Inhibitory Effect of Caffeic Acid Esters on Azoxy-methane-induced Biochemical Changes and Aberrant Crypt Foci Formation in Rat Colon

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ABSTRACT

Previous work from this laboratory established that caffeic acid esters, present in the propolis of honey bee hives, are potent inhibitors of human colon tumor cell growth, suggesting that these compounds may possess antitumor activity against colon carcinogenesis. The present study was designed to investigate (a) the inhibitory effects of methyl caffeate (MC) and phenethyl caffeate (PEC) on azoxymethane (AOM)-induced ornithine decarboxylase (ODC), tyrosine protein kinase (TPK), and arachidonic acid metabolism in liver and colon mucosa of male F344 rats, (b) the effects of caffeic acid, MC, PEC, phenethyl-3-methylcaffeate (PEMC), and phenethyl dimethylcaffeate (PEDMC) on in vitro arachidonic acid metabolism in liver and colon mucosa, and (c) the effects of PEC, PEMC, and PEDMC on AOM-induced aberrant crypt foci (ACF) formation in the colon of F344 rats. At 5 weeks of age, groups of animals were fed diets containing 600 ppm MC or PEC (biochemical study) or 500 ppm PEC, PEMC, or PEDMC (ACF study). Two weeks later, all animals except the vehicle-treated groups were given s.c. injections of AOM, once weekly for 2 weeks. The animals intended for the biochemical study were sacrificed 5 days later and colonic mucosa and liver were analyzed for ODC, TPK, lipoxigenase, and cyclooxygenase metabolites. The animals intended for the ACF study were sacrificed 9 weeks later and analyzed for ACF in the colon. The results indicate that the PEC diet significantly inhibited AOM-induced ODC (P < 0.05) and TPK (P < 0.001) activities in liver and colon. The PEMC diet significantly (P < 0.001) suppressed the AOM-induced lipoxigenase metabolites 8(S)- and 12(S)-hydroxyeicosatetraenoic acid (HETE). The animals fed the MC diet exhibited a moderate inhibitory effect on ODC and 5(S), 8(S), and 12(S)-HETEs and a significant (P < 0.001) effect on colonic TPK activity. However, the MC and PEC diets showed no significant inhibitory effects on cyclooxygenase metabolism. In an in vitro study, caffeic acid and MC showed inhibitory effects on HETE formation only at a 100 µM concentration, whereas PEC, PEMC, and PEDMC suppressed in vitro HETE formation in a dose-dependent manner. AOM-induced colonic ACF were significantly inhibited in the animals fed PEC (55%), PEMC (82%), or PEDMC (81%). The results of the present study indicate that PEC, PEMC, and PEDMC, present in honey, inhibit AOM-induced colonic preneoplastic lesions, ODC, TPK, and lipoxigenase activity, which are relevant to colon carcinogenesis.

INTRODUCTION

Colon cancer is a major public health problem in the United States and other Western countries. It is estimated that, in the United States alone, 155,000 new cases and 60,500 deaths in 1993 are due to cancer of the colon (1). Although the etiology of colon cancer is multifactorial and complex (2–4), in recent years considerable efforts have been made to identify naturally occurring and related synthetic agents that could prevent cancer from developing and also from recurring (5). Cancer chemoprevention, therefore, has become an important emerging area in that, in addition to providing a practical approach to identifying potentially useful novel agents as inhibitors of cancer development, these agents offer opportunities to study the mechanisms of carcinogenesis. Previously, several naturally occurring compounds such as phenols, indoles, isothiocyanates, and dithiolethiones were shown to inhibit several types of cancer, including cancer of the colon, in laboratory animal models (5–7).

