ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1991, p. 1029–1034
0066-4804/91/061029-06$02.00/0

Vol. 35, No. 6

Single-Dose Pharmacokinetics and Tolerance of a Cholesteryl Sulfate Complex of Amphotericin B Administered to Healthy Volunteers

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Received 5 December 1990/Accepted 1 April 1991

Twenty-three healthy volunteer subjects received a single dose of amphotericin B colloidal dispersion or placebo (4:2) in a double-blind, randomized, dose-escalating design. Doses ranged from 0.25 to 1.5 mg/kg of body weight. The medication was administered via intravenous infusion at a rate of 0.5 mg/kg/h. Plasma amphotericin B concentrations increased with increasing doses, resulting in a linear increase in the amphotericin B area under the curve. Concentrations in plasma decreased rapidly upon discontinuation of the infusion, indicating rapid tissue distribution. A log-linear biexponential elimination phase was observed. A three-compartment open model was used to describe the distribution and elimination of amphotericin B. The mean terminal elimination half-life ranged from 86 h at the 0.25-mg/kg dose level to 244 and 235 h at the 1.0- and 1.5-mg/kg dose levels, respectively. Mean total body clearance ranged from 219 to 284 ml/kg/h. The volume of distribution increased with dose, from 3.37 liter/kg at the 0.25-mg/kg dose to 7.92 liter/kg at the 1.5-mg/kg dose. At the lowest dose level, 0.25 mg/kg, the medication was generally well tolerated. Progressive increases in the dose led to increasing side effects. At the 1.5-mg/kg dose level, 50% of the patients on active medication experienced nausea, vomiting, and chills. Physical examinations, ophthalmologic examinations, and clinical laboratory parameters remained within normal limits compared with those obtained during prestudy examinations.

Amphotericin B has been used in the treatment of moderate to severe fungal infections for 30 years (14). The medication continues to be widely used, despite the occurrence of dose-limiting toxicities, primarily renal tubular acidosis (14, 17). In addition, approximately 20 to 50% of patients treated with amphotericin B develop acute reactions such as fever, chills, nausea, and vomiting (5, 14, 17). The pharmacokinetics of amphotericin B are not well known. There have been several reports of studies on limited numbers of patients which have provided basic information on the disposition of the compound (3–5, 16). Adult patients exhibit a large volume of distribution, 3 to 4 liters/kg, and a long terminal elimination-phase half-life, which ranges from 11 to 16 days (3, 5). Studies in children demonstrate a higher clearance rate and shorter half-lives, although concentrations in plasma were measured for a limited time following treatment with amphotericin B (4, 16).

Amphotericin B is available as a desoxycholate micellar solution (17). A new formulation has been developed with the goal of increasing the tolerability of the drug. It consists of a cholesteryl sulfate complex of amphotericin B, the amphotericin B colloidal dispersion (ABCD; Liposome Technology, Inc.). ABCD is a stable complex of amphotericin B and cholesteryl sulfate in a 1:1 molar ratio. The lyophilized product is stable for 6 months at 50°C (10). In vitro incubation studies with fresh human blood have shown that the drug-lipid complex does not result in hemolysis of erythrocytes and that binding to plasma lipoproteins is less than that observed with Fungizone (10). Studies in animals have shown a significantly altered distribution of the compound, particularly in the kidneys (10). In these studies in animals, a reduction in the amount of drug taken up by renal tissue was associated with a reduction in the renal toxicity normally associated with amphotericin B treatment.

The purpose of this study was to assess the tolerance and pharmacokinetics of amphotericin B following a single intravenous infusion of ABCD administered to healthy volunteers.

MATERIALS AND METHODS

Twenty-three healthy male subjects (age range, 19 to 38 years; weight range, 63 to 95 kg) were enrolled in the study. Subjects were within 20% of ideal body weight on the basis of height and body frame (12). Volunteers were accepted into the study on the basis of a prestudy medical history, physical examination, ophthalmologic examination, electrocardiogram, and laboratory tests of blood and urine. The study was approved by the Institutional Review Board of the University of Utah Medical Center. All subjects provided written informed consent.

