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# Pharmacokinetics and Safety of a Unilamellar Liposomal Formulation of Amphotericin B (AmBisome) in Rabbits

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**A unilamellar liposomal formulation of amphotericin B (LAmB) known as AmBisome was safely administered intravenously to 20 rabbits at 0.5, 1.0, 2.5, 5, or 10 mg/kg of body weight, whereas of 12 rabbits given desoxycholate amphotericin B (DAmB) intravenously at 0.5, 1.0, or 1.5 mg/kg, 2 died of acute cardiac toxicity when DAmB was administered at the highest dose. Single-dose LAmB (1 mg/kg) achieved a maximum concentration in serum ($C_{max}$) of 26 ± 2.4 μg/ml and an area under the curve to infinity ($AUC_{0-\infty}$) of 60 ± 16 μg · h/ml, while single-dose DAmB (1.0 mg/kg), by comparison, achieved a lower $C_{max}$ (4.7 ± 0.2 μg/ml; $P = 0.001$) and a lower $AUC_{0-\infty}$ (30.6 ± 2.2 μg · h/ml; $P = 0.07$). Following administration of a single dose of LAmB (10 mg/kg), a disproportionately higher $C_{max}$ (287 ± 14 μg/ml) and $AUC_{0-\infty}$ (2,223 ± 246 μg · h/ml) occurred, indicating saturable elimination. After chronic dosing ($n = 4$) with LAmB at 5.0 mg/kg/day for 28 days or DAmB at 1.0 mg/kg/day for 28 days, LAmB achieved daily peak levels of 122.8 ± 5.8 μg/ml and trough levels of 34.9 ± 1.8 μg/ml, while DAmB reached a peak of only 1.76 ± 0.11 μg/ml and a trough of 0.46 ± 0.04 μg/ml ($P \leq 0.001$). Significant accumulations of amphotericin B into reticuloendothelial organs were observed, with 239 ± 39 μg/g found in the liver after chronic LAmB dosing (5 mg/kg/day), which was seven times higher than the 33 ± 6 μg/g after DAmB dosing (1 mg/kg/day) ($P = 0.002$). Accumulation in kidneys, however, remained 14-fold lower ($P = 0.04$) following LAmB dosing (0.87 ± 0.61 μg/g) than after DAmB dosing (12.7 ± 4.6 μg/g). Nephrotoxicity occurred in only one of four LAmB-treated animals, while it occurred in all four chronically DAmB-treated animals; mild hepatotoxicity with transaminase elevations was seen in one LAmB-treated rabbit. We conclude that LAmB safely achieved higher $C_{max}$s and $AUC_{0-\infty}$s and demonstrated saturable, nonlinear elimination from plasma via reticuloendothelial organ uptake. The reduced nephrotoxicity of LAmB correlated with diminished levels of amphotericin B in the kidneys.**

Despite the toxicity of amphotericin B, it remains the drug of choice for the treatment of many locally invasive and disseminated mycoses. Three novel lipid-containing formulations of amphotericin B have been developed and are being tested in clinical trials. These formulations include, but are not limited to, amphotericin B lipid complex, amphotericin B colloidal dispersion, and small unilamellar vesicle formulations of amphotericin B. The small unilamellar vesicle liposomal amphotericin B (LAmB) formulation, known as AmBisome (Vestar, Inc., San Dimas, Calif.), differs from the others in that it forms true liposomes with uniform, stable, spherical, single-membraned vesicles of <0.1 μm in diameter. Early clinical studies have suggested that LAmB is both safe and effective against cryptococcosis and candidiasis (3, 4, 8, 12).

Little is known, however, about the pharmacokinetics in plasma and the distribution of LAmB in tissue in comparison with those of the conventional desoxycholate amphotericin B (DAmB) after the administration of both single and multiple doses. Moreover, there are a paucity of data comparing the chronic toxicities of DAmB and LAmB at clinically applicable doses. We therefore studied the pharmacokinetics in plasma and distribution in tissue of both LAmB and DAmB in rabbits and performed a chronic toxicity study of rabbits given clinically relevant doses of both compounds.

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## MATERIALS AND METHODS

**Drugs.** LAmB was provided as a lyophilized yellow powder (AmBisome) by Vestar, Inc. The drug's constituents are 53.3% hydrogenated soy phosphatidylcholine, 13.1% cholesterol, 21.1% distearoylphosphatidylglycerol, and 12.5% amphotericin B (by weight, or at a molar ratio of 2:1:0.8:0.4, respectively). The powder was initially reconstituted with sterile water to an initial concentration of 2 mg/ml, and the solution was then heated to 60°C for 10 min to ensure dissolution. The solution was then filtered through a 5-μm-pore-size filter to remove any aggregates that might remain and was diluted to a final concentration of 0.5 mg/ml with 5% glucose in water. DAmB was prepared from commercially available Fungizone (Bristol-Myers Squibb, Princeton, N.J.) by dissolving the initial powder with sterile water and then further diluting the solution with 5% glucose in water to a final concentration of 1 mg/ml.

