Chemoprevention of Azoxymethane-induced Colon Carcinogenesis by Dietary Feeding of S-Methyl Methane Thiosulfonate in Male F344 Rats

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ABSTRACT

Modifying effects of dietary exposure of S-methyl methane thiosulfonate (MMTS) isolated from cauliflower Brassica oleracea L. var. botrytis on rat colon carcinogenesis induced by azoxymethane (AOM) and on the expression of cell proliferation biomarkers were investigated in two experiments. In experiment 1, male F344 rats were given three s.c. injections of AOM (15 mg/kg body weight) and fed 100 ppm MMTS for 5 weeks, starting 1 week before the first dose of AOM. The frequency of colonic aberrant crypt foci was determined at 5 weeks after the start. Feeding of 100 ppm MMTS for 5 weeks significantly decreased the number of aberrant crypt foci/colon. Colonic mucosal ornithine decarboxylase activity and the number of silver-stained nucleolar organizer regions per nucleus in colonic epithelium were significantly decreased by MMTS treatment compared with those of AOM alone. In experiment 2, effects of dietary feeding of MMTS at two doses (20 and 100 ppm) during the postinitiation phase on intestinal tumorigenesis initiated with AOM were investigated by using a long-term experiment in male F344 rats. Incidences of intestinal neoplasms of rats fed MMTS-containing diets after AOM exposure were reduced in a dose-dependent manner. Feeding of MMTS during the postinitiation phase decreased the number of aberrant crypt foci/colon, colonic ornithine decarboxylase activity, 5-bromodeoxyuridine-labeling index in colonic epithelium, and polyamine level in blood compared with those of AOM alone. These results suggest that MMTS might be a possible chemopreventive agent for intestinal neoplasia.

INTRODUCTION

Individuals who consume large amounts of green and yellow vegetables have a lower risk of developing cancer (1). Isothiocyanates and their glucosinolate precursors are widely disturbed in higher plants and are especially prevalent among cruciferous vegetables (2). Certain natural and synthetic aromatic isothiocyanates have been known for more than a decade to inhibit mammary, forestomach, and lung tumorigenesis induced by polycyclic aromatic hydrocarbons in rodents (3). Recently, our colleagues proved that benzyl thiocyanate and benzyl isothiocyanate inhibited hepatocarcinogenesis induced by diethylnitrosamine in rats (4), and these compounds also inhibited intestinal carcinogenesis induced by methylazoxymethanol acetate in rats (5). Some extracts from vegetables induce Phase II detoxification enzymes, such as quinone reductase NAD(P)H (quinone-acceptor) oxidoreductase (EC 1.6.99.2) and glutathione S-transferases (EC 2.5.1.18), in rodent tissues against carcinogens and other toxic electrophiles (6). MMTS2 was newly isolated from cauliflower, Brassica oleracea L. var. botrytis, and its chemical structure was identified by one of the authors (7). MMTS inhibited the UV-induced mutation in Escherichia coli B/r WP2 by activation of the excision-repairing systems.

ACF were first observed in the colon of carcinogen-treated rodents (8). They are putative preneoplastic lesions of colon cancer in both rodent (9) and humans (10) and have been proposed as intermediate biomarkers for colon cancer in many chemopreventive studies (11–16). ODC is the first enzyme in the polyamine biosynthesis pathway (17). Polyamines play essential roles in cell proliferation and differentiation and participate in macromolecular synthesis. The induction of ODC has been implicated as being important to carcinogenesis, and ODC activity is an intermediate biomarker of cell proliferation in chemopreventive studies (11–13, 18). Similarly, AgNOR number/nucleus and BrdUrd-labeling index in the target organ are suggested to be useful biomarkers of cell proliferation. In previous studies, we developed an in vivo short-term model to adapt chemopreventive agents by using a combination of ACF and these cell proliferation biomarkers in rat colon (11, 12). In these studies, we proved that some of the naturally occurring products reduced AOM-induced colonic ACF, ODC activity, AgNOR number, and polyamine levels in our short-term model, and these compounds were proved as chemopreventive agents against rat colon carcinogenesis in the long-term model (15, 19–22).

