Enhancement of Experimental Colon Carcinogenesis by Dietary 6-Phenylhexyl Isothiocyanate

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ABSTRACT
Naturally occurring and related synthetic isothiocyanates are known to exert chemopreventive effects in several organs in rodent models. The present study was designed to investigate the efficacy of 6-phenylhexyl isothiocyanate (PHITC), a potent chemopreventive agent in the lung tumor model in strain A mice, on azoxymethane-induced colon tumorigenesis. Another aim was to study the modulating effect of PHITC on tumor model in strain A mice, on azoxymethane-induced colon tumorigenesis. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Naturally occurring and related synthetic isothiocyanates are known to exert chemopreventive effects in several organs in rodent models. The present study was designed to investigate the efficacy of 6-phenylhexyl isothiocyanate (PHITC), a potent chemopreventive agent in the lung tumor model in strain A mice, on azoxymethane-induced colon tumorigenesis. Another aim was to study the modulating effect of PHITC on tumor model in strain A mice, on azoxymethane-induced colon tumorigenesis. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

INTRODUCTION
Marked variations in dietary habits among populations of different cultures and life styles have been associated with a risk for different types of cancer, including neoplasms of the large bowel and breast cancers (1–3). Among these diet-related cancers, large bowel cancer is one of the leading causes of cancer deaths in Western countries, including North America (4). Although several epidemiological studies and laboratory animal model assays suggest a relationship between the risk of developing colon cancer and certain dietary factors, there is also increasing evidence that minor dietary constituents and/or their related synthetic analogues modulate the risk of colon cancer development. This evidence is supported also by epidemiological as well as by laboratory animal studies (1–5). An attempt to identify naturally occurring as well as synthetic agents responsible for the modulation of tumorigenesis should lead to new strategies for cancer chemoprevention. It is noteworthy that several agents, including ω-3-fatty acids, calcium salts, inorganic and organic selenium compounds, substituted dithiolethiones, polyamine synthetic inhibitors, and AA2 cascade inhibitors have been shown to possess chemopreventive properties in colon carcinogenesis (6, 7). On the other hand, ω-6-fatty acids, secondary bile acids, and phorbol esters, to cite a few, have been shown to have tumor promoter effects (3, 5).

Wattenberg (6) and Kelloff et al. (7) reviewed the results of many chemopreventive agents that have undergone human clinical trials or have been candidates for such trials. Aryl isothiocyanates, such as BITC and PEITC that occur as their glucosinolate precursors in certain cruciferous vegetables (8), have been shown to inhibit carcinogenesis induced by several nitrosamines and polycyclic aromatic hydrocarbons (9–13). In some studies, administration of PEITC before and during carcinogen treatment strongly inhibited lung tumorigenesis in mice and rats (13–15). Recent animal studies further provided evidence that increasing alkyl chain length of isothiocyanates strengthened the tumor inhibitor properties in carcinogen-induced lung tumorigenesis in strain A mice and that it helped to block DNA methylation (14, 15). Thus far, PHITC (Fig. 1) is the most potent of the arylalkyl isothiocyanates tested for the inhibition of lung tumorigenesis in mice (16). With regard to colon carcinogenesis, little information is available on the effect of arylalkyl isothiocyanates. Recently, Sugie et al. (17) reported that feeding of BITC diet before and during the carcinogen treatment significantly inhibited intestinal carcinogenesis in female ACI/N rats. In contrast, BITC administered during the postinitiation stage of carcinogenesis increased the intestinal tumor multiplicity (17). Further, preliminary studies in our laboratory demonstrated that dietary administration of 100 ppm PHITC before and during the carcinogen treatment (initiation stage) inhibited the AOM-induced colon tumor incidence by about 15% (P > 0.05) and tumor multiplicity by 38% (P < 0.05). It is important to further evaluate these potentially useful agents in properly designed preclinical experimental models to provide better understanding for the mechanisms of modulation of tumorigenesis during the initiation and postinitiation stages of carcinogenesis.