**Rabbits.** Female New Zealand White rabbits weighing between 2.5 and 3.0 kg were used for the study. Animals were housed individually and were provided food and water ad libitum. While the rabbits were under general anesthesia, a silastic central venous catheter was surgically placed in each rabbit for repeated, nontraumatic venous access as described previously (13).

**Single-dose study.** Three or four rabbits each received DAmB at 0.5, 1.0, or 1.5 mg/kg of body weight or LAmB at 0.5, 1.0, 2.5, 5.0, or 10 mg/kg of body weight over 5 min by a steady intravenous bolus. Plasma samples were then drawn preinfusion and at 0, 5, 10, 20, 40, and 60 min and then at 2, 3, 4, 6, 8, 10, 12, 18, 24, 48, and 72 h postinfusion.

**Multiple-dose study.** Three rabbits each received either DAmB at 1.0 mg/kg/day for 10 days or LAmB at 5.0 mg/kg/day for 10 days. Plasma samples were drawn just before and 5 min after the administration of each daily dose.

**Chronic dosing study.** Four rabbits each received either DAmB at 1.0 mg/kg/day for 28 days or LAmB at 5 mg/kg/day for 28 days. These regimens represent the highest single dosages not associated with acute toxicity in rabbits. At weekly intervals, plasma samples for determination of peak and trough levels of amphotericin B were drawn just before and 15 min after administration of the dose. Blood samples were also obtained weekly for complete blood count, electrolytes, blood urea nitrogen (BUN), creatinine, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase, bilirubin, triglycerides, and cholesterol. Animals were also weighed once weekly and were clinically evaluated (activity levels, eye color, fur texture, feeding, stool patterns, and water consumption) each day. Given the importance of evaluating central nervous system toxicity during the chronic administration of high dosages of amphotericin B, all rabbits were examined daily for signs of neuromuscular weakness, spasticity, irritability, and seizures. Twenty-four hours after the last dose, all rabbits were sacrificed, and sections of liver, spleen, and kidney were submitted for histologic evaluation as well as for determination of the levels of amphotericin B in tissue.

**Assays.** The analysis of plasma amphotericin B concentrations used a highly sensitive, specific, and reproducible high-performance liquid chromatographic (HPLC) method with UV detection (2). The linear range for the standard calibration curves was 0.05 to 20 μg/ml, and the average interday and intraday variability was less than 6%. The sensitivity was 0.030 μg/ml. Concentrations exceeding the range of the calibration curve were appropriately diluted with rabbit plasma. For analysis of amphotericin B in rabbit tissue, we used the same chromatographic system as we used for analysis of plasma. Extraction of amphotericin B required homogenization of tissue and then acidification to pH 4.0, addition of an extraction solvent consisting of dimethyl sulfoxide-methanol (2:1), centrifugation, and finally, injection onto the HPLC system. The linear range for the standard calibration curves was 2 to 800 μg/g, depending on the tissue type, and the average interday and intraday variability was less than 10%.

**Pharmacokinetic calculations.** Single-dose pharmacokinetic estimates were derived by noncompartmental techniques (5). A nonlinear least-squares regression program was applied to the postdistributive phase of the concentration-time curves to generate an estimate of the elimination rate constant ($k_{el}$). The area under the plasma concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) was calculated by using the linear trapezoidal rule from time zero to the final measured concentration ($C_{last}$). The remaining area was calculated by the relationship $C_{last}/k_{el}$. Plasma clearance (CL) was calculated as dose/$AUC_{0-\infty}$. The volume of distribution at steady-state ($V_{ss}$) was estimated by using the area under the moment curve (AUMC), such that $V_{ss} = CL \cdot (AUMC/AUC_{0-\infty})$. Half-lives ($t_{1/2}$) were calculated as $t_{1/2} = \ln(2)/k_{el}$. Peak ($C_{max}$) and trough ($C_{min}$) concentrations in serum were defined as the concentrations at 5 min and 24 h after the infusion, respectively. All calculations were performed on the data for each rabbit, after which the appropriate arithmetic and harmonic mean values for each dosage group were determined. In order to assess the linearity of the pharmacokinetics of the LAmB formulation, the ratio of $AUC_{0-\infty}$/dose was determined for each rabbit and was plotted against the ratio for the respective dosage group.

