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Pharmacokinetics of Amphotericin B in Children

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Amphotericin B is the most effective agent for the majority of systemic fungal infections but often causes toxicity, and specific dosage guidelines for amphotericin B in pediatric patients are lacking. The purpose of this study was to characterize the pharmacokinetics of amphotericin B in children. Twelve patients (mean age, 6.6 years; range, 4 months to 14 years) receiving amphotericin B, 0.68 ± 0.34 mg/kg per day (mean plus or minus standard deviation), were studied. Four to eight blood samples were collected during a 24-h period and analyzed by high-pressure liquid chromatography. The peak concentration of amphotericin B in serum was 2.9 ± 2.8 μg/ml. The mean total clearance, apparent volume of distribution, and elimination half-life were 0.46 ± 0.20 ml/min per kg, 0.76 ± 0.52 liters/kg, and 18.1 ± 6.6 h, respectively. Total clearance decreased with age ($p < 0.01$). In children aged 8 months to 9 years, the mean total clearance was 0.57 ± 0.15 ml/min per kg, and in children older than 9 years, it was 0.24 ± 0.02 ml/min per kg. Interpatient variation in the clearance and volume of distribution of amphotericin B was greater than threefold and greater than eightfold, respectively. However, pharmacokinetic parameters did not change in two stable patients who were studied again. Because clearance decreased substantially with age, older children may require lower doses of amphotericin B per kilogram to decrease the potential for toxicity.

The introduction of amphotericin B in 1955 represented a major advance in the management of systemic fungal infections. Since that time, there have been few additions to the antifungal armamentarium and none has replaced amphotericin B as the drug of choice for most serious systemic fungal infections. Indications for the use of amphotericin B include systemic infections involving pathogens such as *Candida albicans*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, and *Aspergillus* species. The incidence of infection with these pathogens has increased in recent years because of frequent use of indwelling intravascular catheters, immunosuppressive therapy, broad-spectrum antibiotic therapy, and the occurrence of the acquired immunodeficiency syndrome.

Current clinical use of amphotericin B is based on limited pharmacokinetic information available for this agent. Dosage regimens are often dictated by toxicity rather than by the findings of controlled clinical studies. Decisions on selecting daily dosage, total dosage, duration of therapy, and whether or not to use amphotericin B are often determined by the clinical experience of the physician in treating fungal infections. Even less information is available on the pharmacokinetics of amphotericin B in children. In the last 2 years, the first reports of pharmacokinetic studies of amphotericin B in children have been published (6, 7).

Further studies of amphotericin B are needed to (i) define its pharmacokinetics in older children and adolescents, (ii) evaluate whether changes occur in the pharmacokinetic parameters of amphotericin B over the course of therapy, and (iii) determine the correlation between age and pharmacokinetics or toxicity in pediatric patients. We designed a study to address these issues utilizing a high-pressure liquid chromatography assay (rather than bioassay) for an accurate estimation of the pharmacokinetic parameters of amphotericin B caused by improved sensitivity and specificity.

MATERIALS AND METHODS

The study was approved by the Human Subjects Research Committee of Children's Hospital. All pediatric patients begun on amphotericin B therapy between September 1986 and December 1987 whose parents gave written informed consent were enrolled in the study. Study patients ranged in age from 4 months to 14 years of age (mean, 6.6 years), and the most common underlying disease was acute nonlymphocytic leukemia (6 patients). A total of 12 patients (7 girls, 5 boys), 6 of whom had documented fungal infections, were studied. The demographics of patients entered in the study are presented in Table 1. All decisions to start, alter, or stop amphotericin B therapy were made by the patient's physician. Each patient received a test dose of amphotericin B (Fungizone; E. R. Squibb & Sons) of 0.1 mg/kg which was advanced to a maintenance dosage ranging from 0.25 to 1.5 mg/kg per day (0.68 mg/kg per day ± 0.34 mg/kg per day [mean plus or minus standard deviation]). The drug was administered over 2 to 4.5 h with an infusion pump used for all patients.

