Chemoprevention of Colon Carcinogenesis by Phenylethyl-3-methylcaffeate

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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 A₂, lipooxygenase (LOX), and cyclooxygenase activities in the colon 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 regimens until the termination of the experiment 52 weeks after the carcinogen treatment. Colonic tumors were evaluated histopathologically. Both colon mucosa and tumors were analyzed for PI-PLC, phospholipase A₂, 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 colon mucosal and tumor PI-PLC (about 50%), but PEMC diet had no effect on phospholipase A₂. The production of 5(S), 8(S), 12(S), and 15(S)-hydroxyeicosatetraenoic acids via the LOX pathway from arachidonic acid was reduced in colonic mucosa and tumors (30–60%) of animals fed the PEMC diet as compared to control diet. PEMC had no effect on the formation of colonic mucosal cyclooxygenase metabolites but inhibited the formation in colonic 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).

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1 The abbreviations used are: MC, methyl caffeate; PEC, phenylethyl caffeate; PEMC, phenylethyl-3-methyl caffeate; PEDMC, phenylethyl dimethyl caffeate; AOM, azoxymethane; TPA, 12-O-tetradecanoylphorbol-13-acetate; COX, cyclooxygenase; LOX, lipooxygenase; PLA₂, phospholipase A₂; PI-PLC, phosphatidylinositol-specific phospholipase C; AA, arachidonic acid; PAPC, 1-α-1-palmitoyl-2-α-arachidonyl phosphatidylcholine; HETE, hydroxyeicosatetraenoic acid; PG, prostaglandin; TxA, thromboxane; PIP₃, t-3-phosphatidyl-
linositol 4,5-biphosphate.

2 To whom requests for reprints should be addressed, at Division of Nutritional Carcinogenesis, American Health Foundation, Valhalla, NY 10595.

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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 phenylethyl 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). Pip 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, PGF2α, 6-Keto PGF1α, PGD2, 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 Alttech Associates Inc. (Deerfield, IL), and the reverse phase HPLC (3.9 x 300 mm) μBondpak 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 obtained from Dyets, Inc. (Bethlehem, PA) and were stored at 4°C prior to preparation of diets. Male F344 rats received as weanlings were quarantined for 10 days and had access to modified AIN-76A control diet (31). Following the quarantine, the animals were randomized by weight into various study and control groups and housed in plastic cages with filter tops (3/cage) under controlled conditions of a 12-h light/12-h dark cycle, at 50% relative humidity, and at 21°C. The experimental diet was prepared by adding PEMC to the control diet at the expense of dextrin. PEMC was incorporated into the control diet in a Hobart mixer after it had been premixed with a smaller quantity of diet in a food mixer to ensure its uniform distribution. All control and experimental diets were prepared weekly in our laboratory and were stored in a cold room. The rats had access to food and water at all times; food cups were replenished with fresh diet three times weekly.

The purity and stability of PEMC in the feed samples was determined by HPLC, for which the compound was extracted with 3 volumes of ethylacetate. The organic layer was dried, redissolved in mobile phase containing 0.1 m sodium phosphate (pH 4) and acetonitrile, and injected into the HPLC. The separation on a Waters C18 column with gradient elution was monitored at 254 nm wavelength in a Waters 990 photodiode array detector, indicating that >97% of PEMC can be accounted for in feed samples stored in a cold room for up to 14 days.

Experimental Procedure

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 thereafter. 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 cut into two halves; 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 Pozharski (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.

Fig. 1. Structure of PEMC (phenethyl-3-methylcaffeate or phenethyl ferulate).

Fig. 2. Experimental design for evaluation of chemopreventive efficacy by PEMC. 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.
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.

PLA2 Activity. Membrane-bound PLA2 activity was measured by the method of Blesdale et al. (34) with some modifications using [3H]-PAPC (5 Ci/mmol) as a substrate. PLA2 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]-PAPC (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 to 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]-PAPC/μg protein/15 min.

Lipase 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 PAPC (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, PGF2α, PGI2, 6-keto PGF1α, and TxB2 were detected by their comigration (Rf values) with authentic standards. The area of each metabolite was determined with the Bioscan System 200 image-scanning counter equipped with a β-detector.