Subjects were confined for the duration of the study. The study medication consisted of a 1-mg test dose followed (24 h later) by doses of 0.25, 0.5, 1.0, and 1.5 mg of ABCD or matching placebo (5% glucose with multivitamin) per kg of body weight. Dosing was performed in a double-blind fashion. Neither the subjects nor the clinical staff were able to break the study blind on the basis of the appearance of the dosing solutions. Subjects were randomly assigned to receive active drug or placebo (4:2) in each group at dose levels of 0.25, 0.5, 1.0, and 1.5 mg of ABCD per kg. ABCD was infused at a rate of 0.5 mg/kg/h, resulting in infusion times of 0.5, 1, 2, and 3 h for the 0.25-, 0.5-, 1-, and 1.5-mg/kg

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doses of ABCD, respectively. Vital signs, including blood pressure, pulse, and oral temperature, were measured periodically following each dose. Clinical laboratory tests and urine collections were performed prior to and following administration of each dose.

Blood samples were obtained prior to administration of the study dose; at the midpoint and end of the infusion; and at 15 and 30 min; 1, 2, 4, 7, 10, 24, and 48 h; and 7, 14, 21, and 28 days postinfusion. Samples were collected from the arm opposite the infusion site. Adverse experiences were recorded throughout the study. Subjects were discharged after collection of the blood sample at 48 h, with the remaining samples collected during outpatient visits.

Samples were stored at <-20°C until they were shipped to the analytical laboratory (Bio Research Laboratories, Ltd., Montreal, Quebec, Canada). Samples were analyzed for amphotericin B concentration by reversed-phase high-performance liquid chromatography on a Waters Micro-Bondapak column (0.39 by 30 cm; particle size, 10 μm; Waters, Milford, Mass.) with a reversed-phase, slurry-packed Nucleosil C18 precolumn (Alltech Associates Inc., Deerfield, Ill.). The mobile phase was acetonitrile-water (40:60 [vol/vol]) containing 0.25 mM disodium EDTA at a flow rate of 1.0 ml/min. Amphotericin B was monitored at 328 nm by UV detection (Lambda Max model 481; Waters). Solid-phase extraction on disposable Bond Elut C18 extractions columns (Analytichem, Harbor City, Calif.) were used for initial sample cleanup. Calibration standards and quality control samples were created by spiking blank plasma with amphotericin B in dimethyl sulfoxide-methanol (1:1). The assay was linear over the range of 10 to 2,000 ng/ml. The sensitivity of the assay was defined at the lower limit of detection, i.e., 10 ng/ml. Recovery of amphotericin B from plasma averaged 80%. Interday precision expressed as a relative standard deviation of daily means was less than 10% at 30 ng/ml. Results of the assay were accepted if the within-run quality control samples were within 10% of the known concentration and results for duplicate assays of unknown samples did not deviate by more than 15%.

Subject demographics and incidence of adverse events were summarized by descriptive statistics. Vital signs measurements and laboratory values were analyzed by repeat measures analysis of variance (StatView 512+; Brainpower Inc., Calabasa, Calif.). A repeat measures three-way analysis of variance was performed for subjects, treatment groups (dose level), and days of measurement. The reported P values were corrected for multiple testing by the number of multiple replicate tests i.e., P value times n, analogous to the Bonferroni method (13). Significance was set at $P < 0.05$.

Pharmacokinetic analyses included calculation of noncompartmental parameters and least-squares estimations for multiexponential parameters. Noncompartmental parameters were calculated as follows: C_{max} = maximum measured concentration in plasma; AUC = area under the plasma concentration-time curve determined by the trapezoidal method (the terminal phase was calculated as C_t/k_{last}; see below); C_t = last measured value of the concentration in plasma; k_{last} = terminal first-order rate constant; TBC = total body clearance (CL) = dose/AUC; V_{area} = volume of distribution = CL/k_{last}; V_{ss} = volume of distribution at steady state = dose × (AUMC)/(AUC)^2 − (T × dose)/(2 × AUC), where AUMC is the area under the first moment curve (plasma concentration × time versus time), and T is the time of infusion. AUC was corrected for residual drug present following the 1-mg test dose. When measurable drug was present at time zero, prior to the start of the infusion of the study dose, an amount equal to C_o/k_{last}, where C_o is the concentration at time zero, was subtracted from the AUC.

