ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1995, p. 1885–1887 Vol. 39, No. 8
0066-4804/95/$04.00+0

Liposomal Amphotericin B Therapy of Murine Histoplasmosis

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Received 3 January 1995/Returned for modification 21 February 1995/Accepted 16 May 1995

Liposomal amphotericin B (AmBisome) was compared with amphotericin B deoxycholate for the treatment of disseminated murine histoplasmosis. Liposomal amphotericin B was well tolerated and, milligram for milligram, was as potent as amphotericin B deoxycholate.

The antifungal azoles ketoconazole and itraconazole are effective in the treatment of histoplasmosis (4, 6). However, they may be irregularly absorbed when given orally, and coadministration of several other drugs may accelerate their hepatic degradation (13, 15). Furthermore, ketoconazole is not very effective when administered to AIDS patients with histoplasmosis (17). Fluconazole is better absorbed, but it may be less effective than itraconazole in the treatment of histoplasmosis, particularly for patients with AIDS (10). Additionally, the antifungal azoles may act less rapidly than amphotericin B, and there is concern that they might not be as effective as amphotericin B in the treatment of acute life-threatening histoplasmosis (18).

For those patients who should receive amphotericin B, its toxicity may be reduced by administration of amphotericin B in a lipid vehicle. The vehicle reduces toxicity by preventing deposition in the kidney while allowing amphotericin B to concentrate in the areas of infection (reticuloendothelial tissues). Several lipid-associated amphotericin B derivatives undergoing clinical trials are now available. Among these is liposomal amphotericin B, a unilamellar true liposome preparation of amphotericin B. Liposomal amphotericin B has been found to be effective in a series of studies of animals infected with various fungal pathogens. In one study, both liposomal amphotericin B and colloidal amphotericin B were similarly active in vitro against *Candida albicans* (2). In another study, liposomal amphotericin B was more than eight times less potent in vitro than colloidal amphotericin B (11). In the same study, liposomal amphotericin B was found to be four to eight times less potent than colloidal amphotericin B for the treatment of murine candidiasis caused by *C. albicans*. Similar results were found for mice infected with *Candida lusitaniae* (7). However, the protective effects of liposomal amphotericin B may be evident much longer after treatment is concluded than those of colloidal amphotericin B (5). Liposomal amphotericin B has been well tolerated by patients in initial open clinical studies (16) and has been used successfully to treat antimony-resistant visceral leishmaniasis (14). In the present studies, we compared the efficacy of liposomal amphotericin B with that of commercial amphotericin B deoxycholate (AMB) in a model of murine histoplasmosis in immunodeficient mice.

Congenitally athymic *nu/nu* mice from a BALB/c background were obtained from our breeding facility. These mice are specific pathogen free and raised under barrier conditions.

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Histoplasma capsulatum G217 and a clinical isolate, 93-255, were used. The isolates were maintained in the yeast phase and were plated on blood agar.

Yeast cells were harvested from the colonies, washed, and suspended in sterile normal saline, and the cells were counted with a hemacytometer. Actual inocula were determined by quantitative cultures.

Liposomal amphotericin B was obtained from Vestar, San Dimas, Calif. AMB was obtained from Lyphomed. Prior to administration, liposomal amphotericin B was reconstituted according to the manufacturer's directions. Fresh doses of the drug were prepared daily. Both liposomal amphotericin B and AMB (up to 1 mg/kg of body weight) were administered intravenously in 0.2 ml, but at higher doses, AMB was administered intraperitoneally. This was done because AMB is frequently lethal to mice when given intravenously at doses of more than 1 mg/kg.

The mice were treated on days 2, 3, 5, 9, 12, and 15 after infection, and survival was monitored for 40 days after infection. For tissue counts, the mice were treated on days 2, 3, 5, and 7 and then were sacrificed on day 9. For comparisons, Tukey's multiple comparisons test was used for tissue counts and the log-rank test was used for survival rates.

Survival studies were carried out (Table 1). Doses of 0.3, 0.6, 1.0, and 3.0 mg/kg were used. Groups of 10 to 20 mice were treated. For isolates G217 and 93-255, infecting doses of 5×10^4 and 3×10^6 CFU were given. At doses of 0.3 mg/kg, neither drug prolonged survival significantly over that for the controls (a probability of 0.005 was required for significance, because of multiple comparisons of the treatment groups with the control). At doses of 1 mg/kg and 3 mg/kg, respectively, AMB and lipsomal amphotericin B were effective in extending survival over that of the controls. For isolate 93-255, which was apparently more susceptible to polyene therapy even when given at the higher inoculum of 3×10^6 CFU, all doses extended survival over that of the controls. At a dose of 1 mg/kg, liposomal amphotericin B was significantly more effective than the same dose of AMB. For doses of 3 mg/kg, the same trend was present, but the difference was not significant.

