Chemoprevention of 1,2-Dimethylhydrazine-induced Colon Cancer in Mice by Naturally Occurring Organosulfur Compounds

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ABSTRACT

Organosulfur compounds (OSC) present in garlic and onion oil have been shown to inhibit chemical carcinogenesis. In this study, we compared the chemopreventive efficacy of five lipid- and four water-soluble OSCs using the murine nuclear aberration assay. Administration of diallyl sulfide and S-allyl cysteine p.o. at a dose of 200 mg/kg 3 h prior to i.p. 1,2-dimethylhydrazine (DMH) injection (20 mg/kg) significantly inhibited colonic nuclear damage in female C57BL/6J mice by 47% and 36%, respectively. The inhibitory effect of S-allyl cysteine was found to be dose dependent. The other OSCs did not affect the level of DMH-induced nuclear toxicity. Furthermore, the incidence and frequency of colonic tumors induced by DMH (20 mg/kg, 10 weekly i.p. injections) in female CF-1 mice were significantly inhibited by S-allyl cysteine pretreatment, given 3 h prior to each carcinogen injection. These data indicate that the allyl group coupled to a single sulfur atom might play an important structural role in inhibition of DMH-induced colonic nuclear toxicity and carcinogenesis. OSCs containing allyl groups stimulated glutathione S-transferase activity in both the liver and colon. However, their saturated analogues stimulated little or no hepatic and colonic glutathione S-transferase activity. Induction of hepatic and colonic glutathione S-transferase might assist in detoxification of carcinogens and could be necessary for some aspects of chemoprevention.

INTRODUCTION

Naturally occurring micronutrients in food have been found to have chemopreventive effects, perhaps supporting in part the conclusions from epidemiologic studies that consumption of fresh fruits and vegetables reduces cancer risk (1-5). Our research has focused on unique biochemical and pharmacological OSCs found in garlic and onion. We have reported that DAS, a thioether identified as a flavor component in garlic, inhibited the colonic nuclear toxicity induced by DMH or radiation, DMH-induced colonic cancer in mice and N-nitrosomethylbenzylamine-induced esophageal tumors in rats (6-9). DAS has been shown to inhibit the hepatocarcinogenic responses to DMH- and cyclophosphamide-induced nuclear aberrations in the urinary bladder and hair follicles (10, 11). Furthermore, Belman (12) reported that the topical application of garlic and onion oil inhibited tumor promotion in dimethylbenzanthracene-induced, phorbol-myristate-promoted skin tumors in mice. OSCs containing allyl groups have been shown to inhibit benzo(a)pyrene-induced tumors of the forestomach and lung in A/J mice (13, 14). Thus, a number of oil-soluble OSCs have been known to have chemopreventive effects. Garlic contains water-soluble OSCs as well as oil-soluble OSCs. However, none of the water-soluble OSCs has been investigated for chemopreventive efficacy, the aim of this investigation.

The activity of GST, an enzyme known to assist in the detoxification of many carcinogens, has been demonstrated to be markedly stimulated by OSCs (13-16). Increased GST activity might contribute to the inhibition of carcinogenesis. In this study, we investigated the relationship between OSCs (water and oil soluble), their differing chemical structures, and physical properties for their chemopreventive efficacy. We questioned whether or not the induction of GST activity by OSC is related to the inhibition of nuclear toxicity and tumorigenesis induced by DMH.

MATERIALS AND METHODS

Chemicals. 1,2-Dimethylhydrazine-2-HCl, DAS, DADS, DPS, and DPDS were purchased from Aldrich Chemical Co., Milwaukee, WI. Ajoene, SAC, SPC, and SAMC were kindly provided at high purity by Wakunaga Pharmaceutical Co., Ltd., Osaka, Japan. The chemical structures of these compounds are shown in Fig. 1. Other chemicals were purchased from Sigma Chemical Co., St. Louis, MO. Oil-soluble OSCs were dissolved in corn oil. Water-soluble OSCs were dissolved in 0.1 N HCl and neutralized with 1 N NaOH.

Animals. Six- to 8-wk-old female C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were used for nuclear aberration assays and GST assays (6). Seven-wk-old female CF-1 mice (Charles River Laboratories, Inc., Wilmington, MA) were used for the carcinogenicity experiment. CF-1 mice have a proven susceptibility to DMH-induced cancer (17). They were housed five to a cage and maintained on AIN-76A purified diet (Dyets, Inc., Bethlehem, PA) and water ad libitum. The light-dark cycle was alternated every 12 h.

