Inhibitory Effects of Caffeic Acid Phenethyl Ester on the Activity and Expression of Cyclooxygenase-2 in Human Oral Epithelial Cells and in a Rat Model of Inflammation

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ABSTRACT

We investigated the mechanisms by which caffeic acid phenethyl ester (CAPE), a phenolic antioxidant, inhibited the stimulation of prostaglandin (PG) synthesis in cultured human oral epithelial cells and in an animal model of acute inflammation. Treatment of cells with CAPE (2.5 μg/ml) suppressed phospholipase ester (12-O-tetradecanoylphorbol-13-acetate; TPA) and calcium ionophore (A23187)-mediated induction of COX-2 synthesis. This relatively low concentration of CAPE did not affect amounts of cyclooxygenase (COX) enzymes. CAPE nonselectively inhibited the activities of baccuvirus-expressed hCOX-1 and hCOX-2 enzymes. TPA- and A23187-stimulated release of arachidonic acid from membrane phospholipids was also suppressed by CAPE (4–8 μg/ml). Higher concentrations of CAPE (10–20 μg/ml) suppressed the induction of COX-2 mRNA and protein mediated by TPA. Transient transfections using human COX-2 promoter deletion constructs were performed; the effects of TPA and CAPE were localized to a 124-bp region of the COX-2 promoter. In the rat carrageenain air pouch model of inflammation, CAPE (10–100 mg/kg) caused dose-dependent suppression of PG synthesis. Amounts of COX-2 in the pouch were markedly suppressed by 100 mg/kg CAPE but were unaffected by indomethacin. These data are important for understanding the anticancer and anti-inflammatory properties of CAPE.

INTRODUCTION

Synthesis of PGs depends on the activities of phospholipases and COXs. The former enzymes catalyze the release of arachidonic acid from membrane phospholipids, which can be rate-limiting for the synthesis of PGs. COXs catalyze the conversion of arachidonic acid to PGs. There are two isoforms of COX, one is constitutive (COX-1) and the other is inducible (COX-2). COX-1 and COX-2 are encoded by separate genes (1, 2). COX-2 is the isoform that is up-regulated in transformed cells (3–5) and in malignant tissue (6–11).

Premalignant and malignant tumors form more PGs than the normal tissues from which they arise (12–15). The increased level of PGs in tumors is due, at least in part, to increased expression of COX-2 (6–11). Prostaglandins, such as PGE2, promote angiogenesis (16), stimulate cell proliferation (17), and inhibit immune surveillance (10, 18). Thus, overproduction of PGs favors malignant growth. A possible cause-and-effect connection between the activity of COX-2 and production of PGs, on the one hand, and the induction of tumors, on the other, was highlighted by recent studies of tumor formation in the gut. A null mutation for COX-2 caused a marked reduction in intestinal tumor formation in a murine model of familial adenomatous polyposis, i.e., APC Δ716 knockout mice (19). Furthermore, inhibitors of PG synthesis, such as selective inhibitors of COX-2, protect against carcinogenesis (19–23). One approach to inhibiting carcinogenesis, therefore, is to administer agents that prevent increased PG synthesis in premalignant and malignant tissue.

There are several strategies for inhibiting the synthesis of PGs. Inhibitors of phospholipase A2, for example, will limit the amount of arachidonic acid that is available for PG production. In contrast, NSAIDs reduce levels of PGs because they inhibit the activity of COX. Chemopreventive agents such as retinoids and resveratrol inhibit the production of PGs by suppressing the activation of COX-2 gene expression (24–26).

CAPE (Fig. 1), a phenolic antioxidant derived from honeybee propolis, has anticancer (27–32) and anti-inflammatory (31, 33) properties. It inhibits the development of azoxymethane-induced aberrant crypts in the colon of rats (30), and it blocks tumorigenesis in a two-stage model of skin cancer that was promoted by treatment with phorbol ester (TPA) (32). The anti-inflammatory properties of CAPE have been attributed to suppression of eicosanoid synthesis (33). In the present work, we have extended prior observations concerning the effects of CAPE on PG synthesis by showing that CAPE inhibited the release of arachidonic acid from cell membranes, suppressed the enzyme activities of COX-1 and COX-2, and inhibited the activation of COX-2 gene expression. Decreased arachidonic acid metabolism was observed in cultured human oral epithelial cells and in the rat carrageenain air pouch model of inflammation. These data are important for understanding the chemopreventive and anti-inflammatory properties of CAPE.

