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Tallysomycin S_{10}b: Experimental antitumor activity and toxicity

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Summary. *Tallysomycin S_{10}b (TLM S_{10}b), a structural analog of bleomycin (BLM), was evaluated and compared with BLM for antitumor activity in several murine tumor systems and for toxic effects in mice and rats. Neither TLM S_{10}b nor BLM was effective against IP P388 and L1210 leukemias, whereas both drugs were active against IP P388/J leukemia (a BLM-sensitive subline). TLM S_{10}b and BLM were both active against murine solid tumors, including SC B16 melanoma, IV Lewis lung carcinoma, SC Madison 109 lung carcinoma, SC CD8F$_1$ mammary carcinoma and SC Colon 38 carcinoma. In human tumor xenograft models, TLM S_{10}b was active against a colon tumor and had slight activity against breast and lung tumors. Compared with TLM S_{10}b, BLM had less activity against the colon tumor, comparable activity against the breast tumor, and no effect against the lung tumor. A consensus of the antitumor data indicated that compared with BLM, TLM S_{10}b had comparable or greater activity and was about twice as potent. TLM S_{10}b and BLM had approximately equivalent LD_{50} values in mice. TLM S_{10}b had minimal effects on WBC counts, blood urea nitrogen levels, and serum glutamic pyruvic transaminase levels in mice during the time periods monitored. These effects were comparable to or less pronounced than those of BLM. Both drugs caused dose-related increases in the whole-lung hydroxyproline content in mice, but the dose-response curves were not parallel. TLM S_{10}b caused a larger increase than BLM at the lower doses and a smaller increase than BLM at the highest doses. In rats, TLM S_{10}b and BLM caused comparable, significant decreases in lung mechanics; however, histopathological examination of the lungs indicated that TLM S_{10}b caused less evidence of pulmonary toxicity than did BLM at comparable dose levels. TLM S_{10}b was, therefore, generally comparable to BLM in causing pulmonary toxicity in mice and showed possibly less pulmonary toxicity in rats, while demonstrating approximately equivalent to four-fold greater potency, depending on the test system. It also appeared that TLM S_{10}b caused less pulmonary toxicity than BLM in both mice and rats at doses approaching maximally tolerated levels. TLM S_{10}b is currently undergoing phase I clinical evaluation.*

Introduction

Tallysomycin S_{10}b (TLM S_{10}b) is a new antitumor agent, which is currently undergoing phase I clinical trials. TLM S_{10}b is a member of the TLM series of antibiotics and is related structurally to bleomycin (BLM). The TLMs and BLM have antimicrobial and antitumor activity, but the TLMs tend to be more potent than BLM as antibacterial and antifungal agents in vitro [1, 6] and as antitumor agents in experimental murine tumor models [1, 5, 6, 12].

TLM S_{10}b was prepared by adding the precursor amine 1,4-diaminobutane to the TLM fermentation medium [8]. TLM S_{10}b was selected for further development on the basis of the results of initial studies indicating that it had favorable antitumor activity in murine systems [9]. In this report, we present results from more extensive comparisons of the antitumor activity and toxic effects of TLM S_{10}b and BLM in animals.

The structures of TLM S_{10}b and the BLM complex fractions A_2 and B_2 are shown in Fig. 1.

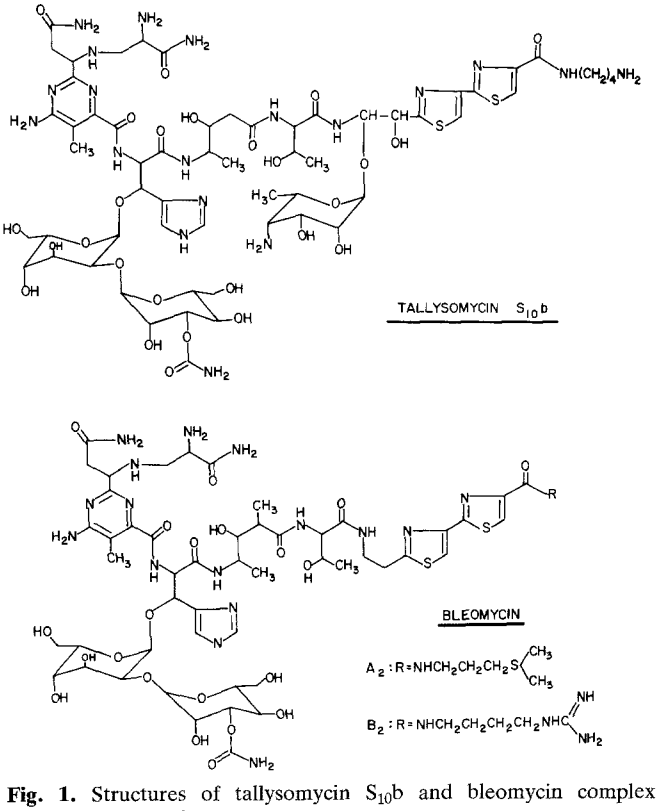

Fig. 1. Structures of tallysomycin S_{10}b and bleomycin complex fractions A_2 and B_2

