IYOMYCIN, A NEW ANTITUMOR ANTIBIOTIC FROM *STREPTOMYCES*. V

EXPERIMENTAL TREATMENT OF SARCOMA 180 WITH IYOMYCIN-B_1 COMPLEX AND ITS DERIVATIVES

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In attempts to reduce the toxicity while maintaining the antitumor activitvy of iyomycin B_1, a variety of complexes and derivatives were prepared and tested.

1. No enhancement of anti-tumor activity was observed with complexes of iyomycin B_1 and human serum albumin prepared at various ratios, and with 6 kinds of metal salts. However, an interesting result was obtained with the magnesium salt of iyomycin B_1.

2. Although the toxicity was reduced in complexes of A and B_1, and in reduced B_1 prepared by the cataltic hydrogenation, their antitumor activities were inferior to that of iyomycin B_1 itself.

3. The acetyl derivative of iyomycin B_1 was found to have lower toxicity and higher antitumor activity than iyomycin B_1 itself. Tumors disappeared entirely in 70 % of the mice treated with the derivative from 3 days after the implantation of the tumor.

Iyomycin is a new antitumor antibiotic produced by a new species, *Streptomyces phaeoverticillatus* isolated by the authors in 1959. The microbiological characteristics of the new species and the procedures of extraction and purification of iyomycins were described in the previous papers[1,2,3].

Iyomycin is composed of iyomycin A, a high-molecular weight substance, and iyomycin B (B_1, B_2, B_3, B_4 and B_5), a mixture of low-molecular weight materials[3]. Among of these, B_1 has the strongest antitumor activity. As reported in the previous papers[4,5], iyomycins A and B_1 inhibit the proliferation of mouse ascites tumors such as Sarcoma 180, EHRLICH carcinoma and Leukemia SN36, and also Sarcoma 180 solid tumor. However, the curative dosage of iyomycin B_1 is close to the LD_{50} value. Accordingly, in attempts to reduce the toxicity without losing its antitumor activity, a variety of complexes and derivatives of B_1 were prepared and were administered to mice bearing Sarcoma 180 (solid tumor). The results of these experiments are described in the present paper.

Experimental Materials

The samples used in the experiments were prepared as follows:

1. Albumin complex[6]: Sixty mg of iyomycin B_1 free base was added to 840 mg of human serum albumin, mixed thoroughly, and triturated with small amounts of distilled water dropwise. The mixture was at first viscous, and became a homogeneous solution

when a final volume of 10 ml had been added. The solution was centrifuged to remove a very small amount of insoluble precipitate, and the supernatant solution was lyophilized to obtain an orange-yellow powder, (Al-15) 1/15 of which was iyomycin B_1 free base. Complexes of iyomycin B_1 and human serum albumin in the ratio of 1 : 30 and 1 : 45 were also prepared (Al-30 and Al-45).

2. Complex of A and B: To prepare a complex of A and B, concentrated solutions of A and B_1-hydrochloride in water were mixed at room temperature and the resulting precipitate was collected, washed with water, and lyophilized to obtain a powder. This powder was dissolved in 1 % sodium bicarbonate solution just before use in the animal experiments. By the criteria of solubility in water and anti-tumor activity, a combination of A and B_1 in the ratio of 20 : 1 resembled closely the naturally occurring iyomycin complex.

3. Metal salts: Iyomycin B_1 was found to form a variety of metallic salts. To prepare such salts, iyomycin B_1 free base, with a molecular weight estimated tentatively to be 1,000, was dissolved in methanol in high concentration, mixed with a concentrated methanol solution of the inorganic (or organic) metallic salt in the mol-ratio of 1 : 2, and the mixture kept overnight in a refrigerator. The resulting precipitate was successively washed with methanol and petroleum ether, and then dried to obtain a powder. The metallic salts were dissolved in water or in M/15 NaH_2PO_4 solution just before use.

4. Reduced iyomycin B_1: Iyomycin B_1 free base was reduced in methanol using platinum black as a catalyst to obtain reduced iyomycin B_1.

5. Acetylated iyomycin B_1[7]: Iyomycin B_1 free base was acetylated with acetic anhydride in the presence of pyridine at 37°C, and separated by alumina column chromatography to obtain purified acetylated iyomycin B_1. For the use of animal experiments, this substance was dissolved in M/2.5 NaH_2PO_4 solution.

Experimental Method

Subcutaneous solid tumors of Sarcoma 180 about 10 days old in *dd* mice were removed aseptically and slices, 2~3 mm^3 in size, were implanted into the axillary cavity of experimental mice with a trochar.

