Chemoprevention of Colon Carcinogenesis by Organosulfur Compounds

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ABSTRACT

It has been reported that several naturally occurring and related synthetic organosulfur compounds exert chemopreventive effects in several target organs in rodent models. The chemopreventive actions of 40 and 80% maximum tolerated doses (MTD) of organosulfur compounds, namely anethole trithione, diallyl disulfide, N-acetylcysteine, and taurine, administered in AIN-76A diet, on azoxymethane (AOM)-induced neoplasia were investigated in male F344 rats. Also, the effects of these agents on the activities of phase II enzymes, namely glutathione S-transferase (GST), NAD(P)H-dependent quinone reductase, and UDP-glucuronosyl transferase, in the liver and colonic mucosa and tumors were assessed. The MTD levels of anethole trithione, diallyl disulfide, N-acetylcysteine, and taurine were determined in male F344 rats and found to be 250, 250, 1500, and 1500 ppm, respectively. At 5 weeks of age, animals were fed the control diet (AIN-76A) or experimental diets containing 40 or 80% MTD levels of each test agent. All animals in each group, except those allotted for vehicle (saline) treatment, were administered AOM s.c. at a dose rate of 15 mg/kg body weight once weekly for 2 weeks. All animals were necropsied during week 52 after the second AOM injection. Colonic mucosal and tumor and liver enzyme activities were measured in animals fed 80% MTD levels of each test agent. Colon tumors were subjected to histopathological evaluation and classified as invasive or noninvasive adenocarcinomas. Colon tumor incidence (percentage of animals with tumors) and tumor multiplicity (tumors/animal) were compared among various dietary groups. The results indicated that administration of 200 ppm (80% MTD) anethole trithione significantly inhibited the incidence and multiplicity of both invasive and noninvasive adenocarcinomas, whereas feeding of 100 ppm (40% MTD) anethole trithione or 100 (40% MTD) or 200 ppm (80% MTD) diallyl disulfide suppressed only invasive adenocarcinomas of the colon. Although diets containing N-acetylcysteine and taurine inhibited colon tumor multiplicity, the effect was somewhat marginal. GST, NAD(P)H-dependent quinone reductase, and UDP-glucuronosyl transferase activities in colonic mucosa and tumor and liver were significantly elevated in animals fed anethole trithione or diallyl disulfide, compared to those fed the control diet. N-Acetylcysteine and taurine slightly but significantly increased only the GST activity in the liver. Although other mechanisms are not excluded, inhibition of AOM-induced colon carcinogenesis by anethole trithione and diallyl disulfide may be associated, in part, with increased activities of phase II enzymes such as GST, NAD(P)H-dependent quinone reductase, and UDP-glucuronosyl transferase in the liver and colon.

INTRODUCTION

Cancer of the colon is a major public health problem in the United States and Canada, as well as other western countries (1). Although a large volume of data derived from epidemiological and laboratory studies suggest that dietary factors play an important role in the etiology of colon cancer (2–5), there is also increasing evidence that chemopreventive properties include inorganic and organic selenium salts, phenoic antioxidants, inositol-6-phosphate, calcium salts, vitamin D, arachidonic acid cascade inhibitors, and difluoromethylornithine, to cite a few (6–17).

Several studies indicate that organosulfur compounds present in cruciferous vegetables (brussels sprouts, cauliflower, and cabbage), such as benzisothiocyanate and dialkylthiolenes, and in Allium species (onion and garlic), such as diallyl disulfide and diallyl sulfide and several of their substituted compounds, have a role in cancer inhibition (6, 7, 9). Diallyl disulfide is a major organosulfur compound present in garlic (18). Pioneering studies by Wattenberg and Bueding (19) and subsequent studies from several laboratories demonstrated that oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione], a substituted dithiolethione, inhibits chemically induced carcinogenesis in several target organs and AFB1-induced hepatotoxicity in rats (20–23). Experiments conducted in our laboratory indicate that oltipraz inhibits chemically induced colon carcinogenesis when administered during the initiation and postinitiation stages (24). Anethole trithione, a substituted dithiolethione, has not been tested as a potential chemopreventive agent in colon cancer models (Fig. 1).

