Comparison of the Efficacies of Various Formulations of Amphotericin B against Murine Visceral Leishmaniasis

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The antileishmanial efficacies of four proprietary amphotericin B (AmB) formulations (Fungizone, AmBi-some, Abelcet, and Amphocil) and an experimental nonionic surfactant vesicle (NIV) formulation were compared in a murine model of acute visceral leishmaniasis. By a multiple-dosing regimen, groups of *Leishmania donovani*-infected BALB/c mice were treated (2.5 mg of AmB per kg of body weight) on days 7 to 11 postinfection with one of the AmB formulations, and parasite burdens were determined on day 18 postinfection. All of the formulations caused significant suppression parasite burdens in spleens ($P < 0.01$ to 0.0005) and livers ($P < 0.0005$) compared with those in the spleens and livers of the controls. In addition, a significant suppression of parasite burdens in bone marrow ($P < 0.0005$) compared to the burdens in the bone marrow of the controls was obtained for all the formulations except Abelcet, which was inactive at this site. On the basis of their overall efficacies (activity against liver, spleen, and bone marrow parasites), the formulations could be ranked as follows: Amphocil = AmBisome > AmB-NIV > Abelcet >> Fungizone. On the basis of spectro-photometric measurements, AmB was shown to exist in a predominantly aggregated state in all of the formulations. Although incubation in 50% serum altered the degree of aggregation, the AmB remained predominantly aggregated, indicating that the AMB-lipid complex in all of the formulations was physically stable. The results of the study showed that antiparasitic efficacy is associated positively with the degree of AmB aggregation in the presence of serum.

Amphotericin B (AmB) is a macrolide polyene antibiotic derived from *Streptomyces nodosus* (9). Introduced in 1958, AmB remains the "gold standard" drug of choice for the treatment of systemic fungal infections and has also been used against antimony-resistant visceral leishmaniasis (3). The fungicidal and leishmanicidal activities of AmB are closely related to its unusual chemical structure, which is characterized by the hydrophilic polyhydroxyl and hydrophobic polyene faces on the long axis (approximately 25 Å) of the molecule. The resulting amphipathic properties provide the driving force for various types of self-association of AmB molecules, and in the environment of a cell membrane, a Van der Walls interaction between the hydrophobic polyene faces of AmB and membrane sterols results in the formation of molecular aggregates of AmB intercalated with sterol. The hydrophilic polyhydroxyl faces of AmB are oriented toward the center of the resultant barrell-like aggregate which, by virtue of repulsion between the opposed hydrophilic faces, is in the form of a water-filled pore (19). Membrane barrier function is compromised, with the loss of intracellular contents and ultimately cell death.

The selectivity of AmB is based on its preferential interaction with 24-substituted sterols, such as ergosterol and epis-terol, which are found in fungal and *Leishmania* cells, respectively; however, damage to host cell membranes is common since AmB will also associate with cholesterol. The membrane activity of AmB underlies its chronic toxic effects of anaphylaxis, cardiac arrhythmias, anemias, and nephrotoxicity. Of

these, nephrotoxicity is the most common side effect which limits therapy (20).

Recently, three novel lipid-based AmB formulations (AmBisome, Abelcet, and Amphocil) have been introduced. By modifying the in vivo distribution of AmB, these formulations have lower toxicities than that of the conventional formulation, Fungizone. The deleterious effects of AmB on nontarget membranes are attributable to the formation of micellar aggregates which interact with cholesterol (1, 17) to cause membrane damage. Monomeric AmB is much less toxic but retains its fungicidal activity. It would appear that the lipid-based formulations are less toxic because they release AmB slowly in vivo and suppress the formation of micellar AmB. The clinical advantage of these new formulations is that compared to Fungizone, higher AmB doses can be tolerated. AmBisome (7, 13), Amphocil (10, 11), and Abelcet (22) have been used against human visceral leishmaniasis (VL).

