Effects of Indole-3-Carbinol and phenethyl isothiocyanate on colon carcinogenesis induced by azoxymethane in rats

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Indole-3-carbinol (I3C) and phenethyl isothiocyanate (PEITC) are breakdown products of the glucosinolates glucobrassicin and gluconasturtiin, respectively, and are thought to reduce carcinogen activation by P450 enzymes. To assess the effects of these compounds on colon cancer risk, rats were divided into five groups and fed the following diets: control diet (AIN-93G), or diets with PEITC or I3C added to the control diet: high-PEITC (3.37 mmols/kg diet—high level of PEITC), low-PEITC (0.67 mmols/kg—low level of PEITC), high-I3C (6.8 mmols/kg—high level of I3C) and low-I3C (1.36 mmols/kg—low level of I3C). Diets were fed for 2 weeks before and 10 weeks after administration of the colon carcinogen azoxymethane. Precancerous lesion (aberrant crypt foci, ACF) number in the distal colon was significantly lower in both high-I3C and low-I3C groups (6.9 ± 0.8 and 5.9 ± 0.59 per cm², respectively) when compared with the control group (10.4 ± 0.9). No significant difference in ACF number was found between the PEITC group and the control group. ACF expressing sialomucin, thought to indicate ACF more likely to progress to tumors, were greater in the high-I3C group (13.2 ± 0.59 per cm²) than in the control (5.6 ± 2). Mucin-depleted ACF, suggested to have the greatest tumorigenic potential, tended to be lower in the low-I3C group (P < 0.06) compared with the control group. Mucosal apoptotic and cell proliferation labeling indices did not differ among groups, suggesting that reduction in the ACF number by I3C does not involve alterations in mucosal cell kinetics. No significant differences were found among the groups in hepatic cytochrome P450 2E1 (CYP2E1) activity, the first enzyme involved in activation of azoxymethane. However, there was increased activity of NADPH- and NADH reductases with high-I3C, which are the enzymes involved in the transfer of reducing equivalents to cytochrome P450. These results suggest that I3C lowers colon cancer risk through a mechanism not involving reduction of carcinogen activation by CYP2E1.

Introduction

It is well established that diet has a major influence on the development of colon cancer (1). Although several foods and dietary constituents have been associated with decreased risk of colon cancer, epidemiological evidence of protection is perhaps strongest regarding consumption of vegetables. Several types of plant constituents, or phytochemicals, have been associated with the protective properties of vegetables against colon cancer, particularly those found in cruciferous vegetables known as glucosinolates (2). Glucosinolates consist of a β-D-thiogluco moiety, a sulfate attached through a C=N bond, and a variable side chain. Upon rupture of the plant tissue, the enzyme myrosinase hydrolyzes glucosinolates at the β-glucosyl moiety yielding breakdown products (3). These have been found to act as blocking and/or suppressing agents against several experimental models of cancers, such as lung cancer (4), mammary tumors (5), liver cancer (6) and colon cancer (7). Although there are many glucosinolate breakdown products, only a few of these compounds have been extensively studied for their chemoprotective activity. Phenethyl isothiocyanate (PEITC) is a breakdown product of gluconasturtiin, a glucosinolate present in high concentration in watercress. It has been found to be a potent chemoprotective agent against lung cancer (4) but its effects on colon cancer risk are less clear. Previous studies have shown both either a reduction or no changes in aberrant crypt foci (ACF) after administration of PEITC (8,9). Indole-3-carbinol (I3C) is one of the breakdown products of the glucosinolate glucobrassicin, found in high levels in cabbages. Its effects on colon cancer risk are uncertain, as it has been shown to function both as an inhibitor and a promoter of colon carcinogenesis (10). Rats treated with the carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline showed decreased ACF when I3C was fed at 7.5 mmols/kg diet (11). In contrast, when I3C was fed to rats before, during and after dimethylhydrazine administration, an increase in colon tumor number was found (12). In addition, feeding rats I3C at 0.1, 0.01 or 0.001% in the diet after initiation with dimethylhydrazine had no effect on incidence or multiplicity of colon tumors (13,14). These findings suggest that the type of colon carcinogen as well as the stage of carcinogenesis may play an important role in the effectiveness of I3C as a chemoprotective agent.

