Comparative Tissue Distribution and Elimination of Amphotericin B Colloidal Dispersion (Amphocil®) and Fungizone® After Repeated Dosing in Rats[1]

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Received August 2, 1994; accepted September 19, 1994

The pharmacokinetic profiles of amphotericin B (AmB) after administration of Amphocil®, an AmB/cholesteryl sulfate colloidal dispersion (ABCD) and the micellar AmB/deoxycholate (Fungizone®) were compared after repeated dosing in rats. After administration of ABCD and Fungizone at an equal AmB dose (1 mg/kg), AmB concentrations in plasma and most tissues were lower for the ABCD dose, especially in the kidneys where reduced drug concentration correlated with reduced nephrotoxicity. In contrast, AmB concentrations in the liver were substantially higher when ABCD was administered; however, without an accompanying increase in hepatotoxicity. Daily administration of ABCD for 14 days did not lead to AmB accumulation in plasma; while a slight accumulation was observed after multiple administration of Fungizone. AmB was eliminated more slowly from the plasma and various tissues and urinary and fecal recoveries of AmB were reduced after ABCD administration. These results suggest that ABCD may be stored in tissues in a form that is less toxic and is eliminated from the systemic circulation by a different mechanism than the free and protein-bound AmB in plasma. AmB accumulation in the spleen was observed when higher doses of ABCD (5 mg/kg) were administered, which could be due to saturation of hepatic uptake of AmB. Comparison of spleen concentrations of AmB between ABCD and Fungizone® at 5 mg/kg AmB doses was not possible because of Fungizone's toxicity in rats. In all other organs, AmB concentrations reached or approached a steady state within two weeks of dosing with ABCD. Urinary and fecal clearances of AmB were not different between ABCD and Fungizone administration. In summary, the distribution and elimination characteristics of AmB in rats were substantially altered when it was administered as ABCD in comparison to Fungizone. Nephrotoxicity of AmB in rats was reduced after administration of ABCD apparently because of the altered tissue distribution pattern. Thus, ABCD (Amphocil®) may be a clinically beneficial formulation of AmB in patients with systemic fungal infections.

KEY WORDS: Amphotericin B; Amphocil®; Fungizone®; Colloidal Dispersion; Tissue Distribution; Pharmacokinetics; Rat.

[1] This work was presented in part at the American Association of Pharmaceutical Scientists Fifth Annual Meeting, November, 1990 in Las Vegas, NV.

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INTRODUCTION

Amphotericin B is a polyene antifungal agent which remains the drug of choice for invasive and disseminated fungal infections, despite its nephrotoxicity and other serious side effects (1, 2). Several lipid-based dosage forms of amphotericin B have recently been developed in attempts to improve the therapeutic index of amphotericin B by altering its plasma and tissue distribution profiles (3-6). Preclinical and clinical studies have demonstrated that lipid-based formulations of amphotericin B retain antifungal efficacy and exhibit significantly reduced toxicity and altered pharmacokinetics profiles of amphotericin B (6-12).

Amphocil® is a stable amphotericin B colloidal dispersion (ABCD) formed with sodium cholesteryl sulfate, a naturally occurring cholesterol metabolite (13). ABCD has been shown to have reduced acute and chronic toxicities compared with conventional deoxycholate-solubilized amphotericin B (Fungizone) in dogs, rats and mice (14) but retains *in vivo* antifungal activity against coccidioidomycosis in mice (15). The pharmacokinetics and tissue distribution of amphotericin B after administration of ABCD and Fungizone have been compared previously in rats after a single intravenous injection (16). When compared to Fungizone, ABCD produced reduced plasma concentrations and reduced (by 3 to 7 fold) kidney concentrations, but increased (by 2 to 3 fold) liver concentrations of amphotericin B in rats at various time points after single administration. In dogs, reduced kidney concentrations of amphotericin B after ABCD administration were associated with reduced renal toxicity (17). Since amphotericin B is given chronically for systemic infections, it is necessary to evaluate whether ABCD also exhibits altered pharmacokinetics and tissue distribution characteristic of amphotericin B after multiple dosing.

In this study, the pharmacokinetics and tissue distribution of amphotericin B after daily intravenous administration of ABCD and Fungizone® at an amphotericin B dose of 1 mg/kg/day for 14 days were compared in Sprague-Dawley rats. ABCD was also administered at a higher dose level, 5 mg/kg/day, for 14 days; however, this was not feasible for Fungizone® due to its toxicity (18). Amphotericin B concentrations in plasma, whole blood, and tissues (liver, kidney, spleen, heart, lung, brain, and skeletal muscle) were analyzed at various time points during the study. Urinary and fecal recoveries of amphotericin B were also determined. This study has defined the pharmacokinetic characteristics and potential therapeutic benefit of ABCD as an improved drug delivery system for repeated dosing of amphotericin B.

MATERIALS AND METHODS

Test Materials

Lyophilized ABCD, amphotericin B colloidal dispersion (Amphocil®, Liposome Technology, Inc., Menlo Park, CA), and micellar amphotericin B (Fungizone®, E.R. Squibb & Sons) were reconstituted with sterile water for injection and used within 48 hours. The reconstituted solutions were diluted daily (immediately prior to dosing) with sterile 5% dextrose for injection to the concentrations required for admin-

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istration to each test group. The unused reconstituted solutions of ABCD and Fungizone were stored refrigerated at about 4 °C and were stable for at least one week. The particle size of reconstituted ABCD, measured by dynamic light scattering, was 93 ± 2.0 nm (mean ± S.D.).

