ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2004, p. 2140–2143 Vol. 48, No. 6
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.6.2140–2143.2004

Efficacies of Amphotericin B (AMB) Lipid Complex, AMB Colloidal Dispersion, Liposomal AMB, and Conventional AMB in Treatment of Murine Coccidioidomycosis

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Received 18 August 2003/Returned for modification 22 September 2003/Accepted 17 February 2004

The therapeutic efficacy of three lipid formulations of amphotericin B was compared with that of conventional amphotericin B in treatment of murine coccidioidomycosis. All treatments prolonged survival compared with the no-treatment group ($P < 0.0001$). Although conventional amphotericin B was more active than lipid formulations on reducing quantitative fungal load on a milligram-per-kilogram basis ($P < 0.003$ to 0.0002), the lipid preparations could be administered at higher doses, sterilizing liver and spleen tissues. The efficacies of the lipid preparations were similar in this murine model of coccidioidomycosis.

Amphotericin B (AMB), a polyene macrolide, has been the agent of choice for the treatment of severe cases of coccidioidomycosis and other systemic mycoses for 40 years. The drug has the broadest spectrum of the activity of any available antifungal agent, but its use is limited by its narrow therapeutic index and its poor tolerability (7, 8). At present, AMB is the only polyene agent administered intravenously to patients with systemic mycoses.

Several strategies have been developed over the past few years in an effort to overcome the disadvantages associated with the clinical use of conventional AMB. The integration of this polyene into phospholipid carriers has proven most useful for agents that have dose-limiting toxicities. The major considerations for developing drug carrier assemblage are related to their in vivo applicability. Drug carrier assemblages appear to be quite similar in that each has a therapeutically active antifungal compound compartment as well as an apparently nontherapeutically active lipid portion. However, the lipid-based polyene products differ widely in the composition of the carrier, the molar ratio of the carrier, the polyene content, the particle size, and the way that the polyene interacts with the lipid (10, 11, 13, 14, 16, 17). The formulations include AMB lipid complex, AMB colloidal dispersion, and liposomal AMB. Some of these preparations have been shown to be valuable for the treatment of coccidioidomycosis in animal models or clinical studies (1, 2, 3, 15). However, no study comparing the efficacies of the three lipid formulations against coccidioidomycosis has been done. There have also been some reports of successful treatment with liposomal AMB in murine histoplasmosis and paracoccidioidomycosis (4, 9). In one study, the three lipid-associated formulations as well as conventional AMB were compared for the treatment of experimental systemic murine cryptococcosis (5). They found that all three preparations prolonged survival and reduced the fungal load in the brain. All three formulations could be administered at higher doses than conventional AMB.

In the present study, we compared the three lipid-based formulations of AMB and the conventional formulation in murine systemic coccidioidomycosis.

MATERIALS AND METHODS

Microorganisms. Two clinical *Coccidioides* isolates were used in this study. They were obtained from the Fungus Testing Laboratory, Department of Pathology, University of Texas, Health Science Center at San Antonio. Isolates were identified as *Coccidioides* spp. by examination of macroscopic and microscopic morphologies and confirmed by DNA probe (Gen-Probe Inc., San Diego, Calif.). In vitro susceptibility testing was evaluated by using the National Committee for Clinical Laboratory Standards broth macrodilution proposed standard reference method M38-P for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi (12). Briefly, the mycelium was overlaid with sterile distilled water, and suspensions were made by gently scraping the colonies with wooden applicators. Heavy fragments were allowed to settle, and the upper, homogenous supernatant was transferred to sterile tubes. The arthroconidial suspensions were vortexed and adjusted to 95% transmittance at the 530-nm setting with a spectrophotometer (Spectronic 21; Milton Roy Company). AMB and its lipid formulations were tested in antibiotic medium 3 (Difco, Detroit, Mich.). The final drug concentrations were 0.03 to 16 μg/ml.