In recent years, there has been a revival of interest in the medical properties of honey because it is thought to exhibit a broad spectrum of activities including antibacterial, antifungal, cytostatic, and anti-inflammatory properties (8–10). Recent studies by Gribel and Pashinskii (11) indicated that honey possessed moderate antitumor and pronounced antimetastatic effects in five different strains of rat and mouse tumors. Furthermore, honey potentiated the antitumor activity of chemotherapeutic drugs such as 5-fluorouracil and cyclophosphamide (11). Honey contains a variety of compounds including caffeic acids, benzoic acid and its esters, substituted phenolic acids and esters, flavonoid glycosides, and beeswax (12). Some of the observed biological activities of honey may be traced to its chemical constituents (13). Wattenburg et al. (14) demonstrated that dietary administration of hydroxycinnamates, constituents of honey, significantly inhibited benzo[a]pyrene-induced neoplasia of the forestomach in mice. Caffeic acid (3,4-dihydroxycinnamic acid) ester derivatives, which are present in honey at levels of 20–25%, are agents thought to exhibit a broad spectrum of activities including possibly tumor inhibition (12). Because of the potential usefulness of caffeic acid ester derivatives in tumor inhibition, we and others previously synthesized several caffeic acid esters such as MC, PEC, and PEDMC and studied their antimutagenic and antitumorigenic activities (13, 15). Our results suggest that MC, PEC, and PEDMC possess antimutagenic activity against 3',2'-dimethyl-4-aminobiphenyl-induced mutagenicity in Salmonella typhimurium strains TA98 and TA100 and inhibit colon adenocarcinoma HT-29 and HCT-116 cell growth (15). These observations raise the possibility that caffeic acid ester derivatives (Fig. 1) may possess antitumor activity against colon carcinogenesis.

Several cellular components that have been associated with cell proliferation, such as polyamines and polyamine synthetic enzyme activities including ODC, are present at high levels in proliferating normal and neoplastic cells (16, 17). Therefore, lowering of polyamine levels through the inhibition of their synthetic enzyme activities may prevent the proliferative activity of neoplastic cells (18). In addition, many kinases, such as TPK, mediate proliferative as well as metabolic signals in the cells. The abnormal expression of TPK activity may be responsible for unlimited growth (19). In addition, increased TPK activity has been observed in neoplastic tissues (20). Based on this realization, TPK blockers in principle are useful anti-proliferative agents (21).

Eicosanoids, the metabolites of arachidonic acid through the lipoxigenase and cyclooxygenase pathways, exert a variety of biological activities. Several studies have shown that HETEs, the metabolites...
of the lipoxygenase pathway, play a modulatory role in inflammatory diseases since they can influence various biological responses including chemotaxis, hormone secretion, ion transport, and various enzymatic pathways (22). For example, 12(S)-HETE promotes tumor cell adhesion to endothelium and subendothelial matrix (23), stimulates tumor cell spreading (24), induces tumor cell release of degradative enzymes, and augments the tumor cell metastatic potential (25). Furthermore, a correlation between the inducibility of 8(S)-HETE and the degree of inflammatory and hyperproliferative responses of mouse skin has been observed in different mouse strains (26). Moreover, clastogenicity and tumor promotion induced by TPA and 8(S)-HETE-induced chromosomal alterations were suppressed by lipoxygenase inhibitors, indicating the mediating role of HETEs in the tumor promotion (27). In this connection, it is also noteworthy that cyclooxygenase metabolites, particularly PGs of the E series, which significantly affect cell proliferation, tumor growth, and immune responses, have been found at high levels in experimental animal tumors as well as in human tumors (28, 29). We and others have shown that cyclooxygenase inhibitors such as indomethacin, piroxicam, sulindac, and ibuprofen suppress colon carcinogenesis in laboratory animal models (30–32). Thus, the possibility exists that the changes in the activities of the cyclooxygenase and lipoxygenase pathways of arachidonic metabolism produced by exogenous agents may alter tumorogenesis.

Studies from various laboratories have suggested that ACF, which are early preneoplastic lesions in the colon, can be induced by carcinogen treatment in laboratory animals (33, 34). In addition, Pretlow et al. (35) also demonstrated the presence of these putative preneoplastic lesions in colonic mucosa of patients with colon cancer. This suggests that the ACF can be used to evaluate novel agents for their potential chemopreventive properties against colon cancer.