MATERIALS AND METHODS

Materials. DMEM/F-12 and FBS were from Life Technologies, Inc. (Grand Island, NY). Keratinocyte basal and growth media were from Clonetics Corp. (San Diego, CA). Sodium arachidonate, Lowry protein assay kits, calcium ionophore A23187, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (thiazolyl blue), carrageenan, TPA, and L-nitrophenol-β-D-galactopyranoside were from Sigma Chemical Co. (St. Louis, MO). Enzyme immunoassay reagents for PGE2, assays were from Cayman Co. (Ann Arbor, MI). [1-32P]dCTP and [3H]arachidonic acid (210 μCi/ml) were from Du Pont NEN (Boston, MA). Pef-3, the cationic lipid used for transfections, was from Invitrogen (San Diego, CA). Reagents for the luciferase assay were from Promega Corp. Boehringer-Mannheim Biochemicals (Indianapolis, IN). Nitrocellulose membranes were from Schleicher & Schuell (Keene, NH). DNA and RNA were prepared using kits from Qiagen (Chatsworth, CA). The 18S RNA cDNA was from Ambion, Inc. (Austin, TX). A rabbit polyclonal anti-human COX-2...
Antiserum was purchased from Oxford Biomedical Research, Inc. (Oxford, MI). A goat polyclonal anti-human COX-1 antiserum was obtained from Santa Cruz Biotechnology, Inc. (San Diego, CA). Western blotting detection reagents (ECL) and [35S]methionine were from Amersham Pharmacia Biotech. CAPE was synthesized by esterification of caffeic acid with phenethyl alcohol in the presence of p-toluenesulfonic acid as detailed previously (27).

**Tissue Culture.** Human 1483 squamous carcinoma cells were described previously (34). Cells were maintained in a 1:1 mixture of DMEM/F-12 supplemented with 10% FBS and 50 μg/ml gentamicin. Cells were grown to 70% confluence, trypsinized with 0.05% trypsin-2 mM EDTA solution, and plated for experimental use in DMEM/F-12 medium without FBS unless stated otherwise. MSK Leuk1 was established from a dysplastic leukoplakia lesion adjacent to a squamous cell carcinoma of the tongue in a 46-year-old non-smoking female (35). Cells were routinely maintained in keratinocyte growth medium and passaged using a 0.125% trypsin-2 mM EDTA solution. In all experiments, 1483 and MSK Leuk1 cells were grown in basem medium for 24 h before treatment. Treatment with vehicle (0.125% DMSO), CAPE, A23187, or TPA was carried out under serum-free conditions. Cellular cytotoxicity was assessed by measurements of cell number, release of lactate dehydrogenase, and trypan blue exclusion, and the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay. Levels of lactate dehydrogenase release were measured according to the manufacturer’s instructions in the supernatants used for PGE2 assay.

**PGE2 Production.** The production of PGE2 was determined two different ways (i.e., spontaneous and arachidonate-stimulated). Cells were plated in six-well dishes at 2.5 × 10^5 cells/well and allowed to grow for 2 days. Fresh medium containing vehicle, TPA (50 ng/ml), or TPA (50 ng/ml) plus CAPE was provided, and the cells were incubated for 4.5 h. Media was collected for 4.5 h. Cultivation of PGE2 by enzyme immunoassay (36). At the end of the treatment period, cells were incubated with fresh medium containing 10 μM sodium arachidonate and 0–5 μg/ml CAPE. After 30 min, the medium was collected for analysis of PGE2. Levels of PGE2 were normalized to protein concentrations.

**Cox Enzyme Assay In Vitro.** The effect of CAPE on the activities of COX-1 and COX-2 was measured using baculovirus-expressed recombinant human COX-1 and COX-2 enzymes as described previously (37). A dose-response curve was generated with CAPE concentrations ranging from 0.005 to 500 μM (1 μg/ml is equivalent to 3.5 μM). CAPE was preincubated with homogenates containing COX-1 or COX-2 for 20 min before addition of arachidonic acid (10 μM). The amount of PGE2 formed in 10 min was detected by enzyme immunoassay.

