Anticancer Effects of a Tertiary Sulfonium Compound, Dimethylsulfoniopropionate, in Green Sea Algae on Ehrlich Ascites Carcinoma-Bearing Mice

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Summary The saline and dimethylsulfoniopropionate (DMSP) solutions at 5, 10 and 20 mM were preliminarily injected intraperitoneally every other day into two control and three DMSP groups of mice (n=8) for 2 wk and thereafter Ehrlich ascites-carcinoma (EAC) cells were peritoneally injected to one control and three DMSP groups of mice, leaving one control group without the EAC injection. Then, the body weight and survival time of all mice were examined over a long rearing time up to 300 d. All EAC-bearing mice, especially the carcinoma control and 5 mM DMSP-carcinoma group mice, rapidly increased their body weights early and then died by day 50 and day 90, respectively. In contrast, the administration of 10 and 20 mM DMSP solutions prolonged the lives of EAC-bearing mice at the survival rate of 50 and 63% respectively up to 300 d without any side effects. Furthermore, the administration of 10 mM DMSP solution proved to activate the delayed-type hypersensitivity of EAC bearing-mice, and the DMSP solutions over the concentrations of 5 to 30 mM to slightly reduce the dead cells in EAC cells on the synthetic medium. Accordingly, the preliminary supplementation of 10 and 20 mM DMSP solutions to EAC-bearing mice was proven to maintain their lives at high survival rates without direct damage to EAC cells for a long time, probably due to the activation of the immune system without any side effects.

Key Words dimethylsulfoniopropionate, Ehrlich ascites carcinoma, survival time, DTH reaction

A tertiary sulfonium compound, dimethylsulfoniopropionate (DMSP), proves to be naturally synthesized and contained in large amounts in green sea algae (1, 2) and sea plankton (3) and to be distributed to various aquatic animals (1). Therefore, DMSP has been habitually ingested in large and small amounts around the world, especially in Japan for a long time (4). However, there is no report on research into the physiological role of DMSP except for compatible solutes (osmoregulant, cryoprotectant (3, 5)) although there are a number of reports concerning its metabolism in micro-, macroalgae and halophytic plants (6). We therefore have examined the effects of DMSP and related compounds on a number of diseased animals, which have verified that the compound ameliorates and/or mitigates a variety of diseases, especially serious contemporary disorders: stress-induced gastric ulcers in rats (7), acute alloxan-diabetes mellitus in rats (8), hyperhomocysteinemia (9, 10), early aging and loss of learning and memory in SAMP-1 and -P8 (Alzheimer’s disease) (11, 12), 3′-methyl-4-dimethylamino-azobenzene-(3′-MeDAB)-induced liver cancer in rats (13) and Parkinson’s disease caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice (14) and SAMP8 (15). Moreover, the amelioration of the early aging and loss of learning and memory in SAMP8 was confirmed by experiments with diets containing 5% of pulverized dry green sea algae (16).

In contrast, other naturally occurring-sulfonium compounds, dimethylsulfonioacetate of animal origin (17) or methylmethionine sulfonium of plant origin (18), prove to be a methyl donor for the methylation reaction of homocysteine to methionine (9, 19) or to be an anti-gastric ulcer agent (20). However, the respective transfer- and the anti-ulcer functions of these compounds are proven to be less effective than those of DMSP (7, 9).

Among a number of diseases, cancer has remained a major cause of death and the number of individuals living with various cancers has continued to expand over a long period. To further examine the multifunction of DMSP, we attempted here to examine the effects of DMSP on a cancer model mouse, Ehrlich ascites carcinoma (EAC)-bearing mice, for a long time.