Organosulfur compounds present in garlic and onions, such as allyl sulfide, allyl disulfide, and allyl methyl di- and trisulfides, and garlic and onion oils have been found to inhibit carcinogenesis at several organ sites (25–30). Allyl methyl sulfide, diallyl sulfide, and diallyl trisulfide administered p.o. prior to carcinogen treatment inhibited benzo[α]pyrene-induced and nitrosodiethylamine-induced forestomach tumors in mice (25, 26), with the most potent compound being diallyl sulfide (Fig. 1). Diallyl sulfide administered prior to carcinogen treatment inhibited DMH-induced colon carcinogenesis and N-nitrosomethylbenzylamine-induced esophageal cancer in rats (27, 28). The inhibitory effects of these compounds may be due to inhibition of carcinogen activation through suppression of oxidative metabolism of carcinogen (31). Administration of diallyl sulfide during the postinitiation phase inhibited DMH-induced colon carcinogenesis in rats (29), but this inhibition of colon carcinogenesis was associated with the retardation of body weight gain.

Several other organosulfur compounds that are formed endogenously in the body, including taurine (2-aminoethanesulfonate) and N-acetylcysteine (Fig. 1), have been shown to ameliorate toxicity induced by a variety of chemicals in various organs (32–34). N-Acetylcysteine has been shown to inhibit cyclophosphamide-induced toxicity such as hemorrhagic cystitis (34). Oral administration of taurine partially protected against lipid peroxidation induced by toxic doses of taumustin (32) and isoprenaline (33). The beneficial effect of taurine may be due, in part, to inhibition of lipid peroxidation and of deterioration of membrane phospholipids (32). Although these observations do not necessarily provide evidence that these compounds act as chemopreventive agents, they raise the possibility that these agents may possess tumor-inhibitory properties.

Although the mechanism of action of many of the chemopreventive agents is poorly understood, it appears that these compounds may inhibit carcinogenesis by blocking the formation of or trapping ultimate carcinogen electrophiles and/or by decreasing activation of car.

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3 The abbreviations used are: AFB1, aflatoxin B1; DMH, 1,2-dimethylhydrazine; AOM, azoxymethane; GST, glutathione S-transferase; NADPH-QR, NADPH:quinone reductase; UDP-GT, UDP-glucuronosyl transferase; MTD, maximum tolerated dose.
cinogens and by suppressing the effect of promoters in the target organ (6). Several organosulfur compounds inhibit carcinogenesis by increasing the metabolism, detoxification, and elimination of carcinogens through phase I and phase II enzymes (6, 35). For example, oltipraz and dithiolethione induce liver GST and NAD(P)H:QR activities in rodents (23–35). Earlier studies from our laboratory demonstrated that the colon tumor-inhibitory effect of oltipraz was associated with an increase in GST, NAD(P)H:QR, and UDP-GT activities of the colon (36). It has been suggested that the protective effects of these organosulfur compounds may be accounted for, at least in part, by their ability to induce GST and other phase II enzymes (6, 35).

Clearly, the influence of organosulfur compounds on carcinogenesis is complex and may depend on the specific compounds, the organ site, and the carcinogen administered. The present study was designed to investigate the modulating effects of organosulfur compounds, namely diallyl disulfide, anethole trithione, N-acetylcysteine, and taurine, in colon carcinogenesis. Dose selection of these compounds was based on MTD values determined in the current study. In addition, the effect of these compounds on colonic mucosal GST, NAD(P)H:QR, and UDP-GT activities was measured. The major goal of this preclinical study was to determine which of these organosulfur compounds could be used as effective chemopreventive agents in colon carcinogenesis.

MATERIALS AND METHODS

Animals, Diets, and Organosulfur Compounds. Weanling male inbred F344 rats were obtained from Charles River Breeding Laboratories (Kingston, NY). Diallyl disulfide, taurine, and N-acetylcysteine were purchased from Sigma Chemical Co. (St. Louis, MO). AOM (CAS 25843–45–2) was purchased from Ash-Stevens (Detroit, MI). All ingredients of the semipurified diets were obtained from Dyets, Inc. (Bethlehem, PA) and stored at 4°C prior to formulation of the experimental diets.