**Arachidonic Acid Release.** Cells (1.5 × 10^5) 1483 cells/well were plated in 24-well tissue culture plates in 1 ml of DMEM/F-12 containing 10% FBS and incubated at 37°C for 2.5 days. The medium in each well was then replaced with 250 μl of fresh medium containing 1 μM/μl of [3H]arachidonic acid, and the plates were incubated for 24 h. Determination of the radioactivity in an aliquot of the labeling medium before and after incubation with the cells showed that [3H]arachidonic acid incorporation was >95%. Subsequently, the labeling medium was removed, and all of the culture wells were washed three times with 1 ml of warm serum-free DMEM/F-12 medium. The experimental solutions (1 ml/well) containing TPA (50 ng/ml), A23187 (2.5 μM), TPA plus A23187, or these agents combined with different doses of CAPE were applied in triplicate to cells, and the plates were incubated for 2 h. Test solutions were collected from the wells into 1.5-ml Eppendorf tubes and centrifuged in a Biofuge A at 10,000 rpm for 5 min at room temperature. Five hundred μl of each test solution were then added to 10 ml of ScintiVerse in vials, and cpm for 3H were measured.

**Western Blotting.** Cell lysates were prepared by treating cells with lysis buffer as described previously (26). Lysates were sonicated for 20 s on ice and centrifuged at 10,000 × g for 10 min to sediment the particulate material. The protein concentrations of supernatants were measured by the method of Lowry et al. (38). SDS/PAGE was performed under reducing conditions on 10% polyacrylamide gels (39). The resolved proteins were transferred onto nitrocellulose sheets as detailed by Towbin et al. (40). The nitrocellulose membrane was then probed with a rabbit polyclonal anti-COX-2 antiserum or a goat polyclonal anti-COX-1 antiserum (26).

**Northern Blotting.** Cells were plated in 100-mm dishes at a density of 3 × 10^5 cells/100-mm dish and allowed to attach for 2 days before experiments. Total RNA was isolated from cell monolayers using the RNA isolation kit from Qiagen, Inc. Seven μg of RNA per lane were electrophoresed in a formaldehyde-containing 1.2% agarose gel and transferred to nylon-supported membranes. The analysis for COX-2 mRNA was performed as described previously (26).

**Plasmids.** A series of human COX-2 promoter-deletion constructs (–1432/59, –327/59, –220/59, –124/59) were used (41). pSV-βgal was obtained from Promega Corp. (Madison, WI). The human COX-2 cDNA was generously provided by Dr. Stephen M. Prescott (University of Utah, Salt Lake City, UT).

**Transient Transfection Assays.** 1483 cells were seeded at a density of 2 × 10^5 cells/well in six-well dishes and grown to 30–40% confluence in DMEM/F-12 containing 10% FBS. For each well, 2 μg of plasmid DNA were introduced into cells using 16 μg of Pax-3 as per the manufacturer’s instruc-
CAPE inhibits prostaglandin synthesis

RESULTS

CAPE Inhibits Phorbol Ester-mediated Increases in the Production of PGE2. As shown in Fig. 2A, treatment of 1483 cells with TPA (50 ng/ml) caused more than a 10-fold increase in the spontaneous production of PGE2 from endogenous arachidonic acid. A dose-dependent suppression of this effect occurred in cells exposed to CAPE. Complete inhibition of TPA-stimulated PGE2 synthesis was achieved at 1 μg/ml CAPE. A similar increase in production of PGE2 was obtained in response to calcium ionophore (A23187, 2.5 μM); the same concentration range of CAPE blocked this effect as well (data not shown). To examine synthesis of PGE2 in more detail, we measured the effects of TPA and CAPE on production of PGE2 when an excess of exogenous arachidonate was added to the reaction. This experiment was done because PGE2 production can be affected by changes in the activity of phospholipase A2, the enzyme that provides substrate for COX-catalyzed reactions. Adding excess arachidonate minimizes any contribution of phospholipase activity to the rate of production of PGE2. As shown in Fig. 2B, treatment with TPA caused a 2-fold increase in the synthesis of PGE2 in the presence of excess arachidonic acid. This effect was suppressed by CAPE at concentrations of 1–2.5 μg/ml. In parallel, we measured levels of COX-1 and COX-2 protein by Western blotting. Under these experimental conditions, enzyme levels were unaffected by CAPE (data not shown). These results suggested that CAPE may directly inhibit COX activity. This idea was tested in separate cell-free assays of COX-1 and COX-2 catalytic activities using baculovirus-expressed, human recombinant enzymes. As shown in Fig. 3, the activities of both COX-1 and COX-2 were inhibited by CAPE with IC50 values of 58 μM (16.6 μM) and 82 μM (23.4 μM), respectively. The fact that lower concentrations of CAPE were required to inhibit PG synthesis in cell culture (Fig. 2) than in this cell-free system (Fig. 3) is likely to reflect the difference in experimental systems.

