Safety, Toxicokinetics and Tissue Distribution of Long-Term Intravenous Liposomal Amphotericin B (AmBisome®): A 91-day Study in Rats

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Purpose. Amphotericin B in small, unilamellar liposomes (AmBisome®) is safer and produces higher plasma concentrations than other formulations. Because liposomes may increase and prolong tissue exposures, the potential for drug accumulation or delayed toxicity after chronic AmBisome was investigated.

Methods. Rats (174/sex) received intravenous AmBisome (1, 4, or 12 mg/kg), dextrose, or empty liposomes for 91 days with a 30-day recovery. Safety (including clinical and microscopic pathology) and toxicokinetics in plasma and tissues were evaluated.

Results. Chemical and histopathologic changes demonstrated that the kidneys and liver were the target organs for chronic AmBisome toxicity. Nephrotoxicity was moderate (urean nitrogen [BUN] ≤51 mg/dl; creatinine unchanged). Liposome-related changes (vacuolated macrophages and hypercholesterolemia) were also observed. Although plasma and tissue accumulation was nonlinear and progressive (clearance and volume decreased, half-life increased with dose and time), most toxic changes occurred early, stabilized by the end of dosing, and reversed during recovery. There were no delayed toxicities. Concentrations in liver and spleen greatly exceeded those in plasma; kidney and lung concentrations were similar to those in plasma. Elimination half-lives were 1–4 weeks in all tissues.

Conclusions. Despite nonlinear accumulation, AmBisome revealed predictable hepatic and renal toxicities after 91 days, with no new or delayed effects after prolonged treatment at high doses that resulted in plasma levels >200 μg/ml and tissue levels >3000 μg/g.

KEY WORDS: amphotericin B; liposomes; pharmacokinetics; toxicokinetics; tissue distribution; toxicity.

INTRODUCTION

Despite the appearance of newer antifungals, amphotericin B remains the most potent broad-spectrum agent for the treatment of disseminated fungal infections. Highly lipophilic and water-insoluble, amphotericin B is conventionally formulated with sodium deoxycholate. This formulation causes frequent, potentially severe side effects including reduced renal function and irreversible tubular damage. Treatment with conventional amphotericin B is often limited by toxicity, rather than by the patient's therapeutic response.

Recently introduced lipid formulations (1) maintain the antifungal activity of amphotericin B while reducing toxicity in animals (2,3) and humans (4–6). AmBisome®, a true liposome, consists of small unilamellar vesicles composed of rigid neutral and charged phospholipids, cholesterol, and amphotericin B, a composition designed to prolong drug residence times in the body (1). AmBisome retains the potency of amphotericin B, increases its safety in animals (7,8) and humans (9–13), and is approved for the treatment of disseminated fungal infections and leishmaniasis, and for empirical therapy of febrile neutropenia (10). AmBisome's pharmacokinetics differ markedly other amphotericin B formulations, exhibiting the highest plasma exposures at a given dose (14). AmBisome is tolerated in patients at doses 15-times higher than the maximum recommended dose of conventional amphotericin B (15), and is safer than amphotericin B deoxycholate and amphotericin B lipid complex (10–12).

Although the safety of intravenous AmBisome was studied after 30-day exposures in rats (16) and dogs (17), systemic fungal infections often require prolonged treatment. The safety of liposomes and liposomal drugs after long-term intravenous exposures is not well understood, but liposomes are known to increase and prolong tissue drug exposures (18). The relationship of AmBisome's increased plasma exposure and altered tissue distribution to its toxicologic profile, especially after repeated high-level dosing, remains unestablished. Because liposomes exhibit nonlinear pharmacokinetics (16,17), short-term studies may be inadequate to predict dose-exposure-response relationships after prolonged exposures. Understanding long-term exposures to amphotericin B is important in guiding the therapy of chronic invasive fungal infections, where high doses are administered over extended periods. For these reasons, we characterized the safety and toxicokinetics of AmBisome at clinically relevant doses during a 91-day intravenous exposure and 30-day recovery in rats to determine the onset, progression, and reversibility of toxic effects and their relationship to drug exposures in plasma and tissues.

MATERIALS AND METHODS

Animals

Sprague-Dawley Cr1:CD® (SD)BR rats (174/sex) weighing 167–211 g (males) or 130–201 g (females) were acclimatized for 14 days prior to testing, and housed in individual stainless steel cages in temperature-, humidity- and light-controlled rooms with free access to water and food, except for overnight fasting prior to blood samples for clinical pathology. The study was conducted in an AAALAS-accredited test facility in compliance with the guidelines described in *Guide for the Care and Use of Laboratory Animals* (National Research Council, Washington, DC).

Test and Control Articles

AmBisome (NeXstar Pharmaceuticals, Inc., San Dimas, CA) is a lyophilized liposomal formulation containing amphotericin B, hydrogenated soy phosphatidylcholine, distear-

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oylphosphatidylglycerol, and cholesterol (approximately 50, 213, 84, and 52 mg/vial, respectively). The formulation also contains sucrose, disodium succinate, and α-tocopherol. AmBisome was reconstituted with sterile water immediately prior to administration to yield an isotonic preparation containing 4 mg/ml amphotericin B and 28 mg/ml total lipids in liposomes with a median diameter <100 nm. Empty liposomes (NeXstar Pharmaceuticals, Inc.), with the same lipid composition as AmBisome but no amphotericin B, were administered at a lipid dose equivalent to the high-dose AmBisome group. Reconstituted AmBisome and empty liposomes were diluted with 5% dextrose.