Treatment was initiated 24 hours after the implantation, by injection into the tail vein once daily for 7 consecutive days. The mice were killed after 11 days and the weight of each tumor was compared with that of the controls. In some experiments, the size of the subcutaneous tumor was measured at given intervals until death of the tumor bearing mice.

Results

1. Human serum albumin-B_1 complexes reduced the toxicity of B_1 to mice considerably. The LD_{50} by intravenous injection to mice was 125 mg/kg with Al-15 and 380 mg/kg with Al-30.

For the evaluation of the antitumor effect, a daily dosage of 1/4~1/8 LD_{50} was administered for 7 consecutive days.

The tumor-weight of each group administered 30 mg/kg (1/4 LD_{50}) of Al-15 and 95 mg/kg (1/4 LD_{50}) of Al-30 as one dose was 38.7 % and 39.7 % of those of the control group, respectively. These antitumor activities were about one half that of the B_1 group in which tumor weight was 21.4 %, as shown in Tables 1 and 3. No significant difference of antitumor effect was noticed between Al-15 and Al-30.

2. The acute LD_{50} of A-B_1 complex, was 75 mg/kg by intraperitoneal injection and 25 mg/kg by intravenous injection to mice.

Table 1. Effect of iyomycin B_1-albumin and A-B_1 complexes on S-180 (solid) in mice.

Substance	Administration route	Dose: Daily (mg/kg)	Dose: Total (mg/kg)	Interval and frequency	No. of mice survived/treated	Average body weight change (g)	Tumor: weight (mg)	Tumor: % of control
Al-15	I.V.	30.0	210	Daily×7	10/10	−0.9±1.14	525	38.7
		15.0	105	〃	9/9	−1.6±1.51	735	54.2
	I.P.	95.0	665	〃	5/8	−3.3±0.65	522	39.4
		47.5	329	〃	8/8	−2.0±0.97	1,180	89.0
Al-30	I.V.	95.0	665	〃	10/10	+0.2±0.92	538	39.7
		47.5	329	〃	10/10	−1.9±2.38	734	54.2
	I.P.	95.0	665	〃	9/9	−3.8±0.87	1,066	80.5
Control	I.V.	Saline		〃	10/10	−2.8±0.97	1,355	—
	I.P.			〃	10/10	−1.2±1.54	1,324	—
A-B_1	I.V.	8.25	58.1	〃	6/8	−2.8±1.7	537	53.8
		4.13	28.7	〃	8/8	−1.7±1.9	733	73.5
	I.P.	25.0	175.0	〃	8/8	−3.0±2.5	225	21.4
		12.5	87.5	〃	8/8	−3.7±1.4	534	50.8
Control	I.V.	Saline		〃	8/8	−1.9±1.1	996	—
	I.P.			〃	8/8	−0.8±0.6	1,051	—

1) Treatment began 24 hours after implantation.
2) Animals sacrificed on the 11th day after implantation.

Table 2. Effect of iyomycin B_1-metal salts on S-180 (solid) in mice.

Substance	Administration route	Dose: Daily (mg/kg)	Dose: Total (mg/kg)	Interval and frequency	No. of mice survived/treated	Average body weight change (g)	Tumor: weight (mg)	Tumor: % of control
B_1-Cu	I.V.	1.4	9.7	Daily×7	8/8	+0.9±1.5	591	60
		0.7	4.9	〃	8/8	+1.3±1.5	720	73
B_1-Ca	I.V.	1.4	9.7	〃	8/8	−0.72±0.8	614	62
		0.7	4.9	〃	8/8	+1.46±2.3	649	66
B_1-Mg	I.V.	1.4	9.7	〃	8/8	−3.1±1.0	320	32
		0.7	4.9	〃	8/8	+0.63±2.7	648	65
B_1-Fe	I.V.	1.4	9.7	〃	8/8	+2.2±1.7	609	62
		0.7	4.9	〃	8/8	+2.6±1.1	620	63
B_1-Co	I.V.	1.4	9.7	〃	7/7	−1.0±1.3	528	53
		0.7	4.9	〃	8/8	+0.8±1.8	709	72
B_1-Hg	I.V.	1.4	9.7	〃	8/8	−0.7±0.9	451	46
		0.7	4.9	〃	8/8	+1.1±1.4	800	81
Control	I.V.	Saline		〃	8/8	−0.8±2.6	990	—

1) Treatment began 24 hours after implantation.
2) Animals sacrificed on the 11th day after implantation.

The effect of A-B_1 complex on S-180 solid tumor is illustrated in Table 1. In the groups administered 1/3 and 1/6 LD_{50} as one dose, the tumor weight was about 50~70 % that of the control group.

The antitumor activity was not enhanced, though the toxicity was reduced.

3. The acute toxicities of various metallic salts of B_1 were all similar to that of B_1 alone. The mice were treated with 6 kinds of metallic salts of B_1 at dosages of 1/3 and 1/6 LD_{50} with the results illustrated in Table 2.

