ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1989, p. 1544–1548
0066-4804/89/091544-05$02.00/0

Vol. 33, No. 9

Therapeutic Evaluation of Free and Liposome-Encapsulated Amphotericin B in the Treatment of Systemic Candidiasis in Mice

JAVED A. GONDAL,[1] RODNEY P. SWARTZ,[2] AND AQUILUR RAHMAN[1]*

Divisions of Medical Oncology[1] and Pulmonary and Critical Care Medicine,[2] Department of Medicine, Georgetown University Medical Center, 3800 Reservoir Road, N.W., Washington, D.C. 20007

Received 21 February 1989/Accepted 19 June 1989

Various doses of amphotericin B encapsulated into unilamellar vesicles of 0.1 μm diameter (lip-AMB) (1.0 to 20.0 mg/kg of body weight) were compared with free amphotericin B (AMB) (0.5 to 2.0 mg/kg of body weight) in a murine model of disseminated candidiasis. CD_2F_1 mice injected intravenously with 3×10^5 *Candida albicans* cells were treated with either single- or multiple-dose regimens. Untreated infected mice had a median survival of 7 days, with all mice dead by 12 days. Single doses of AMB resulted in a median survival range from 18 to 23.5 days, with ≤38% survival by day 42. Single doses of lip-AMB resulted in 88 to 100% survival by day 42. The multiple-dose AMB regimen provided median survival of only 30 to 33 days, with ≤38% survival by day 42. The multiple-dose lip-AMB regimen resulted in >90% survival by day 42. With single-dose regimens, lip-AMB levels in plasma were severalfold higher than AMB levels in plasma. By 10 h, at equivalent doses, lip-AMB levels in plasma were much higher, whereas AMB levels in plasma were not detectable. Compared with normal values, the blood urea nitrogen, serum glutamic pyruvic transaminase, serum glutamic oxaloacetate transaminase, and serum lactate dehydrogenase levels were not significantly altered by high doses of lip-AMB treatment. Viable *C. albicans* was recoverable from the kidneys of some of the lip-AMB-treated mice at day 42. Thus, encapsulation into unilamellar liposomes enhances the antifungal efficacy of amphotericin B while reducing the toxicity normally associated with administration of free amphotericin B.

Disseminated fungal infections remain a leading cause of mortality in patients with leukemia, lymphoma, and immunodeficiency diseases, with *Candida albicans* being a major cause of fatal infections in cancer patients (2, 7, 19). Amphotericin B (AMB) (a polyene antifungal antibiotic produced by *Streptomyces nodosus*) has been shown to be a most effective drug for the treatment of candidiasis (2, 7). However, its clinical use is limited by its side effects (4, 24) and its ineffectiveness in treating fungal infections in neutropenic and immunodeficient patients (19).

In recent years, workers in several laboratories reduced the toxicity of AMB by encapsulating it into multilamellar liposomes (6). Using this technique in animal models, they have demonstrated marked reduction in the acute lethality of AMB, resulting in improved survival of mice with a variety of fungal infections, such as histoplasmosis (21), cryptococcosis (6), and candidiasis (11, 12). Tremblay et al. (22) have shown that the acute dose resulting in 50% lethality of liposome-encapsulated amphotericin B (lip-AMB) given as an intravenous (i.v.) bolus to mice was 11.8 mg/kg of body weight, as opposed to a maximum of 2.3 mg/kg for free AMB.

However, more extensive examination of the effects of lip-AMB are necessary in order to determine the proper regimen of treatment and the resulting side effects on the host with disseminated candidiasis. Therefore, the present study investigated (i) the effects of various doses and the schedule dependency of a new preparation of lip-AMB for therapeutic efficacy as well as toxicity, (ii) the plasma pharmacokinetics and tissue distribution of lip-AMB and AMB, and (iii) whether the lip-AMB resulted in eradication of the infection.

* Corresponding author.

MATERIALS AND METHODS

Animals. Male CD_2F_1 mice, 8 to 10 weeks old, weighing 20 to 25 g, were purchased from Charles River Breeding Laboratories, Inc. (Boston, Mass.). The mice were maintained according to accredited procedures at the Georgetown University animal house facility and fed Purina mouse chow and water ad libitum.