Study Design

Animals were randomized into five groups for the main study: a 91-day exposure followed by a 30-day recovery. These rats received daily intravenous bolus injections of 1 mg/kg AmBisome (30/sex), 4 mg/kg AmBisome (30/sex), 12 mg/kg AmBisome (30/sex), 5% dextrose (15/sex), or empty liposomes (15/sex). In addition, three groups (18/sex/group) of animals received a single intravenous injection of AmBisome (1, 4, or 12 mg/kg of amphotericin B) to obtain single-dose pharmacokinetic data. All injections were made into the lateral tail vein over approximately 1 min in a volume of 5 ml/kg.

Of the animals in each AmBisome-treatment group, 18 per sex were to be sacrificed during the 24-hr period following the final dose of AmBisome (see below), while 4 per sex were to be sacrificed at each of three recovery timepoints (8, 15, and 30 days after the last dose). In the control groups, 10 animals per sex were to be sacrificed 24 h following the final dose of AmBisome, while 5 animals per sex were to be sacrificed 30 days after the last dose.

For toxicokinetic evaluations, blood samples were obtained from the vena cava at the time of sacrifice. Animals receiving a single dose of AmBisome (18/sex/dose) were sacrificed in groups of 3/sex at 15 min, 1, 3, 6, 12, or 24 h after dosing. Animals sacrificed after the last dose of AmBisome (18/sex/dose) were sacrificed in groups of 3/sex at 15 min, 1, 3, 6, 12, or 24 h after their final dose. Blood was obtained from recovery animals sacrificed 8, 15, and 30 days after the last AmBisome dose (4/sex/timepoint). Trough blood samples were obtained from 10 /sex/dose by retro-orbital bleeding 24 h after the 14th, 42nd, and 70th doses. No animal was bled more than once for this purpose. Toxicokinetic blood samples were collected in citrated tubes and cooled on ice. Plasma was separated by centrifugation and frozen at –80°C for amphotericin B analysis. For toxicokinetics, liver (2 g of left lateral lobe), kidney (right), lung (right), and spleen were removed at necropsy, rinsed, blotted, weighed, and frozen (–80°C) for amphotericin B analysis.

For clinical pathology evaluation, retro-orbital blood samples were collected from 5/sex in the control groups and 10/sex in the AmBisome-treated groups 24 h after the 35th, 56th, and 88th doses. No animal was bled more than once for this purpose. Blood was collected from the vena cava from animals sacrificed during the recovery period (8, 15, and 30 days after the last dose of AmBisome, 30 days after the last dose for control animals). Clinical pathology tests included standard hematology and clinical chemistry parameters.

Other safety parameters monitored during the study included daily observation, weekly body weight and food consumption, organ weights at necropsy, and ophthalmologic examination. All animals that were sacrificed or died on study were subjected to a gross necropsy. Forty-four organs and tissues were collected from each animal, and fixed in 10% neutral buffered formalin. Paraffin sections stained with hematoxylin and eosin were examined microscopically. Observed histomorphologic changes were graded as minimal, mild, moderate, or severe.

Statistical Analysis

The statistical significance of differences in safety parameters between groups was evaluated using analysis of variance. Dunnett's test was used to compare the empty liposome control and AmBisome groups to the dextrose controls, and to compare the AmBisome groups to the empty liposome controls.

Amphotericin B Assays

Amphotericin B was determined by a modified HPLC assay (19) that was validated for plasma and liver, and cross-validated for spleen, kidney, and lung. Plasma samples (0.2 ml) were deproteinized with methanol (0.5 ml) containing the internal standard (1-amino-4-nitronaphthalene). Tissue samples (0.5 g for liver, kidney, and lungs, 0.25 g for spleen) were homogenized with methanol (5 ml) containing the internal standard. Methanolic supernatants were diluted 1:1 in mobile phase and injected into a Lichrosphere 5 μm column (250 × 4 mm, Phenomenex, Inc.) at 30°C. These operations were performed under yellow light to minimize photodegradation of amphotericin B. The HPLC system used a mobile phase of methanol/5 mM phosphate, 2.5 mM EDTA buffer (77:23), and UV detection at 405 nm. For plasma, calibration standards were prepared in blank rat plasma at eight concentrations (0.1–100 μg/ml). For tissues, calibration standards were prepared at seven concentrations (0.5–500 μg/g) by homogenizing blank liver in amphotericin B-spiked methanol. The linear range of the assay was 0.1–100 μg/ml (plasma), 0.5–500 μg/g (liver, kidney, lungs), or 1–500 μg/g (spleen). Amphotericin B concentrations in samples were calculated from weighted linear regression fits to the amphotericin B-internal standard peak height ratio. For plasma, intra-assay precision was between 2.6 and 25% and accuracy was within ±14% over the range of the assay. For tissues, intra-assay precision was between 4.9 and 15% and accuracy was within ±8% over the assay range. Recovery of amphotericin B was >90% from plasma over the range tested (1–80 μg/ml) and 73–83% from rat liver over the range tested (1–500 μg/g).

