Effects of Some Fatty Acid Esters on the Viability and Transplantability of Ehrlich Ascites Tumor Cells

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SUMMARY

Fatty acids, monoglycerides, and some esters of fatty acids show antitumor activity against Ehrlich ascites tumor in mice. For investigation of the in vivo mode of action of these lipids, the effects of those fatty acid esters on animal cells in vitro were studied in sheep red blood cells and Ehrlich ascites tumor cells. Throughout these studies, we used fatty acid sucrose esters because they are soluble in water, so that we could obtain reproducible results without interference from the solubility of the samples.

Most of the fatty acids and their esters showed hemolytic action in sheep red blood cells and tumor cell-killing activity in vitro. The tumor cells treated with such agents in vitro lost their transplantability in mice. The sucrose monoester of lauric acid, which showed the strongest in vivo antitumor activity, exhibited a strong in vitro activity, but polyoxyethylene sorbitan monolaurate (Tween 20), which had no antitumor activity, showed the stronger hemolytic and tumor cell-killing activities. These results indicate that antitumor activity of some fatty acid esters cannot be explained only by their physical attack of the tumor cells.

INTRODUCTION

In our screening for antitumor antibiotics, we found that fatty acids and monoglycerides isolated from acetone extracts of some fungal mycelia show antitumor activity in vivo against Ehrlich ascites tumor in mice (3, 5). Chemical study of the fatty acids and the monoglycerides indicated that they are the mixture of the fatty acids or monoglycerides of fatty acids with carbon numbers of 16 and 18 (2, 6).

Antitumor activity of fatty acids was first reported by Nakahara (10–13). He reported that olive oil and fatty acids were effective in increasing the resistance of mice to several Nakanaha (10–13). He reported that olive oil and fatty acids with carbon numbers of 16 and 18 (2, 6). Prior to inoculation into mice, completely suppressed the development of ascitic tumors in mice. They successively investigated the in vitro transplantable mouse leukemia and the formation of ascitic acid from royal jelly suppressed the development of a forms of transplantable tumors.

Mixture of the fatty acids or monoglycerides of fatty acids and the monoglycerides indicated that they are the antitumor activity of mono- and dicarboxylic acids and their derivatives with water solubility, which are inactive against the tumor in vivo. Hemolytic activity and in vitro attack of the tumor cells were studied, and results were compared with in vivo activity. Hemolytic activity and in vitro attack of the tumor cells were studied, and results were compared with in vivo activity. Hemolytic activity and in vitro attack of the tumor cells were studied, and results were compared with in vivo activity. Hemolytic activity and in vitro attack of the tumor cells were studied, and results were compared with in vivo activity. Hemolytic activity and in vitro attack of the tumor cells were studied, and results were compared with in vivo activity. Hemolytic activity and in vitro attack of the tumor cells were studied, and results were compared with in vivo activity. Hemolytic activity and in vitro attack of the tumor cells were studied, and results were compared with in vivo activity. Hemolytic activity and in vitro attack of the tumor cells were studied, and results were compared with in vivo activity.

SUMMARY

Most of the works on the activity of fatty acids and their esters thus far reported were done with water-insoluble materials. All the works by Townsend et al. (21, 22), Morgan et al. (9), and Tolnai et al. (17–19) were carried out with water-insoluble fatty acid esters, and found that L-lysine and L-arginine laurate were effective against the Ehrlich and TN₅ ascites tumor cells in vitro. We examined the sucrose ester of fatty acids that were freely soluble in water.

The antitumor activity of sucrose esters of 7 fatty acids was studied and reported in a previous paper (8). As summarized in Table 1, all the sucrose esters inhibit the tumor growth. This finding of tumor inhibition by fatty acid sucrose esters enabled us to study the mechanism of action of fatty acids and their esters on tumor cells.