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Materials and methods

Animals. DBA/2, C57BL/6, BALB/c, C57BL/6 × DBA/2 F_1 (hereafter called BDF_1), C57BL/6 × C3H F_1 (hereafter called $B6C3F_1$), BALB/c × DBA/2 F_1 (hereafter called CDF_1), and BALB/c × DBA/8 F_1 (hereafter called $CD8F_1$) mice of both sexes, and female athymic (hereafter called nude) Swiss mice were used for the antitumor evaluations. Antitumor tests were conducted at Bristol-Myers Co. or under the auspices of the NCI at various laboratories. Male BDF_1 mice (22–29 g) and male Sprague Dawley rats (200–225 g) were used for the toxicity evaluations. Feed and water were provided ad libitum.

Antitumor testing

Tumors: The P388 leukemias (a parent line and a subline J with modest sensitivity to bleomycin). and L1210 leukemia were maintained in ascitic form in DBA/2 mice and transplanted at weekly intervals. Lewis lung carcinoma (LL) and B16 melanoma (B16) were maintained as SC-growing tumors in C57BL/6 mice, and Madison 109 lung carcinoma (M109) was similarly maintained in BALB/c mice; each of these tumors was transplanted at 2-week intervals. The $CD8F_1$ mammary tumor is a spontaneous tumor arising in female $CD8F_1$ mice. The Colon 38 tumor was maintained as a SC-growing tumor in C57BL/6 mice and transplanted at 3-week intervals. The MX-1 mammary, LX-1 lung, and CX-1 colon human tumor xenograft lines were propagated SC in nude mice.

Experimental design: The route and size of tumor inoculum, host mouse, group size, and drug treatment schedule and route used in each experiment are summarized in Table 1. With regard to the $CD8F_1$ mammary tumor, it should be emphasized that first-generation transplants of the spontaneously occurring tumors were made in $CD8F_1$ mice for the drug evaluation experiments. A saline-treated (or untreated) tumor control group was included in each experiment. In most experiments, BLM and TLM $S_{10}b$ were evaluated concomitantly.

Assessment of drug activity: Antitumor drug activity was determined on the basis of (a) proportion of mice alive on the day the experiment was terminated; and (b) the % T/C using the median survival time (MST) of drug-treated (T) mice divided by the MST of control (C) mice, times 100 (i.e., MST % T/C). L1210, P388, and $CD8F_1$ experiments were terminated on day 30. LL, B16, and M109 experiments were terminated on day 60 (day 65 in one B16 experiment). Colon 38 experiments were terminated on day 20. Human tumor xenograft experiments involving tumor implantation within the subrenal capsule (src) were terminated and evaluated on day 11 (MX-1 and LX-1) or day 15 (CX-1). For human tumor xenograft experiments involving SC tumor implantation, termination and evaluation were on day 33 or 34.

For mice implanted SC with M109, antitumor drug activity was based upon (a) MST % T/C; (b) the relative median time for tumors to reach 1 g in weight in drug-treated as compared to tumor-control groups of mice (i.e., T-C); and (c) the relative median tumor weight (MTW) on day 19 or 20 after implantation among drug-treated and tumor control mice (i.e., MTW % T/C). For mice implanted SC with Colon 38 or $CD8F_1$ mammary tumor cells, MTW % T/C was determined on day 20 and day 30, respectively, and served as the only measure of antitumor activity. For the human tumor xenograft experiments, the assessment of drug activity was based on relative changes in mean tumor size (between day 0 and final evaluation day) for each drug-treated group compared with the control group.

Tumor weight was estimated by converting tumor measurements (determined twice weekly using calipers) to weights by applying the formula: wt (mg) = $a \times b^2/2$, where a = length (mm) and b = width (mm). Mice dying on or before day 10 in the M109 experiments, day 20 in the Colon 38 experiments, day 30 in the $CD8F_1$ experiments, the various termination days in the human tumor xenograft experiments, or day 5 in all other experiments were excluded from all calculations. Therapies resulting in more than 25%–33% lethality by these deadlines were considered as toxic.