Animal Experiments

Male Crl:CD(SD)BR rats weighing 150 to 200 g (Charles River Laboratories, Portage, MI) were housed in accordance with the standards of the National Institutes of Health. Three groups of 26 rats each were randomly assigned to receive either Fungizone (1 mg/kg), or ABCD (1 mg/kg or 5 mg/kg). No attempt was made to administer Fungizone at 5 mg/kg because this dose exceeds the reported 50 % lethal dose of Fungizone in rats (2.4 mg/kg) (18). Test materials were administered daily to the rats by bolus tail vein injection for 14 consecutive days (19). Doses for each animal were adjusted for body weight gain after 7 doses. Groups of five animals per treatment were sacrificed at 24 hours after the 6th, 10th, and 14th doses (Days 6, 10 and 14 samples), respectively. Another five animals per treatment were sacrificed 5 days after receiving the last dose (Day 18 sample) and the other six animals per treatment were sacrificed 14 days after the last dose (Day 28 sample). At the time of sacrifice, blood and tissue samples (brain, heart, lungs, kidneys, liver, spleen, and hind limb skeletal muscle) were collected, weighed and frozen at − 20 °C or below until analysis. Urine and fecal samples were collected daily throughout the study from the groups of six animals sacrificed on Day 28 for each treatment. The volume of pooled urine from six animals per group per day was measured and aliquots of the urine were analyzed. Daily fecal samples were also pooled from six animals per group for analysis.

In a separate experiment for studying plasma profiles, blood samples were obtained from animals, via the retro-orbital sinus as described previously (19), following the first injection (Day 1) for each treatment (n = 4) at pre-dose and 0.5, 1, 2, 4, 6, 8, and 24 hours post dosing. After the last injection (Day 14), blood samples were also obtained at the above time points (n = 4 per treatment). In addition, blood samples collected at 96 hours after the last injection (Day 18 sample, n = 5 per treatment) from the above experiment were used for plasma profile assessments. Plasma was separated by centrifugation and stored frozen at − 20 °C or below until analysis.

Animals were observed daily for signs of toxicity and body weights were recorded pre-study, on Day 7 and immediately prior to sacrifice. Clinical chemistry and hematology evaluations were performed prior to dosing and at necropsy. Urinalysis was performed on samples collected before, during and after the dosing period. Animals were sacrificed by exsanguination following pentobarbital anesthesia.

Amphotericin B Assay

Concentrations of amphotericin B in plasma, blood, tissues, urine and feces were determined using an HPLC method as described previously (20). Plasma (0.5 ml) and urine samples (1-2 ml) were first extracted with a solid-phase column (Bond-Elut™ C-18, 1 ml) and then separated on a reverse-phase column (Waters μBondpack C-18, 30 cm × 3.9 mm, 10 μ) with a mobile phase of 45 % acetonitrile in 2.5 mM Na_2-EDTA. The amphotericin B peak was detected by UV absorbance at 382 nm. The recovery of amphotericin B from spiked plasma was > 90 %. The assay sensitivity was ≤ 5 ng/ml for plasma and was 2.5 ng/ml for urine. The linearity of the assay was shown across the range of the calibration standards (5 - 2500 ng/ml for plasma and 2.5 to 500 ng/ml for urine). Intra- and inter-day assay variabilities were approximately 5 % (coefficient of variation) for plasma and 10 % for urine.

Blood (0.5 ml), tissues (0.3 - 0.5 g) and fecal (total daily collection) samples were first homogenized and extracted with methanol and the supernatant of the methanol extract was loaded onto Bond-Elut™ for further separation as described above. Standard curves for each sample type were constructed by spiking blank samples obtained from untreated rats of similar age and weight. The recoveries of amphotericin B from spiked blood and tissue samples were 70 % and 75 %, respectively. The assay sensitivity was ≤ 25 ng/ml for blood, 50 ng/g for tissues and 5 μg/daily fecal output for feces. The assay was linear up to 5 μg/ml for blood, 500 μg/g for tissues and 1 mg/daily fecal output for feces. Intra- and inter-day assay variabilities were generally < 10 % for all sample types.

Pharmacokinetic and Statistical Analysis

Pharmacokinetic analysis of the plasma concentration vs. time data was performed using noncompartmental methods with the RSTRIP program (Micromath, Salt Lake City, Utah). This program calculates the area under plasma concentration vs. time curve (AUC) using trapezoidal rule and estimates the terminal half-life of amphotericin B in plasma using a non-linear, weighted, least-squares regression. Apparent plasma clearance (CL) and steady-state volume of distribution (V_{ss}) were calculated using data obtained from Day 14 according to the following equations:

$$CL = \text{Dose} / (AUC_{0\text{-}24})_{ss}$$

$$V_{ss} = \text{Dose} \times [(AUMC_{0\text{-}24})_{ss} + 24 \times (AUC_{24\text{-}\infty})_{ss}] / [(AUC_{0\text{-}24})_{ss}]^2$$

where V_{ss} was calculated with correction for multiple dosing (21). $(AUC_{0\text{-}24})_{ss}$ and $(AUMC_{0\text{-}24})_{ss}$ are the AUC and area under the 1st moment curve during the 24-hour dosing interval at steady state (Day 14), respectively. $(AUC_{24\text{-}\infty})_{ss}$ is the AUC from 24 hours post dosing of the last dose at steady state to infinity, which is the sum of $AUC_{24\text{-}96}$ and $AUC_{96\text{-}\infty}$ after the last dose. Values of CL and V_{ss} were not calculated on Day 1 because of the relatively short sampling time (24 hours) as compared to amphotericin B half-life.

