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High-Dose Weekly AmBisome Antifungal Prophylaxis in Pediatric Patients Undergoing Hematopoietic Stem Cell Transplantation: A Pharmacokinetic Study

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ABSTRACT

Disseminated fungal infection causes significant morbidity and mortality in children undergoing hematopoietic stem cell transplantation (HSCT). The widespread use of prophylactic oral triazoles has limitations of poor absorption, interindividual variability in metabolism, and hepatic toxicity. AmBisome (amphotericin B liposomal complex) has a better safety profile than the parent drug amphotericin B and produces higher plasma and tissue concentrations. We hypothesized that once-weekly high-dose AmBisome therapy could provide adequate fungal prophylaxis for immunocompromised children undergoing HSCT. We performed a pharmacokinetic pilot study to determine whether once-weekly high-dose AmBisome administration would result in effective concentrations throughout the dosing interval. A total of 14 children (median age, 3 years, 1 month; range, 4.5 months–9 years, 9 months) undergoing HSCT received once-weekly intravenous AmBisome prophylaxis (10 mg/kg as a 2-hour infusion). Blood samples for pharmacokinetic measurements were drawn around the first and the fourth weekly doses. The concentration of non–lipid-complexed amphotericin in plasma was determined by a validated bioassay. Pharmacokinetic parameters after single doses and during steady state were calculated using standard noncompartmental methods. AmBisome was well tolerated at this dose. Complete pharmacokinetic profiles for weeks 1 and 4 were obtained in 12 patients. The half-life calculated in this pediatric population was shorter on average than reported in adults (45 hours vs 152 hours). The volume of distribution correlated best with body weight (R^2 = .55), and clearance was best predicted by initial serum creatinine level (R^2 = .19). Mean (± standard deviation) individual plasma trough concentrations were 0.23 (0.13) mg/L after single doses and 0.47 (0.41) mg/L after multiple doses. Mean steady-state area under the curve was higher at week 4 than after a single dose ($P < .05$). Single-dose and steady-state pharmacokinetic profiles were similar in 8 patients, whereas in 4 patients the week 4 profile showed nonlinear elimination. However, plasma concentrations at 7 days (Cmin) were not significantly different after the first and fourth doses, suggesting no significant accumulation over the course of therapy. Our data show measurable amphotericin B plasma concentrations 7 days after high-dose infusion of AmBisome. This suggests that once-weekly dosing, as described in this study, may provide useful protection against fungal infections.

KEY WORDS

AmBisome • Fungal prophylaxis • Hematopoietic stem cell transplantation • Pharmacokinetics

INTRODUCTION

Disseminated fungal infection causes significant morbidity and mortality in immunocompromised children. Among those at highest risk are children with leukemias (ie, acute myeloid leukemia [AML], relapsed acute lymphoblastic leukemia [ALL]), bone marrow failure syndromes, or immunodeficiencies, and those undergoing allogeneic hematopoietic stem cell transplantation (HSCT) [1-4]. Graft-versus-host disease (GVHD) after allogeneic HSCT requires fur-

ther immunosuppression and in turn increases the risk of invasive fungal infections. The leading causes of opportunistic fungal infections in these patients are *Candida* and *Aspergillus* species [1,5,6]. Most pediatric series of invasive aspergillosis report an overall survival rate of 15%-34% [1,7]. Very high mortality rates are also reported for infections caused by other filamentous fungi [8].

The life-threatening nature of this complication warrants prophylaxis against fungal infections, which is considered the standard of care for this group of high-risk patients. A number of options are available for prophylaxis, but none has been found to be ideal. Prophylactic oral triazoles are limited by poor oral absorption, interindividual variation in metabolism, and hepatic toxicity, leading to reports of breakthrough infections [9]. Conventional amphotericin B has been used prophylactically [10], but is associated with infusional toxicity, long-term nephrotoxic side effects [11], and need for frequent infusions. AmBisome is a liposomal formulation consisting of amphotericin B in small, unilamellar vesicles. AmBisome has a unique composition, containing cholesterol and charged phospholipids, which stabilize the liposomes and prolong their residence in plasma. AmBisome causes fewer infusional reactions [12-14] and produces higher plasma and tissue concentrations compared with the parent drug amphotericin B [15-19]. The lower toxicity of the liposomal formulation allows the administration of much higher doses. Prophylaxis with AmBisome has been investigated at doses of 1-2 mg/kg/day in high-risk patients in several studies [20-22]. Widespread and long-term use is limited by the need for frequent intravenous administration, however. An alternative approach to prophylaxis is once-weekly high-dose AmBisome therapy. This strategy is simple and convenient for patients, helps ensure compliance in young children in whom frequent oral medication doses can be a challenge, and has the potential to generate high tissue concentrations of amphotericin B.