A total of 636 male F344 rats were used in this study to determine MTD values and efficacy of organosulfur compounds. Male F344 rats received at weaning were maintained in isolation for 10 days and had access to modified AIN-76A (control) diet (Table 1) (37). The animals were then randomly divided by weight into control and experimental dietary groups and transferred to an animal holding room. They were housed in plastic cages with wood chip bedding and filter tops (3 rats/cage) under controlled conditions of a 12-h light/12-h dark cycle, 50% humidity, and 21°C temperature. Animals were allowed free access to food and water at all times, and food cups were replenished with fresh diet three times weekly. Cages were changed weekly. Experimental diets were prepared by mixing test agents with modified AIN-76A diet (Table 1). All control and experimental diets were prepared weekly in our laboratory and stored in a cold room in airtight plastic containers filled with nitrogen.

Determination of Maximum Tolerated Doses of Test Agents. The experimental protocols were as described previously (38). At 35 days of age, groups of male F344 rats (12/group) were fed the modified AIN-76A diet containing the various levels of chemopreventive agents. Anethole trithione and diallyl disulfide were each tested at dose levels of 62.5, 125, 250, 500, and 1000 ppm, whereas N-acetylcysteine and taurine were each tested at concentrations of 125, 250, 1000, 1500, and 2000 ppm in the diet. Body weights were recorded twice weekly for 6 weeks. At the end of 6 weeks, all animals were sacrificed, and colon, small intestine, stomach, liver, and kidney were examined grossly under a dissection microscope for any abnormalities. The MTD is defined as the highest dose that causes no more than 10% weight decrement, compared to the appropriate control diet group, and does not produce mortality or any external signs of toxicity that would be predicted to shorten the natural life span of the animal. These external signs of toxicity include roughened coat, ill-kept appearance, chromodacryorrhea, rhinitis, and prostration, to cite a few. Based on the observation that dietary anethole trithione or diallyl disulfide at dose levels of 500 and 1000 ppm and dietary N-acetylcysteine or taurine at 2000 ppm decreased the body weights by >10%, compared to the control diet (data not shown), the MTDs of diallyl disulfide, N-acetylcysteine, anethole trithione, and taurine were 250, 1500, 250, and 1500 ppm, respectively.

Experimental Procedure for Efficacy Study. The experiment was designed to determine the efficacy of 40 and 80% MTD of each test agent, administered in the modified AIN-76A diet (Table 1), on AOM-induced colon carcinogenesis in male F344 rats. The 40 and 80% MTD levels, respectively, of test agents were as follows: diallyl disulfide, 100 and 200 ppm; N-acetylcysteine, 600 and 1200 ppm; anethole trithione, 100 and 200 ppm; and taurine, 600 and 1200 ppm. Beginning at the fifth week of age, male F344 rats were randomized by weight into various groups and fed one of the diets containing the various levels of chemopreventive agents. Anethole trithione and diallyl disulfide were each tested at dose levels of 0, 40, or 80% MTD levels of each chemopreventive agent (Table 1). After 2 weeks on control or experimental diets, groups of animals intended for carcinogen treatment (36 animals/group) received AOM s.c. once weekly for 2 weeks at a dose rate of 15 mg/kg body weight/week. Animals intended for vehicle treatment (12 animals/group) received an equal volume of normal saline. All animals were fed their respective test agents until the termination of the study. Body weights were recorded every 2 weeks for the first 10 weeks and then every 4–6 weeks. The experiment was terminated at 52 weeks after the second AOM treatment. Both vehicle-treated and AOM-treated animals were sacrificed by CO2 euthanasia as scheduled. Following laparotomy, the entire small intestine, stomach, liver, and kidney were examined grossly under a dissection microscope for any abnormalities. The MTD is defined as the highest dose that causes no more than 10% weight decrement, compared to the appropriate control diet group, and does not produce mortality or any external signs of toxicity that would be predicted to shorten the natural life span of the animal. These external signs of toxicity include roughened coat, ill-kept appearance, chromodacryorrhea, rhinitis, and prostration, to cite a few. Based on the observation that dietary anethole trithione or diallyl disulfide at dose levels of 500 and 1000 ppm and dietary N-acetylcysteine or taurine at 2000 ppm decreased the body weights by >10%, compared to the control diet (data not shown), the MTDs of diallyl disulfide, N-acetylcysteine, anethole trithione, and taurine were 250, 1500, 250, and 1500 ppm, respectively.