In this study, we conducted two experiments to investigate modifying effects of MMTS on AOM-induced rat intestinal carcinogenesis. In experiment 1, modifying effects of MMTS on AOM-induced ACF, ODC activity, and AgNOR number in the colon of male F344 rats by using our in vivo short assay were investigated. In experiment 2, modifying effects of feeding MMTS during the postinitiation phase on AOM-initiated intestinal carcinogenesis in male F344 rats in the long-term assay were investigated. The ultimate goal of this study is to determine whether MMTS has potential as a chemopreventive agent against colon cancer.

MATERIALS AND METHODS

Animals and Diets

Male F344 rats, 4 weeks of age, purchased from Shizuoka Laboratory Animal Center (Hamamatsu City, Japan), were quarantined for 1 week and then randomized into experimental and control groups. All animals were housed in four to five/cage. The housing room was controlled at 23 ± 2°C, 50 ± 10% humidity, and with a 12-h light/12-h dark cycle. Powdered CE-2 (Clea Japan, Inc., Tokyo, Japan) was used as a basal diet during the experiment.

Chemicals

AOM and MMTS (Fig. 1) were purchased from Sigma Chemical Co. (St. Louis, MO).

Experimental Procedure

Experiment 1. A total of 36 male rats was divided into 4 groups (Fig. 2). Starting at 6 weeks of age, rats in groups 1 and 2 were given s.c. injections of AOM (15 mg/kg body weight) once a week for 3 weeks. Animals in groups 2 and 3 were fed the diets containing 100 ppm MMTS for 5 weeks, starting at
Animals were weighed weekly until they reached 14 weeks of age, and then they were weighed every 4 weeks. All animals were sacrificed by decapitation at 36 weeks after the start of the experiment, and complete necropsies were performed on all animals. All organs, especially the intestines, were carefully inspected grossly and all abnormal lesions were examined histologically. Histological examination was done by conventional methods after hematoxylin and eosin staining. Intestinal tumors were diagnosed according to the criteria described by Ward (23).

Identification of ACF

At the termination of the studies, the colons of animals from each group were removed, flushed with saline, slit open longitudinally from the cecum to the anus, placed between two pieces of filter paper, and fixed in buffered 10% formalin for 24 h. Then they were stained with 0.5% methylene blue in saline according to the procedure of Bird (8) to observe ACF. The number of ACF/colon and the number of aberrant crypts in each focus were determined according to the procedure of Bird (8) to observe ACF. The number of aberrant crypts in each focus was counted by eye in a dissecting microscope. The ACF were classified according to the criteria described by Bird (8) as follows.

- Normal crypts: crypts with normal architectural patterns.
- Aberrant crypts: crypts with altered architectural patterns.
- ACF: clusters of aberrant crypts.

The number of ACF/colon was determined by counting the number of foci of ACF in each group. The number of aberrant crypts in each focus was determined by counting the number of aberrant crypts in each focus. The ACF were classified into three categories: (a) increased size, (b) thicker epithelial cell lining, and (c) increased pericryptal zone relative to normal crypts.

ODC Activity

At necropsy of the remaining six animals, their colons were immediately removed. The colon was rinsed in saline, slit open longitudinally, and freed from all the contents. It was laid flat on a glass plate, and the mucosa was scraped with a stainless steel, disposable, microtome-bladed knife, S35 (Feather Safety Razor Co., Ltd., Osaka, Japan). Each colonic mucosa was homogenized in 1.5 ml of homogenizing buffer [250 mM sucrose and 50 mM Tris-HCl (pH 7.4) containing 1 mM DTT, 1 mM EDTA, and 0.4 mM pyridoxal 5'-phosphate] by using a Polytron. The homogenates were centrifuged at 15,000 rpm at 4°C for 30 min. The resulting cytosol fraction was used for determination of ODC activity and protein concentration. ODC activity in the colonic mucosa was determined by the modified method described previously (24). The incubation mixture in a final volume of 40 μl [50 mM N-(2-hydroxyethyl)-piperazine-N'-(2-ethanesulfonic acid (pH 7.4), 1 mM EDTA, 0.25 mM pyridoxal phosphate, 1 mM DTT, and 0.25 μCi of L-[1-14C] ornithine (specific activity, 42.47 mCi/mmol; NEN)] was incubated at 37°C for 1 h. The reaction was stopped by adding 20 μl of 6N HCl, and the 14CO2 released was collected on 10% KOH-saturated discs for another 15 min. 14CO2 in the form of K2CO3 was counted in a scintillation counter. The results were expressed as pmol 14CO2/h/mg protein.