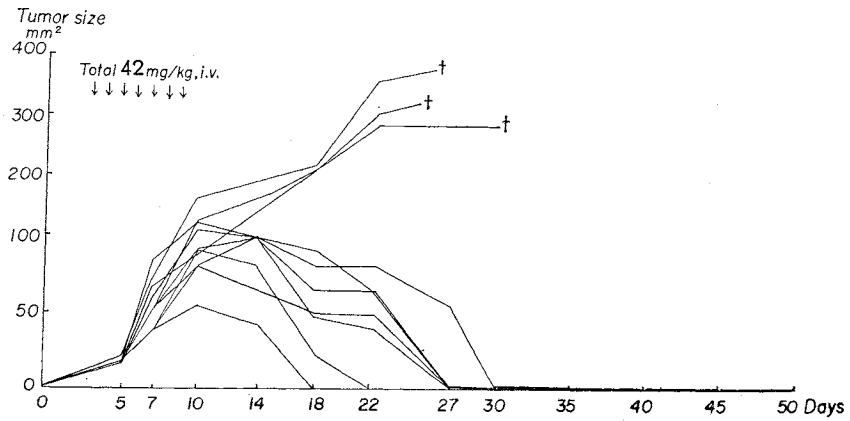
The average weight of the tumors in the group with B_1-Mg salt was 32 % of the control, and those of other salts were 50~60 %, however, the therapeutic effects

Table 3. Effect of iyomycin B compound on S-180 (solid) in mice.

Substance	Administration route	Dose		Interval and frequency	No. of mice survived/ treated	Average body weight change (g)	Tumor	
		Daily (mg/kg)	Total (mg/kg)				weight (mg)	% of control
B_1	I.V.	1.4	9.7	Daily×7	5/8	−1.4±1.0	212	21.4
		0.7	4.9	〃	8/8	−1.5±1.8	771	77.9
Reduced B_1	I.V.	25	175	〃	8/8	−2.5±1.9	528	53.3
		12.5	87.5	〃	8/8	−2.5±1.9	645	65.1
Acetyl B_1	I.V.	13.4	93.8	〃	6/8	−2.5±0.8	137	13.8
		6.7	46.9	〃	8/8	−0.46±1.5	610	61.6
B_4	I.V.	13.3	93.1	〃	8/8	+1.4±1.0	464	46.8
		6.7	46.9	〃	8/8	+2.0±1.8	610	61.6
B_5	I.V.	33.3	233.1	〃	8/8	+1.8±1.4	574	57.9
		16.7	116.9	〃	8/8	+2.0±1.9	743	75.1
Control	I.V.	Saline		〃	8/8	−0.8±2.6	990	——

1) Treatment began 24 hours after implantation.
2) Animals sacrificed on the 11th day after implantation.

Fig. 1. Effect of iyomycin B_1-Ac on S-180 (solid) in mice.
Treatment started 3 days after implantation.



of these metallic salts of B_1 did not exceed that of B_1 alone.

4. Antitumor effects of other B compounds. The LD_{50} in mice by intravenous injection was 75 mg/kg with reduced B_1, 40 mg/kg with acetylated B_1, 40 mg/kg with B_4, and 100 mg/kg with B_5. The therapeutic experiments were carried out using 1/3 and 1/6 LD_{50} of each substance. The tumor growth in the groups treated with acetyl B_1

Fig. 2. Effect of iyomycin B_1-Ac on S-180 (solid) in mice.
Treatment started 5 days after implantation.

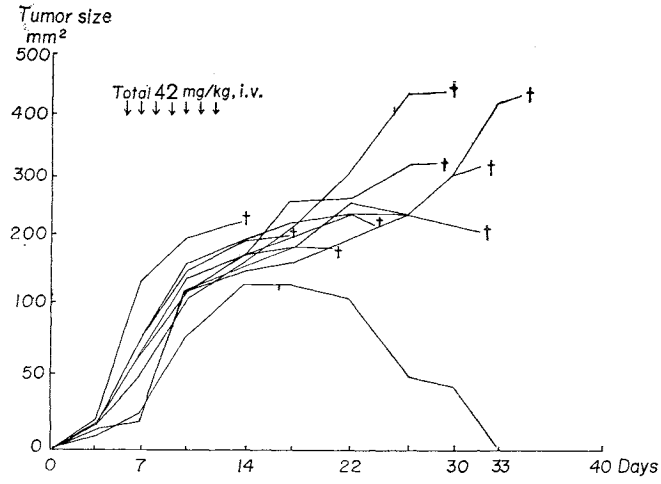
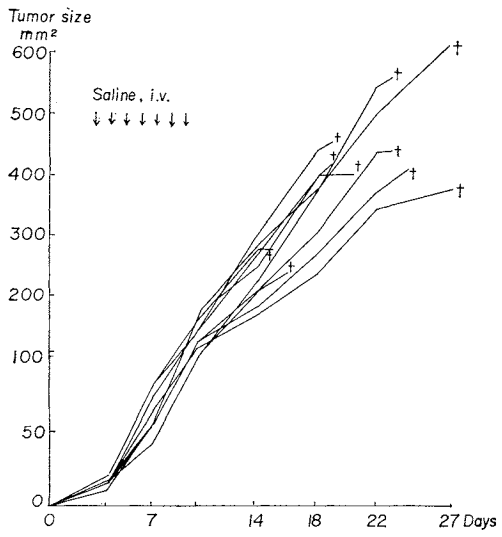