Pharmacokinetic Analysis

Amphotericin B plasma concentration profiles were analyzed using noncompartmental methods. Areas under plasma and tissue concentration vs. time curves (AUCs) were determined by trapezoidal integration, extrapolated from the last measured concentration (C_{last}) as $C_{last} \times \ln 2/t_{1/2}$, where $t_{1/2}$ was the terminal half-life. Clearance was calculated as dose/AUC, using plasma AUC to infinity after the first dose and AUC from 0 to 24 h after the last dose. The volume of distribution (V_d) was calculated as $(CL \times t_{1/2})/\ln 2$. The central compartment volume (V_c) was calculated as dose divided by

plasma concentration at 0.25 h. C_{max} was the highest plasma concentration observed after dosing. The tissue-plasma exposure ratio (K_P) was the ratio of tissue AUC to plasma AUC over the 24 h following the final dose. The steady-state concentration in tissues (C_{ss}) on Day 91 was determined by dividing the tissue AUC from 0 to 24 h by 24 h.

RESULTS

Clinical Observations and Necropsy

Three (0.9%) of 348 animals died during the study. The Day 3 death of one high-dose female was attributed to AmBisome. Histopathologic examination revealed severe multifocal hepatocellular degeneration and moderate hepatocellular necrosis. The deaths of an empty-liposome female (Day 17) and a mid-dose male (Day 63) were unrelated to AmBisome. No other overt clinical or ophthalmologic changes attributable to AmBisome were observed during the study.

Animals in all groups gained weight during the study, but weight gain was 15–30% lower in high-dose animals ($p < 0.05$ vs. dextrose controls) during the first 4-–5 weeks. A trend toward reduced food consumption in the high-dose group was observed, but was not significant ($p > 0.05$). At the end of the 91-day treatment body weights of high-dose males and females were 13% and 9% lower than those of dextrose controls ($p < 0.05$), but after the 30-day recovery there were no differences in body weight between groups. On Day 91, macroscopic changes related to AmBisome were observed in the liver (pale foci or streaks in 6/17 high-dose females and 1/17 mid-dose males), spleen (enlarged in 2/17 high-dose females), and injection site (red or discolored in 10/53 males at all dose levels). No macroscopic changes were observed in animals sacrificed during the recovery period. Organ to body weight ratios on Day 91 were increased ($p < 0.05$ vs. controls) for the kidneys (females, mid- and high-dose males), liver (high-dose females, mid- and high-dose males) and spleen (mid- and high-dose males and females).

Microscopic Pathology

Histopathologic changes related to AmBisome administration were observed on Day 91. Dose-related accumulations of vacuolated macrophages were observed in the liver, spleen, kidneys, and lymph nodes in all AmBisome groups. These macrophages were distributed throughout the liver lobules, with interlobular and subcapsular accumulations also observed in females. Vacuolated macrophages were diffusely distributed within the splenic red pulp, medullary portions of lymph nodes, and in renal glomeruli. Dose-related transitional cell hyperplasia, of minimal to moderate severity, was observed in the renal pelvis and/or bladder in 23% of mid-dose and 100% of high-dose animals. Other renal histopathologic changes, typical of age-related nephropathy in rats, were observed in both treated and control groups. Although not clearly related to AmBisome, minimal to mild tubular dilatation and vacuolation were observed more frequently in high-dose males than in controls (50% vs. 0% on Day 91), and mild pyelitis was observed more frequently in high-dose females than in controls (40% vs. 20% on Day 91). AmBisome-related hepatic changes on Day 91 included multifocal hepatocellular necrosis, of minimal to mild severity in mid-dose animals (24% of males, 22% of females), and mild to moderate severity in high-dose animals (11% of males, 41% of females). Although the time to onset of histopathologic changes could not be determined, the incidence and severity of all observed histopathologic changes lessened during the 30-day recovery period. Except for the persistence of urinary hyperplasia and vacuolated tissue macrophages in high-dose animals, all histopathologic changes observed at Day 91 appeared to be completely reversible.

Clinical Chemistry and Hematology

By Day 89, urea nitrogen (BUN) levels were elevated in the mid- and high-dose groups, in a dose-related manner (Table 1). BUN increased early in the study, then remained relatively constant from Day 36 to Day 89, returning to normal levels during the 30-day recovery (Fig. 1). The degree of

Table 1. Group Mean (± SD) Values of Hematology and Clinical Chemistry Parameters on Day 89 of a 91-Day Study of Intravenous AmBisome in Rats

Group	Sex	BUN (mg/dl)	Creatinine (mg/dl)	Cholesterol (mg/dl)	ALP (IU/l)	ALT (IU/l)	AST (IU/l)	Neutrophils (10^3/mm^3)
Dextrose	M	13 ± 1.4	0.7 ± 0.08	71 ± 7.5	91 ± 11	33 ± 7	80 ± 20	1.8 ± 0.6
	F	17 ± 2.9	0.7 ± 0.0	99 ± 8	40 ± 9	33 ± 14	83 ± 27	1.7 ± 0.5
Empty liposomes	M	12 ± 0.7	0.7 ± 0.04	143 ± 25[a]	87 ± 22	35 ± 8	76 ± 17	2.0 ± 0.7
	F	15 ± 2.9	0.7 ± 0.08	173 ± 16[a]	40 ± 11	28 ± 7	63 ± 8	0.94 ± 0.4
AmBisome 1 mg/kg	M	15 ± 2.1	0.7 ± 0.07	66 ± 16[b]	93 ± 19	28 ± 5	89 ± 12	2.1 ± 1.0
	F	18 ± 2.3	0.8 ± 0.07	97 ± 16[b]	46 ± 14	27 ± 7	84 ± 15	1.2 ± 0.5
AmBisome 4 mg/kg	M	28 ± 7.9[a,b]	0.7 ± 0.09	97 ± 19[b]	101 ± 28	31 ± 10	105 ± 70	2.7 ± 1.3
	F	21 ± 2.6	0.7 ± 0.0	114 ± 21[b]	47 ± 16	29 ± 14	92 ± 31	2.0 ± 0.9
AmBisome 12 mg/kg	M	51 ± 10[a,b]	0.7 ± 0.06	183 ± 45[a]	122 ± 20[a,b]	26 ± 6	89 ± 14	4.3 ± 2.0[a,b]
	F	42 ± 9[a,b]	0.7 ± 0.03	180 ± 35[a]	109 ± 28[a,b]	544 ± 1022	758 ± 884[b]	3.7 ± 1.0[a,b]