Organism. A clinical isolate of *C. albicans*, strain 410, was obtained from the Microbiological Laboratories at the Georgetown University Hospital and maintained on Sabouraud dextrose agar (Emmons, Lenexa, Kan.) at 37°C and 5% CO_2. Fresh 18-h (logarithmic phase) cultures were suspended in Hanks balanced salt solution (HBSS), pH 7.4, washed, enumerated on a hemacytometer, and adjusted to 7.0×10^5 per ml. Mice were infected on day 0 via the tail vein with 0.5 ml of the suspension (3.5×10^5 *C. albicans*) per mouse, resulting in an mean time to death of 7 days. Control mice received 0.5 ml of HBSS as a vehicle control.

Drug. AMB was a clinical preparation, Fungizone (Squibb, Princeton, N.J.). AMB used in liposome encapsulation was provided by LyphoMed, Inc., Rosemont, Ill. The preparation and lyophilization of liposomes were done at Vestar Inc. (San Demas, Calif.). For liposome encapsulation, 2.5 mg of the drug was dissolved in chloroform-methanol (1:1) solvent and mixed with 4.2 mg of distearoyl phosphatidylglycerol. The mixture was evaporated to dryness under N_2, followed by the addition of 10.6 mg of hydrogenated phosphatidylcholine and 2.6 mg of cholesterol in chloroform solution. The solution was vortexed well and centrifuged, and the resulting supernatant was evaporated to dryness to produce a yellow powder. The powder was then reconstituted with sodium succinate buffer and glucose solution and homogenized to produce small unilamellar liposomes. These liposomes were filtered through membranes (0.2-μm pore size) for sterilization, poured into vials,

and subsequently lyophilized. The lyophilized powder was reconstituted with sterile water supplemented with 5% glucose as needed. The liposomes were sized with a Coulter Nanosizer before and after lyophilization and were found to be less than 0.1 μm in size.

Liposome preparations were tested for the presence of contaminating endotoxin by the *Limulus* amebocyte lysate assay (Sigma). Endotoxin levels in all preparations were ≤0.1 ng/ml.

Treatment of infected mice. Mice were infected on day 0, i.v., via the tail vein with 3.5×10^5 *C. albicans*. On day 2 or 3, depending on the treatment, they were randomly divided into groups to receive various doses of either AMB or lip-AMB, i.v., via the tail vein. In a multiple-dose study, AMB and lip-AMB were administered i.v. via the tail vein on days 3, 6, and 9. Randomly chosen control mice were given i.v. HBSS or "empty" liposomes (liposomes without AMB). The injection volume was 2% of body weight (0.2 ml/10 g). Survival was recorded every 24 h. Animals were evaluated until day 42.

***C. albicans* in tissues.** Representative mice from each treatment group were sacrificed on day 42. Blood samples (orbital sinus), kidneys, liver, and spleen from these mice were aseptically removed, weighed, and homogenized in saline. Homogenates (100 μl) were plated onto Sabouraud dextrose agar plates. Colonies were counted after incubation at 37°C for 48 h.

HPLC. Extraction and quantification of AMB were performed by modification of techniques described by Granick et al. (5), which resulted in increased sensitivity (0.01 μg/ml). High-pressure liquid chromatography (HPLC) (Waters Associates, Milford, Mass.) consisted of M6000A and M45 pumps connected to a model 720 system controller. The A_{386} was monitored with a model 450 double-wavelength UV detector. Separation was performed by injecting 100 μl of sample onto a μBondapak C_{18} reverse-phase column (Waters; 3.9 mm by 30 cm). The mobile phase was a mixture of acetonitrile and 0.02 M EDTA (pH 4.5) (45:55, vol/vol) delivered at 1.0 ml/min.

Standard curve. A standard curve was used to calculate the drug concentration in plasma. Standard solutions were prepared from 5-mg/ml stock solutions of AMB or an internal standard (1-amino-4-nitronaphthalene). Reference samples of 0.5, 1.0, and 1.5 μg of AMB per ml of methanol containing 1 μg of internal standard per ml in dimethyl sulfoxide-methanol (1:1) were prepared with control (AMB-free) mouse plasma (2:8, vol/vol). Plasma samples (0.2 ml) were mixed with 0.8 ml of methanol, incubated at 4°C for 20 min, and centrifuged at 4°C, 2,000 × *g*, for 15 min in microcentrifuge tubes. The supernatant was collected and quantitated by the HPLC procedures described above.