Although there has been a great deal of interest in the specific biochemical processes during tumor promotion and progression, it is not known which of these processes are critical and how they con-

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The abbreviations used are: AA, arachidonic acid; BITC, benzyl isothiocyanate; PEITC, phenethyl isothiocyanate; AOM, azoxymethane; PLA2, phospholipase A2; PI-PLC, phosphatidylinositol-specific phospholipase C; PGs, prostaglandins; COX, cyclooxygenase; LOX, lipoxygenase; PHITC, 6-phenylhexyl isothiocyanate; PAF, t-ω-1-palmitolyl-2-arachidonyl phosphatidylcholine; PIP2, t-3-phosphatidylinositol 4,5-bisphosphate; HETEs, hydroxyeicosatetraenoic acids; MTD, maximum tolerated dose; TX, thromboxane.

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Experimental Procedure

The experiment was designed to determine the effects of 320 ppm (40% MTD) and 640 ppm (80% MTD) of PHITC administered before, during, and after carcinogen treatment (during initiation and post-initiation stages) of AOM-induced colon carcinogenesis. Dose selection of PHITC was based on MTD values determined by Chung et al. As indicated in Fig. 2, beginning at 5 weeks of age, groups of animals were fed their respective control diet and experimental diets containing 320 or 640 ppm of PHITC. At 7 weeks of age, animals in the experimental groups received AOM s.c. once weekly for 2 weeks at a dose of 15 mg/kg body weight. Animals in the control group were given an equal volume of normal saline. The rats were maintained on these dietary regimens until termination of the experiment. Body weights were recorded every 2 weeks for the first 10 weeks and then every 4 weeks. Animals were monitored daily for their general health. The experiment was terminated 52 weeks after the second AOM treatment; all animals were sacrificed by CO2 euthanasia. After laparatomy, the entire stomach and small and large intestines were resected and opened longitudinally, and the contents were flushed with normal saline. Using a dissection microscope, tumors of the colon and small intestine were observed grossly for their location, number, and size. The length, width, and depth of each tumor were measured with calipers. Estimates of tumor volume were made using the formula $V = \frac{L \times W \times D}{6}$ (30).

All other organs, including kidney and liver, were also grossly examined under the dissection microscope. Colon tumors with more than 0.5 cm diameter were cut into halves; one portion of the tumor was used to determine PLA2, PI-PLC, and COX and LOX metabolites. The other half of each colon tumor and the remaining tumors of the colon and small intestine were examined histopathologically. Colon mucosa that was free of tumors was harvested from both AOM-treated and saline-treated animals for biochemical analysis according to our previously described method (30). Colon mucosa and portions of tumors intended for biochemical determinations were quickly frozen in liquid nitrogen and stored at -80°C.

Tumors were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed by routine histological procedures. The hematoxylin and eosin-stained sections were examined according to the classification of Pozharisski (31) with minor modifications. Most of the colon tumors were invasive or noninvasive adenocarcinomas. All of the adenocarcinomas in the small intestine were invasive. The invasive adenocarcinomas were mostly of the signet-ring mucinous type, invading the muscularis mucosa deep into the intestinal wall and beyond. The noninvasive adenocarcinomas grew outward toward the intestinal lumen. They were usually well-differentiated adenocarcinomas.

Biochemical Analysis

For PLA2 and PI-PLC assays, samples of colon mucosa and tumors were homogenized in 1:3 (v/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. The homogenates were centrifuged at 100,000×g at 4°C for 1 h. The resulting supernatant fraction was used to determine cytosolic PLA2 activity, and the pellet fraction was redissolved in 30 mM HEPES-NaOH buffer (pH 7.2) containing 0.2% Triton X-100. This solution was used to determine membrane-bound PI-PLC activity.