AgNOR Count and BrdUrd-labeling Index

In experiment 1, the number of AgNORs of the mucosal epithelium of colon in each group was determined. The colon was removed and fixed in 10% buffered formalin and embedded in paraffin; two serial sections (3 μm in thickness) were made. The one section was used for staining AgNOR, and the other was stained with hematoxylin and eosin for histological examination. AgNOR staining was carried out according to the method described previously (25). For determination of AgNOR number on the cell nuclei, 20 well-oriented crypts, in which the base, lumen, and top of crypts could be seen, were used. AgNORs were counted on a AgNOR-stained section by a microscope at ×400. Data were expressed as the number of AgNORs/nucleus. In experiment 2, animals were given an i. p. injection of 5-bromo-2'-deoxyuridine and saline solution (50 mg/kg body weight, Sigma Chemical Co., St. Louis, MO) 1 h before being killed for the measurement of BrdUrd. The colon was removed and divided into three equal portions, named the upper, middle, and lower colon, and fixed in 10% buffered formalin. All portions of colon were embedded in paraffin, and one section (3 μm in thickness) was cut. The section was used for immunohistochemical detection of BrdUrd incorporation by using a Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA). For determination of BrdUrd-labeling index, 20 well-oriented crypts, in which the base, lumen, and top of the crypts could be seen, were observed for each animal in three portions of the colon. The number and the position of the labeled cells in each crypt column were recorded in terms of serial position counting upward from position 1, at the base of crypt, to the mouth of the crypt. The percentage of labeled cells (labeling index) was determined for the whole crypt by calculating the labeled cells/total number of cells ratio × 100.

Polyamine Levels of Rats' Blood

In experiment 2, the polyamines in the blood of rats were measured by means of a new enzymatic method developed by Koide et al. (26). At sacrifice,
blood was collected from rats in each group, and diamine, spermine, and spermidine were determined by a new enzymatic differential assay.

**Statistical Analysis**

The data on tumor incidence (experiment 2) were analyzed statistically by Fisher's exact probability test or the χ² test. The other data were analyzed by Student's t test for paired samples or a two-sample t test with Welch's correction. The results were considered statistically significant if P ≤ 0.05.

**RESULTS**

**Experiment 1**

**General Observations.** The body weight gains during the experiment and liver weights at death are presented in Table 1. No significant effect of AOM and/or MMTS treatment on body and liver weights was observed. Food consumption for MMTS treatment groups was approximately 15.9 g/day/animal. No neoplasms were found on macroscopic and microscopic examinations of all organs of rats in each group. MMTS administration decreased colon mucosal ODC activity, but no significant differences were present. The data on AgNOR enumeration are also shown in Table 3. The mean number of AgNORs/nucleus in group 1 was greater than those of the other groups. MMTS administration significantly decreased the number of AgNORs/nucleus (P < 0.01).

**Colon Mucosal ODC Activity and Number of AgNORs of Colon.** The ODC activity of colon mucosa is indicated in Table 3. The mean ODC activity of colon mucosa of rats in group 1 (AOM alone) was greater than those of the other groups. MMTS administration decreased colon mucosal ODC activity, but no significant differences were present. The data on AgNOR enumeration are also shown in Table 3. The mean number of AgNORs/nucleus in group 1 was greater than those of the other groups. MMTS administration significantly decreased the number of AgNORs/nucleus (P < 0.01).

