ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1991, p. 2144–2146
0066-4804/91/102144-03$02.00/0

Vol. 35, No. 10

Comparative Efficacies of Amphotericin B Lipid Complex and Amphotericin B Deoxycholate Suspension against Murine Blastomycosis

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Received 8 April 1991/Accepted 19 July 1991

Amphotericin B as a lipid complex and as a deoxycholate suspension (Fungizone) was tested against murine blastomycosis. All doses of each form prolonged survival ($P < 0.05$ to 0.001). Fungizone was more effective than lipid complex at doses of 0.8 mg/kg of body weight. However, lipid complex at 12.8 mg/kg was not toxic and superior in efficacy ($P < 0.001$) to 2.0 mg of Fungizone per kg (a toxic dose), and it cleared all animals of infection. Lipid complex is an effective therapy for murine blastomycosis.

The incidence of mycoses that require therapy has risen dramatically. Various factors, such as AIDS, the increased use of immunosuppressive regimens for transplantation, and glucocorticoid use, have contributed to this, making apparent the need for better antifungal therapies. A number of avenues have been explored. Several azole drugs that cause few side effects have shown efficacy in treatment of various mycoses (6, 19). Relapses have been reported (6, 19), demonstrating the need for fungicidal drugs.

Amphotericin B has broad activity and has been the standard antifungal agent against which others are measured (2). However, its application is limited by associated toxicities (2). Mechanisms to lessen the toxicity of amphotericin B, such as chemical derivatization (1, 4, 7, 9) or intercalation into lipid-based carriers (3, 8, 10–13, 15–17, 20, 21), have proven useful. Experimental and clinical studies have shown the utility of administering amphotericin B in lipid carriers that reduce associated toxicities (10, 12, 15, 21). The reduction of toxicities allows greater doses, which compensate for the reduced efficacy of these preparations, to be given. Because of difficulties in preparation and standardization of lipid delivery systems and because of reports of treatment failures (10, 11, 20), an optimal lipid delivery formulation is being sought.

In the present study, we compared amphotericin B lipid complex (Bristol-Myers Squibb Co., Princeton, N.J.) with amphotericin B as a micellar deoxycholate suspension (Fungizone; Squibb) against murine pulmonary blastomycosis. Lipid complex is a preparation of dimyristol-phosphatidylcholine and dimyristol-phosphatidylglycerol in a 7:3 molar ratio, with 33 mol% amphotericin B; the average particle size is 1.6 to 6 μm.

Activities of these drugs in vitro against the isolate used in these studies were similar. The MIC in broth dilution and minimum fungicidal concentration (18) of Fungizone were 0.5 and 1.0 μg/ml, and the MIC and minimal fungicidal concentration of lipid complex were 0.25 and 1.0 μg/ml, respectively.

To initiate the model, 4-week-old male CD-1 mice (Charles River Breeding Laboratories, Inc., Portage, Mich.) (average weight, 26.7 g) were infected intranasally with 19,950 CFU of *Blastomyces dermatitidis* ATCC 26199. This is a modification of the model described previously, which used BALB/c mice (5). This inoculum was used to establish a disease resulting in a 50% lethal dose or greater mortality, with most deaths occurring between days 10 and 28 postinfection, as determined from preliminary studies (data not shown). Therapy was initiated 4 days postinfection and given intravenously (i.v.) or intraperitoneally (i.p.) in 0.1 ml. Groups of 10 infected mice received treatments Monday, Wednesday, and Friday for 2 weeks. Controls were either untreated or given saline, the diluent for lipid complex, alone i.v. No Fungizone diluent controls were included, because preliminary studies showed them to be no different from untreated controls (data not shown). Fungizone was reconstituted per the package insert instructions, diluted in sterile 5% glucose to the desired concentrations, and stored at −20°C until needed. Mice received either 6.3 or 0.63 mg of Fungizone per kg of body weight i.p. or 2.0, 0.8, or 0.2 mg/kg i.v. Lipid complex as a colloidal suspension in saline was stored at 4°C and diluted in saline on the day of treatment. Lipid complex was given i.v. at 12.8, 0.8, or 0.2 mg/kg. The maximum doses of Fungizone given i.p. and lipid complex given i.v. were those shown in prior studies to be without toxicity. All doses are expressed as milligrams of amphotericin B.