The present study was designed to investigate the effects of (a) dietary MC and PEC on AOM-induced ODC and TPK activities and arachidonic acid metabolism (lipoxygenase and cyclooxygenase) in colonic mucosa and liver of male F344 rats and (b) to test the effects of CA, MC, PEC, PEMC, and PEDMC on in vitro lipoxygenase and cyclooxygenase synthetic activities. Beginning at 5 weeks of age, groups of animals were fed the control diet or experimental diets containing 600 ppm MC or PEC. 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. Six animals in each group were used for ODC and TPK enzyme analysis and another six animals in each group were used for determination of lipoxygenase and cyclooxygenase synthetic activities. Animals were killed 5 days after the second AOM or vehicle treatment. Livers were removed, quick frozen in liquid nitrogen, and stored at −80°C. Colonies were rapidly removed, slit open longitudinally, and rinsed in ice-cold normal saline. They were then laid flat on a glass plate and mucosa was scraped with a glass microscope slide. The mucosa was immediately frozen in liquid N2 and stored at −80°C. For the in vitro study, nine animals were fed the control diet for about 4 weeks and sacrificed. Livers and colonic mucosa were obtained and processed as described above.

ODC Analysis. A portion of liver from each rat and colonic mucosa from individual animals was used for ODC assay. The cytosolic fraction was prepared by homogenizing the samples in 1:3 (w/v) volumes of homogenizing buffer (50 mM sodium phosphate buffer, pH 7.2, containing 5 mM dithiothreitol, 0.1 mM EDTA, and 0.1 mM pyridoxal 5-phosphate), using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged at 100,000 × g at 4°C for 1 h and the ODC activity was determined in the cytosolic fraction as described previously (15). The reaction mixture, containing 50 mM sodium phosphate, 0.2 mM L-ornithine, and 0.25 μCi of [2-14C]ornithine (58 Ci/mmol) in a final volume of 250 μl, was incubated at 37°C. The reaction was stopped by adding 200 μl of 2 N sulfuric acid, and the CO2 released was collected for another 45 min. Radioactivity was counted in a Beckman model LS6800 scintillation counter. The activity is expressed as pmol of [14C]ornithine released/mg protein/h.

TPK Analysis. TPK activity was measured in membrane fractions of liver and colonic mucosa as described previously (15). Membrane fractions were prepared by homogenizing liver and colonic mucosa in ice-cold buffer containing 20 mM Tris-HCl (pH 7.4), 0.25 mM sucrose, 1 mM MgCl2, 1 mM EDTA, 50 μg/ml trypsin inhibitor, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride. The homogenates were centrifuged at 100,000 × g at 4°C for 1 h. TPK activity was measured using poly(Glu-Tyr) (4.1:0.9) as substrate. Phosphorylation of membrane proteins (5–10 μg assay) was carried out at 24°C in a total volume of 50 μl reaction mixture, containing 50 mM Tris-HCl (pH 7.4), 20 mM MgCl2, 0.02% Triton X-100, 50 μM sodium orthovanadate,
and 20 μg of poly(Glu-Tyr). The reaction was initiated with 50 μM ATP and 0.4 mCi of [γ-32P]ATP (11.7 Ci/mmol). The reaction was terminated by applying 25 μl of reaction mixture to a 2.3-cm² Whatman no. 3 filter paper disc. The filters were washed several times in 10% trichloroacetic acid containing 10 mM sodium pyrophosphate, rinsed with ethanol and petroleum ether, dried, and counted in 10 ml of scintillation cocktail. Results are expressed as pmol [32P]ATP incorporated/mg protein/min. Protein content was determined by the Bio-Rad method, using bovine serum albumin as the standard.