Toxicity testing

LD_{50} determinations: Mice in groups of 5–10/dose were treated with single IP, single IV or multiple (qd 1 → 9) IP

Table 1. Details of experimental protocols

Tumor	Inoculum size and site or route	Mouse host	Drug treatment schedule and route	Group size
P388	10^6, IP	CDF_1	qd 1 → 9, IP	6
P388/J	10^6, IP	CDF_1	qd 1 → 9, IP	6
L1210	10^5, IP	CDF_1	qd 1 → 9, IP	6
Lewis lung	10^6, IV	BDF_1 or $B_6C_3F_1$	qd 1 → 9, IP	10
	10^6, IP	BDF_1	qd 1 → 9, IP	10
B16 melanoma	10% brei, 0.5 ml, SC	BDF_1 or $B_6C_3F_1$	qd 1 → 9, IP	10
	Tumor fragment, SC	BDF_1 or $B_6C_3F_1$	qd 1 → 9, IP	10
Madison 109	2% brei, 0.1 ml, SC	CDF_1	qd 1 → 4, IP	8
$CD8F_1$ mammary	5×10^6, SC	$CD8F_1$	q7d × 5; d 1, IP	10
Colon 38	Tumor fragment, SC	BDF_1 or $B_6C_3F_1$	d.2 and 9, IP	10
CX-1	Tumor fragment, src[a]	Nude	q4d × 4; d 1, SC	3
	Tumor fragment, SC	Nude	q4d × 3; d 15, IP	5
LX-1	Tumor fragment, src	Nude	q4d × 3; d 1, SC	3
	Tumor fragment, SC	Nude	q4d × 3; d 14, IP	5
MX-1	Tumor fragment, src	Nude	q4d × 3; d 1, SC	3
	Tumor fragment, SC	Nude	q4d × 3; d 15, IP	5

[a] Subrenal capsule

injections of TLM $S_{10}b$ or BLM and observed for 30 days after completion of the schedule. LD_{50} values were then calculated by the Weil method [14].

Hematology and serum chemistry: Mice in groups of 40–50/experiment were bled from the retro-orbital plexus and their individual pretreatment total white blood cell (WBC) counts, blood urea nitrogen (BUN) levels, or serum glutamic pyruvic transminase (SGPT) levels measured as previously described [2, 3]. Serparate groups of mice were used for each parameter. TLM $S_{10}b$ or BLM, or saline for controls, was administered as single IP injections at several dose levels (10 mice/dose). The highest dose of TLM $S_{10}b$ was 188 mg/kg (86% LD_{50}) and the highest dose of BLM was the LD_{50}, 160 mg/kg. Lower doses at 0.75 decrements were tested to determine dose-related effects. The highest dose of TLM $S_{10}b$ was less than the LD_{50} because results from preliminary studies suggested that mice receiving the LD_{50} may not survive the stress of the first post-dose blood sampling.

The total WBC counts were determined 3, 5, and 7 days after dosing, with a decrease in total WBC counts of $\geq$ 35% relative to pretreatment values being considered indicative of drug-induced leukopenia. The BUN and SGPT values were measured 4, 7, and 11 or 1, 3, and 5 days after dosing, respectively. BUN values $\geq$ 30 mg% and SGPT values $\geq$ 40 international units (IU) were considered indicative of drug-induced nephrotoxicity or hepatotoxicity, respectively.

Lung hydroxyproline: TLM $S_{10}b$ was administered SC to 10 mice/dose at doses of 1.25, 2.5, and 5 mg/kg per injection twice weekly for 4 weeks. BLM was administered SC according to the same schedule at doses of 2.5, 5, and 10 mg/kg per injection. Control mice were injected SC with saline. The BLM doses were based on those used by Sikic et al. [11] in this model. The dose selection for TLM $S_{10}b$ was based on lethality data [6, 8] which suggested that a 2 : 1 (BLM : TLM $S_{10}b$) dose ratio was appropriate. At the end of 12 weeks, 8 weeks after the last injection, the mice were sacrificed; the thorax was opened and the lungs dissected from their bronchi and blood vessels. The hydroxyproline content of the lungs from each mouse was determined as described previously [10].

Pulmonary mechanics: Rats, in groups of five/dose, received TLM $S_{10}b$, 3.9 or 5.25 mg/kg per injection, or BLM, 10.5 or 14 mg/kg per injection SC three times per week for 5 weeks. Preliminary studies had indicated that, based on lethality, the high dose of each drug approximated the maximum tolerated dose (MTD) for this schedule. Control rats received saline SC. During the 6th week, the rats were anesthetized with IP sodium pentobarbital and prepared for measurement of lung quasistatic compliance (Cst) and vital capacity (VC). The trachea was cannulated and the rats allowed to breathe spontaneously. Transpulmonary pressure (PTP) was measured with a water filled Validyne differential pressure transducer (MP45-1), one side connected to the tracheal cannula and the other to a water-filled esophageal cannula. The air flow rate was measured from the tracheal cannula with a Fleisch pneumotachograph (No. 00). The PTP and air flow signals were fed into an analog computer (Buxco Electronics, Sharon, CT), which monitored PTP and the lung volume above functional residual capacity.