**Statistical comparisons.** Differences between the means of the pharmacokinetic variables in plasma were evaluated by two-tailed Student's unpaired *t* test. Differences between chemistry values in serum during the chronic dosing study were evaluated by Student's paired *t* test. Evaluation of dose linearity was performed by the Student-Newman-Keuls test and one-way analysis of variance. For all tests, a two-tailed *P* value of less than 0.05 was considered significant.

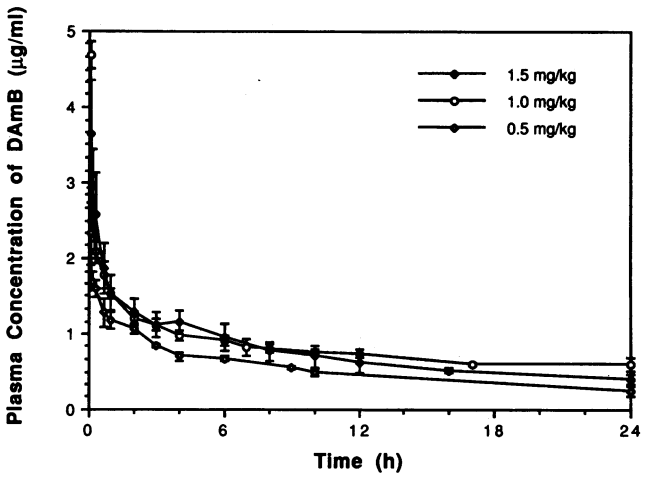


FIG. 1. Plasma amphotericin B concentration-versus-time curve for three or four rabbits each receiving a single 0.5-, 1.0-, or 1.5-mg/kg intravenous dose of DAmB. Each point plots the mean and standard errors of the level of amphortericin B in plasma at that time. Note the rapid decline from peak levels of <5.0 to ≤1.0 μg/ml within 2 h postdosing and then a long terminal $t_{1/2}$.

## RESULTS

**Pharmacokinetics of DAmB.** The plasma concentration-versus-time curves for DAmB are shown in Fig. 1, and calculated pharmacokinetic values are given in Table 1. DAmB was well tolerated at 0.5 and 1.0 mg/kg, but two of six animals succumbed to acute cardiac toxicity when they received DAmB at 1.5 mg/kg. Despite the rapid infusion, the $C_{max}$ achievable with DAmB was less than 5 μg/ml, and levels in plasma declined rapidly to less than 1 μg/ml within 4 h postdose and then declined more slowly, with a long $t_{1/2}$ (12.7 to 18.3 h; overall mean, 15 ± 1.1 h). $C_{min}$ at 24 h postdose averaged 0.4 μg/ml and did not change significantly with dose. The $V_{ss}$ was equivalent to that of total body water. Although both $C_{max}$ and $AUC_{0-\infty}$ approximately doubled as the dose was doubled from 0.5 to 1.0 mg/kg, there was little change in these values with dose increases from 1.0 to 1.5 mg/kg. This coincided with an increase in clearance from 0.088 to 0.144 liter/h. Consequently, the plasma concentration-versus-time curves were nearly identical for DAmB at 1.0 and 1.5 mg/kg.

**Pharmacokinetics of LAmB.** The plasma concentration-versus-time curves for LAmB are shown in Fig. 2. LAmB was well tolerated at all doses from 0.5 to 10 mg/kg. The pharmacokinetics of LAmB displayed saturable elimination over the dose range studied ($P < 0.05$), as depicted in Fig. 3. As the LAmB dose was increased 20-fold, the $AUC_{0-\infty}$s increased 111-fold. Consequently, only the $AUC_{0-\infty}$s, $C_{max}$s, and $C_{min}$s are reported. Calculation of CL and $V_{ss}$ would contradict the underlying assumption of noncompartmental analysis, which is a requirement for linear pharmacokinetics. The $C_{max}$ following administration of LAmB rose proportionately with increasing dose up to nearly 300 μg/ml after administration of a dose of