Table 1 Percentage composition of experimental semipurified diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control diet</th>
<th>Experimental diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>52.0</td>
<td>52.0</td>
</tr>
<tr>
<td>Dextrose</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Alphacel</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Mineral mix, AIN</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mix, AIN revised</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Anethole trithione</td>
<td>0</td>
<td>200 or 100 ppm</td>
</tr>
<tr>
<td>Diallyl disulfide</td>
<td>0</td>
<td>200 or 100 ppm</td>
</tr>
<tr>
<td>N-Acetylcysteine</td>
<td>0</td>
<td>1200 or 600 ppm</td>
</tr>
<tr>
<td>Taurine</td>
<td>0</td>
<td>1200 or 600 ppm</td>
</tr>
</tbody>
</table>

*Adopted from American Institute of Nutrition Reference Diet AIN-76A with modification of the source of carbohydrate (38).

*Test agents at 40 and 80% MTD levels were added to the diets at the expense of corn starch.

Fig. 1. Structures of organosulfur compounds.
stomach, small intestine, and large intestine were resected and opened longitudinally, and the contents were flushed with normal saline. Using a dissection microscope, tumors were noted grossly for their location, number, and size. All other organs, including kidney and liver, were also grossly examined under the microscope. In addition, colon tumors with a diameter of >0.5 cm were cut approximately into two halves; one portion of the tumor was used for enzyme assays and the other for histopathology. Colonic mucosa was scraped from each animal for biochemical analysis according to our previously described method (24). Portions of colonic tumors and colonic mucosa intended for biochemical determinations were quickly frozen in liquid nitrogen until analyses.

For histopathological confirmation, tumors were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed by routine histological methods using hematoxylin and eosin stains. The stained sections were examined histologically for tumor types, using histological criteria described previously (14).

Biochemical Study. Liver and colonic mucosal and tumor samples were homogenized in 4 volumes (w/v) of 50 mM Tris buffer (pH 7.4) containing 0.25 M sucrose, with a Polytron homogenizer. Homogenates were centrifuged at 105,000 × g; the resulting supernatant was used for GST and NAD(P)H:QR assays and the membrane fraction was used for assay of UDP-GT activity. GST activity was assayed according to the method of Habig et al. (39), using 1-chloro-2,4-dinitrobenzene as substrate. Dicoumarol-inhibited NAD(P)H:QR activity was assayed according to the modified method of Benson et al. (40), using 2,6-dichloroindophenol as final electron acceptor. UDP-GT activity was assayed according to the method of Temple et al. (41), using p-nitrophenol as substrate. Protein was determined by the Bio-Rad method.

Statistical Analysis. Tumor incidence is expressed as percentage of animals with tumors and was compared between the groups by χ² test. Tumor multiplicity is expressed as mean number of tumors/animal and was compared by the Student’s t test. Body weights and enzyme values for the control and experimental groups were analyzed by analysis of variance. Differences were considered statistically significant at P < 0.05.

RESULTS

The body weights of animals fed the chemopreventive agents were comparable to those of the animals fed the control diet in AOM- and saline-treated groups, except that, at termination, the animals fed 200 ppm (80% MTD) diallyl disulfide showed slightly but significantly lower (about 5%) body weights than did those fed the control diet (Fig. 2). Saline-treated animals weighed more than those treated with AOM in all groups (data not shown).

