Antitumor Activity of Cytogenin

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(Received for publication August 31, 1994)

Antitumor effect of cytogenin against IMC carcinoma in mice was investigated. Since cytogenin did not show cytotoxicity against tumor cells in vitro at 50 μg/ml and toxicity at more than 2,000 mg/kg i.p., it was considered that the antitumor effect is due to host mediated events. Cytogenin showed antitumor activity against a syngeneic murine transplantable tumor, IMC carcinoma by oral administration depending upon schedule of administration. The optimum effect was observed by the administration starting day 8 after transplantation of tumor cells, every other day for 10 times or every 2nd day for 7 times. The antitumor effect was reduced in immunosuppressed mice given anti-asialo GM1 serum and in athymic mice, but not in mice irradiated with X ray. The antitumor effector cells activated by cytogenin were determined to be macrophages and T cells.

Cytogenin was found in cultured broth of Streptoverteicillum eurocidicum MI43-37F11 as an antitumor antibiotic exhibiting antitumor activity against Ehrlich carcinoma in mice by oral administration1). Since cytogenin did not show cytotoxicity at 50 μg/ml against cultured tumor cells and acute toxicity to mice at more than 2,000 mg/kg, i.p., it has been considered that the antitumor activity might be due to host mediated events. Therefore, we investigated the antitumor activity of cytogenin against a syngeneic murine transplantable tumor, IMC carcinoma, in normal and immunosuppressed mice and its effect on generation of antitumor effector cells.

Materials and Methods

Mice
CDF1 mice (female, 6 weeks old) were purchased from Charles River Japan Inc. (Kanagawa, Japan), and were maintained under specific pathogen-free conditions at 23.0±1°C and 55±5% humidity. Balb/c nu/nu(−) mice (female, 6 weeks old) were purchased from Japan SLC Inc. (Shizuoka, Japan), and were kept in a clean rack under the conditions mentioned above. These mice were used for experiments at 8 to 10 weeks old.

Cytogenin
Cytogenin was prepared by Mercian Co., Ltd. (Tokyo, Japan) according to the methods reported previously1).

Antitumor Activity against IMC Carcinoma
IMC carcinoma was maintained by a serial transplantation i.p. weekly in CDF1 mice and in cultures. CDF1 mice were inoculated with 10⁶ IMC carcinoma cells at groin and given cytogenin p.o. on different schedules as shown in results. Immunocompromised mice were prepared as follows: CDF1 mice were injected i.p. with 5 μl of anti-asialo GM1 serum2) 2 days before and 4, 10 and 16 days after tumor cell inoculation and employed as anti-asialo GM1 treated mice. CDF1 mice were irradiated with 400 rad of X ray by SOFRON (SOKEN Co., Ltd., Tokyo, Japan), 3 and 1 days before tumor cell inoculation and employed as X ray-irradiated mice. Those mice were implanted with 10⁶ IMC carcinoma cells s.c. as same as normal mice. Balb/c nu/nu(−) mice were implanted with 5 x 10⁵ tumor cells.

Antitumor activity was monitored by measuring tumor volume and determined on day 31 after implantation of tumor cells by measuring tumor weight. Tumor volume and the percentage of inhibition of tumor weight were calculated as follows:

Tumor volume (mm³) = length (mm) x width (mm)² x 0.5

Inhibition (%) = 

\[
\frac{1 - \frac{\text{Mean tumor weight of cytogenin treated group}}{\text{Mean tumor weight of control group}}}{100}
\]

Winn Assay
Antitumor effector cells were assessed by a Winn assay according to the method described in previous report3). Briefly, peritoneal exudate cells (PEC) and spleen cells were collected from tumor bearing mice on day 31 after tumor inoculation and splenic T cells were prepared by the methods using nylon wool column as reported previously4). PEC, spleen cells and splenic T cells were admixed with 5 x 10⁶ tumor cells at a ratio of 20:1, 20:1 and 2:1, respectively. Then, 0.1 ml of the mixture was inoculated s.c. to CDF1 mice and the antitumor activity was assessed by measuring tumor weight on day 32 after the inoculation.
**Results**

Antitumor activity of cytogenin against IMC carcinoma on different schedules is shown in Table 1. The optimum activity was observed on the schedule starting day 8 after tumor inoculation although the effect was shown slightly in daily treatment on days 1 to 7, but it was not significant in this tumor system. The effect in every 2nd day was better than that in other schedules. The dose response on the schedule in every 2nd day is shown in Fig. 1. The administration of cytogenin at 0.39 to 6.25 mg/kg p.o. showed a significant antitumor effect with a bell-shaped dose response curve. In this case, the most effective dose was 1.56 mg/kg. The antitumor activity of cytogenin at 1.56 mg/kg in every 2nd day starting day 8 after tumor inoculation was monitored. As shown in Fig. 2, cytogenin retarded the tumor growth in days 14 to 32 significantly.

In immunocompromised mice, the antitumor effect of cytogenin was examined. As shown in Table 2, in comparison to normal mice, the antitumor effect was reduced in mice treated with anti-asialo GM1 serum and in athymic mice but not in mice irradiated with X ray.

Since results shown above indicated that the antitumor

<table>
<thead>
<tr>
<th>Cytogenin (mg/kg)</th>
<th>Administration schedules and tumor weight (mg ± SD)</th>
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<tbody>
<tr>
<td></td>
<td>Days 1-7</td>
</tr>
<tr>
<td>0</td>
<td>3,364 ± 1,536 (0)*</td>
</tr>
<tr>
<td>1.56</td>
<td>2,388 ± 1,210 (29)</td>
</tr>
<tr>
<td>6.25</td>
<td>2,281 ± 879 (32)</td>
</tr>
</tbody>
</table>

*Inhibition rate (%), *P < 0.05, **P < 0.01 and ***P < 0.001 against control group.