Nuclear Aberration Assay. Five C57BL/6J mice in each group were given OSC p.o. at the molar equivalent dose of 200 mg/kg of body weight DMH 3 h prior to DMH injection (20 mg/kg, i.p.) in 1 mM EDTA, pH 6.5. The maximum inhibition by DAS has been previously observed at this time point (6). Controls received vehicle solutions (10 ml/kg). In a dose-response experiment, five mice at each dose were given SAC p.o. at doses of 50, 100, 200, and 400 mg/kg 3 h prior to DMH injection. All mice were sacrificed at 24 h after DMH administration. The colon was removed and fixed with 10% buffered formalin. Paraffin sections were stained by the Feulgen reaction with a Fast Green counterstain. Nuclear aberrations were enumerated per crypt, as previously described (18).

GST Assay. Ten C57BL/6J mice in each group were given OSCs p.o. at the molar equivalent dose of 200 mg/kg of DAS. Controls received vehicles (10 mg/kg). Five mice each in groups were sacrificed at 24 h and 48 h after p.o. chemical administration. The liver and colon were removed and stored in liquid nitrogen until assayed. The activity of the cytosolic GST was determined spectrophotometrically at 25°C with 1-chloro-2,4-dinitrobenzene as a substrate by the method of Di Ilio et al. (19). The reaction mixture (1 ml) contained 100 mM potassium phosphate buffer (pH 6.8), 1 mM 1-chloro-2,4-dinitrobenzene, 2 mM glutathione, 1 mM EDTA, and an aliquot (100 µl) of cytosol.

Carcinogenicity Experiment. A total of 150 CF-1 mice were randomly distributed by weight into seven groups. Groups 4, 5, 6, and 7 (30 mice per group) were given 10 weekly i.p. injections of DMH at a dose of 20 mg/kg, and Groups 1, 2, and 3 (10 mice per group) were given injections of the vehicle as the control. Three h prior to each weekly injection of DMH and vehicle, Groups 2, 5, and 6 were given p.o. SAC...
CHEMOPREVENTION AND ORGANOSULFUR COMPOUNDS

Oil-soluble compounds

\[
\begin{align*}
\text{CH}_2\text{=CH-CH}_2\text{-S-CH}_2\text{-CH=CH}_2 & \quad \text{DAS} \\
\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-S-CH}_2\text{-CH}-
\end{align*}
\]

Water-soluble compounds

\[
\begin{align*}
\text{CH}_2\text{=CH-CH}_2\text{-S-CH-COOH} & \quad \text{SAC} \\
\text{CH}_3\text{-CH}_2\text{-CH}-
\end{align*}
\]

Fig. 1. Organosulfur compounds used in this study.

Fig. 2. Effect of oil-soluble OSCs on DMH-induced colonic nuclear aberrations in mice. OSCs at the molar equivalent dose of 200 mg/kg of DAS were given p.o. 3 h prior to DMH (20 mg/kg, i.p.) administration. The control received corn oil (10 ml/kg). Columns, mean (n = 5); bars, SE. D, vehicle treatment; III, DMH treatment. *, significantly different from the DMH-alone group (P < 0.05).

Fig. 3. Effect of water-soluble OSCs on DMH-induced colonic nuclear aberrations in mice. OSCs at the molar equivalent dose of 200 mg/kg of DAS were given p.o. 3 h prior to DMH (20 mg/kg, i.p.) administration. The control received the vehicle (10 ml/kg). Columns, mean (n = 5); bars, SE. D, vehicle treatment; III, DMH treatment. *, significantly different from the DMH-alone group (P < 0.05).

Fig. 4. Dose-dependent inhibition of DMH-induced colonic nuclear aberrations in mice by SAC pretreatment. SAC at graded doses was given p.o. 3 h prior to DMH (20 mg/kg, i.p.) administration. Points, mean (n = 5); bars, SE. *, significantly different from the vehicle-treated group (P < 0.05).

at a dose of either 200 mg/kg (Group 5) or 400 mg/kg (Groups 2 and 6); Groups 3 and 7 were given p.o. 400 mg/kg of cysteine; Groups 1 and 4 were gavaged with the vehicle alone. Twenty-five wk after the initiation of DMH injection, all animals were sacrificed by cervical dislocation and fully necropsied. Gross lesions, as well as normal-appearing tissues, were fixed in 10% buffered formalin. The paraffin sections (4.5 µm in thickness) were stained with hematoxylin and eosin and examined histologically.

Statistics. The data obtained were evaluated by Student’s t test or χ² analysis.