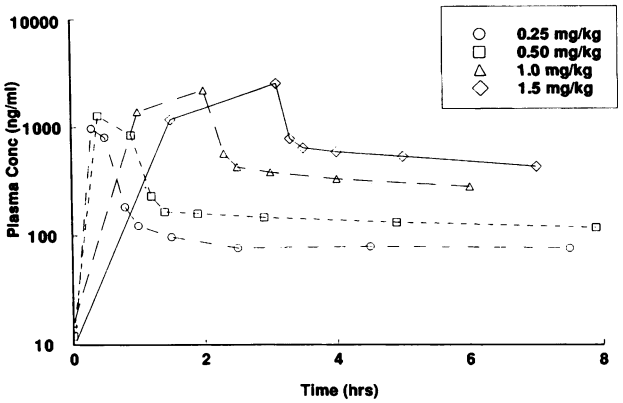

FIG. 1. Mean concentrations of amphotericin B in plasma over the first 8 h following the start of intravenous infusions.

Pharmacokinetic stripping and least-squares fitting of the pharmacokinetic model were performed on individual concentration-in-plasma data (RSTRIP and MINSQ; Micromath Scientific Software, Salt Lake City, Utah). The postinfusion data were fit initially to multiple polyexponential functions with weighting to produce the best fit. Weights varied from zero to the squared reciprocal of the measured concentration in plasma. Individual weighting schemes were selected on the basis of visual best fit of the model to the data, in addition to minimization of the sum of squared deviations between the model predicted and the measured concentrations in plasma. Following initial estimation, the data were then fit to the following multicompartment model: C_t = Sum ($R_n \times e^{(-k_n \times t_1)}$) − Sum ($R_n \times e^{(-k_n \times t)}$) where C_t = concentration in plasma at time t; t = time after the start of infusion; R_n = macroconstant (analogous to intercept values obtained from data stripping); k_n = first-order rate constants; $t_1 = t - T_{iv}$ for $t > T_{iv}$ and = 0 for $t < T_{iv}$; and T_{iv} = time of infusion. Parameters were then derived from the pharmacokinetic model according to the following equations: AUC = sum (R_n/k_n); TBC (total body clearance) = dose/AUC; V_{area} = dose/(AUC × k_{last}); and V_1 = volume of the central compartment = dose/(sum Rn). The AUC calculated from the model was also corrected for drug present at the time of administration of the study dose in the same manner as described above for the trapezoidal AUC.

The goodness of fit of the pharmacokinetic model was evaluated on the basis of the coefficient of determination. The coefficient of determination was a measure of the fraction of the total variance accounted for by the model (15).

RESULTS

Mean plasma amphotericin B concentrations for the four dosing groups are shown in Fig. 1 and 2. Amphotericin B was rapidly distributed, leading to large changes in the concentration in plasma over short periods of time after the end of the infusion. Concentrations in plasma at the end of infusion were quite variable because of 1- to 4-min delays in the collection of the blood sample at this time point (Table 1). Distribution was followed by a biexponential elimination phase. The terminal elimination phase was log-linear (Fig. 2).

