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Bleomycin Therapy of Experimental Disseminated Candidiasis in Mice

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Bleomycin, an antineoplastic agent, was found to be very effective in vitro against a variety of fungi, including *Candida albicans*. Mice were infected with *C. albicans* intravenously and then treated with various doses of bleomycin. No efficacy was shown by either prolongation of survival or reduction of tissue counts.

Bleomycin is an antineoplastic agent which acts by disrupting glycoside linkages in mammalian DNA (12). When fungal cells are exposed to bleomycin, DNA is disrupted but in addition there is increased fragility of cell walls, with greatly facilitated formation of spheroplasts (6, 8). Bleomycin-treated *Saccharomyces cerevisiae* cells may also develop increased susceptibility to peroxide and ionizing radiation (6). Time to formation of spheroplasts after exposure to bleomycin is sharply reduced (5). Some of the effects of bleomycin appear to be accomplished through a direct effect of bleomycin on anchorage of mannoproteins of the cell wall, facilitating their release (1). Electron microscopy demonstrates both large breaks and small holes in walls of *Saccharomyces* cells treated with bleomycin (8). Damage is potentiated by ferrous ion as well (7). Most of these and other studies have been involved with elucidating mechanisms of DNA damage and repair, but activity of bleomycin against *S. cerevisiae* might be turned to clinical advantage against more pathogenic fungi.

In addition to direct antifungal activity, bleomycin stimulates production and release of several host cytokines, among them tumor necrosis factor and transforming growth factor β (2, 10, 11). These immunologic effects have been associated with an adverse event, the development of pulmonary fibrosis in bleomycin-treated mice. However, proinflammatory activity by bleomycin might also augment host macrophage activity in killing fungi. For these reasons we evaluated bleomycin as a potential antifungal antibiotic. Our studies included in vitro testing against a variety of yeast isolates and in vivo therapy in a model of murine candidiasis.

Fungal yeast species were maintained on Sabouraud's agar and transferred to brain heart infusion broth for culture at 37°C overnight. Before infection, *Candida albicans* was washed and diluted in normal saline to 7×10^5 CFU per mouse.

Bleomycin was purchased from Bristol Myers Squibb, Princeton, N.J. For in vitro studies aqueous dilutions of bleomycin were tested by the National Committee for Clinical Laboratory Standards method against a number of yeast pathogens (9). The minimum lethal concentration was determined by subculturing the clear tubes of the 24-h MIC study and recording as the minimum lethal concentration that value above which there were no fungal colonies isolated. As shown in Table 1, bleomycin was highly effective against the isolates tested, with the MIC at 24 h of incubation generally <1.25 μg/ml. Of 16 isolates, for 15 the 24-h MIC was <1 μg/ml and for 9 the 48-h MIC was <2 μg/ml. For seven isolates the MIC rose to ≥ 6 μg/ml by 48 h of incubation. This rise in the 48-h MIC and in the minimum lethal concentration is a common phenomenon with multiple other antifungal drugs. In our prior experience with fluconazole therapy of murine cryptococcal meningitis, we were able to correlate the 24-h MIC but not the 48-h MIC with the in vivo response (13).

In studies of two isolates of *C. albicans* (R-1590) and *Cryptococcus neoformans* (95) a parallel in vitro MIC was determined with mouse serum in the medium. This was to determine whether serum binds or inactivates bleomycin. Bleomycin was prepared in water at 20× the final concentration. In one study the bleomycin was diluted 1:1 in water, and in the serum set the dilution was 1:1 into mouse serum. These 10× concentrations were then diluted 10 times into the buffered RPMI medium. The MIC was consistently markedly increased by incubation with serum, reflecting either binding to or inactivation by the serum. Because bleomycin is a complex of multiple active constituents, we did not attempt to develop an assay to measure any of these components.

For in vivo studies outbred ICR mice were given food and water ad libitum. Uninfected toxicity control mice were treated for 5 days with bleomycin, 5 mg/kg of body weight subcutaneously, and observed for 2 weeks. The dose of 5 mg/kg was chosen because this dose is biologically active in that it stimulates cytokine production in rats (4). The mice tolerated this dose well, with no apparent toxicity.

For survival studies groups of 10 mice were infected intravenously with 1.7×10^5 to 7.3×10^5 CFU of *C. albicans* R-1590 per mouse. Mice were treated with bleomycin from days 1 through 5. For studies 1 and 2 bleomycin was diluted in normal saline, and for study 3 bleomycin was diluted in sterile water. Water was used in studies because of a concern that saline might cause rapid degradation of bleomycin. Table 2 shows the results of survival studies. For studies of survival the log rank test and the Wilcoxon test of life tables were used for statistical comparison, with $P \leq 0.01$ determining significance. There was no significant effect of bleomycin on survival of mice in any of the studies, which included subcutaneous dosing (study 1) and intravenous dosing (studies 2 and 3).

Four similar studies were conducted to assess the effect of bleomycin on tissue burden (Table 3). Groups of 10 mice were infected intravenously. Bleomycin was administered in saline for studies 1, 2, and 3, and for study 4 bleomycin was diluted in sterile water. In studies 1 and 2 mice were treated subcutaneously (to slow the rate of clearance), and in study 3 mice were treated intravenously (to provide maximal concentration in a bolus). In study 1 a sublethal inoculum of 2.7×10^4 *Candida* CFU was used, to be sure that there was no inoculum-dependent resistance. Studies 2 and 3 had a lethal inoculum of approximately 7×10^5 *Candida* CFU. Mice were treated on days 1 through 5 and sacrificed on day 6 for tissue counts.