Drug samples containing 0.1 ml of each drug were inoculated with 0.9-ml volumes of the suspensions. A drug-free growth control tube was included for each isolate. The tubes were incubated at 35°C. The MIC and the minimum lethal concentration (MLC) were read at 48 h. The MIC was defined as the drug concentration in the first tube that was optically clear. The MLC was determined by streaking 100 μl of broth from tubes with concentrations above the MIC and incubating them at 35°C. The MLC was proposed as the lowest concentration of an antifungal compound that allowed the growth of five or fewer colonies. A *Paecilomyces variotii* control strain, UTHSC 90-459, was included for testing.

Antifungal agents. Drugs were obtained from their manufacturers. They included AMB (Fungizone; Bristol-Myers Squibb, Princeton, N.J.), AMB lipid complex (Abelcet; The Liposome, Princeton, N.J.), AMB colloidal dispersion (Amphocil; Seqqus Pharmaceuticals, Menlo Park, Calif.), and liposomal AMB (AmBisome; NeXstar Pharmaceuticals, San Dimas, Calif.).

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TABLE 1. In vitro activities of conventional AMB and its lipid formulations against *Coccidioides* spp.

Compound	MIC (μg/ml) at 48 h		MLC (μg/ml) at 48 h	
	Strain 98–1037	Strain 98–293	Strain 98–1037	Strain 98–293
AMB lipid complex	0.125	0.25	0.5	2
AMB colloidal dispersion	0.06	0.125	0.5	2
Liposomal AMB	0.5	1	1	4
AMB	0.25	0.5	0.25	0.5

Mice. Outbred male ICR mice 4 to 6 weeks old and weighing 25 to 30 g at the start of the study were purchased from Harlan Sprague Dawley Laboratory. Ten mice were randomly assigned to each treatment or control group for each survival and tissue burden study. They were housed five to a cage. Mice were provided food and water ad libitum.

Experimental coccidioidomycosis. A previously described model of systemic coccidioidomycosis was used (6). Mice received an intravenous injection of 200 *Coccidioides* arthroconidia. Quantitative cultures by serial dilution were used to confirm the inoculum size. All the formulations were reconstituted in accordance with the instructions of the manufacturers. Dilutions for intravenous or intraperitoneal administration to mice were prepared in sterile 5% dextrose. The formulations were administered in 0.2-ml volumes three times per week for 2 weeks, which is a complete therapy regimen of six doses. AMB was given at 0.5 mg/kg intravenously and 1and 5 mg/kg/dose intraperitoneally. The lipid formulations were given at 1, 5, and 10 mg/kg/dose intravenously. The control group received 5% dextrose intravenously. Deaths were recorded through 50 days postinfection. Moribund mice were terminated, and their deaths were recorded as occurring on the next day. At the end of the study, survivors were sacrificed by inhalation of Metofane, followed by cervical dislocation. The spleens and livers of the dead mice and the survivors that had been terminated were removed aseptically. The organs were homogenized in 2 ml of sterile saline, and the entire organs were plated onto potato dextrose agar and incubated at 35°C for 1 week.

For the tissue burden study, treatment with the lipid formulations was given at 1 mg/kg/dose intravenously, AMB was given at 0.5 mg/kg/dose intravenously and 1 mg/kg/dose intraperitoneally. The drug regimen was three times per week for 2 weeks. The control group of infected mice received 5% dextrose intravenously. Deaths were recorded through day 24. The livers, spleens, and lungs of dead mice and sacrificed survivors were removed, weighed, and homogenized in 1 to 5 ml of sterile saline, and serial 10-fold dilutions were placed on potato dextrose agar plates and incubated at 35°C for 1 week to determine the number of viable CFU in each organ.

Analysis of data. For survival studies, the log rank and Wilcoxon tests were used. The *P* values for determining significance varied because of correction for multiple comparisons. For the tissue burden studies, Dunnett's two-tailed *t* test was used, and a *P* value of ≤ 0.05 determined the significance of differences compared with controls only.