RESULTS

Nuclear Aberration Study. The effect of oil- and water-soluble OSCs on DMH-induced nuclear aberrations in the mouse colon is shown in Figs. 2 and 3, respectively. Among the five oil-soluble OSCs, only DAS, which has been previously shown to inhibit chemical carcinogen-induced nuclear aberration and tumors (6, 8, 11), significantly suppressed the occurrence of nuclear aberrations. Of the water-soluble OSCs, SAC was also active in preventing genotoxic events induced by DMH, but its saturated analogue, SPC, was inactive. SAMC and cysteine did not suppress DMH-induced nuclear aberrations. Moreover, SAC showed dose-dependent inhibition (Fig. 4). None of the oil- and water-soluble OSCs themselves induced nuclear toxicity in the colon.

GST Activity Study. All oil-soluble OSCs increased GST activity in the liver (Table 1). Among the four water-soluble OSCs, only SAC stimulated hepatic GST activity. Colonic GST activity was stimulated by DAS, DADS, ajoene, SAC, and SAMC. Increased GST activity by OSCs in the liver was higher at 48 h versus 24 h after the administration, whereas in the colon, no difference was observed at 24 h and 48 h. OSCs containing the allyl group and monosulfide induced higher GST activity than did those containing the propyl group and/or disulfide linkage.

Carcinogenesis Study. The body weight of animals is shown in Fig. 5. DMH-treated animals gained less weight than did the vehicle-treated animals. However, SAC and CySH administration did not affect the growth of mice treated with DMH or the vehicle.

The incidence and histology of colonic tumors are presented in Table 2. Pretreatment with SAC before each DMH injection significantly inhibited the development of colonic tumors. The incidence of colon tumors in the DMH-alone group was 63.3%, whereas it was 33.3% and 26.7% in SAC-administered groups.
given 200 and 400 mg/kg, respectively. However, cysteine (400 mg/kg), and the vehicle (10 ml/kg) were administered p.o. 3 h prior to each weekly injection of DMH and the vehicle.

**DISCUSSION**

We have previously shown that DAS inhibited nuclear aberration and development of tumors induced by DMH in the colon and by N-nitrosomethylbenzylamine in the esophagus (6–8). In the present study, SAC, a water-soluble OSC derived from garlic, inhibited DMH-induced nuclear aberrations and tumors in the mouse colon in a dose-dependent manner. It alone was active, as the other OSCs used in the study did not inhibit the nuclear toxicity by DMH. These data suggest that an allyl group coupled to a sulfur atom is the essential in inhibition of DMH-induced colon carcinogenesis. Ajoene contains an allyl group coupled to a sulfur atom. However, it did not inhibit DMH-induced colonic nuclear aberrations. Since some chemopreventive compounds have been known to be chemical-reducing agents, the allyl group coupled to sulfoxide which is a strong oxidant might not be effective against carcinogenesis. The importance of an allyl group and number of composite sulfur atoms in OSCs has been shown by other groups as follows (a) Sparnins et al. (14) reported that OSCs containing allyl groups (allyl methyl trisulfide, diallyl trisulfide, allyl methyl disulfide, diallyl sulfide) inhibited benzo[a]pyrene-induced forestomach tumors, whereas the saturated analogues containing propyl groups did not; and (b) Goldberg (20) demonstrated that none of five analogues (DADS, DPS, allyl methyl sulfide, dimethyl sulfide, and dipropyl sulfone) of DAS was as effective as SAC in inhibiting DMH-induced colonic nuclear toxicity.

Recent interest in the potential activity of OSC in humans was heightened by a case-control study in northern China that demonstrated inverse trends in stomach cancer risk with dietary intake of allium vegetables (garlic, scallions, Chinese chives, and onion) (21, 22). This finding persisted after adjustment for intake of other fresh vegetables, yet it remains to be determined whether these data can be explained by the activity of specific allium OSCs in humans. Garlic consumption showed a more protective effect than did onion (22). Garlic contains OSCs with allyl groups, and onion contains propyl groups (23, 24). Therefore, garlic could have a stronger and wider range of chemopreventive effects than onion.