TABLE 1. Pharmacokinetics of DAmB versus those of LAmB in plasma[a]

| Drug (dose [mg/kg]) | $C_{max}$ (μg/ml) | $C_{min}$ (μg/ml) | $t_{1/2}$ (h) | $AUC_{0-\infty}$ (μg · h/ml) | $V_{ss}$ (liter/kg) | CL (liter/h) |
|---|---|---|---|---|---|---|
| DAmB | | | | | | |
| 0.5 | 2.3 ± 0.4 | 0.25 ± 0.06 | 12.7 ± 2.5 | 18 ± 3.0 | 0.45 ± 0.01 | 0.087 ± 0.02 |
| 1 | 4.7 ± 0.17[b] | 0.6 ± 0.09 | 18.3 ± 1.31[c] | 31 ± 2.2[d] | 0.77 ± 0.02[e] | 0.088 ± 0.007[f] |
| 1.5 | 4.8 ± 0.7 | 0.4 ± 0.006 | 14.3 ± 0.0 | 35 ± 1.7 | 0.87 ± 0.05 | 0.144 ± 0.018 |
| LAmB | | | | | | |
| 0.5 | 11 ± 2.2 | <0.025[g] | —[h] | 20 ± 8.4 | — | — |
| 1 | 26 ± 2.4[b] | 0.1 ± 0.1 | — | 60 ± 16[d] | — | — |
| 2.5 | 53 ± 3.0 | 0.8 ± 0.3 | — | 207 ± 18 | — | — |
| 5 | 132 ± 4.4 | 1.7 ± 1.0 | — | 838 ± 96 | — | — |
| 10 | 287 ± 14 | 21 ± 2.7 | — | 2223 ± 246 | — | — |

[a] All values are expressed as means ± standard errors of the mean.
[b] $P = 0.001$ in comparison with DAmB at 1 mg/kg by Student's $t$ test.
[c] $P = 0.0003$ in comparison with DAmB at 1 mg/kg by Student's $t$ test.
[d] $P = 0.07$ in comparison with DAmB at 1 mg/kg by Student's $t$ test.
[e] $P = 0.00001$ in comparison with DAmB at 1 mg/kg by Student's $t$ test.
[f] $P = 0.02$ in comparison with DAmB at 1 mg/kg by Student's $t$ test.
[g] Values were below the detection limit of the assay (0.025 μg/ml) for all rabbits.
[h] —, the pharmacokinetics of LAmB displayed saturable elimination over the dosage range studied. Consequently, only the $AUC_{0-\infty}$s, $C_{max}$s, and $C_{min}$s are reported. Calculation of $t_{1/2}$, $V_{ss}$, and CL would violate the underlying assumption of noncompartmental analysis, which is a requirement for linear pharmacokinetics.

10 mg/kg. The $C_{min}$s following administration of doses of 2.5 mg/kg or less were <1.0 μg/ml, and after administration of a 10-mg/kg dose, the $C_{min}$s exceeded 20 μg/ml.

**Comparison of LAmB and DAmB.** Table 1 illustrates several striking differences between the pharmacokinetic properties of LAmB and DAmB. At the same doses of 1 mg/kg, LAmB had at least a fivefold greater $C_{max}$ ($P = 0.001$) and a twofold greater $AUC_{0-\infty}$ than DAmB ($P = 0.07$). Moreover, as the LAmB dose was increased 10-fold to 10 mg/kg, greater LAmB:DAmB ratios were achievable (>60-fold higher $C_{max}$s and $AUC_{0-\infty}$s for LAmB at 10 mg/kg versus DAmB at 1 mg/kg), reflecting the nonlinear rise in LAmB levels in plasma with increasing dose.

**Multiple-dose study.** All rabbits tolerated the drugs well over the 10-day course of treatment. Trough levels continued to rise over the period and did not achieve steady state. DAmB levels in the first 3 days were 0.24 ± 0.03 μg/ml predose and rose to 0.39 ± 0.04 μg/ml during days 8 to 10, while LAmB levels were 3.50 ± 0.53 and 9.17 ± 1.1 μg/ml, respectively. However, peak levels did not change appreciably, with DAmB averaging 1.72 ± 0.16 μg/ml and LAmB achieving 95.4 ± 2.2 μg/ml at 5 min after the administration of each dose. This continued accumulation of amphotericin B in plasma following LAmB administration suggests either that a third compartment existed or that the rate of elimination of amphotericin B was changing over time.

**Chronic dosing study.** Four rabbits per group received either DAmB at 1.0 mg/kg/day for 28 days or LAmB at 5 mg/kg/day for 28 days. These regimens represent the highest single dosages not associated with acute toxicity in rabbits. All rabbits

