GILVOCARCINS, NEW ANTITUMOR ANTIBIOTICS

3. ANTITUMOR ACTIVITY

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Gilvocarcin V, isolated from a *Streptomyces* culture showed activity against experimental tumors such as sarcoma 180, Ehrlich carcinoma, Meth 1 fibrosarcoma, MH134 hepatoma and lymphocytic leukemia P388. In particular, 40% of treated mice survived for 60 days, after intraperitoneal administration of gilvocarcin V to mice bearing Ehrlich ascites carcinoma. But it was marginally active against B16 melanoma and did not produce prolongation of lifespan of mice bearing Lewis lung carcinoma.

Gilvocarcin, a group of antibiotics with a novel carbon skeleton[1~3] was isolated from the culture broth of *Streptomyces gilvotanareus*. This antibiotic is strongly active against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, but is weakly active against Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae*. In the previous report[1], the preliminary results of antitumor activity of gilvocarcin against P388 leukemia and sarcoma 180 were described.

This paper describes the antitumor activity against various murine tumors and the effect against the number of peripheral white blood cells. The activity of gilvocarcins V and M was compared in the antitumor activity and the cell growth in tissue culture.

Materials and Methods

Animals

Male mice of *dd*Y, BDF_1 hybrid strain (BALB/c, female × DBA/2, male) weighing 18~22 g, male mice of CDF_1 hybrid strain (C57BL/6, female × DBA/2, male) weighing 20~22 g, male mice of C3H/He/J weighing 18~22 g and male C57BL/6 weighing 20~22 g were obtained from Shizuoka Agricultural Co-operative Association for Laboratory Animals (Hamamatsu).

Tumor

Sarcoma 180 ascites type obtained from National Cancer Center (Tokyo) has been maintained by intraperitoneal passage in *dd*Y mice. For antitumor test against solid type of sarcoma 180, 5×10^6 cells were inoculated subcutaneously at the axillary region of *dd*Y mice. P388 cells (1×10^6) donated by the Cancer Chemotherapy Center were inoculated intraperitoneally into CDF_1 mice. Ehrlich carcinoma cells (5×10^6), were transplanted intraperitoneally into *dd*Y mice. Lewis lung carcinoma from Cancer Chemotherapy Center (Tokyo) was inoculated by trocar into C57BL/6 mice for passage and into BDF_1 mice for antitumor test. BDF_1 mice were inoculated with 0.2 ml of 10% homogenate of B16 melanoma tumor. MH134 hepatoma was inoculated into C3H/He mice with 5×10^6 cells intraperitoneally. Ascites tumor of fibrosarcoma originally induced in BALB/c mice with 3-methylcholanthrene (Meth 1)[4] has been passaged in the same strain[4]. For the experiment of antitumor activity, 1×10^6 cells were inoculated intraperitoneally into CDF_1 mice.

Gilvocarcins were prepared according to the method of NAKANO *et al*[1]. Mitomycin C (Kyowa Hakko Kogyo) and adriamycin (Kyowa Hakko Kogyo) were used as reference antitumor agents.

With the ascites tumors, antitumor activity was evaluated by comparing the mean survival time of the treated animal (T) with that of the control tumor bearing animals (C) *i.e.*, percentage increase in lifespan (ILS); $(T/C-1)\times 100$ (%). Each of the experiments was terminated on the 60th day after inoculation. For the solid tumor, the antitumor activity was evaluated in terms of the inhibitory ratio of tumor growth, comparing the mean size in the treated animals (T) with that in the control (C); *i.e.* T/C. Tumor sizes were measured periodically with calipers. Tumor volume was calculated by the formula represented in the NCI Protocols for screening[5]: Volume (mm^3)$=1/2\ ab^2$, where a and b represent the larger and smaller diameter, respectively.

LD_{50} was calculated from the number of survivors at 14 days after a single intravenous or intraperitoneal administration into *dd*Y mice.

