

## Pharmacokinetic Profile of ABELCET (Amphotericin B Lipid Complex Injection): Combined Experience from Phase I and Phase II Studies

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Received 13 December 1996/Returned for modification 21 May 1997/Accepted 21 July 1997

**Amphotericin B (AmB) has been the most effective systemic antifungal agent, but its use is limited by the dose-limiting toxicity of the conventional micellar dispersion formulation (Fungizone). New formulations with better and improved safety profiles are being developed and include ABELCET (formerly ABLC), but their dispositions have not been well characterized; hence, the reason for their improved profiles remains unclear. This report details the pharmacokinetics of ABELCET examined in various pharmacokinetic and efficacy studies by using whole-blood measurements of AmB concentration performed by high-pressure liquid chromatography. The data indicated that the disposition of AmB after administration of ABELCET is different from that after administration of Fungizone, with a faster clearance and a larger volume of distribution. It exhibits complex and nonlinear pharmacokinetics with wide interindividual variability, extensive distribution, and low clearance. The pharmacokinetics were unusual. Clearance and volume of distribution were increased with dose, peak and trough concentrations after multiple dosings increased less than proportionately with dose, steady state appeared to have been attained in 2 to 3 days, despite an estimated half-life of up to 5 days, and there was no evidence of significant accumulation in the blood. The data are internally consistent, even though they were gathered under different conditions and circumstances. The pharmacokinetics of ABELCET suggest that lower concentrations in blood due to higher clearance and greater distribution may be responsible for its improved toxicity profile compared to those of conventional formulations.**

Since its introduction in 1956, amphotericin B (AmB) has remained the most effective systemic therapy for serious fungal infections (4). Despite its proven clinical efficacy, its use has been limited by the narrow therapeutic index of the conventional formulation (32), which is prepared as a mixed micellar dispersion with deoxycholate (Fungizone; Bristol-Myers Squibb), because of its high lipophilicity and the fact that it is practically insoluble in water. This formulation exhibits dose-limiting toxicity, and the maximal tolerated dose has been suggested to be about 0.7 to 1.0 mg/kg of body weight/day (13). This toxicity prevents the administration of higher doses even to those patients for whom insufficient availability of drug is suspected as the cause of ineffective therapy. This has led to the development of new formulations that use either liposomes or phospholipid complexes as drug carriers and that limit the availability of free AmB (5, 7, 13). The incorporation of AmB into liposomes was designed to decrease the adverse effects of this drug, enhance its activity, and provide site-specific delivery of high doses of the drug (5, 21).

Different formulations with liposomal or phospholipid-complex delivery vehicles for AmB have been developed, and at least five formulations have already been tested in humans (5, 7, 13). They include dimyristoyl phosphatidylcholine-dimyris-

toylphosphatidylglycerol liposomes, intralipid AmB, AmB colloidal dispersion, AmBisome, and ABELCET (formerly ABLC). These preparations continue to undergo extensive evaluations in *in vitro*, whole-animal, and clinical studies (3, 11, 14, 20–23, 25, 26, 30, 31, 33, 35). ABELCET is a suspension in sodium chloride (0.9% wt/vol) of very fine particles of AmB complexed in a 1:1 molar ratio with a mixture of the phospholipids 1- $\alpha$ -dimyristoylphosphatidylcholine and 1- $\alpha$ -dimyristoylphosphatidylglycerol (7:3 molar ratio). Its clinical efficacy and reduced toxicity compared to those of the conventional Fungizone formulation have been demonstrated previously (2), but its disposition characteristics and the basis of its better safety profile are not well understood.

Studies with animals have indicated that the tissue distribution and pharmacokinetics of ABELCET and other lipid-based formulations are different from those of the conventional drug formulation (6, 13, 27). In general, they indicate a larger volume of distribution (*V*), greater systemic clearance (CL), and substantial differences in the levels of accumulation in regional organs. Specifically, for ABELCET, it accumulates in the reticuloendothelial system (RES), similar to other particulate formulations (29), with the total concentrations of AmB in liver and spleen being higher than those achieved with the conventional formulation. Also, the distribution in the kidney is different between these two formulations, with comparable concentrations of AmB in the kidneys being obtained with a dose of ABELCET that was 10-fold higher than those achieved with the conventional formulation. This difference is suggested

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to be partly responsible for the reduced toxicity of ABELCET to the kidneys (27).

On the basis of encouraging results in preclinical and emergency-use studies, ABELCET has been approved for use in the treatment of aspergillosis in patients who are refractory to conventional AmB therapy, and it is undergoing clinical evaluation for other indications. There are considerable challenges in evaluating the pharmacokinetics of drugs such as ABELCET. These include (i) the inherent toxicity of AmB, which, although substantially reduced with ABELCET, still restricts the use of healthy volunteers in pharmacokinetic studies in which there is no potential benefit; (ii) the wide variety of underlying conditions in patients prone to systemic fungal infections; and (iii) the fact that most patients receiving ABELCET in clinical trials are seriously ill, limiting the ability to collect an adequate number of samples in a consistent fashion. The design and conduct of a conventional pharmacokinetic study are therefore seriously limited and compromised. Thus, the studies used to evaluate this new formulation were conducted under different protocols with small numbers of subjects, and this report is a compilation of the pharmacokinetic data obtained from those studies. Despite these limitations, an interesting, internally consistent pharmacokinetic profile of the concentrations of amphotericin B in the whole blood of patients receiving ABELCET has emerged from these studies, a profile that any of the component studies alone could not provide. This report represents the first detailed profile of the disposition of ABELCET after administration to humans. The clinical reports obtained from these studies are presented elsewhere.