All patients were studied within 7 days of the first dose of amphotericin B, and three patients had a repeat study done 13 to 21 days later. Blood samples were obtained from each patient by heel or finger prick or via an indwelling intravenous catheter. Samples (0.5 ml) were obtained at the beginning of infusion (0 h), and at 2, 4, 6, 8, 12, 18, and 24 h from the beginning of the infusion. Samples were analyzed by a high-pressure liquid chromatography method published by Granich and co-workers (4), a reverse-phase method which uses simple protein precipitation and UV light detection. The between-day coefficient of variation in our laboratory at an amphotericin B concentration of 0.2 μg/ml was less than 11%, and the within-day coefficient of variation was less than 7%. Similar coefficients of variation were found at an am-

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TABLE 1. Patient characteristics

Patient	Age (yr)	Wt (kg)	Organism (site)	Underlying disease	Outcome
1	5	18.8	*Malassezia furfur* (blood)	Acute lymphocytic leukemia	Death[a]
2	1.6	10.0	None	Transposition of great arteries	Death[b]
3	0.3	4.0	*Candida albicans* (urine)	Ureteral reflux/nephrostomy	Cure
4	6	18.5	*Aspergillus* sp. (trachea)	Acute nonlymphocytic leukemia	Cure
5	1.6	12.0	None	Acute nonlymphocytic leukemia	—[b]
6	9	27.0	*Candida albicans* (cerebrospinal fluid)	Trauma	Cure
7	0.7	5.0	None	Severe combined immune deficiency	—
8	8	21.0	*Aspergillus* sp. (sputum)	Acute nonlymphocytic leukemia	Death
9	12	43.0	*Candida albicans, Aspergillus* sp. (sputum)	Acute nonlymphocytic leukemia	Death
10	14	40.0	None	Ewing's sarcoma	—
11	13	39.0	None	Acute nonlymphocytic leukemia	—
12	8	21.0	None	Acute nonlymphocytic leukemia	—

[a] Patient died from complications of bacterial pneumonia. No clinical signs of fungal infection were present at the time of death.
[b] —, Fungal infection was not documented.

photericin B concentration of 2 μg/ml; the limit of detection was 0.1 μg/ml.

Pharmacokinetic evaluation was possible in 9 of 12 patients enrolled. Two patients (patients 3 and 5) had inadequate serum samples for an area under the curve (AUC) calculation (multiple finger pricks refused by patients or parents), and one (patient 2) had an elevated bilirubin concentration in serum which interfered with the measurement of amphotericin B, leaving an insufficient volume of sample to repeat the analysis (4).

The elimination rate constant (β) was calculated from the linear regression analysis of serum concentration-versus-time data. The AUC from 0 h to infinity was calculated by the trapezoidal method and extrapolation to infinity. The AUC from 0 h to infinity was corrected by subtracting the AUC contributed by the previous dose, as calculated by dividing the predose concentration by β. These parameters were then used to calculate total clearance (CL), apparent volume of distribution (V), and half-life ($t_{1/2}$) by employing the following equations: CL = dose/AUC; V = CL/β; and $t_{1/2}$ = 0.693/β.

Adverse effects of amphotericin B therapy were monitored in each of the 12 patients during amphotericin B therapy by monitoring complete blood counts with differential, serum electrolytes (sodium, potassium, chloride, and bicarbonate), serum creatinine, and blood urea nitrogen at least weekly and urinalyses, aspartate aminotransferase, and alkaline phosphatase at least monthly. The patients were monitored daily by one of the investigators (J.M.B.) during the entire hospitalization, even after amphotericin B therapy had been stopped, to identify the development of late toxicity.

Correlations between pharmacokinetic parameters and age, time dosages or outcome were analyzed with the Pearson correlation coefficient. An a priori P value of less than 0.05 was considered significant.

RESULTS

The pharmacokinetic parameters for all patients are presented in Table 2. There was considerable variation in the peak concentrations in serum among patients (Fig. 1). Peak concentrations in serum occurred at the end of the infusion and ranged from 0.78 to 10.02 μg/ml. No correlation was found between dosage and peak concentration in serum, even when the dosages were corrected for body weight. We examined five patients who each had received doses of 0.5 mg/kg infused over 2 to 4 h and found no correlation between peak concentration in serum and infusion rate. Furthermore, no correlation was found between patient age and either peak concentration in serum or peak-to-dose ratio. We also did not find correlations between peak concentrations in serum and measures of clinical outcome such as cure, failure, relapse, or death.

Total CL, V, and $t_{1/2}$ ranged from 0.22 to 0.79 ml/min per kg, 0.23 to 1.91 liters/kg, and 11.9 to 33.2 h, respectively. We found a significant negative correlation between patient age and CL of amphotericin B ($r = -0.91$, $P < 0.01$; Fig. 2). Mean CL for patients 8 months to 9 years was 0.57 ± 0.15 ml/min per kg, and for children older than nine years, it was 0.24 ± 0.02 ml/min per kg. No other correlations with age were found.