Lipoxygenase and Cyclooxygenase 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) with the use of a Polytron tissue homogenizer. The samples were then centrifuged at 9000 X g for 10 min. The 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 Bleasdale 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) containing 0.2% Triton X-100, which was used for the analysis of membrane-bound PI-PLC activity.

Cyclooxygenase Activity. The COX activity of cytosolic mucosa and of tumors was measured by previously published methods (15, 37). Briefly, 150-μl reaction mixture containing 12 μM [14C]-AA (420,000 dpm), 1 mM epinephrine, 1 mM glutathione in 50 mM phosphate buffer, 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, redissolved in chloroform, and subjected to TLC with Silica G. The TLC plates were developed with a solvent system containing chloroform:methanol:acetic acid:water (100:15:1.25:1, 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, 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 the Bioscan System 200 image-scanning counter equipped with a β-detector.

Table 1. Effect of PEMC 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>
</thead>
<tbody>
<tr>
<td>Control (AIN-76A)</td>
<td>30</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>Saline treated</td>
<td>12</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>
<td>456 ± 5.4</td>
</tr>
</tbody>
</table>

RESULTS

General Observations. The body weights of animals treated with vehicle or AOM and fed the control or PEMC diets were comparable throughout the study (Table 1). In vehicle-treated animals, the feeding of PEMC 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 (percentage of animals with tumors), and multiplicity (number of tumors/animal). There were no tumors in vehicle-treated animals fed the control or PEMC diet. Administration of PEMC significantly inhibited the AOM-induced incidences of invasive (P < 0.02), noninvasive (P < 0.03), 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 PEMC diet had fewer small intestinal tumors...
similarly treated animals. PEMC diet showed no significant inhibitory control diet when compared to these levels in the colonie mucosa of the colonie mucosal and tumor 5(5)-, 8(5)-, 12(5)-, and 15(5)-HETEs with vehicle and fed similar diets (data not shown). The levels of PGs and TxB2 were observed in the colonie tumors of animals fed the formation to more than 30-60%. Markedly increased levels (3-5-fold) of the colonie mucosa and in tumors but had no significant effect on the PLA2 activity. The effect of dietary PEMC on LOX and COX metabolites in tumors when compared to surrounding colonie mucosa. Long-term 3-4-fold increase in the activities of PI-PLC and PLA2, irrespective of dietary regimen. It is interesting that there was a addition had a minimal effect on the production of LOX and COX metabolites in the colonie mucosa and tumors are summarized in Table 5. AOM administration elevated the activities of colonie mucosal PI-PLC and PLA2 activity is expressed as pmol of 14C-labeled arachidonic acid released/mg protein/min at 37°C. "PI-PLC activity is expressed as pmol [3H]inositol 1,4,5-triphosphate formed from [3H]PIP2/mg protein/15 min at 37°C. "Values are mean ± SEM (n = 6—8). "Significantly different from control group by Student’s t test, P < 0.01 (UHM) 0.0001. "Intestine includes colon plus small intestinal adenocarcinomas. "Values in the parenthesis are percentage inhibition when compared to control group.

Table 2. Effect of PEMC on AOM-induced intestinal and ear duct tumor incidence in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Colon</th>
<th>Small intestine</th>
<th>Ear duct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Invasive</td>
<td>Noninvasive</td>
<td>Totala</td>
</tr>
<tr>
<td>AOM treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>38 (44.4)</td>
<td>67 (32.6)</td>
<td>81 (37.5)</td>
</tr>
<tr>
<td>750 ppm PEMC</td>
<td>13' (65.7)</td>
<td>(P &lt; 0.02)</td>
<td>40' (40.3)</td>
</tr>
<tr>
<td>Saline treated</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

a Total includes invasive plus noninvasive adenocarcinomas.
b Intestine includes colon plus small intestinal adenocarcinomas.
c Significantly different from control group, by χ2 test using Z-probability.
d Values in the parenthesis are percentage inhibition when compared to control group.

effect on colonie mucosal COX metabolite formation but had a minimal inhibitory effect on colonie 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 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.
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produced 100% inhibition of ear duct tumors induced by AOM, suggesting that the chemopreventive (antimutagenic) 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 administration of hydroxycinnamates, nonesterified caffeic acid derivatives, significantly inhibited benzo[a]pyrene-induced neoplasia of the forestomach in mice. A recent study from our laboratory showed that dietary administration of curcumin, structurally similar to PEMC, significantly inhibited AOM-induced colon carcinogenesis in male F344 rats (36).