RESULTS

In vitro susceptibility testing. Table 1 displays the in vitro susceptibility of *Coccidioides* spp. to AMB and its lipid formulations. AMB lipid complex and AMB colloidal dispersion were more active than conventional AMB with regard to the MIC. However, conventional AMB displayed the strongest activity with regard to the MLC. The 48-h *Paecilomyces variotii* control strain MIC and MLC were 0.5 and 1 μg/ml, respectively.

Survival study. The results of the survival study with strains 98-1037 and 98-293 are displayed in Table 2. All mice in the control group (strain 98-1037) died between days 14 and 24. All therapy regimens significantly prolonged survival ($P < 0.0001$) over that in the control group. No differences in survival occurred between drug regimens. The tissue burden of the entire livers and spleens of mice that died or that were obtained on day 50 postinfection for those who survived were determined. Cultures of homogenized organs showed 100% sterilization in both organs when mice were treated with either lipid formulation at 10 mg/kg and 50 to 70% sterilization in both organs when mice were treated with 5 mg/kg. Ten percent sterilization in the spleen and liver was found when AMB colloidal dispersion was administered at 1 mg/kg, but this effect was not observed with either AMB lipid complex or liposomal AMB. AMB showed 20% sterilization at 5-mg/kg doses. Results with strain 98-293 were similar to those obtained with strain 98-1037. However, two deaths occurred 15 min after application of the fourth and fifth dose of AMB at 0.5 mg/kg (intravenous) and 1 mg/kg (intraperitoneal), respectively.

TABLE 2. Survival of mice with systemic coccidioidomycosis treated with different formulations of AMB

Isolate and formulation	Dose (mg/kg)	% Survival	% Sterilization	
			Spleen	Liver
98–1037				
Control	None	0	0	0
AMB lipid complex	1	100	40	0
	5	100	60	50
	10	100	100	100
AMB colloidal dispersion	1	90	50	10
	5	100	80	60
	10	100	100	100
Liposomal AMB	1	100	50	0
	5	100	80	60
	10	100	100	100
AMB	0.5	100	70	30
	1	100	60	10
	5	100	60	20
98–293				
Control	None	10	0	0
AMB lipid complex	1	100	50	0
	5	100	60	50
	10	100	100	100
AMB colloidal dispersion	1	90	60	20
	5	90	80	50
	10	90	100	100
Liposomal AMB	1	90	60	0
	5	100	80	70
	10	100	100	100
AMB	0.5	90	60	30
	1	90	50	10
	5	100	60	30

Tissue burden study. Table 3 shows the results of the quantitative tissue fungal burden test for the spleen, liver, and lungs of mice that died or on day 24 postchallenge for those who survived. The organs in the control group had high numbers of *Coccidioides* CFU per organ.

Comparisons of the *Coccidioides* fungal burden showed that the regimen (1 mg/kg) with the lipid formulations and AMB (0.5 and 1 mg/kg) significantly reduced the burdens over those in the control group ($P \leq 0.05$). However, mice given AMB at 0.5 and 1 mg/kg had a lower *Coccidioides* fungal load in all organs than mice given an equivalent dose of either lipid formulation. There were statistically significant differences in the results of the tissue burden studies between AMB and all of the lipid formulations ($P = 0.003$ to 0.0002)

TABLE 3. Fungal burden in spleens, livers, and lungs of mice infected with 200 arthroconidia of *Coccidioides* sp. strains 98–1037 and 98–293[a]