In the present study the antileishmanial activities of four proprietary AmB formulations (Fungizone, AmBisome, Abelcet, and Amphocil) and an experimental nonionic surfactant vesicle (NIV) formulation were compared in an experimental murine model of VL.

MATERIALS AND METHODS

Materials. AmB was obtained from Bristol-Meyers Squibb, Moreton, United Kingdom. Proprietary AmB formulations (AmBisome, Abelcet, Amphocil, and Fungizone) were purchased direct from wholesalers. The nonionic surfactant tetraethylene glycol mono-*n*-hexadecylether was purchased from Chesham Chemicals Ltd., Herrow, United Kingdom. Dicetyl phosphate and ash-free cholesterol were obtained from Sigma, and all other reagents were of analytical grade.

Animals and parasites. Age-matched, 8- to 10-week-old, in-house-inbred female BALB/c mice were used throughout the study. *Leishmania donovani* MHOM/ET/67:LV82 was maintained (4) by serial passage through in-house-bred Golden Syrian hamsters (*Mesocricetus auratus*). Mice were infected by

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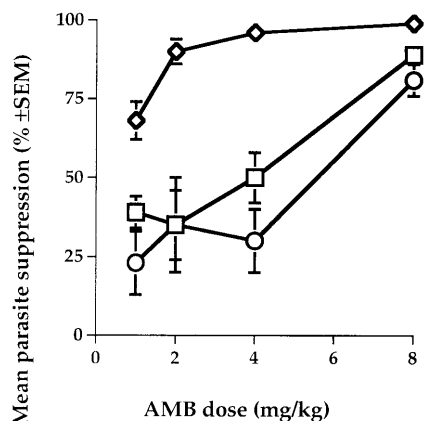
DISCUSSION

If it is assumed that the antiparasitic activities of these AmB formulations depend on the delivery of the drug to the infected phagocytes of the mononuclear phagocytic system (MPS), the efficacy ranking Amphocil = AmBisome > AmB-NIV > Abelcet represents the ability of the different formulations to deliver AmB to the parasites.

It must also be assumed that these formulations are transported via the systemic circulation from the site of administration (tail vein) to the infected target tissues of the MPS. It is apparent that efficacy differences among these particulate formulations is dependent on the behaviors of the particles en route to the MPS. Thus, low efficacy would be the result of partial loss of the the AmB payload due to exchange interactions with blood lipoprotein. Similarly, uptake by non-MPS tissue such as the large capillary beds of the lung, which the particles must traverse before they gain access to the arterial vessels leading to the MPS, would compromise efficacy.

The antileishmanial activities of the AmB formulations were similar to those of various sodium stibogluconate formulations (4–6) in that liver parasites were most susceptible and bone marrow parasites were the least susceptible to drug therapy. It is well established (21) that the uptake of vesicular systems by the various parts of the of the MPS (spleen, liver, and bone marrow) is a function of particle size. The low activity of Abelcet against bone marrow parasites is a consequence of its large particle size (2,196 $\pm$ 138.6 nm) which favors uptake by the liver and spleen at the expense of uptake by the bone marrow. Although AmB-NIV, AmBisome, and Amphocil suspensions had comparable sizes, the activity of AmB-NIV in the spleen and liver was significantly lower than those of the other two formulations, yet in bone marrow all three were equally active. This suggests that differences in AmB entrapment or in the composition of the lipid complex, or both, also influence antileishmanial activity.

The results of this study suggest that antiparasitic efficacy is also associated with a high A_{348}/A_{409} ratio since both Amphocil and AmBisome, which were the most effective formulations, had the highest absorbance ratios in both dextrose solution and serum. The high A_{348}/A_{409} ratio observed for all of the formulations was to be expected since AmB in vesicles, discs, micelles, and ribbons is aggregated. However, the significance of the magnitude of the A_{348}/A_{409} ratio after incubation in serum is as a predictor of the strength of the association between AmB and the lipid components of a formulation in vivo. A stable AmB-lipid complex would be more likely to resist opsonic challenge in the systemic circulation and deliver the AmB to the MPS. Although all of the lipid formulations were susceptible to challenge with serum, the major part of the AmB remained aggregated.