Tumor Data. In the present study, >90% of colon tumors were adenocarcinomas and the remainder were benign adenomas. Since there were no differences in the incidence or multiplicity of colon adenomas between the control and experimental groups, Table 2 summarizes only the results for adenocarcinomas. Adenocarcinomas were classified as invasive or noninvasive type based on invasion into muscularis mucosa. Tumors invading beyond the submucosa into muscularis mucosa were classified as invasive carcinomas, whereas those that had developed exophytically with the roots above the muscularis mucosa were termed as noninvasive carcinomas. Daily administration of 200 ppm anethole trithione significantly inhibited the incidence and multiplicity of both invasive and noninvasive adenocarcinomas of the colon, whereas anethole trithione at 100 ppm suppressed the incidence and multiplicity of invasive adenocarcinomas. The results also indicate that daily feeding of 100 or 200 ppm diallyl disulfide significantly reduced the incidence and multiplicity of invasive colon adenocarcinomas but had no effect on noninvasive adenocarcinomas. Diets containing 600 or 1200 ppm N-acetylcysteine or taurine also decreased slightly the incidence of invasive adenocarcinomas of the colon, but the difference did not reach statistical significance (P > 0.05). The multiplicity of invasive adenocarcinomas was significantly inhibited in animals fed 600 or 1200 ppm taurine, but N-acetylcysteine had no effect on the multiplicity of invasive adenocarcinomas.

Biochemical Data. The activities of GST, NAD(P)H:QR, and UDP-GT were analyzed in liver and colonic mucosa and tumors of AOM-treated animals fed the control diet and diets containing 80% MTD each of anethole trithione, diallyl disulfide, N-acetylcysteine, and taurine and in liver and colonic mucosa of saline-treated animals (Table 3). In general, AOM treatment had no measurable effect on these enzyme activities in liver and colonic mucosa. Long term administration of anethole trithione and diallyl disulfide significantly increased the activities of GST, NAD(P)H:QR, and UDP-GT in liver and colonic mucosa and tumors, compared to control diet (P < 0.01–0.0001). The degree of enhancement of enzyme activities was more pronounced in animals fed anethole trithione, compared to those...
Chemopreventive properties of organosulfur compounds in an established model of colon carcinogenesis (36). Indeed, the observations of our current and previous studies with colon cancer (36) and the results of earlier studies with mammary gland, liver, pancreas, forestomach, bladder, and skin cancer models (15, 23, 35) collectively suggest that the substituted dithiolethiones appear to possess chemopreventive properties against several classes of carcinogens in many target organs. Diallyl disulfide has been shown to be effective in the prevention of carcinogenesis in a number of animal models including DMH-induced colon carcinogenesis (29) and diethylnitrosamine-induced forestomach and lung tumor development (25). In the present study, 100 and 200 ppm diallyl disulfide significantly inhibited AOM-induced invasive adenocarcinomas of the colon. In contrast, the effects of N-acetylcysteine and taurine in modulating colon tumor inhibition were somewhat marginal. Collectively, the results of current and earlier studies (36) indicate that, (a) of all organosulfur compounds tested, oltipraz and anethole trithione are the most effective chemopreventive agents in reducing the risk of colon cancer, followed by diallyl disulfide, and (b) the dithiolethione nucleus of substituted dithiolethiones may be a contributing factor to colon cancer chemopreventive activity.

Although the mechanisms by which anethole trithione and diallyl disulfide exert their inhibitory effects in AOM-induced colon carcino-

Table 2 Effect of organosulfur compounds on AOM-induced colon adenocarcinomas in male F344 rats

<table>
<thead>
<tr>
<th>Chemopreventive agents tested</th>
<th>Incidence (% of animals with tumors)</th>
<th>Multiplicity (tumors/animal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Invasive⁵ Noninvasive⁶ Total⁷</td>
<td>Invasive Noninvasive Total</td>
</tr>
<tr>
<td>Control diet</td>
<td>34 53 78</td>
<td>0.63 ± 0.18⁸ 1.03 ± 0.20 1.66 ± 0.21</td>
</tr>
<tr>
<td>Anethole trithione, 200 ppm</td>
<td>8.3³ 30² 42¹</td>
<td>0.11 ± 0.06⁸ 0.53 ± 0.15⁸ 0.64 ± 0.15⁸</td>
</tr>
<tr>
<td>Anethole trithione, 100 ppm</td>
<td>11² 47 58</td>
<td>0.28 ± 0.14⁸ 0.72 ± 0.17 1.00 ± 0.18⁸</td>
</tr>
<tr>
<td>Diallyl disulfide, 200 ppm</td>
<td>5.3³ 58 64</td>
<td>0.11 ± 0.07⁸ 0.94 ± 0.12 1.05 ± 0.12⁸</td>
</tr>
<tr>
<td>Diallyl disulfide, 100 ppm</td>
<td>11² 53 64</td>
<td>0.30 ± 0.17⁸ 1.03 ± 0.19 1.33 ± 0.22</td>
</tr>
<tr>
<td>N-Acetylcysteine, 1200 ppm</td>
<td>19 42 61</td>
<td>0.41 ± 0.17 0.69 ± 0.11 1.10 ± 0.21¹</td>
</tr>
<tr>
<td>N-Acetylcysteine, 600 ppm</td>
<td>22 44 64</td>
<td>0.41 ± 0.15 0.66 ± 0.15 1.08 ± 0.19¹</td>
</tr>
<tr>
<td>Taurine, 1200 ppm</td>
<td>17 47 61</td>
<td>0.25 ± 0.11¹ 0.75 ± 0.16 1.00 ± 0.17¹</td>
</tr>
<tr>
<td>Taurine, 600 ppm</td>
<td>16 61</td>
<td>0.24 ± 0.09¹ 1.03 ± 0.17 1.27 ± 0.18¹</td>
</tr>
</tbody>
</table>