#### MATERIALS AND METHODS

All studies were conducted after the protocols were approved by the respective ethical committees of each institution or site, and informed consent was obtained from the subjects or their legal representatives.

**Escalating-dose study of ABELCET pharmacokinetics in subjects infected with HIV.** A study was designed as an open-label pharmacokinetic study of the pharmacokinetics of ABELCET at various single low doses in groups of asymptomatic human immunodeficiency virus (HIV)-positive subjects. The intention was to enroll five patients infected with HIV in each of the following three groups: group 1, five subjects receiving 0.6 mg of ABELCET per kg over 18 min; group 2, five subjects receiving 1.2 mg of ABELCET per kg over 18 min; and group 3, five subjects receiving 0.3 mg of Fungizone per kg over 72 min. Blood samples (5 ml each) were collected at timed intervals: before the infusion and at 10, 20, and 30 min and 1, 1.5, 2, 4, 8, 24, 48, 72, and 96 h after the infusion. In addition to routine clinical and biochemical safety screens, tests for altered immune function were incorporated.

**Single-dose study of ABELCET pharmacokinetics in healthy and renally impaired subjects.** A pharmacokinetic study was designed as a single-dose (2.5-mg/kg), open-label study to compare the pharmacokinetics of ABELCET in patients with various degrees of renal function. The intention was to enroll six subjects in each of the following three groups: group I, normal renal function (creatinine clearance [ $CL_{CR}$ ],  $>70$  ml/min/1.73 m<sup>2</sup>); group II, moderate renal impairment ( $CL_{CR}$ , 30 to 70 ml/min/1.73 m<sup>2</sup>); and group III, severe renal impairment ( $CL_{CR}$ ,  $<30$  ml/min/1.73 m<sup>2</sup>). Each subject received a single dose of 2.5 mg of ABELCET per kg infused over 1 h, with blood samples (5 ml each) collected before and at 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 240, 336, 504, 672, and 840 h after the infusion.

**Seven-day dose-ranging pharmacokinetic study of ABELCET in patients receiving antineoplastic therapy.** A 7-day dose-ranging pharmacokinetic study of ABELCET in patients receiving antineoplastic therapy was designed as part of an open-label, maximally tolerated, multiple-dose and pharmacokinetic study with a three-tier dose escalation in patients prior to receiving cancer chemotherapy. It was hypothesized that the AmB in ABELCET would enhance the activities of the antineoplastic agents (24). The intention was to enroll six to eight patients in each of the following groups: tier 1, 2.5 mg/kg/day for 7 days; tier 2, 5.0 mg/kg/day for 7 days; and tier 3, 6.0 mg/kg/day for 7 days. In each instance, the same dose of ABELCET was administered daily for 7 days prior to administration of chemotherapy to cancer patients. The initial design was to randomize the duration of infusion between 2 and 1 h in each subject in a balanced crossover design. Blood samples (3 ml each) were obtained at timed intervals (before the infusion and at 5 and 30 min and 1.5, 3, 6, 9, 12, and 24 h after the infusion) to define an area under the concentration-time curve from 0 to 24 h ( $AUC_{0-24}$ ) on days 1 and 7 and to obtain trough and peak levels in blood 5 min before and 5

TABLE 1. Numbers of patients and treatments for patients with mucocutaneous leishmaniasis

Group no.	Dosage (mg/kg/day)	Duration of treatment (days)	Formulation	No. of subjects studied	No. of subjects for whom kinetic data were available
1	0.6	42	ABELCET	15	8
2	1.2	21	ABELCET	15	8
3	2.5	10	ABELCET	15	8
4	5.0	5	ABELCET	15	8
5	0.6	42	Fungizone	12	5

min after each infusion, respectively, on days 2 to 6. In addition, blood samples were obtained on days 1, 5, and 10 after administration of the last dose.

**Seven-day pharmacokinetic study of ABELCET in patients with neutropenia and presumed or proven fungal infection.** A 7-day pharmacokinetic study of ABELCET in patients with neutropenia and presumed or proven fungal infection was designed as part of an open-label, dose-escalating efficacy and safety study with neutropenic patients with presumed or proven fungal disease. The study was designed as a six-tier dose escalation study of ABELCET, with each dose being given daily over 2 h for 7 days. The dosage range was planned to start at 5 mg/kg/day and to increase to 6.5, 8, 10, 12.5, and 15 mg/kg/day.

Seven blood samples in addition to the one taken before the dose were collected for the first dose interval (sampling times, 1, 2, 3, 4, 8, 12, and 24 h after the infusion) to determine the maximum concentration of AmB in blood ( $C_{max}$ ) and  $AUC_{0-24}$ . Additional blood samples were collected to determine peak and trough levels daily for 6 days to permit an assessment of whether steady state is reached in this 6-day time interval. Also, seven samples were further collected within 24 h of administration of the last dose (1, 2, 3, 4, 8, 12, and 24 h after the infusion), with subsequent blood samples obtained at 48, 72, 96, 120, 168, and 240 h after the infusion to define the kinetics of the last dose and a terminal elimination rate constant.