**Experiment 2**

**General Observations.** One rat from group 2, which was moribund and necropsied on the 239th day, had a small intestinal tumor (adenocarcinoma). Therefore, the rats alive on that day were counted as effective animals.

The mean body weight, liver weight, and relative liver weight (g/100 g body weight) in each group are indicated in Table 4. The average body weights of rats receiving AOM with MMTS (groups 2 and 3) were lower than those weights of rats given AOM alone (group 1); this was especially true for rats given AOM with MMTS at 100 ppm for which average body weights were significantly lower than that of rats in group 1 (P < 0.001). The mean liver weights and relative

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### Table 1 Mean body and liver weights of rats in each group (experiment 1)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Mean body weight (g)</th>
<th>Mean liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>90.5 ± 5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>121.2 ± 6.6</td>
</tr>
<tr>
<td>2</td>
<td>AOM + MMTS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.1 ± 3.1</td>
<td>120.8 ± 5.4</td>
</tr>
<tr>
<td>3</td>
<td>MMTS alone</td>
<td>91.2 ± 6.1</td>
<td>125.1 ± 5.7</td>
</tr>
<tr>
<td>4</td>
<td>No treatment</td>
<td>85.5 ± 5.1</td>
<td>116.8 ± 6.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SD.
<sup>b</sup> Chemical was given at the level of 100 ppm in the diet.

### Table 2 Effect of MMTS on AOM-induced ACF in rat colon (experiment 1)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Incidence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of ACF/colon</th>
<th>No. of aberrant crypts/colon</th>
<th>No. of aberrant crypts/focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>6/6</td>
<td>131.3 ± 16.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>234.6 ± 63.2</td>
<td>1.94 ± 0.13</td>
</tr>
<tr>
<td>2</td>
<td>AOM + MMTS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6/6</td>
<td>86.8 ± 23.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>145.2 ± 42.0</td>
<td>1.67 ± 0.08</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of rat colons with ACF/total number of rat colons scored.
<sup>b</sup> Mean ± SD.
<sup>c</sup> Chemical was given at the level of 100 ppm in the diet.
<sup>d</sup> Significantly different from group 1 by Student's t test (P < 0.05).

### Table 3 ODC activity, number of cells/crypt column, and AgNORs count of the colonic epithelium of rats in each group (experiment 1)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>No. of rats examined</th>
<th>Colonic ODC activity (pmol &lt;sup&gt;14&lt;/sup&gt;C&lt;sub&gt;O&lt;/sub&gt;H&lt;sub&gt;2&lt;/sub&gt;/mg protein)</th>
<th>No. of cells/crypt column</th>
<th>AgNORs count/nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>6</td>
<td>37.7 ± 20.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>31.9 ± 4.9</td>
<td>1.73 ± 0.25</td>
</tr>
<tr>
<td>2</td>
<td>AOM + MMTS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6</td>
<td>17.1 ± 5.8</td>
<td>32.6 ± 5.5</td>
<td>1.25 ± 0.24</td>
</tr>
<tr>
<td>3</td>
<td>MMTS alone</td>
<td>6</td>
<td>7.2 ± 8.3</td>
<td>30.5 ± 4.8</td>
<td>1.09 ± 0.29</td>
</tr>
<tr>
<td>4</td>
<td>No treatment</td>
<td>6</td>
<td>4.9 ± 3.5</td>
<td>36.7 ± 4.9</td>
<td>1.01 ± 0.28</td>
</tr>
</tbody>
</table>

<sup>e</sup> Mean ± SD.
<sup>b</sup> Chemical was given at the level of 100 ppm in the diet.

