C]-AA corresponding to PGE₂, PGE₃, PGD₂, 6-keto PGF₁α, and TxB₂ were detected by their comigration (R₀ values) with authentic standards. The area of each metabolite was determined with the Bioscan System 200 image-scanning counter equipped with a β-detector.

Statistical Analysis

Body weights, tumor incidence, tumor multiplicity, tumor volume, and biochemical parameters were compared between the animals fed the control and PECM diets. Tumor incidence, expressed as percentage of animals with tumors, was analyzed by χ² test. Tumor multiplicity, expressed as the 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 by Student’s t test and ANOVA. 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 or PECM diets were comparable throughout the study (Table 1). In vehicle-treated animals, the feeding of PECM did not produce any gross changes in liver, kidney, stomach, intestine, or lungs, nor any kind of histopathological changes in the liver or intestine attributable to toxicity.

Tumor Incidence. Tables 2 and 3 summarize the AOM-induced colon, small intestinal and ear duct tumor incidence (percentages of animals with tumors), and multiplicity (number of tumors/animal). There were no tumors in vehicle-treated animals fed the control or PECM diet. Administration of PECM significantly inhibited the AOM-induced incidences of invasive (P < 0.02), noninvasive (P < 0.003), and total (invasive plus noninvasive) adenocarcinomas (P < 0.002; Table 2), and multiplicity of invasive (P < 0.01), noninvasive (P < 0.05), and total (P < 0.004) adenocarcinomas of the colon (Table 3). Animals fed PECM diet had fewer small intestinal tumors

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

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of animals</th>
<th>Wk 0</th>
<th>Wk 3</th>
<th>Wk 6</th>
<th>Wk 14</th>
<th>Wk 22</th>
<th>Wk 32</th>
<th>Wk 42</th>
<th>Wk 52</th>
</tr>
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<tbody>
<tr>
<td>AOM treated</td>
<td></td>
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</tr>
<tr>
<td>Control diet</td>
<td>36</td>
<td>73 ± 0.8</td>
<td>171 ± 1.8</td>
<td>232 ± 2.5</td>
<td>317 ± 3.3</td>
<td>369 ± 4.0</td>
<td>428 ± 4.5</td>
<td>438 ± 5.6</td>
<td>449 ± 5.3</td>
</tr>
<tr>
<td>(AIN-76A)</td>
<td>750 ppm PECM</td>
<td>30</td>
<td>73 ± 0.8</td>
<td>172 ± 1.7</td>
<td>235 ± 2.6</td>
<td>323 ± 2.9</td>
<td>375 ± 3.8</td>
<td>435 ± 4.4</td>
<td>444 ± 5.3</td>
</tr>
<tr>
<td>Saline treated</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>12</td>
<td>73 ± 1.1</td>
<td>168 ± 2.2</td>
<td>239 ± 2.7</td>
<td>320 ± 4.1</td>
<td>378 ± 4.9</td>
<td>432 ± 6.3</td>
<td>456 ± 7.2</td>
<td>468 ± 7.1</td>
</tr>
<tr>
<td>750 ppm PECM</td>
<td>12</td>
<td>73 ± 0.8</td>
<td>170 ± 1.9</td>
<td>235 ± 2.2</td>
<td>319 ± 4.2</td>
<td>375 ± 5.1</td>
<td>428 ± 6.2</td>
<td>450 ± 7.1</td>
<td>461 ± 7.3</td>
</tr>
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</table>
similarly treated animals. PEMC diet showed no significant inhibitory effect on colon tumor PGs (25-29%) and TxB2 (15%) compared to these levels in the colonic mucosa of the colonic mucosal and tumor tissue. The levels of PGs and TxB2 were observed in the colonic tumors of animals fed the control diet when compared to surrounding colonic mucosa. Long-term feeding of PEMC significantly suppressed PI-PLC activities in colonic mucosa and tumors but had no significant effect on the PLA2 activity. The effect of dietary PEMC on LOX and COX metabolites in the colonic mucosa and tumors is shown in Table 6. AOM feeding of PEMC significantly suppressed PI-PLC activities in colonic mucosa and tumors but had no significant effect on the PLA2 activity. The effect of dietary PEMC in the diet significantly suppressed PI-PLC activities in co-
tumors when compared to surrounding colonic mucosa. Long-term feeding of PEMC significantly suppressed PI-PLC activities in colonic mucosa and tumors but had no significant effect on the PLA2 activity. The effect of dietary PEMC on LOX and COX metabolites in the colonic mucosa compared to those treated with vehicle and fed similar diets (data not shown). The levels of HETEs were significantly higher in colonic tumors than in colonic mucosa. Administration of PEMC in the diet significantly inhibited the formation of colonic mucosal PI-PLC and PLA2, irrespective of dietary regimen. It is interesting that there was a 3-4-fold increase in the activities of PI-PLC and PLA2 in colon tumors when compared to surrounding colonic mucosa. Long-term feeding of PEMC significantly suppressed PI-PLC activities in colonic mucosa and tumors but had no significant effect on the PLA2 activity. The effect of dietary PEMC on LOX and COX metabolites in the colonic mucosa and tumor tissue is shown in Table 6. AOM administration had a minimal effect on the production of LOX and COX metabolites in the colonic mucosa and tumors but had no significant effect on the PLA2 activity. The effect of dietary PEMC on LOX and COX metabolites in the colonic mucosa was observed in the colonic tumors of animals fed the control diet when compared to these levels in the colonic mucosa of similarly treated animals. PEMC diet showed no significant inhibitory effect on colonic mucosal COX metabolite formation but had a minimal inhibitory effect on colonic tumor PGs (25-29%) and TxB2 (15%).