**Pharmacokinetic study of chronic constant total dose of ABELCET given by variable dosing regimen in treatment of patients with mucocutaneous leishmaniasis.** A pharmacokinetic study was designed as part of a phase I and phase II safety and efficacy study comparing ABELCET with Fungizone treatment in patients with mucocutaneous leishmaniasis. The study was designed as an open-label, parallel-group, sequential-ascending-dose comparison of ABELCET to one fixed dose of Fungizone. In each of the ABELCET groups, the total cumulative dose was a constant, and the time to reach this amount varied; thus, the dose per daily infusion was varied. The numbers of patients for whom data were available for pharmacokinetic evaluation and details about their treatments are presented in Table 1.

The pharmacokinetic study was a 10-day detailed profile of the level of AmB in blood obtained from each subject after the last dose of drug with blood samples (5 ml each) obtained before and at 1, 5, 15 and 30 min and 1, 2, 4, 8, 12, 16, 24, 36, 48, 60, 72, 144, and 240 h after stopping the infusion.

**Determination of AmB levels in blood by HPLC.** Human whole-blood samples containing AmB were analyzed by high-pressure liquid chromatography (HPLC). The samples were collected in heparinized tubes and shipped frozen from the different study sites shortly after collection to the laboratories that performed the analysis. An aliquot (0.2 to 1.0 ml) of the sample was extracted with an organic solvent (dimethyl sulfoxide-methanol [2:1] or chloroform-acetonitrile-dimethyl sulfoxide [3:3:4]) containing the internal standard (*N*-acetyl-AmB) by vortex mixing for 30 s, allowing the mixture to sit for 1 h, and repeating the mixing. The mixture was centrifuged, and the supernatant was filtered through a 0.45- $\mu$ m-pore-size filter before an aliquot (50 to 100  $\mu$ l) of the supernatant was injected onto the HPLC column. Extraction recoveries by this method were similar for both ABELCET and free AmB. Separation was achieved on a Waters  $\mu$ Bondapak C<sub>18</sub> column (10- $\mu$ m particle size) (3.9 by 150 mm) or an equivalent column with a mobile phase consisting of a mixture of acetonitrile and pH-adjusted, aqueous EDTA (pH 4.2) (36:64) at a flow rate of 1.0 ml/min, with detection by measurement of the absorbance at 405 nm. The assays were specific for AmB, had coefficients of variation of less than 11%, and could quantitate the level of AmB down to 0.075  $\mu$ g/ml. The calibration curves were linear ( $r = 0.998$ ) over the range of 0.05 to 5  $\mu$ g/ml.

**Pharmacokinetic analysis.** The pharmacokinetic data were all analyzed by noncompartmental methods. AUC and the area under the first moment of the concentration-time curve (AUMC) were determined by trapezoidal and log trapezoidal methods, half-life ( $t_{1/2}$ ) was estimated by log-linear regression of the concentration-time data in the terminal elimination phase. CL was estimated as the ratio of dose to the AUC, the volume of distribution at steady state ( $V_{ss}$ ) was estimated as the ratio of (dose  $\cdot$  AUMC) to (AUC  $\cdot$  AUC) (this was adjusted for the infusion time by subtracting the product of the mean input time [infusion time/2] and CL from this ratio when the drug was given by short infusion), and the volume of distribution in the terminal phase ( $V_{\beta}$ ) was estimated as the ratio of the CL to the terminal elimination rate constant.

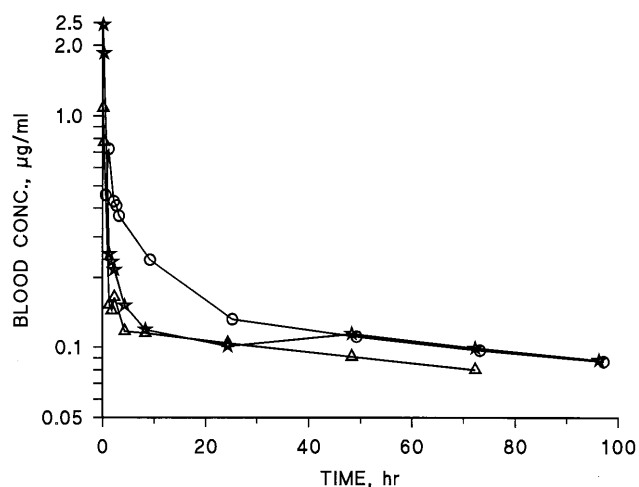


FIG. 1. Concentration-time profiles of AmB in blood after the administration of ABELCET (0.6 mg/kg [ $\Delta$ ] and 1.2 mg/kg [ $\circ$ ]) or Fungizone (0.3 mg/kg [ $\square$ ]).

**Statistical analysis.** Data were compared by the nonparametric Mann-Whitney test (for two groups) or the Kruskal-Wallis test with post hoc group comparisons (for more than two groups), with the level of significance set at a  $P$  value of  $<0.05$ .

## RESULTS

### Determination of AmB concentration in biological fluid.

The characterization of the pharmacokinetics of AmB after administration of ABELCET is influenced by the biological fluid that is used. Preliminary experiments demonstrated that when whole blood was spiked in vitro with ABELCET and then centrifuged to separate the plasma, the majority of the AmB ( $>90\%$ ) was located in the pellets. In contrast, when conventional AmB was used, there was complete recovery of the AmB in the plasma. Similarly, when plasma was spiked and centrifuged, the AmB from ABELCET was mostly associated with the pellets. This suggests that the use of plasma as the biological fluid for measurement of AmB concentrations will greatly underestimate the concentrations of AmB after ABELCET administration. Consequently, all analyses were conducted with whole blood.