BUN, urea nitrogen; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase. Other hematologic (hemoglobin, hematocrit, MCV, MCH, MCHC, leucocyte, platelet, RBC, and differential counts) and clinical chemistry parameters (potassium, sodium, calcium, phosphorus, chloride, glucose, bilirubin, LDH, total protein, albumin, globulin, triglycerides) did not change significantly.

[a] Significantly different ($p < 0.05$) from dextrose control group.

[b] Significantly different ($p < 0.05$) from liposome control group.

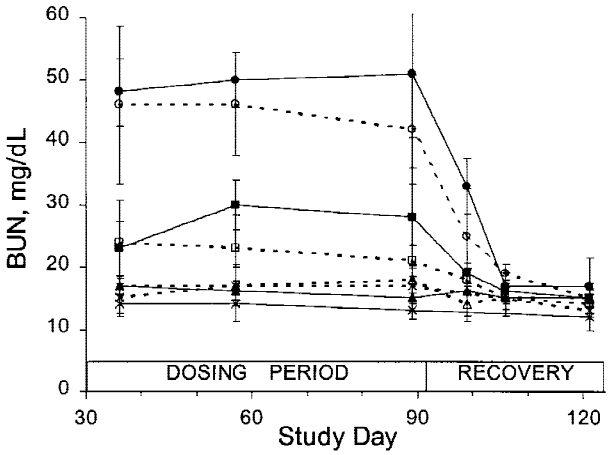

Fig. 1. Urea nitrogen (BUN) levels (mean ± SD) in rats during and after 91 consecutive daily doses of intravenous AmBisome at 1 mg/kg (triangles), 4 mg/kg (squares), 12 mg/kg (circles), or dextrose controls (X-symbols). Control group not measured on day 99 or 106. Males, solid lines; females, dotted lines.

renal impairment due to AmBisome was limited in magnitude. Mean BUNs did not exceed 30 mg/dl in the mid-dose group, and 51 mg/dl in the high-dose group, whereas serum creatinine remained unchanged during the entire study (Table 1).

Elevated alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were observed during the study (Table 1). ALP increases were statistically significant in high-dose males and females, but of limited clinical significance due to their magnitude. Clinically significant changes in ALT and AST were observed only in high-dose females. Levels of these enzymes rose in 60–80% of females by Day 36, but did not progressively increase over the remainder of the study, with similar values on Day 57 and Day 89, and returned to normal levels in all animals during the first 8 days of recovery. Mean ALT and AST values for high-dose females were influenced by a single animal, whose ALT and AST both exceeded 3000 IU/L. The median ALT and AST levels in this group were only 208 and 399 IU/L, respectively. When sacrificed at the end of the recovery period, this animal had normal levels of ALT (23 IU/L) and AST (53 IU/L) and no histopathologic evidence of hepatocellular toxicity.

A significant dose-related hypercholesterolemia was observed in high-dose AmBisome and empty-liposome groups (Table 1). Cholesterol increased steadily during the dosing period, then returned to normal values during recovery. Total bilirubin and triglyceride levels were unchanged during the study, with no differences observed between the treated groups and dextrose controls. AmBisome-related changes in hematologic parameters were limited to a slight neutrophilia in high-dose animals, which resolved completely during the recovery period.

Plasma Pharmacokinetics of Amphotericin B

The plasma pharmacokinetics after a single dose of AmBisome (1, 4, or 12 mg/kg) were consistent with those previously observed after single doses of 1, 3, and 9 mg/kg in rats (16). Plasma pharmacokinetics were biexponential (Fig. 2), with similar terminal half-lives (6.6–8.8 h) at all dose levels (Table 2). As previously observed, plasma concentrations and AUCs rose more than dose-proportionally, resulting in clearances that fell by 30–40% as the dose increased 12-fold. The volume of distribution (V_d) also decreased with increasing dose. No apparent gender differences in pharmacokinetic parameters were observed after a single dose.