In Vitro and In Vivo Lipoygenase and Cyclooxygenase Activities

Lipoygenase Activity. Liver and colonic mucosal lipoygenase activity in cytosolic fractions was determined by the method of Fischer et al. (26) and Huang et al. (37), with some modifications. Briefly, this method involved HPLC measurement of 14C-labeled 5(S), 8(S), 12(S), and 15(S)-HETEs that were formed from 14C-arachidonic acid. 14C-Arachidonic acid (6 mmol, 480,000 dpm) was incubated at 37°C for 15 min with cytosolic fractions of colon or for 45 min with liver cytosolic fractions (300–500 μg of protein), in a total volume of 200 μl reaction mixture containing 100 mM Tris-HCl (pH 7.2) and 2 mM CaCl₂. The reaction was terminated by the addition of 12 μl of 0.2 M HCl, and the metabolites of 14C-arachidonic acid were extracted with 0.5 ml of ethyl acetate 3 times. The HETEs were analyzed by normal phase HPLC using two serially connected 5-μm silica columns (250 x 4.6 mm) with a mobile phase of n-hexane:isopropanol:acetic acid (96:4:0.1, v/v/v) and an isocratic flow rate of 1.0 ml/min. The column eluate was monitored first at 235 nm with a Waters 990 photodiode array detector and then with a β-Flow radiometric detector. The radioactive profiles from the β-Flow detector were compared with the 235-nm UV absorption profiles of authentic HETEs analyzed under identical conditions. The in vitro effects of various caffeic acid esters were tested using the procedure described above, by incorporating various concentrations of inhibitor into the reaction mixture.

Cyclooxygenase Activity. The cyclooxygenase activity of liver and colonic mucosa was measured by using a slight modification of previously published methods (37, 38). In brief, the 150-μl reaction mixture contained 12 μM 14C-arachidonic acid (420,000 dpm), 1 mM epinephrine, and 1 mM glutathione (420,000 dpm) was incubated at 37°C for 15 min with colonic mucosa or for 30 min for liver, the reaction was terminated by the addition of 40 μl of 0.2 M HCl. The cyclooxygenase metabolites of arachidonic acid were extracted with 0.5 ml of ethyl acetate 3 times. The combined extracts were evaporated to dryness under N₂, redissolved in chloroform, and subjected to TLC on pre-coated plastic TLC plates (silica G 60; 20 x 20 cm; layer thickness, 150 μm). The TLC plates were developed with a solvent system containing chloroform:methanol:acetic acid:water (100:15:1:25:1.0, v/v/v/v/v) and were exposed in an iodide chamber for 5 min for visualization of the standards. The metabolites of 14C-arachidonic acid 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 (Bioscan Inc., Washington, DC) equipped with a β-detector.

EFFECT OF CAFFEIC ACID ESTERS ON COLON CARCINOGENESIS

Table 1 Effect of caffeic acid esters on AOM-induced colonic mucosal ODC and TPK activities in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group*</th>
<th>ODC activity (pmol 14CO₂ released/mg protein/h)</th>
<th>TPK activity (pmol [32P]ATP/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colonic mucosa</td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>12.7 ± 2.8a</td>
<td>8.57 ± 2.3</td>
</tr>
<tr>
<td>Saline-treated control</td>
<td>63.3 ± 12.8b</td>
<td>47 ± 10.1c</td>
</tr>
<tr>
<td>AOM-treated control</td>
<td>48 ± 9.7</td>
<td>42 ± 5.36</td>
</tr>
<tr>
<td>AOM + MC</td>
<td>39 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>AOM + PEC</td>
<td>41.3 ± 8.5</td>
<td>26.2 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>124 ± 19.5c</td>
<td>71.9 ± 7.5c</td>
</tr>
<tr>
<td></td>
<td>75 ± 15c</td>
<td>50.3 ± 9.1c</td>
</tr>
<tr>
<td></td>
<td>64 ± 13c</td>
<td>40.5 ± 7.2c</td>
</tr>
</tbody>
</table>

*Significantly different from saline-treated control, P < 0.0001.

*Significantly different from AOM-treated control, P < 0.001.