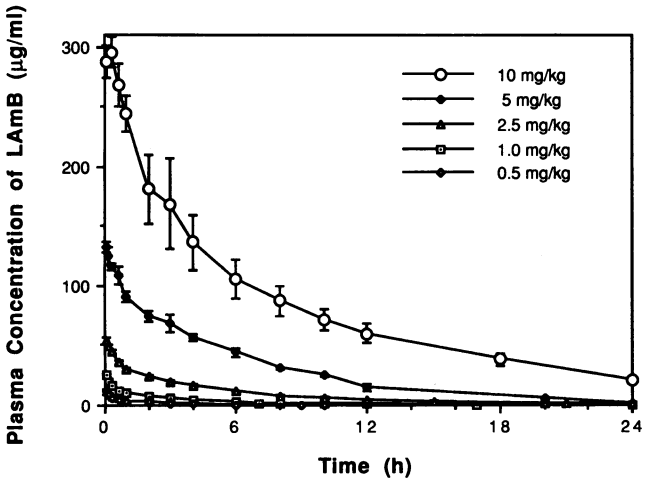


FIG. 2. Plasma amphotericin B concentration-versus-time curve for three or four rabbits each receiving a single intravenous dose of LAmB of 0.5, 1.0, 2.5, 5, or 10 mg/kg. Each point plots the mean and standard errors of the level of amphotericin B in plasma at that time. Note the much greater levels in plasma (peaks of nearly 300 μg/ml and troughs of 20 μg/ml after administration of a dose of 10 mg/kg) achieved with LAmB versus those achieved with DAmB.

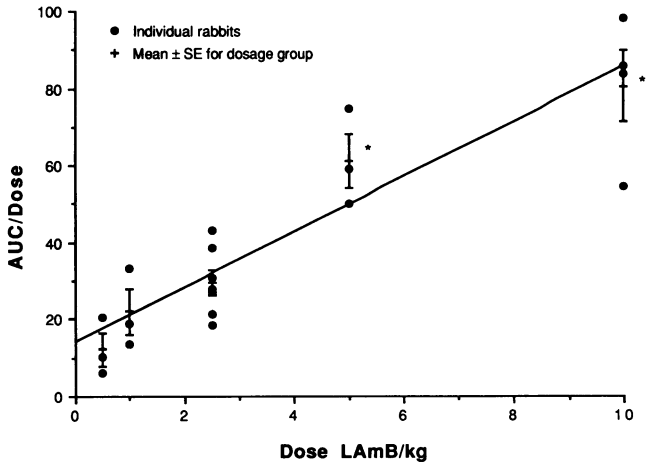


FIG. 3. Plot of the ratios of AUC:dose for each rabbit (●) and the mean ± standard errors (bars) within each dosage group. For a drug that follows linear kinetics, such a plot would have a slope of 0. For LAmB, however, the significantly higher (*, $P < 0.05$; Student-Newman-Keuls) AUC:dose ratios at the 5- and 10-mg/kg dosages, as well as the significantly positive slope of the means ($P < 0.001$; analysis of variance), indicates its nonlinear kinetics.

TABLE 2. Levels in plasma following chronic dosing with DAmB at 1 mg/kg/day versus those following chronic dosing with LAmB at 5 mg/kg/day[a]

| Time (days) | Concn (μg/ml) | | | |
|---|---|---|---|---|
| | DAmB | | LAmB | |
| | Predose | Postdose | Predose | Postdose |
| 1 | 0 | 1.78 ± 0.27[b] | 0 | 91.7 ± 4.2[c] |
| 8 | 0.40 ± 0.08[b] | 1.90 ± 0.17 | 15.5 ± 4.5[d] | 102.0 ± 6.0 |
| 16 | 0.47 ± 0.03 | 1.54 ± 0.14 | 34.3 ± 2.4 | 119.4 ± 3.7 |
| 22 | 0.46 ± 0.08 | 1.83 ± 0.25[b] | 35.6 ± 3.0 | 126.1 ± 11.0[c] |
| 29 (presacrifice) | 0.46 ± 0.04[b] | | 34.9 ± 1.8[d] | |
| Steady-state[e] | | 1.76 ± 0.11[f] | | 122.8 ± 5.8[f] |

[a] Four rabbits each received either DAmB at 1 mg/kg/day for 28 days or LAmB at 5 mg/kg/day for 28 days. These regimens represent the highest single dosages not associated with acute toxicity in rabbits. All levels of amphotericin B in plasma are expressed as mean ± standard errors.
[b] No significant differences between first and last trough and peak values, which is indicative of a lack of accumulation of DAmB in plasma.
[c] $P = 0.02$ (Student's paired $t$ test); comparison between the differences in peak values over time, which is indicative of the accumulation of LAmB in plasma.
[d] $P = 0.002$ (Student's paired $t$ test); comparison between the differences in trough values over time, which is indicative of the accumulation of LAmB in plasma.
[e] Steady-state values are averages of all values from days 16, 22, and 29.
[f] $P < 0.00001$ (Student's unpaired $t$ test), comparing steady-state plasma levels of DAmB and LAmB in plasma.

