AZINOMYCINS A AND B, NEW ANTITUMOR ANTIBIOTICS

III. ANTITUMOR ACTIVITY

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Azinomycins A and B, isolated from the culture broth of *Streptomyces griseofuscus* S 42227, were examined for antitumor activities against P388 leukemia, P815 mastocytoma, B-16 melanoma, Ehrlich carcinoma, Lewis lung carcinoma and Meth A fibrosarcoma. Azinomycin B was markedly effective against the intraperitoneally inoculated tumors such as P388 leukemia, B-16 melanoma and Ehrlich carcinoma. The intraperitoneal administration of azinomycin B showed 57% survivors for 45 days and 193% ILS against P388 leukemia. For Ehrlich carcinoma, azinomycin B gave 161% ILS and 63% survivors for 45 days, but solid tumors such as Lewis lung carcinoma and Meth A fibrosarcoma were not susceptible to repeated injection of this substance. Azinomycin A was somewhat less effective than azinomycin B for the tumor systems tested.

In the course of screening for new antitumor substances, azinomycins A (**1**) and B (**2**) were discovered in the culture broth of strain S 42227. In the previous paper[1], the taxonomy of the producing organism, fermentation, isolation, physico-chemical properties, antimicrobial activity and *in vitro* cytotoxicity of azinomycins were reported. This paper describes the antitumor activity of these substances against various murine tumors.

Materials and Methods

Animals

Male mice of CDF_1 (BALB/c×DBA/2) and BDF_1 (C57BL/6×DBA/2) were purchased from Charles River Japan Inc. Female mice of ICR were obtained from Clea Japan, Inc. These mice, fed a standard diet (MF, made of Oriental Yeast Co.) and housed in plastic cages, were used at 6 and 10 weeks old.

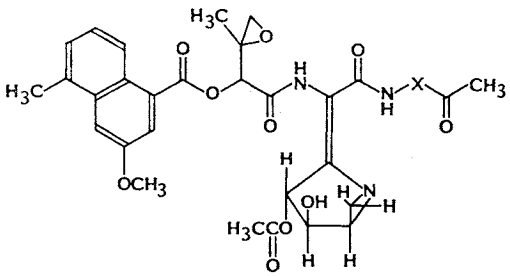
Tumors

P388 Leukemia, B-16 melanoma and Lewis lung carcinoma were obtained from Cancer Chemotherapy Center. Ehrlich carcinoma and Meth A fibrosarcoma were donated from National Cancer Center. P815 Mastocytoma was presented from Institute of Microbial Chemistry.

P388 Leukemia and P815 mastocytoma have been maintained by intraperitoneal passage in DBA/2 mice, and 1×10^6 cells of each tumor were inoculated intraperitoneally to CDF_1 mice. Ehrlich carcinoma cells (1×10^6) were intraperitoneally injected into ICR mice. B-16 Melanoma was transplanted by trocar into C57BL/6 mice for passage. BDF_1 mice were inoculated intraperitoneally with 0.1 ml of 50% homogenate of B-16 melanoma. Lewis lung carcinoma was maintained in C57BL/6 mice and for antitumor test 5×10^5 viable cells were implanted subcutaneously in axillary region of BDF_1 mice. For experiment on solid type of Meth A, 1×10^6 cells were transplanted subcutaneously in axillary region of CDF_1 mice.

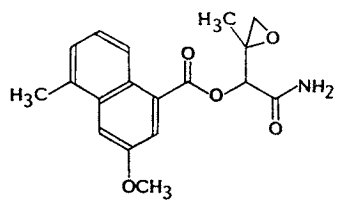
Antitumor activity against the intraperitoneally inoculated tumors and Lewis lung carcinoma was evaluated by comparing the mean survival time (days) of the treated group (T) with that of the

Fig. 1. Structure of azinomycins A, B and a related metabolite.



Azinomycin A (**1**) X = CH_2

Azinomycin B (**2**) X = C=C(OH)H



Related metabolite (**3**)

control (C) *i.e.*, percent increase in lifespan (ILS); (T/C−1)×100 (%). Each experiment was terminated on the 45th or 60th day after inoculation. Furthermore, the lifespan of mice surviving even at the end of the experiment were calculated as the value of the observation period.

With the solid tumors, the antitumor activity was determined as the percent inhibition of the weight of tumors which were weighed on the 21st day after transplantation.

Drug treatments were carried out by intraperitoneal administration daily for 10 days from the day following tumor inoculation.

Acute toxicities of test substances were estimated as LD_{50} values, which were calculated from the mortality for 14 days after a single intraperitoneal injection into *dd*Y mice.