**Escalating-dose study of ABELCET pharmacokinetics in subjects infected with HIV.** A total of 15 subjects in three groups were studied, with 5 subjects in each group receiving ABELCET at 0.6 mg/kg over 18 min or 1.2 mg/kg over 18 min or Fungizone at 0.3 mg/kg over 72 min. The concentration-time profiles in blood are presented in Fig. 1, and the values of the pharmacokinetic parameters are presented in Table 2. At the end of infusion, AmB concentrations after the high-dose ABELCET infusion (1.2 mg/kg) were approximately twice those after the low-dose infusion (0.6 mg/kg). Because the infusion times were the same (18 min), this implies equivalent distributional characteristics over this dose range. There was an extremely rapid initial decrease in the concentration of AmB after the administration of both doses of ABELCET. This initial decrease was less with Fungizone (Fig. 1).

The estimated terminal  $t_{1/2}$ s were similar for both doses of ABELCET and Fungizone and were on the order of 4 to 8 days. The  $AUC_{0-24}$  and the AUC from time zero to infinity ( $AUC_{0-\infty}$ ) following ABELCET administration were increased by only 36 and 44%, respectively, when the dose of ABELCET was doubled from 0.6 to 1.2 mg/kg, and both AUCs were less

TABLE 2. Pharmacokinetic parameters of AmB in whole blood after administration of either ABELCET (over 18 min) or Fungizone (over 72 min) to patients infected with HIV

Treatment, dose (no. of subjects)	$C_{max}$ ( $\mu\text{g/ml}$ )	$AUC_{0-24}$ ( $\mu\text{g} \cdot \text{h/ml}$ )	$AUC_{0-\infty}$ ( $\mu\text{g} \cdot \text{h/ml}$ )	AUC/dose	$t_{1/2}$ (h)	CL (ml/h/kg)	$V_{ss}$ (ml/kg)	$AUMC_{0-96}$ ( $\mu\text{g} \cdot \text{h}^2/\text{ml}$ )
ABELCET, 0.6 mg/kg ( $n = 5$ )	$1.28 \pm 0.19$	$3.29 \pm 0.41$	$19.56 \pm 2.69$	$0.40 \pm 0.06$	$107.19 \pm 24.46$	$31.14 \pm 4.46$	$4,605.1 \pm 671.7$	$3,028.3 \pm 1,019.2$
ABELCET, 1.2 mg/kg ( $n = 5$ )	$2.72 \pm 0.96$	$4.38 \pm 0.66$	$28.13 \pm 23.69$	$0.34 \pm 0.30$	$144.31 \pm 136.85$	$67.44 \pm 42.58$	$7,930.8 \pm 1,124.3$	$9,043.6 \pm 14,183.5$
Fungizone, 0.3 mg/kg ( $n = 5$ )	$0.72 \pm 0.22$	$6.14 \pm 0.65$	$40.24 \pm 25.74$	$2.02 \pm 1.12$	$190.70 \pm 141.01$	$9.45 \pm 4.18$	$1,802.2 \pm 383.3$	$14,403.7 \pm 19,717.6$

TABLE 3. Pharmacokinetic parameters in whole blood of healthy subjects and patients with renal disease after a single intravenous infusion of 2.5 mg of ABELCET per kg over 1 h

Subject group (no. of subjects)	Dose (mg)	$C_{\max}$ ( $\mu\text{g/ml}$ )	$t_{1/2}$ (h)	$\text{AUC}_{0-\infty}$ ( $\mu\text{g} \cdot \text{h/ml}$ )	$\text{AUMC}_{0-\infty}$ ( $\mu\text{g} \cdot \text{h}^2/\text{ml}$ )	CL (ml/min)	$V_{\text{ss}}$ (liters)
Healthy subjects ( $n = 4$ )	207.25 (25.04) <sup>a</sup>	3.72 (0.79)	139.9 (67.5)	46.95 (18.6)	8,607.0 (7,123.0)	81.7 (29.0)	676 (122)
Moderate renal disease ( $n = 1$ )	203.5	4.47	385.0	89.22	42,001.0	38.0	1,073
Severe renal impairment ( $n = 1$ )	181	2.92	147.5	52.59	9,773.4	57.3	638

<sup>a</sup> Values are means (standard deviations).

than those for Fungizone given at a dose of 0.3 mg/kg (Table 2). When the independent pharmacokinetic variables that determine AUC and  $t_{1/2}$  were considered, the CL and  $V$  of ABELCET were higher than those of Fungizone. Furthermore, both CL and  $V_{\text{ss}}$  were almost doubled by increasing the dose of ABELCET from 0.6 to 1.2 mg/kg. This suggests an unusual pattern of nonlinear kinetics, with increased distribution and CL with increasing dose.

**Single-dose study of ABELCET pharmacokinetics in healthy and renally impaired subjects.** A total of six subjects were enrolled in the single-dose (2.5-mg/kg) study, including four with normal renal function (group I;  $\text{CL}_{\text{CR}}$ ,  $>70$  ml/min/ $1.73$  m<sup>2</sup>), one with moderate renal impairment (group II;  $\text{CL}_{\text{CR}}$ , 30 to 70 ml/min/ $1.73$  m<sup>2</sup>), and one with severe renal impairment (group III;  $\text{CL}_{\text{CR}}$ ,  $<30$  ml/min/ $1.73$  m<sup>2</sup>). Concentration-time profiles of drug in blood over the period of time when the AmB level was measurable in these subjects showed multiexponential characteristics, and the estimated pharmacokinetic parameters are presented in Table 3. Although there was only one patient in each renal impairment group, it could be noted that the patient with mild renal impairment had an apparent higher maximum blood AmB concentration, AUC, and AUMC, a longer  $t_{1/2}$ , and a smaller CL than those for the healthy subjects. By contrast, the pharmacokinetic parameters for the patient with severe renal impairment were comparable to those for the healthy subjects (Table 3).