The amount of amphotericin B recovered in each organ or tissue was calculated as the product of the measured organ or tissue weight and drug concentration. Total volume and weight of blood and skeletal muscle were estimated as 6.5 % and 49 % of total body weight, respectively. The half-life of amphotericin B removal (elimination) from each organ or tissue was calculated from the slope of a log-linear curve of the Day 14 to Day 28 tissue concentration vs. time data. Renal clearance (CL_r) of amphotericin B was calculated as $CL_r = A_u / AUC_{0\text{-}24}$, where A_u is the 24-hour urinary recov-

Table I. Pharmacokinetic Parameters of Amphotericin B in Rats after Single (Day 1) and Multiple (Day 14) Intravenous Administration of Fungizone and ABCD

	Day 1			Day 14		
	Fungizone (1 mg/kg)	ABCD (1 mg/kg)	ABCD (5 mg/kg)	Fungizone (1 mg/kg)	ABCD (1 mg/kg)	ABCD (5 mg/kg)
C_{min}* (ng/ml)	56.5 ± 6.9	36.9[b] ± 6.9	69.5[a] ± 9.9	82.9 ± 14.9	44.8[b] ± 9.6	70.8[a] ± 10.0
$T_{1/2}$, β (hr)	14.1	33.6	82.1	19.7	47.4	62.0
AUC_{0-24} (ng · h/ml)	2282	1296	2534	3210	1327	2641
AUC_{0-96} (ng · h/ml)				5315	4450	9980
CL (L/h/kg)				0.29	0.69	1.76
V_{ss} (L/kg)				24.1	46.8	147

* C_{min} was measured at 24 hrs after dosing on both days. Values presented are mean ± SD.
[a] Significantly different from Fungizone and from 1 mg/kg ABCD, $p < 0.001$.
[b] Significantly different from Fungizone, $p < 0.05$.

ery of amphotericin B and AUC_{0-24} is the area under the plasma concentration-time curve over the 24-hour dosing period at steady state. Clearance of amphotericin B via fecal excretion (fecal clearance) was calculated in a manner similar to CL_r. Mass balance of total drug recoveries on Days 6, 10, 14, 18 and 28 were calculated as the sum of the amount of amphotericin B in each of the organs/tissues examined and the cumulative urinary and fecal recoveries at each time point.

Statistical analysis was performed to compare data between formulations and between dose levels using ANOVA and a two-sample t-test (one-sided) with unequal variance.

RESULTS

Plasma Concentrations of Amphotericin B

Pharmacokinetic parameters of amphotericin B calculated after the first dose and fourteenth dose of ABCD and Fungizone are presented in Table I. The plasma concentration vs. time profiles and the calculated pharmacokinetic parameters of amphotericin B obtained on Day 1 were similar to those obtained on Day 14 in all treatment groups (Figure 1). In addition, plasma amphotericin B concentration-time profiles obtained during Day 14 to Day 28 showed terminal half-lives similar to those obtained on Day 1. At an equal dose level, Fungizone showed about 2 fold higher plasma amphotericin B concentrations than ABCD, but the terminal half-life of amphotericin B was 2.5 times shorter after Fungizone administration. The Day 14 AUC_{0-96} ratio between ABCD (1 mg/kg) and Fungizone was close to 1. A fivefold increase in the dose of ABCD did not produce a proportional increase in plasma concentrations of amphotericin B, but resulted in a longer terminal half-life. The plasma amphotericin B concentration-time profiles after 5 mg/kg ABCD were similar to those found after 1 mg/kg Fungizone (Figure 1). The Day 14 AUC_{0-96} ratios between 5 mg/kg ABCD and Fungizone, and between 5 mg/kg ABCD and 1 mg/kg ABCD were approximately 2.

Based on the C_{min} values, no accumulation of amphotericin B in plasma was found after multiple dosing with ABCD, but a slight accumulation (~ 50 %) was observed after multiple administration of Fungizone. The extent of amphotericin B accumulation in plasma after multiple dosing of Fungizone was consistent with the accumulation index (1.45) calculated using the terminal half-life (14.1 hours) obtained after the first dosing. However, the terminal half-lives observed after ABCD administration on Day 1 (33.6 and 82.1 hours at the 1 and 5 mg/kg doses, respectively), would predict accumulation indices of 2.56 and 5.45 at these doses. The lower observed values (1.21 and 1.02) suggest non-linear kinetics in amphotericin B disposition.

The values for the apparent plasma clearance (CL) and

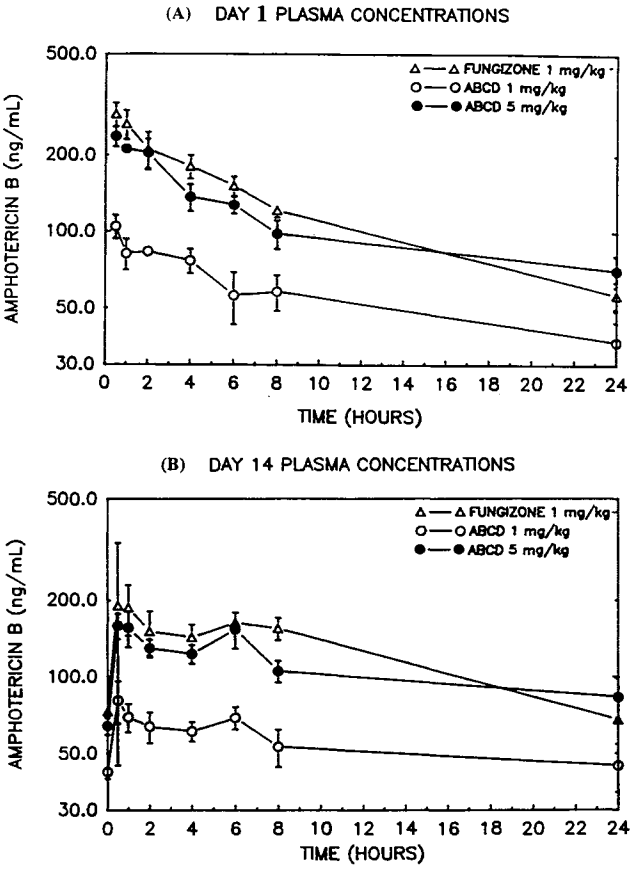

Figure 1. Plasma concentrations of amphotericin B in rats on Day 1 (A) and on Day 14 (B) following intravenous administration of Fungizone and ABCD. Values are expressed in mean ± S.D. (n = 4). (Δ) Fungizone 1 mg/kg, (○) ABCD 1 mg/kg and (●) ABCD 5 mg/kg.

steady-state volume of distribution (V_{ss}) of amphotericin B were both formulation and dose dependent. At an equal dose level, higher CL and V_{ss} values (2 to 2.5 fold) were found after ABCD administration. A fivefold increase in the dose of ABCD resulted in 2.5 to 3 fold increase in the CL and V_{ss} values.