**DISCUSSION**

The major aim of this investigation, which is a part of a large-scale study on the identification of naturally occurring compounds with potential chemopreventive properties, was to elucidate the inhibitory role of PEMC present in propolis, a natural resin produced by honey bees, on the formation of colon tumors in rats. This investigation clearly demonstrated the colon cancer chemopreventive effect of PEMC. Previous studies in our laboratory have demonstrated that dietary administration of PEMC significantly inhibited the formation of AOM-induced preneoplastic aberrant crypt foci in the colon of male F344 rats (15) and also inhibited human colon adenocarcinoma cell growth (14). The results of the present study further support our previous observations that, indeed, caffeic acid esters such as PEMC possess antitumorigenic activities. Further, administration of PEMC...
produced 100% inhibition of ear duct tumors induced by AOM, suggesting that the chemopreventive (antitumorigenic) activities of this compound are not limited to cancer of the colon. Although there are no other studies in animal models to suggest a chemopreventive role for PEMC or related caffeic acid esters such as MC, PEC, and PEDMC, Wattenberg et al. (38) have demonstrated that dietary ad

The precise mechanism by which these agents inhibit predominantly colon tumorigenesis in rodents (16, 27). Thus, the inhibition of colon adenocarcinomas by PEMC was consistent with the reduction of particular LOX metabolites in the colonic tumors, suggesting that the chemopreventive action of PEMC may be mediated through the inhibition of formation of metabolites of LOX rather than of COX. The exact mechanism by which these agents inhibit predominantly LOX metabolites is not clearly known. Recently, Glasgow et al. (43) have shown that 2,5-dihydroxyxycinnamic acid methyl ester nonspecifically inhibited formation of LOX metabolites but not of COX metabolites in Syrian hamster embryo cells. Previous studies from our laboratory showed that administration of curcumin, structurally similar to PEMC, significantly inhibited AOM-induced colon carcinogenesis in male F344 rats (36).

Several previous studies have shown that PEMC significantly inhibits AOM-induced colonic mucosal and tumor lipoxigenase and cyclooxygenase metabolism in male F344 rats.

<table>
<thead>
<tr>
<th>Lipoxygenase activity*</th>
<th>COLONIC MUCOSA</th>
<th>TUMORS</th>
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<tbody>
<tr>
<td>Control diet</td>
<td>PEMC</td>
<td>Control diet</td>
</tr>
<tr>
<td>5(S)-HETE 211 ± 12b</td>
<td>148 ± 9 (36)2</td>
<td>313 ± 13</td>
</tr>
<tr>
<td>8(S)-HETE 312 ± 21</td>
<td>165 ± 12 (47)</td>
<td>348 ± 16</td>
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<tr>
<td>12(S)-HETE 266 ± 14</td>
<td>122 ± 10 (54)</td>
<td>585 ± 28</td>
</tr>
<tr>
<td>15(S)-HETE 308 ± 22</td>
<td>204 ± 13 (34)</td>
<td>428 ± 31</td>
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Cyclooxygenase activity

<table>
<thead>
<tr>
<th></th>
<th>COLONIC MUCOSA</th>
<th>TUMORS</th>
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<tbody>
<tr>
<td>Control diet</td>
<td>PEMC</td>
<td>Control diet</td>
</tr>
<tr>
<td>PGE2 329 ± 13</td>
<td>302 ± 15 (8.2)</td>
<td>1577 ± 38</td>
</tr>
<tr>
<td>PGE2 355 ± 16</td>
<td>328 ± 13 (7.6)</td>
<td>936 ± 29</td>
</tr>
<tr>
<td>PGE2 243 ± 13</td>
<td>248 ± 16 (0)</td>
<td>550 ± 20</td>
</tr>
<tr>
<td>6-2-keto-PGF1α 378 ± 14</td>
<td>336 ± 14 (11)</td>
<td>1193 ± 46</td>
</tr>
<tr>
<td>TXB2 260 ± 12</td>
<td>254 ± 14 (2.3)</td>
<td>973 ± 33</td>
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<table>
<thead>
<tr>
<th></th>
<th>Control diet</th>
<th>PEMC</th>
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<tbody>
<tr>
<td>1 µmol of HETEs produced from 14C-labeled arachidonic acid/mg protein/15 min at 37°C.</td>
<td></td>
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<tr>
<td>a Values in horizontal rows are significantly different from control group, either in colonic mucosa or in tumors, P &lt; 0.05 to 0.0001.</td>
<td></td>
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<tr>
<td>b Values in the parenthesis are percentage inhibition from their respective control diet group, either in colonic mucosa or tumors.</td>
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<td></td>
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<tr>
<td>c Values in the parenthesis are percentage inhibition from their respective control diet group.</td>
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<tr>
<td>d Values in the parenthesis are percentage inhibition from their respective control diet group.</td>
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In conclusion, the study described here demonstrates that dietary PEMC significantly inhibits AOM-induced colon tumorigenesis in F344 rats and that this inhibition is mediated through the suppression of PI-PLC and LOX activities in colonic mucosa and tumor tissues. Although the exact mechanisms of chemopreventive action of PEMC remain to be elucidated, modulation of PI-PLC-dependent signal transduction pathways and AA metabolism by PEMC are likely playing a role in its inhibitory action. Our current efficacy study and the availability of PEMC as a natural product strongly suggest that further studies should be pursued to test PEMC for its potential chemopreventive properties in human clinical trials.

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