Aberrant Crypt Foci

At 5 weeks of age, groups of animals were fed the modified AIN-76A diet containing 0 or 500 ppm PEC, PEMC, or PEDMC. At 7 weeks of age all animals except the vehicle-treated animals 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 continued on experimental diets until the termination of the experiment at 16 weeks of age. All animals were sacrificed by CO₂ euthanasia. The colons were removed, flushed with Krebs Ringer solution, opened from cecum to anus, and fixed flat between two pieces of filter paper in 10% buffered formalin. Microscope slides were placed on the top of the filter paper to act as a extra weight, to ensure that the tissue remained flat during fixation. After a minimum of 24 h in formalin, the colons were cut into 2-cm segments, starting at the anus, and were placed in a petri dish containing 0.2% methylene blue (dissolved in Krebs Ringer solution) for 5–10 min. They were then placed, mucosal side up, on a microscope slide and observations were made with a light microscope. Recording of ACF was carried out according to standard procedures (33, 34).

Statistical Analysis

Data were analyzed by analysis of variance and Duncan's multiple range test, using P < 0.05 as the level of significance.

RESULTS

General Observations. There were no significant differences in body weights or organ weights of animals fed the control or 500 or 600 ppm caffeic acid ester diets throughout the study (data not shown).

Biochemical Observations. Table 1 summarizes the activities of ODC and TPK in liver and colon of animals fed the MC and PEC diets. AOM treatment significantly increased the ODC (4–5-fold) and TPK (3–4-fold) activities in colon and liver. Animals fed the 600 ppm MC diet showed a moderate inhibition of ODC activity in colon (P ≤ 0.05) and a nonsignificant effect on liver ODC activity. However, 600 ppm PEC significantly inhibited the ODC activity in both colon and liver (P < 0.01). Dietary MC and PEC significantly inhibited the AOM-induced membrane TPK activity in both colon and liver.

Fig. 2 shows the HPLC separation of HETEs. Compared to previous methods (26, 38), this method provided a clear separation of 12(S)- and 15(S)-HETEs and direct analysis of HETEs with the β-Flow detector. In the liver and colonic mucosal samples we did not find any detectable levels of 9(S)-HETE or 11(S)-HETE. The effect of dietary MC and PEC on colonic mucosal lipoygenase metabolites in vivo is shown in Table 2. Administration of AOM significantly elevated the levels of HETEs in colonic mucosa, compared to animals treated with saline and fed similar diets. The MC diet produced little (13–18% inhibition) or no effect on AOM-induced colonic mucosal HETE formation. Feeding of 600 ppm PEC significantly inhibited colonic mucosal 8(S)- and 12(S)-HETE (39–48%) formation but had a lesser effect on 5(S)- and 15(S)-HETE formation.

Table 1 Effect of caffeic acid esters on AOM-induced colonic mucosal ODC and TPK activities in male F344 rats

*Significantly different from saline-treated control, P < 0.0001.

*Significantly different from AOM-treated control, P < 0.001.

*Mean ± SD (n = 6).

*Significantly different from saline-treated control, P < 0.0001.

*Significantly different from AOM-treated control, P < 0.001.

*Significantly different from AOM-treated control, P < 0.05.
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This investigation clearly demonstrated the inhibitory effects of MC, PEC, PEMC, and PEDMC on AOM-induced biochemical changes that are relevant to colon cancer. The results indicate that feeding of 600 ppm MC or PEC inhibited the AOM-induced ODC activity in liver moderately and activity in the colonic mucosa very significantly. ODC, a rate-limiting enzyme in polyamine biosynthesis, has been correlated with the rate of cell proliferation in several tissues (16, 17). ODC has been shown to increase in several tissues upon exposure to carcinogens and promoting agents (17, 18). Previous studies in different cancer models indicated an important role for ODC in tumor promotion (17, 39). It was also demonstrated that agents which inhibit ODC activity are effective tumor inhibitors (17, 40). With regard to the caffeic acid esters, recent studies showed that PEC significantly inhibited TPA-induced epidermal ODC activity in mouse (41).