Deaths were scored for 49 days postinfection. At the end of this period, all survivors were killed by cervical dislocation and were necropsied. The lungs were removed aseptically, weighed, and homogenized in 5 ml of saline with a Tissumizer (Tekmar Co., Cincinnati, Ohio). Diluted suspensions were placed onto sheep blood agar plates. The residual burden of *B. dermatitidis* was determined by counting the number of colonies arising and was expressed as the number of CFU ($\log_{10}$) per lung. Differences in treatment group mortalities were analyzed by Wilcoxon rank sums tests, and differences in organ burdens were analyzed by Mann-Whitney U tests (14).

The cumulative mortalities of the mice in the various groups are presented in Fig. 1. All mice given Fungizone at 6.3 mg/kg i.p. or 0.8 mg/kg i.v. or lipid complex at 12.8 mg/kg i.v. survived through day 49. Other treatment regimens were less effective. One death, due to acute toxicity, occurred in

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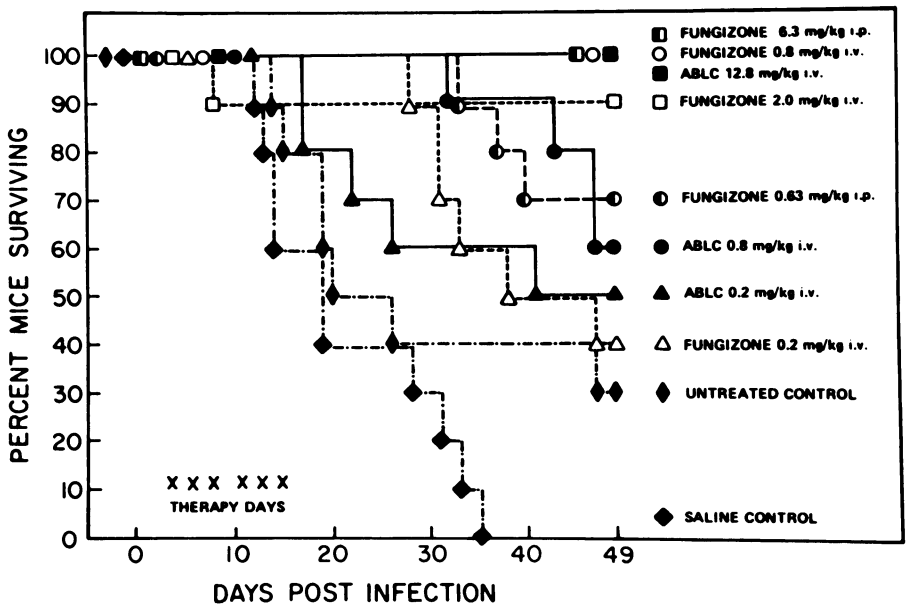

FIG. 1. Cumulative mortalities of CD-1 mice infected intranasally with *B. dermatitidis*. Abbreviation: ABLC, amphotericin B lipid complex.

the group given 2.0 mg of Fungizone per kg i.v. All therapy regimens except doses of 0.2 mg of Fungizone or lipid complex per kg prolonged survival of treated groups over that of untreated controls ($P < 0.05$ or 0.01, dependent on the dose), and all regimens prolonged survival of treated groups over that of saline controls ($P < 0.001$, except with lipid complex at 0.2 mg/kg, for which P was <0.05). Fungizone at 6.3 but not at 0.63 mg/kg i.p. was superior to 0.2 mg of Fungizone per kg i.v. or 0.2 mg of lipid complex per kg ($P < 0.05$). Fungizone at 0.8 mg/kg i.v. was equivalent to 0.8 mg of lipid complex per kg but superior to 0.2 mg of either drug per kg ($P < 0.05$). The 0.2-mg/kg regimens were inferior to 12.8 mg of lipid complex per kg ($P < 0.05$).

To assess further the efficacy of the treatment regimens, the residual CFU of *B. dermatitidis* in the lungs of surviving mice were determined (Table 1). No surviving untreated controls were free of infection. In contrast, 80 and 70% of mice given 6.3 or 2.0 mg of Fungizone per kg, respectively, were free of infection. Lower doses cleared 10 or 20% of the mice of infection. Treatment with 12.8 mg of lipid complex per kg cleared all mice of *B. dermatitidis*. However, lower doses were much less effective.

