TALLYSOMYCIN, A NEW ANTITUMOR ANTIBIOTIC COMPLEX RELATED TO BLEOMYCIN

III. ANTITUMOR ACTIVITY OF TALLYSOMYCINS A AND B

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The antitumor activity of tallysomycins A and B was determined in five experimental tumor systems in mice. Tallysomycins A and B were highly active against B16 melanoma, sarcoma 180 ascites tumor and LEWIS lung carcinoma, and moderately active against P388 leukemia but were without effect on lymphoid leukemia L1210. The antitumor activity of tallysomycin A was 2~3 times that of tallysomycin B and 3~17 times that of bleomycin. Tallysomycin A was about 1.5 and 4 times more toxic for mice than tallysomycin B and bleomycin, respectively, in terms of subacute LD_{50} values.

Tallysomycin is a new glycopeptide antibiotic complex produced by an unusual actinomycetes strain No. E465-94. Major components A and B of the tallysomycin complex were isolated and characterized, and their antimicrobial properties have been reported[1]. The structures of tallysomycins A and B (Fig. 1) have been determined; they are closely related to bleomycin (Fig. 2), differing only in the amino acid composition and by the fact that they possess an additional unique sugar moiety, 4-amino-4,6-dideoxy-L-talose[2]. This paper reports the antitumor activity of tallysomycins A and B in five experimental tumor systems.

Materials and Methods

Antibiotics:

Copper-free preparations of tallysomycins A and B were used throughout the present study. They were prepared from the copper-chelated form of tallysomycins A and B by the method described in a previous report[1]. Copper-free preparations of bleomycin complex (consisting mainly of bleomycin A_2)[3] and bleomycin A_5[4] which were supplied by Bristol Laboratories, Syracuse, New York, were used as reference agents. Bleomycin A_5 (Fig. 2) was included because of its structural similarity to tallysomycin B but the amount available was only enough to test against B16 melanoma. All materials were dissolved in sterile 0.9% saline and administered to experimental animals intraperitoneally or intravenously.

Animals:

Female BDF_1 (C57BL/6×DBA/2) and male dd-strain mice weighing 18~22 g were used for the experiments.

Tumors:

Melanotic melanoma B16 was implanted subcutaneously into BDF_1 mice at an inoculum size of 5×10^5 cells per mouse. Sarcoma 180 ascites tumor was inoculated intraperitoneally into dd mice with 2.5×10^6 cells per mouse. LEWIS lung carcinoma, lymphocytic leukemia P388 and lymphoid leukemia L1210 were implanted intraperitoneally into BDF_1 mice using an inoculum of 5×10^5, 3×10^5 and 10^6 cells per mouse, respectively.

Treatment:

Twenty-four hours after the implantation of tumor cells, graded doses of test compounds were

Fig. 1. Structures of tallysomycins A and B

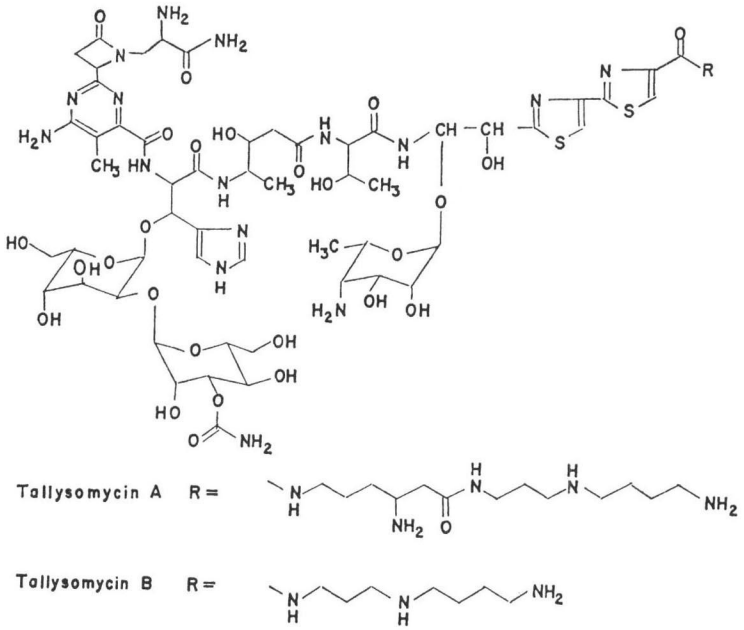
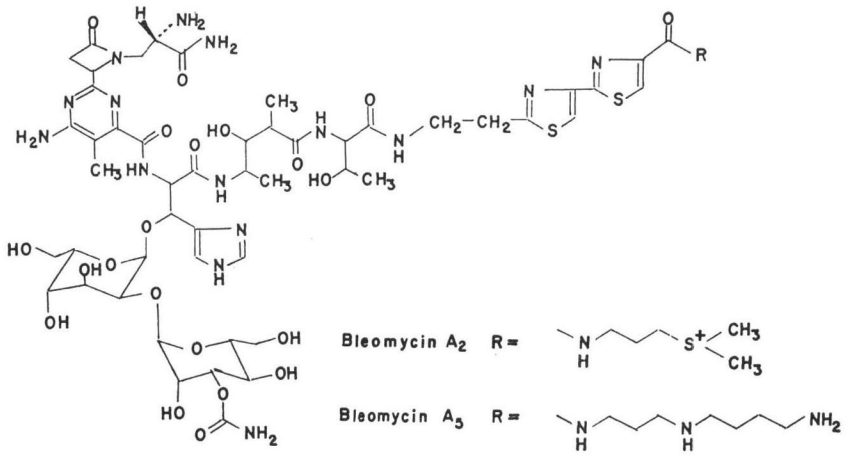


