Population pharmacokinetics of amphotericin B in children with malignant diseases

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Aims To construct a population pharmacokinetic model for the antifungal agent, amphotericin B (AmB), in children with malignant diseases.

Methods A two compartment population pharmacokinetic model for AmB was developed using concentration-time data from 57 children aged between 9 months and 16 years who had received 1 mg kg⁻¹ day⁻¹ doses in either dextrose (doseform = 1) or lipid emulsion (doseform = 2). P-Pharm (version 1.5) was used to estimate the basic population parameters, to identify covariates with significant relationships with the pharmacokinetic parameters and to construct a Covariate model. The predictive performance of the Covariate model was assessed in an independent group of 26 children (the validation group).

Results The Covariate model had population mean estimates for clearance (CL), volume of distribution into the central compartment (*V*) and the distributional rate constants (*k*₁₂ and *k*₂₁) of 0.88 l h⁻¹, 9.97 l, 0.27 h⁻¹ and 0.16 h⁻¹, respectively, and the intersubject variability of these parameters was 19%, 49%, 55% and 48%, respectively. The following covariate relationships were identified: CL (l h⁻¹) = 0.053 + 0.0456 weight^{0.75} (kg) + 0.242 doseform and *V* (l) = 7.11 + 0.107 weight (kg). Our Covariate model provided unbiased and precise predictions of AmB concentrations in the validation group of children: the mean prediction error was 0.0089 mg l⁻¹ (95% confidence interval: -0.0075, 0.0252 mg l⁻¹) and the root mean square prediction error was 0.1245 mg l⁻¹ (95% confidence interval: 0.1131, 0.1349 mg l⁻¹).

Conclusions A valid population pharmacokinetic model for AmB has been developed and may now be used in conjunction with AmB toxicity and efficacy data to develop dosing guidelines for safe and effective AmB therapy in children with malignancy.

Keywords: amphotericin B, children, population pharmacokinetics, P-Pharm

Introduction

Children with malignant diseases receive many immuno-suppressive agents as part of their treatment and are therefore vulnerable to fungal infections. When these children experience fever that persists in spite of broad spectrum antibiotic therapy, they are administered a standard amphotericin B (AmB) dose of 1 mg kg⁻¹ day⁻¹ to prevent fungal superinfection or to control clinically detected or undetected fungal infection. This dose has

evolved from clinical experience and dose escalation studies [1] and is generally well tolerated and effective. However, there are occasions where the standard dose is insufficient to prevent fungal infection and there are also occasions when renal impairment occurs. A rational basis for modifying the standard dose would help to ensure optimal outcomes. This may be achieved by developing a better understanding of AmB pharmacokinetics and its relationships with toxicity and efficacy. There have been few studies on the pharmacokinetic behaviour of AmB in children [2–5] and these studies have generally used a traditional approach to the pharmacokinetic analysis employing many blood samples and few patients. Two previous studies [6, 7] have used a population pharmacokinetic approach to examine the pharmacokinetics of lipid-based formulations of AmB. The patient groups

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studied were adults with HIV (AIDS) [7] and bone marrow recipients (5 children under 13 years, 70 adults) [6]. A population approach is especially suited for pharmacokinetic investigations in children since it is possible to study the disposition of the drug in situations where the data are sparse [8, 9]. The aim of this paper is to use a population approach to understand better the pharmacokinetics of AmB in children with malignant disease.

Methods

Patients

Eighty-three children with malignant diseases who received a total of 122 courses of AmB for fever/neutropenia were included in this analysis. A course of AmB consisted of a period of time (usually 6 days) in which AmB was administered daily. A total of 27 children received more than one course of AmB (18 patients had two courses, 6 children had three courses and 3 children had four courses). The average time between courses was 3.3 ± 3.2 months (range: 1–13 months, $n=39$). Fungal infection was demonstrated in 20 courses: blood culture (10), fungal lesions in lung (3), stool specimen (3), mouth swab (4). The pathogens were *Candida* (15), *Aspergillus* (1), *Xanthomonas* (1) and unidentified (3). Nephrotoxicity, as previously defined (abnormal increase in plasma creatinine $>100\%$ above baseline) [10], only occurred in two courses.