PLA2 Activity

Cytosolic PLA2 activity was measured by a method published previously using $[^{14}C]PAPC (40–60 mCi/mmol)$ as a substrate (32). The PLA2 assay activity was carried out in 100 μl of the reaction mixture containing 50 mM sodium HEPES (pH 7.3), 0.8 mM CaCl2, 0.02% Triton X-100, and 20–30 μg cytosolic protein. The reaction was initiated by adding 40 μM PAPC (10 μCi/μmol), the mixture was incubated at 37°C in a shaking water bath for 10 min, and then stopped by the addition of 0.5 ml of a chloroform/methanol mixture (2:1). The lipids were extracted by the method described by Folch et al. (33). After centrifugation, the chloroform phase was recovered and subjected to HPLC as described above.
The area of each [14C]AA metabolite was measured with a Bioscan system precoated plastic TLC plates (Silica G). These plates were developed with a solvent system consisting of chloroform:methanol:acetic acid:water (90:12:2:1, volumes) and exposed in an iodide chamber for 5 min to visualize AA. The TLC plate was developed in a solvent system composed of chloroform:methanol:acetic acid:water (90:12:2:1, volumes) and exposed in an iodide chamber for 5 min to visualize AA. The area of each [14C]AA metabolite was measured with a Bioscan system 200 image-scanning counter (Bioscan, Inc., Washington, DC) equipped with a β-detector. Protein content was determined by the Bio-Rad method. Results are expressed as pmol [14C]AA released/mg protein/min.

**PI-PLC Activity.** Membrane-bound PI-PLC activity was measured by the method of Rao et al. (32) using [3H]PIP2 (1-5 Ci/mmol) as a substrate. The assay for PI-PLC activity of membrane proteins (100-200 µg) was carried out in 250 µl of the reaction mixture containing 30 mM HEPES-NaOH buffer (pH 7.2), 5 mM dithiothreitol, 4 mM CaCl2, 2 mM EGTA, 0.9 mM MgSO4, and 50 µM [3H]PIP2 (50 µCi/mmol). The reaction was initiated by adding the radioactive substrate to the mixture and incubating it at 37°C for 20 min in a shaking water bath. The reaction was terminated by adding first 0.2 ml 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 aqueous layer, containing [3H]inositol 1,4,5-triphosphate, was transferred into a scintillation vial with 10 ml of scintillation cocktail; radioactivity was counted in a Beckman model LD6800 scintillation counter. The activity is expressed as pmol [3H]inositol 1,4,5-triphosphate formed from [3H]PIP2/mg protein/15 min.

**Prostaglandin E2 Content**

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

**COX and LOX Activities**

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

**Statistical Analysis**

Body weights, tumor incidence, tumor multiplicity, tumor volume, and biochemical parameters were assessed for all animals fed the control and PHITC diets. Tumor incidence, which is expressed as the percentage of animals with tumors, was analyzed statistically by chi² test. Tumor multiplicities, expressed as the mean number of tumors/animal, and the mean number of tumors/tumor-bearing animal were 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 AOM and fed the control and experimental diets were comparable throughout the study (P > 0.05; data not shown). In vehicle-treated groups, however, dietary PHITC at 640 ppm decreased the body weight slightly but significantly (P < 0.05) compared to weights of the animals on control diet only. In vehicle-treated animals, 640 ppm PHITC did not produce any gross or histopathological changes in liver, kidney, or lungs. However, this high dose of PHITC induced some bleeding in the cecum and intestine in about 30% of the animals.

Tumor Incidence. The effects of dietary PHITC on AOM-induced colon and small intestinal tumor incidence are summarized in Table 1. There was no evidence of tumors in vehicle-treated animals. AOM-induced adenocarcinomas of the colon in about 81% of animals fed the control diet; 38% of these animals had invasive and 67% had noninvasive adenocarcinomas. Feeding of 320 ppm PHITC had no significant effect on the incidence of invasive, noninvasive, and total (invasive and noninvasive) adenocarcinomas of the colon. Administration of 640 ppm PHITC in the diet increased the incidences of invasive (53%), noninvasive (12%), and total adenocarcinomas (16%) of the colon. The incidence of total intestinal (colon plus small intestine) adenocarcinomas was significantly (P < 0.03) increased in animals fed 640 ppm PHITC in the diet by comparison to the control diet group.