Over the course of the 91-day study, C_{max} increased by less than 2-fold, while trough (24 h) concentrations rose between 2- and 15-fold (Fig. 2). This was associated with an apparent decrease in the magnitude of the initial, rapid disappearance phase of the plasma pharmacokinetic profile, to the extent that elimination after the final dose appeared nearly monoexponential in high-dose animals. As a result, plasma AUCs were markedly larger after the last dose than after a single dose, even though plasma half-lives (6.0–11.9 h)

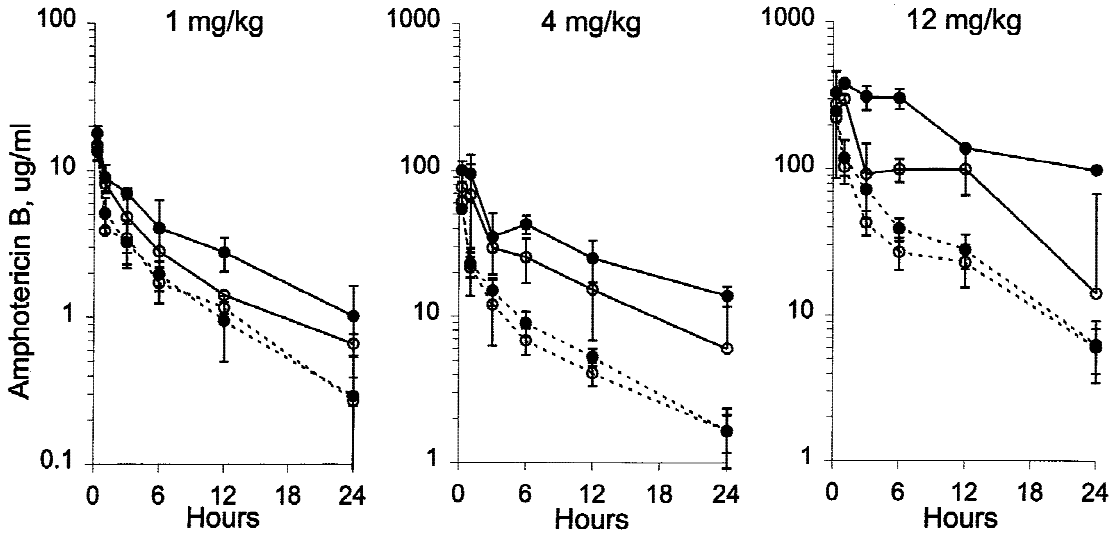

Fig. 2. Plasma concentrations of amphotericin B (mean ± SD) after a single dose (dotted lines) and 91 consecutive daily doses (solid lines) of intravenous AmBisome at three dose levels in rats. Males, solid circles; females, open circles. Dose groups, shown top to bottom, are 12, 4, and 1 mg/kg.

Table 2. Noncompartmental Pharmacokinetic Parameters of Amphotericin B After a Single Dose and 91 Daily Doses of Intravenous AmBisome in Rats

Day	Dose (mg/kg)	Sex	$t_{1/2}$ (h)	C_{max} (μg/ml)	C_{24} (μg/ml)	AUC_{0-24} (μg h ml^{-1})	V_d (ml/kg)	V_c (ml/kg)	CL (ml h^{-1} kg^{-1})
1	1	M	6.6	13.5 ± 1.7	0.29 ± 0.25	42.7	209	73.9	22.0
		F	6.6	14.8 ± 3.2	0.27 ± 0.02	43.1	207	67.6	21.9
	4	M	7.3	54 ± 2	1.6 ± 0.72	201	193	73.8	18.3
		F	8.8	60 ± 6.8	1.7 ± 0.48	175	260	66.4	20.4
	12	M	6.6	245 ± 10	6.3 ± 2.8	961	112	49.0	11.8
		F	7.9	223 ± 14	6.0 ± 2.0	753	167	53.8	14.6
91	1	M	8.9	17.8[a]	1.0 ± 0.11	90.2	125	59.6	9.7
		F	8.9	14.9 ± 2.2	0.66 ± 0.63	61.9	182	70.2	14.2
	4	M	11.4	99.6 ± 19	13.9 ± 7.4	781	65	46.7	4.0
		F	9.5	76.9 ± 16	6.7 ± 2.2	507	91	57.0	6.7
	12	M	11.9	381 ± 19	97.8 ± 54	4698	32	51.7	1.88
		F	6.0	298 ± 13	14.1[b]	2236	44	46.2	5.09

Mean ± SD shown for parameters calculated in individual animals, other parameters were calculated from pooled data.
Abbreviations: $t_{1/2}$, half-life; C_{max}, maximum observed plasma concentration; C_{24}, trough plasma concentration 24 h after dosing; AUC_{0-24}, Area under plasma concentration vs. time curve during 24 h dosing interval; V_d, volume of distribution; V_c, volume of the central compartment; CL, total clearance.
[a] $n = 2$.
[b] $n = 1$.

increased by less than 2-fold over the 91-day study. As observed after a single dose, both clearance and volume (V_d) fell with increasing dose on Day 91. In addition, the Day 91 values for these parameters were lower than the single dose values at all doses. The magnitude of these changes appeared to be larger in males than in females. The volume of the central compartment (V_c) remained relatively constant with dose and time. Plasma concentrations measured in recovery animals 8 days after the last dose were detectable (>0.1 μg/ml) only in the 12 mg/kg AmBisome group (0.21 ± 0.01 μg/ml for males, 0.57 ± 0.94 μg/ml for females). In this group, the apparent plasma half-lives of amphotericin B during the first week of the recovery period were 19 h for males and 36 h for females.