Fig. 2. Structures of bleomycins A_2 and A_5



administered to mice intraperitoneally in an injection volume of 0.02 ml per gram of body weight. A logarithmic dilution series consisting of one-half log unit increments of the antibiotics was used for the dose-response study. Treatments were given once daily for 9 days (*qd* 1→9 schedule) except for the mice inoculated with LEWIS lung carcinoma which were treated for 11 days (*qd* 1→11).

Evaluation of antitumor effects:

Death or survival of the treated and non-treated (control) animals was recorded daily during the observation period of 45 days after the implantation of tumor cells, and the median survival time (MST) was calculated for each of the test (T) and control (C) groups. A T/C value equal to or greater than 125% indicates that a significant antitumor effect was achieved. The actual dose giving a T/C of 125% was estimated by linear regression analysis[5] and defined as the effective $dose_{125}$ or ED_{125}.

In the B16 melanoma experiment the tumor size (long axis × short axis × thickness) was measured on day 16 after subcutaneous tumor inoculation, and the dose giving 50% inhibition of tumor growth (ID_{50}) when compared with that of control group was calculated from regression lines.

Toxicity determination:

Graded doses of test compounds were administered to groups of 8~12 dd mice intraperitoneally or intravenously. The injection was given one time only or once daily for 9 consecutive days in a volume of 0.02 ml per gram of body weight. Death or survival of the animals was recorded daily for 30 days after the last dose of test compound, and the LD_{50} was calculated according to the method of VAN DER WAERDEN[6].

Results

Melanotic Melanoma B16

The antitumor activities of tallysomycin A, tallysomycin B, bleomycin complex and bleomycin A_5 determined in the B16 melanoma system are shown in Table 1. Tallysomycins A and B showed a dose-related inhibition of tumor growth, the ID_{50} being 0.26 mg/kg/day for tallysomycin A and 0.46 mg/kg/day for tallysomycin B. The mice treated with tallysomycin also showed a significant prolongation of life span; the increase of MST was more apparent in animal groups in which tumor growth was inhibited greater than 50%. The highest T/C value obtained with tallysomycin A was 183% at a dose of 1 mg/