Pharmacologic studies. Normal mice were injected i.v. via the tail vein with either AMB (1 mg/kg of body weight) or lip-AMB (1 or 5 mg/kg). At specific time points, blood from three mice from each group was obtained by retroorbital exsanguination after induction of anesthesia. The blood was collected into heparinized tubes (10 U/ml), placed on ice, and centrifuged at 1,500 × *g* for 10 min to isolate the plasma. The liver, kidneys, spleen, lung, heart, and brains were collected. All plasma and tissues were frozen at −70°C until extraction and measurement of the drug by HPLC.

A standard curve for drug concentration in tissue was prepared by using tissues taken from control mice spiked with AMB and internal standard at 1 μg/ml each. These tissue standards were extracted, and AMB levels were calculated by HPLC. The concentration of AMB or lip-AMB in the various organs was determined by a slight modification of the method of Nilsson et al. (16) (see HPLC section above). Each organ was homogenized in 3 volumes of methanol with a Polytron homogenizer (Brinkman, Westbury, N.J.). The homogenate was centrifuged at 4°C, 2,000 × *g*, for 15 min in 1.5-ml microcentrifuge tubes. The supernatant was collected and stored at −70°C until quantitation by HPLC.

Toxicology studies. Serum biochemical evaluations were performed on mice sacrificed on day 42 following injection with *Candida* cells and various treatment schedules with either AMB or LIP-AMB. Blood urea nitrogen, serum glutamic pyruvic transaminase, serum glutamic oxaloacetate transaminase, and serum lactate dehydrogenase levels were determined at the clinical laboratories of the Georgetown University Hospital by established methods.

Statistical analysis. All experiments were repeated at least three times, and representative results are presented. The significance of the difference between means was determined by analysis of variance (3).

RESULTS

The effect of AMB or lip-AMB on the survival of mice infected with *C. albicans* and treated with single or multiple doses is illustrated in Tables 1 and 2. Untreated infected mice (HBSS controls) had a median survival time of 7 days, with 100% dead by day 12.

Mice treated on day 2 (Table 1) with AMB had a median survival time ranging from 18 days for the 1.0- and 1.5-mg/kg doses to 23.5 days for the 0.5-mg/kg dose. Survival to day 42 ranged from 12.5% (1.0 and 1.5 mg/kg) to 37.5% (0.5 mg/kg). In contrast, the lip-AMB group median survival was 34.5 days, with a 37.5% survival rate for the 1.0-mg/kg dose and ≥75% survival to day 42 for all other doses.

Mice treated on day 3 (Table 1) with AMB had a median survival ranging from 8.5 days (1.5 mg/kg) to 18 days (1.0 mg/kg). There were no survivors at day 42 in any of the groups treated with AMB. The lip-AMB median survival was 7 days (1.0 mg/kg), 26 days (2.5 mg/kg), and 42 days (5.0 and 20.0 mg/kg). Survival to day 42 varied from 0% (1 mg/kg) to 100% (10.0 and 15.0 mg/kg). The doses of AMB (1.5 mg/kg) and lip-AMB (20 mg/kg) used were nontoxic in normal, noninfected mice.

Data for mice treated with multiple doses of AMB on days 3, 6, and 9 following infection are illustrated in Table 2. Median survival was 33 days for the 1.0-mg/kg dose, with 37.5% surviving to day 42. Median survival was 30 days for the 1.5-mg/kg dose, with 25% surviving to day 42. In contrast, survival to day 42 was ≥92% for the 5.0- and 6.5-mg/kg doses and 100% for the 8.5-mg/kg dose of lip-AMB.