Results

Antitumor Activity on Murine Transplantable Tumors

P388 Leukemia

Table 1 shows the antitumor activity of azinomycins A and B on P388 leukemia. Azinomycin B was effective in a range of 2 to 32 μg/kg almost dose-dependently. At a dose of 32 μg/kg, the maximum values were obtained on survival time (193% ILS) and survivors (4/7). Azinomycin A also showed a significant increase in survival time with a lesser potency than azinomycin B. Mitomycin C (1 mg/kg/day), used as a reference antitumor agent, showed 204% ILS and gave 4 survivors in 7 mice.

P815 Mastocytoma

Azinomycin B prolonged the survival period (46% ILS) only at a dose of 30 μg/kg, but not at the other dose levels. Azinomycin A gave no prolongation of the lifespan of treated mice. P815 mastocytoma was not susceptible to azinomycins (Table 2).

B-16 Melanoma

Table 3 shows the inhibitory effect of azinomycins A and B against B-16 melanoma. Azinomycin B increased the survival time markedly and showed more than 35% ILS among 5 dose ranges from 2 to 32 μg/kg. The maximal value in prolongation of lifespan was 64%, and 12.5 or 25% of mice treated with a dose range from 4 to 32 μg/kg survived for more than 60 days. Azinomycin A produced 75% ILS at a dose of 16 μg/kg, but its effective dose range was narrower than that of azinomycin B.

Ehrlich Carcinoma

As shown in Table 4, azinomycin B exhibited a dose-related increase in the lifespan. Especially

Table 1. Antitumor effect of azinomycins A and B on P388 leukemia.

Drugs	Dose (μg/kg/day)	Survival time[a] (days; mean±SD)	ILS (%)	Survivors[b] (45 days)
Control	—	12.3±1.4	—	0/7
Azinomycin A	1	16.6±5.2	35	0/7
	2	15.4±1.1	25	0/7
	4	18.8±1.8	53	0/7
	8	18.3±2.9	49	0/7
	16	21.6±6.7	76	0/7
	32	14.4±9.2	17	0/7
Azinomycin B	1	15.1±1.2	23	0/7
	2	17.9±2.2	46	0/7
	4	24.6±14.6	100	2/7
	8	20.7±10.9	68	1/7
	16	36.0±9.6	193	3/7
	32	36.0±13.1	193	4/7
	64	26.3±9.1	114	0/7
Mitomycin C	1 mg/kg/day	37.4±11.0	204	4/7

Each mouse was inoculated with 1×10^6 cells. The drugs were administered daily for 10 days from the next day after the inoculation.

ILS: Increase in lifespan.

[a] Animals surviving more than 45 days were calculated as 45 days survivors.

[b] Survivors for over 45 days per total numbers treated.

Table 2. Antitumor effect of azinomycins A and B on P815 mastocytoma.

Drugs	Dose (μg/kg/day)	Survival time[a] (days; mean±SD)	ILS (%)	Survivors[b] (45 days)
Control	—	12.0±1.2	—	0/6
Azinomycin A	0.77	14.5±3.3	21	0/6
	1.9	14.5±0.8	21	0/6
	4.8	14.2±1.6	18	0/6
	12	14.7±2.5	23	0/6
	30	14.8±1.3	23	0/6
Azinomycin B	0.77	11.8±1.7	−2	0/6
	1.9	14.3±1.4	19	0/6
	4.8	15.3±1.2	28	0/6
	12	14.5±2.6	21	0/6
	30	17.5±3.3	46	0/6
	75	13.8±2.9	15	0/6

Each mouse was inoculated with 1×10^6 cells. The drugs were administered daily for 10 days from the next day after the inoculation.

ILS: Increase in lifespan.

[a] Animals surviving more than 45 days were calculated as 45 days survivors.

[b] Survivors for over 45 days per total numbers treated.

at the dose range from 8 to 32 μg/kg, more than 2-fold ILS (maximum ILS was 161%) and several survivors for over 45 days were obtained. Azinomycin A also showed an obvious antitumor activity in this experiment. Treatment of azinomycin A (2~16 μg/kg) resulted in more than 100% of ILS and some survivors for over 45 days.

Lewis Lung Carcinoma

Table 5 shows the inhibitory effect of azinomycin B against subcutaneously transplanted Lewis

Table 3. Antitumor effect of azinomycins A and B on B-16 melanoma.

Drugs	Dose (μg/kg/day)	Survival time[a] (days; mean±SD)	ILS (%)	Survivors[b] (60 days)
Control	—	21.9±3.3	—	
Azinomycin A	1	26.4±8.1	21	0/8
	2	25.6±3.6	17	0/8
	4	27.0±2.7	23	0/8
	8	31.8±12.1	45	1/8
	16	38.4±14.8	75	2/8
Azinomycin B	1	25.9±10.7	18	0/8
	2	29.9±10.7	37	0/8
	4	30.5±12.5	39	1/8
	8	36.0±15.1	64	2/8
	16	31.4±12.0	43	1/8
	32	33.7±12.7	54	1/8

Each mouse was inoculated with 0.1 ml of 50% homogenate of the tumor. The drugs were administered daily for 10 days from the next day after the inoculation.