ACF have been widely used as early markers of colon cancer risk in animal studies. ACF exhibit distinct preneoplastic features such as dysplasia, abnormal proliferation (15) and K-ras mutations (16). However, relatively few ACF present in the colon must develop into tumors, as at a given carcinogen dose, the number of tumors is always small relative to the number of ACF found. Furthermore, several studies have reported a lack of correlation between ACF number and eventual tumor formation (17,18). This has prompted interest in identifying subgroups of ACF that may be more predictive of eventual tumorigenesis. Two of these potential new markers are based
on mucin production by the colonic mucosal cells: sialomucin-producing foci (SPF), and mucin-depleted foci (MDF). It has been shown that ACF producing sialomucins have a higher rate of cell proliferation, higher degree of dysplasia and increased distortion of the luminal opening than ACF producing sulfomucins (19,20). Based on this evidence, sialomucin-producing ACF have been proposed to be a better predictor of colon cancer risk than the measurement of total ACF. Recently, MDF, which are characterized by the absence or scant production of mucin, were discovered in the colons of rats treated with azoxymethane (AOM). These lesions were found to be histologically more dysplastic than mucin-producing ACF, and to occur in the same order of magnitude as tumors, leading to the suggestion that these foci are the direct precursors of tumors (21).

The aim of this study was to determine the effects of dietary PEITC or I3C on colon cancer risk in rats, and to provide a possible mechanism by which these compounds may influence colon cancer risk. To assess the risk of colon cancer, ACF were used as the primary marker, but SPF and MDF were also quantified as additional measures of risk.

Materials and methods

Chemicals
AOM was purchased from Ash Stevens (Detroit, MI), I3C, PEITC and p-nitrophenol were purchased from Sigma-Aldrich (St Louis, MO).

Animals and diets
Male Wistar rats, 3–4 wk of age, were purchased from Harlan Sprague–Dawley (Indianapolis, IN). Animals were housed individually in wire-bottomed cages in rooms maintained at 20 ± 2°C with a relative humidity of 50 ± 10%, and a 12 h light–dark cycle. This study was approved by the University of Minnesota Committee on Animal Care and Use. Food and water were available ad libitum throughout the study. The rats were divided in five groups ([n = 15] upon arrival, and each group received a different experimental diet: control diet (AIN-93G) (22), low-I3C diet (AIN-93G + 1.36 mmoles I3C/kg diet or 36.7 μmol/day) high-I3C diet (AIN-93G + 6.8 mmoles I3C/kg diet or 183.6 μmol/day), low-PEITC diet (AIN-93G + 0.67 mmoles PEITC/kg diet or 18 μmol/day) and high-PEITC diet (AIN-93G + 3.37 mmoles PEITC/kg diet or 91 μmol/day). The diets were freshly prepared every 2 weeks, and stored at 4°C.

Experimental design

The rats were fed the experimental diets for two weeks prior to the first carcinogen treatment. AOM was administered subcutaneously, once a week for two consecutive weeks at a dose of 15 mg/kg body wt. After the second AOM injection, the animals were fed the experimental diets for an additional 8 weeks. Body weights and food intake were recorded periodically throughout the study.

Determination of ACF and mucin production

Animals were anesthetized with ethyl ether and colons removed and flushed with PBS. Aberrant crypts (ACs) and ACF were enumerated by a modification of the method of Bird (14). Briefly, animals were anesthetized with ethyl ether and colons removed and flushed with PBS, then slid onto a 2 ml glass pipette, and submerged in 10% formalin–PBS (phosphate buffered saline) for 5 min. The colons were cut open and fixed flat between filter paper submerged in 10% formalin–PBS. A 2.5 cm² section of the colon, ~2 cm from the anal end, was stained with 0.2% methylene blue chloride–PBS and enumerated under a microscope at 100× magnification. After ACF determination, colons were kept in 10% formalin solution, and later processed in high-iron diamine Alcian blue staining (HID-AB) for visualization of mucin production (19). Scoring of colons was carried out at 40× according to criteria described by Caderni et al. (21) Briefly, to be considered a MDF, a focus had to show absence or very little production of mucin in addition to fulfilling two of the following criteria: (i) distortion of the opening of the lumen compared to normal surrounding crypts; (ii) elevation of the lesion above the surface of the colon; and (iii) multiplicity > 3.