Phorbol Ester-mediated Induction of COX-2 Is Inhibited by CAPE. TPA was shown previously to induce COX-2 protein in 1483 cells (24). Fig. 4A shows that cotreatment of 1483 cells with TPA and a range of CAPE concentrations (10–20 μg/ml) caused dose-dependent suppression of TPA-mediated induction of COX-2 protein. We also determined whether CAPE suppressed the induction of COX-2 by TPA in a premalignant oral epithelial cell line, designated MSK Leuk1. As shown in Fig. 4B, CAPE inhibited TPA-mediated induction of COX-2 in this cell line as well (Fig. 4B).

To further investigate the effects of CAPE on TPA-mediated induction of COX-2, steady-state levels of COX-2 mRNA were evaluated by Northern blotting. As shown in Fig. 5, TPA markedly induced COX-2 mRNA levels in both the transformed (1483) and premalignant (MSK Leuk1) cell lines.

Fig. 3. In vitro inhibition of COX-1 and COX-2 catalytic activity by CAPE. CAPE (0.005–500 μM) was incubated with membranes containing baculovirus-expressed human recombinant COX-1 (hCOX-1) or COX-2 (hCOX-2) for 20 min. The reaction was initiated by adding arachidonic acid (10 μM) for 10 min. COX activity was measured as PGE2 formed/μg protein. Percent control was determined by comparing levels of synthesis of PGE2 in control incubations with levels observed in incubation mixtures containing the indicated concentrations of CAPE. Bars, SD.

Fig. 4. CAPE causes dose-dependent inhibition of TPA-mediated induction of COX-2. A, lysate protein was from 1483 cells treated with vehicle (Lane 1), TPA (50 ng/ml; Lane 2) or TPA (50 ng/ml; Lane 2) and CAPE (10, 15, 20 μg/ml; Lanes 3–5) for 4.5 h. B, lysates were from premalignant oral epithelial (MSK Leuk1) cells treated with vehicle (lane 2), TPA (50 ng/ml; lane 3), or TPA (50 ng/ml) and CAPE (5, 10, and 15 μg/ml; Lanes 4–6) for 4.5 h. Lane 1, an ovine COX-2 standard. Cellular lysate protein (25 μg/lane) was loaded onto a 10% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose. Immunoblots were probed with antibody specific for COX-2.

Fig. 5. TPA-mediated induction of COX-2 mRNA is suppressed by CAPE. A, 1483 cells were treated with vehicle (Lane 1), TPA (50 ng/ml; Lane 2) or TPA (50 ng/ml) and CAPE (10, 15, and 20 μg/ml; Lanes 3–5) for 3 h. B, premalignant oral epithelial (MSK Leuk1) cells were treated with vehicle (Lane 1), TPA (50 ng/ml; Lane 2), or TPA (50 ng/ml) and CAPE (5, 10, 15, and 20 μg/ml; Lanes 3–6) for 3 h. Total cellular RNA was isolated. Each lane contained 7 μg of RNA. The Northern blot was probed sequentially with probes that recognized COX-2 mRNA and 18S rRNA.
After transfection, cells were treated with vehicle (0.1% DMSO), TPA (10 ng/ml, 20 ng/ml), or TPA (50 ng/ml) and CAPE (15 µg/ml). Reporter activities were measured in cellular extract 6 h later. Luciferase activity represents data that have been normalized with β-galactosidase activity. Six wells were used for each of the conditions. Columns, means; bars, SD. *, P < 0.01 compared with TPA treatment. IL6, interleukin 6; CRE, cyclic AMP response element.

nificant oral epithelial cells (MSK Leuk1). Again, this effect was suppressed by CAPE in both cell lines. The inhibitory effects of CAPE were dose dependent in MSK Leuk1 cells, whereas maximal suppression was observed with 10–15 µg/ml CAPE in 1483 cells.