At day 42, representative mice from each treatment group were killed, and several tissues were assayed for the presence of viable *C. albicans*. No viable colonies of *C. albicans* were detectable in the blood, liver, or spleen of infected mice in either the AMB or lip-AMB single- or multiple-dose treatment group. The number of mice that had viable *C. albicans* recovered from their kidneys is shown in Tables 1 and 2. In the single-dose, day 2 regimen, the kidneys of mice treated with AMB at 0.5 mg/kg had viable *C. albicans* present, but *C. albicans* was not detectable in the kidneys of mice treated with higher doses of AMB (1.0, 1.5, or 2.0 mg/kg). Viable *C. albicans* was recovered from the kidneys of mice treated with 1.0, 2.5, 5.0, and 10.0 mg of lip-AMB per kg but not in the kidneys of mice treated with 7.5, 15, or

TABLE 1. Effect of single doses of AMB or lip-AMB on survival of CD_2F_1 mice infected with *C. albicans*[a]

Group	Dose (mg/kg)	Day 2 treatment			Day 3 treatment		
		Median survival (days)	No. of survivors by day 42	*Candida* outgrowth in kidney (no. positive/no. tested)	Median survival (days)	No. of survivors by day 42	*Candida* outgrowth in kidney (no. positive/no. tested)
Control		7.0	0		7.0	0	ND[b]
AMB	0.5	23.5	3	2/3	11.5	0	ND
	1.0	18.0	1	0/1	18.0	0	ND
	1.5	18.0	1	0/1	8.5	0	ND
	2.0	21.5	2	0/2	ND	ND	ND
Lip-AMB	1.0	34.5	3	3/3	7.0	0	
	2.5	—[c]	7*†	2/3	26.0	3	3/3
	5.0	—	7*†	1/3	42.0	4*†	3/3
	7.5	—	7*†	0/3	—	6*†	0/3
	10.0	—	8*†	1/3	—	8*†	3/3
	15.0	—	6*†	0/3	—	8*†	2/3
	20.0	—	8*†	0/3	42.0	4*†	1/3

[a] CD_2F_1 mice (eight mice per group) were injected with 3.5×10^5 *C. albicans*, i.v., via the tail vein. On day 2 or 3 postinfection, mice were treated i.v. via the tail vein with either HBSS (control), AMB, or lip-AMB. Symbols: *, $P < 0.02$ versus control; †, $P < 0.02$ versus AMB at 0.5 mg/kg.
[b] ND, Not determined.
[c] —, >42 days.

20 mg of lip-AMB per kg. In the single-dose, day 3 regimen, viable *C. albicans* was recovered from the kidneys of mice in each lip-AMB group except the 7.5-mg/kg dose group. When multiple doses were used, viable *C. albicans* was recovered from the kidneys of both AMB- and lip-AMB-treated mice. When infected control mice (no AMB) were examined on day 7 in a separate experiment, viable *C. albicans* was recovered from the blood, liver, spleen, and kidneys.

The maximum tolerated dose of AMB was 2.0 mg/kg, and the 50% lethal dose was 2.5 mg/kg, causing acute toxic reactions leading to severe loss of weight, sickness, and death of the mice. Surviving mice, treated with lower doses of AMB, examined at day 42 showed no weight loss but did show acute nephrotoxicity in the form of nephrocalcinosis, kidney atrophy, and diffuse interstitial edema.

Figure 1 indicates the plasma pharmacokinetics of AMB (1 mg/kg) and lip-AMB (1 or 5 mg/kg). Following i.v. administration, AMB levels in plasma peaked (1.5 μg/ml) within 5 min, diminishing below detectable levels (<0.01 μg/ml) within 12 h. Lip-AMB levels in plasma peaked (8 μg/ml for 1 mg/kg, 50 μg/ml for 5 mg/kg) within 5 min and diminished very slowly thereafter. It appears that approximately 80 to 90% of the lip-AMB administered circulated in plasma for at least 1 h. The value at 24 h with the 1-mg/kg dose was 0.3 μg/ml, and with the 5-mg/kg dose it was 15 μg/ml. The enhanced level in plasma may in part be responsible for

TABLE 2. Effect of multiple doses of AMB or lip-AMB on survival of CD_2F_1 mice infected with *C. albicans*[a]

Group	Dose (mg/kg)	Median survival (days)	No. of survivors at day 42/no. in group	*Candida* outgrowth in kidney (no. positive/no. tested)
Control		7.0	0/8	
AMB	1.0	33.0	3/8	2/3
	1.5	30.0	2/8	3/3
Lip-AMB	5.0	—[b]	12/13*†‡	2/3
	6.5	—	12/13*†‡	2/3
	8.5	—	13/13*†‡	1/3

[a] CD_2F_1 mice were injected with 3.5×10^5 *C. albicans*, i.v., via the tail vein. At 3, 6, and 9 days postinfection, mice were treated i.v. via the tail vein with either HBSS (control), AMB, or lip-AMB. Symbols: *, $P < 0.02$ versus control; †, $P < 0.02$ versus AMB at 1.0 mg/kg; ‡, $P < 0.01$ versus AMB at 1.5 mg/kg.
[b] —, >42 days.