Tissue Pharmacokinetics of Amphotericin B

Amphotericin B concentrations in liver, spleen, kidneys, and lungs were measured for 30 days following AmBisome administration (Fig. 3, Table 3). As previously observed, reticuloendothelial system (RES) tissues had the highest amphotericin B concentrations (16,17). After 91 days of AmBisome, concentrations in liver and spleen reached 1000–10,000 μg/g in the mid- and high-dose groups. Concentrations in the kidneys and lungs were lower, reaching 100 μg/g only in the high-dose group. During recovery, the elimination of amphotericin B from tissues was slower than its disappearance from plasma, with apparent tissue half-lives of 1–4 weeks. In the high-dose group, tissue concentrations in the spleen, kidneys, and lungs were 2- to 3-fold higher in males than in females. Tissue exposures increased more than dose-proportionally but were proportional to plasma exposures at all doses, except in the livers of high-dose males (Fig. 4). In the kidneys, tissue AUC was nearly equal to the plasma AUC (overall mean $K_P = 1.09$). In the lungs, tissue AUC was slightly lower than the plasma AUC (Table 4). The liver and spleen had tissue AUCs 16- to 108-fold higher than the AUC in plasma. The tissue-plasma exposure ratio (K_P) in the liver appeared to decrease with increasing dose, and to be higher in females than males. The elevations in BUN, when correlated with concentrations of amphotericin B in the kidneys, appeared to be less than proportional (Fig. 5).

DISCUSSION

The safety of AmBisome following a 91-day intravenous exposure in rats was similar to the profile observed after 30 days (16), with no evidence of additional target tissues or delayed effects. At 1, 4, and 12 mg/kg/day, rats tolerated Am-

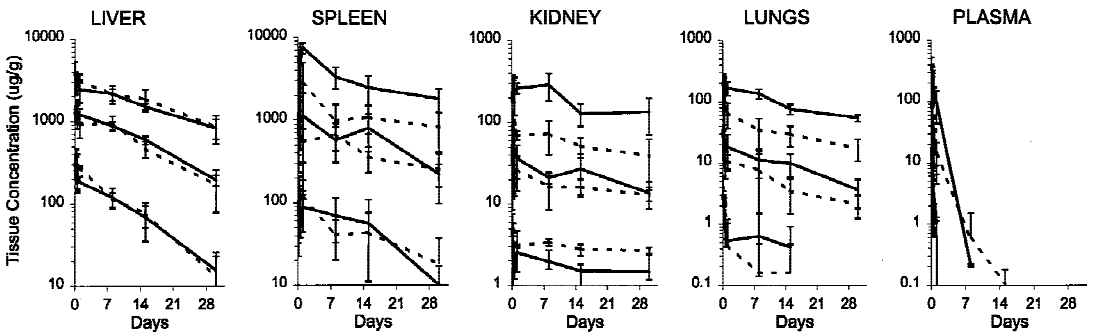

Fig. 3. Concentrations of amphotericin B (mean ± SD) in tissues (mg/g) and plasma (mg/ml) measured from 0.25 h to 30 days following the last of 91consecutive daily doses of intravenous AmBisome (1, 4, or 12 mg/kg) in rats. Solid lines, males; dotted lines, females.

Table 3. Mean (SD) Amphotericin B Concentrations (μg/g) in Tissues at Three Timepoints Following Administration of the Last of 91 Daily Doses of AmBisome[a]

Dose (mg/kg)	Sex	Liver			Spleen			Kidneys			Lungs		
		0.25 h	24 h	720 h	0.25 h	24 h	720 h	0.25 h	24 h	720 h	0.25 h	24 h	720 h
1	M	312	183	15.5	104	89	10	4.1	2.5	1.5	3.0	0.53	[b]
		(76)	(32)	(7)	(39)	(36)	(7)	(1.8)	(0.4)	(0.9)	(0.5)	(0.5)	
	F	299	277	13	74	116	18	3.5	3.1	2.7	4.0	0.44	[b]
		(108)	(11)	(13)	(41)	(72)	(19)	(1.2)	(1.6)	(0.2)	(2.1)	(0.8)	
4	M	1274	1205	193	1203	1130	222	33	36	14	34	18	3.6
		(285)	(227)	(27)	(544)	(991)	(67)	(2.1)	(16)	(5)	(8.6)	(11)	(1.8)
	F	1045	913	168	610	547	252	30	27	13	20	11	2.1
		(100)	(279)	(90)	(178)	(236)	(155)	(21)	(12)	(2.0)	(8.1)	(2.8)	(0.9)
12	M	3709	2452	829	5264	7495	1804	185	262	134	191	167	55
		(1623)	(764)	(229)	(2356)	(1147)	(581)	(48)	(40)	(63)	(40)	(41)	(6.9)
	F	2556	3095	853	1798	2773	818	84	70	39	81	66	17
		(280)	(656)	(320)	(849)	(2220)	(425)	(51)	(8.9)	(23)	(18)	(29)	(6.7)

[a] At 0.25 and 24 h time points, $n = 3$. At 720 h time point, $n = 4$.
[b] Below assay limit of quantitation.

Bisome with a spectrum of changes that did not increase in severity over the latter part of the exposure period, and that were largely reversible during the 30-day recovery. At the highest dose, all but one of 60 rats survived the 91-day study, with minimal changes in renal function, at a daily dose 5 times higher than the reported single-dose LD_{50} for conventional amphotericin B (20). Other changes, including urinary epithelial hyperplasia, renal tubular changes, elevated hepatic enzyme levels, and hepatocellular necrosis were of a nature and magnitude similar to those observed previously in rats exposed to AmBisome and other amphotericin B formulations (16,21,22). Changes associated with the administration of liposomes, including vacuolated tissue macrophages and hypercholesterolemia, were observed with other liposome formulations (23,24), and were not demonstrated to be of toxic consequence.

