Antitumor Activity of Cytogenin

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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 *Streptoverticillium eurocidicum* MI43-37F11 as an antitumor antibiotic exhibiting antitumor activity against Ehrlich carcinoma in mice by oral administration[1]. 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

CDF_1 mice (female, 6 weeks old) were purchased from Charles River Japan Inc. (Kanagawa, Japan), and were maintained under specific pathogen-free conditions at $23.0 \pm 1°C$ and $55 \pm 5\%$ 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 previously[1].

Antitumor Activity against IMC Carcinoma

IMC carcinoma was maintained by a serial transplantation i.p. weekly in CDF_1 mice and in cultures. CDF_1 mice were inoculated with 10^6 IMC carcinoma cells at groin and given cytogenin p.o. on different schedules as shown in results. Immunocompromised mice were prepared as follows: CDF_1 mice were injected i.p. with 5 μl of anti-asialo GM1 serum[2] 2 days before and 4, 10 and 16 days after tumor cell inoculation and employed as anti-asialo GM1 treated mice. CDF_1 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 10^6 IMC carcinoma cells s.c. as same as normal mice. Balb/c nu/nu(−) mice were implanted with 5×10^5 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:

$$\text{Tumor volume (mm}^3\text{)} = \text{length (mm)} \times \text{width (mm)}^2 \times 0.5$$

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Mean tumor weight of cytogenin treated group}}{\text{Mean tumor weight of control group}}\right) \times 100$$

Winn Assay

Antitumor effector cells were assessed by a Winn assay according to the method described in previous report[3]. 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 previously[4]. PEC, spleen cells and splenic T cells were admixed with 5×10^5 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 CDF_1 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.

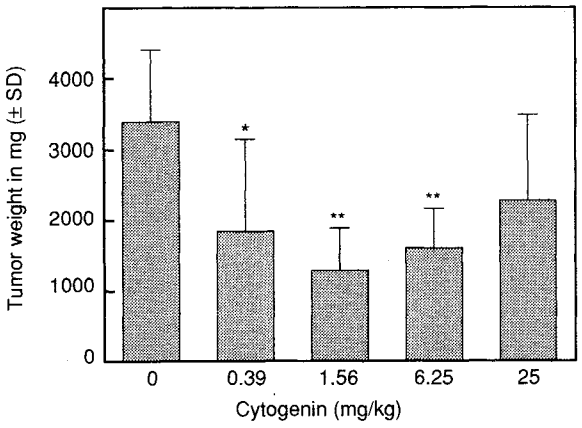
Since results shown above indicated that the antitumor

Table 1. Antitumor effect of cytogenin in different schedules on IMC carcinoma.

Cytogenin (mg/kg)	Administration schedules and tumor weight (mg±SD)			
	Days 1~7	Days 8~12	Days 8~26 (Q1D×10)	Days 8~26 (Q2D×7)
0	3,364±1,536 (0)[a]	3,387±1,019 (0)	3,387±1,019 (0)	3,387±1,019 (0)
1.56	2,388±1,210 (29)	1,160± 777* (51)	1,934± 353** (43)	1,606± 522** (53)
6.25	2,281± 879 (32)	2,015± 767* (41)	1,239± 606** (63)	1,289± 593** (62)

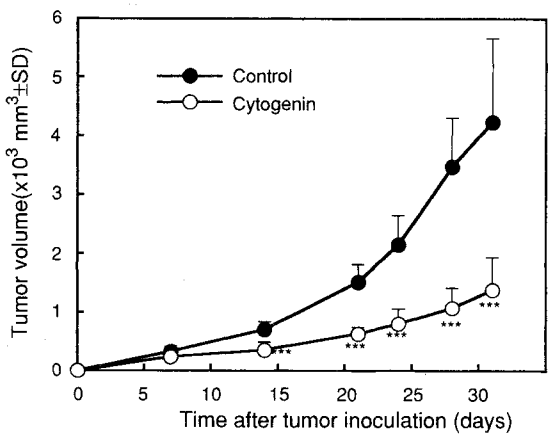
1×10^6 IMC carcinoma cells were inoculated sc to CDF_1 mice on day 0. Cytogenin was administered po on days indicated. Mean tumor weight were measured on day 31 after inoculation.
[a] Inhibition rate (%), * $P<0.05$, ** $P<0.01$ against control group.

Fig. 1. Antitumor activity of cytogenin against IMC carcinoma.



1×10^6 IMC carcinoma cells were inoculated sc to CDF_1 mice on day 0. Cytogenin was administered po from day 8 (Q2D×7). Mean tumor weight were measured on day 31.
* $P<0.05$, ** $P<0.01$ against control group.

Fig. 2. Inhibitory effect of cytogenin on growth of IMC carcinoma.



1×10^6 IMC carcinoma cells were inoculated sc to CDF_1 mice on day 0. Cytogenin (1.56 mg/kg) was administered po from day 8 (Q2D×7).
*** $P<0.001$ against control group.

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

Cytogenin (mg/kg)	Tumor weight (mg±SD)			
	CDF_1 mice	CDF_1 mice treated with anti-asialo GM1 serum	Balb/c nu/nu(−) mice	X-ray irradiated mice
0	3,737± 979 (0)[a]	4,484±2,025 (0)	3,661±1,653 (0)	7,038±2,039 (0)
0.39	2,114± 858* (43)	2,760±1,238 (38)	3,590± 724 (2)	4,321±1,055* (39)
1.56	1,372± 411*** (63)	3,111± 940 (31)	4,306± 330 (−18)	3,521± 997** (50)
6.25	2,560±1,136 (31)	3,681±1,821 (18)	3,000± 194 (18)	3,603± 597** (49)

[a] 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.

Effector cells[a]	Cytogenin[b]	Tumor weight[c] (mg±SD)	T/C[d] (%)	No. of tumor-free mice
Peritoneal exudate cells	−	1,925± 813	100	0/5
Peritoneal exudate cells	+	704± 460**	37	0/7
Spleen cells	−	2,571± 227	100	0/10
Spleen cells	+	1,598±1,109	62	0/10
Splenic T cells[e]	−	1,905±1,553	100	0/9
Splenic T cells[e]	+	599± 356*	31	0/8
Splenic T cells	+	0	0	5/5

[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 5×10^5 IMC carcinoma cells at a ratio of 20 : 1.
[b] Cytogenin (1.56 mg/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) × 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 *Streptoverticillium eurocidicum* MI43-37F11 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 50 μg/ml and acute toxicity to mice at 2,000 mg/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 ubenimex[5], forphenicinol[6] and conagenin[7]. 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 immunocommpromised 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-1[8,9]. It was reported that cytogenin enhanced monokine production of macrophages[1]. Furthermore, it will be reported that cytogenin modulates macrophage functions to stimulate IL-1α production[10]. 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, the antitumor effect was detected in PEC and T cells. As reported[7,11], in IMC carcinoma bearing mice cytotoxic T cells (CTL) were generated and IMC carcinoma cells were sensitive to CTL and macrophages but not NK cells. In this case, the activation of macrophages leads to stimulate generation of CTL in tumor-bearing mice. From above mentioned results, it can be concluded that cytogenin exerts antitumor effect by its immunomodulating activity exhibiting activation or modulation of macrophages.

Acknowledgments

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