The present study also demonstrated distinct stimulation of liver and colonic mucosal TPK activity by AOM and inhibition of AOM-induced TPK activity by dietary MC and PEC. The mechanism by which these agents inhibit TPK activity is not clearly known. There is, however, a possibility that the mechanism of inhibition of TPK activity by these compounds might be similar to that of structurally related tyrosine analogues such as dihydroxyccinnamates, cinnamides, erbastin, tyrphostins, and methyl-2,5-dihydroxycinnamic acid-like TPK inhibitors (42, 43). These agents inhibit TPK activity by blocking the peptide site of the EGF receptor, as well as by stimulating auto-phosphorylation of the EGF receptor. Our previous results showed

### Table 2 Effect of caffeic acid esters on colonic mucosal lipoxygenase metabolism in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>5(5)-HETE</th>
<th>8(5)-HETE</th>
<th>12(5)-HETE</th>
<th>15(5)-HETE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-treated control</td>
<td>149 ± 13^b</td>
<td>218 ± 30</td>
<td>222 ± 27</td>
<td>191 ± 38</td>
</tr>
<tr>
<td>AOM-treated control</td>
<td>225 ± 26 (+51)^c</td>
<td>526 ± 38 (+141)^d</td>
<td>574 ± 47 (+158)^d</td>
<td>372 ± 28 (+94)^e</td>
</tr>
<tr>
<td>AOM + MC</td>
<td>193 ± 28 (+149)^f</td>
<td>430 ± 47 (-18)^f</td>
<td>502 ± 49 (-13)^f</td>
<td>322 ± 29 (-13)^f</td>
</tr>
<tr>
<td>AOM + PEC</td>
<td>187 ± 24 (-17)^f</td>
<td>272 ± 28 (-48)^f</td>
<td>350 ± 32 (-30)^f</td>
<td>328 ± 36 (-13)^f</td>
</tr>
</tbody>
</table>

^a Saline-treated control animals include those fed the control diet and administered vehicle (saline); AOM-treated control animals include those fed control diet and administered AOM; AOM + MC and AOM + PEC groups include those fed diets containing 600 ppm MC or PEC and administered AOM.

^b Mean ± SD (n = 6).

^c Values in parentheses represent percentage increase (+) from the saline-treated control group.

^d Values in parentheses represent percentage decrease (–) from the AOM-treated control group.

^e Significantly different from saline-treated control, P < 0.0001.

^f Significantly different from AOM-treated control, P < 0.001.
EFFECT OF CAFFEIC ACID ESTERS ON COLON CARCINOGENESIS

Table 3 Effect of caffeic acid esters on liver lipoxygenase metabolism in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>5(S)-HETE (pmol/mg protein/45 min)</th>
<th>8(S)-HETE (pmol/mg protein/45 min)</th>
<th>12(S)-HETE (pmol/mg protein/45 min)</th>
<th>15(S)-HETE (pmol/mg protein/45 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-treated control</td>
<td>64 ± 100b</td>
<td>61 ± 11</td>
<td>53 ± 12</td>
<td>72 ± 11</td>
</tr>
<tr>
<td>AOM-treated control</td>
<td>120 ± 28c</td>
<td>278 ± 53a</td>
<td>168 ± 38e</td>
<td>210 ± 47c</td>
</tr>
<tr>
<td>AOM + MC</td>
<td>82 ± 14</td>
<td>209 ± 28 (24)c</td>
<td>129 ± 13 (23)c</td>
<td>153 ± 28 (27)c</td>
</tr>
<tr>
<td>AOM + PEC</td>
<td>85 ± 13 (29)c</td>
<td>128 ± 19 (53)c</td>
<td>67 ± 13 (59)c</td>
<td>170 ± 24 (19)c</td>
</tr>
</tbody>
</table>

* Saline-treated control animals include those fed the control diet and administered vehicle (saline); AOM-treated control animals include those fed the control diet and administered AOM; AOM + MC and AOM + PEC groups include those fed diets containing 600 ppm MC or PEC and administered AOM.