Tissue Concentrations of Amphotericin B

Concentrations of amphotericin B in 3 major organs/tissues (liver, spleen, and kidneys) are shown in Figure 2. Due to space limitation, amphotericin B concentration-time profiles in other tissues are not presented, but are discussed in the text along with those in these 3 major organs. Tissue concentrations generally approached a steady state by the end of the two-week dosing period. Day 14 tissue levels were either lower than or only slightly higher than Day 10 and/or Day 6 levels in all tissues and in all dosing groups. The only exception was in the spleen after administration of 5 mg/kg ABCD, where tissue levels increased steadily throughout the dosing period. The highest concentrations of amphotericin B were observed in the liver and spleen (up to 200 μg/g of tissue), followed by the lungs and kidneys (1 - 10 μg/g), and then the heart, skeletal muscles, brain and blood (< 1 μg/g or μg/ml). This order of magnitude generally held for both formulations, except that after Fungizone administration the highest tissue concentrations were found in the spleen but not liver.

The mean (±S.D.) concentrations of amphotericin B in various tissues on Day 14 for the 3 dosing groups are presented in Table II. Again, due to space limitation, only mean concentrations on Day 14 are presented. The relationships of amphotericin B tissue concentrations between the three dosing groups were similar on all sampling days. ABCD (1 mg/kg) administration resulted in lower amphotericin B concentrations in the kidneys, lungs, spleen, heart, skeletal muscle, brain and blood, as compared to Fungizone ($p < 0.01$). ABCD produced approximately 2 to 3 fold lower amphotericin B concentrations in all tissues except liver, as compared to Fungizone. In contrast, amphotericin B concentrations in liver were approximately 5 fold higher after ABCD administration as compared to an equal dose of Fungizone. Tissue amphotericin B concentrations increased with the dose of ABCD in all tissues studied ($p < 0.05$), but the magnitude of this increase varied considerably between tissues (Table II). The five-fold increase in dose of ABCD caused 2 to 4 fold increase in amphotericin B concentration in most tissues except spleen (18 fold) and lungs (6.6 fold). Amphotericin B concentrations in red blood cells appeared to exceed those in plasma on Day 14 in all three dosing groups. The ratios of blood to plasma amphotericin B concentration on Day 14 were 1.32, 1.22, and 2.82 for Fungizone, 1 mg/kg ABCD and 5 mg/kg ABCD, respectively.

Amphotericin B concentrations in all tissues declined steadily during the two-week washout period following the last dose (from Days 14 to 28) for all three dosing groups, with one exception which was in the spleen after the 1 mg/kg ABCD administration (Figure 2). The half-lives of amphotericin B removal (washout) from various organs/tissues are shown in Table III. At an equal dose level (Fungizone vs. ABCD), there was no significant differences between Fungizone and ABCD in the rate of amphotericin B washout except from the liver and lungs. The washout of amphotericin B from liver and muscle appeared to be slower after the administration of 5 mg/kg ABCD when compared to 1 mg/kg ABCD.