tolerated both drugs well over the course of 28 days. There was no significant weight loss or change in clinical signs. Both drugs appeared to achieve steady state after the administration of 16 doses. As shown in Table 2, DAmB at 1.0 mg/kg/day for 28 days achieved a $C_{max}$ of 1.76 ± 0.11 μg/ml and a $C_{min}$ of 0.46 ± 0.04 μg/ml, while 5-fold higher doses of LAmB reached a 70-fold higher $C_{max}$ (122.8 ± 5.8 μg/ml) and an 80-fold higher $C_{min}$ (34.9 ± 1.8 μg/ml). As shown in Table 3, concentrations of amphotericin B in liver were 75 times greater than trough levels in plasma ($P = 0.00005$) in DAmB-treated animals and 7 times greater than the levels in plasma ($P = 0.0001$) in LAmB-treated animals, suggesting substantial uptake into reticuloendothelial system (RES) organs for both formulations. The concentration of amphotericin B in liver tissue after the administration of LAmB (239 ± 39 μg/g) was seven times greater ($P = 0.002$) than that after DAmB administration (33 ± 6 μg/g). Nevertheless, non-RES organs, like the kidney, had over 14 times lower concentrations of amphotericin B ($P = 0.04$) after LAmB administration (0.87 ± 0.61 μg/g) than after DAmB administration (12.7 ± 4.6 μg/g), suggesting preferential uptake of LAmB over DAmB by the RES.

Table 4 shows the results of toxicity studies for each group of rabbits. Rabbits showed no significant changes in complete blood count, electrolytes, alkaline phosphatase, bilirubin, triglycerides, or cholesterol during the 4-week period. All tissue sections of liver, spleen, and kidney were histologically normal.

Moderate nephrotoxicity was evident in all DAmB-treated rabbits after the administration of 14 daily doses, with an average rise in BUN levels of 96% over the baseline and an average rise in creatinine levels of 60% over the baseline. This azotemia improved somewhat by the end of the 28-day period (BUN, 58% over the baseline; creatinine, 38% over the baseline). No significant rise in hepatic transaminases occurred during DAmB treatment, with only one of four animals having a slightly elevated SGOT level (79 IU/liter) at the end of the study.

For LAmB-treated rabbits, however, azotemia developed after the administration of 14 daily doses in three of four rabbits, with an average rise in BUN of 54% over the baseline, but no rabbit had a rise in creatinine of more than 0.3 mg/dl (27%) over the baseline. By the end of 28 days, when rabbits had received a total amphotericin B dose of 140 mg/kg, the average BUN level remained elevated to 33 mg/dl (54% over baseline), but creatinine levels remained elevated (by 0.3 mg/dl) in just one of the four rabbits. Indeed, the median creatinine level (1.1 mg/dl) was not elevated over the baseline. On the other hand, two rabbits had rises in hepatic transaminases at the end of the study, although in only one (with an

TABLE 3. Levels in tissues following chronic dosing with DAmB at 1 mg/kg/day versus those following chronic dosing with LAmB at 5 mg/kg/day[a]

| Tissue | DAmB concn (μg/ml) | $P$ for tissue versus plasma[b] | LAmB concn (μg/ml) | $P$ for tissue versus plasma[c] | LAmB:DAmB[d] | LAmB versus DAmB[e] |
|---|---|---|---|---|---|---|
| Liver | 33.4 ± 6.4 | 0.00005 | 239 ± 39 | 0.0001 | 7.1 | 0.002 |
| Liver:plasma[f] | 75.9 | | 7.1 | | | |
| Kidney | 12.7 ± 4.6 | 0.005 | 0.87 ± 0.61 | <0.00001 | 0.068 | 0.04 |
| Kidney:plasma[f] | 34.2 | | 0.022 | | | |
| Cerebrum | <1.0 | NA | 2.32 ± 0.51 | <0.00001 | >2.3 | NA |
| Cerebrum:plasma[f] | <2 | | 0.066 | | | |
| Trough plasma | 0.46 ± 0.04 | | 34.9 ± 1.8 | | 76 | <0.00001 |

[a] Four rabbits each received either DAmB at 1 mg/kg/day for 28 days or LAmB at 5 mg/kg/day for 28 days. These regimens represent the highest single dosages not associated with acute toxicity in rabbits. All levels of amphotericin B in tissue are expressed as means ± standard errors. Trough levels of amphotericin B in plasma (at sacrifice) are expressed as means ± standard errors.
[b] $P$ values compare the mean tissue vs mean plasma level of amphotericin B following DAmB by Student's $t$ test.
[c] $P$ values compare the mean levels of amphotericin B in tissue versus those in plasma following LAmB (by Student's $t$ test).
[d] Ratios are the concentrations of amphotericin B in tissue following administration of LAmB divided by that following administration of DAmB.
[e] $P$ values compare the levels in tissue following administration of LAmB versus those in tissue following administration of DAmB as well as trough levels in plasma (by Student's $t$ test).
[f] Ratios are the c oncentration of amphotericin B in tissue divided by the mean trough level in plasma at the time of sacrifice.