Gallamine triethiodide, 10 mg/kg, was administered IV to arrest spontaneous respiration and the rats were artificially ventilated with room air for about 2 min. Cst and VC were then determined using a modification of the method of Snider et al. [13]. A 30-ml syringe was attached to the pneumotachograph and the lungs slowly inflated to a PTP of 35 cm H_2O. The lungs were slowly deflated to a PTP of 0 cm H_2O and a negative pressure of −20 cm H_2O was then applied. The syringe volume change between +35 cm H_2O and −20 cm H_2O was the VC. Cst was measured as the slope of the steepest portion of the deflation pressure-volume curve, which was usually between 2.5 and 10 cm H_2O.

After these measurements were made, the rats were sacrificed by exsanguination. The intact lungs and trachea were removed and inflation-fixed with 10% neutral, buffered formalin. Sections of each of the lobes were prepared for microscopy so as to present the maximum amount of pulmonary tissue for examination. Paraffin-embedded sections were cut at 5 μm thickness and stained with hematoxylin and eosin; selected sections were stained with Masson's trichrome. Histological examinations were conducted by routine light microscopy.

The histopathological lesion classification scheme used for pulmonary toxicity evaluation utilized a grading scale of 0–4 for five different morphologic parameters – numbers of alveolar macrophages, alveolitis, alveolar cell hypertrophy/hyperplasia, alveolar proteinosis, and pulmonary fibrosis. This method of classification was based on previous experience with this or similar models in our laboratory and is comparable to that used by other investigators [7]. The following grades were incorporated: grad 0, not noticeable; grade 1, minimal changes in a few, focal subpleural areas; grade 2, focal areas of slightly greater degree and not limited to subpleural areas; grade 3, involvement of significant areas of pulmonary tissue throughout the lung and some lesion confluence; grade 4 (not attained in this study), diffuse changes throughout all lobes and considerable lesion confluence.

Drugs. TLM $S_{10}b$ was obtained from the Bristol-Myers Research Institute, Tokyo, Japan. The BLM was from various lots of outdated clinical ampules which contained 15 units BLM sulfate (Blenoxane)/vial (1.75 U/mg). TLM $S_{10}b$ and BLM were dissolved in saline. For studies in mice, the drug concentrations were adjusted according to the average weight of each experimental group so that the appropriate dose could be administered in 0.5 ml/mouse, or the drug doses were administered on an individual mouse basis on a volume of 0.01 ml/g body weight. In the rat studies the drug concentrations were calculated so that individual rats received the appropriate dose in a volume of 0.1 ml/100 g body weight.

Results

Antitumor testing

The antitumor test results obtained for TLM $S_{10}b$ and BLM in several experimental tumor models are summarized in Table 2.

TLM $S_{10}b$ and BLM were not effective (T/C < 125%) against P388 or L1210 leukemias. Against P388/J leukemia, a BLM-sensitive subline, TLM $S_{10}b$ achieved a maximum T/C value of 150% whereas BLM caused a maximum T/C of 131%. The optimal dose of TLM $S_{10}b$ was one-half that of BLM in this experiment.

TLM $S_{10}b$ and BLM were evaluated in parallel against a number of murine solid tumors. Nine experiments were performed in which TLM $S_{10}b$ was evaluated against SC B16; in eight of these experiments BLM was included for compar-

Table 2. Effect of tallysomycin $S_{10}b$ and bleomycin against several experimental tumors