Peripheral white blood cells of mice bearing solid sarcoma were counted with Micro Cell Counter (Toa Medical Electronics) 4 or 7 days after tumor transplantation.

For the effect on the growth of KB cells, 1×10^4 cells were incubated in 1 ml of Eagle's minimum essential medium (Nissui) supplemented with 10% calf serum. After 24 hours, drugs were added and cell numbers were counted after 72 hours incubation. The dose of 50% inhibition of cell growth (IC_{50}) was determined.

Results

Acute Toxicity of Gilvocarcin V

The LD_{50} of gilvocarcin V by a single intraperitoneal and intravenous dose were higher than 1,000 mg/kg and 375 mg/kg respectively. For gilvocarcin M, the LD_{50} was 450 mg/kg by a single intravenous administration.

Effect on Sarcoma 180

Table 1 shows the inhibitory effect of gilvocarcin V on the growth of solid sarcoma 180 implanted *in dd*Y mice. At a single injection of 400 mg/kg, gilvocarcin V inhibited tumor growth with T/C of 0.48. Successive injections at a dose of 100 mg/kg/day and 200 mg/kg/day for 5 days, starting 24 hours after

Table 1. Effect of gilvocarcin V on sarcoma 180.

Drugs	Dose (mg/kg/day)	Schedule[a]	T/C[b] (Day 7)	Body wt.[c] change (g)	WBC[d] (/mm^3)
Control	—	—	—	3.9	7,170*
Gilvocarcin V	50	Day 1	0.90	3.8	11,500*
	100	Day 1	0.85	4.1	
	200	Day 1	0.72	3.4	
	400	Day 1	0.48	1.2	
	25	Day 1~5	0.85	3.5	10,900**
	50	Day 1~5	0.72	2.8	13,400**
	100	Day 1~5	0.37	0.5	12,100**
	200	Day 1~5	0.34	−0.8	10,800**
Mitomycin C	1.0	Day 1~5	0.60	2.7	9,130*
	2.0	Day 1~5	0.40	−0.1	4,470*

a) Sarcoma 180 cells (5×10^6) were inoculated subcutaneously into *dd*Y mice on day 0.
b) Mean tumor volume of control mice on day 7 was $872\pm 308\ mm^3$.
c) Body weight change shows the difference of the body weight measured on day 1 and 7.
d) White blood cell counts on day 4 (*) and 7 (**).

Table 2. Effect of gilvocarcin V on survival of mice bearing i.p. Ehrlich carcinoma.

Exp.	Drugs	Dose (mg/kg)	Schedule[a]	Survival days	ILS[b] (%)	Survivors (60 days)
1.	Control	—	—	15.2±1.2	—	0/5
	Gilvocarcin V	12.5	Day 1	18.8±4.4	24	0/5
		25	Day 1	18.4±3.1	21	0/5
		50	Day 1	17.4±3.6	14	0/5
		100	Day 1	23.3±3.6	53	1/5
		200	Day 1	27.0±9.4	78	2/5
		400	Day 1	29.7±9.1	95	2/5
	Mitomycin C	2.1	Day 1	18.6±4.4	22	0/5
		4.2	Day 1	23.5±6.8	55	1/5
		5.6	Day 1	18.6±4.4	22	0/5
2.	Control	—	—	19.0±3.4	—	—
	Gilvocarcin V	5	Day 1~5	20.4±3.6	7	0/5
		10	Day 1~5	22.3±3.3	17	0/5
		20	Day 1~5	30.0±0	50	2/5
		40	Day 1~5	43.0±2.0	126	3/5
		80	Day 1~5	57.0	200	4/5

a) Tumors (5×10^6 cells) were inoculated intraperitoneally into *ddY* mice and injections were started at 24 hours after inoculation.

b) ILS (%) were calculated from the average lifespans of mice which died within 60 days.

the tumor implantation, gave T/C of 0.37 and 0.34 respectively. Five successive injections of 200 mg/kg/day showed slight decrease of body weight, but did not show lethal toxicity. None of the mice treated with gilvocarcin V showed a decrease in white blood cell counts even at the dose of 200 mg/kg/day for successive 5 days which gave 0.34 of T/C against solid sarcoma 180.