TABLE 4. Comparison of chronic toxicity of DAmB at 1 mg/kg/day versus that of LAmB at 5 mg/kg/day[a]

| Day | BUN (mg/dl) | | Creatinine (mg/dl) | | SGOT (IU/liter) | | SGPT (IU/liter) | |
|---|---|---|---|---|---|---|---|---|
| | DAmB | LAmB | DAmB | LAmB | DAmB | LAmB | DAmB | LAmB |
| 0 | 20.0 (18–27) | 24.0 (18–26) | 0.85 (0.8–1.5) | 1.20 (0.0–1.3) | 13.5 (10–23) | 18.5 (15–28) | 29.5 (20–43) | 41.0 (30–52) |
| 14 | 40.5 (36–46)[b] | 39.0 (21–40) | 1.5 (1.3–1.8)[b] | 1.2 (0.8–1.5) | 15.0 (8–27) | 15.0 (15–39) | 27.5 (21–39) | 47.5 (43–87) |
| 28 | 33.0 (27–37)[b] | 35.5 (21–39) | 1.3 (1.1–1.6) | 1.1 (0.8–1.4) | 17.5 (11–79) | 28.5 (11–327) | 33.5 (23–55) | 67.5 (35–283) |

[a] Four rabbits each received either DAmB at 1 mg/kg/day for 28 days or LAmB at 5 mg/kg/day for 28 days.
[b] $P \leq 0.05$ versus values on day 0 (by paired Student's $t$ test).

SGOT level of 327 IU/liter and an SGPT level of 283 IU/liter) was the increase greater than the upper limit of normal for rabbits (SGOT, ≤75 IU/liter; SGPT, ≤89 IU/liter).

Throughout the chronic administration of high doses of LAmB and DAmB, there were no signs of irritability, focal neuromuscular weakness, spasticity, or seizures.

## DISCUSSION

The study described here showed that LAmB is safe and well-tolerated both after the administration of single dosages up to 10 mg/kg and after chronic administration of total dosages of 140 mg/kg. It also showed that the pharmacokinetic properties of LAmB in plasma are strikingly different from those of DAmB in that (i) LAmB achieves much higher $C_{max}$s and $AUC_{0-\infty}$s; (ii) LAmB elimination appears to be a saturable process over the dosage range studied, in contrast to DAmB, which had an apparent linear increase in concentrations in plasma between dosage groups of 0.5 and 1.0 mg/kg and a modest increase between dosage groups of 1.0 and 1.5 mg/kg (doses of DAmB greater than 1.5 mg/kg were lethal to rabbits and therefore were not studied); and (iii) although both drugs accumulated in RES organs, preferential accumulation of LAmB into the RES was observed along with increased hepatotoxicity but decreased nephrotoxicity.

The single-dose pharmacokinetic characteristics of LAmB in rabbits were similar to those seen in mice and rats, in which $C_{max}$s of 87 and 118 μg/ml, respectively, and $AUC_{0-\infty}$s of 351 and 391 μg · h/ml, respectively, have been found (10). Similarly, the preferential uptake observed for LAmB into RES organs (two- to threefold greater concentrations of amphotericin B after administration of LAmB than after administration of equal doses of DAmB), although it spared the kidneys (six times lower levels in LAmB-treated animals than in DAmB-treated animals), was also observed in rats after chronic dosing (10).

The high $C_{max}$s and $AUC_{0-\infty}$s of LAmB in comparison with those of DAmB could be explained by the following model. DAmB rapidly binds to plasma lipoproteins as well as to erythrocytes shortly after dosing, but then it is taken up into tissue sites, largely by binding to the membranes of tissues. Then, a slow release of free amphotericin B from the erythrocyte- and tissue-bound fractions likely accounts for the long terminal $t_{1/2}$ of DAmB. LAmB, however, consists of small (0.1-μm) unilamellar vesicles in which the amphotericin B moiety is tightly associated with the vesicle lipids, and these vesicles are quite stable in plasma. Moreover, these lipids in LAmB markedly slow the rate of transfer of the amphotericin B molecules to plasma lipoproteins and cell membranes (6). Thus, following the administration of a bolus of LAmB, the unilamellar vesicles may impede the binding of amphotericin B to erythrocytes and other tissues and the subsequent removal from plasma, resulting in higher $C_{max}$s and $AUC_{0-\infty}$s of amphotericin B. The apparent elimination of LAmB from plasma is via phagocytic uptake of these vesicles by macrophages of the RES. Such phagocytic uptake by the RES is likely to be a saturable process. Moreover, given that the relatively little free amphotericin B has become tissue-bound, the long terminal $t_{1/2}$ observed with DAmB may not be observed after a single dose of LAmB. Indeed, computation of apparent $t_{1/2}$s (defined here as those obtained under nonlinear conditions) for LAmB at each of the doses ranged from 3.6 to 7.7 h. Perhaps plasma sampling beyond 24 h could reveal a deeper tissue compartment and a longer terminal $t_{1/2}$.