Immunohistochemistry

A 0.8 cm section of the distal end of the colon was taken prior to fixing. This section was cut in half and embedded in paraffin, and processed for apoptosis and cell proliferation assays. The apoptosis assay was performed using a commercial kit (ApoTag Peroxidase In Situ Oligo Ligation Kit, Serologicals, NY, 10577). This kit is based upon the biochemical specificity of the enzyme T4 DNA ligase, which joins the fragmented DNA in the sample with the biotinylated oligonucleotide supplied. Detection is achieved by binding of a peroxidase conjugate of streptavidin, and consequent deposition from solution of diaminobenzidine that can be visualized by microscopy. Mucosal cell proliferation was quantitated by immunohistologic detection of proliferating cell nuclear antigen (PCNA) using a PCNA-specific mouse antibody (Oncogene Research Products, Boston, MA). The antigen–antibody complex was labeled with diaminobenzidine, which produces a golden-brown color in proliferating cells when visualized by microscopy. Both the apoptotic labeling index (ALI), and the cell proliferation labeling index (CPLI) were calculated as the ratio of the number of labeled cells to the total number of cells present in one side of a colonic crypt, multiplied by 100. A total of 25 crypts per animal were counted.

Preparation of microsomes

Microsomal isolation was performed using the method described by Prasad et al. (23) Livers were perfused in situ with ice-cold 50 mM Tris–150 mM KCl buffer (pH 7.5) and homogenized in Tris–KCl buffer containing phenylmethyl-sulphonyl fluoride (1 mM final concentration). The microsomal fraction was obtained by ultracentrifugation of the 10 000 g supernatant at 105 000 g for 1 h. The microsomal pellet was resuspended in Tris–glycerol buffer (pH 7.5) plus 0.01% butylated hydroxytoluene (BHT), and stored at −18°C. Prior to analyses, microsomes were washed by ultracentrifugation at 105 000 g with 0.1 mM sodium pyrophosphate/1 mM EDTA buffer.

Enzyme assays

Activity of microsomal cytochrome P450 2E1 (CYP2E1) was determined by a kinetic assay of p-nitrophenol hydroxylase activity (24), as modified by Reinke and Moyer (25). Briefly, 0.1 mM p-nitrophenol in phosphate buffer (pH 6.8), and the microsomal fraction (0.2 mg protein/ml) were incubated for 5 min at 37°C. The reaction was started by adding NADPH to a final concentration of 0.1 mM. The absorbance of p-nitrophenol was measured at 480 nm for 10 min. Enzyme activity was calculated using extinction coefficients of 3.57 mM⁻¹cm⁻¹ for p-nitrophenate. Measurements of NADH-ferricyanide reductase and NADPH-cytochrome c reductase were as described previously (26). Enzyme activities were calculated using extinction coefficients of 21.1 mM⁻¹cm⁻¹ for cytochrome c and 1.02 mM⁻¹cm⁻¹ for ferricyanide.

Statistical analyses

The data were analyzed by one-way analysis of variance using the SAS System for Windows, release 8.2 (SAS Institute, Cary, NC). Comparisons among groups were made using the Tukey–Krammer test. All p-values reported were adjusted for multiple comparisons.

Results

Food consumption and body weights were similar in all diet groups throughout the study (data not shown). At the end of the study, average body weights and daily food intake were 496 ± 11 and 27 ± 5 g, respectively.

