ANTITUMOR ACTIVITY OF A NEW ANTITUMOR ANTIBIOTIC, STUBOMYCIN

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The antitumor activity of a new antibiotic, stubomycin, against various murine tumors was studied. Stubomycin showed antitumor activity against Sarcoma 180, IMC-carcinoma and Meth-A tumor. In particular, all of the mice inoculated i.p. with IMC-carcinoma were cured when they were given nine successive injections of stubomycin. Stubomycin also showed remarkable antitumor effects against Sarcoma 180 and Ehrlich ascites carcinoma when administered concomitantly with bleomycin.

A new antitumor antibiotic, stubomycin, was isolated from the culture broth and mycelia of *Streptomyces* strain No. KG-2245 by UMEZAWA *et al.* in 1981.[1] The antibiotic is active against Gram-positive bacteria, some fungi and HeLa cells *in vitro*. The molecular formula was determined as $C_{29}H_{35}NO_5$. Structural studies suggested that the antibiotic contains a polyene amide group (–NH–CO–$(C=C)_n$–) and a phenyl group.

In the previous report, the preliminary results of antitumor activity of stubomycin against Ehrlich ascites carcinoma and leukemia P-388 were described. This paper describes the antitumor activity of stubomycin against various murine tumors.

Materials and Methods

Animals

Male ICR, *dd*Y, CDF_1, BDF_1, and BALB/c mice 5 ~ 7 weeks of age were obtained from the Shizuoka Agricultural Cooperative Association (Hamamatsu).

Tumors

Lewis lung carcinoma and B-16 melanoma were obtained from Dr. TSUKAGOSHI of the Cancer Chemotherapy Center (Tokyo) and these were inoculated by trocar into C57BL/6 mice for passage. IMC-carcinoma obtained from the Institute of Microbiological Chemistry was maintained in CDF_1 mice. Sarcoma 180 obtained from the Sasaki Institute (Tokyo) was maintained in ICR mice and Meth-A tumor was maintained in BALB/c mice.

Antitumor activity was evaluated by the increase in life span (ILS): $(T/C-1)\times 100\%$, where "T" is the median survival days (MSD) of the treated group and "C" is the MSD of the control group. Survival of mice was scored 60 days after implantation of tumors and mice remaining alive after this period of observation were considered cured. For the solid tumor, major and minor diameters of the tumor were measured with calipers and the antitumor activity was evaluated as the inhibitory ratio of tumor growth by comparing the mean size in the treated mice with that in the controls.

Agents

Stubomycin isolated in our laboratory was suspended in 0.5% carboxymethyl cellulose (CMC). The anticancer agents used in this study were mitomycin C (MMC, Kyowa Hakko Kogyo Co.), 5-fluorouracil (5-FU, Kyowa Hakko Kogyo Co.), cyclophosphamide (CY, Shionogi Pharma. Co. Ltd.) and bleomycin (BLM, Nippon Kayaku Co.). 5-FU was suspended in 0.5% CMC and all other agents were dissolved in sterile physiological saline. They were administered at a volume of 0.1 ml/10 g of body weight.

Results

Effect on Sarcoma 180

As shown in Table 1, a single i.p. injection of stubomycin at a dose of 150 mg/kg or 300 mg/kg resulted in an ILS value of 175% or 300% respectively; one or three out of seven mice respectively survived for over 60 days. Nine successive administrations of 150 mg/kg or 300 mg/kg also gave an ILS of 300% or 275% respectively and three out of seven mice survived for over 60 days.

Effect on IMC-carcinoma

Table 2 shows the antitumor activity of stubomycin on IMC-carcinoma inoculated i.p. Nine injections of stubomycin on consecutive days at a total dose of 150 mg/kg or 300 mg/kg gave ILS of over 173% and all of mice survived for over 60 days. With single injection of 300 mg/kg, four out of seven mice survived for over 60 days.

Effect on Meth-A Tumor

As shown in Table 3, stubomycin was also effective against Meth-A tumor inoculated i.p. Single or nine successive administrations of stubomycin at a total dose of 75 mg/kg to 300 mg/kg produced ILS values of 30% to 80%.

Table 1. Effect of stubomycin on Sarcoma 180.

Dose (mg/kg/day)	Treatment schedule	MSD	ILS (%)	60-day survivors
—	—	12	0	0 / 7
75	Day 1	33	175	3 / 7
150		33	175	1 / 7
300		48	300	3 / 7
8.3	Days 1~9	20	67	1 / 7
16.7		49	308	1 / 7
33.3		45	275	3 / 7

Sarcoma 180 cells (1×10^5) were inoculated i.p. into IRC mice on day 0.

Table 2. Effect of stubomycin on IMC-carcinoma.

Dose (mg/kg/day)	Treatment schedule	MSD	ILS (%)	60-day survivors
—	—	22	0	0 / 7
75	Day 1	20	−9	0 / 7
150		24	9	1 / 7
300		>60	>173	4 / 7
8.3	Days 1~9	21	−5	1 / 7
16.7		>60	>173	7 / 7
33.3		>60	>173	7 / 7

IMC-carcinoma cells (1×10^4) were inoculated i.p. into CDF_1 mice.

Table 3. Effect of stubomycin on Meth-A tumor.