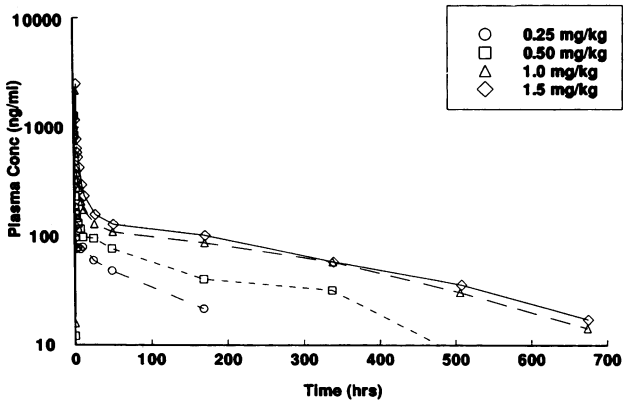

FIG. 2. Mean concentrations of amphotericin B in plasma showing the log-linear terminal elimination phase following distribution.

Mean concentrations in plasma and noncompartmental parameters are given in Table 1. Concentrations in plasma increased with increasing dose in an approximately linear fashion. Comparison of the samples at 168 h showed an increase from 21.5 ng/ml at a dose of 0.25 mg/kg to 40, 86.75, and 101.25 ng/ml at doses of 0.5, 1.0, and 1.5 mg/kg, respectively. Direct comparison of concentrations in plasma at earlier time points is misleading because of the various durations of the intravenous infusions. AUC increased in a linear fashion (r = 0.99043). The mean terminal elimination-phase half-life increased from a mean of 86 h at a dose of 0.25 mg/kg to 235 h at the 1.5-mg/kg dose level. The mean total body clearance remained relatively constant, ranging from 21.9 ml/kg/h (1.0-mg/kg dose) to 28.4 ml/kg/h (0.25- and 1.5-mg/kg doses). The V_{ss} increased with dose, from a mean of 3.37 liters/kg at 0.25 mg/kg to 7.92 liters/kg at 1.5 mg/kg.

Measurable concentrations in plasma were present in 11 of the 15 subjects prior to the start of the study infusion as a result of the 1-mg test dose. Concentrations ranged from 11 to 24 ng/ml. Although a correction to the AUC was performed, the area contributed by the test dose was less than 0.2% of the total AUC. Further corrections to pharmacokinetic parameters were unnecessary.

A three-term exponential model provided the best least-squares fit for most subjects. Distribution and elimination rate constants for the three-compartment analysis are given in Table 2. The coefficients of determination indicated that an adequate fit of the data was obtained for all subjects. In some subjects, insufficient data were available for the three-compartment model; therefore, a two-compartment model was used. Two subjects from the 1.5-mg/kg dosing group (subjects 19 and 21) exhibited a large decline in the concentration in plasma following the end of the infusion which led to large estimates for the distribution rate constant. These two estimates were not included in the mean data for this dosing group (Table 2). An example of the three-compartment fit of the individual concentration in plasma data is shown in Fig. 3. Model-derived estimates of AUC, clearance, and V_{ss} compared well with noncompartmental parameters (Table 2).

The safety of ABCD was assessed by physical examination, ophthalmological examination, laboratory tests, vital signs, and monitoring of adverse events. Seventy-three percent (11 of 15) of the volunteers on active drug experienced one or more adverse events. A total of 48 adverse events were reported for the volunteers on active drug, with 40 events reported as mild and 8 events reported as moderate. Fifty percent (four of eight) of the volunteers on placebo reported one or more adverse events. Five subjects reported mild (n = 4) to moderate (n = 1) pain at the sites of their study dose infusion. The infusion site became occluded in four subjects during the infusion of medication and required a change in infusion site. The most common adverse events are listed in Table 3. No clinically significant changes were noticed in the poststudy physical examinations, with the exception of ongoing phlebitis for subjects 19 and 24.

