Efficacy of Escalating Doses of Liposomal Amphotericin B (AmBisome) against Hematogenous *Candida lusitanae* and *Candida krusei* Infection in Neutropenic Mice

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Immunosuppressed CF1 mice were infected intravenously with two strains of *Candida krusei* and four strains of *Candida lusitanae* (two of which were resistant to amphotericin B). Mice were treated with 1 or 2 mg of amphotericin B desoxycholate per kg of body weight per day or escalating doses of liposomal amphotericin B (8 to 30 mg/kg/day) or were left untreated. Higher doses of liposomal amphotericin B were as effective as standard dose of amphotericin B desoxycholate in prolonging survival but were significantly more effective in reducing the fungal burden in the kidneys of animals infected with both *C. krusei* strains and the *C. lusitanae* strains that were susceptible to amphotericin B desoxycholate. This advantage of liposomal amphotericin B therapy could not be demonstrated in mice infected with the *C. lusitanae* strains that were resistant to amphotericin B desoxycholate.

Fungal infections represent a major life-threatening complication in immunocompromised patients (2). Disseminated candidiasis caused by *Candida albicans* is the most frequently encountered infection, although several other species such as *Candida lusitanae* and *Candida krusei* are emerging as significant pathogens (5, 9). Encapsulation of amphotericin B into liposomes is a novel approach that enables the safe administration of increased doses of the drug, which it is hoped would translate into a better therapeutic efficacy (1, 8). In the study described here we examined the efficacies of escalating doses of liposomal amphotericin B (LiAmB) in immunocompromised CF1 mice with hematogenous infections caused by *C. lusitanae* and *C. krusei* strains and compared it with that of amphotericin B desoxycholate (AmB).

Two *C. krusei* strains (2506 and 4935) and three *C. lusitanae* strains (1706, 2819, and 524) were randomly selected isolates recovered from the blood of patients cared for at The University of Texas M. D. Anderson Cancer Center in Houston. A fourth *C. lusitanae* strain (strain 5W31) was a clinical isolate with known resistance to AmB; strain 5W31 was kindly provided by W. G. Merz (Department of Laboratory Medicine, The Johns Hopkins Hospital, Baltimore, Md.). All six strains were tested for their in vitro susceptibilities by a broth dilution micromethod (4). We have recently shown that the results of in vitro testing obtained by this method are comparable to those obtained by using the proposed standard method of the National Committee for Clinical Laboratory Standards and correlate well with the in vivo outcome in mice with experimental candidiasis (3). The MICs of AmB were between 0.125 and 0.3125 µg/ml for the “susceptible” strains *C. krusei* 2506 and 4935 and *C. lusitanae* 1706 and 524 and 1.25 and 2.5 µg/ml for the “resistant” strains *C. lusitanae* 5W31 and 2819, respectively. The MICs of LiAmB were identical to those of AmB. Isolates were maintained in water stock, subcultured onto

Sabouraud's dextrose agar plates, and incubated for 24 h at 35°C. The yeast cells were then suspended in normal saline and were adjusted to the desired spectrophotometric transmission to produce each infecting inoculum.

Four-week-old outbred CF1 male mice (Harlan Sprague-Dawley, Indianapolis, Ind.) weighing 20 to 25 g were used. Mice were immunosuppressed and were infected with either a *C. lusitanae* or a *C. krusei* inoculum as described previously (3). One hour after inoculation groups of 20 animals each were randomized to receive treatment with AmB (Fungizone; E. R. Squibb & Sons Inc., Princeton, N.J.) at 1 mg/kg of body weight intravenously or 2 mg/kg intraperitoneally or different dose schedules of LiAmB (AmBisome; Vestar, Inc., San Dimas, Calif.) or were left untreated. LiAmB was given intravenously at a low (8 or 10 mg/kg), intermediate (15 mg/kg), or high (30 mg/kg) dose. Treatment was given once daily for 3 consecutive days. Twenty-four hours after the end of therapy, the kidneys of five randomly selected mice from each group were examined for fungal burden and the number of CFU per gram of kidney tissue was calculated. The survival of the remaining mice was monitored daily for up to 21 days postchallenge. The Mann-Whitney U test was used to analyze survival and clearance of the organisms from the kidneys, with significance defined as a *P* value of ≤0.05.