Table 4 Effect of caffeic acid esters on colon mucosal lipoxygenase metabolism in vitro

<table>
<thead>
<tr>
<th>Concentration of caffeic acid esters (µM)</th>
<th>HETE formed (pmol/mg protein/15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5(S)-HETE</td>
</tr>
<tr>
<td>Control</td>
<td>168 ± 22a</td>
</tr>
<tr>
<td>CA</td>
<td>172 ± 28</td>
</tr>
<tr>
<td>10</td>
<td>134 ± 21 (20.2)b</td>
</tr>
<tr>
<td>50</td>
<td>96 ± 15 (42.8)</td>
</tr>
<tr>
<td>100</td>
<td>159 ± 31 (5.4)</td>
</tr>
<tr>
<td>MC</td>
<td>128 ± 22 (24)</td>
</tr>
<tr>
<td>PEC</td>
<td>84 ± 16 (50)</td>
</tr>
<tr>
<td>PEMC</td>
<td>144 ± 25 (14)</td>
</tr>
<tr>
<td>PEDMC</td>
<td>94 ± 12 (44)</td>
</tr>
<tr>
<td>100</td>
<td>56 ± 11 (67)</td>
</tr>
<tr>
<td>10</td>
<td>154 ± 26 (8.3)</td>
</tr>
<tr>
<td>50</td>
<td>98 ± 17 (41)</td>
</tr>
<tr>
<td>100</td>
<td>44 ± 10 (74)</td>
</tr>
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</table>

Table 5 Effect of caffeic acid esters on colon mucosal cyclooxygenase metabolism in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>PGs and TXB₂ produced from [14C]arachidonic acid (pmol/mg protein/15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PGF₁₂₀</td>
</tr>
<tr>
<td>Saline-treated control</td>
<td>299 ± 36a</td>
</tr>
<tr>
<td>AOM-treated control</td>
<td>2014 ± 121 (6.7)c</td>
</tr>
<tr>
<td>AOM + MC</td>
<td>1875 ± 160 (6.9)d</td>
</tr>
<tr>
<td>AOM + PEC</td>
<td>1538 ± 124 (24)c, d</td>
</tr>
</tbody>
</table>

* Saline-treated control animals include those fed the control diet and administered vehicle (saline); AOM-treated control animals include those fed the control diet and administered AOM; AOM + MC and AOM + PEC groups include those fed diets containing 600 ppm MC or PEC and administered AOM.

that MC, PEC, and PEDMC significantly inhibit the TPK activity of human colon adenocarcinoma HT-29 cells (15). Umezawa et al. (44) observed that the EGF-induced cell growth was significantly inhibited by methyl-2,5-dihydroxycinnamate in quiescent normal rat kidney cells. Su et al. (45) showed that adenovirus type 5E1 A-induced transfection was suppressed by PEC in rat embryo fibroblast cells in a dose-dependent manner. These results suggest that caffeic acid esters probably act on the receptor tyrosine kinases and modulate cell growth.

In the present study we compared the inhibitory effects of MC and PEC on AOM-induced colonic mucosal arachidonic acid metabolism and also compared the inhibitory effects of CA, MC, PEC, PEMC, and PEDMC on the in vitro metabolism of arachidonic acid via the lipoxygenase and cyclooxygenase pathways. The results indicate that feeding of the 600 ppm PEC diet significantly inhibited the AOM-induced lipoxygenase products 8(S)- and 12(S)-HETE, whereas MC showed little or no effect on HETE formation in the colon. However, in the in vitro studies, CA and MC induced moderate inhibition of HETE formation at 100 µM concentration. Dietary PEC, PEMC, and PEDMC produced strong inhibitory effects on HETE formation, in a dose-dependent manner. In contrast to these results, MC and PEC had little or no effect on liver and colonic mucosal cyclooxygenase activities, but in in vitro studies PEC, PEMC, and PEDMC at 100 µM concentrations induced moderate inhibition (30-40%) of PG and
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Fig. 3. Unsectioned methylene blue-stained rat colon. A, topographic view (X 40) of mucosa of colon obtained from a saline-treated control animal; no ACF were identified. B, several ACF (X 40). C, single multicrypt foci (X 100) with 5 crypt/foci and D, single multicrypt foci (X 100) with more than 5 crypt/foci identified in AOM-treated rat colon; the surrounding appeared to be normal.