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Table 1
Antitumor activity of some esters of lauric acid against Ehrlich ascites tumor in mice

About 1 million of the tumor cells were implanted i.p. into 5-week-old ddY mice. A solution or suspension of the samples in 0.86% NaCl solution was administered once daily for 5 successive days. Six mice were used for each group. Control mice were given injections of 0.2 ml of the NaCl solution.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose (mg/mouse/day)</th>
<th>Tumor gain (g)</th>
<th>Body weight gain (g)</th>
<th>Survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monolaurin</td>
<td>10.0</td>
<td>–</td>
<td>+1.3</td>
<td>&gt;29</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>–</td>
<td>+4.0</td>
<td>&gt;29</td>
</tr>
<tr>
<td>Methyl laurate</td>
<td>20.0</td>
<td>–</td>
<td>+2.0</td>
<td>&gt;29</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>–</td>
<td>+2.9</td>
<td>&gt;29</td>
</tr>
<tr>
<td>Sorbitan laurate</td>
<td>10.0</td>
<td>+++</td>
<td>+3.7</td>
<td>&gt;29</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>+++</td>
<td>+4.2</td>
<td>&gt;29</td>
</tr>
<tr>
<td>Tween 20</td>
<td>10.0</td>
<td>+++</td>
<td>+6.6</td>
<td>&gt;29</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>+++</td>
<td>+6.9</td>
<td>&gt;29</td>
</tr>
<tr>
<td>Sucrose laurate</td>
<td>16.0</td>
<td>–</td>
<td>+2.9</td>
<td>&gt;29</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>–</td>
<td>+1.4</td>
<td>&gt;29</td>
</tr>
<tr>
<td>Sucrose myristate</td>
<td>20.0</td>
<td>–</td>
<td>+1.8</td>
<td>&gt;29</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>–</td>
<td>+3.0</td>
<td>&gt;29</td>
</tr>
<tr>
<td>Sucrose palmitate</td>
<td>10.0</td>
<td>–</td>
<td>+1.5</td>
<td>&gt;29</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>–</td>
<td>+2.9</td>
<td>&gt;29</td>
</tr>
<tr>
<td>Sucrose linoleate</td>
<td>18.0</td>
<td>–</td>
<td>+1.4</td>
<td>&gt;29</td>
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<tr>
<td></td>
<td>4.5</td>
<td>–</td>
<td>+1.2</td>
<td>&gt;29</td>
</tr>
<tr>
<td>Control</td>
<td>++</td>
<td>+8.4</td>
<td>++</td>
<td>&gt;29</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Sucrose Monoesters of Fatty Acids. These were synthesized according to the method of Osipow et al. (15). About 3 moles of sucrose are dissolved in dimethylformamide, and 1 mole of methyl ester of fatty acid is added with 0.1 mole of potassium carbonate as an alkaline catalyst. The reaction mixture is maintained at 90–95° under 80 to 100 mm Hg pressure. After 6 to 9 hr, the solvent is distilled off and the residue is dried in a vacuum to obtain sucrose ester, monoester being the main component.

Other Esters of Fatty Acids. These were all available as chemicals or industrial detergents.

Assay for Antitumor Activity in Vivo. Antitumor activity was assayed with the use of Ehrlich ascites tumor in mice. About 1 million of the tumor cells were implanted i.p. in ddY mice, 5 weeks old, weighing 18 to 22 g. A solution or suspension of the samples in 0.86% NaCl solution was administered once daily for 5 successive days, and the tumor growth and body weight gain after 7 days and life-span up to 30 days were observed. The life-span was shown as the average life-span of 10 mice in each group.

Assay for Hemolytic Activity. Hemolytic activity of the esters of fatty acids was assayed with the use of sheep red blood cells. Sheep red blood cells were collected from the commercial sheep red blood cell suspension (Toshiba Kagaku, Tokyo, Japan) by centrifugation at 1500 rpm for 5 min, washed with 0.86% NaCl solution 3 times, and suspended in 100 volumes of the NaCl solution. Samples were dissolved or suspended in the 0.86% NaCl solution at the serial dilutions, and an equal volume of the red cell suspension was added. After incubation at 37°, for 4 hr, the hemolytic activity was determined. The activity was expressed by the highest dilution in which hemolysis was observed visually.