**Seven-day dose-ranging pharmacokinetic study of ABELCET in patients receiving antineoplastic therapy.** Pharmacokinetic data were available for three subjects in group 1 (2.5 mg/kg/day), five subjects in group 2 (5.0 mg/kg/day), and one subject in group 3 (6.0 mg/kg/day). For the first two subjects in group I who were randomized and treated with 1- and 2-h infusions of ABELCET, there were differences in the peak concentrations and initial declines in the levels of AmB in blood between the two durations of drug administration. The peak concentration and decline after the 2-h infusion were less than those after the 1-h infusion, suggesting that the 2-h infusion may have less potential than the 1-h infusion to cause toxicity due to the presence of high AmB concentrations. All subsequent data were therefore obtained only for the 2-h infusion, and only the data from the 2-h infusion were used to compile the summary results for all subjects. The  $C_{\max}$  of AmB after administration of the first dose of ABELCET was observed immediately following drug infusion. At each dose, there was between a two- and a threefold intersubject variation that was not explained by differences in body weight. A significant overlap in  $C_{\max}$  was attained between the groups after administration of the dose, although the mean  $C_{\max}$  increased when the dose increased from 2.5 mg/kg ( $1.14 \pm 0.17$   $\mu\text{g/ml}$ ) to 5.0 mg/kg ( $1.62 \pm 0.12$   $\mu\text{g/ml}$ ), and the  $C_{\max}$  increased marginally upon a further increase in the dose to 6 mg/kg ( $1.71 \pm 0.40$   $\mu\text{g/ml}$ ), although this last value was only for one subject.

In each of the study groups, there was no evidence of significant increases in either peak or trough levels of AmB in

blood during successive days of therapy (Fig. 2). It is interesting that a 100% increment in dose from 2.5 to 5.0 mg/kg was associated with only 46 and 42% increases in trough and peak levels ( $0.37 \pm 0.06$  versus  $0.54 \pm 0.07$   $\mu\text{g/ml}$  and  $1.14 \pm 0.07$  versus  $1.62 \pm 0.12$   $\mu\text{g/ml}$ ), respectively. For each dose, peak and trough levels appeared to have reached steady state by day 2 to 3, and mean peak levels were only 18% higher than the peak level after the administration of the first dose for the 2.5-mg/kg dose and 16.5% higher than the peak level after the administration of the 5-mg/kg dose. By the end of the dosing interval, the concentrations in blood had fallen to about 33% of the peak concentration for the 2.5- and 5.0-mg/kg doses.  $\text{AUC}_{0-24\text{h}}$  were only modestly higher (34 to 41%) on day 7 than on day 1 for both doses, giving day 7/day 1 ratios of 1.40 and 1.34 for the 2.5- and 5.0-mg/kg doses, respectively. The limited sampling conducted to determine a terminal  $t_{1/2}$  at the end of therapy suggested a multiexponential elimination with a very long and poorly defined terminal  $t_{1/2}$ .

**Seven-day pharmacokinetic study of ABELCET in patients with neutropenia and presumed or proven fungal infection.** The 7-day pharmacokinetic study of ABELCET in patients with neutropenia or fungal infections was designed to be a dose escalation study. However, only the first tier of the dose range was used. Of the 24 subjects entered into this first tier, i.e., receiving 5 mg of ABELCET per kg per day for 7 days, evaluable data were available for six subjects. The available pharmacokinetic parameters are summarized in Table 4.

The  $C_{\max}$  following administration of the first dose was reasonably reproducible between subjects, ranging from 1.36 to 3.83  $\mu\text{g/ml}$ . The initial  $\text{AUC}_{0-24}$  after administration of the first dose of ABELCET was also reasonably consistent between

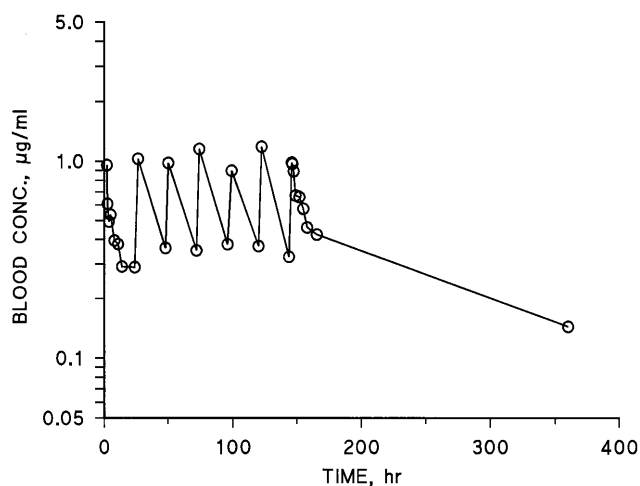


FIG. 2. Concentration-time profile of AmB in blood after administration of ABELCET at a dosage of 2.5 mg/kg/day by a 2-h infusion for 7 days.