Tumor	Experiment no.	Site of implant	Drug	Optimal dose, IP (mg/kg per injection)	MST % T/C	MTW % T/C
Murine leukemias						
P388	6905	IP	TLM $S_{10}b$	2	124	–
			BLM	None	NA[a]	–
P388/J	6067	IP	TLM $S_{10}b$	6.4	150	–
			BLM	12.8	131	–
L1210	7363	IP	TLM $S_{10}b$	None	NA	–
			BLM	None	NA	–
Murine solid tumors						
B16	Nine[b]	SC	TLM $S_{10}b$	3.4[b]	172[b]	–
	Eight[b]		BLM	8.0[b]	167[b]	–
Lewis lung	234	IV	TLM $S_{10}b$	16	148	–
			BLM	16	140	–
	268	IV	TLM $S_{10}b$	8	176	–
			BLM	8 and 16	143	–
	142	IP	TLM $S_{10}b$	0.8	168	–
Madison 109 Lung	34	SC	TLM $S_{10}b$	16	105	0
			BLM	36	113	15
	81	SC	TLM $S_{10}b$	16	174	0
			BLM	24	146	12
$CD8F_1$ Mammary	226	SC	TLM $S_{10}b$	16	–	16
			BLM	32	–	42
	356	SC	TLM $S_{10}b$	8 and 32	–	5
			BLM	64	–	2
	470	SC	TLM $S_{10}b$	8	–	0
			BLM	32	–	0
Colon 38	150	SC	TLM $S_{10}b$	32	–	4
			BLM	128	–	10
	276	SC	TLM $S_{10}b$	64	–	9
			BLM	32	–	14
Human xenografts in mice						
Colon	6500	src	TLM $S_{10}b$	32	–	28
			BLM	None	–	≥113
	1112	SC	TLM $S_{10}b$	16	–	7
			BLM	16	–	28
Lung	5900	src	TLM $S_{10}b$	32	–	39
			BLM	None	–	≥ 62
	1113	SC	TLM $S_{10}b$	None	–	≥ 77
			BLM	None	–	≥ 56
Breast	5800	src	TLM $S_{10}b$	32	–	47
			BLM	50	–	46
	1092	SC	TLM $S_{10}b$	32	–	21
			BLM	32 and 64	–	27

Details involving host mice, group size, drug treatment schedule and tumor inocula are provided in Table 1

[a] Not active

[b] The optimal dose and maximum T/C values shown represent averages from nine and eight experiments, respectively, involving TLM $S_{10}b$ and BLM

ison. TLM $S_{10}b$ was active (T/C ≥ 130%) in all nine experiments and the average maximum T/C value achieved was 172%. BLM was active in six of the eight experiments in which it was included, and yielded an average maximum T/C of 167%. The average optimal dose of TLM $S_{10}b$ was about half that of BLM, but the actual difference varied substantially between experiments.

Both drugs were active against IV LL. The optimal doses of TLM $S_{10}b$ and BLM were approximately equivalent. In one study (expt. 234) the T/C values at the optimal doses were comparable and in the other study TLM $S_{10}b$ achieved a higher T/C value (176%) than BLM (143%). TLM $S_{10}b$ was also evaluated against IP LL and was active (T/C: 168%).

In M109 experiment 34, administration of TLM $S_{10}b$ at 16 mg/kg per injection resulted in only one of eight mice growing a tumor to 1 g before dying (death is due to metastatic disease in such instances). In comparison, BLM at 36 mg/kg per injection caused a 15-day delay (T-C) in the median time to reach 1-g tumors. On day 20, the median tumor weight was 15% of the tumor weight in the control mice. Neither drug

caused a significant extension of life-span. In the second M109 experiment (no. 81), treatment with 16 mg/kg TLM S_{10}b per injection resulted in a T-C for a 1 g tumor of 31.5 days and a MST % T/C of 174%. Optimal treatment with BLM, 24 mg/kg per injection, resulted in a T-C for a 1-g tumor of 16 days and a MST % T/C of 146%.

Three experiments were performed in mice with implanted $CD8F_1$ mammary tumor. TLM S_{10}b was active (MTW % T/C $\leq$ 42%) in all the studies, as reflected by median tumor weights in treated mice of between 0 and 16% those of control mice. BLM had minimal activity (MTW % T/C of 42%) at 32 mg/kg per injection in the first study, but showed activity comparable to TLM S_{10}b in the other two studies. The optimal doses of BLM (32–64 mg/kg per injection) were about twice to four times the optimal doses for TLM S_{10}b (8–16 mg/kg per injection).

Against Colon 38, both TLM S_{10}b and BLM were highly effective. The degree of tumor inhibition caused by each drug was comparable in both experiments. MTW % T/C values ranged between 4%–9% of control values for TLM S_{10}b and 10%–14% for BLM. Neither showed a clear potency advantage in this tumor model.

Experiments using human tumor xenografts involved both SC and src tumor implantation. Against the human colon tumor xenograft models, TLM S_{10}b displayed a therapeutic advantage over BLM regardless of the site of tumor implantation (note: only a MTW % T/C of $\leq$ 20% is usually considered indicative of significant drug activity, and such a degree of tumor inhibition was obtained by TLM S_{10}b against the SC tumor model). Against the lung tumor xenografts, neither TLM S_{10}b nor BLM was effective in the SC model and only TLM S_{10}b showed slight activity (MTW % T/C of 39%) in the src model. Finally, both drugs displayed comparable effects (slight activity) against the breast tumor xenografts implanted SC.