To date, no pharmacokinetic data for AmBisome in children have been reported in the literature. To investigate the potential of weekly prophylactic administration, we performed a pilot pharmacokinetic study of once-weekly high-dose (10 mg/kg) AmBisome therapy in pediatric patients undergoing HSCT.

METHODS

This study was a prospective open-label single-center observational clinical trial aimed at studying the pharmacokinetics of once-weekly high-dose AmBisome therapy in children. Patients receiving HSCT in whom antifungal prophylaxis was clinically indicated were eligible for the study. A total of 14 children with various hematologic disorders, metabolic disorders, and immunodeficiency syndromes undergoing HSCT were enrolled (Table 1). The study included only children age ≤10 years, because the goal of our study was to evaluate the pharmacokinetics of AmBisome in small children. The median age was 3 years, 1 month (range, 4.5 months–9 years, 9 months), and the male-to-female ratio was 2:1. None of the patients had a history of previous fungal infection.

The study design was approved by the Cincinnati Children's Hospital Medical Center's Institutional Review Board (IRB), and consent was obtained from each child's parents before the child was enrolled in the study.

AmBisome (Fujisawa Healthcare, Deerfield, IL), a lyophilized liposomal preparation of amphotericin B, was reconstituted according to the manufacturer's in-

Table 1. *Patient Demographics*

Patient #	Age	Weight (kg)	Sex	Diagnosis
1	9 months	9.0	Male	SCID
2	3 years, 2 months	13.0	Male	DBA, ALL, SCID
3	9 years, 9 months	44.7	Female	Fanconi's anemia
4	3 years, 9 months	12.4	Male	Neuroblastoma
5	8 years, 2 months	20.6	Female	Fanconi's anemia
6	1 year, 4 months	19.9	Male	HLH
7	2 years, 2 months	13.5	Female	Amegakaryocytic thrombocytopenia
8	3 years	14.1	Female	Neuroblastoma
9	4.5 months	5.8	Male	SCID
10	4 years, 5 months	22.0	Male	Sideroblastic anemia
11	11 months	9.1	Male	WAS
12	1 year, 5 months	13.0	Male	Congenital erythropoietic porphyria
13	6 years, 8 months	22.0	Female	Niemann-Pick disease
14	4 years, 5 months	18.7	Male	Glanzmann's thrombasthenia
Median (range)	3 years, 1 month (4.5 months-9 years, 9 months)	13.8 (5.8-44.7)	5/9 (F/M)	

SCID indicates severe combined immunodeficiency; DBA, Diamond-Blackfan anemia; ALL, acute lymphoblastic leukemia; HLH, hemophagocytic lymphohistiocytosis; WAS, Wiskott-Aldrich syndrome.

structions to give a 4-mg/mL solution. Drug dilutions for injection were prepared as needed with 5% dextrose. All patients received once-weekly intravenous AmBisome prophylaxis at a dose of 10 mg/kg.

Pharmacokinetic Sampling

Serial blood samples were drawn around the first and the fourth weekly doses. Venous blood samples (2.0 mL) were obtained from an indwelling catheter immediately before AmBisome administration (ie, time = 0) and then at 0.5, 1, 2, 2.5, 3, 4, 6, 24, 60, 96, 120, 144, and 168 hours after administration.

Amphotericin Assay

The concentration of non–lipid-complexed amphotericin in plasma was determined by validated bioassay with *Paecilomyces variotii* as an indicator organism [23] in the laboratory of Dr. David Stevens, Division of Infectious Diseases, Santa Clara Valley Medical Center, San Jose, California. The lower limit of detection by this assay was 0.03 μg/mL. The intraday and interday coefficients of variation were <10% and ranged from 2.3% to 9.6%.