Table 2 summarizes the results on multiplicity of tumors of the colon and small intestine. Interestingly, administration of 320 or 640 ppm PHITC in the diet significantly (P < 0.05 to 0.01) increased the multiplicities of colon adenocarcinomas in terms of tumors/animal and also tumors/tumor-bearing animal in a dose-dependent manner. Compared to the control diet, adding 640 ppm PHITC significantly (P < 0.01) increased the number of total intestinal adenocarcinomas per animal.

Data summarized in Table 3 and Fig. 3 demonstrate that the volume of colon tumors was significantly larger (1.93- to 4.3-fold) in animals receiving dietary doses of either 320 or 640 ppm PHITC. Four animals in the higher-dose group and two animals among those getting 320 ppm PHITC had large prolapsing colon tumors 38 weeks after AOM treatment (Fig. 3a). Also, 30% of the animals in the high-dose PHITC group showed large tumors of the cecum with associated bleeding (Fig. 3c). It is noteworthy that colon tumors of more than 1 cm in diameter were found far more frequently in animals administered 640 ppm PHITC than in those fed the control diet.

Biochemical Studies. The activities of PLA₂ and PI-PLC and PG₂ levels in colonic mucosa and tumors are summarized in Table 4. Carcinogen administration significantly elevated the activities of PLA₂ and PI-PLC in the colonic mucosa in both control and PHITC groups. Also, there was a 3- to 4-fold increase in the activities of PLA₂ and PI-PLC in colon tumors compared to the activities in the surrounding colonic mucosa. Interestingly, animals fed PHITC showed significantly (P < 0.05 to 0.0001) increased PLA₂ activity in the colonic mucosa and in the tumors. However, administration of PHITC had no significant effect on PI-PLC activity in either mucosa or tumors of the colon. AOM treatment also caused an increase in PG₂ levels in the mucosa of the animals as compared to their vehicle-treated counterparts. However, PG₂ levels were markedly higher in colon tumors than in mucosa. Administration of PHITC significantly elevated the PG₂ in the colonic tumors and mucosa of animals treated with AOM and in the colonic mucosa of vehicle-treated animals as compared to their respective control groups.

The effect of PHITC on LOX and COX activities in AOM-induced colonic tumors is summarized in Table 5. Markedly increased levels (2- to 5-fold) of PG₂ and TXB₂ were found in colon tumors compared to the surrounding mucosa on the control diet. Administration of 640 ppm PHITC in the diet significantly increased the formation of PG₂ (50–100%) and TXB₂ (50%) in the colonic mucosa and in tumors. In control animals, the levels of 5(S), 12(S), and 15(S)-HETEs formed were significantly (P < 0.001) higher in colon tumors than in the mucosa. PHITC in the diet significantly enhanced the formation of 12(S)- and 15(S)-HETEs in colonic mucosa and that of 8(S), 12(S)- and 15(S)-HETEs in tumors; however, 5(S)-HETE formation was not significantly (P > 0.05) affected by PHITC.