Toxicity testing

The acute and multiple dose (qd 1 → 9) LD_{50} values for TLM S_{10}b and BLM in male BDF_1 mice are listed in Table 3. The acute IP LD_{50} of TLM S_{10}b was about 1.4 times that of BLM, with overlapping confidence limits. Interestingly, the qd 1 → 9 IP and acute IV LD_{50} values of TLM S_{10}b were identical with those for BLM.

The results of the hematology and serum chemistry studies are summarized in Table 4. TLM S_{10}b caused a reduction in the total WBC counts of 41% at the highest dose tested, 188 mg/kg, but had little or no effect at lower doses. Reductions in the total WBC counts induced by BLM were all less than 35%. There were some incidences of BUN values $\geq$ 30 mg% with both drugs. In the SGPT studies there were some incidences of SGPT $\geq$ 40 IU at all dose levels of TLM S_{10}b, with one incident in the control group. In contrast, almost all of the BLM-treated mice had SGPT values $\geq$ 40 IU on day 5.

The results of the mouse whole-lung hydroxyproline study are shown in Fig. 2. Both TLM S_{10}b and BLM caused dose-related increases in whole-lung hydroxyproline content. The TLM S_{10}b dose-response curve was to the left of the curve of BLM and had a slope which was less pronounced than that of the BLM curve. The lack of parallelism precluded determination of the relative potency of TLM S_{10}b and BLM. The increase in whole-lung hydroxyproline content produced by TLM S_{10}b at 1.25 mg/kg per injection (12%) was comparable to that produced by BLM at 5 mg/kg per injection (13%). However, the increase produced by TLM S_{10}b at 5 mg/kg per injection (22%) was less than that produced by BLM at 10 mg/kg per injection (32%).

The effects of TLM S_{10}b and BLM on lung Cst and VC in rats are summarized in Fig. 3. Both drugs caused significant

Table 3. LD_{50} values of tallysomycin S_{10}b and bleomycin in male BDF_1 mice

Drug	LD_{50} (mg/kg per injection)		
	Single dose IP	qd 1 → 9 IP	Single dose IV
Tallysomycin S_{10}b	219 (195–246)[a]	9.8 (7.5–13)	187 (153–228)
Bleomycin	160 (117–218)	9.8 (7.5–13)	185 (164–207)

[a] 95% confidence limits

Table 4. Effects of tallysomycin S_{10}b and bleomycin on total WBC counts, BUN, and SGPT in male BDF_1 mice[a, b]

Drug	Dose (mg/kg IP)	Max. % decrease in WBC (day)	Highest incidence	
			BUN ≥ 30 mg % (day)	SGPT ≥ 40 IU (day)
TLM S_{10}b	188[c]	− 41 (5)	0/10	2/10 (1)
	142	− 14 (5)	1/7 (4)	2/10 (1)
	106	0	1/10 (4)	2/10 (1)
	80	0	0/10	3/10 (3)
Vehicle	–	0	0/10	1/10 (1)
BLM	160[d]	− 22 (5)	1/8 (7)	6/6 (5)
	120	− 15 (3)	2/10 (4)	8/8 (5)
	90	− 32 (3)	0/10	7/10 (5)
Vehicle	–	− 8 (3)	0/10	0/10

[a] Treatment: Single dose, day 0, 10 mice/dose. Separate groups of mice were used for each test

[b] Schedules for measurements: BUN on days −2, 4, 7, 11; WBC on days −2, 3, 5, 7; and SGPT on days −2, 1, 3, 5

[c] 86% LD_{50}

[d] LD_{50}

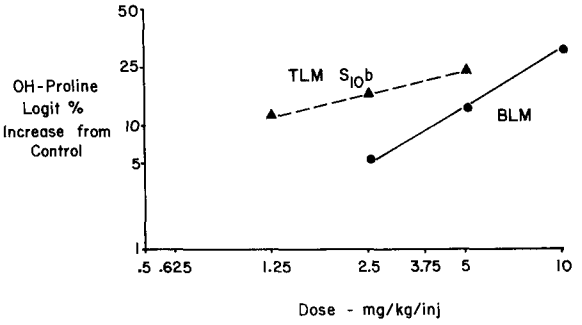

Fig. 2. Effect of tallysomycin $S_{10}b$ and bleomycin on whole-lung hydroxyproline content in BDF_1 mice. The drugs were administered SC twice a week for 4 weeks. The hydroxyproline content of the lungs was determined at the end of the 12th week (8 weeks after the last dose). Each *point* is the average value from 10 mice and is significantly different ($P < 0.05$) from controls based on analysis of variance