Assay for Viability of Tumor Cells. Ascitic tumor cells of Ehrlich carcinoma maintained by weekly i.p. transplantation were washed 3 times with PBS1 and suspended at a

Table 1. Hemolytic activity of fatty acid esters. Samples were dissolved or suspended in 0.86% NaCl solution at serial dilutions starting with 1 mg/ml, and an equal volume of the sheep red blood cell suspension in the NaCl solution was added. After the incubation at 37° for 4 hr, the hemolytic activity was determined. The activity was expressed by the highest dilution in which hemolysis was observed. PG, propylene glycol; MG, monoglyceride; FA, fatty acid; Me, methyl.

1 The abbreviation used is: PBS, phosphate-buffered saline.
concentration of $1 \times 10^6$ cells/ml. Equal volumes of the sample in PBS and the tumor cell suspension were mixed and incubated at $37^\circ$ for 1 hr. The viability of the treated tumor cells was determined by staining the cells with safranin dye according to the method described by Oda (14). To the reaction mixture, safranin solution in PBS was added to a final concentration of 0.05%. The percentage of the dead cells in the mixture was determined by counting the number of cells microscopically.

**Assay for Transplantability of Tumor Cells.** Ehrlich ascites tumor cells were treated as described for assay for viability except that the tumor cells were suspended at a concentration of $1 \times 10^7$ cells/ml. After the incubation at $37^\circ$ for 1 hr, 0.2 ml of the mixture was implanted i.p. into a ddY mouse. The survival time and tumor growth of the each mouse were observed. Ten mice were used for each group, and the control mice were given injections of untreated tumor cells.

### RESULTS

In vivo antitumor activity of some of the fatty acid esters is shown in Table 1. While monolaurin and sucrose monolaurate show activity, sorbitan laurate and polyoxyethylene sorbitan monolaurate (Tween 20) have no activity. Studies on the activity of other esters (8) showed that most of the sucrose esters of fatty acids and the propylene glycol ester of myristic acid were the most effective against the tumor tested and that Twenses, sorbitan esters, and most of the methyl esters were ineffective.

Hemolytic activity of some of the fatty acid esters was studied by the method described in “Materials and Methods.” The purpose of this experiment was to examine whether there would exist a parallel relationship between hemolytic activity and in vivo antitumor activity. The result is presented in Chart 1. Hemolytic activity was expressed by the highest dilution in which hemolysis was observed. As can be seen from the chart, some of the esters with strong in vivo activity show strong hemolytic activity while others show very weak activity.
Tween 20, which showed no in vivo activity, demonstrated strong hemolytic activity. Thus there is no relationship between antitumor activity and hemolytic activity.

Next, we examined in vitro action of those lipids on the tumor cells, thinking that each lipid might have specific affinity to the tumor cells and that those with strong affinity attack the cell and show antitumor activity. In vitro attack of the cell was measured by viability and transplantability of the treated tumor cells. The method is described in “Materials and Methods.” In Table 2, viability of the tumor cells treated with some esters at 3 doses is presented with the reference to their antitumor activity. Here again, we see no strict relationship between in vitro and in vivo activities. Table 3 shows the viability and transplantability of the tumor cells treated with sucrose and propylene glycol esters of fatty acids and Tweens at concentrations of 2500 and 500 μg/10^7 cells. At the higher concentration, none of the treated cells were viable; when inoculated into mice, these cells did not produce tumors. When the concentration was reduced to 500 μg/10^7 cells, none of the sucrose esters and propylene glycol ester showed any cytotoxic effects on the tumor cells. However, 90% of the cells treated with Tween 20 at the same concentration, 500 μg/10^7 cells, lost their viability and consequently their transplantability.

**DISCUSSION**

It is significant that fatty acid esters with strong in vivo activity were less effective in vitro than Tween 20, which did not show antitumor activity in vivo. Thus antitumor activity shown by sucrose monoesters and some other esters of fatty acids cannot be explained only by their physical attack of the tumor cells. Differences in physiological and biochemical properties of the esters should differentiate their antitumor activity. Further studies are under way.

This is the first report in which sucrose esters of fatty acids are used in biological studies. Our results suggest the possibility that sucrose esters of fatty acids could be used as water-soluble derivatives of fatty acids in many experiments.

**ACKNOWLEDGMENTS**

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**REFERENCES**


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