Note that although Fungizone was pushed to toxicity at the 2.0-mg/kg dose given i.v., it was not curative for all mice (Fig. 1 and Table 1). Upon gross examination, all survivors in this group had evidence of renal damage in the forms of partial atrophy and capsular paleness as well as paleness of the myocardium consistent with loss of tissue mass. No tissue damage in any lipid complex-treated mice or in mice treated with other regimens of Fungizone was noted.

In statistical comparisons of the residual burdens, a value of $\log_{10}$ 8 was assigned to datum points missing because of the death of the animal during the study, except if death was associated with acute toxicity. This value is the approximate number of CFU in the lungs just prior to death, and it was established from previous studies (data not shown). It also ensures that the rank assigned to animals that die is lower than that assigned to those that survive with any size burden. Analysis of the residual burdens of *B. dermatitidis* with the various therapy regimens demonstrated that 12.8 mg of lipid complex per kg or 6.3 mg of Fungizone per kg i.p. was superior to control treatments ($P < 0.001$) as well as to Fungizone at 0.63 ($P < 0.001$), 0.8 ($P < 0.001$ or 0.01), or 0.2 ($P < 0.001$) mg/kg and to lipid complex at 0.8 or 0.2 mg/kg ($P < 0.001$). Similarly, 2.0 mg of Fungizone per kg i.v. reduced residual burdens ($P < 0.01$ to 0.001) better than the latter five regimens and control treatments. However, both acute and chronic toxicities were observed with this regimen, indicating that it is not desirable. At doses of 0.8 mg of amphotericin B per kg, Fungizone was superior to lipid complex ($P < 0.01$). Fungizone at 0.2-mg/kg doses was not superior.

The Fungizone results were confirmed in another experiment of the same design (data not shown) in which 90% of

TABLE 1. Recovery of *B. dermatitidis* from the lungs of surviving mice

Treatment and dose (mg/kg)	Route	Total no. surviving (no. surviving and culture free)[a]	Geometric mean $\log_{10}$ CFU in lungs
None		3 (0)	4.20
Saline	i.v.	0	
Fungizone	i.p.		
6.3		10 (8)	0.24
0.63		7 (1)	4.24
Fungizone	i.v.		
2.0		9 (7)	0.27
0.8		10 (2)	3.69
0.2		4 (1)	4.90
Lipid complex	i.v.		
12.8		10 (10)	0
0.8		6 (0)	5.62
0.2		5 (1)	4.46

[a] Of a total of 10 mice.

mice treated with 5% glucose and 100% of untreated controls died. Fungizone at 1.2, 0.8, 0.6, 0.4, and 0.2 mg/kg per dose produced survival rates of 100, 100, 80, 60, and 0%, respectively, and sterilized 70, 30, 0, 0, and 0% of surviving mice, respectively.

In summary, lipid complex was found to be efficacious as a treatment for pulmonary murine blastomycosis. Lipid complex caused no overt toxicity at a dose of 12.8 mg/kg and is estimated to be greater than 6.4-fold less toxic than Fungizone. On a basis of milligrams per kilogram, lipid complex and Fungizone were not equivalent. Lipid complex was less active than Fungizone; with the same dosage, Fungizone caused a greater reduction in residual burden. However, even though less active, lipid complex could be given in greater doses that proved to be efficacious, clearing all mice of residual burdens, whereas neither 2.0 mg of Fungizone per kg given i.v., a clearly toxic dose, nor 6.3 mg per kg given i.p. cleared all animals of infection. Lipid complex shows promise for the treatment of systemic mycoses and should be tested in other animal models and in clinical trials.

We thank Linda H. Hanson and Alon M. Perlman for technical assistance during parts of these studies.

These studies were funded in part by a grant from Bristol-Myers Squibb.