Kidney fungal titers were reduced by AmB and LiAmB in mice infected with *C. krusei* strains (*P* ≤ 0.05). The use of intermediate and high doses of LiAmB resulted in significantly fewer CFU per gram of tissue compared with the use of AmB or low-dose therapy with LiAmB (*P* ≤ 0.001). The survival of mice infected with *C. krusei* was significantly improved by treatment with AmB and low and intermediate doses of LiAmB, whereas treatment with high-dose LiAmB had no beneficial effect (Table 1). The response to therapy was variable in mice infected with *C. lusitanae* strains. AmB and LiAmB treatment improved survival (62 to 100% of treated mice were still alive by day 21, whereas 12 to 25% of untreated ones were still alive by day 21) and reduced the fungal titers only in mice infected with *C. lusitanae* 1706 and 524. A striking reduction in the number of fungi recovered from the kidneys of infected mice was observed with intermediate and high doses

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TABLE 1. Activities of AmB and LiAmB in neutropenic CF1 mice infected with two *C. krusei* and four *C. lusitanae* isolates

Therapy ^a	<i>C. krusei</i> 2506		<i>C. krusei</i> 4935		<i>C. lusitanae</i> 1706		<i>C. lusitanae</i> 524		<i>C. lusitanae</i> 2819		<i>C. lusitanae</i> 5W31	
	Median survival (days [%]) ^b	Mean CFU/g ^c	Median survival (days [%])	Mean CFU/g	Median survival (days [%])	Mean CFU/g	Median survival (days [%])	Mean CFU/g	Median survival (days [%])	Mean CFU/g	Median survival (days [%])	Mean CFU/g
None	9 (32)	6.65	7 (20)	6.10	7 (23)	6.71	7 (12)	7.38	8 (23)	6.44	7 (23)	6.90
AmB												
2 mg/kg i.p.	21 (87) ^d	4.99 ^d	21 (100) ^d	5.35 ^d	NT ^e	NT	NT	NT	NT	5.54	NT	NT
1 mg/kg i.v.	NT	NT	NT	NT	21 (78) ^d	3.73 ^d	21 (95) ^d	3.21 ^d	8 (23)	6.04	8 (23)	6.04
LiAmB												
8 mg/kg i.v.	21 (72) ^d	4.67 ^d	17 (80) ^d	4.54 ^d	NT	NT	NT	NT	NT	5.42 ^d	NT	NT
10 mg/kg i.v.	NT	NT	NT	NT	21 (100) ^d	3.81 ^d	21 (92) ^d	3.07 ^d	10 (40)	6.76	8 (0)	6.76
15 mg/kg i.v.	21 (70) ^d	3.70 ^{d,f}	21 (75) ^d	4.07 ^{d,f}	NT	NT	21 (80) ^d	1.00 ^{d,f}	NT	NT	NT	NT
30 mg/kg i.v.	7 (27)	3.62 ^{d,f}	NT	NT	21 (62) ^d	1.42 ^{d,f}	NT	NT	10 (23)	5.33 ^d	7 (20)	5.81 ^d

^a i.p., intraperitoneally; i.v., intravenously.^b Percent overall survival.^c Mean log₁₀ CFU per gram of kidney tissue.^d $P < 0.05$ versus no therapy (Mann-Whitney U test).^e NT, not tested.^f $P < 0.05$ versus AmB therapy (Mann-Whitney U test).

of LiAmB compared with that with AmB ($P < 0.001$). A number of kidneys from infected mice were sterilized by LiAmB therapy. The prolongation of survival was not seen in animals infected with *C. lusitanae* 5W31 and 2819 and treated with either AmB or LiAmB (Table 1). In this case high-dose LiAmB reduced the fungal burden in the kidneys, but this effect was moderate (<1-log-unit reduction) and similar to that observed with AmB.

AmB and LiAmB were active against experimental hematogenous infection caused by *C. krusei* and AmB-susceptible *C. lusitanae* in immunosuppressed mice. Escalation of the LiAmB dose resulted in a dose-related reduction in fungal titers in tissue. Higher LiAmB doses were more effective than AmB. Treatment with high-dose LiAmB was associated with early mortality, most likely secondary to toxicity, since a good antifungal effect was documented in the clearance of fungi from the kidneys. These observations indicate the need for further investigations to determine the maximum tolerated dose of the newer lipid formulations of AmB.

The impressive effect of LiAmB on the clearance of yeasts from kidneys at a time when mice were profoundly neutropenic (data not shown) suggests that LiAmB may be useful in the treatment of granulocytopenic patients with disseminated candidiasis. On the other hand, LiAmB, even at doses as high as 30 mg/kg, failed to improve the outcomes in mice infected with AmB-resistant *C. lusitanae*. This indicates that the resistance of *Candida* isolates to AmB may not be overcome by the administration of much higher doses of LiAmB.

Multiple lipid formulations of AmB such as multilamellar (8) or unilamellar (10) vesicles, lipid complexes (6), and colloidal dispersions (7) have been tested against hematogenous candidiasis in normal and immunosuppressed rodents. In most of those studies the lipid formulations of AmB were better tolerated than the free drug and overall were more effective when administered at higher doses. Our results are in agreement with those findings. However, our study is the first to examine the effect of administering high-dose LiAmB to neutropenic animals infected with AmB-resistant *Candida* species and to show the limited benefit of the lipid formulation in this setting.

In conclusion, high doses of LiAmB are significantly more active than AmB against experimental candidiasis in immunosuppressed mice infected with AmB-susceptible *C. lusitanae* and *C. krusei* strains. No advantage of the liposomal formulation of the drug over the free drug could be demonstrated in mice infected with AmB-resistant strains.

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