Table I. Effect of I3C and PEITC on AC and ACF of rat colon

<table>
<thead>
<tr>
<th>Group</th>
<th>AC/cm² of colon</th>
<th>ACF/cm² of colon</th>
<th>Large ACF/cm² of colon</th>
<th>AC/large ACF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.6 ± 1.9a</td>
<td>10.4 ± 0.9a</td>
<td>1.2 ± 0.2</td>
<td>5.3 ± 0.8</td>
</tr>
<tr>
<td>High-PEITC</td>
<td>19.9 ± 2.5a</td>
<td>9.3 ± 1.1</td>
<td>1.0 ± 0.2</td>
<td>5.0 ± 1.0</td>
</tr>
<tr>
<td>Low-PEITC</td>
<td>20.5 ± 1.6b</td>
<td>9.5 ± 0.7b</td>
<td>1.1 ± 0.2</td>
<td>4.7 ± 0.8</td>
</tr>
<tr>
<td>Low-I3C</td>
<td>16.6 ± 0.9ab</td>
<td>6.9 ± 0.8a</td>
<td>1.3 ± 0.1</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>Low-I3C</td>
<td>13.7 ± 1.3b</td>
<td>5.9 ± 0.6b</td>
<td>0.9 ± 0.1</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>Control</td>
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<td>5.0 ± 1.0</td>
</tr>
<tr>
<td>Low-PEITC</td>
<td>20.5 ± 1.6b</td>
<td>9.5 ± 0.7b</td>
<td>1.1 ± 0.2</td>
<td>4.7 ± 0.8</td>
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</tr>
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<td>Low-I3C</td>
<td>13.7 ± 1.3b</td>
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<td>4.3 ± 0.7</td>
</tr>
</tbody>
</table>

aValues are mean ± SEM, n = 15 per group. Means within the same column having no common superscripts are significantly different (P < 0.05).
Aberrant crypt foci
Rats fed the diets containing I3C had significantly fewer ACF in the distal colon compared to the control and PEITC-fed groups (P < 0.01) (Table I). The total number of AC was also significantly lower in the low-I3C group compared to the control and the PEITC groups (P = 0.013). Although there was a trend toward fewer AC in the high-I3C group compared to the control group, this difference did not reach statistical significance. There were no significant differences among groups in large ACF (ACF with >3 AC) or AC multiplicity (AC/ACF) within large ACF.

Mucin staining of ACF
Figure 1 shows the effect of the diets on the total number of SPF, which includes both foci producing a combination of sulfomucins and foci producing siaiomucins only (black bars). There were no significant differences among the diet groups in SPF number. Figure 1 also depicts the effect of diet on the total number of foci producing siaiomucins only (SOF) (white bars). There was a significantly greater number of SOF in the high-PEITC group compared to the control group (P < 0.05). However, there were no significant differences among any of the other groups.

There was a modest positive but statistically significant correlation between total ACF and total SPF (R² = 0.29, P < 0.0001).

Table II. Effect of I3C and PEITC on the ALI and CPLI of rat colon³

<table>
<thead>
<tr>
<th>Group</th>
<th>ALI (%)</th>
<th>CPLI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.65 ± 0.17</td>
<td>6.94 ± 1.95</td>
</tr>
<tr>
<td>High-PEITC</td>
<td>0.45 ± 0.08</td>
<td>6.56 ± 1.70</td>
</tr>
<tr>
<td>Low-PEITC</td>
<td>0.70 ± 0.15</td>
<td>8.24 ± 1.68</td>
</tr>
<tr>
<td>High-I3C</td>
<td>0.45 ± 0.08</td>
<td>6.83 ± 1.52</td>
</tr>
<tr>
<td>Low-I3C</td>
<td>0.75 ± 0.25</td>
<td>8.58 ± 2.11</td>
</tr>
</tbody>
</table>

³Values are mean ± SEM, n = 15 per group.

Discussion
Previous studies on the chemoprotective effects of I3C against colon cancer have shown inconsistent results (12,13,27,28). These inconsistencies may be explained, in part, by the different experimental designs employed, especially with regard to dosage of I3C and stage of carcinogenesis during which I3C was administered. Since the stage at which I3C may be protective is not known, in this study rats were fed I3C before, during and after azoxymethane treatment in order to potentially affect every stage. Two dietary concentrations of I3C were used. The high dose approximated the dose used by several other investigators, whereas the low doses were intended to represent a dietary concentration potentially attainable in the human diet.