Effect on Ehrlich Ascites Carcinoma

Gilvocarcin V was intraperitoneally injected into mice bearing Ehrlich ascites carcinoma on day 1 and day 1~5. The dose effect of gilvocarcin V depended on the administration schedule. And the consecutive divided dose gave higher activity than a single administration. Single injection of 200 mg/kg produced 78% ILS, but 40 mg/kg/day for 5 days from day 1~5 gave 126% ILS. Gilvocarcin V displayed stronger activity than mitomycin C against Ehrlich carcinoma by both single and multiple dose treatments (Table 2). Gilvocarcin V was also more effective than mitomycin C and adriamycin when therapy was delayed until day 4 after tumor transplantation (Table 3).

Table 3. Effect of gilvocarcin V on advanced Ehrlich ascites carcinoma.

Drugs	Dose[a] (mg/kg)	Survival days	ILS (%)	Survivors (60 days)
Control	—	14.5±3.1	—	0/6
Gilvocarcin V	12.5	16.3±2.4	12	0/6
	25	16.5±2.7	14	0/6
	50	18.0±2.4	24	0/6
	100	20.7±4.5	43	0/6
	200	20.0±3.2	38	0/6
	400	27.3±8.7	89	0/6
Mitomycin C	4.2	16.7±3.6	15	0/6
	5.6	18.5±2.4	24	0/6
Adriamycin	3.5	20.0±5.6	38	0/6
	7.0	22.2±4.4	53	0/6
	14.0	19.3±7.2	33	0/6

a) Tumor cells (5×10^6) were intraperitoneally inoculated into *ddY* mice and drugs were injected intraperitoneally on day 4 after tumor transplantation.

Table 4. Effect of gilvocarcin V on Meth 1 fibrosarcoma.

Drugs	Dose[a] (mg/kg)	Schedule	ILS[b,c] (%)	Survivors (60 days)
Gilvocarcin V	25	Day 1	42	0/5
	50	Day 1	95	0/5
	100	Day 1	136	0/5
	200	Day 1	180	1/5
	400	Day 1	153	4/5
Mitomycin C	4.2	Day 1	109	0/5
	5.6	Day 1	115	1/5

a) Meth 1 tumor cells (1×10^6) were inoculated intraperitoneally into CDF_1 mice (20~22 g).
b) Mean survival time of control mice was 15.0±2.2 days.
c) For the calculation of ILS (%), mice which survived for 60 days were omitted.

Table 5. Effect of gilvocarcin V on MH 134.

Drugs	Dose[a] (mg/kg)	Survival days	ILS[b] (%)	Survivors (60 days)
Control	—	16.5± 1.1	—	0/6
Gilvocarcin V	25	17.5± 3.5	6	0/6
	50	21.3± 3.6	29	0/6
	100	31.5± 4.5	91	2/6
	200	53.0± 7.8	221	3/6
	400	39.5± 9.1	139	2/6
Mitomycin C	2	36.1± 4.6	119	0/6
	4	45.0±10.3	173	0/6
	6	17.8±13.9	7	0/6

a) MH 134 Hepatoma (10^6 cells) was inoculated intraperitoneally into C3H/HeJ mice and intraperitoneal injection was on day 1.
b) For the calculation of ILS (%), the mean survival days of mice died within 60 days after tumor inoculation was used as treated group.

Table 6. Effect of gilvocarcin V on survival of mice bearing i.p. P388 leukemia.