All of the patients in this investigation were part of a previously reported clinical study which was a single centre, randomized, open-labelled controlled comparison of the clinical tolerance, toxicity and pharmacokinetics of AmB infused in either 5% dextrose or in lipid emulsion [5]. The study was approved by The Children's Hospital at Westmead Ethics Committee and the parents of all children gave informed consent.

Dose administration

AmB desoxycholate (Fungizone, Bristol-Myers-Squibb) was reconstituted with water for injection and diluted with either 5% dextrose (the conventional method of administration) or parenteral lipid emulsion (20% Intralipid, Kabi Pharmacia). The AmB dose of 1 mg kg^{-1} was administered as a 2 h intravenous infusion. Doses of 40 mg or less were given in 100 ml of infusate (maximum concentration 0.4 mg kg^{-1}). Doses of over 40 mg (with a maximum of 50 mg) were given in 250 ml of infusate.

All patients had an indwelling double lumen central line catheter into the right atrium, so that one lumen could be used for drug administration and one for blood collections. Patients received the AmB dose daily for up to 8 days.

Figure 1 Frequency histogram of blood collection times in the total data cohort.

Blood collection and analysis

Heparinized whole blood samples (1–2 ml) were collected throughout the period of AmB therapy and the times of blood collection and dose administration were recorded. Figure 1 shows the blood sampling times for the whole data cohort. Blood sampling after the first dose was performed in 35 patients at specific times including the end of the infusion (2 h), and then at 3, 4, 6, 12, 18 and 24 h after the infusion start. For subsequent doses blood was generally taken towards the end of the dosing interval, but approximately 20% of samples were collected towards the beginning of the dosing interval.

Plasma samples were separated by centrifugation for 10 min at 4°C at $3000 \text{ rev min}^{-1}$ in a Beckmann GS-6R centrifuge, stored at -40°C and analysed within 1 week of collection. AmB was determined in plasma using a high performance liquid chromatographic assay method published previously [5]. This assay was linear over AmB concentrations ranging from 0.1 to $6 \mu\text{g ml}^{-1}$. The between-day coefficient of variation was 15% for $0.5 \mu\text{g ml}^{-1}$ concentration ($n=83$) and 11% for a $1.5 \mu\text{g ml}^{-1}$ ($n=83$) concentration. The limit of detection of the AmB assay was $0.1 \mu\text{g ml}^{-1}$.

Clinical and pharmacokinetic data

The pharmacokinetic data that was collected included AmB dose, time of dose, blood collection time and AmB concentration. The clinical data that was recorded included both continuous covariates (patient age, height, weight, weight^{0.75}) and categorical covariates (gender, diagnosis, method of AmB administration, history of prior bone marrow transplant, coadministration of total parenteral nutrition, concomitant medications including

cyclosporin, diuretics, acyclovir, morphine, ondansetron or promethazine).

Data sets

The total data cohort (807 concentration–time observations) consisted of pharmacokinetic and covariate data from 83 children who received 122 courses of AmB. For the purposes of developing and validating the population pharmacokinetic model the data were randomly split in a ratio of 2:1, as described in the FDA guidelines on population pharmacokinetics [11]. Microsoft Excel (version 5.0) was used to randomly select approximately two thirds of the children whose data would be used for model building. This, the Model Development Data Set, consisted of pharmacokinetic and covariate data from 57 children who were administered 83 courses of AmB (581 concentration–time observations). The data from the remainder of the children were used for model validation. This, the Model Validation Data Set, consisted of pharmacokinetic and covariate data from 26 children who were administered 39 courses of AmB (226 concentration–time observations).