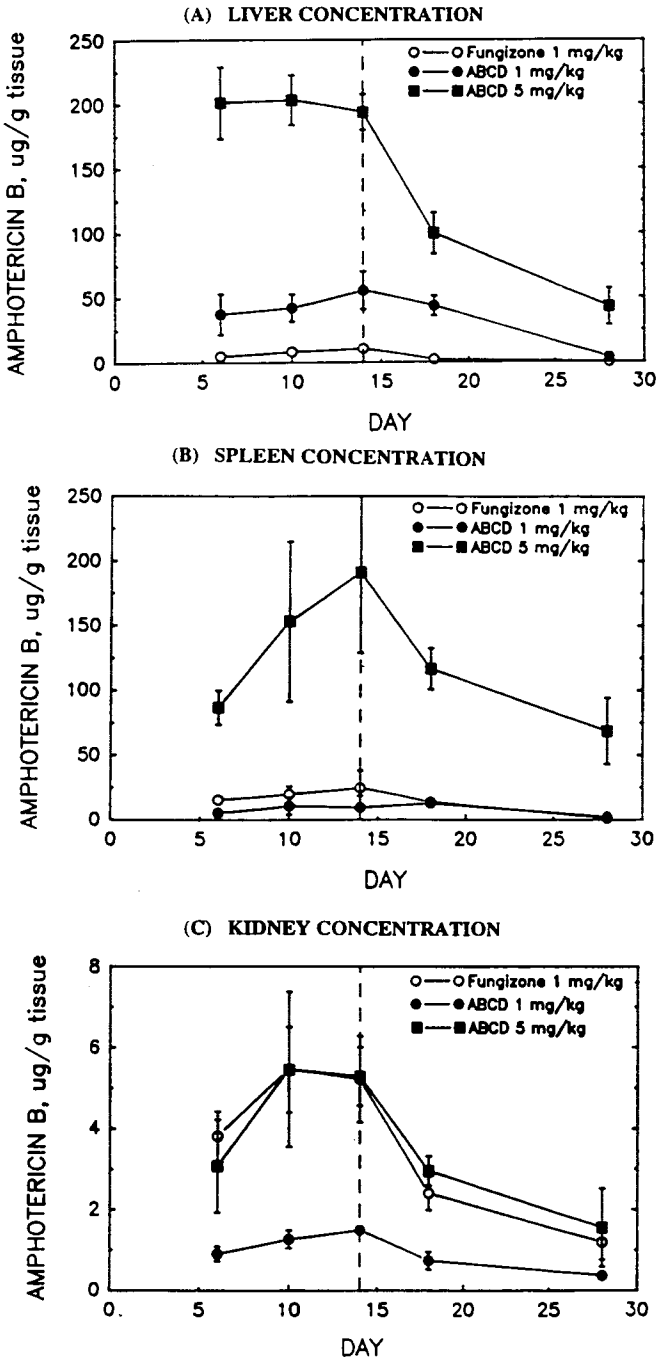

Figure 2. Concentrations of amphotericin B in liver (A), spleen (B) and kidneys (C) during and after 14-day repeated administration of Fungizone and ABCD in rats. Values shown are mean ± S.D. (n = 5 or 6). (△) Fungizone 1 mg/kg, (○) ABCD 1 mg/kg and (●) ABCD 5 mg/kg.

Urinary and Fecal Recoveries of Amphotericin B

The total cumulative urinary recovery of amphotericin

Table II. Mean (±S.D.) Amphotericin B Concentrations* (μg/g or μg/ml) in Various Rat Biological Matrices on Day 14 Following Multiple Daily Administration of Fungizone and ABCD

Sample type	1 mg/kg Fungizone[a]	1 mg/kg ABCD[a,b]	5 mg/kg Fungizone[b]
Liver	10.6 ± 3.7	55.4 ± 14.6	193.7 ± 13.8
Spleen	24.8 ± 13.3	9.42 ± 9.12	191.1 ± 62.4
Kidney	5.21 ± 1.06	1.49 ± 0.15	5.29 ± 0.72
Lung	3.16 ± 0.57	1.10 ± 1.15	7.37 ± 2.90
Brain	0.078 ± 0.015	0.031 ± 0.006	0.119 ± 0.069
Muscle	0.21 ± 0.06	0.12 ± 0.04	0.27 ± 0.12
Heart	0.43 ± 0.11	0.12 ± 0.03	0.55 ± 0.12
Blood	0.11 ± 0.02	0.055 ± 0.015	0.20 ± 0.11
Plasma	0.083 ± 0.015	0.045 ± 0.010	0.071 ± 0.010

* All concentrations measured at 24 hours following the 14th dose.

[a] Amphotericin B concentrations in all sample types are significantly different between Fungizone and 1 mg/kg ABCD groups ($p < 0.05$).

[b] Amphotericin B concentrations in all sample types are significantly different between 1 mg/kg ABCD and 5 mg/kg ABCD ($p < 0.05$).

B from Day 1 to Day 28 was 3.95 % of the total dose administered for 1 mg/kg/day Fungizone, 2.13 % for 1 mg/kg/day ABCD and 0.85 % for 5 mg/kg/day ABCD (Table IV). During the dosing period (Days 1 to 14), the daily urinary recovery of amphotericin B ranged from 2.6 % to 5.2 % of the daily dose administered for 1 mg/kg/day Fungizone, from 1.0 % to 2.7 % for 1 mg/kg/day ABCD and from 0.5 % to 0.9 % for 5 mg/kg/day ABCD. Renal clearances of amphotericin B determined on Day 14 were similar between all dosing groups which were 16.9, 12.7 and 10.4 ml/hr/kg in rats treated with 1 mg/kg Fungizone, 1 mg/kg ABCD and 5 mg/kg ABCD, respectively. The total cumulative fecal recovery of amphotericin B from Day 1 to Day 28 was 20 % of the total dose administered for 1 mg/kg/day Fungizone, 10.9 % for 1 mg/kg/day ABCD and 4.1 % for 5 mg/kg/day ABCD. During the dosing period (Days 1 to 14), the daily fecal recovery of amphotericin B ranged from 11.3 % to 27.0 % for Fungizone, from 3.4 % to 13.4 % for 1 mg/kg ABCD and from 1.7 % to 4.1 % for 5 mg/kg ABCD, respectively. Fecal clearances of amphotericin B determined on Day 14 were 77.3, 90.3, and 68.6 ml/hr/kg for the Fungizone, ABCD 1 mg/kg and ABCD 5 mg/kg groups, respectively.

Total Recovery of Amphotericin B

The total recovery of amphotericin B from the urine, feces and the various tissues samples analyzed on Days 6, 14, and 28 for each dosing group (Mean ± S.D.) are presented in Table IV as the percent of the cumulative dose administered at the time of sampling. Recovery data obtained on Day 10 and Day 18 are omitted from presentation; however, Day 10 data are similar to those obtained on Day 14. In all cases, less than one third of the total cumulative dose administered was accounted for in all samples analyzed. After administration of 5 mg/kg ABCD, the total recovery of amphotericin B appeared to decrease with time (from 32.7 % on Day 6 to 16.4 % on Day 14 and then to 9.2 % on Day 28); while for Fungizone the total recovery initially increased with time and then maintained at a constant level (from 13.2 % on Day 6 to 25.6 % on Day 14 and then to 24.1 % on Day 28). At all time points measured; the majority of the amphotericin B dose administered as Fungizone was recovered in the urine and feces (47 - 99.3 % of the total recovery); while the majority of the amphotericin B dose administered as ABCD was recovered in the tissues (50 - 96 % of the total recovery), primarily in the liver. At all times, the percent of total recovery of amphotericin B in urine or feces followed the order: Fungizone 1 mg/kg > ABCD 1 mg/kg > ABCD 5 mg/kg. Most of the amphotericin B dose recovered from tissues was found in the liver for both ABCD and Fungizone. Very little amphotericin B was recovered in the brain, heart and blood.