It should be noted that $t_{1/2}$ calculated for three of nine patients exceeded the sampling period, and for eight of nine patients, the calculated $t_{1/2}$s were greater than one-half the sampling interval. In general, the sampling period should include two $t_{1/2}$s to accurately estimate the pharmacokinetic parameters.

Of the three patients studied twice, one (patient 8) had significant changes in pharmacokinetic parameters between the first and second evaluations. Total CL and V of amphotericin B both increased more than fourfold at the time of the second study, compared with the initial evaluation in this patient. Total CL increased from 0.35 to 1.47 ml/min per kg, and V increased from 0.37 to 1.64 liters/kg, while $t_{1/2}$

TABLE 2. Pharmacokinetics of amphotericin B

Patient	Maintenance dose (mg/kg per day)	Peak concn (μg/ml)	Total CL (ml/min per kg)	Vol of distribution (liters/kg)	$t_{1/2}$ (h)
1	0.5	1.05	0.03	0.71	15.9
2	0.5				
3	0.5				
4	1.5	1.99	0.67	1.91	33.2
5	1.0				
6[a]	1.0	2.22	0.54	1.04	22.4
	1.0	2.29	0.42	1.04	28.6
7	0.5	0.78	0.79	1.0	14.7
8[a]	0.7	3.21	0.35	0.37	12.0
	1.0	1.14	1.47	1.64	12.9
9[a]	0.25	1.64	0.26	0.47	20.6
	0.5	2.35	0.23	0.80	40.3
10	0.5	3.88	0.25	0.35	16.3
11	0.75	10.02	0.22	0.23	11.9
12	0.5	1.60	0.55	0.75	15.7

[a] These patients were studied twice during extended therapy.

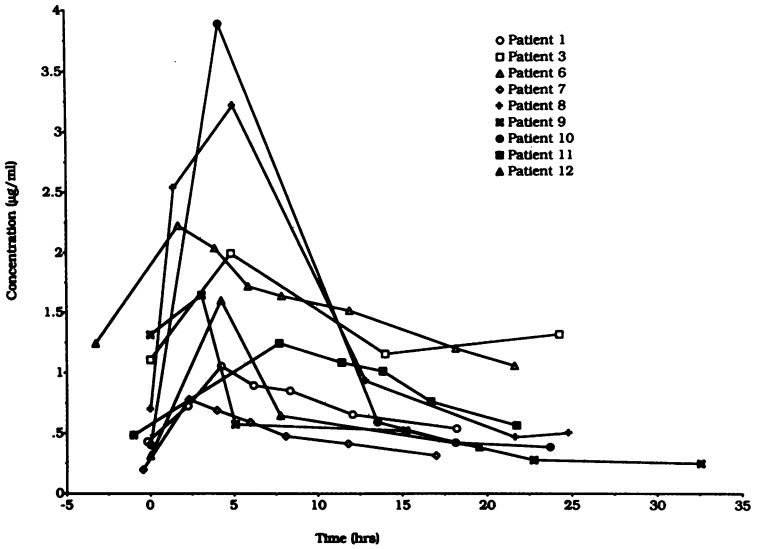

FIG. 1. Serum amphotericin B concentrations in nine patients. Time, Time from the beginning of amphotericin B infusion. A concentration of 10.02 μg/ml at 5.17 h for patient 11 was presumed to be erroneous and was omitted.

remained relatively constant at 12.0 and 12.9 h at the times of the first and second studies, respectively. At the time of the second study, this patient had developed fluid overload and decreases in renal and hepatic functions and was comatose. The other two patients (6 and 9) remained clinically stable between the two evaluations and had relatively little change in their pharmacokinetic parameters.

All 12 patients experienced some degree of renal compromise during amphotericin B therapy, as evidenced by an average increase of 210% in serum creatinine concentrations during therapy (Table 3). The serum creatinine returned to baseline in six of eight surviving patients within 7 days after therapy and was not evaluated after therapy in one patient. It should be noted that all patients received either concomitant or recent previous courses of other potentially nephrotoxic drugs, usually gentamicin or tobramycin, although these were closely monitored by our therapeutic drug monitoring service to maintain concentrations in serum within the therapeutic range (peak, <8 μg/ml; trough, <2 μg/ml). There was no correlation between increases in serum creatinine and either total dosages or peak concentrations of amphotericin B in serum.

