Antitumor Activity of Glyceryl Ethers

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SUMMARY

Antitumor activity of fatty alcohols and α-glyceryl ethers of fatty alcohols was examined with Ehrlich carcinoma in mice. Significant antitumor activity was exerted against Ehrlich ascites carcinoma by i.p. administration of capryl, lauryl, and myristyl α-glyceryl ethers. Capryl and lauryl glyceryl ethers suppressed the growth of solid tumor when administered through various routes. Administration s.c. was the most effective.

INTRODUCTION

Tsujimoto and Toyama (24) isolated glyceryl ethers of higher fatty alcohols from the unsaponifiable fractions of some fish liver oils. Chimyl, batyl, and selachyl alcohols are naturally occurring glyceryl ethers of n-hexadecanol, n-octadecanol, and n-octadecenol, respectively. It has been reported that the lipid classes containing α-glyceryl ethers are present in neutral and phospholipid fractions of animal cells including tumor cells (21). Biological properties of the glyceryl ethers, however, have not yet been extensively studied.

We screened for antitumor antibiotics and found that fatty acids and α-monoglycerides of higher fatty acids, both of which were extracted with acetone from some fungal mycelia, exerted significant antitumor activity against Ehrlich ascites tumor (4, 16). The fatty acids and the fatty acid moieties of the monoglycerides with antitumor activity consist of oleic and linoleic acids as major constituents (2, 18). Evaluation of antitumor activity of fatty acids, with each higher fatty acid as well as the fungal fatty acids, revealed that antitumor activity is not a specific property of the fungal fatty acids but a common property of some higher fatty acids (3). Antitumor activity of some esters of fatty acids was also studied, and the results were previously reported (17). Among the esters of fatty acids tested, sucrose monoesters of lauric, myristic, oleic, linoleic, and elaidic acids, and propylene glycol ester of myristic acid, were highly active against Ehrlich ascites tumor.

From these observations, we became interested in antitumor properties of lipids, and our study has been extended to fatty alcohols and their α-monoglycerol ethers.

A recent series of papers by Snyder et al. (22) clearly demonstrated that tumor cells are rich in the lipid classes containing α-glyceryl ethers in neutral and phospholipid fractions. It was assumed that these increased levels of glyceryl ethers might be related to abnormal growth of neoplasm.

In this paper, we report antitumor activity of some glyceryl ethers against Ehrlich carcinoma; this is the first report that glyceryl ethers of higher fatty alcohols show significant antitumor activity against Ehrlich carcinoma.

MATERIALS AND METHODS

Fatty Alcohols. The higher fatty alcohols used in this study were straight-chain primary alcohols with even carbon numbers ranging from 10 to 18. These were purchased from Tokyo Kasei Co., Ltd., Tokyo, Japan. The purity of these chemicals was satisfactory from the gas chromatograms, so they were used without further purification.

Synthesis of α-Glyceryl Ethers. α-Monoglycerol ethers were synthesized by the method of Kornblum and Holmes (20). Fatty alcohol moieties of the glyceryl ethers were straight-chain primary alcohols with even carbon numbers ranging from 10 to 18. Although naturally occurring glyceryl ethers were optically active, the glyceryl ethers thus synthesized were optically inactive crystalline needles.

Preparation of the Aqueous Suspensions of the Glyceryl Ethers Used in Antitumor Study. The glyceryl ethers tested were insoluble in water; therefore, aqueous suspensions were used when the ethers were administered to mice. For preparation of uniform suspensions, 0.2 part of Tween 80 was added to 1 part of the agents to be tested, and the mixtures were heated gently to melt crystalline materials. Then distilled water was added to the mixture drop by drop with vigorous shaking until the final volume was reached. The suspensions thus prepared were stable for several months at 5°C.

Mice. Swiss albino ddY mice were maintained in a thermostatically controlled room at 24°C with a 12-hr light cycle. They were fed an ordinary pellet diet (Japan Krea Co., Ltd.) and tap water ad libitum.