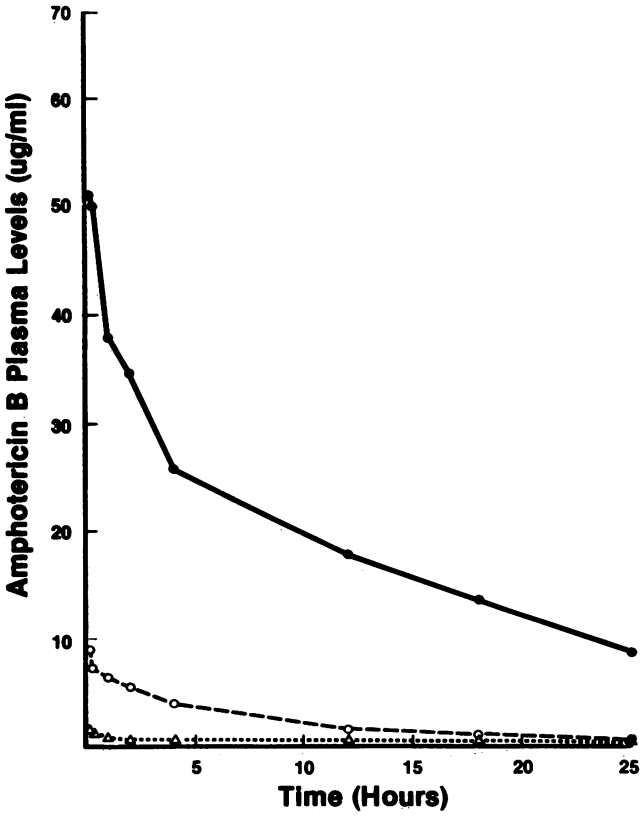

FIG. 1. Plasma pharmacokinetics of AMB and lip-AMB. Mice were injected i.v. with either AMB (1 mg/kg of body weight) (△), lip-AMB (1 mg/kg) (○), or lip-AMB (5 mg/kg) (●). Blood was collected by retroorbital exsanquination at intervals following injection, and levels of AMB in plasma were evaluated by HPLC. Values are means for three determinations.

TABLE 3. Organ concentration of AMB after i.v. injection of AMB and lip-AMB into normal mice[a]

Time postinjection (h)	AMB concn (μg/g)																	
	Liver			Spleen			Kidney			Brain			Heart			Lung		
	AMB	Lip-AMB		AMB	Lip-AMB		AMB	Lip-AMB		AMB	Lip-AMB		AMB	Lip-AMB		AMB	Lip-AMB	
		1	5		1	5		1	5		1	5		1	5		1	5
0.08	2.7	2.8	6.5	3.0	3.0	6.0	1.8	1.5	3.5	ND[b]	ND	0.03	0.02	ND	0.9	1.8	3.3	18.5
0.25	2.7	3.6	8.5	3.4	5.7	9.8	1.2	1.2	3.6	ND	ND	0.03	ND	ND	1.0	2.7	7.6	13.6
1	2.2	3.8	13.3	4.2	8.0	16.6	0.68	0.90	2.7	ND	ND	0.45	ND	ND	1.2	3.0	3.6	13.0
2	2.0	4.4	14.5	5.3	8.4	22.7	0.45	0.87	1.8	ND	ND	0.61	ND	ND	0.44	2.5	3.3	13.4
4	1.5	5.4	20.2	1.96	8.3	11.2	0.40	0.77	1.2	ND	ND	0.32	ND	ND	0.03	2.6	2.5	9.4
12	1.0	5.4	25.7	0.12	5.6	9.0	0.03	0.50	0.8	ND	ND	0.04	ND	ND	0.03	0.09	1.2	8.4
24	0.9	4.4	29.7	ND	4.2	6.6	0.03	0.50	0.7	ND	ND	0.01	ND	ND	0.02	0.06	0.09	3.4
48	ND	3.8	21.2	ND	2.1	5.3	ND	0.54	0.6	ND	ND	ND	ND	ND	ND	ND	ND	3.1