This mechanism of saturable elimination of LAmB from plasma via clearance by the RES is consistent with the saturability of RES uptake of small unilamellar liposomal vesicles demonstrated in earlier RES-blocking studies (11). If phagocytosis of LAmB vesicles by the RES was saturable, then this could explain the nonlinear rise in $C_{max}$ and $AUC_{0-\infty}$. Accordingly, at low doses (≤1.0 mg/kg), LAmB may easily be taken up by RES macrophages, but at higher doses, these sites may become saturated (i.e., too many vesicles for a limited number of macrophages or limited capacity to bind and engulf the vesicles). Thus, the vesicles would stay in the circulation longer, leading to higher $C_{max}$s and $AUC_{0-\infty}$s.

Further support for this mechanism of elimination of LAmB from plasma via RES phagocytic uptake comes from previous studies in both mice and rats demonstrating the high concentration of amphotericin B in liver and spleen (49 and 92 μg/g, respectively) 24 h after infusion of a dose of 5 mg/kg (10). Indeed, uptake by the RES has been described for most other liposomal formulations of amphotericin B, including amphotericin B lipid complex, amphotericin B colloidal dispersion, and multilamellar vesicles of Abra and Hunt (1) and Lopez-Berestein et al. (7). The rate of RES uptake of LAmB, however, appears to be much slower than that of these larger liposomes, which may completely disappear from circulation within a few hours postdose. It has been suggested that the small size of the LAmB vesicle with its tightly packed stable phospholipids may diminish adsorption of opsonins and decrease the rate of phagocytosis by RES macrophages (6, 10).

The preferential uptake of LAmB by the RES may partly explain the greater incidence of hepatotoxicity and the reduced incidence of nephrotoxicity observed following chronic LAmB dosing. Rats given liposomes without amphotericin B did not develop any hepatotoxicity, while those rats receiving 50 to 75 mg of amphotericin B per kg/day developed elevations in SGOT and SGPT values (10). Given that empty liposomes from LAmB have not been associated with hepatotoxicity, the rise in transaminase levels in serum may be reasonably ascribed to the amphotericin B component. Moreover, hepatotoxicity caused by high doses of DAmB has been reported (9).

The lowered rate of nephrotoxicity from LAmB may have been related to the diminished efflux of amphotericin B into the kidneys, in part because of decreased filtration of free amphotericin B into the tubules, or to a decreased transfer and

accumulation of amphotericin B into renal tubular cell membranes. Further consistent with the reduced chronic toxicity of LAmB is the trend of blood urea nitrogen to be elevated in the absence of elevated serum creatinine (Table 4). By comparison, both blood urea nitrogen and serum creatinine were significantly elevated in rabbits receiving DAmB.

In conclusion, the liposomal preparation of amphotericin B described here demonstrated pharmacokinetic properties markedly different from those of conventional amphotericin B as well as diminished chronic renal toxicity and acute cardiac toxicity. The high $C_{max}$s and $AUC_{0-\infty}$s achievable with this compound in plasma, especially at doses of 5 and 10 mg/kg, suggest potential uses of LAmB for the treatment of disseminated fungal infections, especially intravascular infections like fungemias, fungal endocarditis, or prosthetic device infections, or possibly for infections by even such angioinvasive organisms like *Aspergillus* species. It remains to be determined whether the high levels in plasma are pharmacodynamically important or whether rapid penetration into tissues will be the more important determinant of antifungal efficacy. Nevertheless, there may be an advantage to achieving elevated trough levels of amphotericin B (in excess of 20 μg/ml 24 h after administration of a single 10-mg/kg dose of LAmB) in order to quickly target amphotericin B against intravascular fungal infections. It also remains to be seen whether the high levels in RES tissues achieved with chronic dosing can lead to better antifungal efficacy against such refractory mycoses as hepatosplenic candidiasis.

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