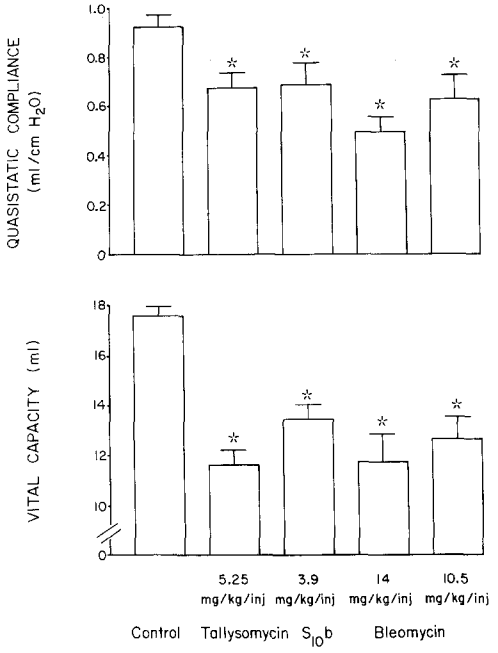

Fig. 3. Effect of tallysomycin $S_{10}b$ and bleomycin on quasistatic compliance and vital capacity in rats. The drugs were administered SC three times per week for 5 weeks. The rats were evaluated during the 6th week. The values are means and the *vertical bars* denote SE. There were five rats per dose with 10 controls. *Significantly different ($P < 0.05$) from controls (Student's *t*-test)

decreases in Cst and VC at the doses tested. The decrease in Cst produced by TLM $S_{10}b$ at 5.25 mg/kg per injection appeared to be less pronounced than that produced by BLM at 14 mg/kg per injection; however, the difference was not significant.

The results of the histopathiological evaluation of the lungs from these rats are summarized in Table 5. Compared with the control lungs, the lungs from the rats receiving the high doses of TLM $S_{10}b$ and BLM had higher lesion scores for alveolar macrophages, alveolar cell hypertrophy/hyperplasia, alveolar proteinosis, and fibrosis; BLM also caused some alveolitis. The lesion score for histological fibrosis, the single most definitive alteration evaluated, in rats receiving the high dose of TLM $S_{10}b$ was only slightly greater than for the control rats and there

Table 5. Mean histopathological changes in the lungs of tallysomycin $S_{10}b$- and bleomycin-treated rats[a]

		TLM $S_{10}b$ (mg/kg per injection)		BLM	
Lesion	Control	5.2	3.9	14	10.5
Alveolar macrophages	1.2	2.3	1.4	2.9	2.6
Alveolitis	0.1	0	0.2	1	0.4
Alveolar cell hypertrophy/hyperplasia	0	0.2	0	1.2	0.8
Alveolar proteinosis	0	0.6	0.2	0.6	0.8
Fibrosis	0	0.6	0	2.4	1.6
Total mean score	1.3	3.7	1.8	8.1	6.2

[a] Treatment: SC 3×/week for 5 weeks. Five rats/dose with 10 controls. Scoring system: 0, not noticeable; 1, minimal; 2, mild; 3, moderate; 4, severe

was no difference between low-dose TLM $S_{10}b$ and controls. Rats receiving either the low or the high dose of BLM had higher scores for fibrosis than either TLM $S_{10}b$ dose group.

The lungs from the rats receiving the low dose of TLM $S_{10}b$ had a total mean score which was similar to that in the control lungs. BLM at both low and high doses resulted in total mean scores that were greater than controls and both TLM $S_{10}b$ dose groups.

Discussion

The results of these studies demonstrate that TLM $S_{10}b$ has antitumor activity against a broad spectrum of experimental tumor models. Included among the more susceptible murine tumors are LL, B16, M109, $CD8F_1$ mammary, and Colon 38. Against human tumor lines grown as xenografts in nude mice, TLM $S_{10}b$ displayed definite activity against a colon tumor and had some mild effects against a human breast tumor.

TLM $S_{10}b$ had comparable or occasionally superior activity to BLM in every tumor model that was used to evaluate both drugs. These comparisons were made on the basis of concomitant testing (and not historical data) conducted over more than 2 years in several different laboratories.

These results are in general agreement with existing published reports. Miyaki et al. [9] found that TLM $S_{10}b$ had antitumor activity against P388, B16, LL, and Sarcoma 180 tumors. In comparison with BLM, which was included in the LL and B16 tests, TLM $S_{10}b$ had a slight activity advantage against both tumors.