Table III. The Half-Lives (days) of Amphotericin B Washout from Various Tissues

Tissue Type	Fungizone 1 mg/kg	ABCD 1 mg/kg	ABCD 5 mg/kg
Liver	1.7 (1.3–2.7)*	3.4 (2.9–4.0)	5.8 (4.8–7.2)
Spleen	2.8 (2.3–3.8)	4.3 (3.0–7.5)	8.6 (6.4–13.0)
Kidney	6.2 (4.9–8.4)	5.7 (4.3–8.5)	3.7 (2.9–5.2)
Lung	1.8 (1.3–2.7)	5.2 (3.5–10.5)	3.1 (2.5–3.9)
Brain	2.5 (2.1–3.0)	3.5 (2.7–5.1)	4.0 (2.2–17.9)
Muscle	1.6 (1.3–2.0)	1.7 (1.4–2.1)	4.2 (3.3–5.8)
Heart	1.7 (1.1–3.4)	2.4 (1.8–3.8)	3.8 (3.3–4.5)

* Values in parentheses are 95% confidence intervals. Half-Lives were determined using data obtained on Days 14, 18 and 28.

Tissue Concentration and Target Organ Toxicity

The relationships between amphotericin B concentrations and organ-specific clinical pathology parameters in two target organs (kidneys and liver) on Day 14 are shown in Figure 3. Serum urea nitrogen concentrations were measured as an indicator of nephrotoxicity, and alanine aminotransferase concentrations as an indicator of hepatotoxicity (22). Mean (± S.D.) serum urea nitrogen values after 14 daily repeated dosings of ABCD (18 ± 4.5 and 28 ± 8.1 mg/dL for the 1 and 5 mg/kg groups, respectively) and Fungizone (34 ± 6.0 mg/dL) all significantly exceeded the undosed control value (12 ± 1.7 mg/dL, $p < 0.05$). Serum urea nitrogen levels also differed significantly between the two

Table IV. Total Cumulative Recoveries of Amphotericin B from Various Biological Matrices in Rats on Days 6, 14, and 28 after Multiple Administration of Fungizone and ABCD

Sample Type	Percent of Total Dose Administered (%, Mean ± S.D.) Fungizone[a] (1 mg/kg)	ABCD[a,b] (1 mg/kg)	ABCD[b] (5 mg/kg)
Day 6			
Liver	3.49 ± 0.47	25.99 ± 14.56	29.18 ± 6.99
Spleen	0.72 ± 0.24	0.24 ± 0.12	0.85 ± 0.17
Kidney	0.78 ± 0.14	0.17 ± 0.03	0.12 ± 0.05
Lung	0.28 ± 0.07	0.03 ± 0.01	0.09 ± 0.01
Brain	0.01 ± 0.002	0.004 ± 0.001	0.002 ± 0.006
Muscle	1.63 ± 0.45	0.24 ± 0.06	1.04 ± 1.13
Heart	0.001 ± 0.001	0.00	0.001 ± 0.001
Blood	0.09 ± 0.01	0.03 ± 0.03	0.04 ± 0.03
Urine[c]	1.04	0.63	0.24
Feces[c]	5.17	3.06	1.14
Total (mean)	13.21	30.39	32.70
Day 14			
Liver	2.85 ± 1.40	14.76 ± 3.23	11.53 ± 2.87
Spleen	0.44 ± 0.24	0.22 ± 0.16	0.76 ± 0.24
Kidney	0.45 ± 0.10	0.13 ± 0.02	0.09 ± 0.02
Lung	0.15 ± 0.15	0.04 ± 0.04	0.06 ± 0.03
Brain	0.004 ± 0.007	0.002 ± 0.0003	0.001 ± 0.006
Muscle	0.77 ± 0.27	0.42 ± 0.15	0.20 ± 0.09
Heart	0.01 ± 0.004	0.004 ± 0.001	0.003 ± 0.001
Blood	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Urine[c]	3.55	1.74	0.66
Feces[c]	17.30	8.64	3.07
Total (mean)	25.57	26.00	16.39
Day 28			
Liver	0.02 ± 0.01	1.61 ± 0.60	3.91 ± 0.93
Spleen	0.03 ± 0.03	0.05 ± 0.05	0.26 ± 0.12
Kidney	0.11 ± 0.04	0.03 ± 0.01	0.03 ± 0.02
Lung	0.01 ± 0.001	0.005 ± 0.001	0.003 ± 0.001
Brain	0.00	0.00	0.00
Muscle	0.00	0.00	0.02 ± 0.01
Heart	0.00	0.00	0.00
Blood	0.00	0.00	0.00
Urine[c]	3.95	2.13	0.85
Feces[c]	20.00	10.90	4.09
Total (mean)	24.12	14.72	9.16

[a] Recoveries of AmB from all tissues are different between 1 mg/kg ABCD and Fungizone groups ($p < 0.05$).

[b] Recoveries of AmB from all tissues are different between 1 and 5 mg/kg ABCD groups ($p < 0.05$).

[c] No S.D. data because of pooled data.

formulations at the same dose level ($p < 0.001$). Amphotericin B concentration in the kidneys after 14 daily doses of 1 mg/kg Fungizone was 3.5 times higher than that after dosing with 1 mg/kg ABCD. These data suggest a correlation between kidney amphotericin B concentration and renal toxicity as reflected in serum urea nitrogen level. A higher urea nitrogen level was associated with a higher amphotericin B concentration in the kidney, regardless of the type of formulation administered (Figure 3). Both serum urea nitrogen level and the kidney amphotericin B concentration were not significantly different between animals receiving 14 daily doses of Fungizone 1 mg/kg and ABCD 5 mg/kg.