Materials and Methods

Dimethylsulfoniopropionate (DMSP) was synthesized by refluxing dimethylsulfide and 3-bromopropionic acid and purified to 99.8% purification (according to element analysis) by washing with ethyl ether and crystal-
lizing from methanol (21). Ehrlich ascites carcinoma (EAC) cells were kindly donated by Cancer Cell Repository, Research Institute for Tuberculosis and Cancer, Tohoku University and maintained in vivo by weekly intraperitoneal transplantation. Bovine serum albumin (Lyophilized) and Freund incomplete adjuvant (10 mL \( \times 5\)A) were purchased from Wellcome Co. Ltd., USA (through Wako Pure Chemical Industries, Ltd., Japan). Four-week-old ICR/Jcl male mice were purchased from CLEA Japan, Inc., Japan. The test mice were freely given distilled water and solid diets (“MF,” CLEA Japan, Inc.) and reared under 12 h/d light cycles at around 60% relative humidity and 24±2°C during the experimental period. After preliminarily acclimation for 1 wk, these mice were divided into 5 groups (8 animals in each group). Thereafter, the saline solution, 5, 10 and 20 mM DMSP saline solutions (1 mL in each) were peritoneally injected every other day into two control and three DMSP groups of mice for 2 wk. Then, 0.5 mL mixture (5\( \times 10^3\) cells/mL) of EAC cells which had been washed twice with and suspended in Ham’s medium (22) were peritoneally injected into one control group (Carcinoma group) and the DMSP groups (DMSP-Carcinoma group) of mice. Thereafter, the body weight and number of surviving mice in all the groups were measured and counted up to 300 d after the injection of the EAC cell suspension. Simultaneously, to examine the delayed-type hypersensitive (DTH) immune activity, the same type of mice as stated above were preliminarily housed, divided into three groups \((n=6)\) and subjected to the injection of the saline solution, 5 and 10 mM DMSP solutions and EAC cells in the same way as mentioned above. Then, the mixture (0.2 mL) of equal volume of bovine serum albumin saline solution (10 mg/mL) and Freund incomplete adjuvant solution were subcutaneously injected into the center of dorsal portions in three groups of mice. After 3 wk, the saline solution (3.5 mL, pH 6.3) containing bovine serum albumin (20 mg) was freshly mixed with 1 mL of 10% aluminum sulfate \((K^+)\) and centrifuged, which steps were repeated three times. The mixture (0.02 mL) of thus treated bovine serum albumin (0.5 mg/mL) was injected into the skin of the rear foot arch in test mice. After 24 h, the vertical height from the foot instep to foot arch was measured with slide and outside calipers (5 times each) and the swelling in the foot arch was expressed as the ratio (%) \((\text{means±SD}, n=10)\) of the height of the swollen portion versus the initial height to the arch from the instep. Moreover, EAC cells freshly taken from the ascites fluid in EAC-bearing mice were suspended in Ham’s medium containing 8% fetal bovine serum and centrifuged. The washed EAC cells were suspended in the Ham’s medium containing DMSP at indicated concentrations \((2.5\times 10^4\text{ cells/mL})\) and incubated in a humidified atmosphere of 5% \(CO_2\) in air at 37°C for 5 h in a closed and sterilized box \((\text{Ch-16, Hitachi Co., Ltd., Japan})\). One milliliter was drawn from the incubation mixture at indicated times. Then, dead cells were counted in a Neubauer haematocytometer using trypan blue dye and expressed in terms of the rate (%) \((\text{means±SD}, n=5)\) of dead cells versus all the EAC cells in each group. All other chemicals used were of the best available quality. The care and treatment of the experimental animals were in accordance with the guidelines of the Animal Center of the National Research Council for the Care and Use of Laboratory Animals (23). The statistical analyses were performed using ANOVA and the Scheffe test (Fig. 1), and Fisher’s PLSD test (Tables 1, 2) (StatView-SAS Institute, Inc, version 5 software).

**Results**

The effects of DMSP solutions at the concentrations of 5, 10 and 20 mM on the body weight were examined with increasing rearing times up to 300 d. The results are given in Fig. 1. The body weight appeared to rapidly increase in order of the mice in the Carcinoma, 5 mM DMSP-Carcinoma, 20 mM DMSP- and 10 mM DMSP-Carcinoma and Control groups, especially in the former two groups, and in this order reached maxima on the 40th, 44th, 44th and 48th day. Thereafter, the body weight of all the mice rapidly decreased except for the Control and 5 mM DMSP-Carcinoma group mice. However, the decline of body weights in the Carcinoma...
group mice ceased on the 47th day and that in the 10 mM and 20 mM DMSP-Carcinoma group mice simultaneously stayed even on 54th day but not that in the Control and 5 mM DMSP-Carcinoma group mice. Thereafter, the 10 and 20 mM DMSP-Carcinoma group mice exhibited almost the same growth curve as that in the Control group mice up to 300 d. The results of the survival of all the groups are given in Fig. 2, which clearly indicates that all the mice in the Carcinoma and the 5 mM DMSP-Carcinoma group died by the 50th and 90th day, respectively. Four mice in the 10 mM DMSP-Carcinoma group died by the 64th day but thereafter the residual four mice continued to live up to 300 d (50% survival rate). Three mice in the 20 mM DMSP-Carcinoma group died by the 53rd day and the residual five mice survived up to 300 d (63% survival rate).