REFERENCES

1. **Ellis, W. G., R. A. Sobel, and S. L. Nielsen.** 1982. Leukoencephalopathy in patients treated with amphotericin B methyl ester. J. Infect. Dis. **146**:125–137.
2. **Gallis, H. A., R. H. Drew, and W. W. Pickard.** 1990. Amphotericin B: 30 years of clinical experience. Rev. Infect. Dis. **12**:308–329.
3. **Graybill, J. R., P. C. Craven, R. L. Taylor, D. M. Williams, and W. E. Magee.** 1982. Treatment of murine cryptococcosis with liposome-associated amphotericin B. J. Infect. Dis. **145**:748–752.
4. **Graybill, J. R., and S. R. Kaster.** 1984. Experimental murine aspergillosis: comparison of amphotericin B and a new polyene antifungal drug, SCH 28191. Am. Rev. Respir. Dis. **129**:292–295.
5. **Harvey, R. P., E. S. Schmid, C. C. Carrington, and D. A. Stevens.** 1978. Mouse model of pulmonary blastomycosis: utility, simplicity, and quantitative parameters. Am. Rev. Respir. Dis. **117**:695–703.
6. **Hay, R. J.** 1990. Fluconazole. J. Infect. **21**:1–6.
7. **Hoeprich, P. D.** 1982. Amphotericin B methyl ester and leukoencephalopathy: the other side of the coin. J. Infect. Dis. **146**:173–176.
8. **Hopfer, R. L., K. Mills, R. Mehta, G. Lopez-Berestein, V. Fainstein, and R. L. Juliano.** 1984. In vitro antifungal activities of amphotericin B and liposome-encapsulated amphotericin B. Antimicrob. Agents Chemother. **25**:387–389.
9. **Lawrence, R. M., and P. D. Hoeprich.** 1976. Comparison of amphotericin B and amphotericin B methyl ester: efficacy in murine coccidioidomycosis and toxicity. J. Infect. Dis. **133**:168–174.
10. **Lopez-Berestein, G.** 1988. Liposomes as carriers of antifungal drugs. Ann. N.Y. Acad. Sci. **544**:590–597.
11. **Lopez-Berestein, G., G. P. Bodey, V. Fainstein, M. Keating, L. S. Frankel, B. Zeluff, L. Gentry, and K. Mehta.** 1989. Treatment of systemic fungal infections with liposomal amphotericin B. Arch. Intern. Med. **149**:2533–2536.
12. **Patterson, T. F., P. Miniter, J. Dijkstra, F. Szoka, J. L. Ryan, and V. T. Andriole.** 1989. Treatment of experimental invasive aspergillosis with novel amphotericin B/cholesterol-sulfate complexes. J. Infect. Dis. **159**:717–724.
13. **Shirhoda, A., G. Lopez-Berestein, J. M. Holbert, and M. A. Luna.** 1986. Hepatosplenic fungal infection: CT and pathologic evaluation after treatment with liposomal amphotericin B. Radiology **159**:349–353.
14. **Sokal, R. R., and F. J. Rohlf.** 1981. Biometry, 2nd ed., p. 432–435. W. H. Freeman & Co., San Francisco.
15. **Szoka, F. C., D. Milholland, and M. Barza.** 1987. Effect of lipid composition and liposome size on toxicity and in vitro fungicidal activity of liposome-intercalated amphotericin B. Antimicrob. Agents Chemother. **31**:421–429.
16. **Taylor, R. L., D. M. Williams, P. C. Craven, J. R. Graybill, D. J. Drutz, and W. E. Magee.** 1982. Amphotericin B in liposomes: a novel therapy for histoplasmosis. Am. Rev. Respir. Dis. **125**:610–611.
17. **Tremblay, C., M. Barza, C. Fiore, and F. Szoka.** 1984. Efficacy of liposome-intercalated amphotericin B in the treatment of systemic candidiasis in mice. Antimicrob. Agents Chemother. **26**:170–173.
18. **Tucker, R. M., L. H. Hanson, E. Brummer, and D. A. Stevens.** 1989. Activity of ICI 195,739, a new oral triazole, compared with that of ketoconazole in the therapy of experimental murine blastomycosis. Antimicrob. Agents Chemother. **33**:573–575.
19. **Tucker, R. M., P. L. Williams, E. G. Arathoon, and D. A. Stevens.** 1988. Treatment of mycoses with itraconazole. Ann. N.Y. Acad. Sci. **544**:451–470.
20. **Weber, R. S., and G. Lopez-Berestein.** 1987. Treatment of invasive *Aspergillus* sinusitis with liposomal-amphotericin B. Laryngoscope **97**:937–941.
21. **Wiebe, V. J., and M. W. DeGregorio.** 1988. Liposome-encapsulated amphotericin B: a promising new treatment for disseminated fungal infections. Rev. Infect. Dis. **10**:1097–1101.