Exp.	Drugs	Dose (mg/kg)	Schedule[a]	Survival days	ILS[b] (%)
1.	Control	—	—	9.4±0.5	—
	Gilvocarcin V	50	Day 1	12.6±1.0	34.0
		100	Day 1	12.8±0.7	36.1
		200	Day 1	14.0±1.5	48.9
		400	Day 1	14.8±0.7	57.4
	Mitomycin C	4.2	Day 1	20.2±3.5	114.9
2.	Control	—	—	9.2±0.4	—
	Gilvocarcin V	12.5	Day 1~5	12.8±1.5	39.1
		25	Day 1~5	15.6±1.4	69.6
		50	Day 1~5	14.8±1.9	60.8
		100	Day 1~5	15.0±2.3	63.0
		200	Day 1~5	15.6±0.8	69.6
	Mitomycin C	4.2	Day 1	18.0±0.7	11.3
3.	Control	—	—	9.2±0.4	—
	Gilvocarcin V	50	Day 1	10.8±0.7	11.3
		100	Day 1	12.4±0.8	27.8
		200	Day 1	12.8±1.2	32.0
	Gilvocarcin M	25	Day 1	10.0±0	3.1
		50	Day 1	10.2±0.4	5.2
		100	Day 1	10.6±0.5	9.3
		200	Day 1	10.4±0.4	7.2
		400	Day 1	11.0±1.1	19.6

a) Leukemia P388 (1×10^6 cells) was inoculated intraperitoneally into CDF_1 mice on day 0.
b) Increased lifespan.

Effect on Methylcholanthrene Induced Fibrosarcoma

Meth 1 fibrosarcoma (1×10^6 cells) was inoculated intraperitoneally. Gilvocarcin V or mitomycin C were administered by a single intraperitoneal injection at 24 hours after tumor inoculation. The maximum value of ILS% produced by gilvocarcin V was 180%, but ILS % produced by mitomycin C was 115% at a dose of 5.6 mg/kg, almost equal to optimal dose. Gilvocarcin V was also shown more effective than mitomycin C, since 4 mice out of 5 treated mice survived for 60 days (Table 4).

Effect on MH134

Mice bearing ascites MH134 were given a single injection of gilvocarcin V on the day after tumor inoculation. Gilvocarcin V produced fairly good ILS value; a dose 200 mg/kg showed the maximum percentage of increase of lifespan with higher than 221% and 60% of treated mice survived more than 60 days. Mitomycin C was optimal at a dose of 4 mg/kg with 173% ILS, but no mice survived until 60 days (Table 5).

Effects on P388

Gilvocarcin V was intraperitoneally injected day 1 or day $1\sim5$ beginning 24 hours after inoculation of 10^6 tumor cells. As shown in Table 6, a single injection of 400 mg/kg gave 57% ILS, and the administration of 200 mg/kg/day for 5 consecutive days produced 69% ILS. The compound was less effective against P388 than mitomycin C which gave 114 and 96% ILS by single and 5 successive administrations respectively. Gilvocarcin M did not show antitumor activity against P388 at doses examined (Exp. 3 in Table 6).

Effect on B16 Melanoma

Gilvocarcin V was effective against ascites tumor of B16 melanoma and produced 40% ILS at a dose of 400 mg/kg on day 1. But the activity of gilvocarcin V was lower than that of mitomycin C which gave 72% of the maximum increase in lifespan by a single intraperitoneal injection (Table 7).

Table 7. Effect on B16 melanoma.

Drugs	Dose[a] (mg/kg)	Survival days (Mean±S.D.)	ILS[b] (%)
Control	—	20.5±0.8	—
Gilvocarcin V	25	21.5±1.8	5
	50	25.0±5.0	22
	100	23.3±2.2	14
	200	27.2±6.7	33
	400	28.6±6.3	40
	800	27.5±5.3	34
Mitomycin C	2.1	29.5±3.3	44
	4.2	35.2±6.4	72

a) Test compounds were administered intraperitoneally on day 1.
b) Increased lifespan.