[a] Values are means for three mice. The AMB dose was 1 mg/kg, and the lip-AMB dose was either 1 or 5 mg/kg.
[b] ND, Not detectable.

higher therapeutic activity. From 0.5 to 4 h, the clearance rate was greater with AMB, with drug levels reaching negligible amounts by 24 h. The half-lives at α and β phase ($t_{1/2\alpha}$ and $t_{1/2\beta}$) in plasma of the 1-mg/kg dose of AMB were 36 min and 11 h, respectively. The $t_{1/2\alpha}$ and $t_{1/2\beta}$ in plasma of the 1-mg/kg dose of lip-AMB were 1.6 and 17 h, respectively. The $t_{1/2\alpha}$ and $t_{1/2\beta}$ in plasma of the 5-mg/kg dose of lip-AMB were 1.6 and >24 h, respectively. It appears that with higher doses of lip-AMB, enhanced terminal half-life is achieved because of slow clearance of lip-AMB from plasma.

The organ distribution of AMB and lip-AMB is illustrated in Table 3. After i.v. administration of AMB or lip-AMB at a 1-mg/kg dose, no drug was detectable in the brain or heart. At the 5-mg/kg dose of lip-AMB, both the heart and brain showed detectable levels of drug between 5 min and 24 h, diminishing to undetectable levels within 48 h. Both the 1- and 5-mg/kg doses of lip-AMB resulted in peak levels in the kidneys and lungs immediately and in the spleen and liver by 2 and 4 h, respectively. Drug levels in the kidney and lung declined at various rates, remaining measurable for 24 h or longer. The concentration of lip-AMB in liver and spleen was substantially higher than that of AMB 4 h after injection, whereas AMB was undetectable.

The levels of biochemical indices in the serum of treated mice in all groups were determined at day 42 (data not shown). Control animals were noninfected, nontreated normal mice. Lactate dehydrogenase levels in all treatment groups (AMB and lip-AMB) were not significantly different from control levels. Blood urea nitrogen, glutamic pyruvic transaminase, and glutamic oxaloacetate transaminase levels in serum in all treatment groups (AMB and lip-AMB) were only slightly elevated above control levels.

DISCUSSION

The majority of fungal infections in cancer patients are caused by *C. albicans* (2). Aspergillosis, cryptococcosis, and histoplasmosis usually exhibit the same organ tropism as candidiasis, i.e., kidney, spleen, liver, brain, and lung (14, 15, 23, 24). Tissue necrosis and local inflammation are the characteristic organ damage seen in disseminated candidiasis (15).

It has been shown previously that liposomal encapsulation decreases the toxicity associated with the administration of AMB while maintaining its therapeutic efficacy in experimental candidiasis (12, 22). Compared with AMB, lip-AMB produced higher survival in treated mice. We evaluated our preparation of lip-AMB against AMB 2 and 3 days after infection of the mice with *C. albicans*. The maximum tolerated dose of AMB, 2 mg/kg, led to severe loss of weight and sickness, resulting in death of the animals, while a 10-fold increase in dose was achieved with lip-AMB without any apparent toxicity. When organism growth was higher (on day 3), AMB substantially lost its efficacy at a dose of 1 mg/kg. In contrast, lip-AMB fully maintained its effectiveness at doses of 5 to 20 mg/kg, providing 100% survival at some doses (Table 2). However, the survival of mice receiving the highest dose of AMB (1.5 mg/kg) or lip-AMB (20 mg/kg) was decreased, perhaps due to the increased severity of the disseminated candidiasis, altering their sensitivity to the toxic side effects of AMB. The results of our study are in general agreement with those reported by other workers, demonstrating an improved therapeutic index with lip-AMB used in mice with acute candidiasis and histoplasmosis (12, 21).

Unlike AMB, several doses of lip-AMB provided 100% survival by day 42, showing that a single high dose or multiple doses of lip-AMB are more effective than multiple low doses of AMB. However, our study did not demonstrate complete eradication of the *Candida* infection in the kidneys of all groups following lip-AMB treatment in long-term survivors of *Candida*-infected mice. This incomplete eradication of the infection may be clinically significant and introduces concern for future clinical use of this liposomal preparation of AMB.