Differences in levels of COX-2 mRNA could reflect altered rates of transcription in response to CAPE treatment. To investigate this possibility, transient transfections were performed using a series of human COX-2 promoter deletion constructs ligated to luciferase (−1432/59, −327/59, −220/59, and −124/59) and 0.2 µg of pSV-β-gal. After transfection, cells were treated with vehicle (□), TPA (50 ng/ml, ▲), or TPA (50 ng/ml) and CAPE (15 µg/ml). Reporter activities were measured in cellular extract 6 h later. Luciferase activity represents data that have been normalized with β-galactosidase activity. Six wells were used for each of the conditions. Columns, means; bars, SD. *, P < 0.01 compared with TPA treatment. IL6, interleukin 6; CRE, cyclic AMP response element.

Fig. 6. Localization of region of COX-2 promoter that mediates the effects of TPA and CAPE. A, shown is a schematic of the human COX-2 promoter. B, 1483 cells were cotransfected with 1.8 µg of a series of human COX-2 promoter deletion constructs ligated to luciferase (−1432/59, −327/59, −220/59, and −124/59) and 0.2 µg of pSV-β-gal. After transfection, cells were treated with vehicle (□), TPA (50 ng/ml, ▲), or TPA (50 ng/ml) and CAPE (15 µg/ml). Reporter activities were measured in cellular extract 6 h later. Luciferase activity represents data that have been normalized with β-galactosidase activity. Six wells were used for each of the conditions. Columns, means; bars, SD. *, P < 0.01 compared with TPA treatment. IL6, interleukin 6; CRE, cyclic AMP response element.

To determine the capacity of CAPE to inhibit the synthesis of PGs in vivo, we used the carrageenan air pouch model of inflammation. In this model, inflammatory stimuli induce COX-2 and PG synthesis. I.p. administration of CAPE caused dose-dependent inhibition of PGE2 production in the pouch fluid with an ED50 of 23 mg/kg (Fig. 7). Treatment with CAPE also caused similar dose-dependent inhibition of 6-keto-PGF1α synthesis (data not shown). To evaluate whether the inhibitory effects of CAPE on PG synthesis reflected decreased expression of COX-2, amounts of COX-2 were measured in pouch tissue. CAPE at a dose of 100 mg/kg led to a marked decrease in amounts of COX-2 protein. This dose of CAPE also completely blocked the synthesis of PGs. In contrast, the NSAID indomethacin, when given at a dose of 2 mg/kg, almost completely inhibited the synthesis of PGs but had no effect on levels of COX-2 (Fig. 7).

DISCUSSION

CAPE and other esters of caffeic acid have anticancer and anti-inflammatory properties. The present data link these effects of CAPE to inhibition of PG synthesis and possibly other products derived from the oxidation of arachidonic acid, e.g., hydroxyeicosatetraenoic acid. With regard to the synthesis of PGs, we have shown that CAPE inhibits TPA- and carrageenan-mediated induction of COX-2, directly inhibits the activities of COX-1 and COX-2, and inhibits the release of arachidonic acid from membrane phospholipids. The suppressive effects of CAPE occur in vivo. In fact, this is the first report of a compound other than dexamethasone that can be given in vivo to decrease the expression of COX-2.

The chemopreventive properties of CAPE can be understood, in part, from the known relationship between arachidonic acid metabolism and carcinogenesis. Prostaglandins formed by COX impair immune surveillance and killing of malignant cells (18). COXs and their end-products also inhibit apoptosis (43, 44), which could prolong the