TABLE 4. Pharmacokinetic parameters of AmB after administration of multiple doses of ABELCET in patients with neutropenia and proven or suspected fungal infection<sup>a</sup>

Subject no.	Kinetics on day 1				Kinetics after administration of last dose	
	Dose (mg)	C <sub>max</sub> (μg/ml)	AUC <sub>0-24</sub> (μg · h/ml)	Apparent t <sub>1/2</sub> (h)	AUC (μg · h/ml)	t <sub>1/2</sub> (h)
104	375	1.96	10.75	5.9		
107	425		7.19	6.3		
108	530	1.36	26.90	18.2	23.96 <sup>b</sup>	17.3
114	420	2.19	16.57			
115 <sup>c</sup>	485	3.83	15.33		15.43 <sup>b</sup>	182.4
117	370	1.67	14.79	4.5		
Mean	434.2	2.42	15.26	8.0		
SD	62.7	0.93	6.68	7.0		

<sup>a</sup> ABELCET was given at a dosage of 5 mg/kg/day given over 2 h for 7 days.

<sup>b</sup> AUCs after administration of the last dose were calculated over the same time interval used for the initial doses: 24 h for subject 108 and 10 h for subject 115.

<sup>c</sup> The full kinetic study was performed after administration of the second dose on day 2.

subjects, ranging from 7.19 to 26.90 μg · h/ml (Table 4). The terminal elimination could not be determined within a dosing interval, but there was evidence of a rapid reduction in the concentration in blood over the first dosing interval. There was marginal elevation of peak and trough levels on successive days of daily therapy, with an approximately 17% increase in the trough concentration (from 0.65 ± 0.38 to 0.76 ± 0.31 μg/ml) and a 27% increase in the peak level (from 1.54 ± 0.49 to 1.96 ± 1.17 μg/ml) between day 2 and day 6, suggesting that the rate of increase was leveling off over this short time interval.

Data that could be used to compare the kinetics of the first dose with those of the last dose over each subsequent 24 h were available for only one subject (subject 108) (Table 4). The AUCs over these time periods were similar: 26.90 μg · h/ml on day 1 and 23.96 μg · h/ml on the last day. These areas suggest a CL of approximately 330 ml/min. Given a dose of 530 mg/day, a predicted mean steady-state concentration would be estimated to be 1.12 μg/ml. This can be compared with the observed concentrations, which ranged from 0.81 to 1.56 μg/ml.

For another subject (subject 115), only two samples were drawn on day 1, but a full profile and AUC were available for day 2, as were data for a complete study at the discontinuation of drug therapy. These data also did not indicate evidence of drug accumulation on repeated therapy because comparable C<sub>max</sub>s were observed on day 2 and day 6 (3.83 versus 3.74 μg/ml), and comparable AUCs were observed over the 10 h immediately postdosing (15.33 versus 15.43 μg · h/ml).

**Pharmacokinetic study of chronic constant total dose of AmB given by various dosing regimens in the treatment of patients with mucocutaneous leishmaniasis.** A total of 72 subjects with mucocutaneous leishmaniasis were enrolled in a pharmacokinetic study of a chronic constant total dose of AmB; this number represented the full planned enrollment. Pharmacokinetic data were available for eight subjects receiving each dose of ABELCET and five subjects receiving Fungizone. Two series of pharmacokinetic comparisons are available. First, the same dosages of ABELCET and Fungizone (0.6 mg/kg/day) were given to eight subjects for 42 days for ABELCET and five subjects for Fungizone. After administration of the last dose, peak blood AmB concentrations were similar (0.86 and 1.06 μg/ml, respectively) (Table 5). However, the subsequent concentration-time profiles in blood were markedly different, with the AmB levels after the administration of ABELCET decreasing much more rapidly and to much lower levels than those after the administration of Fungizone (Fig. 3), consistent with a fivefold larger V and a fourfold greater CL in comparison with those after the administration of Fungizone (Table 5).

The second series of comparisons can be made between the concentration-time profiles in blood after the administration of four doses of ABELCET, with the realization that each study was undertaken at a different time after starting therapy with the first dose. These data are also summarized in Table 5. These data indicate the nonlinearity of observed values for all measured or estimated variables. C<sub>max</sub> did increase between dosages of 0.6 and 1.2 mg/kg/day, but further increments in the

TABLE 5. Pharmacokinetic parameters for AmB after the administration of four doses of ABELCET to four groups of patients with mucocutaneous leishmaniasis for various periods of time<sup>a</sup>

Drug (dosage [mg/kg/day])	No. of daily doses	No. of subjects	C <sub>max</sub> (μg/ml)	AUC <sub>0-24</sub> (μg · h/ml)	t <sub>1/2</sub> (h)	CL (ml/min)	V <sub>β</sub> (liters/kg)
Fungizone (0.6)	42	5	1.06 ± 0.14	17.06 ± 5.03	91.1 ± 40.9	34.1 ± 14.3	5.1 ± 2.6
ABELCET							
0.6	42	8	0.86 ± 0.31	4.45 ± 0.90 <sup>b</sup>	113.1 ± 20.6	132.7 ± 33.4 <sup>b</sup>	23.4 ± 8.6 <sup>b</sup>
1.2	21	8	2.21 ± 0.81	6.72 ± 1.02	77.2 ± 20.8	166.2 ± 39.1	20.7 ± 7.6
2.5	10	8	2.41 ± 0.78	6.77 ± 0.92	187.2 ± 88.2	349.6 ± 84.8	105.5 ± 64.1
5.0	5	8	1.70 ± 0.83	9.50 ± 1.36	173.4 ± 78.0	476.3 ± 72.2	131.0 ± 57.7

<sup>a</sup> Values of pharmacokinetic parameters are means ± standard deviations.