Effect on Lewis Lung Carcinoma

Lewis lung carcinoma was implanted subcutaneously into BDF_1 mice and drug treatment was started on the day after implantation. Gilvocarcin V was given on 5 consecutive days and the tumor volume was measured on days shown in Table 8. At a dose of 400 mg/kg/day, gilvocarcin V caused marked inhibition of tumor growth on day 12 to day 18. However the prolongation of the lifespan of the treated mice was not produced in this administration schedule. Mitomycin C which showed a suppressive effect of tumor growth at a dose of 6 mg/kg, also did not produce the prolongation of the lifespan of treated mice.

Effect on KB Cells

IC_{50} was 0.012 mcg/ml for gilvocarcin V, 0.52 mcg/ml for gilvocarcin M and 0.64 mcg/ml for gilvocarcin A which was prepared from gilvocarcin V as described in the previous report[1].

Table 8. Effect on Lewis lung carcinoma.

Drugs	Dose[a] (mg/kg/day)	Day 12		Day 15		Day 18	
		Tumor volume[b] (Mean±S.D.)	T/C	Tumor volume[b] (Mean±S.D.)	T/C	Tumor volume[b] (Mean±S.D.)	T/C
—	—	916±379	—	1,681±582	—	4,767±1,468	—
Gilvocarcin V	50	864±430	0.91	1,640±591	0.98	5,034±1,371	1.05
	100	673±440	0.73	1,357±506	0.81	4,197± 716	0.88
	200	702±170	0.77	1,266±191	0.75	3,801± 728	0.80
	400	318±164	0.35	594±283	0.35	2,065± 682	0.43
Mitomycin C	4	706±179	0.77	1,616±310	0.96	4,347± 829	0.91
	6	269± 99	0.29	593±259	0.35	2,480± 899	0.53

[a] Gilvocarcin V was administered intraperitoneally in 5 divided doses (days 1~5) and mitomycin C was administered intraperitoneally on day 1 after tumor transplantation.

[b] Mean tumor volume (mm^3).

Discussion

It is apparent from the present results that gilvocarcin V has a broad spectrum of activity against a number of experimental neoplasms in mice. In particular, gilvocarcin V is markedly effective against the ascites tumor. Among the various tumor lines examined, ascitic Ehrlich carcinoma and MH134 responded especially well to gilvocarcin V. For Ehrlich ascites carcinoma, it produced 200% ILS and gave four 60-day survivors per 5 mice. But mitomycin C gave 173% ILS and no 60-day survivors. For MH134, the optimal dose of gilvocarcin V made 50% of treated mice survive more than 60 days, but no mice survived after administration of mitomycin C. Solid tumor was less susceptible to gilvocarcin V than ascites tumor and significant activity was obtained only by successive administration schedule.

As the LD_{50} of gilvocarcin V was higher than 1,000 mg/kg by a single intraperitoneal administration and optimal dose was not determined in several tumor systems, the correct therapeutic ratio (Optimal dose/Dose of 30% ILS) could not be calculated. The safety margin of gilvocarcin V might be very high in an ascites tumor system, because, in the i.p.-i.p. system of P388, a typical example of the i.p.-i.p. system, the dose range of gilvocarcin V which gave ILS over 30% was shown from 12.5 mg/kg to 400 mg/kg.

Myelosuppressive activity of gilvocarcin V was not observed in *dd*Y mice bearing sarcoma 180. On the contrary, a slight increase in the number of peripheral white blood cells was observed after 5 successive injections of gilvocarcin V. The result might suggest an irritating property of gilvocarcin V in mice.

As gilvocarcin V was virtually insoluble in water, the therapeutic efficacy of the p.o. administration was a subject of discussion. But it was not effective against sarcoma 180 by oral administration.

Gilvocarcin M, 8-methylisomer of gilvocarcin V, and gilvocarcin A, 8-aldehydeisomer of gilvocarcin V were not active against P388 (i.p.-i.p.) (unpublished observation). Gilvocarcins M and A were 50 times less effective than gilvocarcin V on the growth of KB cells. These results may indicate the importance of the 8-vinyl group for the antitumor activity, but the difference in solubility of gilvocarcins M, A and V may also contribute to their activity.

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