Strategy for the population pharmacokinetic analysis

The population pharmacokinetic analysis was conducted using the population pharmacokinetic modelling software, P-Pharm version 1.5 (Innaphase, Philadelphia, USA), which implements non-linear mixed effects modelling using a parametric EM-like algorithm [12]. For the purpose of the population pharmacokinetic analysis, each course of AmB was considered as a separate individual.

The population pharmacokinetic analysis strategy involved a three-step approach. In step 1, an initial P-Pharm analysis provided the population pharmacokinetic parameters without taking into account potentially explanatory covariates. In the second step, an exploratory analysis was used to investigate the relationships between patient covariates and the posterior Bayesian estimates of individual pharmacokinetic parameters. In step 3, the covariates found to be influential using stepwise multiple linear regression were included in the analysis and population pharmacokinetic parameters were again determined.

Development of the population pharmacokinetic model without covariates (Step 1)

A Basic Population Pharmacokinetic Model for AmB (without covariates) was developed using the Model Development Data Set and initial pharmacokinetic parameter estimates derived from a previous study [5]. A range of different population pharmacokinetic and

pharmacostatistical models were generated that varied in the following: (1) the number of compartments (one *vs* two) (2) the distribution of the intersubject variability of the pharmacokinetic parameter estimates (normal *vs* lognormal) and (3) the residual error variance (heteroscedastic *vs* homoscedastic). A number of evaluation criteria were then used to select the most appropriate model including (1) a low estimate for sigma (the residual error term which comprises the intra-subject variability, the assay error and the model misspecification error), (2) low estimates of intersubject variability in the pharmacokinetic parameters, (3) a low estimate for the Akaike Information Criterion (AIC), (4) a high estimate for Maximum Likelihood (ML), (5) good agreement between model-predicted and measured AmB concentrations, (6) residuals with a mean that was not significantly different from zero using a Student's *t*-test and (7) residuals with a distribution that was not significantly different from that of normal with a mean of zero and a variance of 1 using the Kolmogorov–Smirnov test.

The primary amphotericin B pharmacokinetic parameters of interest were clearance (CL), volume of distribution into the central compartment (*V*), and the distributional first order rate constants (*k*₁₂ and *k*₂₁). The population mean and variance of the primary pharmacokinetic parameters were determined from the model and individual posterior Bayesian estimates of the primary pharmacokinetic parameters were generated using Bayesian forecasting.

A number of additional pharmacokinetic parameters were derived using the individual posterior Bayesian estimates of the primary pharmacokinetic parameters. Clearance and volume of distribution were normalized for weight by dividing the individual Bayesian parameter estimates by patient weight. The elimination rate constant (*k*) was calculated by dividing the estimates of CL by the estimates of *V*. Volume of distribution at steady state (*V*_{ss}) was determined using the equation: $V_{ss} = V(1 + k_{12}/k_{21})$. The area under the AmB concentration–time curve (AUC) was determined by dividing the dose (mg) by the individual posterior Bayesian estimates of CL (l h^{−1}). The mean residence time (MRT) was determined by dividing *V*_{ss} by CL. The distributional half-life (*t*_{1/2,λ₁}) and the elimination half-life (*t*_{1/2,λ_z}) were calculated by dividing 0.693 by λ₁ and λ_z, respectively, where

$$\lambda_1 = \frac{k_{12} + k_{21} + k + \sqrt{(k_{12} + k_{21} + k)^2 - (4 \cdot k_{21} \cdot k)}}{2}$$

$$\lambda_z = \frac{k_{12} + k_{21} + k - \sqrt{(k_{12} + k_{21} + k)^2 - (4 \cdot k_{21} \cdot k)}}{2}$$

Exploratory analysis to identify influential patient covariates (Step 2)

Microsoft Excel (version 5.0) was used to explore graphically the relationships between the individual posterior Bayesian estimates of the pharmacokinetic parameters and the continuous patient covariates and to determine significance using Pearson's correlation coefficient. A number of covariate data transformations (including the natural logarithm and the inverse) were also tested. The Mann–Whitney *U*-test (SPSS version 8.0) was used to test whether there were differences in the pharmacokinetic parameters when binary categorical covariates were varied.