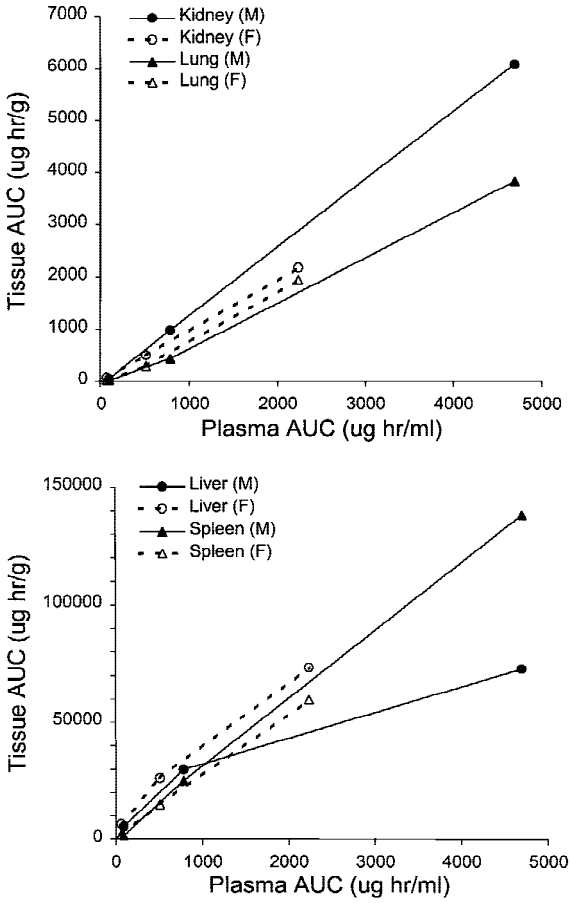

Fig. 4. Relationship between amphotericin B exposures (AUC from 0–24 h) in plasma to those in tissues following the last of 91 daily injections of AmBisome (1, 4, or 12 mg/kg) in rats.

Amphotericin B nephrotoxicity may involve hemodynamic, glomerular, and tubular mechanisms that produce complex changes in overall renal function not easily assessed by any single indicator of renal function (22,25). However, the minimal, nonprogressive elevations in BUN, the lack of changes in creatinine, and the absence of marked histomorphologic changes in the tubules suggest that no significant renal functional changes occurred even after 91 days of AmBisome at 12 mg/kg/day. AmBisome's renal effects appeared to be self-limiting, and did not involve marked reductions in GFR or tubular function observed with conventional amphotericin B (25).

The pharmacokinetic parameters (AUC, clearance, half-life, and V_d) we observed after 91 days were similar to those observed in a 30-day rat study (16), suggesting that the reduced clearance and V_d observed after repeated-dosing with AmBisome occurred largely during the first month, with little subsequent change upon continued exposure. Tissue concentrations on Day 91 were higher than those observed in the 30-day study, especially in the liver and spleen, suggesting that these tissues continued to accumulate drug with continuing exposure to AmBisome. Kidney AUCs were similar to plasma AUCs in both the 30-day and 91-day studies. This, and the observation that renal impairment increases less than proportionally as kidney amphotericin B concentrations increase (Fig. 5), help to explain the lack of progressive nephrotoxicity observed during the 91-day study, as renal pharmacokinetic and toxicologic endpoints plateau after approximately 1 month of daily dosing. Although the liver and spleen continued to accumulate amphotericin B throughout the study, there was little evidence of progressive toxicity in these tis-

Table 4. Amphotericin B Exposures (AUC_{0-24}) and Tissue/Plasma Exposure Ratios (K_P) in RES Tissues (Liver, Spleen), Kidneys, and Lungs after 91 Daily Doses of Intravenous AmBisome in Rats

		Liver		Spleen		Kidneys		Lungs	
Dose (mg/kg)	Sex	AUC_{0-24} ($\mu g\ h\ g^{-1}$)	K_P	AUC_{0-24} ($\mu g\ h\ g^{-1}$)	K_P	AUC_{0-24} ($\mu g\ h\ g^{-1}$)	K_P	AUC_{0-24} ($\mu g\ h\ g^{-1}$)	K_P
1	M	5623	62.3	1779	19.7	59	0.65	27	0.30
	F	6682	107.9	2343	37.9	84	1.36	23	0.37
4	M	29835	38.2	25072	32.1	984	1.26	437	0.56
	F	26299	51.9	14989	29.6	501	0.99	294	0.58
12	M	72761	15.5	138110	29.4	6091	1.30	3837	0.82
	F	73411	32.8	59614	26.7	2177	0.97	1944	0.87

AUC_{0-24}, Area under the tissue concentration vs. time curve during the 24 h interval after the last of 91 daily intravenous doses of AmBisome; K_p, ratio of tissue to plasma AUC_{0-24}.

sues. Females showed no increase in ALP, AST, or ALT levels during the last month of the study, and these enzymes were not elevated in males at any time, even though liver concentrations of amphotericin B exceeded 3000 μg/g in these animals. This study confirms the ability of phagocytically active tissues such as the liver and spleen to accumulate large quantities of liposomal drugs without severe toxicity (2,26), and suggests that the prolonged tissue residence times we observed result from sequestration of the liposomal drug in tissue compartments in which its diffusional and toxicologic activities are reduced. Compared to conventional amphotericin B, AmBisome both increased and prolonged amphotericin B concentrations in the liver and spleen. Half-lives of AmBisome during elimination from RES tissues in this study were 1–-2 weeks. Tissue half-lives reported for conventional amphotericin B were 2–3 days (26). The eventual elimination of drug from these deep compartments via excretion or metabolism, and its influence on the duration of AmBisome activity and safety, remain to be determined. Although this study was performed in healthy animals, these data suggest AmBisome may also increase and prolong tissue concentrations in infected tissues.