Table 1. Effects of tallysomycins A and B on B16 melanoma

	Dose (mg/kg/day)	Tumor size at day 16			Survival time			No. survivors at day 45/tested
		Mean±S.E. (cm^3)	Inhibition (%)	ID_{50} (mg/kg/day)	MST/range (days)	T/C (%)	ED_{125} (mg/kg/day)	
Control	—	5.81±0.47	—	—	23.0/21~43	—	—	0/20
Tallysomycin A	3	0.15±0.08	97	0.26	36.5/22~38	159	0.042	3/10*
	1	1.26±0.24	78		42.0/26~43	183		2/10
	0.3	2.78±0.39	52		37.0/19~39	161		2/10
	0.1	4.02±0.65	31		32.0/22~42	139		1/10
	0.03	4.84±0.75	17		27.5/16~42	120		0/10
Tallysomycin B	3	0.98±0.21	83	0.46	>45.0/31~38	>196	0.11	7/10
	1	1.92±0.18	67		36.5/36~41	159		1/10
	0.3	3.41±0.49	41		29.0/22~44	126		0/10
	0.1	5.88±0.49	−1		29.5/21~42	128		0/10
	0.03	6.40±0.91	−10		25.0/16~42	109		0/10
Bleomycin complex	10	0.66±0.14	89	0.91	37.5/14~45	163	0.28	0/10
	3	1.79±0.37	69		37.0/19~39	161		0/10
	1	2.34±0.50	60		33.0/27~39	143		2/10
	0.3	4.28±0.51	26		30.5/24~43	133		0/10
	0.1	6.06±0.77	−4		24.0/18~36	104		0/10
Bleomycin A_5	10	0.72±0.10	88	1.3	>45.0/43~45	>196	0.45	2/4
	3	2.06±0.09	65		35.5/31~41	154		0/4
	1	2.89±0.59	50		31.0/20~39	135		0/4
	0.3	4.69±0.65	19		28.5/21~31	124		0/4
	0.1	5.86±1.33	−1		20.5/20~40	89		0/4

* 3 of 10 animals died due to toxicity.

kg/day. With tallysomycin B the T/C was >196% at 3 mg/kg/day with 7/10 mice still surviving at the termination of the 45-day observation period. The ED_{125} of tallysomycins A and B in this tumor system was 0.042 and 0.11 mg/kg/day, respectively. Two reference compounds, bleomycin complex and bleomycin A_5, were also active against B16 melanoma, showing ID_{50} values of 0.91 and 1.3 mg/kg/day and ED_{125} values of 0.28 and 0.45 mg/kg/day, respectively. These values indicate that tallysomycins A and B are approximately 7 and 2.5 times as potent as bleomycin complex, respectively, in this tumor system.

Sarcoma 180 Ascites Tumor

As shown in Table 2, sarcoma 180 was very sensitive to tallysomycins A and B, the ED_{125} being 0.02 and 0.042 mg/kg/day. The animals receiving 0.3 mg/kg/day of tallysomycin A or 1 mg/kg/day of tallysomycin B showed the greatest T/C value (>293% for tallysomycin A and >318% for tallysomycin B), with one half of the animals receiving these doses still surviving after 45 days. Tallysomycin A was approximately twice as active as tallysomycin B which in turn was about 8 times more potent than bleomycin in terms of ED_{125} values.

Table 2. Effects of tallysomycins A and B on sarcoma 180 ascites tumor

	Dose (mg/kg/day)	MST/range (days)	T/C (%)	ED_{125} (mg/kg/day)	No. survivors at day 45/tested
Control	—	14.0/10~23	—	—	1/20
Tallysomycin A	3	19.0/11~21	136	0.020	0/6
	1	23.0/14~37	164		3/12
	0.3	>41.0/24~37	>293		6/12
	0.1	24.0/12~25	171		4/12
	0.03	17.5/13~25	125		4/12
	0.01	16.0/ 7~24	114		2/12
Tallysomycin B	3	23.0/19~26	164	0.042	0/6
	1	>44.5/20~44	>318		6/12
	0.3	36.0/12~45	257		5/12
	0.1	19.5/13~20	139		5/12
	0.03	17.5/11~23	125		2/12
Bleomycin complex	3	27.0/16~27	193	0.33	5/12
	1	19.0/16~20	136		5/12
	0.3	18.5/11~39	132		4/12
	0.1	13.0/10~25	93		1/12

Lewis Lung Carcinoma

Table 3 shows the effects of tallysomycins A and B and bleomycin complex on Lewis lung carcinoma. Significant prolongation of MST was noted in groups of animals receiving tallysomycin A at a daily dose of 0.1~3 mg/kg/day, the ED_{125} being 0.068 mg/kg/day. Although tallysomycin B was about one-half as active as tallysomycin A in terms of the ED_{125} (0.16 mg/kg/day for tallysomycin B), it showed a greater T/C value at 3 mg/kg/day (215%) than was achieved with any dosage of tallysomycin A. Bleomycin also showed significant prolongation of MST in the dose range of 0.3~3 mg/kg/day, the ED_{125} being 0.24 mg/kg/day. The greatest survival rate (5/16) was noted in animals receiving