The effects of 5 and 10 mM DMSP solution on the DTH immune reaction in EAC bearing-mice were examined by the footpad test. The results are given in Table 1. The preliminary administration of saline and 5 and 10 mM DMSP solutions, especially the last solution, proved to activate the DTH immune system of the diseased mice. Moreover, the effects of DMSP at the concentrations of 5 to 30 mM on the EAC cells were examined on synthetic medium for 5 h. The results are given in Table 2, which indicated that the dead cells decline in a dose-dependent manner of DMSP, compared to the mean values among these groups.

**Discussion**

DMSP occurs widely throughout the world in a number of sea creatures at varying levels: plankton, algae, prawns, shellfish, fish, whales and so on, along the “food chain” in the sea. In contrast, the long term administration of DMSP at 0.5 mM proves to show no toxicity in SAM but rather to provide a new lease on life (11). Furthermore, a direct dose of DMSP as an aqueous paste (7 g net wt./kg body wt. (n/H11005/5)) to the stomach in rats exhibited slight toxicity: loss of appetite, slow movement, diarrhea, ruffled hair or hypothermia for several hours. However, these symptoms rapidly disappeared and the same behavior as that in normal rats was displayed without mortality even after several months.

Table 1. Effects of 5 and 10 mM DMSP solutions on the delayed-type hypersensitive immune reaction of EAC bearing-mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Delayed-type hypersensitivity</th>
<th>Swelling ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.11±0.12</td>
</tr>
<tr>
<td>DMSP (5 mM)</td>
<td></td>
<td>0.52±0.23*</td>
</tr>
<tr>
<td>(10 mM)</td>
<td></td>
<td>2.49±0.45*</td>
</tr>
</tbody>
</table>

Values are the mean±SD of six mice (determinations/mice:10 times). DMSP, dimethylsulfoniopropionate; EAC, Ehrlich ascites carcinoma. * Significantly different (p<0.05) from the values in the control and 5 mM DMSP group.

Table 2. Effects of several concentrations of DMSP on EAC cells on the synthetic culture medium.

<table>
<thead>
<tr>
<th>Group</th>
<th>Incubation time (h)</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2.04±0.52</td>
<td>4.26±0.43</td>
<td>5.31±0.52</td>
<td></td>
</tr>
<tr>
<td>DMSP (5 mM)</td>
<td></td>
<td>0.78±0.35*</td>
<td>1.65±0.44*</td>
<td>1.74±0.52*</td>
<td></td>
</tr>
<tr>
<td>(10 mM)</td>
<td></td>
<td>0.58±0.28*</td>
<td>1.31±0.21*</td>
<td>1.45±0.42*</td>
<td></td>
</tr>
<tr>
<td>(20 mM)</td>
<td></td>
<td>0.35±0.26*</td>
<td>1.13±0.30*</td>
<td>1.13±0.61*</td>
<td></td>
</tr>
<tr>
<td>(30 mM)</td>
<td></td>
<td>0.34±0.19*</td>
<td>0.45±0.32*#@$</td>
<td>0.48±0.43*#@$</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean±SD (n=5). DMSP, dimethylsulfoniopropionate; EAC, Ehrlich ascites carcinoma. *, #, $ Significant difference (p<0.05) between values in the control and the 5, 10 and 20 mM DMSP groups at indicated times.
Therefore, we attempted here to investigate the ameliorating effects of DMSP on EAC-bearing mice by examining the fluctuation of body weight and survival time (a greatly significant measure for evaluating the anticancer efficacy) longer that we have ever examined in this line of work. DMSP was preliminarily added every second day for 2 wk before the injection of EAC cells because we have more or less eaten habitually a variety of sea creatures, especially green sea algae, as a foodstuff for a long time (4). The obtained results clearly represented that the intraperitoneal supplementation (free cell cancer) but not the subcutaneous supplementation (solid cancer) of EAC cells without DMSP rapidly increases the ascites fluid resulting in an increase of body weight and all the mice in this group die by day 50 under the experimental conditions. The mice treated with EAC cells were thus proven to be a serious and significant vehicle for anticancer research. The preliminary administration of 10 or 20 mM DMSP solution proved to maintain the lives of half the EAC-bearing mice or more up to 300 d and further, they continued to survive without untoward side effects as well as the Control mice did. However, there were large differences in the effects on the survival of EAC bearing-mice between 5 and 10 mM DMSP solutions. In contrast, the administration of not 5 but 10 mM DMSP solution was proven to largely elicit the activation of the DTH immune system in the footpad test for EAC bearing-mice. This is considered to cause the large differences in the survival effects between the two solutions. Furthermore, the in vitro experiments with the synthetic medium demonstrated that DMSP at the concentrations of 5 to 30 mM gives no damage to EAC cells and rather slightly reduces the death of EAC cells almost in parallel with the increased administrations. These results represent that DMSP does not directly damage EAC cells living in the abdominal cavity of diseased mice.