Dose (mg/kg/day)	Treatment schedule	MSD	ILS (%)	60-day survivors
—	—	20	0	0 / 7
75	Day 1	28	40	0 / 7
150		31	55	0 / 7
300		36	80	0 / 7
8.3	Days 1~9	26	30	0 / 7
16.7		30	50	0 / 7
33.3		26	30	0 / 7

Meth-A tumor cells (1×10^5) were inoculated i.p. into BALB/c mice.

Table 4. Effect of stubomycin on Lewis lung carcinoma and B-16 melanoma.

Dose (mg/kg/day)	Treatment schedule	MSD		ILS(%)	
		L.L.	B-16	L.L.	B-16
—	—	29	19	0	0
75	Day 1	29	28	0	47
150		30	22	3	16
300		34	20	17	5
8.3	Days 1~9	29	25	0	31
16.7		34	21	17	11
33.3		13	13	−55	−32

Lewis lung (L.L.) carcinoma cells (5×10^5) or B-16 melanoma cells (5×10^5) were inoculated s.c. into BDF_1 mice.

Table 5. Combined effect of stubomycin and known antitumor agents on Sarcoma 180.

Antitumor agent (mg/kg)	Stubomycin dose (mg/kg)	MSD	ILS (%)	60-day survivors
—	—	18	0	0 / 7
	25	22	22	0 / 7
	50	26	44	1 / 7
MMC (0.125)	—	22	22	0 / 7
	25	26	44	2 / 7
	50	23	28	0 / 7
5-FU (100)	—	23	28	0 / 7
	25	27	50	0 / 7
	50	19	6	0 / 7
CY (50)	—	19	6	1 / 7
	25	27	50	1 / 7
	50	36	100	1 / 7
BLM (12.5)	—	22	22	0 / 7
	25	>60	>233	4 / 7
	50	>60	>233	4 / 7

Sarcoma 180 cells (1×10^6) were inoculated i.p. into ICR mice on day 0. Stubomycin and antitumor agents were administered i.p. on day 1.

Table 6. Combined effect of stubomycin and bleomycin on Ehrlich ascites carcinoma.

BLM dose (mg/kg)	Stubomycin dose (mg/kg)	MSD	ILS (%)	60-day survivors
—	—	20	0	0 / 7
	25	20	0	0 / 7
	50	51	155	3 / 7
12.5	—	20	0	0 / 7
	25	>60	>200	4 / 7
	50	>60	>200	4 / 7
25	—	41	105	2 / 7
	25	>60	>200	4 / 7
	50	>60	>200	5 / 7

Ehrlich ascites carcinoma (1×10^6) were inoculated i.p. into *dd*Y mice on day 0. Stubomycin and bleomycin were administered i.p. on day 1.

Effect on Lewis Lung Carcinoma and B-16 Melanoma

The effect of stubomycin on Lewis lung carcinoma and B-16 melanoma inoculated s.c. was examined. Stubomycin was slightly effective against B-16 melanoma. It resulted in an ILS of 47% at a dose of 75 mg/kg on day 1 (Table 4).

Combined Effect on Sarcoma 180

Sarcoma 180 (1×10^6) cells were inoculated into ICR mice on day 0. Then stubomycin and known antitumor agents were administered i.p. on day 1. As shown in Table 5, potentiation of the antitumor effect was observed with a combination of stubomycin and bleomycin, *i.e.*, a single i.p. injection of stubomycin at dose of 25 mg/kg or 50 mg/kg gave ILS values of 22% or 44% respectively while bleomycin at a dose of 12.5 mg/kg resulted in a 22% ILS. In comparison with a single drug, a combination of stubomycin and bleomycin yielded a ILS value of over 233% and four out of seven mice survived for over 60 days.

Combined Effect on Ehrlich Ascites Carcinoma

Table 6 shows the synergistic effect of stubomycin and bleomycin on Ehrlich ascites carcinoma. A single intraperitoneal injection of 25 mg/kg or 12.5 mg/kg of stubomycin or bleomycin respectively on day 1 caused no prolongation of the life span of the treated mice. However, simultaneous injections of stubomycin and bleomycin resulted in an ILS of more than 200% and four out of seven mice survived for over 60 days.

Discussion

Stubomycin showed remarkable antitumor effects against various murine tumors. In particular, all seven mice bearing IMC-carcinoma survived for more than 60 days at an optimal dose. It was suggested that stubomycin has a polyene amide moiety in its structure like viridenomycin[2,3] and vario-

tin.[4,5] However, the biological characteristics of stubomycin were remarkably different from viridenomycin and variotin, since these did not show the antitumor activity. A remarkable combined effect of stubomycin and bleomycin was observed. Stubomycin has a polyene structure but does not belong to the polyene macrolide group which potentiated the effect of known antitumor agents.[6~9] However, since stubomycin inhibits the growth of fungi as in the case of polyene macrolide antibiotics, it is considered that the mechanism of action in combined therapy is partially similar to that of known antibiotics. In this experiment, mice were given with stubomycin by intraperitoneal injection because the antibiotic is hardly soluble in water and most organic solvent. Then, we are studying the drug design for intravenous injection, and the result will be reported elsewhere.

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