⁵ Invasive adenocarcinomas include those with invasion into the muscularis mucosa of colon.
⁶ Noninvasive adenocarcinomas include those that do not invade into muscularis mucosa.
⁷ Total tumors represent invasive and noninvasive adenocarcinomas.

Table 3 Effect of organosulfur compounds on liver and colonic mucosal and tumor enzyme activities

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Colonic</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GST (μmol/mg/min)⁹</td>
<td>NAD/Pi/HQR (μmol/mg/min)</td>
<td>UDP-GT (nmol/min)</td>
</tr>
<tr>
<td>AOM-treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>720 ± 114⁸ 158 ± 34 3.3 ± 0.6</td>
<td>372 ± 50 450 ± 57 1.7 ± 0.36</td>
<td>409 ± 44 336 ± 38 1.8 ± 0.2</td>
</tr>
<tr>
<td>Anethole trithione, 200 ppm</td>
<td>6196 ± 90⁶ 889 ± 127³ 10.9 ± 1.7⁷</td>
<td>2010 ± 262² 2702 ± 381³ 3.6 ± 0.6²</td>
<td>1302 ± 171² 2501 ± 421² 3.7 ± 0.6²</td>
</tr>
<tr>
<td>Diallyl disulfide, 200 ppm</td>
<td>2662 ± 372² 277 ± 33³ 5.8 ± 0.7³</td>
<td>1089 ± 174² 660 ± 49² 2.0 ± 0.2</td>
<td>960 ± 117² 642 ± 54² 2.5 ± 0.4²</td>
</tr>
<tr>
<td>N-Acetylcysteine, 1200 ppm</td>
<td>1076 ± 135⁵ 194 ± 24 4.0 ± 0.7²</td>
<td>353 ± 36 450 ± 55 1.6 ± 0.3²</td>
<td>347 ± 50³ 478 ± 82 1.9 ± 0.3³</td>
</tr>
<tr>
<td>Taurine, 1200 ppm</td>
<td>953 ± 120¹</td>
<td>219 ± 42 3.3 ± 0.5²</td>
<td>415 ± 32 440 ± 41 1.5 ± 0.3²</td>
</tr>
<tr>
<td>Saline-treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>678 ± 128 176 ± 36 3.0 ± 0.6</td>
<td>336 ± 64 438 ± 64 1.6 ± 0.3</td>
<td>1894 ± 106² 2424 ± 283³ 3.9 ± 0.4³</td>
</tr>
<tr>
<td>Anethole trithione, 200 ppm</td>
<td>5476 ± 1156³ 889 ± 127⁷ 10.0 ± 1.5³</td>
<td>1894 ± 106² 2424 ± 283³ 3.9 ± 0.4³</td>
<td></td>
</tr>
<tr>
<td>Diallyl disulfide, 200 ppm</td>
<td>2533 ± 296² 264 ± 43³ 5.4 ± 0.6³</td>
<td>1098 ± 114³ 649 ± 49² 2.0 ± 0.4²</td>
<td></td>
</tr>
<tr>
<td>N-Acetylcysteine, 1200 ppm</td>
<td>1103 ± 173³ 185 ± 43 3.6 ± 0.5³</td>
<td>358 ± 50 449 ± 58 1.9 ± 0.2³</td>
<td></td>
</tr>
<tr>
<td>Taurine, 1200 ppm</td>
<td>988 ± 102³ 181 ± 44 2.9 ± 0.5²</td>
<td>414 ± 67 448 ± 39 1.5 ± 0.3²</td>
<td></td>
</tr>
</tbody>
</table>