In the present study, rats fed I3C developed significantly fewer ACF in the distal colon compared to the control group. Colonic ACF are recognized as early preneoplastic lesions (29,30), and since their discovery have been widely used as early markers of colon cancer risk. Recently, additional markers of colon cancer risk have been suggested, based on their
correlation with tumor formation and/or histological changes reflecting DNA mutation. These markers include SPF (19,30), MDF (21,31), β-catenin accumulated crypts (32,33) and flat dysplastic ACF (34), all of which are indicative of dysplastic ACF. Several recent studies strongly suggest that dysplastic ACF, which represent only a small fraction of total ACF, are the direct precursors to adenomas and carcinomas (34,35). This has led to the thought that the majority of ACF, which are hyperplastic, do not progress to adenomas and that therefore their number are not indicative of tumorigenic potential. Consequently, in the present study, SPF and MDF were assessed as estimates of the number of dysplastic ACF. In contrast to the reduction in total ACF seen with feeding of I3C, no changes in SPF were detected. Interestingly, there was a modest but statistically significant correlation between ACF and SPF (data not shown), suggesting the possibility that the number of dysplastic ACF are somewhat proportional to total ACF. We also found a strong trend towards a lower number of MDF with feeding of a low dose of I3C (P < 0.06). As MDF have been shown to exhibit a greater degree of dysplasia than SPF (21), MDF may be the most predictive of eventual tumor formation of the types of ACF measured here. Although the number of ACF and MDF did not correlate in this study, probably in part due to the low number of MDF present in any one animal, both were decreased after feeding of I3C, suggesting a protective effect of this compound, especially at the low dose (1.36 mmols/kg diet). It also reinforces the possibility that the numbers of dysplastic ACF are proportional to the total number of ACF.

Two previous studies have examined the effects of PEITC on colon cancer risk in rats. In one, in which PEITC was fed before AOM treatment at a dose of 1.2 or 2.4 mmols/kg diet, no differences were found in total ACF per colon, compared with rats fed the control diet (9). In contrast, another study reported a significant decrease in AC number in rats given either PEITC or sulforaphane, another isothiocyanate, three times a week for 8 weeks, by gavage, at doses varying between 5 and 20 μmols per day, before or after administration of the carcinogen (8). In the present study, there were no changes in total ACF number but a slight tendency toward fewer MDF after feeding PEITC at ~18 or 91 μmols/day. There was, however, a significantly greater number of SOF in the group fed the high dose of PEITC compared with the control group. It is possible that at this dose PEITC was cytotoxic and possibly carcinogenic. PEITC has a propensity to react with sulf-hydryl groups due to the electrophilic character of the carbon atom of the isothiocyanate and could, therefore, deplete intracellular glutathione. Further, at approximately the same dose as the high-PEITC diet used in the present study, PEITC was shown to acutely inhibit liver aldehyde dehydrogenase, an enzyme containing cysteine at the active site, and thus may inhibit aldehyde metabolism (36). Acetaldehyde, a normal constituent of the colonic contents (37), is mutagenic and carcinogenic in animal studies (38) and caused hyperproliferation in Caco-2 cells (39). However, the significance of a greater number of SOF in rats fed the high-PEITC diet is unclear, given the lack of difference in total ACF and MDF from the control group.

An increased rate of apoptosis in colonic cells has been associated with a decreased risk of colon cancer in rats (40,41). At the doses fed, PEITC and I3C did not influence apoptosis in the colonic mucosa relative to the control diet. To our knowledge, this is the first study examining apoptosis in rat colon after feeding of PEITC or I3C. In vitro assays using human colorectal adenocarcinoma cell lines suggest a possible increase in apoptosis after treatment with PEITC or I3C (42). One recent study in rats showed an increase in apoptosis in the colon after feeding them raw Brussels sprouts, which contain the precursor of I3C (43). However, several different glucosinolates are present in Brussels sprouts, and the effect on apoptosis could be attributed to a combination of compounds. It should be noted that different techniques were used in these in vitro and animal studies to measure apoptosis in the colonic crypts, which vary from morphological to very specific immunohistochemical assays. In the present study the method used is specific enough to prevent labeling of necrotic cells, a problem with other methods that reduces the reliability of the results.

No significant effect on cell proliferation in colonic crypts was found after feeding PEITC or I3C. These results suggest that these compounds do not alter cell proliferation in the colonic mucosa.

Induction of colonic tumors by AOM depends on its metabolic activation to form an unstable compound, methylyazoxy-methanol, which decomposes into formaldehyde and a diazonium ion capable of forming DNA adducts (44,45). CYP2E1 is thought to be the main mixed-function oxidase to activate AOM in rat liver (44). In this study, we examined whether I3C and PEITC might provide chemoprotection by decreasing carcinogen activation. Overall, there was a low but statistically significant correlation between hepatic CYP2E1 activity and both the number of large ACF (ACF with ≥ 4 AC) and total number of AC in large ACF, but not with the other ACF parameters. As several studies suggest that large ACF may be precursors of colon tumors (46,47), this supports the evidence, obtained using CYP2E1-null mice, that changes in CYP2E1 activity may influence tumorigenesis in animals treated with AOM (48).