A_{348}/A_{409} values for the commercially available formulations are unavailable in the literature. However, from spectra provided by Janoff et al. (14) for an experimental Abelcet-like formulation, an A_{348}/A_{409} ratio of 5.9 can be calculated. Although this is higher than the value found in this study, it confirms the aggregated state of AmB. The difference between the values for Abelcet and the values for the experimental formulation of Janoff et al. (14) may be attributable to processing differences, which have been shown to affect the AmB aggregation state (14).

Available pharmacokinetic data on the proprietary formulations of AmB indicate that each has a different maximum concentration in serum, half-life, clearance, and volume of distribution (15). Dose-dependent variations in pharmacokinetic parameters occur due to the nonlinear behavior of AmB

at doses higher than 50 mg (9). Janknegt et al. (15) reported that the area under the plasma concentration-versus-time curve (AUC) after administration of the same AmB dose was highest for AmBisome, while Amphocil and Abelcet had two- and fivefold lower AUCs, respectively. The low antiparasitic activity of Abelcet is not at variance with these observations. However, in the present study AmBisome and Amphocil had comparable antiparasitic activities, despite the twofold difference in their AUCs (15).

With a model of acute VL in BALB/c mice, Gangneux et al. (12) showed that AmBisome and Abelcet formulations (up to 12 mg of AmB/kg) were better tolerated than Fungizone or AmB emulsified with 20% Intralipid (0.8 to 1.2 mg of AmB/kg). Six 12-mg/kg doses of AmB as AmBisome or Abelcet (total cumulative dose, 72 mg of AmB/kg) eradicated *Leishmania infantum* from the lungs, liver, and spleen, an effect that was maintained throughout the 14-week follow-up study. In the present study, the lower cumulative dose of 12.5 mg of AmB/kg in the form of AmBisome and Amphocil significantly suppressed liver (99.5% $\pm$ 0.2% and 100%, respectively) and splenic (86% $\pm$ 3% and 96% $\pm$ 2%, respectively) *L. donovani* burdens, indicating that parasite eradication occurs at lower cumulative doses than those used by Gangneux et al. (12). Unfortunately Gangneux et al. (12) did not examine bone marrow burdens, which precludes further comparison, but the fact that the liver was not recolonized at 14 weeks posttreatment suggests that the parasites in bone marrow were suppressed.

The future role of AmB-lipid formulations in the treatment of *Leishmania* infections will remain uncertain until the cost-effectiveness of such treatments can be established. At present, on a drug weight basis, the three AmB-lipid formulations are some 22 to 74 times more expensive than Fungizone. In a single-center study, AmBisome was shown to be as cost-effective as Fungizone in the treatment of systemic mycoses in liver transplant patients (23). No such data exist in the literature for any of the other formulations in the treatment of *Leishmania* infection. However, given the cost of AmB-lipid formulations, they are unlikely to become standard antileishmanial agents in the developing countries. In Western Europe, liposomal AmB is of proven value against VL in human immunodeficiency virus-infected individuals (16, 24), although relapses following apparently successful treatment have been observed (8).

In summary, the results of this study indicated that Amphocil and AmBisome are equally effective and, of the formulations tested, are the most active against *L. donovani* in the murine model of acute VL. Recent studies on the clinical use of these types of AmB formulation indicate, however, that in humans, Abelcet has good antileishmanial activity. It should be stressed that the present study considered only one dosing regimen in a murine model of acute VL and did not take into account the maximum tolerated AmB dose for each formulation and the various alternative dosage schedules, which could have a profound effect on efficacy. In addition, Amphocil and Abelcet are, at present, significantly cheaper than AmBisome, and cost-effectiveness is an important consideration. Studies with both humans and animals confirm the superiority of the lipid formulations of AmB over Fungizone and emphasize the importance of efficient drug delivery in therapy for VL.

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