The organ localization and concentration of AMB and lip-AMB in our studies differed somewhat from that reported by Lopez-Berestein et al. (13). However, the protracted drug presence is in agreement with their study. The higher values reported for tissue concentration may well be explained by lower levels of the drug in the murine plasma. However, our study agrees with the versatility of liposomal carrier systems for AMB to induce an improved therapeutic efficacy of this important antifungal agent. The detectable levels of lip-AMB in brain tissue in our study may well be related to the smaller size distribution of our liposomal preparation or to the presence of intracranial blood in the brain tissue at the time of drug measurement. The presence of higher lip-AMB concentrations in the liver, lung, and spleen, as seen in our study, may be helpful in improving the therapeutic efficacy of the drug, because these are the organs most severely affected by disseminated fungal infections.

The decreased toxicity of lip-AMB may be due to a reduction in interaction of the encapsulated drug with the host membranes. This may result in a greater interaction of

the drug with fungal ergesterol than with the cholesterol of animal cell membranes. While one study showed no improvement when ergesterol was incorporated into the liposomal preparation (1), Szoka et al. have shown that liposome size and composition significantly modulate in vitro cytotoxicity and in vivo lethality in a murine model (20).

Liposomes have been shown to be taken up by reticuloendothelial cell-rich organs (8), which in turn are the organs most frequently affected by disseminated candidiasis (12), possibly explaining the presence of *Candida* cells in the kidney of long-term "recovered" mice in our study. In addition, the enhanced levels in plasma achieved with lip-AMB doses of 1 and 5 mg/kg may result in substantial therapeutic advantage. The level of drug circulating in plasma even at 25 h with the 5-mg/kg dose was 15 μg/ml, providing a higher terminal half-life. Interestingly, this 30-fold-higher level of lip-AMB than of AMB in plasma does not appear to elicit any acute or chronic toxicity in mice. This advantage in plasma concentration may be related to the use of small unilamellar liposomes and/or their lipid composition. The detectable levels of AMB in the brain tissue, in contrast with other studies (13), may be due to the presence of intracranial blood at the time of extraction and the high sensitivity of the detection methods used in this study. An extension of our work is in general agreement with our hypothesis that an improved therapeutic index of lip-AMB can be achieved in mice with acute cryptococcosis (E. J. McManus, A. Rahman, J. Gondal, and J. E. Bennett, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 318, 1988).

The potential advantage of the liposomal preparation clearly rests on its lower toxicity when administered by rapid i.v. injection, as demonstrated by this study and others (6, 12, 21). Preliminary results indicate that our preparation may be less toxic but therapeutically effective against pulmonary aspergillosis in a heart transplant patient (N. M. Katz, P. F. Pierce, R. A. Anzeck, M. S. Visner, H. G. Canter, M. L. Foegh, D. L. Pearle, C. Tracy, and A. Rahman, J. Heart Transplant, in press). This is in agreement with Sculier et al., who observed higher serum antifungal activity with none of the common side effects of AMB when they used lip-AMB for treatment of 15 cancer patients with fungal infections (18). Further studies are needed to test the clinical efficacy of our preparation in comparison to the preparations of others (8, 9, 11, 19).

ACKNOWLEDGMENTS

This work was supported in part by funds from Lypho-Med, Inc., Rosemont, Ill.; and Vestar Companies, Inc., San Demas, Calif.

We thank Henry Yeager, Division of Pulmonary Disease and Critical Care Medicine, for the use of his facilities in culturing *C. albicans*; and Clinical Biochemistry, Georgetown Hospital, for evaluation of serum biochemical indices.