Development of a population pharmacokinetic model including covariates (Step 3)

Within P-Pharm, the relationships between the posterior Bayesian estimates of the pharmacokinetic parameters and the patient covariates were analysed by multiple linear regression with stepwise inclusion and deletion of covariates with the threshold value of the *F* statistic set to 5. Covariates demonstrating a significant *F*-test ($P < 0.05$) were retained to produce the Covariate Population Pharmacokinetic Model. The likelihood ratio test was then used to assess whether this model was significantly better than the Basic Population Pharmacokinetic Model. The values for AIC and sigma (residual variability) were also compared for the Basic and Covariate Models, as well as the estimates of the intersubject variability in the population pharmacokinetic parameters.

Validation of the population pharmacokinetic model for AmB

The Basic and Covariate Models were validated by examining the bias and precision of the model-predicted AmB concentrations using the Model Validation Data Set. Bias (mean prediction error) and precision (root mean square prediction error) and their 95% confidence intervals were determined as previously described [13].

Comparison with other models

Some additional models were developed and compared with the Basic and Covariate Population Pharmacokinetic Models. One was based upon dextrose AmB administration only (46 children, 55 courses, 375 concentration–time observations). Another combined all courses for each child (57 children, 83 courses, 581 concentration–time observations).

Results

Comparison of the model development and model validation data sets

There were no significant difference between the Model Development and Model Validation Data Sets in the patient demographic characteristics shown in Table 1.

Selection of the pharmacokinetic and pharmacostatistical model

A two compartment pharmacokinetic model with zero-order input, first order distributional rate constants and first-order elimination provided a better description of the AmB concentration–time data than a one compartment model. The two compartment model had lower values for residual error (0.027 *vs* 0.064 mg l⁻¹) and AIC (0.0017 *vs* 0.31), higher values for ML (6.99 *vs* -174), a favourable pattern of residuals, good predictions of the observed concentration data and acceptable intersubject variability for the pharmacokinetic parameter estimates. The residual error was homoscedastic. A normal distribution best described the intersubject variability in CL while lognormal distributions best described the intersubject variabilities in *V*, *k*₁₂ and *k*₂₁.

The Basic Population Pharmacokinetic Model for AmB

The population parameters for the two compartment Basic Population Pharmacokinetic Model are shown in Table 2. The population mean estimates of AmB CL, *V*, *k*₁₂ and *k*₂₁ were 0.86 l h⁻¹, 8.73 l, 0.26 h⁻¹ and 0.14 h⁻¹, respectively, and their intersubject variabilities were 35%, 53%, 57% and 61%, respectively.

Exploratory analysis

In the exploratory analysis, significant positive correlations were identified between the individual posterior Bayesian estimates of CL (l h⁻¹) and the patient characteristics of weight ($r^2 = 0.304$, $P < 0.001$), weight^{0.75} ($r^2 = 0.306$, $P < 0.001$), age ($r^2 = 0.244$, $P < 0.001$) and height ($r^2 = 0.260$, $P < 0.001$). Significant positive correlations were also identified between the individual posterior Bayesian estimates of *V* (l) and the patient characteristics of weight ($r^2 = 0.072$, $P < 0.05$), weight^{0.75} ($r^2 = 0.067$, $P < 0.05$), age ($r^2 = 0.057$, $P < 0.05$) and height ($r^2 = 0.067$, $P < 0.05$). The method of AmB administration significantly influenced CL (l h⁻¹), but not *V* (l). A number of covariates did not significantly affect AmB pharmacokinetic parameters including gender, prior

Table 1 Characteristics of the children in the Model Development and Model Validation Data Sets.