In contrast, alanine aminotransferase levels were not significantly different from the undosed control value in any of the treatment groups. The mean (±S.D.) values of alanine aminotransferase were 43 ± 7.5, 37 ± 3.6, 31 ± 3.9 and 33 ± 4.8 IU/L in control animals and in animals dosed with 1 mg/kg ABCD, 5 mg/kg ABCD and 1 mg/kg Fungizone, respectively. Although amphotericin B concentrations in the liver differed significantly between all dosing groups ($p < 0.001$), the levels of alanine aminotransferase were not significantly different.

DISCUSSION

This study demonstrates that the pharmacokinetics and

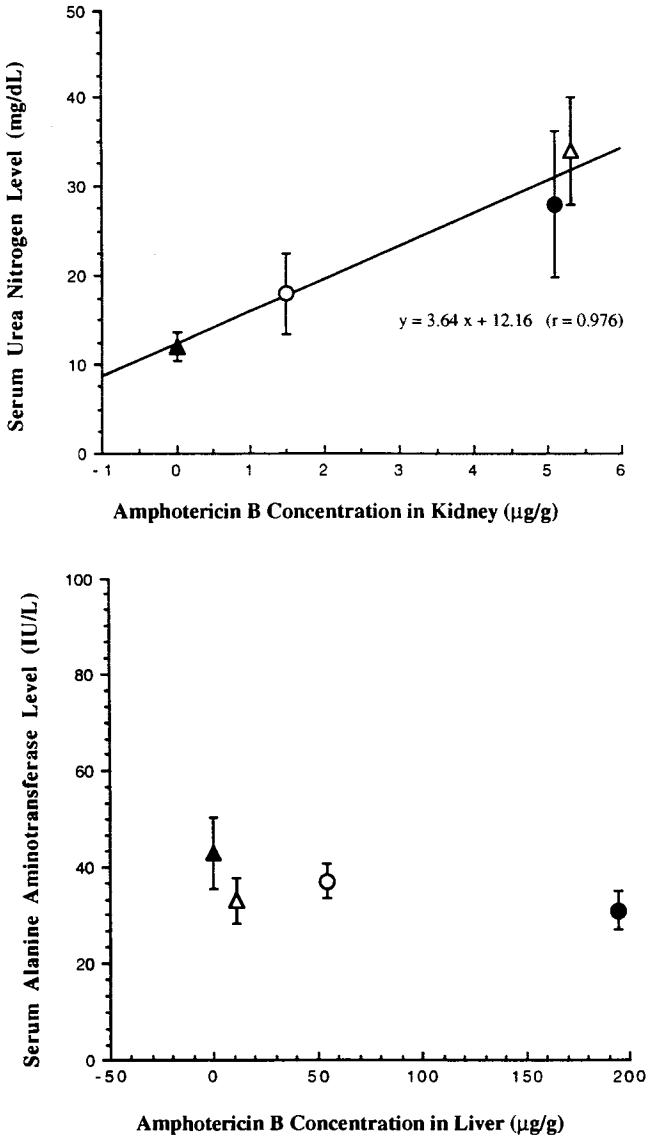

Figure 3. The relationships between amphotericin B concentrations and organ-specific clinical pathology parameters in kidney and liver on Day 14 following administration of Fungizone and ABCD. (▲) Control, (△) Fungizone 1 mg/kg, (○) ABCD 1 mg/kg and (●) ABCD 5 mg/kg. Values shown are mean ± S.D. (n = 5 or 6).

tissue distribution profiles of amphotericin B in rats after repeated administration of ABCD differ significantly from those observed after administration of Fungizone. At an equivalent dose level, ABCD produced significantly lower amphotericin B concentrations in the plasma, kidneys, lungs, heart, brain, skeletal muscle and blood than Fungizone. Amphotericin B concentrations in the spleen were similar between the Fungizone and ABCD groups; while in the liver amphotericin B concentrations were significantly higher after ABCD administration. These results are in contrast to the reported tissue distribution of a phospholipid-solubilized amphotericin B preparation, which resulted in increased amphotericin B levels in the kidneys, liver, spleen and lungs in mice (23). However, our study results are similar to those reported for another liposomal formulation of amphotericin B in mice and rats (11).

The plasma concentration vs. time profiles of amphotericin B obtained after a single administration of either formulation were similar to those after 14 daily doses. Daily administration of ABCD at either dose level did not lead to amphotericin B accumulation in plasma; while a slight accumulation (< 50 %) was observed after multiple administration of Fungizone. However, if one used linear kinetics and the plasma half-life values of amphotericin B after ABCD administration on Day 1 (34 hours), the accumulation factor for amphotericin B in plasma after multiple daily administration would be 2.6. The lack of accumulation of amphotericin B in plasma is likely due to a non-linearity (e.g. time-dependent kinetics) in amphotericin B disposition after multiple administration of ABCD. It is also possible that amphotericin B administered as ABCD distributes rapidly and extensively into the tissues and then is stored in the tissues from which amphotericin B is slowly released into the plasma. Thus, the rate limiting step in amphotericin B disposition after ABCD administration may be the redistribution of amphotericin B out of the tissues where the drug is stored. The higher V_{ss} values for amphotericin B administered as ABCD also suggest that this form of amphotericin B distributes more extensively into the tissues, which is consistent with the results of the tissue recoveries of amphotericin B, especially in the liver (Table IV). Similar to previous studies in rodents (11, 16) using lipsomal formulation of amphotericin B, dose-dependent kinetics for amphotericin B was observed.

Compared with Fungizone, ABCD significantly reduced amphotericin B distribution to the kidneys during repeated dosing. In addition, ABCD significantly decreased the degree of azotemia accompanying the administration of this nephrotoxic agent. Since serum urea nitrogen levels on Day 14 correlated with concurrently measured renal amphotericin B concentrations, it is proposed that the mechanism by which ABCD spares the kidneys from amphotericin B-induced toxicity is related to the ability of this dosage form to alter the distribution of its amphotericin B payload away from the kidneys. Thus, ABCD was tolerated by rats in this multiple dosing study at a dose level five times higher than that of Fungizone without indication of increased nephrotoxicity. Reduced toxicity of amphotericin B associated with lipid-based formulations was also observed in rodents (6, 11) and dogs (17).