Pharmacokinetic Analysis

Pharmacokinetic analyses of single-dose and steady-state data were conducted using standard noncompartmental methods (WinNonlin Professional version 4.0; Pharsight, Mountain View, CA). Individual plasma trough concentrations were determined by visual inspection of the plasma concentration–time profiles. The apparent terminal elimination rate constant (λz) for AmBisome was estimated for each subject by nonlinear regression analysis. The area under the plasma concentration–time curve (AUC0-τ; AUC0-∞) was determined using the linear trapezoidal method. Total body clearance (CL), volume of distribution (Vz), and terminal half-life (T½) were calculated using standard equations.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). The 2-tailed Student *t*-test for paired data was used to compare pharmacokinetic estimates after single and multiple doses. A *P* value <.05 was considered significant. Associations between pharmacokinetic measures and patient data (eg, age, height [Ht], body weight [Wt], body surface area [BSA], body mass index) were evaluated using Spearman's correlation coefficient at the .05 significance level. Statistical analyses were performed using SPSS version 11.5 for Windows (SPSS, Chicago, IL).

RESULTS

Fourteen patients completed week 1, and 12 completed both the week 1 and week 4 pharmacokinetic studies. One participant did not complete the week 4 study because of problems with venous access, and another developed infusion toxicity (fever, rash, and leg cramps) requiring withholding of the week 4 dose. AmBisome was well tolerated at this dose in all of the other subjects. The mean change in serum creatinine level between week 1 and week 4 was 0.07 mg/dL (P = .12). None of the patients developed hypokalemia, hypomagnesemia, or increased alkaline phosphatase or tranaminase levels.

The mean concentration-time profiles of nonliposomal amphotericin B in plasma after the first dose (week 1) and during steady state (week 4) are shown in Fig. 1. As a result of measuring nonliposomal amphotericin B as opposed to total liposomal content, the initial drug concentrations did not increase in a linear fashion with dose and continued to rise after the end of AmBisome infusion in some patients, due to release of active amphotericin from the liposomal vehicle. Peak concentrations were observed between 1 and 6 hours after the start of the AmBisome infusion and ranged from 2.1 to 3.4 mg/mL after week 1 and from 2.6 to 3.8 mg/L at week 4. The elimination half-life ranged from 28.5 to 107.5 hours, shorter than that previously described in adults [24].

Pharmacokinetic parameter estimates after a single dose (week 1) and during steady state (week 4) for the 12 patients who completed both parts of the study are summarized in Table 2. Parameter estimates for the 2 patients who completed only the first week of the study were similar. Comparing the parameters after week 1 and week 4 revealed no statistically significant differences except for the AUC (Table 2). The AUC, as determined by the trapezoidal rule, ranged from 79 to 275 mg · h/L in week 1 and 105 to 462 mg · h/L in week 4 (P = .03).

Plasma levels at 7 days (C_{min}) were not significantly different after the first and fourth doses, sug-

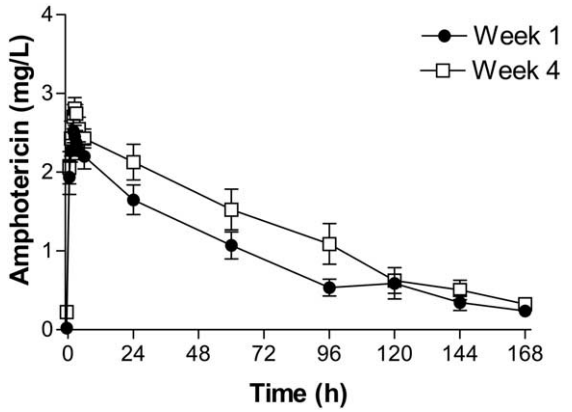

Figure 1. Pharmacokinetic profiles for week 1 and week 4 (mean ± SD): Mean concentration–time profiles of nonliposomal amphotericine B in pediatric HSCT patients. Mean data points (±SD) are graphically connected for each course of therapy in week 1 and week 4.

Table 2. *Mean (SD) Pharmacokinetic Parameter Estimates after Week 1 and Week 4 Therapy in 12 Patients*

Parameters (unit)	Week 1 (n = 12)	Week 4 (n = 12)	P Value
Cmax (mg/L)	2.71 (0.47)	3.02 (0.39)	NS
Tmax (h)	2.52 (1.42)	2.33 (0.99)	NS
Trough (mg/L)	0.23 (0.13)	0.47 (0.41)	NS
$t_{½}$ (h)	43.42 (14.11)	54.99 (24.32)	NS
AUC0-∞ (hr*mg/L)	156.21 (69.73)	255.29 (133.74)	.03
Vz (L/kg)	4.19 (1.14)	4.24 (2.85)	NS
CL (L/hr/kg)	0.0744 (0.0338)	0.0593 (0.0407)	NS