Table 3. Effects of tallysomycins A and B on LEWIS lung carcinoma

	Dose (mg/kg/day)	MST/range (days)	T/C (%)	ED_{125} (mg/kg/day)	No. survivors at day 45/tested
Control	—	17.0/13~38	—	—	0/32
Tallysomycin A	3	28.0/16~36	165	0.068	0/11
	1	27.0/23~39	159		0/17
	0.3	25.0/19~42	147		1/17
	0.1	21.5/16~27	126		2/17
	0.03	19.5/17~35	115		0/6
Tallysomycin B	3	36.5/25~43	215	0.16	2/12
	1	26.0/19~35	153		1/18
	0.3	25.0/15~34	147		1/18
	0.1	19.5/15~34	115		0/18
	0.03	18.5/17~34	109		0/6
Bleomycin complex	3	34.0/21~42	200	0.24	2/16
	1	26.5/19~38	156		5/16
	0.3	22.0/16~43	129		0/16
	0.1	18.0/16~32	106		0/12

a dose of 1 mg/kg/day of bleomycin. Tallysomycins A and B were about 3.5 and 1.5 times more active than bleomycin, respectively, in terms of ED_{125} values.

Lymphocytic Leukemia P388

Table 4 shows the antitumor activity of tallysomycins A and B and bleomycin complex against P388 leukemia. Significant prolongation of life span was seen with doses in the range of 0.3~3 mg/kg/day of tallysomycin A, 1~3 mg/kg/day of tallysomycin B or 3~10 mg/kg/day of bleomycin. The

Table 4. Effects of tallysomycins A and B on P388 leukemia

	Dose (mg/kg/day)	MST/range (days)	T/C (%)	ED_{125} (mg/kg/day)	No. survivors at day 45/tested
Control	—	9.5/ 7~13	—	—	0/16
Tallysomycin A	3	14.0/11~15	147	0.27	0/6
	1	13.0/11~20	137		0/12
	0.3	12.0/11~16	126		0/12
	0.1	11.0/ 8~16	116		0/12
	0.03	11.0/ 8~15	116		0/12
Tallysomycin B	3	12.0/11~14	126	0.89	0/6
	1	12.0/10~18	126		0/12
	0.3	11.0/10~18	116		0/12
	0.1	11.0/ 9~13	116		0/12
	0.03	10.0/ 7~13	105		0/12
Bleomycin complex	10	12.5/10~16	132	3.3	0/12
	3	12.0/10~15	126		0/12
	1	11.0/ 9~15	116		0/12
	0.3	10.5/10~13	111		0/12

dose-response curves of the three test compounds in the P388 experiments were rather flat, and the maximum T/C values obtained were only 147% for tallysomycin A, 126% for tallysomycin B and 132% for bleomycin. None of the animals receiving these compounds survived until day 20. The ED_{125} values were determined to be 0.27, 0.89 and 3.3 mg/kg/day for tallysomycin A, tallysomycin B and bleomycin, respectively. This indicates that tallysomycins A and B were about 12 and 4 times more potent, respectively, than bleomycin.

Lymphoid Leukemia L1210

As shown in Table 5, L1210 leukemia was quite insensitive to tallysomycins A and B. The greatest T/C values obtained were only 112% for tallysomycin A (at 0.3 mg/kg/day) and 106% for tallysomycin B (at 1~10 mg/kg/day). All animals treated with tallysomycins A or B died within 10 days, the longest survival period of control animals. Bleomycin was also ineffective against this tumor.

Table 5. Effects of tallysomycins A and B on L1210 leukemia

	Dose (mg/kg/day)	MST/range (days)	T/C (%)	ED_{125} (mg/kg/day)	No. survivors at day 45/tested
Control	—	8.5/ 7~10	—	—	0/10
Tallysomycin A	3	9.0/ 8~10	106	>3	0/6
	1	9.0/ 7~10	106		0/6
	0.3	9.5/ 7~10	112		0/6
	0.1	8.0/ 7~10	94		0/6
Tallysomycin B	10	9.0/ 8~10	106	>10	0/6
	3	9.0/ 9~10	106		0/6
	1	9.0/ 9	106		0/6
	0.3	8.0/ 7~ 9	94		0/6
Bleomycin complex	10	9.0/ 8~10	106	>10	0/6
	3	7.0/ 7~10	82		0/6
	1	7.0/ 7~ 9	82		0/6
	0.3	7.0/ 7~ 8	82		0/6