Other infusion-related adverse effects such as phlebitis, chills, rigors, nausea, and vomiting were also seen to some extent in all patients. These were controlled by using central venous catheters for drug infusion (nine patients); by decreasing the rate of infusion; by administering acetaminophen, ibuprofen, diphenhydramine, meperidine, heparin, or hydrocortisone; or by combinations of these measures.

Six patients had documented fungal infections, and three of these had clinical and microbiological resolution of their infections with no relapse from 1 to 20 weeks after therapy (Table 1). The other six patients were empirically treated (fungal infection never confirmed) with amphotericin B for continued fever, despite 5 to 7 days of broad-spectrum antibacterial therapy.

Four patients died during or shortly after completing amphotericin B therapy. Two patients (8 and 9) expired during therapy because of severe pulmonary aspergillosis in the presence of neutropenia, and one (patient 1) died of gram-negative bacterial pneumonia in the presence of neutropenia 7 days after completing a course of empiric amphotericin B therapy. Patient 2 received empiric amphotericin B for continued fever in the presence of multiple antibacterial drugs and expired 8 days after the completion of antifungal therapy from complications resulting from her underlying

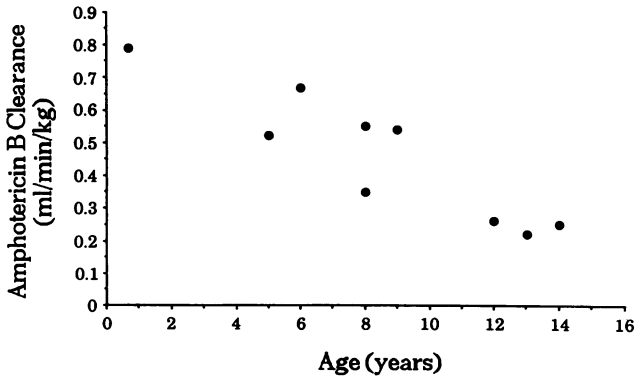

FIG. 2. Relationship between age and total CL of amphotericin B in nine patients.

TABLE 3. Effect of amphotericin B on renal function

Patient	Cumulative dose (mg/kg)	Serum creatinine values (mg/dl)		
		Baseline	Maximal (% increase)	Posttherapy (days[a])
1	8.3	0.4	0.8 (100)	0.5 (1)
2	3.0	0.2	1.7 (750)	—[b]
3	4.2	0.4	0.6 (50)	0.2 (3)
4	30.0	0.2	0.6 (200)	—
5	8.6	0.1	0.3 (200)	<0.1 (0)
6	30.0	0.5	0.9 (80)	0.5 (1)
7	2.5	0.2	0.3 (50)	0.2 (2)
8	13.8	0.5	1.2 (140)	—
9	6.9	0.4	1.9 (375)	0.5 (41)
10	4.0	0.3	0.9 (200)	—
11	5.9	0.6	1.6 (167)	—
12	5.2	0.2	0.6 (200)	0.2 (0)

[a] Number of days after amphotericin B therapy was discontinued.
[b] —, Not determined.

disease (transposition of the great arteries). No autopsies were performed.

DISCUSSION

Peak concentrations of amphotericin B in serum in our patients were higher than the MICs reported for most fungi (3). However, there was a lack of correlation between concentrations in serum and clinical outcome. There was also considerable variation in peak concentrations among patients, indicating that different dosages may be needed to achieve a given target concentration in different patients. The importance of this finding is unclear, since a therapeutic concentration in serum has yet to be clearly identified for amphotericin B.

We found a strong inverse correlation between patient age and CL of amphotericin B. At least two interpretations of this finding are possible. First, younger children with higher CL perhaps receive inadequate dosages of amphotericin B for the treatment of fungal infections. This interpretation is not supported by our clinical experience, since there does not appear to be a higher failure rate in young children treated with amphotericin B for fungal infections, compared with that in older children. The second interpretation is that older children (more than 9 years of age) are being exposed to excessive dosages of amphotericin B. We found no correlation between changes in serum creatinine and patient age, CL, total dosage, or duration of therapy. The absence of these correlations may be due to the low number of patients studied, which would increase the chance of type 2 error. The relationship between age and CL of amphotericin B should be studied further to define its clinical significance.