Table 1 CAPE inhibits TPA, calcium ionophore, and TPA plus calcium ionophore-stimulated release of [3H]arachidonic acid from 1483 cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CPM ± SD</th>
<th>P by t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>8,006 ± 640</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>TPA</td>
<td>13,087 ± 1,297</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TPA, CAPE (2 µg/ml)</td>
<td>12,839 ± 1,265</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TPA, CAPE (4 µg/ml)</td>
<td>10,870 ± 1,266</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TPA, CAPE (6 µg/ml)</td>
<td>9,061 ± 737</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TPA, CAPE (8 µg/ml)</td>
<td>8,628 ± 423</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Solvent</td>
<td>3,082 ± 1,038</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A23187</td>
<td>10,856 ± 1,678</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A23187, CAPE (2.5 µg/ml)</td>
<td>8,816 ± 2,188</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A23187, CAPE (5.0 µg/ml)</td>
<td>6,624 ± 904</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A23187, CAPE (7.5 µg/ml)</td>
<td>6,776 ± 532</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Solvent</td>
<td>3,233 ± 50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TPA</td>
<td>6,108 ± 125</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A23187</td>
<td>11,285 ± 971</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TPA, A23187</td>
<td>18,378 ± 422</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TPA, A23187, CAPE (7.5 µg/ml)</td>
<td>7,326 ± 671</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* p = 3
a DMSO (0.01%).
≤ 5 µM.
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(10–100 mg/kg; six rats/dose) and indomethacin (2 mg/kg; six rats) were given by i.p. injection 2 h before carrageenan. Carrageenan (1%) was then injected into the air pouch. Three h later, pouch exudates were collected to determine levels of PGE2, by enzyme immunoassay. The effect of CAPE and indomethacin on COX-2 expression in the air pouch lining was determined by immunoprecipitation as described in “Materials and Methods.”

Survival of cells containing damaged DNA. Consequently, compounds like CAPE that inhibit COX should enhance apoptosis (45) and immune surveillance, which tend to destroy initiated cells. Moreover, because PGs are mediators of inflammation and chronic inflammation increases the risk of malignancy (46), the inhibition of PG synthesis by CAPE is likely to contribute to both its anti-inflammatory and chemopreventive activity. Arachidonic acid can also be converted to understanding how CAPE inhibits the metabolism of arachidonic acid via lipoxygenase to other eicosanoids, e.g., hydroxyeicosatetraenoic acid, that modulate inflammation and tumorogenesis (47). Our observation that CAPE blocks TPA- and A23187-mediated release of arachidonic acid from cell membranes contributes, therefore, to understanding how CAPE inhibits the metabolism of arachidonic acid via lipoxygenase (30), as well as COX pathways.

Although NSAIDs inhibit COX and are chemopreventive, it is important to recognize the potential limitations of this single type of treatment for preventing cancer. Cellular transformation enhances the transcription of COX-2 (3–5). Consequently, an overabundance of COX-2 enzyme could be synthesized in transformed cells, which could override the positive therapeutic effects of direct NSAID-dependent inhibition of enzyme activity. Additionally, whereas NSAIDs inhibit the production of PGs, most do not inhibit the peroxidase activity of COX, which can generate proinflammatory lipoxins (48). Hence, up-regulation of COX-2, as a consequence of chronic inflammation or cellular transformation, may increase the risk of carcinogenesis despite administration of NSAIDs. In theory, it should be possible to overcome these potential limitations of NSAID therapy by using compounds such as CAPE that down-regulate the expression of COX-2. Thus, a chemoprevention regimen combining CAPE with a NSAID may be more effective in preventing and/or treating tumors than a NSAID alone. This prediction is supported by a recent study in which the antitumor activity of a selective COX-2 inhibitor was potentiated by an antioxidant that inhibited the expression of COX-2 in vitro (49).

With regard to the mechanism by which CAPE suppresses the induction of COX-2, CAPE inhibits protein kinase C activity (50) and the activation of nuclear transcription factor NF-kB (51). It is unclear, however, whether either of these effects accounts for the suppression of COX-2 after stimulation by TPA in vitro or carrageenan in vivo. Indeed, localization of the inductive effects of TPA to a 124-bp region (~124/59) of the COX-2 promoter (Fig. 6), which does not contain an NF-kB site (52), suggests that the suppressive effect of CAPE is unlikely to result from its blocking the activation of NF-kB in this system. Another interesting question is what structural properties of CAPE account for inhibition of COX-2 transcription and COX enzyme activity, respectively. Structural analogues of CAPE have been synthesized (53). These can be used to determine the relationship between its structure and these different functions for the purpose of developing new and possibly more potent chemopreventive agents.

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