DISCUSSION

The major purpose of this study was to elucidate the role of arylalkyl isothiocyanates in colon carcinogenesis. The known inhib-
itor activities of the arylalkyl isothiocyanate PHITC in certain experimental tumorigenesis models provided a rationale for investigating the effect of this agent in a well-established colon cancer model. Arylalkyl isothiocyanates such as BITC, PEITC, and PHITC administered either before or during carcinogen treatment have proved to inhibit carcinogenesis in the lung (10, 13, 15), esophagus (12), liver (35), forestomach (9, 10), and mammary gland (9, 36). This suggests that these agents possess antiinitiating activity in various organs. The results of the present study are of great interest because long-term feeding of PHITC at levels of both 320 and 640 ppm (approximately 40 and 80% MTD) during the initiation and postinitiation phases of carcinogenesis enhanced colon tumorigenesis. These findings are in agreement with those by Sugie et al. (17), who showed that administration of BITC during the postinitiation stage increased the methyloxazoxymethanol acetate-induced colon tumor formation in female ACI/N rats. Importantly, results with PEITC in a recent short-term bioassay in our laboratory indicate that this agent significantly \((P < 0.001)\) increases the colonic lymphatic nodules associated with aberrant crypt foci, which are preneoplastic lesions in the colon.5 Further, long-term dietary administration of allyl isothiocyanate elicited hyperplasia and papillomas of the urinary bladder, and also leukemia, as well as histopathological changes in the liver of male and female rats (37). Also, PHITC enhanced the benzo[a]pyrene-induced skin tumor multiplicity in A/J mice (38), and a similar effect was observed with PEITC in DMBA-induced rat mammary tumorigenesis (39). The colon tumor-promoting activity of PHITC observed in this study is contrary to the reports of anticarcinogenic effects of PHITC and related arylalkyl isothiocyanates against the chemically induced cancers of the lung (14–16) and esophagus (12). These divergent findings may be due to differences in the mode of administration of the arylalkyl isothiocyanates. In most of the earlier studies, these compounds were applied during the initiation stage, whereas we gave them during the postinitiation stage. Additional studies are needed to understand the mode of action of PHITC and related arylalkyl isothiocyanates in the colon.
dietary PHITC throughout the study, i.e., during initiation and post-initiation phases. The results of our preliminary study and those of Sugie et al. (17) support that administration of arylalkyl isothiocyanates before and during carcinogen treatment inhibits colon carcinogenesis. It is, therefore, important that potential chemopreventive dietary PHITC throughout the study, i.e., during initiation and post-initiation stages of carcinogenesis. There is evidence that the type III cytochrome P450s are involved in the metabolism of steroids, corticosteroids, vitamin D, and bile acids, HETEs, PCs, TXs, and leukotrienes (47, 48). One reason for tumor inhibition by PHITC in lung and esophageal carcinogenesis when the agent is administered during the initiation phase may lie in the ability of the agent to modulate carcinogen metabolism (13-15, 42-44). The precise mechanism by which PHITC inhibits colon tumorigenesis when administered during the initiation phase is not entirely known; but it is well known that isothiocyanates, such as allyl isothiocyanate, BITC, and PElTIC, are major constituents of regularly consumed mustard, watercress, and cruciferous vegetables (8, 40, 41).

One reason for tumor inhibition by PHITC in lung and esophageal carcinogenesis when the agent is administered during the initiation phase may lie in the ability of the agent to modulate carcinogen metabolism (13-15, 42-44). The precise mechanism by which PHITC inhibits colon tumorigenesis when administered during the initiation phase is not entirely known; but it is well known that isothiocyanates are potent modulators of cytochrome P450 enzymes, which play a key role in various carcinogen activation pathways (37, 45), including the metabolism of AOM (46). It is, therefore, reasonable to assume that these agents are predominantly initiation blockers, as shown in previous studies (9-16). Our observation that dietary PHITC at the 100-ppm level inhibits colon carcinogenesis when administered during the initiation stage further supports its modulating effect on the carcinogen metabolism. However, additional studies are needed to examine whether the higher doses evaluated in the current study would also inhibit colon tumorigenesis when administered only during the initiation phase.