The previous report with dietary supplementation (0.06%) of 3′-MeDAB demonstrated that the drug clearly induces liver cancer in rats, but that the oral administration of 10 mM DMSP solution to the diseased rats significantly restores the increased liver weight and the elevated activity of the marker enzyme of liver, γ-glutamyltranspeptidase, in the serum to the normal levels and activates the immune system (delayed-type hypersensitive (DTH) immune reaction with phytohemagglutinin (PHG) in ear skin) lowered by the cancer to the same levels as those in the control group rats treated with PHG in the 33rd week (1,3). Accordingly, these findings may indicate that DMSP induces the activation of the immune system, which plays a significant role in ameliorating and/or mitigating not only 3′-MeDAB-induced liver cancer (solid cancer) in rats (1,3) but also EAC (free cell cancer) in mice without any side effects.

The present experiments may represent that the injection of 1 mL of 10 mM DMSP solution (1.7 mg DMSP) is equivalent to the ingestion of ca. 0.28 g of wet algae (6.1 mg DMSP/g (2)) or ca. 0.04 g dry algae (41.2 mg DMSP/g) per 40 g body wt. (mouse) per day, which may require the ingestion of ca. 350 g wet algae (2.1 g DMSP) or ca. 50 g dry algae (2.1 g DMSP) per 50 kg body wt. (man), every other day for 2 wk. In contrast, the amounts of DMSP in the commercially available wet and dry algae appear to be capable of being elevated about 5–10 fold by a newly developed dry procedure, in which the biosynthesis of DMSP probably proceeds in the course of drying the wet and dry algae (unpublished data). The methods may reduce by 1/5–1/10 the amounts of these algae needed for humans every second day for 2 wk. Moreover, the obtained results indicated that the injection of 10 mM DMSP solution (1 mL) every other day for 2 wk ameliorates Ehrlich ascites carcinoma at a high rate in mice, and that the free ingestion of 10 mM DMSP solution for 33 wk reinstates the 3′-MeDAB-induced liver cancer in rats (1,3). Our preliminary experiments further revealed that the ingestion of 70 mM DMSP solution as a drink solution ad libitum, of great interest, does not affect the growth of the mice at the age of 4 wk for 15 d. Therefore, all the above results and findings may support claims that the corresponding amounts (2.1 g DMSP) to man of 1 mL of 10 mM DMSP solution (1.7 mg DMSP) to mouse hold no toxicing for humans. Similary, the relation of pure compounds and related natural products is also found in the cases of garlic and green tea exerting significant healing effects on various cancers, in which the amounts of garlic and green tea needed for the improvement of cancers correspond to about 10–100 garlic cloves (24) and 10 cups of green tea per day per person (25), respectively. However, the fact that large amounts of natural products are needed for the healing of cancers are believed not to make light of further investigation into the effects and action mechanisms of pure compounds on cancers (25, 26).

The activation mechanisms of DMSP on the immune system of EAC-bearing mice need to be elucidated in more detail.

REFERENCES