Although variable, and often based on only a limited number of experiments for each treatment schedule, the optimal dose (that associated with the greatest increase in life-span or degree of tumor inhibition with acceptable levels of lethality) of TLM $S_{10}b$ was generally lower than that of BLM. Our assessment of the antitumor potency of TLM $S_{10}b$ compared with BLM, based on a consensus of all the antitumor data available to us, is that TLM $S_{10}b$ is almost twice as potent as BLM.

The results of the lethality studies indicate that in male BDF_1 mice, TLM $S_{10}b$ and BLM had approximately equivalent LD_{50} values when administered IP in single or multiple (qd $1 \rightarrow 9$) doses and IV in single doses. With both drugs, the single dose LD_{50} values were higher (1.5- to 5-fold) and the qd

$1 \rightarrow 9$ LD_{50} values somewhat lower (1.2- to 1.8-fold) than the values reported by the Bristol-Myers Research Institute [5, 6, 8, 9] in a different strain of mice. Moreover, the results from their evaluations suggest that the single dose and qd $1 \rightarrow 9$ LD_{50} values for BLM were approximately 2-fold higher than those for TLM S_{10}b.

In the hematology and serum chemistry studies in mice, TLM S_{10}b produced slight decreases in WBC counts and minimal elevations in BUN and SGPT levels. Similarly, BLM had minimal effects on WBC counts and BUN levels; however, BLM caused increased SGPT levels in nearly all the mice tested. Therefore, during the observation periods used in these studies, TLM S_{10}b was comparable to BLM in its effects on WBC counts and BUN levels, but had less effect than BLM on SGPT levels.

TLM S_{10}b has shown evidence of nephrotoxicity in studies using longer posttreatment observation periods. In mice treated with single or multiple (qd $1 \rightarrow 5$) IV doses of TLM S_{10}b (50% LD_{10} to LD_{50} range), proximal tubular degeneration and interstitial fibrosis were observed approximately 4 weeks after the end of the treatment [4]. This toxicity was not predicted well by measurements of BUN levels. Miyaki et al. [9] treated rats IP with TLM S_{10}b at 1.5 and 5 mg/kg per day for 9 days and found mild to moderate tubular degeneration and necrosis 3 weeks after the last treatment. BLM was not included in either of these studies, so that a direct comparison of relative nephrotoxic effects is not possible. In single-dose studies in dogs, TLM S_{10}b at 12 mg/kg IV was found to be slightly more nephrotoxic than BLM at 13.5 mg/kg IV according to clinicopathological and histological evaluations during a 4-month observation period [4]. Although the results of the longer-term studies indicate that TLM S_{10}b caused nephrotoxicity in animals, which was delayed in onset and poorly reversible, the comparative nephrotoxicity of TLM S_{10}b and BLM in these animals has not been established.

TLM S_{10}b and BLM caused pulmonary toxicity in both mice and rats. However, their relative pulmonary toxic effect varied depending on the animal and the parameter used to quantitate the toxicity. In mice TLM S_{10}b and BLM caused nonparallel, dose-related increases in lung hydroxyproline content. The lowest dose of TLM S_{10}b, 1.25 mg/kg per injection, was comparable in effect to BLM at 5 mg/kg per injection indicating an apparent four-fold difference in potency. This difference in potency decreased with increasing doses, the effect of TLM S_{10}b at 5 mg/kg per injection being smaller than that of BLM at 10 mg/kg per injection. The nonparallel responses suggest differences in the pulmonary toxicity of TLM S_{10}b and BLM in mice which could be related to as yet undetermined differences in mechanism, pharmacokinetics, or metabolism.

In rats, TLM S_{10}b and BLM caused comparable reductions in lung Cst and VC, TLM S_{10}b being approximately two or three times more potent than BLM. TLM S_{10}b did not have a greater effect than BLM at the lower doses, as observed in the mouse hydroxyproline studies; however, the dose range was much narrower. Histopathological examination of the lungs from these rats indicated that the MTD of TLM S_{10}b showed less evidence of pulmonary toxicity than either dose of BLM, and that the lower dose of TLM S_{10}b had essentially no effect. Therefore, TLM S_{10}b appeared to be less than twice as potent as BLM in causing histopathological changes in the lungs of the rats in these studies.

Based on the results from these evaluations of pulmonary toxicity, we conclude that TLM S_{10}b was generally comparable to BLM in causing pulmonary toxicity in mice and showed possibly less pulmonary toxicity in rats. There were indications that TLM S_{10}b caused less pulmonary toxicity than BLM at doses approaching MTD levels. Relative to BLM, TLM S_{10}b appeared to have approximately equivalent to four-fold greater potency, depending on the test system.

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