Vital signs were within normal limits for the majority of subjects. Statistically significant differences between subject groups were observed for diastolic blood pressure and oral

TABLE 1. Amphotericin B concentrations in plasma and noncompartmental pharmacokinetic parameters

Sample time (h)	Concn (ng/ml) at the following dose level (mg/kg)[a]:			
	0.25	0.5	1.0	1.5
0	8 ± 9	12 ± 12	16 ± 3	10 ± 7
Mid-infusion	975 ± 238	1,277 ± 277	1,390 ± 202	1,168 ± 263
End infusion	806 ± 394	843 ± 155	2,191 ± 487	2,534 ± 1,049
0.25	184 ± 56	231 ± 31	566 ± 84	775 ± 237
0.5	123 ± 26	165 ± 34	426 ± 52	636 ± 83
1	97 ± 17	159 ± 12	380 ± 40	586 ± 92
2	76 ± 14	146 ± 32	329 ± 37	529 ± 93
4	79 ± 6	131 ± 4	278 ± 35	425 ± 62
7	76 ± 3	117 ± 17	206 ± 34	296 ± 41
10	80 ± 2	98 ± 17	174 ± 45	233 ± 20
24	60 ± 10	95 ± 9	129 ± 16	157 ± 15
48	48 ± 14	77 ± 8	109 ± 10	128 ± 22
168	22 ± 8	40 ± 19	87 ± 17	101 ± 27
336	0 ± 0	32 ± 16	58 ± 17	58 ± 22
504	0 ± 0	8 ± 7	31 ± 10	36 ± 14
672	0 ± 0	0 ± 0	14 ± 10	18 ± 12
$t_{1/2}$[b] (h)	86 ± 20	157 ± 68	244 ± 49	235 ± 74
AUC (μg · h/ml)	9.4 ± 1.8	21.0 ± 2.6	46.3 ± 7.1	57.3 ± 14.3
V_{ss} (liters/kg)	3.4 ± 0.7	5.7 ± 1.7	7.2 ± 0.9	7.9 ± 1.0

[a] Values are means ± standard deviations.
[b] $t_{1/2}$, Terminal-phase elimination half-life.

TABLE 2. Model-derived pharmacokinetic parameters

Parameter[a]	Value at the following dose level (mg/kg) (n)[b]:			
	0.25 (4)	0.5 (3)	1.0 (4)	1.5 (4)
α (h^{-1})	21.03 ± 12.49	23.19 ± 13.76	8.73 ± 1.52	18.07 ± 12.55[c]
β (h^{-1})	2.4081 ± 2.4803	0.2265 ± 0.2219	0.2476 ± 0.1119	0.2012 ± 0.0401
γ (h^{-1})	0.0085 ± 0.0026	0.0050 ± 0.0022	0.0029 ± 0.0005	0.0033 ± 0.0013
Coefficient of determination	0.9860 ± 0.0158	0.9741 ± 0.0428	0.9599 ± 0.0554	0.9423 ± 0.0359
AUC (μg · h/ml)	9.1 ± 1.8	20.0 ± 4.1	45.6 ± 7.4	56.8 ± 14.9
CL (ml/kg/h)	28.3 ± 6.4	25.7 ± 20.3	21.9 ± 2.9	28.4 ± 9.9
V_{area} (liters/kg)	3.38 ± 0.29	5.48 ± 1.34	7.58 ± 0.73	8.88 ± 0.90
V_1 (ml/kg)	40 ± 15	30 ± 18	41 ± 4	32 ± 2[c]

[a] α, distribution phase; β and γ, elimination phases; AUC, area under the concentration-time curve; CL, clearance; V_{area}, dose/(AUC × k_{last}); V_1, volume of the central compartment.
[b] Values are means ± standard deviations.
[c] Values for subjects 19 and 21 were eliminated from the calculation.

temperature. Mean diastolic blood pressure was 62 mm Hg for subjects receiving 1.0 mg/kg versus the overall mean of 67 mm Hg (P = 0.008). Mean temperature reached a maximum value 2 h following the end of the infusion and was elevated for subjects in the 1.5-mg/kg dosing group, 37.8°C versus an overall mean of 37.0°C (P = 0.0016). Systolic blood pressure and heart rate were not statistically different (P = 0.0628 and P = 0.0832, respectively).

Clinical laboratory parameters were assessed daily. There were no clinically significant changes in complete blood count, serum chemistry, or urinalysis during the study. All four dose levels were administered in the study without delay or interruption caused by reported or observed adverse events.