In contrast to the observed reduction in renal distribution, there was a rapid and extensive uptake of a major portion of the amphotericin B dose administered as ABCD by the liver. Although liver amphotericin B concentrations were significantly higher after ABCD administration ($p < 0.01$, see Figure 2(a)), no corresponding increase in a liver-specific toxicity indicator (alanine aminotransferase) was observed. It is possible that this uptake of ABCD was the result of rapid phagocytosis of the ABCD colloidal particles by liver macrophages. In any case, the liver appears to serve as the body's primary reservoir of amphotericin B administered as ABCD in which amphotericin B is stored in a form or location which reduces its toxicological activity and from which it is slowly released into the systemic circulation (24).

The bile salt-solubilized amphotericin B formulation (Fungizone) dissociates rapidly after intravenous administration and the amphotericin B released becomes bound to

plasma lipoproteins (25, 26). In contrast, ABCD consists of relatively stable discoidal particles about 100 nm in diameter which may remain intact for some time after administration (13). The high liver concentrations of amphotericin B may result from the high capacity of this organ to remove exogenous particulates from the circulation. If ABCD is taken up by the liver as intact particles, the apparent lack of hepatotoxicity associated with the relatively high liver concentration of amphotericin B may be explained. This is because the amphotericin B-lipid complex (such as ABCD) may behave in a different manner toxicologically than free amphotericin B, which reaches the liver as lipoprotein bound drug. Although the relationship between amphotericin B concentrations in plasma or tissues and antifungal activity has not been clearly established, the fact that ABCD is toxicologically less active than Fungizone in the kidneys and liver raised the issue that this dosage form may also have diminished antifungal activities in these and other organs. However, studies in animals have shown that colloidal or lipid-based amphotericin B maintained the ability to clear *Asperigillus fumigatus* infections from the liver, kidneys and lungs in rabbits (27); *Coccidioides immitis* infections from the liver, spleen and lungs in mice (15); and systemic infections in mice caused by *Candida albicans, Cryptococcus neoformans,* and *Histoplasma capsulatum* (6).

Amphotericin B concentrations in all tissues reached or were approaching steady state by the end of the two-week dosing period for both formulations. When the dose of ABCD was increased to 5 mg/kg, amphotericin B concentrations still approached steady state in all tissues except the spleen. Since liver amphotericin B concentrations did not increase significantly after Day 6 for 5 mg/kg ABCD, continuously rising spleen concentrations may reflect saturation of hepatic capacity to remove circulating particulates. The increasing spleen concentrations of amphotericin B might also be due to the increased distribution of amphotericin B to red blood cells at the 5 mg/kg ABCD dose. However, it is not possible to determine if this phenomenon was unique to ABCD, since a higher dose of Fungizone could not be administered.

Upon cessation of dosing, the washout of amphotericin B from most tissues occurred at approximately the same rate for both formulations. In the liver and lungs, amphotericin B washout appeared to occur more slowly when administered as ABCD. The difference in the washout rates may imply that ABCD is stored as a different form or in a physically different compartment (such as different cell types) within these tissues. This would be possible if the subcellular localization of the intact ABCD is different from that of free or lipoprotein bound amphotericin B.

Urinary excretion of amphotericin B accounted for less than 4 % of the total dose administered for both formulations. The majority of the excreted amphotericin B was collected in the feces for both formulations. Studies in dogs (28) and primates (29) indicate that biliary excretion is an important elimination pathway for amphotericin B. In this study, since biotransformation or autoxidation of the drug could have occurred during transit through the gut, the actual biliary excretion could exceed the measured fecal recovery. The similarity of fecal clearance between ABCD and Fungizone suggests that most of the amphotericin B in the liver after ABCD administration exists in a pool that is not readily accessible to biliary excretion. The total recovery of amphotericin B from all biological matrices assayed accounted for only about 30 % of the total cumulative dose administered for either formulation. The ultimate fate of amphotericin B in the body is poorly understood and previous studies have also failed to account for a majority of the amphotericin B dose administered (29, 30). This has been generally attributed to the slow washout of amphotericin B from the "deep" tissue sites and/or metabolism of amphotericin B. Although metabolism of amphotericin B has not been reported in the literature, the data from this study suggest that hepatic metabolism of amphotericin B occurred. Degradation of amphotericin B could occur due to autoxidation at the polyene moiety via a free radical mechanism (31).

In summary, there were significant differences in the pharmacokinetics and tissue distribution profiles of amphotericin B after repeated dosing with the two amphotericin B formulations. As compared to Fungizone® (a micellar suspension), a lipid-based colloidal dispersion of amphotericin B, (ABCD) exhibited reduced plasma concentrations of amphotericin B and reduced distribution to kidneys, the major target organ for amphotericin B toxicity. The altered pharmacokinetics of amphotericin B administered as ABCD appeared to be related to the rapid and extensive uptake of ABCD by the liver, which may act as a reservoir for amphotericin B. The results of the study also suggest that ABCD is sequestered from the systemic circulation in a form or location whose activity and fate differ from the free and protein-bound amphotericin B in plasma. The present study extends the results of a previous single-dose study of ABCD in rats (16) and demonstrates the similar disposition characteristics of ABCD in both rats and dogs (17). It is possible that the disposition of amphotericin B after administration of ABCD in humans is similar to that observed in dogs and rats, where reduced plasma and target organ concentrations lead to decreased toxicity of amphotericin B in this colloidal dosage form.

ACKNOWLEDGEMENT

The authors thank Liposome Technology, Inc. for the financial support of this project.

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