	Model development	Validation	Significance
Total number of children	57	26	
Total number of courses	83	39	
Total number of observations	581	226	
Number of concentration observations per patient			
Mean $\pm$ s.d. (range)	6.9 $\pm$ 3.8 (1–19)	6.2 $\pm$ 2.6 (1–11)	NS ¹
Method of AmB administration			
Number in dextrose/number in lipid	43/40	14/25	NS ²
Age (months)	74.5	96.5	
Median (range)	(9–190.5)	(12–219)	NS ¹
Weight (kg)			
Mean $\pm$ s.d.	21.6 $\pm$ 10.2	25.2 $\pm$ 12.1	NS ¹
Height (cm)			
Mean $\pm$ s.d.	113.8 $\pm$ 24.7	122.2 $\pm$ 27.7	NS ¹
Gender			
Number of males/number of females	49/34	26/13	NS ²
Number of children with the following diagnoses:			
ALL	22	13	
AML	13	5	
Neuroblastoma	13	1	
Rhabdomyosarcoma	16	2	
Ewings sarcoma	0	3	
Osteosarcoma	1	1	
Synovial sarcoma	0	2	
Wilm's tumour	2	2	
Non Hodgkins lymphoma	6	1	
Germ cell tumours	4	2	
Aplastic anaemia	0	1	
Other	6	6	
Number of courses after BMT			
Yes/No/unknown	31/52/0	10/25/2	NS ²
Number of courses where TPN was administered			
Yes/No/unknown	52/31/0	18/18/1	NS ²

ALL = acute lymphoblastic leukaemia, AML = acute myeloid leukaemia, BMT = bone marrow transplant, TPN = total parenteral nutrition.

¹Significance assessed using the Mann-Whitney-*U*-test.

²Significance assessed using the chi square test.

bone marrow transplantation, total parenteral nutrition administration or the administration of acyclovir, diuretics, morphine, ondansetron and cyclosporin.

The Covariate Population Pharmacokinetic Model for AmB

In the Covariate Model, weight^{0.75} and method of AmB administration were found to be influential determinants of CL (l h⁻¹) using stepwise multiple linear regression, while patient weight was found to be an influential determinant of *V* (l). After inclusion of these covariates in the population pharmacokinetic model, the population mean estimates of CL, *V*, *k*₁₂ and *k*₂₁ were 0.88 l h⁻¹, 9.97 l, 0.27 h⁻¹ and 0.16 h⁻¹, respectively, and their

intersubject variabilities were 19%, 49%, 55% and 48%, respectively (Table 2). The regression equations describing the relationships between the pharmacokinetic parameters and the covariates that were retained in the final Covariate Population Pharmacokinetic Model are shown in Table 3.

The Covariate Model had a higher AIC (0.0333 *vs* 0.00017) and a lower Maximum Likelihood (–11.3 compared with 6.99) than the Basic Model. The inclusion of covariates in the population pharmacokinetic model for AmB did not significantly improve the model based on the likelihood ratio test but did reduce the intersubject variability in CL (46%), *V* (8%), *k*₁₂ (4%) and *k*₂₁ (21%).

Table 2 Population pharmacokinetic parameters of AmB.

Parameter	Mean	Basic Model		Mean	Covariate Model	
		% CV	95% CI		% CV	95% CI
CL (l h ⁻¹)	0.86	35	0.77, 0.95	0.88	19	0.83, 0.93
V (l)	8.73	53	7.31, 10.15	9.97	49	8.47, 11.47
k ₁₂ (h ⁻¹)	0.26	57	0.21, 0.31	0.27	55	0.22, 0.32
k ₂₁ (h ⁻¹)	0.14	61	0.11, 0.17	0.16	48	0.14, 0.18
Sigma (mg l ⁻¹)	0.027			0.027		
ML	6.99			-11.3		
AIC	0.0017			0.0333		

% CV = intersubject variability expressed as percent coefficient of variation.