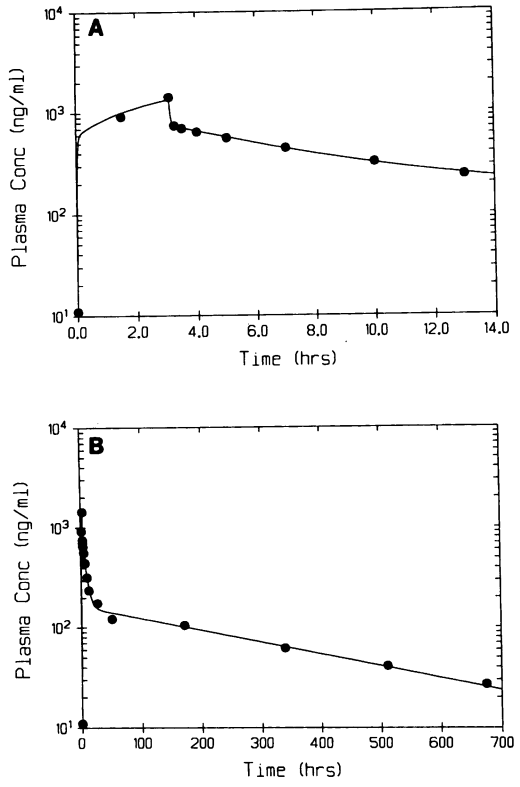

FIG. 3. Concentrations of amphotericin B in plasma for an individual subject receiving ABCD (1.5 mg/kg) over 3 h. (A) Time period surrounding the infusion. (B) All datum points.

DISCUSSION

The disposition of amphotericin B following intravenous ABCD dosing was similar to those described for amphotericin B in previous reports (3–5, 16). Measurement of concentrations in plasma for 4 weeks following a single dose of ABCD represents a more complete assessment of amphotericin B pharmacokinetics than those provided in previous reports. The three-compartment model provided the best fit of the observed distribution and elimination phases of the compound. The initial distribution of amphotericin B occurred rapidly at all dose levels. Although few blood samples were collected during the distribution phase, most subjects exhibited a distribution-phase half-life of 2 to 5 min. An improved characterization of the distribution phase could be achieved by increasing the number of samples collected in the first few minutes following the end of the infusion. The poor fit of the three-term exponential function in subjects 19 and 21 was most likely due to this inadequate number of samples and the timing of the samples and does not indicate pharmacokinetic differences in these subjects. The biexponential elimination phase has been observed in previous studies of amphotericin B (3–5, 16). Concentrations in plasma were measured for 12 and 18 days following the final dose of medication in two patients, as reported by Atkinson and Bennett (3). The elimination-phase half-life of amphotericin B in these two patients was approximately 15 days (360 h). Both patients, however, exhibited renal insufficiency, with creatinine clearances of 28 and 37 ml/min. Two other studies reporting pharmacokinetic data for amphoter-

TABLE 3. Summary of the most common adverse events

Adverse event	No. (%) with adverse event after:	
	Active treatment (n = 15)	Placebo (n = 8)
Nausea	8 (53)	2 (25)
Headache	6 (40)	2 (25)
Pain at infusion site	5 (33)	0 (0)
Vomiting	3 (20)	0 (0)
Chills, feverish	3 (20)	1 (13)
Lower back pain	2 (13)	0 (0)
Lip, tongue numb	2 (13)	0 (0)

icin B collected concentration-in-plasma data for approximately 24 h following a dose; these data provided limited information on the terminal elimination of the drug (4, 16). A half-life of 11 days was reported by Chabot et al. (5) in a series of 14 cancer patients receiving doses ranging from 0.5 to 0.8 mg of Fungizone per kg. Chabot et al. (5) reported a V_{ss} of 3.2 liters/kg, which was similar to the value observed at the ABCD dose level of 0.25 mg/kg. The increase in the apparent terminal elimination-phase half-life between doses and the corresponding increase in the V_{ss} may have indicated a dose-related change in disposition. Because of the low concentrations in plasma at the lower doses, there may have been an inadequate characterization of the terminal elimination phase. The ability to measure concentrations in plasma for up to 4 weeks in the 1.0- and 1.5-mg/kg dosing groups allowed an accurate estimate of the terminal elimination-phase half-life. The linear increase in the AUC provided some evidence against dose-related changes in disposition; however, this possibility cannot be ruled out by the results of the present study. A comparison of elimination rates based on plasma samples collected between 48 and 168 h indicates an increase in half-life with increasing dose.

