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Distribution of Lipid Formulations of Amphotericin B into Bone Marrow and Fat Tissue in Rabbits

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The distribution of the three currently available lipid formulations of amphotericin B (AmB) into bone marrow and fat tissue was evaluated in noninfected rabbits. Groups of four animals each received either 1 mg of AmB deoxycholate (D-AmB) per kg of body weight per day or 5 mg of AmB colloidal dispersion, AmB lipid complex, or liposomal AmB per kg per day for seven doses. Plasma, bone marrow, fat, and liver were collected at autopsy, and AmB concentrations were determined by high-performance liquid chromatography. At the investigated dosages of 5 mg/kg/day, all AmB lipid formulations achieved at least fourfold-higher concentrations in bone marrow than did standard D-AmB at a dosage of 1 mg/kg/day. Concentrations in bone marrow were 62 to 76% of concurrent AmB concentrations in the liver. In contrast, all AmB formulations accumulated comparatively poorly in fat tissue. The results of this study show that high concentrations of AmB can be achieved in the bone marrow after administration of lipid formulations, suggesting their particular usefulness against disseminated fungal infections involving the bone marrow and against visceral leishmaniasis.

Three lipid formulations of amphotericin B (AmB) are available for use in North America and Western Europe. These compounds offer a therapeutic alternative to decrease nephrotoxicity and infusion-related reactions while maintaining the efficacy of AmB deoxycholate (D-AmB) (10, 11).

Several investigators have described the pharmacokinetics and organ distribution of a single lipid formulation versus conventional AmB (5, 13, 16, 21). A head-to-head comparison of the central nervous system disposition of all four AmB formulations has been reported recently (A. Groll, N. Giri, C. Gonzales, T. Sein, J. Bacher, S. Piscitelli, and T. J. Walsh, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-90, p. 19, 1997). However, the distribution of the lipid formulations into bone marrow has not been addressed yet. This tissue compartment is important since it is a site of infection for fungal diseases, especially disseminated histoplasmosis (20, 22). The bone marrow also is a site of infection in visceral leishmaniasis (2, 14), for which AmB has evolved into an important therapeutic modality (2, 7, 14).

The mononuclear phagocytic system (MPS) assumes a pivotal role in the tissue uptake and tissue distribution of the lipid formulations (9, 15). The bone marrow, however, is rich not only in mononuclear phagocytes but also in fat cells. Whether encapsulation of AmB into a lipid formulation leads to enhanced concentrations in fat tissues is not known. We therefore investigated the distribution of all currently available AmB formulations into the bone marrow, using adipose tissue as a control for non-MPS fatty tissue and liver tissue to serve as a control for MPS-rich, nonfatty tissue.

Experimental design. Four groups of four rabbits each were studied. Each group was administered either D-AmB (dosage, 1 mg of AmB per kg of body weight per day) at 0.4 mg/min or one of three lipid formulations (dosage, 5 mg of AmB per kg/day) at 1.2 mg/min once daily for a total of seven doses. Dosage selection of the lipid formulations was based on results from previous infection models with rabbits that have demonstrated equivalent to superior activity in comparison to conventional AmB at 5 but not at 1 mg/kg/day (1, 8) (J. W. Lee, M. Allende, P. Francis, J. Peter, V. Thomas, A. Francesconi, C. A. Lyman, P. A. Pizzo, and T. J. Walsh, Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 579, 1991). Animals were sacrificed 30 min after the last dose by intravenous pentobarbital anesthesia. Immediately prior to euthanasia, a plasma sample was obtained. Perirenal fat, bone marrow (femur), and liver were collected at autopsy and stored at −80°C until assay of drug concentrations.

Animals. Female New Zealand White rabbits (Hazleton, Denver, Pa.) weighing 2.5 to 3.5 kg were used in all experiments. They were housed and maintained according to National Institutes of Health guidelines for laboratory animal care and in fulfillment of American Association for Accreditation of Laboratory Animal Care criteria (6). Vascular access was established in each rabbit by the placement of a subcutaneous silastic central venous catheter (19).

Antifungal therapy. D-AmB (Fungizone; 50-mg vials; Bristol-Myers Squibb, Princeton, N.J.) was reconstituted with 10 ml of distilled water, maintained at 4°C, and diluted 1:4 (vol/vol) with sterile 5% dextrose in water immediately before use to a 1-mg/ml concentration. AmB colloidal dispersion (ABCD or Amphotec; Sequus Pharmaceuticals, Menlo Park, Calif.) was provided as lyophilized sterile powder (100 mg/vial). Prior to use, the powder was dissolved in 20 ml of sterile water and then further diluted with 5% dextrose in sterile H_2O (D5W) to a final concentration of 1 mg/ml. AmB lipid complex (ABLC or Abelcet; The Liposome Company, Princeton, N.J.) was provided as a 5-mg/ml solution in 20-ml vials and further diluted to a 1-mg/ml solution with D5W prior to use. Liposomal AmB (L-AmB or AmBisome; Fujisawa USA, Deerfield, Ill.) was prepared from lyophilized powder. The powder was reconstituted initially with 12 ml of sterile water to a 4-mg/ml solution. This solution was then heated to 60°C for 10 min, filtered through a 5-μm-pore-size filter, further diluted with D5W to a

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TABLE 1. Concentrations of AmB in bone marrow, liver, perirenal fat, and plasma 30 min after the seventh dose of either D-AmB, ABCD, ABLC, or L-AmB