The exact mechanism(s) by which long-term chronic administration of PHITC enhances colon carcinogenesis are not clearly understood. However, the involvement of cytochrome P450 isoforms should be considered because several of them play a pivotal role in the metabolism of a wide array of xenobiotics and have been shown to be involved in the metabolism of steroids, corticosteroids, vitamin D, and biosynthesis of fatty acids, bile acids, HETEs, PGs, TXs, and leukotrienes (47, 48). It has also been established that several of the above-mentioned metabolites are generated by the actions of cytochrome P450s significantly influence tumorigenesis (20-25, 48). There is evidence that the type III cytochrome P450s are involved in the catabolism of eicosanoid metabolites (20-22, 48). This is supported by our finding that the administration of PHITC significantly increased the induction of PGE2 and COX and LOX activities in colonic mucosa and in tumors, suggesting that PHITC enhances AA metabolism. The role of COX metabolites, particularly PGE2, in colon tumor promotion has been well established (21, 48, 49). Previous studies by us and others have shown that PG synthesis inhibitors such as piroxicam, indomethacin, aspirin, and sulindac decrease colon tumorigenesis in rodents (49-52). LOX metabolites such as 12(S)-HETE promote tumor cell adhesion, stimulate the spreading of tumor cells, and increase the metastatic potential of tumor cells (53, 54). Furthermore, a positive correlation was observed between the levels of 8(S)-HETE and degree of inflammation, hyperproliferation, clastogenicity, and tumor promotion by TPA (55). Also, the activities of 5(S)- and 15(S)-HETEs, which are potent modulators of inflammation, were suppressed by lipooxygenase inhibitors, thus indicating that HETEs mediate tumor promotion (56). The enhancement of colon adenocarcinomas by PHITC in the present study was thus consistent with the increase in most of the COX and LOX metabolites in the colonic mucosa and tumors, suggesting that the colon cancer-promoting activity of PHITC is likely mediated through the increase in COX and LOX metabolite production. The promoting effect of PHITC on colon tumorigenesis in the current study could conceivably also be due to low-grade toxicity induced by chronic feeding of high dietary levels of PHITC. It needs to be emphasized that the 40 and 80% MTD levels of PHITC used in this study were based on subchronic toxicity assays.

We analyzed PLA2 and PI-PLC activities which are dominant pathways for the AA release in both the colonic mucosa and tumor tissues. One of the pathways leading to generation of AA involves a direct action of PLA2 on a phospholipid that could include diacyl- or arachidonic acid. Arachidonic acid promotes tumor cell adhesion, stimulates the spreading of tumor cells, and increases the metastatic potential of tumor cells (53, 54). The second pathway, mediated by PI-PLC, involves the degradation of phosphatidylinositol-4,5-biphosphate via a sequence of reactions beginning with PI-PLC, followed by diglyceride and monoglyceride lipases (24). Furthermore, PI-PLC activity is responsible for diacylglycerol formation, PKC-dependent signal transduction, and cell proliferation (25, 57). We demonstrated that dietary PHITC significantly enhanced the PLA2 activity in the colonic mucosa and in tumors, but that it had little or no significant effect on the PI-PLC activity. The exact mechanism by which PHITC increases the PLA2 activity is not clear. It is possible that PHITC may exert its enhancing effect by directly acting on PLA2 or, alternatively, by acting on the regulators of PLA2, resulting in increased levels of AA and its metabolites. Further, in recent studies we observed that animals fed PHITC and treated with AOM had an increased expression.

### Table 4 Effect of PHITC on colonic mucosal and tumor PLA2, PI-PLC, and PGE2 levels in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Saline-treated</th>
<th>AOM-treated</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosolic PLA2 activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Control diet 4.8 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.7 ± 1.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71 ± 5.6</td>
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<tr>
<td>640 ppm PHITC 25.6 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.6 ± 1.9&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>189 ± 11.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>PI-PLC activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Control diet 22.5 ± 2.5</td>
<td>32.5 ± 2.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>128 ± 6.3</td>
</tr>
<tr>
<td>640 ppm PHITC 18.2 ± 1.6</td>
<td>28.7 ± 1.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>112 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>PGE2 levels&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Control diet 0.24 ± 0.04</td>
<td>0.63 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.74 ± 0.52</td>
</tr>
<tr>
<td>640 ppm PHITC 0.49 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.13 ± 0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.12 ± 0.90</td>
<td></td>
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</table>

<sup>a</sup> PLA2 activity is expressed as pmol of [14C]arachidonic acid released/mg protein/min at 37°C, and PI-PLC activity is expressed as pmol [3H]inositol 1,4,5-triphosphate formed from [3H]inositol 1,4,5-triphosphate/mg protein/15 min at 37°C. PGE2 levels were expressed as ng of PGE2/mg protein.