Cmax indicates peak plasma concentration; Tmax, time to peak plasma concentration; $t_{½}$, elimination half-life; AUC0-∞, area under the plasma concentration-time curve extrapolated to infinity; Vz, volume of distribution; CL, total body clearance; NS, not significant.

gesting no accumulation over the course of therapy. The half-life measured in our study group was shorter than that reported in adults (43-55 hours vs 152 hours) [24]. The volume of distribution (Vz) and clearance (CL) values in our group were higher than those reported in adults and correlated positively with body weight Vz > CL (R^2 = .55 for Vz; R^2 = .19 for CL) (Figs. 2 and 3). The volume of distribution was significantly correlated with Wt, Ht, and BSA for both week 1 and week 4. There was a trend toward a correlation between Wt and clearance (week 1) that did not achieve statistical significance (Table 3).

Only one patient (patient 3) developed evidence of fungal infection. This child had a single pulmonary nodule removed, and even though all cultures remained negative, pathological examination suggested fungal infection. The patient was treated with empiric antifungal therapy and remains well 9 months post-transplantation.

DISCUSSION

In this study we evaluated the pharmacokinetics of high-dose AmBisome given once weekly to young children (age <10 years). Our interest in weekly administration of AmBisome came from 2 sources. First, animal studies have suggested that this dosing schedule may be effective. Garcia et al [25] used a mouse model of *Candida albicans* and *Histoplasma capsulatum* infection [25] to demonstrate the efficacy of a single prophylactic dose of AmBisome (1-20 mg/kg) given 7 days before challenge. These and recent data using invasive *Aspergillosis* as a model suggest that biologically relevant blood and tissue levels of drug may be present 7 days after administration of a single dose of AmBisome [26]. The second impetus for the study was the clinical challenge of administering long-term oral antifungal prophylaxis (eg, with azoles) to small children. Small children may not accept oral administration, and hepatic toxicity is common. Alternative therapy with an echinocandin (eg, caspofungin) requires daily intravenous administration. We reasoned that if weekly dosing provided reasonable plasma levels of AmBisome, then this would provide a simple prophylaxis regimen that could be administered for long periods on an outpatient basis. In addition, if a weekly schedule was feasible, then the strategy could be applied to other populations needing long-term prophylaxis, such as children with bone marrow failure syndromes, AML or high-risk ALL, and immune deficiencies.

Our study has demonstrated a consistent pharmacokinetic profile for AmBisome administered at this dose, with nonliposomal amphotericin plasma levels detectable on the seventh day before redosing and no accumulation found with repeated dosing. Previous in vitro time-kill and post-antifungal effect (PAFE) studies with amphotericin B have demonstrated concentra-

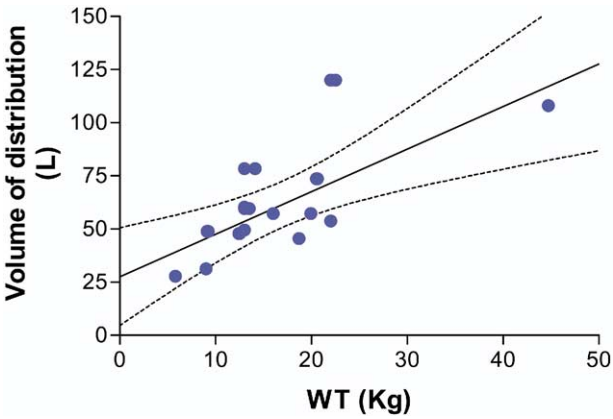

Figure 2. Correlation between body weight and volume of distribution (Vz): Correlation plot of individual estimates for volume of distribution (in L) versus body weight (WT in Kg). The solid line is the line of best fit for the data (R^2 = .55). The dashed line represents the 95% confidence intervals.

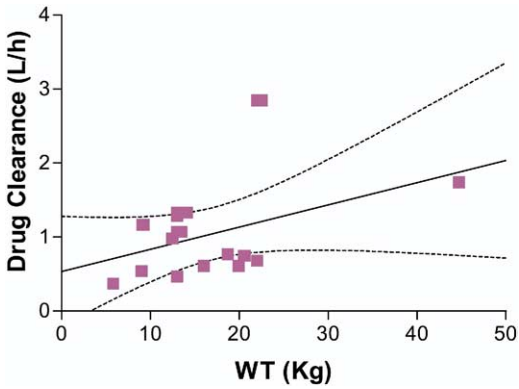

Figure 3. Correlation between body weight and clearance (CL): Correlation plot of individual estimates for clearance (in L/h) versus body weight (WT in Kg). The solid line is the line of best fit for the data (R^2 = .19, not significant). The dashed line represents the 95% confidence intervals.