Determination of Antitumor Activity against Ehrlich Ascites Tumor. The mice used were 5 weeks old, weighing 20 to 23 g. Ehrlich ascites tumor cells were maintained by serial transplantation every 10 days. The ascites tumor cells (1 X 10⁶ cells/mouse) were implanted i.p. into the mice. Treatment was initiated 24 hr after implantation, the agents being given once daily for 5 consecutive days. All mice were sacrificed 30 days after implantation unless otherwise stated and were examined whether or not a tumor was present. Antitumor activity was expressed as (mean survival time of treated group/mean survival time of untreated control group) X 100. In some experiments, mitomycin C was used as a positive control agent.

Determination of Antitumor Activity against Ehrlich Solid Tumor. Ascites tumor cells (4 X 10⁶ cells/mouse) were
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Table 1

Antitumor activity of fatty alcohols against Ehrlich ascites tumor

Four mice were used in each dose in Experiment 1, and 6 mice were used in Experiment 2. The untreated control group contained 20 mice in each experiment. All mice were sacrificed 30 days after tumor implantation.

<table>
<thead>
<tr>
<th>Fatty alcohols</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (mg/mouse/day)</td>
<td>Mean survival time (days)</td>
<td>Prolongation of life-span (%)</td>
<td>Dose (mg/mouse/day)</td>
<td>Mean survival time (days)</td>
<td>Prolongation of life-span (%)</td>
</tr>
<tr>
<td>Capryl (C₁₀)</td>
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<td>3.5</td>
<td>18</td>
<td>2</td>
<td>9.0</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>7.5</td>
<td>41</td>
<td>4</td>
<td>&gt;21.0</td>
<td>&gt;146</td>
</tr>
<tr>
<td>Lauryl (C₁₃)</td>
<td>10</td>
<td>5.0</td>
<td>27</td>
<td>2</td>
<td>13.0</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>&gt;30.0</td>
<td>&gt;157</td>
<td>4</td>
<td>18.3</td>
<td>127</td>
</tr>
<tr>
<td>Myristyl (C₁₄)</td>
<td>10</td>
<td>18.0</td>
<td>98</td>
<td>2</td>
<td>&gt;26.0</td>
<td>&gt;194</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>&gt;16.5</td>
<td>&gt;90</td>
<td>4</td>
<td>18.0</td>
<td>125</td>
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<td>Palmityl (C₁₆)</td>
<td>10</td>
<td>&gt;29.5</td>
<td>&gt;156</td>
<td>2.5</td>
<td>19.9</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>12.0</td>
<td>66</td>
<td>10</td>
<td>&gt;23.2</td>
<td>&gt;161</td>
</tr>
<tr>
<td>Stearyl (C₁₈)</td>
<td>10</td>
<td>18.0</td>
<td>98</td>
<td>2.5</td>
<td>14.1</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>&gt;25.5</td>
<td>&gt;139</td>
<td>10.0</td>
<td>15.7</td>
<td>109</td>
</tr>
<tr>
<td>Untreated control</td>
<td>18.3</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2

Antitumor activity of glyceryl ethers against Ehrlich ascites tumor

Four mice were used in each dose. Twenty mice were used in the untreated control group.

<table>
<thead>
<tr>
<th>Glyceryl ethers</th>
<th>Dose (mg/mouse/day)</th>
<th>Mean survival time (days)</th>
<th>Prolongation of life-span (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capryl</td>
<td>2</td>
<td>&gt;24</td>
<td>&gt;152</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>&gt;24</td>
<td>&gt;152</td>
</tr>
<tr>
<td>Lauryl</td>
<td>4</td>
<td>&gt;23</td>
<td>&gt;144</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>&gt;27</td>
<td>&gt;158</td>
</tr>
<tr>
<td>Myristyl</td>
<td>2</td>
<td>&gt;20</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>&gt;26</td>
<td>&gt;164</td>
</tr>
<tr>
<td>Palmityl</td>
<td>2</td>
<td>20</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>&gt;22</td>
<td>&gt;139</td>
</tr>
<tr>
<td>Stearyl</td>
<td>2</td>
<td>22</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>23</td>
<td>144</td>
</tr>
<tr>
<td>Untreated control</td>
<td>15.8</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

implanted s.c. at the left groins of 5-week-old mice weighing 20 to 23 g. The dose schedule was the same as that of the ascites tumor. All mice were sacrificed 10 days after implantation of cells. The solid tumor was carefully dissected out and weighed. The wet weight of the treated group was compared with that of the untreated control group. Mycocphenolic acid was used as a positive control agent. To our knowledge, this antibiotic is the most effective against Ehrlich solid tumor, although it was completely ineffective against the ascites tumor (23). The tumor inhibition rate was expressed as follows: (1 – mean tumor weight of treated group/mean tumor weight of untreated control group) X 100%.