GST has been shown to assist in the detoxification of many carcinogens (15, 16). Sparnins et al. (13, 14) reported that GST activity in the liver, forestomach, small intestine, and lung was enhanced at 96 h after p.o. administration of OSCs. However, Hayes et al. (10) reported no influence of DAS on GST activity at 18 h after being added to primary cultured rat hepatocytes. We have found that the increase in mouse hepatic GST by OSCs was more elevated at 48 h than at 24 h after gavage. These results indicate that the stimulation of hepatic GST by OSCs may require quite a long lag time to demonstrate an effect. Colonic GST activity was also stimulated by p.o. OSC administration in our study but without a significant difference between 24 h and 48 h after treatment. Also, the GST response to OSC showed organospecificity. OSCs containing allyl groups were more significantly stimulatory for GST activity in the liver compared to OSCs containing propyl groups (23, 24).

<table>
<thead>
<tr>
<th>Organosulfur compounds</th>
<th>GST activity (μmol/60 min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Oil soluble</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.62 ± 0.06*</td>
</tr>
<tr>
<td>DAS</td>
<td>1.92 ± 0.02*</td>
</tr>
<tr>
<td>DADS</td>
<td>1.85 ± 0.16</td>
</tr>
<tr>
<td>DPS</td>
<td>1.73 ± 0.05</td>
</tr>
<tr>
<td>DPDPS</td>
<td>1.87 ± 0.15</td>
</tr>
<tr>
<td>Ajoene</td>
<td>2.06 ± 0.13*</td>
</tr>
<tr>
<td>Water soluble</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.59 ± 0.07</td>
</tr>
<tr>
<td>CySH</td>
<td>1.67 ± 0.03</td>
</tr>
<tr>
<td>SAC</td>
<td>1.94 ± 0.08*</td>
</tr>
<tr>
<td>SPC</td>
<td>1.53 ± 0.07</td>
</tr>
<tr>
<td>SAMC</td>
<td>1.81 ± 0.11</td>
</tr>
</tbody>
</table>

* Mean ± SE (n = 5).
* P < 0.01 versus control group.
* P < 0.001 versus control group.
* P < 0.05 versus control group.

Table 2 Inhibition by S-allyl cysteine of DMH-induced colon tumorigenesis in mice

Animals were given either 10 weekly i.p. injections of DMH at a dose of 20 mg/kg of body weight or the vehicle (10 ml/kg). SAC (200 and 400 mg/kg), CySH (400 mg/kg), and the vehicle (10 ml/kg) were administered p.o. 3 h prior to each weekly injection of DMH and the vehicle.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of effective mice</th>
<th>No. of tumor-bearing mice</th>
<th>No. of adenoma</th>
<th>No. of adenocarcinoma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + vehicle</td>
<td>10</td>
<td>0</td>
<td>23</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>SAC, 400 mg/kg, + vehicle</td>
<td>10</td>
<td>0</td>
<td>11</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>CySH, 400 mg/kg, + vehicle</td>
<td>10</td>
<td>0</td>
<td>7</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>SAC, 200 mg/kg, + DMH</td>
<td>30</td>
<td>19 (63.3)*</td>
<td>22</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>SAC, 400 mg/kg, + DMH</td>
<td>30</td>
<td>10 (33.3)*</td>
<td>8</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>CySH, 400 mg/kg, + DMH</td>
<td>30</td>
<td>8 (26.7)*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentages.
* P < 0.05 versus vehicle + DMH group.
* P < 0.01 versus vehicle + DMH group.
and colon, whereas their saturated analogues (propyl group) produced little or no induction. These results agree with the observation reported by Sparnins et al. (14). Our preliminary results showed that GST activity in the liver was stimulated by DMH as has been shown for other carcinogens, such as benzo(a)pyrene and 3-methylcholanthrene (15), but not in the colon (data not shown). Induction of GST activity in the liver and colon by OSCs may account for some aspects of chemoprevention. Since some OSCs which stimulated GST activity did not inhibit DMH-induced nuclear aberrations in the colon, we conclude that induction of GST activity itself is not sufficient to explain full chemopreventive efficacy. Yet, Brady et al. (25) have shown that DAS inhibits hepatic monooxygenase, which catalyzes the oxidation of azoxymethane to methylazoxymethanol. It is therefore possible that some OSCs could be speculated to partially inhibit chemical carcinogenesis through both stimulation of detoxifying enzymes and inhibition of metabolic activating enzymes. Determination of relationship between OSC and enzymes related to carcinogen metabolism will require further investigation.

An increasing number of animal studies have reported inhibitory effects of OSCs derived from garlic on the development of tumors (see Ref. 26 for a review). Future epidemiological studies may clarify the cancer-preventive role for allium vegetables in humans. In any event, OSCs in garlic have proven to be interesting investigational compounds for studying chemoprevention of carcinogenesis. It is hoped that they will ultimately provide some avenues of cancer protection in humans.

REFERENCES

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