Drug	Concn in tissue or fluid[a]:			
	Marrow* (μg/g)	Liver* (μg/g)	Fat** (μg/g)	Plasma (μg/ml)
D-AmB	8.0 ± 1.7 (5.7)	26.9 ± 4.8 (19.2)	1.2 ± 0.3 (0.86)	1.4 ± 0.3
ABCD	53.1 ± 21.5 (54.7)	69.5 ± 22 (71.6)	1.1 ± 0.24 (1.3)	0.97 ± 0.08
ABLC	35.4 ± 12.7 (42.1)	57.9 ± 5.3 (68.9)	2.1 ± 1.3 (2.5)	0.84 ± 0.10
L-AmB	39.5 ± 4.7 (0.66)	59.8 ± 6.9 (1.0)	8.9 ± 1.9 (0.15)	59.5 ± 1.8

[a] All values are given as means ± standard deviations. Values in parentheses are the tissue/plasma ratios. *, $P < 0.05$ for the comparison between D-AmB and ABCD, ABLC, and L-AmB; **, $P < 0.05$ for the comparison between L-AmB and D-AmB, ABCD, and ABLC (Mann-Whitney U test).

final concentration of 2 mg/ml, and administered at ambient temperature.

Analytical methods. AmB concentrations in plasma and tissues were determined as total unassociated (free) AmB after methanol extraction by an internally validated reversed-phase high-performance liquid chromatographic method (4). In order to reduce contamination with blood, all solid-tissue specimens were thoroughly rinsed prior to homogenization with phosphate-buffered saline and blotted to dryness with Micro Wipes (Scott Paper Company, Philadelphia, Pa.). The mobile phase consisted of methanol–acetonitrile–0.0025 M Na-EDTA (500:350:200; all provided by Fisher Scientific, Fair Lawn, N.J.), delivered at 1.6 ml/min. The injection volume was 100 μl. AmB was detected by UV absorbance at 382 nm using a C_{18} analytical column (Waters, Milford, Mass.) in conjunction with a new Guard C_{18} in-line precolumn filter (Perkin-Elmer, Norwalk, Conn.). Quantification was based on the peak area-concentration response of the external calibration standard. Six-point standard curves, prepared in drug-free plasma, bone marrow, or tissue homogenates, were linear with r^2 values of greater than 0.99. Inter- and intraday variability (precision) was ≤7.5%, and accuracies were within 12% for all matrixes. The lower limit of quantitation was 0.04 μg/ml in plasma.

Results and discussion. Concentrations of AmB in bone marrow and, for comparison, plasma, fat tissue, and liver 30 min after the last of seven daily doses are shown in Table 1. At the investigated dosage of 5 mg/kg/day, all lipid formulations achieved at least fourfold-higher concentrations in bone marrow than did conventional AmB administered at the standard dosage of 1 mg/kg/day. Similarly, concentrations in liver tissue were approximately twofold higher with the lipid formulations. ABCD demonstrated the greatest degree of distribution into bone marrow with a tissue-to-blood ratio of 54.7 and the highest absolute mean concentration (53.1 μg/g). Compared to that in bone marrow and liver, accumulation in fat tissue was relatively poor, with L-AmB achieving the highest absolute concentrations.

Incorporation of AmB into novel, biodegradable amphiphilic lipid carriers greatly reduces nephrotoxicity, thus allowing for the safe administration of therapeutically effective dosages of this important antifungal agent (10, 11). However, the detailed distribution of these lipid formulations has not been completely elucidated, and comparative data describing how formulation alters tissue penetration are limited. After intravenous administration, AmB incorporated into lipid structures is thought to be preferentially taken up by organs of the MPS (15), but their circulation in the bloodstream varies according to physiochemical properties of the vehicle such as lipid composition, particle size, and charge. In comparison to D-AmB, both ABCD and ABLC are more rapidly cleared from the bloodstream and achieve lower peak concentrations and areas under the concentration-time curve in plasma; in contrast, L-AmB is more slowly taken up by the MPS, has a longer circulation half-life, and achieves strikingly high peak concentrations and areas under the concentration-time curve (3, 9). However, whether and how these distinct pharmacokinetic characteristics translate into different pharmacodynamic properties in vivo are yet largely unknown.

Multiple dosing of all four AmB formulations achieved concentrations in bone marrow that exceeded the MICs for most clinically relevant fungal pathogens severalfold. With the exception of D-AmB, concentrations in bone marrow were very similar to concomitant concentrations in liver tissue, whereas concentrations in fat tissue were generally low. These findings suggest that cells of the MPS were responsible for the preferential distribution of the lipid formulations into bone marrow.

Uptake of lipid-formulated AmB in non-MPS cells is not well elucidated. Several mechanisms have been proposed including endocytosis, absorption, fusion, and exchange of lipids with the target cell (3, 9). Based on the data from this study, it does not appear that incorporation of AmB into a lipid moiety specifically increases the uptake into fat tissue. Whether the comparatively higher fat tissue concentrations after administration of L-AmB represented truly tissue-bound drug or were due to high drug concentrations in the microcirculation remains open. We cautiously suggest that dosing in obese patients may be based on lean body weight plus a factor accounting for the expanded blood volume in these patients. This approach would be analogous to that proposed for aminoglycoside dosing in obesity (12, 17, 18).

Animal models offer an attractive alternative to examine tissue distribution into sites which are difficult to sample in humans. The findings of this study suggest that high concentrations of AmB can be achieved in the bone marrow after administration in lipid formulations at the dosages used in this study. This would predict that lipid formulations of AmB would be particularly active against disseminated fungal infections involving the bone marrow and against visceral leishmaniasis. At the same time, the potential impact of such enhanced marrow concentrations on hematopoietic functions remains to be elucidated.

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