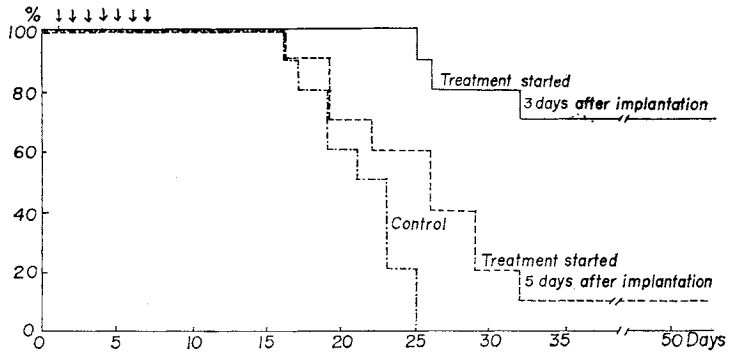
Fig. 3. Growth of solid tumor in mice (control)



was 13.8% and 61.6% of controls with each dosages described above. These results were superior to those obtained with B_1 itself, which gave values of 21.4% and 77.9%. On the contrary, reduced B_1, B_4, and B_5 showed less effect than B_1 itself.

Using acetyl B_1, initiation of treatment was delayed until 3 or 5 days after the implantation of S-180 solid tumor. Daily therapy of 6 mg/kg was administered for 7 consecutive days. When the initiation of treatment was delayed 3 days, the tumor disappeared entirely in 7 of 10 mice as shown in Fig. 1. In the group with treatment delayed until 5 days, one of 10 mice survived for more than 50 days with no detectable tumor, and remarkable prolongation of survival time was noticed in the other mice an shown in Fig. 2.

Fig. 4. Life prolongation of S-180 bearing mice treated with iyomycin B_1-Ac



The growth curves of the tumors in the treated groups were similar to those of the control group (Figs. 3) up to about 10 days after the implantation, but thereafer growth of the tumors was suppressed and disappeared finally, whereas all the control mice died of tumors 16~25 days after implantation.

Discussion

In the past ten years, about 10 high-molecular weight antitumor antibiotics were reported[8~16]. Iyomycin is also a high-molecular weight substance which is remarkably effective on ascites tumors of animals, but less effective on solid tumors.

Iyomycin B_1, which was separated and purified from the iyomycin complex or extracted from culture broth, had a growth-inhibitory effect on both ascites and subcutaneous solid tumors, but its therapeutic effect was so low that tumors could be suppressed entirely only when doses close to the LD_{50} were administered.

Iyomycin complex is less toxic, and effective on both ascites and solid tumors. As it is a high-molecular substance, however, there is a probability of antibody formation *in*

vivo after repeated injections. As far as the experiments concern, however, the production of antibody could not be observed.

In order to avoid such risk, and to reduce its toxicity, complexes of B_1 and human serum albumin were prepared and tested for their antitumor effects. No superior effect was observed, though the toxicity was reduced considerably. After our publication (1965)[6] on iyomycin B_1 complex with human serum albumin, H. UMEZAWA *et al.* (1966)[17,18] reported pluramycin A complex with human serum albumin and plurallin. Plurallin consists of a pluramycin-like prosthetic group and a glyoprotein. While, the authors reported in 1964 that the iyomycin A of high molecular weight was combined readily with B, especially with B_1, *in vitro*. A combined substance of A and B_1 in the ratio of 20 : 1 seemed to resemble the naturally occurring iyomycin complex. But the anti-cancer activity of B_1 alone was superior to the complex with A.

It was an interesting finding that acetylated B_1 did not inhibit the proliferation of the tumor for 7 days of administration, but the tumor began to decrease in size a few days after the final dose and finally disappeared in many cases.

Such a phenomenon has never been observed with other antitumor agents[19], but is a characteristic of acetyl iyomycin B_1.

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