### Table 4 Mean body, liver, and relative liver weights of rats in each group (experiment 2)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>No. of effective rats&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Relative liver weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>30</td>
<td>330 ± 26&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.0 ± 1.3</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>AOM → MMTS, 20 ppm</td>
<td>24</td>
<td>320 ± 17</td>
<td>8.7 ± 1.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.7 ± 0.5&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>AOM → MMTS, 100 ppm</td>
<td>28</td>
<td>288 ± 37&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.5 ± 1.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.6 ± 0.5&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>No treatment</td>
<td>29</td>
<td>351 ± 48</td>
<td>11.7 ± 2.9</td>
<td>3.3 ± 0.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Rats that survived for more than 239 days.
<sup>b</sup> Mean ± SD.
<sup>c</sup> Significantly different from group 1 by Student's t test (P < 0.005).
<sup>d</sup> Significantly different from group 1 by Welch's t test (P < 0.05).
<sup*e</sup> Significantly different from group 1 by Welch's t test (P < 0.001).
CHEMOPREVENTION BY S-METHYL METHANE THIOSULFONATE

Table 5 Incidences of neoplasms in small and large intestines of rats in each group (experiment 2)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>No. of effective ratsa</th>
<th>Entire intestine</th>
<th>Small intestine</th>
<th>Large intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total ADb ADc</td>
<td>Total AD ADc</td>
<td>Total AD ADc</td>
</tr>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>30</td>
<td>19 (63)</td>
<td>8 (27)</td>
<td>15 (50)</td>
</tr>
<tr>
<td>2</td>
<td>AOM → MMTS, 20 ppm</td>
<td>24</td>
<td>10 (42)</td>
<td>4 (17)</td>
<td>7 (29)</td>
</tr>
<tr>
<td>3</td>
<td>AOM → MMTS, 100 ppm</td>
<td>28</td>
<td>6 (21)</td>
<td>3 (11)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>4</td>
<td>No treatment</td>
<td>29</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

a Rats that survived for more than 239 days.
b AD, adena; ADC, adencarcinoma.
c Significantly different from group 1 by \( P < 0.05 \).
d Significantly different from group 1 by Fisher's exact probability test (\( P < 0.005 \)).
e Significantly different from group 1 by Student's \( t \) test (\( P < 0.001 \)).

No. of rats with neoplasms (%)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>No. of rats with neoplasms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>10/10</td>
</tr>
<tr>
<td>2</td>
<td>AOM → MMTS, 20 ppm</td>
<td>10/10</td>
</tr>
<tr>
<td>3</td>
<td>AOM → MMTS, 100 ppm</td>
<td>10/10</td>
</tr>
<tr>
<td>4</td>
<td>No treatment</td>
<td>0/10</td>
</tr>
</tbody>
</table>

Table 6 Effect of MMTS on AOM-induced ACF in rat colons (experiment 2)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Incidencea</th>
<th>No. of ACF/colon</th>
<th>No. of aberrant crypts/colon</th>
<th>No. of aberrant crypts/focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>10/10</td>
<td>192.5 ± 55.4</td>
<td>701.3 ± 230.5</td>
<td>3.62 ± 0.40</td>
</tr>
<tr>
<td>2</td>
<td>AOM → MMTS, 20 ppm</td>
<td>10/10</td>
<td>170.6 ± 48.4</td>
<td>609.6 ± 173.9</td>
<td>3.60 ± 0.32</td>
</tr>
<tr>
<td>3</td>
<td>AOM → MMTS, 100 ppm</td>
<td>10/10</td>
<td>131.4 ± 43.6</td>
<td>471.1 ± 172.5</td>
<td>3.54 ± 0.31</td>
</tr>
</tbody>
</table>

a Number of rat colons with ACF/Total number of rat colons scored.
b Mean ± SD.
c Significantly different from group 1 by Student's \( t \) test (\( P < 0.005 \)).