⁹ GST activity is expressed as μmol 1-chloro-2,4-dinitrobenzene conjugated/mg protein/min. NAD/Pi/HQR activity is expressed as μmol 2,6-dichloroindophenol reduced/mg protein/min, and UDP-GT activity is expressed as nmol ρ-nitrophenol conjugated/mg protein/min.

Values are mean ± SD (n = 6).
genesis remain to be elucidated, the inhibition of tumorigenesis by these agents can proceed through several mechanisms, as discussed by Wattenberg (6) and Talalay (35) for other chemopreventive agents. A tenable possibility is that these agents alter the metabolism of carcinogens in the target organs. The metabolic activation of AOM to a reactive species capable of alkylating DNA occurs through the hydroxylation of AOM to methylazoxymethanol in the liver, and the metabolism of methylazoxymethanol to a highly reactive electrophile, namely methyldiazonium ion, which can methylate cellular nucleophiles including DNA occurs in both liver and colon (42, 43). Several colon tumor inhibitors, such as pyrazole, disulfiram or its metabolites, oltipraz, and benzylselenocyanate, have been shown to alter AOM metabolism and/or DNA adduct formation in the colon, thereby inhibiting carcinogenicity (24, 44-46). The mechanistic studies carried out by Kensler et al. (23) on the inhibition of AFB₁-induced liver tumorigenesis by oltipraz demonstrated that this agent reduced the binding of AFB₁ to DNA in male F344 rats. It is, therefore, likely that anethole trithione and diallyl disulfide might increase the rate of detoxification of AOM in liver and colon, thereby decreasing the formation of carcinogen metabolites and reducing AOM-induced colon carcinogenesis by these agents.

Another possible mechanism of the colon tumor-inhibitory action of these agents is through an increase in the levels of detoxifying enzymes in the liver and colon. Substituted dithiolethiones such as oltipraz and anethole dithione have been shown to increase glutathione levels, GST activity, and other detoxifying enzyme activities (23, 35, 47). It was also reported that, in AFB₁-induced liver carcinogenesis, modifications of AFB₁ metabolism and disposition produced by alterations in the activities of both phase I and phase II enzymes are major components of the chemopreventive activity of dithiolethiones in this model (23). Our recent study demonstrated that dietary oltipraz induced colonic and liver GST, NAD(P)H:QR, and UDP-GT activities (37). As observed in the present study, administration of anethole trithione strikingly increased the activities of GST, NAD(P)H:QR, and UDP-GT in the liver and colonic mucosa and tumors of animals treated with AOM and the liver and colonic mucosa of saline-treated animals. Although diallyl disulfide significantly increased the activities of GST and NAD(P)H:Q in liver and colonic mucosa and tumors, the degree of enhancement of these enzyme activities was more profound in anethole trithione-fed animals, compared to animals fed diallyl disulfide. This observation is supported by the finding in the current study that the degree of colon tumor inhibition for both invasive and noninvasive adenocarcinomas is more pronounced in anethole trithione-fed animals than in animals fed diallyl disulfide. Taken together, these observations corroborate the inhibitory effect of anethole trithione and diallyl disulfide on invasive and/or noninvasive adenocarcinomas of the colon.

In conclusion, the results of this study demonstrate that dietary anethole trithione and diallyl disulfide inhibit colon carcinogenesis and that the degree of inhibition is more pronounced with anethole trithione than diallyl disulfide. Although the exact mechanisms involved in their protective effects against colon carcinogenesis are not clearly understood at present, the results for colonic mucosal and liver GST, NAD(P)H:QR, and UDP-GT activities should provide a stimulus to design additional studies on the mechanisms of colon tumor inhibition by anethole trithione and diallyl disulfide.

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