Figure 1 presents the spleen and kidney counts for mice infected with 3×10^6 CFU per mouse. Groups of five to seven mice were used. At a treatment dose of 0.6 mg/kg, neither drug was effective in reducing spleen or kidney counts. At doses of 1 mg/kg, lipsomal amphotericin B significantly reduced spleen tissue counts while AMB significantly reduced kidney counts ($P < 0.005$).

On the basis of the studies discussed above, it is apparent that both AMB and liposomal amphotericin B are efficacious for the treatment of histoplasmosis in athymic mice. AMB at these doses is curative in immunocompetent mice (9). The

TABLE 1. Survival of mice with disseminated histoplasmosis treated with AmBisome or amphotericin B

Isolate and group	Dose (mg/kg)	Mean days survival	SEM[a]
G217			
Control	None	29	2.1
Ampho B	0.3	37	4.0
	1.0	46	—**
	3.0	37	3.6
AmBisome	0.3	30	3.1
	1.0	28	3.9
	3.0	46	—**
93-255			
Control	None	6	0.2
Ampho B	0.3	9	0.9*
	0.6	14	2.7*
AmBisome	0.3	9	0.2*
	0.6	14	1.6*
None			
Control	None	7	0.1
Ampho B	1.0	10	0.9**
	3.0	23	2.9**
AmBisome	1.0	16	2.5***
	3.0	29	0.8**

[a] —, none (all mice survived the study); *, $P < 0.005$ compared with control; **, $P < 0.001$ compared with controls; ***, $P < 0.02$ compared with amphotericin B (Ampho B) at 1.0 mg/kg, and <0.001 compared with control.

course of histoplasmosis in immunocompetent BALB/c mice is that of an acute infection with either death before week 3 or complete resolution. This clearly reflects the contribution of host-cell-mediated immune defenses (1, 8, 19). Athymic mice are vulnerable to much lower infecting doses of *H. capsulatum*, and the infection is progressive, ending in death from widely disseminated disease. Therefore, the athymic mouse is a difficult test for antifungal therapy, and it is encouraging that liposomal amphotericin B is about as effective as AMB. Nevertheless, despite similar survival benefits, at intermediate doses there are subtle differences, which are reflected in the tissue counts. Liposomal amphotericin B produces much higher concentrations of amphotericin B in serum than AMB, and tissue distribution is altered to reflect less deposition in the kidneys and more deposition in the reticuloendothelial tissues (14). Because *H. capsulatum* resides within macrophages and monocytes, it is advantageous to concentrate the antifungal drug at the site of infection. This may be why the AMB effect on tissue counts is seen primarily in the kidney and the liposomal amphotericin B effect is seen primarily in the spleen.

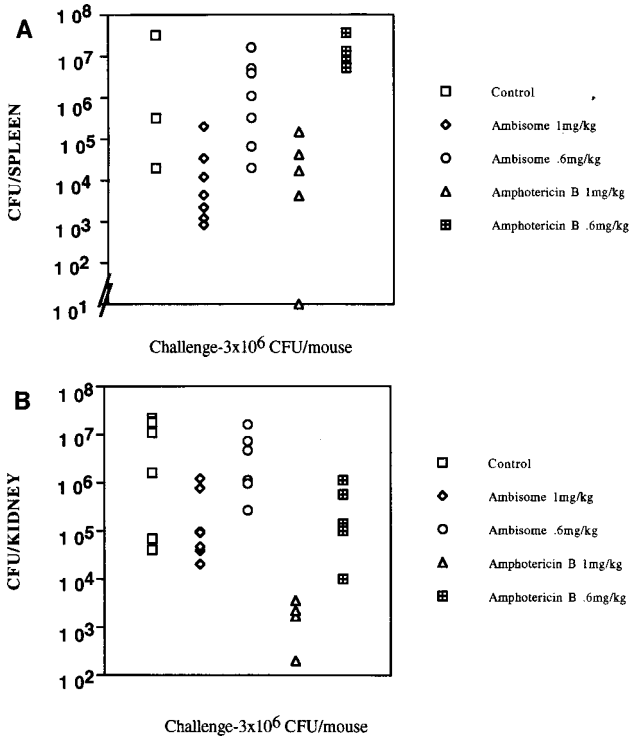

FIG. 1. Tissue counts for the spleens (A) and kidneys (B) of mice infected with 3×10^6 CFU of *H. capsulatum* 93-255 and treated until day 7. The mice were sacrificed on day 9. AmBisome is the trade name of liposomal amphotericin B.

Other studies of lipid-associated formulations of amphotericin B have shown consistently reduced toxicity but also consistently reduced efficacy. The major benefit of these drugs appears to be a shift of the therapeutic ratio (3, 12). This shift is enough for the much higher doses of lipid-associated polyenes that are used still to be given with tolerance better than that for AMB. The present studies differ with others in that liposomal amphotericin B appears to be as efficacious as AMB in this model of histoplasmosis. These studies support clinical trials of liposomal amphotericin B in histoplasmosis.

This work was supported by NIAID contract NOI-A1-25141.

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