Table 7 ODC activity, number of cells/crypt column, and BrdUrd-labeling index of the colonic epithelium of rats in each group (experiment 2)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>No. of rats examined</th>
<th>Colonic ODC activity (pmol (^14)CO(_2)/mg protein)</th>
<th>No. of cells/crypt column</th>
<th>BrdUrd-labeling index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>6</td>
<td>60.3 ± 58.9</td>
<td>40.9 ± 4.8</td>
<td>13.6 ± 5.1</td>
</tr>
<tr>
<td>2</td>
<td>AOM → MMTS, 20 ppm</td>
<td>6</td>
<td>36.8 ± 30.9</td>
<td>39.2 ± 6.9</td>
<td>4.5 ± 4.9*</td>
</tr>
<tr>
<td>3</td>
<td>AOM → MMTS, 100 ppm</td>
<td>6</td>
<td>20.6 ± 28.6</td>
<td>43.9 ± 6.6</td>
<td>3.4 ± 3.1b</td>
</tr>
<tr>
<td>4</td>
<td>No treatment</td>
<td>6</td>
<td>6.4 ± 5.1</td>
<td>43.5 ± 5.0</td>
<td>2.5 ± 1.8</td>
</tr>
</tbody>
</table>

a Mean ± SD.
b Significantly different from group 1 by Student's \( t \) test (\( P < 0.05 \)).

Table 8 Polyamine concentrations of rats' blood in each group (experiment 2)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Diamine</th>
<th>Spermidine</th>
<th>Spermine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM alone</td>
<td>0.7 ± 1.1</td>
<td>24.0 ± 4.1</td>
<td>6.4 ± 2.2</td>
<td>31.1 ± 2.9</td>
</tr>
<tr>
<td>2</td>
<td>AOM → MMTS, 20 ppm</td>
<td>1.3 ± 1.6</td>
<td>22.4 ± 3.6</td>
<td>5.5 ± 2.3</td>
<td>29.2 ± 2.1a</td>
</tr>
<tr>
<td>3</td>
<td>AOM → MMTS, 100 ppm</td>
<td>0.9 ± 1.0</td>
<td>19.9 ± 4.7</td>
<td>6.0 ± 2.2</td>
<td>26.8 ± 3.7f</td>
</tr>
<tr>
<td>4</td>
<td>No treatment</td>
<td>0.3 ± 0.3</td>
<td>18.7 ± 4.2</td>
<td>6.1 ± 1.0</td>
<td>25.0 ± 3.8</td>
</tr>
</tbody>
</table>

a Mean ± SD.
b Significantly different from group 1 by Student's \( t \) test (\( P < 0.05 \)).
c Significantly different from group 1 by Student's \( t \) test (\( P < 0.01 \)).
d Significantly different from group 1 by Student's \( t \) test (\( P < 0.001 \)).
MMTS (groups 2 and 3), mean colonic ODC activities were lower than that of group 1, but no significant differences were present. The data on BrdUrd-labeling index are also shown in Table 7. The mean value of BrdUrd-labeling index in group 1 was greater than those of the other groups. The mean values of BrdUrd-labeling indexes of rats treated with AOM and MMTS were significantly smaller than that of group 1 ($P < 0.05$).

**Blood Polyamine Levels.** Polyamine concentrations in blood are indicated in Table 8. Spermidine, spermine, and total polyamine levels in the blood of rats in group 1 were higher than those of the other groups. Total polyamine levels of rats treated with AOM and MMTS at both doses were significantly smaller than that in group 1 ($P < 0.05$ for 20 ppm and $P < 0.001$ for 100 ppm). The spermidine level in rats treated with AOM and MMTS at 100 ppm was significantly smaller than in group 1 ($P < 0.01$).

**DISCUSSION**

In this study, MMTS administration during the initiation phase decreased AOM-induced colonic ACF formation and AgNOR count/nucleus by using in vivo short-term assay (experiment 1). Feeding of MMTS during the postinitiation phase decreased the incidences of neoplasms in intestines induced by AOM and also decreased colonic ACF formation, BrdUrd-labeling index, and blood polyamine levels in the long-term experiment (experiment 2). These results indicate that MMTS inhibited the development of both preneoplastic and neoplastic colonic lesions induced by AOM.