Comparison of ABCD pharmacokinetics with those of liposomal forms of amphotericin is difficult because of the limited information available on the liposomal formulations. The relationship between the reported improved therapeutic index for liposomal amphotericin B and alterations in distribution or elimination are unknown. Several studies have reported an improved therapeutic index for liposomal forms of the drug in animal models of infection and patients with systemic fungal infections (1, 8, 11). A recent report by Kan et al. (9) compared the pharmacokinetics of amphotericin B following administration of Fungizone and a liposomal preparation. That study reported an amphotericin B half-life of 30 and 50 h for 0.1- and 0.25-mg/kg doses of Fungizone, respectively. The half-life of the liposomal complex increased from 19 h at the 0.1-mg/kg dose to 45 h at the 0.5-mg/kg dose. The short half-lives that were reported may indicate only short-term sampling following the infusions of medication. The reported V_{ss} increased with dose to approximately 4 liters/kg at a 0.5-mg/kg dose of the liposomal complex. This is somewhat lower than the V_{ss} of 5.7 liters/kg observed following the 0.5-mg/kg dose of ABCD.

Amphotericin B is highly bound to the lipoproteins, erythrocytes, and cholesterol present in plasma and in tissues throughout the body (6, 7, 14). The primary storage site appears to be the liver (6). Liposomal forms of the drug are believed to be rapidly taken up by the reticuloendothelial system, thereby reducing binding to cholesterol and plasma membranes (17). The cholesteryl sulfate complex should achieve a similar decrease in plasma membrane lipoprotein binding and lead to reduced associated toxicity, as has been observed in animal studies (10).

The acute side effects seen after administration of ABCD were those typically seen with Fungizone (7). No unique side effects were reported. The incidence of side effects increased with dose level. At the highest dose level, 1.5 mg/kg, two volunteers experienced nausea, vomiting, and chills. These acute side effects are commonly seen in premedicated patients treated with Fungizone at dose levels two to three times lower, 0.5 to 0.75 mg/kg (14, 17). When a dose of 0.25 mg of Fungizone per kg was given to healthy volunteers, side effects similar to those reported at the 1.5-mg/kg ABCD level were reported (9). In the 1.5-mg/kg ABCD dosing group, three subjects complained of pain at the site of infusion. This could be related to local irritation. Tachycardia and fever were present in two subjects in the 1.5-mg/kg dosing group.

Laboratory tests assessing renal and hepatic status were of particular interest. There were no clinically significant differences in blood urea nitrogen, creatinine, urine osmolality, or liver chemistry tests. Blood urea nitrogen and creatinine were measured approximately 4 h after administration of the dose of medication in an attempt to detect any transient effects. They were also measured daily during the study. The first voided urine sample obtained following administration of the dose of medication also showed no significant changes. No changes were observed in the post-study physical and ophthalmologic examinations, with the exception of ongoing phlebitis for two of the volunteers, who reported pain at the infusion site. The phlebitis resolved slowly following completion of the study.

The pharmacokinetics of amphotericin B following infusions of ABCD do not differ significantly from those of Fungizone. The cholesteryl complex of amphotericin B may reduce renal tissue binding, which appears to lead to the renal tubular acidosis which develops with amphotericin B treatment. Future trials in patients with systemic fungal infections will determine the usefulness of this new formulation.

ACKNOWLEDGMENT

This study was supported by a grant from Liposome Technology, Inc.

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