Table 3. *Associations Between Pharmacokinetic Measures and Patient Data*

	Week 1			Week 4		
	Vz (L)	CL (L/h)	AUC	Vz (L)	CL (L/h)	AUC
Age	0.546*	0.383	0.306	0.574	0.431	0.445
Weight	0.740†	0.443	0.304	0.641*	0.305	0.574
Height	0.692†	0.543*	0.147	0.643*	0.357	0.517
BSA	0.679†	0.437	0.279	0.643*	0.357	0.517
Serum creatinine	0.747†	0.612*	−0.123	0.363	0.00	0.550

Vz indicates volume of distribution; CL, total body clearance; AUC, area under the plasma concentration-time curve; BSA, body surface area.
*Correlation is significant at the 0.05 level (2-tailed).
†Correlation is significant at the 0.01 level (2-tailed).

tion-dependent activity and significant PAFE against a variety of yeasts [27]. These and other studies have demonstrated that the peak serum level/minimum inhibitory concentration (MIC) ratio was the pharmacokinetic and pharmacodynamic parameter that most strongly correlated with the efficacy of amphotericin B. In addition, serum drug concentrations have been shown to be a relatively good surrogate of tissue concentrations. The concentrations of nonliposomal amphotericin B at 7 days (the end of interval in this study) are around the MICs for susceptible strains (*Candida*, 0.25-1 mg/L; *Aspergillus*, 0.5-2 mg/L) [28].

A limitation of our study was the absence of measured tissue AmBisome concentrations. Most of the clinical benefit of AmBisome likely requires tissue dispersion of the drug. A recent study found that a once-weekly 15-mg/kg AmBisome dose given to adult patients undergoing HSCT achieved high, sustained tissue concentrations, similar to those achieved with conventional (1 mg/kg) daily dosing. The mean tissue-to-plasma concentration ratio of AmBisome on day 7 was 16.3, suggesting markedly higher tissue drug levels compared with measured plasma levels [29]. These data indicate that in adults, AmBisome can be given safely in high doses and support our hypothesis that once-weekly AmBisome dosing may provide effective antifungal prophylaxis in small children.

Data collected in adults receiving therapy for proven or likely fungal infections have demonstrated that Ambisome doses as high as 15 mg/kg are well tolerated [30]. A maximum tolerated dose (MTD) was not reached in this dose escalation study, and the major toxicities of infusion reactions and renal impairment did not appear to be dose-related. Of interest, this study demonstrated nonlinear pharmacokinetics of AmBisome at doses >10 mg/kg, with no further increase in Cmax or AUC with a dose of 12.5 or 15 mg/kg. These data suggest the uptake of AmBisome in the reticuloendothelial system, the major pathway for extraction of AmBisome from the plasma, with accumulation of the drug in tissues. This finding has the potential to offer protection to the liver and spleen, and possibly the lungs at higher doses, sites particularly vulnerable to mold or yeast infection.

We selected a dose of 10 mg/kg for our study due to the nonlinear pharmacokinetics of AmBisome at higher doses as well as data indicating that this dose is below the MTD of AmBisome, at least in adults, in whom doses of 15 mg/kg/day did not reach MTD. In our study, 10 mg/kg dosing was well tolerated, with a single case of infusion toxicity and no renal, liver, or other toxicities, suggesting that this is a safe strategy for long-term use. Weekly AmBisome therapy carries a significant cost, higher than oral therapy but (in most hospitals) is less expensive than daily echinocandin therapy. Weekly AmBisome therapy offers an option for long-term prophylaxis in children unable to tolerate oral azole therapy.

Liposomal amphotericin B has been shown to have a long terminal half-life (approximately 152 hours) in normal adults [24]. In our population of young children, we found a significantly shorter half-life for nonliposomal amphotericin B. In addition, the volume of distribution was associated with weight, suggesting some pharmacokinetic differences between small children and adults. When interpreting these data, it is important to note that in contrast to many previous reports that describe the pharmacokinetics of total amphotericin B (the sum of liposomal, plasma protein–bound, and free or unbound drug), we have measured the pharmacokinetics of the active portion of the drug or non–lipid-complexed amphotericin, making it difficult to directly compare our results with earlier pharmacokinetic results. Although our study was not designed to prove the efficacy of this approach, the data provide support for a clinical study of this prophylaxis strategy.

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