LITERATURE CITED

1. **Andreoli, T. E.** 1973. On the anatomy of amphotericin B-cholesterol pores in lipid bilayer membranes. Kidney Int. **4:** 337–345.
2. **Bodey, G. P.** 1977. Infectious complications in cancer patients. Curr. Prob. Cancer **1:**3–61.
3. **Brown, W. B., Jr., and M. Hollander.** 1977. Statistics: a biomedical introduction. John Wiley & Sons., Inc., New York.
4. **Edwards, J. E., R. I. Lehrer, E. R. Stiehm, T. J. Fischer, and L. S. Young.** 1978. Severe candidal infections: clinical perspective, immune defense mechanisms, and current concepts of therapy. Ann. Intern. Med. **89:**91–106.
5. **Granich, G. G., G. S. Kobayash, and D. J. Krogstad.** 1986. Sensitive high-pressure liquid chromatographic assay for amphotericin B which incorporates an internal standard. Antimicrob. Agents Chemother. **29:**584–588.
6. **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.
7. **Inagaki, J., V. Rodriguez, and G. P. Bodey.** 1974. Causes of death in cancer patients. Cancer **33:**568–573.
8. **Juliano, R. L.** 1982. Interactions of liposomes with the reticuloendothelial system: implications for the controlled delivery of drugs, p. 405–417. *In* H. Gungard, A. B. Hansen, and H. Kofud (ed.), Optimization of drug delivery. Monskgaard, Copenhagen.
9. **Lopez-Berestein, G., R. L. Hopfer, R. Mehta, K. Mehta, E. M. Hersh, and R. L. Juliano.** 1984. Liposome-encapsulated amphotericin B for treatment of disseminated candidiasis in neutropenic mice. J. Infect. Dis. **150:**278–283.
10. **Lopez-Berestein, G., R. L. Hopfer, R. Mehta, K. Mehta, E. M. Hersh, and R. L. Juliano.** 1984. Prophylaxis of *Candida albicans* infection in neutropenic mice with liposome-encapsulated amphotericin B. Antimicrob. Agents Chemother. **25:**366–367.
11. **Lopez-Berestein, G., R. Mehta, R. Hopfer, K. Mehta, E. M. Hersh, and R. L. Juliano.** 1983. Effects of sterols on the therapeutic efficacy of liposomal amphotericin B in murine candidiasis. Cancer Drug Deliv. **1:**37–42.
12. **Lopez-Berestein, G., R. Mehta, R. L. Hopfer, K. Mills, L. Kasi, K. Mehta, V. Fainstein, M. Luna, E. M. Hersh, and R. Juliano.** 1983. Treatment and prophylaxis of disseminated infection due to *Candida albicans* in mice with liposome-encapsulated amphotericin B. J. Infect. Dis. **147:**939–945.
13. **Lopez-Berestein, G., M. G. Rosenblum, and R. Mehta.** 1984. Altered tissue distribution of amphotericin B by liposomal encapsulation: comparison of normal mice to mice infected with *Candida albicans*. Cancer Drug Deliv. **1:**199–205.
14. **Meyer, R. D., L. S. Young, D. Armstrong, and B. Yu.** 1973. Aspergillosis complicating neoplastic disease. Am. J. Med. **54:**6–15.
15. **Myerowitz, R. I., G. J. Pazin, and C. M. Allen.** 1977. Disseminated candidiasis. Changes in incidence, underlying disease and pathology. Am. J. Clin. Pathol. **68:**29–38.
16. **Nilsson-Ehle, I., T. Y. Yoshikawa, J. E. Edwards, M. C. Schotz, and L. B. Guze.** 1977. Quantitation of amphotericin B with use of high-pressure liquid chromatography. J. Infect. Dis. **135:** 414–422.
17. **Rahman, A., G. White, N. More, and P. F. Schein.** 1985. Pharmacological, toxicological, and therapeutic evaluation in mice of doxorubicin entrapped in cardiolipin liposomes. Cancer Res. **45:**796–803.
18. **Sculier, J.-P., A. Coune, F. Meunier, C. Brassinne, C. Laduron, C. Hollaert, N. Collette, C. Heymans, and J. Klastersky.** 1988. Pilot study of amphotericin B entrapped in sonicated liposomes in cancer patients with fungal infections. Eur. J. Cancer Clin. Oncol. **24:**527–538.
19. **Singer, C., M. H. Kaplan, and D. Armstrong.** 1977. Bacteremia and fungemia complicating neoplastic diseases. Am. J. Med. **62:**731–742.
20. **Szoka, F. C., Jr., 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.
21. **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.
22. **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.
23. **Williams, D. M., J. A. Drick, and J. S. Remington.** 1976. Pulmonary infection in the compromised host. Am. Rev. Respir. Dis. **114:**359–394.
24. **Young, L. S.** 1981. Nosocomial infections in the immunocompromised adult. Am. J. Med. **70:**398–404.