RESULTS

Table 1 shows antitumor activity of fatty alcohols. Administrations i.p. of capryl, lauryl, and myristyl alcohols prolonged the life-span of the treated mice. These alcohols, however, were toxic, since excess administrations resulted in severe diarrhea and loss of body weight. The antitumor study of fatty alcohols was repeated 3 times, and similar results were obtained.

Antitumor activity of glyceryl ethers is demonstrated in...
Table 2. In Experiment 1, glyceryl ethers were given at doses of 2 and 8 mg/mouse/day for 5 days, and it was found that glyceryl ethers of capryl, lauryl, and myristyl alcohols were very active, i.e., 60 to 70% of the treated mice were completely cured at a dose of 8 mg/mouse/day for 5 days. Although these ethers were less toxic than the corresponding alcohols, body weight gain was slightly suppressed during treatment. This inhibition of body weight gain soon reversed after cessation of administration. Positive control agent, mitomycin C, was highly active at a dose of 40 µg/mouse/day for 5 days; 9 out of 10 mice survived longer than 30 days after implantation.

From these data, we selected capryl and lauryl glyceryl ethers for further evaluation of antitumor activity. Chart 1 shows survival and weight gain curves when capryl and lauryl glyceryl ethers were administered i.p. at a dose of 6 mg/mouse/day for 5 days. When lauryl glyceryl ether was administered, 80% of the treated mice survived longer than 30 days after the tumor implantation, and 3 out of 10 mice were completely cured. Body weight gain was slightly suppressed during the treatment, but diarrhea was not observed at this dose. Capryl glyceryl ether was also effective; 8 out of 10 mice survived longer than 30 days, and 5 out of 10 were completely cured. In this experiment, the activity of capryl glyceryl ether was more effective than that of lauryl glyceryl ether; however, repeated experiments revealed that their activities are approximately the same.

Table 3 shows effects of treatment of more advanced Ehrlich ascites carcinoma with glyceryl ethers. The treatment was initiated 3 days after tumor cell implantation (2 × 10⁶ cells/mouse), the agents being given i.p. once daily for 5 consecutive days. Mitomycin C was effective against the advanced tumor, whereas treatment by the 2 glyceryl ethers resulted in only slight prolongation of life-span. Therefore, the 2 glyceryl ethers are less effective against advanced tumor than mitomycin C.

Table 4 demonstrates the effects of administration routes on antitumor activity of capryl glyceryl ether on ascites and solid tumor. When the administration routes were compared against the solid tumor, administration s.c. was the most effective, showing a tumor inhibition rate of 81.9%. The mean tumor weight of the untreated control group was 846 mg/mouse, whereas that of the positive control group was 119 mg/mouse (inhibition rate 85.6%). Necrosis was often seen at the injected loci of the skin as a side effect through this route. Destruction of the tumor occurred if the agent was injected directly into tumor. This phenomenon seemed to be tumor specific to some extent because i.m. administration brought about no such effect to muscle. Both i.p. and i.m. administration reduced the tumor weights at the rate of 47.3 and 30.4%, respectively.

Administration i.p. was the most effective against the ascites tumor. The treated mice showed a life-span prolonged over 171% when compared with that of untreated control, and 5 out of 10 mice were completely cured. Neither s.c. nor i.m. administration showed antitumor activity against the ascites tumor.

Table 5 shows the effects of administration routes of lauryl glyceryl ether on Ehrlich solid tumor. The tumor weight of the untreated control group was 519 mg/mouse, and that of the positive control group was 108 mg/mouse. The tumor growth...
at the left groin of male Swiss albino ddY mice 5 weeks of age and of each group during this experiment. Mycophenolic acid, a positive weighed. Body weight gain is expressed as mean body weight increase of each group during this experiment. Mycophenolic acid, a positive control agent, was administered at a dose of 6 mg/mouse/day according to the same dose schedule as the agent tested.