In our study, patient 8 showed a significant decline in clinical status with simultaneous increases in CL and V, and thus a marked decrease in peak concentration of amphotericin B in serum, between the first and second studies. These changes may have resulted from this patient's hepatic and renal failure which may have decreased binding of amphotericin B to serum lipoproteins and cholesterol (decreased binding sites or displacement from sites), resulting in an increase in V. If amphotericin B is restrictively cleared by the liver or kidney, CL also would increase if there were decreased binding. Since amphotericin B appears to accumulate in tissues and is released very slowly (terminal $t_{1/2}$ may approach 15 days [1]), renal or hepatic mechanisms may in fact be primary routes for amphotericin B elimination, as it is released from tissue depots, and thereby be influenced by changes in binding. Unfortunately, this hypothesis was not tested since binding studies were not performed. Failure to find significant contributions of hepatic or renal mechanisms in the elimination of amphotericin B by investigators (2) may be due to the difficulty of accurately measuring the extremely prolonged elimination rate of this drug.

The means of CL and V in our patients were similar to those reported in a recent study of children (6) but were different from other studies of children (7) and two adults (1) (Table 4). These differences may be partly due to differences in patient age (among the pediatric studies), although other factors such as the analytical method used to determine amphotericin B concentrations may have played a role. It should be noted that the elimination $t_{1/2}$ of 15 days reported by Atkinson and Bennett (1) was calculated by measuring amphotericin B concentrations over several days after cessation of therapy, and represents a terminal-phase $t_{1/2}$. This value cannot be compared with the β $t_{1/2}$ reported by us or other investigators.

TABLE 4. Pharmacokinetic parameters of amphotericin B[a]

Investigators (reference)	No. of patients studied	CL (ml/min per kg)	V (liters/kg)	$t_{1/2}$ (h)
Atkinson and Bennett (1)	2	0.43 ± 0.08	3.99 ± 0.40	—[b]
Starke et al. (7)	9[c]	3.72 ± 3.91	3.10 ± 2.32	17.7 ± 17.6
Koren et al. (6)	13	0.43 ± 0.08	0.38 ± 0.02	9.93 ± 1.50
Benson and Nahata (this work)	9	0.46 ± 0.20	0.76 ± 0.52	18.1 ± 6.65

[a] Values are means ± standard deviations.
[b] β $t_{1/2}$ not reported; terminal $t_{1/2}$ was 15 days.
[c] Excluding one neonate, aged 17 days, whose CL, V, and $t_{1/2}$ values were 0.16 ml/min per kg, 9.44 liters/kg, and 693 h, respectively.

The V we calculated is similar to that reported for the volume of the central compartment by Atkinson and Bennett (0.44 liter/kg) (1). The method we used to calculate V may be a better estimate for the volume of the central compartment than the total volume, since amphotericin B is very highly tissue bound.

Considerable variation in the pharmacokinetic parameters of amphotericin B has been reported in the literature (5–7). Some authors recommend routine monitoring of concentrations of amphotericin B in serum in some or all patients to minimize toxicity and improve efficacy. Although we also found a high degree of variability in the CL, V, and peak concentrations of amphotericin B in serum in our patients, we believe it is premature to advocate routine serum concentration monitoring at this time. (Patient 11 had a peak concentration of 10.02 μg/ml in serum, but all previous and subsequent concentrations were much lower and this measurement may have been erroneous.) Until clear relationships between concentrations of amphotericin B in serum and either toxic manifestations or clinical efficacy are established, measurement of concentrations in serum will provide little clinically useful information. It is our recommendation that therapy continue to be guided by criteria such as location and severity of the infection, the infecting organism, the immune status of the patient, and measures of toxicity until other monitoring parameters that consistently correlate with outcome are found (2).

We found considerable variability in concentrations in serum and in the pharmacokinetic parameters of amphotericin B among pediatric patients. A significant inverse correlation was observed between patient age and CL of amphotericin B. Although a study involving a large patient population is needed to evaluate the clinical significance of this finding, it is tempting to speculate that lower dosages of amphotericin B may be needed in patients older than 9 years of age to achieve optimal efficacy and minimize dose-related toxicity. The pharmacokinetics of amphotericin B are poorly understood, and additional studies looking specifically at the mechanism of elimination are needed to better define the effects of disease and age on amphotericin B disposition.

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