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TABLE 1. In vitro susceptibilities of fungi to bleomycin

Species	Isolate	MIC (μg/ml) at:		24-h MLC[a] (μg/ml)
		24 h	48 h	
Candida glabrata	ATCC 90030	0.78	0.78	25
	94-94	<0.19	0.39	25
	94-97	0.39	0.39	50
Candida krusei	94-51	0.78	1.57	12.5
Candida lusitaniae	94-69	0.78	12.5	25
	94-84	0.39	1.57	6.25
Candida parapsilosis	94-79	0.39	12.5	50
	94-89	1.57	6.25	25
Candida tropicalis	92-120	0.39	6.25	12.5
Candida albicans	94-75	0.39	12.5	50
	94-77	<0.19	0.39	50
	94-101	0.39	6.25	12.5
	94-104	<0.19	1.57	12.5
	R-1590	<0.19	12.5	ND[b]
	R1590 + serum	6.25	>100	ND
Cryptococcus neoformans	94-181	0.78	1.57	3.23
	95	<0.19	1.57	
	95 + serum	1.57	3.13	

[a] MLC, minimum lethal concentration.
[b] ND, not done.

Study 4 mice received a low dose of 5.6×10^4 *C. albicans* CFU. In study 4 mice were infected and 2 days later received subcutaneous Alzet osmotic pumps loaded with water (control) or bleomycin in water, set to deliver 10 mg/kg/day for 7 days. Mice for study 4 were sacrificed on day 10 after infection. Tissue counts were compared by Tukey's studentized range test. In none of the four studies was there a significant difference in *C. albicans* counts in either kidneys or spleens between the control and treated mice.

It is not clear why bleomycin appears highly effective in vitro but ineffective in vivo. Bleomycin-induced damage to *S. cerevisiae* tRNA is antagonized by physiologic concentrations of magnesium (3). Perhaps this ameliorates antifungal activity in vivo. Drug delivery might have been relatively ineffective for subcutaneously infected mice. If this was true, intravenous dosing should have provided higher peak concentrations and perhaps more effect, but again no benefit was shown. We considered the possibility that therapeutic benefit and toxicity combine to show no net effect of bleomycin. There was no evidence for this in that (i) uninfected mice showed no acute toxicity with the maximal dose used and (ii) we explored doses down to 0.1 mg/kg, with no difference in results. We also administered bleomycin via constant delivery through an Alzet pump. Finally, even if direct bleomycin antifungal activity were neutralized in vivo (the in vitro study suggested that considerable activity was lost when bleomycin was mixed with serum), the doses used were still sufficient to stimulate production of multiple cytokines and perhaps augment host clearing of *Candida* infection by that mechanism (10). This also did not occur.

In summary, despite excellent in vitro susceptibility, in vivo studies did not support a role for bleomycin in treatment of murine candidiasis. These studies illustrate yet another situation in which there does appear to be a discrepancy between in vitro and in vivo results.

TABLE 2. Survival after treatment with bleomycin for murine candidiasis

Study	Group (mg of bleomycin/kg/day)[a]	Mean survival (days)	SEM (days)
1 (bleomycin in saline, subcutaneously)	0 (control)	5.9	0.7
	0.1	6.7	0.5
	1	4.8	0.5
	5	6.8	0.5
2 (bleomycin in saline, intravenously)	0 (control)	7.4	0.7
	5	7.3	1.3
3 (bleomycin in water, intravenously)	0 (control)	11.3	2.3
	5	8.7	2.6

[a] Ten mice per group. Mice receiving bleomycin were treated from days 1 through 5.

TABLE 3. Tissue counts of *C. albicans* in infected mice treated with bleomycin

Tissue	Study	Group (mg of bleomycin/kg/day)[a]	Tissue count (10^5 CFU/g)	
			Median	Range
Kidneys	1	0 (control)	3.6	1.3–9.2
		1	2.4	0.2–14
		3	2.2	0.9–39
	2	0 (control)	11	1.7–14
		0.1	6.4	1.2–33
		1	21	1.6–70
		3	20	1.9–45
		5	12	3.5–65
	3	0 (control)	12	0.3–63
		5	1.0	0.8–27
	4	0 (control)	33	150–6.8
		10	280	530–45
Spleen	1	0 (control)	0.001	<0.001–0.003
		1	0.001	<0.001–0.004
		3	0.001	<0.001–0.003
	2	0 (control)	0.004	0.002–0.006
		0.1	0.007	0.02–<0.001
		1	0.03	0.002–0.03
		3	0.05	0.02–0.2
		5	0.007	0.002–0.07
	3	0 (control)	0.02	0.006–0.1
		5	0.03	0.006–0.05
	4	0 (control)	0.01	0.001–0.31
		10	0.16	0.009–5.5

[a] Ten mice per group. For studies 1 and 2 bleomycin was given subcutaneously, and for study 3 it was given intravenously. For study 4, bleomycin was given by Alzet pump.

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