<table>
<thead>
<tr>
<th>Routes</th>
<th>Body weight gain (g/mouse)</th>
<th>Mean tumor weight (mg/mouse)</th>
<th>Tumor inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.p.</td>
<td>3.4</td>
<td>461</td>
<td>11.0</td>
</tr>
<tr>
<td>p.o.</td>
<td>8.5</td>
<td>331</td>
<td>43.9</td>
</tr>
<tr>
<td>s.c.</td>
<td>5.0</td>
<td>119</td>
<td>75.1</td>
</tr>
<tr>
<td>i.m.</td>
<td>4.9</td>
<td>200</td>
<td>61.4</td>
</tr>
<tr>
<td>Untreated control</td>
<td>8.8</td>
<td>519</td>
<td>0</td>
</tr>
<tr>
<td>Positive control</td>
<td>7.2</td>
<td>108</td>
<td>77.5</td>
</tr>
</tbody>
</table>

was most effectively suppressed when the agent was given s.c. Both i.m. and p.o. administrations also resulted in a significant decrease of the tumor weight gain. However, i.p. injection showed only slight inhibition, although body weight gain was considerably suppressed.

**DISCUSSION**

Glyceryl ethers were found by Tsujimoto and Toyama (24) in 1921 in the unsaponifiable fraction of some fish liver oils. They have since been found in such diverse sources as the body cavity fat and liver oils of rats (15), the yolk of chicken eggs (15), the yellow bone marrow of cattle (14), and, in man, in the bone marrow, spleen, and milk (12), the arteriosclerotic arteries (13), and the fetal meconium (6). In 1948, Berger (5) reported that chymil and batyl alcohols were only slightly active.

Recently, Wood and Snyder (25) isolated an unknown lipid class from Ehrlich ascites tumor cells and spectrometrically identified it as glyceryl ether diesters. Subsequent studies by Snyder (21) revealed that a high level of a lipid class of glyceryl ether diesters is characteristic for a variety of transplantable rodent tumors, as well as human tumors. Ether-linked phosphoglycerides are also rich in minimum-deviation hepatoma 7777 and in LM-cells, that is, a malignant variant derived from Earle's L-cells (1, 22). A series of papers suggested that high levels of lipid classes containing glyceryl ethers would be related to neoplasia.

Our studies showed that antitumor activity was exerted by the glyceryl ethers with shorter alkyl chains rather than naturally occurring ones; that is, the glyceryl ethers with C10 to C14 alkyl chains were very active against Ehrlich ascites tumor, whereas chymil and batyl alcohols were only slightly active.

It is well known that some detergents, such as fatty acids, attack cell membranes and cause lysis of cells. Glyceryl ethers are weak surface-active agents, and sheep RBC and the protoplast of Bacillus megaterium lysed only at high concentrations of the ethers. The antitumor property of the glyceryl ethers might be partly due to the surface activity because the ethers showed antitumor activity against Ehrlich ascites tumor only when the ethers were given i.p., i.e. where the agents would be able to contact directly with tumor cells. However, the fact that the unnatural glyceryl ethers are more active than naturally occurring ones suggests the presence of additional modes of action. This idea was supported by the data of the present authors (19) that Ehrlich ascites tumor cells lost transplantability when the cells were incubated in vitro with Tween 20, a surface-active agent, whereas Tween 20 itself is completely ineffective against the tumor in vivo at high doses.

Capryl and lauryl glyceryl ethers inhibit the growth of Ehrlich solid tumor. Marked inhibition was observed with s.c. administration, although necrosis of the injected loci occurred in some cases. On the other hand, p.o. administration showed normal body weight gain and resulted in significant reduction of the tumor growth. Therefore, the possibility exists that the glyceryl ethers, once given p.o., were transported to the solid tumor and specifically suppressed the tumor growth without affecting the host.

The antitumor property of the glyceryl ethers discussed in this paper might be related to elevated levels of lipid classes containing glyceryl ethers in tumor cells.

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