ACF are one of the earliest hallmarks of colon carcinogenesis (27). They are readily utilizable as intermediate end points in colon cancer prevention studies because they are easily and rapidly quantified topographically in a methylene blue-stained whole colon from rodents fed test chemicals (12, 16). In addition, ACF have been reported to be associated with gene alterations in rats (28–32) and in human (33, 34). In this study, MMTS administration inhibited the development of ACF in the short-term assay. Similarly, feeding of MMTS during the postinitiation phase reduced the number of ACF in the long-term assay. These results suggest that MMTS has blocking effects on the formation of ACF and suppressing effects on the growth of ACF.

Polyamines, spermidine, spermine, and their precursor diamine putrescine are ubiquitous in eukaryotes. Polyamines play essential roles in normal cell proliferation, differentiation, and malignant transformation (17). The precise cellular function of polyamines is not known, although the effects attributed to these molecules are extensive. ODC, which decarboxylates ornithine to putrescine, is characterized by its inducibility and rapid turnover rate. ODC is the key enzyme in mammalian polyamine synthesis. Several studies have demonstrated that tumor promoters induce ODC activity in mouse skin and colonic mucosa, suggesting a relationship between the tumor promotion and the induction of ODC activity in their respective target organs (35–37). Previous studies have also demonstrated that the specific irreversible enzyme-activated or suicide inhibitor of ODC, namely DFMO, can inhibit colon tumors (38–41) or other organs (18, 42) induced by carcinogens. Recently, Jasnis et al. (43) demonstrated that the depletion of polyamines by DFMO results in a significant suppression of the angiogenic activity of tumor cells or spleen lymphocytes of tumor-bearing mice. In our previous study, colonic ODC activity and polyamine levels are very useful intermediate biomarkers in colon carcinogenesis (12). In this study, MMTS administration slightly decreased colonic ODC activity in both experiments. In the long-term assay, MMTS also reduced polyamine synthesis in blood. Malignant cell proliferation is associated with an increase in intracellular polyamine metabolism, which itself appears to be in equilibrium with the extracellular circulating polyamine compartments (44).

MMTS has the potential to inhibit polyamine synthesis like DFMO and might have antipromotive effects. Cell kinetic analysis in the colonic mucosa of individuals at increased risk for colon cancer has revealed an anomalous expansion of epithelial cells within the colonic crypts. We have demonstrated that AgNOR and the BrdUrd-labeling index are very useful biomarkers for cell proliferation in colon carcinogenesis (19). In the present study, MMTS decreased the number of the AgNOR count/nucleus in the short-term assay, and feeding of MMTS during the postinitiation phase decreased the BrdUrd-labeling index in colonic mucosa in the long-term assay. Thus, MMTS had blocking effects against carcinogens through reduction of the formation of ACF, cell proliferation, and ODC activity. MMTS also had suppressive effects through reduction of ODC activity and inhibition of polyamine synthesis with the reduction of cell proliferation.

With regard to the toxicity of MMTS, rats fed MMTS at 1000 mg/kg body weight did not have any acute toxic signs within 7 days. In this study, MMTS had no effects on body and liver weights of rats in the short-term assay; however, feeding of MMTS at 100 ppm during the postinitiation phase decreased body and liver weights of rats in the long-term assay. The mortality rate for rats treated with AOM and 100 ppm MMTS was lower than that for rats treated with AOM and 20 ppm MMTS. These phenomena were caused by lowered food consumption of MMTS mixed diet, especially 100 ppm MMTS diet. This action may lead some restriction effects on the tumor incidence. However, in the present study, animals given MMTS showed no evidence of toxicity (45).

In conclusion, dietary administration of MMTS, isolated from cauliflower, during the postinitiation phase inhibited the incidences of intestinal neoplasms induced by AOM in rats. Also, MMTS reduced the formation and the growth of colonic ACF and inhibited expression of several cell proliferation biomarkers. These results suggest that MMTS might be a possible chemopreventive agent for colon cancer.

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**REFERENCES**


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Chemoprevention of Azoxy methane-induced Colon Carcinogenesis by Dietary Feeding of S-Methyl Methane Thiosulfonate in Male F344 Rats

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