ILS: Increase in lifespan.

[a] Animals surviving more than 60 days were calculated as 60 days survivors.

[b] Survivors for over 60 days per total numbers treated.

Table 4. Antitumor effect of azinomycins A and B on Ehrlich carcinoma.

Drugs	Dose (μg/kg/day)	Survival time (days; mean±SD)	ILS (%)	Survivors (45 days)
Control	—	14.3±3.1	—	
Azinomycin A	1	12.1±4.6	−15	0/8
	2	30.0±13.7	110	2/8
	4	31.6±9.7	121	2/8
	8	36.6±7.5	156	3/8
	16	34.6±10.1	142	2/8
Azinomycin B	1	18.4±11.2	29	0/8
	2	25.8±10.8	80	0/8
	4	26.4±10.4	85	1/8
	8	31.3±9.9	119	2/8
	16	35.8±10.6	150	4/8
	32	37.3±10.9	161	5/8

Experimental conditions are the same as in Table 1.

Table 5. Antitumor effect of azinomycin B on Lewis lung carcinoma.

Drugs	Dose (μg/kg/day)	Survival time[a] (days; mean±SD)	ILS (%)
Control	—	28.1±3.3	—
Azinomycin B	0.77	28.8±5.1	2
	1.9	28.5±4.8	1
	4.8	27.7±5.0	−1
	12	25.1±5.4	−11
	30	23.4±5.7	−17

Each mouse was implanted subcutaneously with 5×10^5 cells. The drug was administered daily for 10 days from the next day after the tumor implantation.

ILS: Increase in lifespan.

[a] No animal survived for over 38 days.

Table 6. Antitumor effect of azinomycins A and B on Meth A fibrosarcoma.

Drugs	Dose (μg/kg/day)	Tumor weight (g; mean±SD)	Inhibition (%)
Control	—	8.95±2.28	—
Azinomycin A	0.77	10.29±1.13	−15
	1.9	9.47±1.26	−6
	4.8	9.41±1.91	−5
	12	8.02±1.38	10
	30	5.54±2.44	38
Azinomycin B	0.77	10.19±2.09	−14
	1.9	10.26±2.51	−15
	4.8	9.46±1.50	−6
	12	8.94±1.25	0
	30	6.06±2.65	32

Each mouse was implanted subcutaneously with 1×10^6 cells. The drugs were administered daily for 10 days from the next day after tumor implantation. Tumors were weighed on 21st day after the implantation.

lung carcinoma. None of the mice treated with azinomycin B showed the prolongation of the lifespan and the suppression of the tumor growth (data not shown).

Meth A Fibrosarcoma

No suppression of the tumor growth was obtained on mice treated with azinomycins A and B (Table 6).

Acute Toxicity

The LD_{50} of azinomycin B by a single intraperitoneal administration was 190 μg/kg.

Discussion

Azinomycin B showed a significant antitumor activity against ascites tumor systems with a broad spectrum in this study. In particular, P388 leukemia and Ehrlich carcinoma inoculated into the abdominal cavity of mice were susceptible to azinomycin B, which gave about 60% survivors over the observation period. On B-16 melanoma, this substance also produced the marked prolongation of lifespan, whereas no effect was observed on Lewis lung carcinoma and Meth A fibrosarcoma by administration of azinomycin B. Azinomycin A was also effective against some intraperitoneally inoculated tumors, but showed no effect on solid tumor of Meth A fibrosarcoma. However the antitumor potency of azinomycin A was somewhat less than azinomycin B and the antitumor spectrum was also narrower.

Azinomycin A was more cytotoxic than azinomycin B against L5178Y cells *in vitro* and various bacteria[1]. On the other hand, azinomycin B was more potent than azinomycin A with respect to antitumor activity *in vivo*. These are unique antibiotics which have 1-azabicyclo-[3.1.0]-hexane ring system in the chemical structure (Fig. 1)[2]. However, the structurally related metabolites which were deprived of the ring system coproduced with azinomycins (3) had no antibacterial and antitumor activities (data not shown). Thus the partial structure consisting of 1-azabicyclo-[3.1.0.]-hexane ring system in these antibiotics may be the essential constituent for antitumor activity.

Furthermore, as the antitumor effect of azinomycin B on P388 leukemia was almost comparable to mitomycin C which has been used clinically, further studies of this antibiotic might be interesting.

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