<sup>b</sup> Mean ± SEM (n = 6).

<sup>c</sup> Significantly different from their respective control groups by Student’s t test; P < 0.05.

<sup>d</sup> Significantly different from their saline-treated groups by Student’s t test; P < 0.05.

### Table 5 Effect of PHITC on AOM-induced colonic mucosal and tumor COX and LOX metabolism in F344 rats

<table>
<thead>
<tr>
<th>AA metabolism</th>
<th>Control diet</th>
<th>640 ppm PHITC</th>
<th>Control diet</th>
<th>640 ppm PHITC</th>
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<tr>
<td>COX activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Control diet 329 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>685 ± 31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1577 ± 238</td>
<td>3463 ± 103</td>
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<tr>
<td>640 ppm PHITC 355 ± 16</td>
<td>496 ± 37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>938 ± 29</td>
<td>1784 ± 54&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>PGI2 &lt;sup&gt;c&lt;/sup&gt; 243 ± 13</td>
<td>387 ± 29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>550 ± 20</td>
<td>973 ± 43&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>PGD2 378 ± 14</td>
<td>421 ± 18</td>
<td>1193 ± 46</td>
<td>1942 ± 84&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>6-Keto PGF1α &lt;sup&gt;d&lt;/sup&gt; 260 ± 12</td>
<td>342 ± 21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>973 ± 33</td>
<td>1579 ± 72&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>TXB2 &lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>LOX activity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Control diet 211 ± 12</td>
<td>278 ± 17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>313 ± 13</td>
<td>412 ± 18&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>640 ppm PHITC 312 ± 21</td>
<td>359 ± 26</td>
<td>348 ± 16</td>
<td>508 ± 32&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>12(S)-HETE 266 ± 14</td>
<td>524 ± 37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>585 ± 28</td>
<td>1647 ± 73&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>15(S)-HETE 308 ± 22</td>
<td>493 ± 32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>428 ± 31</td>
<td>1836 ± 64&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

<sup>a</sup> Pmol of PG and TXB2 formed from [14C]arachidonic acid/mg protein/15 min at 37°C.

<sup>b</sup> Mean ± SEM (n = 6).

<sup>c</sup> Values in horizontal columns are significantly different from their respective control diet group by Student’s t test; P < 0.05-0.0001.

<sup>d</sup> Pmol of HETEs produced from [14C]arachidonic acid/mg protein/15 min at 37°C.

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of ras p21 protein as well as K-ras mutations in the colonic mucosa and in tumors compared to those in animals fed control diet and treated with AOM (58). On the basis of these results, it is reasonable to state that PHITC modifies not only PL2A to alter endogenous AA availability but also COX and LOX activities.

In conclusion, the present study documents that dietary PHITC, administered to male F344 rats during initiation and postinitiation stages, significantly increases AOM-induced colon tumorigenesis in a dose-dependent manner. Also, long-term feeding of PHITC significantly increases PL2A, COX, and LOX activities in colonic mucosa and in tumors. These changes are relevant to colon carcinogenesis. Although the exact mechanism(s) of colon tumor promotion by feeding of PHITC remains to be elucidated, it would appear that modulation of AA metabolism by PHITC may play a direct or indirect role. The results of current and earlier investigations in animal models emphasize that the studies for preclinical evaluation of potential chemopreventive compounds must be designed to include long-term administration during initiation and postinitiation stages if they are to contribute to realistic and meaningful testing for the potential use in humans. It would be irresponsible to rely solely on results obtained with tests of the potential chemopreventive agents only during the initiation phase of carcinogenesis. These critical aspects should govern further development of isothiocyanates or any other novel compounds as potential chemopreventive agents.

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