Toxicity

The acute and subacute toxicities of tallysomycins A, B and bleomycin were determined in mice and the LD_{50} values obtained are summarized in Table 6. Tallysomycin A was 3.1 and 4.1 times more toxic than bleomycin in single intravenous (iv) and intraperitoneal (ip) administrations, respectively. Tallysomycin A was also more toxic ($\times 1.8 \sim 2.4$) than tallysomycin B. In the multiple dosing schedule (ip, *qd* 1→9), tallysomycin A was about 1.5 times more toxic than tallysomycin B which in turn was 2.6 times more toxic than bleomycin.

Discussion

Among the five experimental tumor systems examined in the present study, tallysomycins A and B were highly active against B16 melanoma, sarcoma 180 ascites tumor and LEWIS lung carcinoma, moderately active against P388 leukemia, but inactive against L1210 leukemia. The tallysomycins have also been reported to be active against WALKER carcinosarcoma 256 in rats[7]. The antitumor effects of tallysomycins A and B were compared with bleomycin complex and their relative activities are sum-

Table 6. Comparison of antitumor activity and toxicity

	Antitumor activity or acute toxicity in mg/kg/day (relative activity or toxicity)			
	Tallysomycin A	Tallysomycin B	BLM complex	BLM A_5
B16 melanoma (ID_{50})	0.26 (3.5)	0.46 (2.0)	0.91 (1.0)	1.3 (0.70)
B16 melanoma (ED_{125})	0.042 (6.7)	0.11 (2.5)	0.28 (1.0)	0.45 (0.62)
Sarcoma 180 (ED_{125})	0.020 (17)	0.042 (7.9)	0.33 (1.0)	—
LL carcinoma (ED_{125})	0.068 (3.5)	0.16 (1.5)	0.24 (1.0)	—
P388 leukemia (ED_{125})	0.27 (12)	0.89 (3.7)	3.3 (1.0)	—
L1210 leukemia (ED_{125})	>3 (—)	>10 (—)	>10 (—)	—
IV LD_{50} (single dose)	17 (3.1)	30 (1.8)	53 (1.0)	—
IP LD_{50} (single dose)	19 (4.1)	46 (1.7)	77 (1.0)	—
IP LD_{50} (*qd* 1→9)	4.4 (4.1)	6.8 (2.6)	18 (1.0)	—

marized in Table 6. Tallysomycin A was generally 2~3 times more potent than tallysomycin B, and 3~17 times more potent than bleomycin. Tallysomycin A was about 1.5 and 4 times more toxic than tallysomycin B and bleomycin, respectively, when given in a multiple dosing schedule for 9 days. The therapeutic index was obtained for each compound from the ratio of multiple dose LD_{50} (ip, *qd* 1→9) to the ED_{125} for each of tumor systems (Table 7). Tallysomycin A showed better therapeutic indices than tallysomycin B in all of the tumor systems tested. Bleomycin complex showed somewhat better therapeutic index than that of tallysomycins A and B against LEWIS lung carcinoma. Against B16 melanoma, sarcoma 180 and P388 leukemia, the therapeutic indices of tallysomycin A were 2~4 times superior to those of bleomycin.

Table 7. Comparison of therapeutic indices

	Therapeutic indices*				
	B16	S180	LL	P388	L1210
Tallysomycin A	105	220	65	16	<1.5
Tallysomycin B	62	162	43	7.6	<0.68
Bleomycin complex	64	55	75	5.5	<1.8

* IP LD_{50} (*qd* 1→9)/ED_{125}

Tallysomycin B is structurally related to bleomycin A_5[4], both having the same terminal amine moiety. The antitumor activity of tallysomycin B was compared with bleomycin A_5 only in the B16 melanoma system. Tallysomycin B showed 3~4 times greater activity than bleomycin A_5, suggesting some favorable effect of the presence of the aminodeoxytalose moiety and/or the carbinolamine structure[2] in the molecule.

Since tallysomycin A differs structurally from tallysomycin B only in that it contains β-lysine[2], the greater biological activity (antitumor and antibacterial[1]) of tallysomycin A over tallysomycin B suggests an important role for this amino acid.

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