The precise mechanism by which PEMC inhibits colon tumorigenesis has not been established; it would appear that the possible modes of action involve suppression of oxidative processes, tyrosine phosphorylation, and modulation of AA metabolism. Frenkel et al. (39) showed that PEMC, a demethoxy form of PEMC, inhibits TPA-induced cell proliferation. Also, the present study demonstrated that PEMC had no significant effect on PLA2 activity. We are not aware of any previous studies in which PEMC or other caffeic acid esters were tested on these enzyme activities.

The levels and production of HETEs and PGs in colon tumors are higher than in colon mucosa, suggesting an increased synthesis of LOX and COX metabolites in tumors. LOX metabolites such as 12(S)-HETE promote tumor cell adhesion, stimulate tumor cell spreading, and augment the metastatic potential of the tumor cells (18-20). Also, a positive correlation was observed between the levels of 12(S)-HETE and degree of inflammation, hyperproliferation, clastogenicity, and tumor development induced by TPA (41). In addition, the activities of 5(S)- and 15(S)-HETEs, which are potent modulators of inflammation, have been shown to be suppressed by lipoxygenase inhibitors, indicating a mediating role of HETEs in tumor promotion (37, 42). Also, the role of COX metabolites, particularly PGE2, in colon tumor promotion has been established (16, 29, 30). Earlier studies from our laboratory and others have shown that PG inhibitors such as piroxicam, indomethacin, sulindac, and aspirin inhibit 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 colon 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-dihydroxybenzoinic 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 caffeic acid esters MC, PEC, PEMC and PEDMC predominantly inhibited AOM-induced LOX metabolites rather than COX metabolites in the colon mucosa (15).

In the present experiment, we studied the levels of PLA2 and PI-PLC, which are dominant pathways for the AA release, in the colon mucosa and tumors. Also, PI-PLC is responsible for diacylglycerol transduction and cell proliferation (40). 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 phosphatidylethanolamine. The second pathway, mediated by PI-PLC, involves the degradation of phosphatidylinositol 4,5-bisphosphate via a sequence of reactions beginning with PI-PLC, followed by diglyceride lipase and monoglyceride lipase (22). Our study demonstrates that dietary PEMC significantly inhibits the PI-PLC activity in the colon mucosa and tumor tissue. The exact mechanism by which PEMC inhibits these enzyme activities is not clear. It may be possible that PEMC exerts inhibitory activity by directly acting on PI-PLC or, alternatively, by acting on the regulators of PI-PLC, thus decreasing the levels of AA and/or protein kinase C-mediated signal transduction functions and cell proliferation. Also, the present study demonstrated that PEMC had no significant effect on PLA2 activity. We are not aware of any previous studies in which PEMC or other caffeic acid esters were tested on these enzyme activities.

<table>
<thead>
<tr>
<th>Table 6: Effect of PEMC on AOM-induced colon mucosal and tumor lipoxygenase and cyclooxygenase metabolism in male F344 rats</th>
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<tbody>
<tr>
<td>Lipoygenase activity</td>
</tr>
<tr>
<td>5(S)-HETE</td>
</tr>
<tr>
<td>8(S)-HETE</td>
</tr>
<tr>
<td>12(S)-HETE</td>
</tr>
<tr>
<td>15(S)-HETE</td>
</tr>
<tr>
<td>Cyclooxygenase activity</td>
</tr>
<tr>
<td>PGE2</td>
</tr>
<tr>
<td>PGG2</td>
</tr>
<tr>
<td>PGD2</td>
</tr>
<tr>
<td>6-5H-PGF1a</td>
</tr>
<tr>
<td>TXB2</td>
</tr>
</tbody>
</table>

*a Values in horizontal rows are significantly different from control diet, either in colonic mucosa or tumors, P < 0.05 to 0.0001.

*b Values in the parenthesis are percentage inhibition from their respective control diet group, either in colonic mucosa or tumors.

*c Values in the parenthesis are percentage inhibition from their respective control diet group, either in colonic mucosa or tumors.

*d Values in the parenthesis are percentage inhibition from their respective control diet group, either in colonic mucosa or tumors.

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 colon 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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