95% CI = lower and upper limits of the 95% confidence interval.

Sigma = residual error.

ML = Maximum Likelihood.

AIC = Akaike Information Criterion.

Table 3 Regression models which describe relationships between AmB pharmacokinetic parameters and influential patient covariates.

Regression model	Regression coefficients (θ) mean (s.d.)	F-test	Significance
CL (l h ⁻¹) = $\theta_1 + \theta_2 \text{WT}^{0.75} + \theta_3 \text{DF}$	θ_1 : 0.053 θ_2 : 0.046 (0.006) θ_3 : 0.242 (0.040)	44.5	$P < 0.005$
V (l) = $\theta_4 + \theta_5 \text{WT}$	θ_4 : 7.106 θ_5 : 0.107 (0.043)	6.3	$P < 0.025$

WT = weight (kg), $\text{WT}^{0.75}$ = weight^{0.75} (kg), DF = method of AmB administration: dextrose infusion was coded 1 and lipid emulsion infusion was coded 2.

θ = regression coefficient, s.d. = standard deviation.

Performances of the Basic and Covariate Population Pharmacokinetic Models

The performances of both the Basic and Covariate Population Pharmacokinetic Models were excellent. There was very close agreement between observed and posterior Bayesian predicted concentrations using the Model Development Data Set as shown in Figure 2a and b. The regression line had a slope of 0.95 and a correlation coefficient of 0.97 for the Basic Model and the slope was 0.95 and correlation coefficient 0.97 for the Covariate Model. The residuals for both the Basic and Covariate Models were normally distributed, as indicated by a Kolmogorov-Smirnov test, and were not significantly different from zero using a Student's *t*-test.

Validation of the Basic and Covariate Population Pharmacokinetic Models

The Basic and Covariate Models were validated by examining the bias and precision of model-predicted concentrations using the Validation Data Set. As shown in Table 4, both models generated unbiased and precise predictions of AmB concentrations. Mean prediction errors (bias) were not significantly different from zero

using the Student's *t*-test, 95% confidence intervals for bias included zero and the values for root mean square prediction error (precision) were low. There was also close correlation between model-predicted *vs* observed concentrations for both Basic and Covariate Models (Figure 3a and b, respectively).

Comparison of AmB administration in dextrose and lipid emulsion

The individual posterior Bayesian estimates of the pharmacokinetic parameters (obtained after step 1) for the children receiving AmB in dextrose (57 courses) and the children receiving AmB in lipid emulsion (65 courses) are compared in Table 5. Administration of AmB in lipid emulsion resulted in significantly higher posterior Bayesian estimates of CL (l h⁻¹ kg⁻¹), V (l kg⁻¹) and V_{ss} (l kg⁻¹) than administration in dextrose (37%, 41% and 47%, respectively), with there being no significant difference in age or weight between the two groups. Clearance (l h⁻¹) was also significantly higher (17%) for AmB administered in lipid emulsion. The distributional rate constants, $t_{1/2, \lambda_1}$, $t_{1/2, \lambda_2}$, MRT and V (l) were unaffected by method of AmB administration.

AmB exposure for children of different weights

There was a significant positive correlation between the individual posterior Bayesian estimates of AUC and patient weight ($r^2=0.40$, $P<0.001$, Figure 4) with lighter children tending to have lower AUCs than heavier children.

Figure 2 Scatterplots of observed and individual Bayesian predicted AmB concentrations in the Model Development Data Set a) using the Basic Population Pharmacokinetic Model and b) using the Covariate Population Pharmacokinetic model.