TxB₂ formation. The induction of tumor promotion in colon mediated by TPA or several colon carcinogens is believed to be governed by lipoxygenase- and cyclooxygenase-produced metabolites of arachidonic acid such as PGs and HETEs (46-48). Such an assumption is strongly supported by studies showing that inhibitors of these metabolites suppress colon tumor growth (29-32). These studies support the involvement of arachidonic acid metabolism pathways in colon tumor promotion (29-32, 46-48). Furthermore, it was found that tumor promoters such as TPA and bile acids stimulate cell proliferation and increase the production of HETEs and PGs (46, 49). Even though the exact role of individual HETEs in colon tumor promotion is not clearly established, in the present study 8(S)-HETE and 12(5)-HETE were significantly suppressed by caffeic acid esters. Unlike the colon lipoxygenase activity, which is altered by the caffeic acid esters, the cyclooxygenase activity was shown to be little affected in the animals fed the 600 ppm PEC diet. Based on in vitro studies, we believe that the higher concentrations of PEC and its analogues may be required to inhibit the cyclooxygenase products.

The role of cyclooxygenase metabolites, particularly PGE₂, in colon tumor promotion has been clearly established (28, 29). Previous studies from our laboratory and those of others have shown that PGE₂ inhibitors such as aspirin, indomethacin, piroxicam, ibuprofen, and sulindac suppress colon tumorigenesis in rodents (30-32). With regard to caffeic acid esters, the exact mechanism by which these agents inhibit the arachidonic acid pathway is not clearly known. Recently, Glasgow et al. (50) showed that methyl-2,5-dihydroxycinnamate non-specifically inhibits the lipoxygenase metabolism of Syrian hamster embryo cells. Huang et al. (51) showed that topical application of curcumin, chlorogenic acid, caffeic acid, and ferulic acid, which are structurally very much similar to caffeic acid esters, produces inhibition of epidermal arachidonic acid metabolism. In a related study, Katiyar et al. (52) showed that polyphenolic compounds extracted from green tea possess similar inhibitory activities against epidermal PG and HETE formation and tumor incidence.

To assess the cancer chemopreventive effects of caffeic acid esters, we studied the inhibitory effects of PEC, PEMC, and PEDMC on AOM-induced colonic ACF. Several previous observations (33-35) and the results of the present study suggest that the ACF are early preneoplastic lesions in the colon which are not present in untreated laboratory animals but are induced by colon carcinogens. The results indicate that PEC, PEMC, and PEDMC inhibit AOM-induced ACF formation to 55.2%, 82.1%, and 81.3%, respectively. These results are significant because ACF represents the precursor lesions of chemically induced colon cancer (33). Inhibition of such precursor lesions by PEC, PEMC, and PEDMC in the current study suggests that these agents might possess inhibitory activity against colon cancer.

In summary, the results of the present study suggest that MC, PEC, PEMC, and PEDMC, present in honey, possess inhibitory activities against AOM-induced colonic ODC, TPK, and lipoxygenase activities and ACF formation, which are relevant to colon carci-
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Preclinical efficacy studies are warranted to test these compounds for their potential chemopreventive properties in laboratory animal models.

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REFERENCES

Inhibitory Effect of Caffeic Acid Esters on Azoxymethane-induced Biochemical Changes and Aberrant Crypt Foci Formation in Rat Colon

Chinthalapally V. Rao, Dhimant Desai, Barbara Simi, et al.


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