The similarity of amphotericin B concentrations in the kidneys and lungs to those in plasma after AmBisome exposure suggests that concentrations in these tissues could be predicted from plasma drug level monitoring in patients, and that these tissue concentrations approach steady-state along with concentrations in plasma. Although the half-life of AmBisome in the kidneys was longer than in plasma, nephrotoxicity rapidly reversed during recovery. The elimination of AmBisome from the kidneys was slower than that of conventional amphotericin B (26), suggesting that the kidneys also sequester AmBisome in compartments with diminished distributional and toxicologic activity. This hypothesis is supported by the observation that rats tolerated higher concentrations of amphotericin B in the kidneys than those reported for other amphotericin B formulations at similar levels of nephrotoxicity (26). Thus, AmBisome's ability to alter the disposition of amphotericin B appears to protect the kidneys from the drug's nephrotoxic effects.

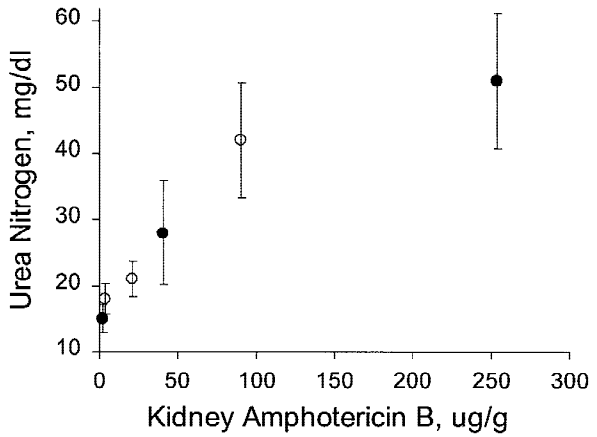

Fig. 5. Relationship between urea nitrogen (BUN) levels (mean ± SD, Day 89) and amphotericin B concentrations (Css) in the kidney after the last of 91 daily injections of AmBisome (1, 4, or 12 mg/kg). Males, solid circles; females, open circles.

This study examined the unique pharmacokinetic properties of AmBisome in rats. The plasma AUC after a single 1 mg/kg dose of AmBisome (43 $\mu g\ h^{-1}\ ml^{-1}$) was an order of magnitude higher than AUCs after similar doses of amphotericin B deoxycholate (4.0 $\mu g\ h^{-1}\ ml^{-1}$) or amphotericin B colloidal dispersion (4.4 $\mu g\ h^{-1}\ ml^{-1}$) in rats (27,28). AmBisome Day 91 tissue exposures in the liver, spleen, kidneys, and lungs were higher than those reported for other amphotericin B formulations (3,26). The critical association of the specific pharmacokinetic profile of AmBisome with its improved safety is demonstrated by comparison with a similarly sized liposomal amphotericin B that did not contain the charged phospholipids and cholesterol present in AmBisome. This formulation did not prolong plasma residence, and caused nephrotoxicity similar to conventional amphotericin B in rats (22).

Studies in rats, dogs, and humans have shown nonlinearity in the pharmacokinetics of AmBisome (9,16,17). In this study, both clearance and volume of distribution (V_d) decreased in proportion to the total dose administered, as observed in dogs (17). Plasma half-lives also increased over the course of the 91-day study. In contrast, the central compartment volume (V_c) appeared to change little with dose or time. This, and the observation that V_d approaches V_c as the plasma profile becomes more monoexponential at higher doses, suggest that the rapid initial phase of the plasma concentration profile represents a saturable clearance process. As the total dose of AmBisome is increased, the contribution of the rapid clearance process is reduced, resulting in the observed decreases in apparent clearance and volume of distribution. The plasma half-life (6–12 h) appears to change less markedly across doses, durations, species, and genders, sug-

gesting the existence of a slower, less saturable clearance mechanism. The observed apparent clearance varies as the relative contributions of the two clearance mechanisms changes with dose and time. This explanation is consistent with previous reports that RES uptake is saturable (29), that liposome uptake can occur by both saturable and nonsaturable pathways (30), and that liposome clearance may decrease in proportion to the total exposure to liposomal lipid (30). Our observation that the liver-plasma exposure ratio (K_P) falls with dose also suggests that liver uptake of AmBisome becomes saturated with increasing dose and time. The saturation of hepatic uptake was more apparent in males, who had lower liver K_P values than females. This is consistent with the lower total clearance and higher concentrations in spleen, kidneys, and lungs observed in males. The relationship of this gender difference to the increased incidence of hepatotoxicity in females is unknown. Gender differences in AmBisome toxicity have not been reported in other species (10,17).

The results of this study support the safety of daily administration of intravenous AmBisome for 91 days at clinically relevant doses. Although drug accumulation in plasma and some tissues was nonlinear and progressive, toxicologic changes associated with AmBisome reached a plateau early in the study, with no evidence of delayed onset. Despite prolonged residence times of drug in tissues after AmBisome exposure, all toxic changes demonstrated reversibility during the recovery period. The unique ability of AmBisome to sequester amphotericin B in stable liposomes within the plasma and tissue compartments for an extended time appears to modulate the drug's toxicity, protecting the kidneys from the nephrotoxic effects of amphotericin B, and allowing higher and longer exposures to be tolerated without severe toxicity.

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