Treatment (dose)	Mean $\log_{10}$ no. of CFU/organ (no. of animals free of infection) [95% confidence interval]/*P* value[b]					
	Strain 98–1037			Strain 98–293		
	Spleen	Liver	Lungs	Spleen	Liver	Lungs
None (control) lipid complex	6.14 (0) [5.2–6.7]	6.81 (0) [5.5–6.9]	6.11 (0) [4.9–6.8]	6.27 (0) [5.9–6.9]	6.45 (0) [5.3–6.8]	6.91 (0) [5.2–7.2]
AMB (1 mg/kg)	1.47 (3) [0–2.3]/0.003	3.41 (0) [2.2–4.5]/0.0006	3.78 (1) [0.04–4.2]	1.52 (2) [0–2.2]/0.0002	3.33 (0) [2.6–4.1]/0.0002	3.98 (0) [2.7–4.3]/0.0002
AMB colloidal dispersion (1 mg/kg)	1.21 (5) [0–1.8]/0.0006	2.62 (2) [0–4.1]/0.4418	3.15 (4) [0–4.7]	1.04 (4) [0–1.6]/0.0002	2.87 (2) [0.7–3.3]/0.0002	3.01 (4) [0–3.6]/0.0002
Liposomal AMB (1 mg/kg)	1.39 (4) [0–2.1]/0.0006	3.11 (0) [2.5–4.2]/0.0006	3.29 (3) [0.6–3.9]	1.16 (3) [0–2.6]/0.0002	3.07 (0) [2.8–3.9]/0.0002	3.31 (3) [0–4.1]/0.0002
AMB						
0.5 mg/kg intravenous	0.35 (5) [0–0.7]	1.39 (2) [0–3.2]	1.08 (2) [0–2.1]	0.31 (4) [0–0.9]	1.17 (2) [0–3.1]	1.48 (2) [0–2.6]
1 mg/kg intraperitoneal	0.41 (5) [0–0.9]	2.45 (1) [0–3.6]	2.48 (2) [0–2.9]	0.39 (5) [0–2.2]	1.98 (2) [0–2.7]	1.86 (2) [0–2.5]

[a] There were 10 animals per group. Survivors were sacrificed on day 24.
[b] Compared with AMP-treated group (1 mg/kg).

DISCUSSION

AMB remains the therapy of choice for coccidioidomycosis as well as other systemic mycoses, mainly due to its strong antifungal activity and a low tendency for inducing resistance. In order to increase the therapeutic efficacy of AMB and reduce its associated toxicity, lipid-based formulations have been developed and have become available commercially. At present, their use is appropriate in patients following treatment failure with conventional AMB or other systemic antifungals as well as in patients who become intolerant to conventional AMB due to adverse reactions, those who have underlying kidney diseases, or those who receive other nephrotoxic drug.

A 0.5-mg/kg dose of conventional AMB was as effective as a dose of 1 mg/kg in prolonging survival and lowering fungal burden over these values in controls. Conventional AMB administered either intravenously or intraperitoneally was very active in lowering the fungal load, more so than a comparable dose of any of the lipid formulations ($P = 0.003$ to 0.0002).

The three lipid-associated preparations of AMB could be administered at higher doses, and a clear response from 1 to 10 mg/kg showed that all three preparations sterilized tissues at 10 mg/kg, a dose that could not be used for conventional AMB.

The results of our study point to the different lipid formulations of AMB as having utility as a treatment for coccidioidomycosis, since they can be given at higher doses that were well tolerated and achieved sterilization of tissues. Furthermore, within the limitations of this study, we found no significant differences among these preparations. Such differences might be shown with much lower *Coccidioides* inocula. However, the relevance of such low doses to the clinical situation is unclear. Patients with disseminated coccidioidomycosis generally have heavy fungal burdens, and regimens which are effective in that situation are of more practical value. Thus, despite a greater milligram-per-milligram potency in reducing tissue burden, AMB was too toxic to allow administration at a dose as high as 10 mg/kg, a dose which has been lethal to mice in our hands. However, the lipid formulations do not in fact have a maximum tolerated dose and have been administered clinically at doses of up to 15 mg/kg for months. Thus, we believe that any of these preparations, pushed to high doses, is beneficial, and we are not likely be able to show that one has advantages in efficacy over the other.

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