<sup>b</sup> Significantly different (P < 0.001) from the value for Fungizone at the same dosage (0.6 mg/kg/day).

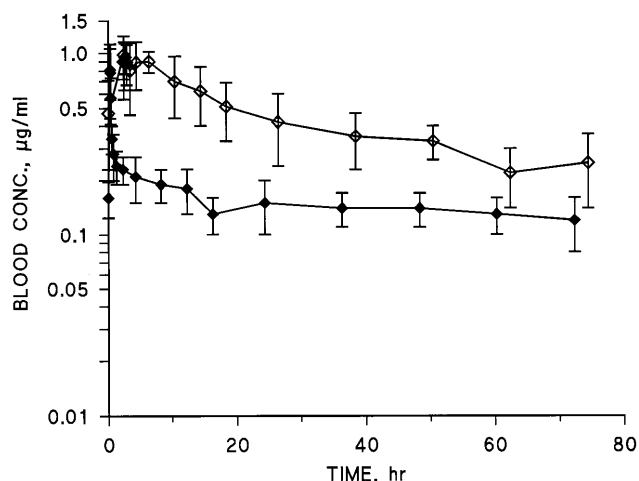


FIG. 3. Concentration-time profiles of AmB in blood after administrations of the same dose (0.6 mg/kg) of ABELCET (◆) or Fungizone (◇).

dosage were not associated with any further increase in peak AmB levels in blood. A comparison of the  $AUC_{0-24}$  at the two lowest doses indicated that a doubling of the dose was only associated with a 51% increase in the AUC. There was no change in the AUC on further doubling of the dose, and there was only a modest increase when the dose was once again doubled. This nonlinearity could be attributed to changes in two independent pharmacokinetic variables. First, CL increased with increasing dose, with an approximate two- to threefold increase occurring between the two lower doses and the two upper doses. Second,  $V$  was approximately fivefold greater after administration of the two higher doses in comparison with that after administration of the two lower doses. With concomitant changes in CL and distribution,  $t_{1/2}$  was essentially unchanged. The mean  $t_{1/2}$  of 5.7 days for this entire study reflects the best estimate of the terminal elimination of AmB following chronic ABELCET therapy, because the sampling times in the study design were adequate to cover two  $t_{1/2}$ s and provide concentration-time profiles in blood consistent with a terminal exponential elimination.

## DISCUSSION

The series of studies detailed in this report represent the most comprehensive evaluations of the pharmacokinetics of ABELCET to date. They include studies of the pharmacokinetics of AmB in whole blood obtained from dose escalation studies following the administration of both single and multiple doses of ABELCET, with direct comparison with the results of studies with the conventional formulation of AmB, Fungizone. The studies have evaluated ABELCET in patients with a variety of disease states, including HIV-positive patients, mucocutaneous leishmaniasis patients, cancer patients prior to chemotherapy, and cancer patients with neutropenia and presumed or proven fungal infection, and limited studies have been performed with patients with renal dysfunction. Together, these studies provide insight into the pharmacokinetic profile of ABELCET, despite the incompleteness of some of the studies as designed due to problems in recruiting patients or obtaining appropriate blood samples. The studies indicate that ABELCET is distinctly different from Fungizone.

ABELCET exhibits very complex pharmacokinetic characteristics that showed wide interindividual variability that cannot be explained by differences in body weight. In many re-

spects, the disposition is unusual and indicates that it is a distinctly different entity from the conventional formulation of AmB, Fungizone. When given in equivalent doses, Fungizone achieved higher AUCs per unit dose than ABELCET (Tables 2 and 5). This was a consequence of the higher CL and larger  $V$  for ABELCET than for Fungizone. This difference between ABELCET and Fungizone may partly be due to the particulate nature of ABELCET. It has been demonstrated that particulate formulations such as liposomes or lipid complexes are rapidly cleared by the RES in the body (16, 18, 29). Thus, the more rapid removal of ABELCET and the faster decline in the concentration of AmB in blood could have resulted from the affinity of the RES for the lipid complex. The resultant  $t_{1/2}$ s were comparable. It is obvious from the data that AmB distributes into a very large volume, far in excess of body size, and has a very low CL.

The pharmacokinetics of ABELCET have an unusual non-linear profile. In single-dose studies, the pharmacokinetic parameters were found to be dose dependent. Nonlinearity due to saturation of an active or capacity-limited process usually produces greater than proportional increases in AUC with increasing doses. Such a profile has been observed with some liposomal preparations (1, 9). However, by contrast, the ABELCET AUC increased less than proportionately with the dose (Tables 2 and 5) as a consequence of apparent increases in both CL and  $V$  as a function of dose. The reason for this unusual behavior of ABELCET is unclear. However, an unusual pattern of liposome clearance by the RES has been reported previously (10, 15). In those studies, hepatic CL was reported to increase with increasing concentration at any particular dose. An unusual non-Michaelis-Menten kinetics of saturable uptake of liposomes by RES was invoked as an explanation, whereby hepatic uptake CL was suggested to be AUC dependent (10). It was speculated that nonlinearity was due to a change in hepatic uptake CL as a function of time as well as concentration rather than as a function of concentration alone. It is unclear if the observations with ABELCET are also related to this kind of phenomenon. Other factors that might be relevant in affecting the uptake of liposomes by tissue and the distribution of liposomes in tissue are liposomal composition (19, 28, 34, 36, 38) and size (8, 12, 17). It is unclear if size or composition contributes to this unusual observation with ABELCET.