Comparison with other models

The Basic Population Pharmacokinetic Model, which included data from both dextrose and lipid emulsion administrations and treated each course separately, was far superior to a model using only dextrose data (AIC: 0.144, ML: -46.2 , residual error: 0.034 mg l^{-1}) and to a model which combined courses for each patient (AIC: 0.182, ML: -97.1 , residual error: 0.055 mg l^{-1}). A limited

Figure 3 Scatterplots of model-predicted and individual observed AmB concentrations in the Model Validation Data Set a) using the Basic Population Pharmacokinetic Model and b) using the Covariate Population Pharmacokinetic model.

Table 4 Validation of the Basic and Covariate Models: Errors in predictions of AmB concentrations using the Validation Data Set ($n=226$).

Parameter	Basic model	Covariate model ¹
Prediction error (mg l^{-1})	0.0057	0.0089
Mean (95% CI)	(-0.0093 , 0.0208)	(-0.0075 , 0.0252)
Root square prediction error (mg l^{-1})	0.1145	0.1245
Mean (95% CI)	(0.1034 , 0.1587)	(0.1131 , 0.1349)

95% CI=lower and upper limits of the 95% confidence interval.

¹The data were modelled with clearance as a function of weight^{0.75} and method of administration and volume of distribution as a function of weight.

Table 5 Comparison of the posterior Bayesian estimates of the pharmacokinetic parameters (mean $\pm$ s.d.) of AmB administered in dextrose and AmB administered in lipid emulsion.

Parameter	Dextrose (n = 57)	Lipid (n = 65)	Significance ¹
Weight (kg)	23.3 $\pm$ 11.3	21.4 $\pm$ 10.5	NS
CL (l h ⁻¹)	0.79 $\pm$ 0.23	0.96 $\pm$ 0.24	P < 0.005
CL (l h ⁻¹ kg ⁻¹)	0.038 $\pm$ 0.015	0.052 $\pm$ 0.021	P < 0.0005
V (l)	8.51 $\pm$ 3.26	9.72 $\pm$ 4.24	NS
V (l kg ⁻¹)	0.41 $\pm$ 0.20	0.58 $\pm$ 0.43	P < 0.05
V _{ss} (l kg ⁻¹)	1.18 $\pm$ 0.57	1.74 $\pm$ 1.35	P < 0.05
t _{1/2} λ_1 (h)	1.46 $\pm$ 0.33	1.45 $\pm$ 0.26	NS
t _{1/2} λ_z (h)	26.4 $\pm$ 11.6	26.1 $\pm$ 12.8	NS
k ₁₂ (h ⁻¹)	0.28 $\pm$ 0.13	0.28 $\pm$ 0.14	NS
k ₂₁ (h ⁻¹)	0.15 $\pm$ 0.05	0.14 $\pm$ 0.03	NS
MRT (h)	33.0 $\pm$ 15.5	32.4 $\pm$ 17.8	NS

s.d. = standard deviation.

¹The pharmacokinetics of AmB infused in dextrose and lipid emulsion were compared using the Mann–Whitney–U-test.

Figure 4 The relationship between the individual posterior Bayesian estimates of AUC and patient weight.

covariate model, developed using only dextrose data, identified weight^{0.75} as a significant predictor of clearance and weight as a significant predictor of volume of distribution, so had very similar characteristics to the final model which included data from both dextrose and lipid administrations.

Discussion

To improve knowledge of AmB pharmacokinetics in children with malignant diseases we have developed a two compartment Basic Population Pharmacokinetic Model which provided unbiased and precise predictions of AmB concentrations. There have been only two previous population studies of AmB pharmacokinetics [6, 7] and both of these also found that a two compartment model was the most appropriate. We were able to include a wide variety of sparse data in this Basic Model which we had not been able to use in a previous traditional pharmacokinetic

analysis [5]. We also found that by (a) treating each course as a separate subject and by (b) including data from two different formulations of AmB (lipid and dextrose), there were marked improvements in the model, based upon AIC and Maximum Likelihood. Dextrose and lipid administrations were randomized, some patients receiving separate courses of each, so it was not possible to separate interoccasion variability from intersubject variability or method of administration. Karlsson & Sheiner have pointed out that neglecting interoccasion variability may lead to biased population parameter estimates, especially when the study design is poor [14]. Randomization of method of administration and accurate recording of pharmacokinetic and covariate data in this study undoubtedly minimized but would not eliminate such effects.