Table 2. Reduction of antitumor activity of cytogenin in immunocompromised mice.

<table>
<thead>
<tr>
<th>Cytogenin (mg/kg)</th>
<th>CDF1 mice</th>
<th>CDF1 mice treated with anti-asialo GM1 serum</th>
<th>Balb/c nu/nu (−) mice</th>
<th>X-ray irradiated mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3,737 ± 979 (0)*</td>
<td>4,484 ± 2,025 (0)</td>
<td>3,661 ± 1,653 (0)</td>
<td>7,038 ± 2,039 (0)</td>
</tr>
<tr>
<td>0.39</td>
<td>2,114 ± 858* (43)</td>
<td>2,760 ± 1,238 (38)</td>
<td>3,590 ± 724 (2)</td>
<td>4,321 ± 1,055* (39)</td>
</tr>
<tr>
<td>1.56</td>
<td>1,372 ± 411*** (63)</td>
<td>3,111 ± 940 (31)</td>
<td>4,306 ± 330 (−18)</td>
<td>3,521 ± 997** (50)</td>
</tr>
<tr>
<td>6.25</td>
<td>2,560 ± 1,136 (31)</td>
<td>3,681 ± 1,821 (18)</td>
<td>3,000 ± 194 (18)</td>
<td>3,603 ± 597** (49)</td>
</tr>
</tbody>
</table>

*Inhibition rate (%), *P < 0.05, **P < 0.01 and ***P < 0.001 against control group.
Table 3. Antitumor activity of effector cells taken from cytogenin-treated IMC carcinoma bearing mice on IMC carcinoma.

<table>
<thead>
<tr>
<th>Effector cellsa</th>
<th>Cytogeninb</th>
<th>Tumor weightc (mg± SD)</th>
<th>T/Cd (%)</th>
<th>No. of tumor-free mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritoneal exudate cells</td>
<td>−</td>
<td>1,925 ± 813</td>
<td>100</td>
<td>0/5</td>
</tr>
<tr>
<td>Peritoneal exudate cells</td>
<td>+</td>
<td>704 ± 460**</td>
<td>37</td>
<td>0/7</td>
</tr>
<tr>
<td>Spleen cells</td>
<td>−</td>
<td>2,571 ± 227</td>
<td>100</td>
<td>0/10</td>
</tr>
<tr>
<td>Spleen cells</td>
<td>+</td>
<td>1,905 ± 1,109</td>
<td>62</td>
<td>0/10</td>
</tr>
<tr>
<td>Splenic T cellsf</td>
<td>−</td>
<td>599 ± 356*</td>
<td>31</td>
<td>0/8</td>
</tr>
<tr>
<td>Splenic T cellsf</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>5/5</td>
</tr>
</tbody>
</table>

a Effector cells were taken from IMC carcinoma-bearing mice treated with or without cytogenin on day 28 after tumor inoculation. These cells were mixed with 5 x 10^5 IMC carcinoma cells at a ratio of 20:1.
b Cytogenin (1.56mg/kg) was administered po from days 8 to 26 every 2nd day after tumor inoculation.
c Tumor weight were measured on day 35 after tumor inoculation.
d (Cytogenin treated group/Non-treated group) x 100.
e T cell: IMC carcinoma, 2:1.

P<0.05 and ** P<0.01 against non-treated group.

Effect of cytogenin is host mediated, the antitumor effector cells activated by cytogenin were investigated. As shown in Table 3, PEC and splenic T cells obtained from mice given cytogenin had significant antitumor activity against that of non-treated tumor bearing mice.

Discussion

Cytogenin was found in products of Streptoverticillum eurocidicum M143-37F11 as an antitumor antibiotic exhibiting antitumor effect by oral administration against an allogenic murine solid tumor, Ehrlich carcinoma. Since cytogenin did not show cytotoxicity on cultured murine and human tumor cells at 50μg/ml and acute toxicity to mice at 2,000mg/kg i.p., it was considered that the antitumor activity of cytogenin might be due to host mediated events. Thus, we investigated the antitumor activity against a syngeneic murine tumor in detail. The antitumor activity of cytogenin against IMC carcinoma was strictly dependent on schedule as well as other low molecular immunomodulators such as ubenimex5), forphenicinol6) and conagenin7). The most effective schedule showed that the administration should be starting day 8 after tumor inoculation. It can be considered that cytogenin may be effective in activating concomitant immunity induced in tumor bearing mice.

It was supported that the antitumor effect of cytogenin reduced markedly in immunocompromised mice treated with anti-asialo GM1 serum and in athymic mice although it did not reduced in mice irradiated with X ray. Since those mice except mice irradiated with X ray could not be induced concomitant tumor immunity after transplantation of tumor cells and could not generate antitumor effector cells, the antitumor activity of cytogenin was not shown. It is of note that the antitumor activity of cytogenin did not reduce in mice irradiated with X ray. It is well known although X ray irradiation affects bone marrow cells, it can be protected by some cytokines like IL-18.10). It was reported that cytogenin enhanced monokine production of macrophages1). Furthermore, it will be reported that cytogenin modulates macrophage functions to stimulate IL-1α production10). In this context, cytogenin might be effective in protecting dysfunction of bone marrow cells affected by X ray irradiation and might protect the generation of antitumor effector cells. It will be reported that cytogenin has the radio and chemoprotective activity in treatment of tumor bearing mice with antitumor agents.

Antitumor effector cells activated in mice given cytogenin was determined. As shown in Table 3, PEC and splenic T cells obtained from mice given cytogenin had significant antitumor activity against that of non-treated tumor bearing mice.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

References

3) Winn, H. J.: Immune mechanisms in homotransplanta-