No significant changes in hepatic CYP2E1 activity were found amongst the groups, suggesting that the mechanism by which I3C lowers colon cancer does not involve reduction of carcinogen activation. It is possible that I3C exerts its

<table>
<thead>
<tr>
<th>Group</th>
<th>NADPH-β-nitrophenol hydroxylase (nmols/min/mg P)</th>
<th>NADPH-cytochrome c reductase (nmols/min/mg P)</th>
<th>NADPH-ferricyanide cytochrome b5 reductase (nmols/min/mg P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.80 ± 1.96</td>
<td>1.24 ± 0.08</td>
<td>69.91 ± 2.90ab</td>
</tr>
<tr>
<td>High-PEITC</td>
<td>16.85 ± 1.45</td>
<td>1.34 ± 0.07</td>
<td>62.11 ± 2.46</td>
</tr>
<tr>
<td>Low-PEITC</td>
<td>19.33 ± 1.35</td>
<td>1.15 ± 0.10</td>
<td>65.03 ± 3.86ab</td>
</tr>
<tr>
<td>High-I3C</td>
<td>23.10 ± 2.97</td>
<td>2.01 ± 0.11</td>
<td>80.70 ± 6.05*</td>
</tr>
<tr>
<td>Low-I3C</td>
<td>18.14 ± 2.33</td>
<td>1.45 ± 0.09</td>
<td>66.57 ± 3.29ab</td>
</tr>
</tbody>
</table>

Low-PEITC, 0.67 mmols/kg diet; High-PEITC, 3.37 mmols/kg diet; Low-I3C, 1.36 mmols/kg diet; High-I3C, 6.8 mmols/kg diet.

*Values are means ± SEM, n = 15 per group. Means within the same column having no common superscripts are significantly different (P < 0.05).
chemoprotective role through induction of phase II enzymes, such as glutathione S-transferases, as previously shown by Manson et al. (49) Previous studies have shown a decrease in CYP2E1 activity after PEITC administration (50–52). It should be noted, however, that two of these were in vitro studies, in which PEITC was added to an incubation mixture containing microsomes (50,51), and the other study delivered PEITC either by gavage or intraperitoneally (52), which most likely increased the peak exposure of the animals to this compound.

Interestingly, feeding the high dose of I3C resulted in increased NADPH- and NADH-reductase activities in the present study. To our knowledge, this is the first time the activity of these reductases has been examined after dietary treatment with I3C or PEITC. The P450 reductases are key enzymes in the transfer of electrons from NADPH and NADH to the substrates of cytochrome P450’s. NADPH-P450 reductase is involved in the first electron transfer of the P450 catalytic cycle (53). It is not known whether the second electron transfer is mediated by the NADH- or the NADPH-reductase. An increase in the activity of NADPH-P450 reductase suggests that the first electron transfer in the P450 catalytic cycle (and possibly the second electron transfer, as well) is not a limiting factor for P450 reactions. An increase in the activity of NADH-reductase may also facilitate this cycle. Regardless, in the present study, the increased activity of these reductases after feeding of high-I3C did not seem to translate into a lower colon cancer risk.

Thus, dietary I3C, but not PEITC, decreased the risk of colon cancer, as assessed by a significant decrease in ACF number, and a trend toward fewer MDF. Based on the present results, the mechanisms by which I3C decreased the number of ACF does not seem to involve increased apoptosis, decreased cell proliferation or decreased carcinogen activation through CYP2E1. PEITC did not decrease colon cancer risk at the doses provided in the diet. However, given that others have reported reductions in ACF number with PEITC administration (8), further studies of the chemoprotective effect of this isothiocyanate may be warranted.

While this study was designed to focus on the isolated breakdown products of glucosinolates, humans are exposed to these products by consuming the vegetables in which their precursors are found. Most cruciferous vegetables contain more than one type of glucosinolate, which leads to a number of breakdown compounds, in addition to the ones described here. It will be of interest in future studies to examine feeding of whole vegetables rather than isolated compounds, which will provide a better understanding of human exposure to these compounds.

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Conflict of Interest Statement: None declared.

References


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