After testing a wide variety of patient characteristics and clinical factors, only weight and method of administration were found to influence AmB pharmacokinetics, and a second population pharmacokinetic model was developed which included these covariates. Compared with our Basic Model, this Covariate Model had reduced intersubject/interoccasion variability in all four of the primary pharmacokinetic parameters, although there was no significant improvement based upon AIC or likelihood ratio. A similar situation for a *p*-aminohippurate covariate model has been previously reported [15]. The predictive performance of our Covariate Model was very similar to that of the Basic Model and both provided an excellent description of the concentration–time data.

Method of AmB administration affected clearance and this relationship was included as part of the Covariate Model. The Basic Model posterior Bayesian estimates of clearance were higher for lipid compared with dextrose administration but there was no significant difference in mean residence time and the distributional rate constants. Posterior Bayesian estimates for volume of distribution (l) were lower for dextrose compared with lipid administration but the difference was significant only when the comparison was made on a weight basis (l kg⁻¹). Consequently, stepwise multiple linear regression failed to identify a significant relationship between method of administration and volume of distribution (l) for inclusion in the Covariate Model. Our results compared very favourably with previous traditional pharmacokinetic studies comparing AmB administered in lipid and dextrose [5, 16]. A possible reason for the pharmacokinetic differences between the two methods of administration is that the larger particle size of AmB in lipid emulsion [17, 18] may lead to greater uptake by the organs of the reticuloendothelial system [5]. Administration of AmB in lipid emulsion is no longer recommended because the product is inconsistent and undesirable [17–19] and we have previously observed no significant advantages in safety or tolerance [5].

Our study population had a broad weight range (6.4–56 kg) and we found that volume of distribution was a function of weight whilst clearance was a function of weight^{0.75}. Holford [20] has recommended using 3/4 power of weight to predict clearance and we found that weight^{0.75} gave slightly better predictions of clearance than weight. In children, clearance and volume of distribution are commonly weight-related and weight has been included in covariate population pharmacokinetic models for gentamicin [21], meropenem [22], ketotifen [23] and ondansetron [24]. Villani *et al.* [7], did not identify weight or any other factor as influencing their population model for AmB administered in lipid emulsion to adult AIDS patients where there was a relatively narrow weight range from 42 to 89 kg. Amantea *et al.* [6] administered a colloid dispersion of AmB in varying doses (0.5–8 mg kg⁻¹) to bone marrow recipients, including some children and, in this case, clearance (l h⁻¹ kg⁻¹) and volume of distribution (l kg⁻¹) were related to both weight and dose. In a traditional pharmacokinetic study of AmB administered to 10 infants and children in dextrose, clearance (ml min⁻¹) and volume of distribution (l) were both found to be fractional power functions of weight [4]. Our Covariate Population Pharmacokinetic Model for AmB is consistent with these other studies. A uniform dose of 1 mg kg⁻¹ AmB was used throughout our study and dose-related effects were not observed.

AmB exposure (AUC) was relatively lower in lighter, younger children suggesting that younger children are relatively underdosed on the 1 mg kg⁻¹ dose and may be at greater risk for developing fungal infections. Older, heavier children, with relatively higher exposure to AmB, may be relatively overdosed and at greater risk for developing toxic side-effects. Additional studies, investigating the relationships between AmB exposure and toxicity and/or efficacy are now needed to identify a safe and effective target AUC. It will then be possible to use our population pharmacokinetic model to develop a new dosing strategy to ensure that children of different ages achieve equivalent exposure to AmB, whilst optimizing outcomes and minimizing toxic side-effects.

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