An alternative, equally plausible explanation is that ABELCET acts as a depot preparation that is completely or nearly completely extracted by tissue on the first pass through an organ or tissue, with subsequent delayed release of drug from this depot. The complete or nearly complete extraction by tissue would manifest as an apparent increase in uptake by tissue with increasing dose, which is consistent with previous observations with some lipid-based particulate systems in which increased uptake by tissue with an increase in the dose was reported (18, 37). This increased uptake can be tissue (18) or cell type (37) specific. For example, an increase in hepatic uptake was mostly accounted for by uptake by parenchymal cells, with little change in uptake by non-parenchymal cells (37). Subsequent to distribution into tissue, free AmB may be released at a delayed rate. Thus, as the dose is increased, the rapid extraction of ABELCET and the delayed release of free AmB at low levels would manifest as a relative decrease in composite AUC with increasing dose. However, insufficient information is available from these studies to confirm this suggestion.

The unusual characteristics and the nonlinearity of ABELCET pharmacokinetics were also displayed from multiple-dose, multiday dosings. At different individual doses, the max-

imum concentrations and peak and trough levels increased less than proportionately with the dose. In fact, over an eightfold increase in dose, the maximum concentration increased only two- to threefold (Table 5). The  $t_{1/2}$ s were comparable at the different doses and were sufficiently long that drug accumulation could be anticipated with daily drug dosing. However, another unusual observation was that steady state appeared to have been reached within 2 to 3 days of starting dosing, despite an estimated terminal  $t_{1/2}$  of about 5 days. Also, there was no evidence of significant accumulation of AmB in the body with continued dosing, with the result that the peaks and troughs on day 1 were comparable to those on subsequent days (Fig. 2).

Collectively, these data are also consistent with the hypothesis that ABELCET is a rapidly distributed depot preparation with a rate of release that is not necessarily proportional to the total dose that is administered or the total amount that is accumulated in the tissues. This hypothesis is supported by preclinical data that indicated that ABELCET is rapidly distributed into different tissues including the liver, spleen, and lungs, in which high concentrations are maintained for at least 24 h (27, 29). It is also supported by the observation that high levels in liver were not different between 1 and 6 h after dosing. The results for all of these organs are indicative of the slow release from these depot sites. Thus, ABELCET appears to be acting as a drug depot in the body that limits the availability of free AmB. An implication of this hypothesis is that it is possible that equivalent levels of free AmB in blood may be provided at lower doses and at longer dosing intervals. This would create a potential for delivering effective therapy with a lower risk of toxicity and cost. The extensive distribution of ABELCET in tissue and the slow release of free AmB from these tissues may offer advantages for antifungal therapy. Not only may low systemic concentrations reduce toxicity, but a relatively high local availability and the presence of AmB from the local tissue may be more effective in treating localized infections than an otherwise more uniformly systemically distributed drug. More studies will be required to elucidate the validity of this hypothesis.

It is recognized that the proposed explanation for the unusual nonlinear kinetics of AmB concentrations measured in whole blood after administration of ABELCET needs to be made with caution. This is because the whole-blood assay method used in these studies cannot distinguish between the free and complexed forms of AmB. It is therefore difficult to relate the characterized pharmacokinetics to the observed pharmacological and/or toxic effects because the disposition or level of the active species is not known. There is therefore a need to develop new analytical techniques that can be used to discriminate between chemical entities and that can be applied to future studies.

The present collation of multiple pharmacokinetic observations reflects the type of information that can be obtained during accelerated development of agents that are often being used on a compassionate basis. Despite the focus of studies on efficacy and safety as well as differences in the way in which the information was collected (i.e., from different studies or from different clinical-use situations) and the multiple study sites, subjects, patients, and investigators involved, observations that were internally consistent across a study series were obtained. Collectively, they constitute an initial and detailed description of the pharmacokinetics of ABELCET in humans and allow for a direct comparison of the pharmacokinetics of ABELCET with those of Fungizone.

In summary, measurement of AmB levels in whole blood after the administration of ABELCET have indicated that ABELCET exhibits unusual pharmacokinetic characteristics.

It shows wide interindividual variability, extensive distribution in tissue, a low CL, and a long  $t_{1/2}$ . Compared to Fungizone, it is more extensively distributed and more rapidly cleared but has a similar  $t_{1/2}$ . It exhibits dose-dependent, nonlinear kinetics, but contrary to the usual pattern, CL and  $V$  increased with increases in dose. More unusual was the finding that on multiple dosing with a dosing interval that was less than the  $t_{1/2}$ , there was very little accumulation of the drug in the body. Together, these data have been consistent across several studies conducted at different sites with several different populations of healthy subjects and patients. They also indicate that disease appears to have very little effect on its disposition. We suggest that ABELCET may act as a depot preparation that rapidly distributes to tissue stores, from which it releases active or free AmB, although further studies will be needed to fully elucidate this. A full understanding of the relationship between the pharmacokinetics and activity and toxicity profiles of ABELCET will require an ability to distinguish the free and bound forms of AmB after the administration of ABELCET.

#### ACKNOWLEDGMENTS

The studies described here were supported by The Liposome Company, Inc., and research in the laboratory of one of the authors (R.A.B.) is supported in part by PHS grant GM47124 from NIH.

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