Population Pharmacokinetics of Liposomal Amphotericin B in Immunocompromised Children

Running Title: Pharmacokinetics of liposomal amphotericin in children

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Conflicts of Interest

WWH has acted as consultant, received research support for Merck, Pfizer Inc., Astellas, Gilead Sciences, F2G.
TJW receives research grants for experimental and clinical antimicrobial
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AHG has received research grants from Gilead and Merck, Sharp & Dohme, and Pfizer; is a
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Keywords

Liposomal, amphotericin B, children, pediatrics, pharmacokinetics, invasive fungal disease
Abstract

Background Liposomal amphotericin B (LAmB) is widely used in the treatment of invasive fungal disease (IFD) in adults and children. There are relatively limited PK data to inform optimal dosing in children that achieves systemic drug exposures comparable to those of adults.

Objectives To describe the pharmacokinetics of LAmB in children aged 1-17 years with suspected or documented IFD.

Methods Thirty-five children were treated with LAmB at dosages of 2.5-10 mg kg\(^{-1}\) daily. Samples were taken at baseline and at 0.5-2.0 hourly intervals for twenty-four hours after receipt of the first dose (n=35 patients) and on the final day of therapy (n=25 patients). LAmB was measured using high performance liquid chromatography (HPLC). The relationship between drug exposure and development of toxicity was explored.

Results An evolution in PK was observed during the course of therapy resulting in a proportion of patients (n=13) having significantly higher maximum serum concentration (C\(_{\text{max}}\)) and area under the concentration time curve (AUC\(_{0-24}\)) later in the course of therapy, without evidence of drug accumulation (C\(_{\text{min}}\) accumulation ratio, AR < 1.2). The fit of a 2-compartment model incorporating weight and an exponential decay function describing volume of distribution best described the data. There was a statistically significant relationship between mean AUC\(_{0-24}\) and probability of nephrotoxicity (OR 2.37; 95% CI 1.84-3.22, p=0.004).

Conclusions LAmB exhibits nonlinear pharmacokinetics. A third of children appear to experience a time-dependent change in PK, which is not explained by weight, maturation or observed clinical factors.
Introduction

The small unilamellar liposomal formulation of amphotericin B (LAmB; AmBisome®) is widely used for the treatment of invasive fungal disease (IFD) in adults and children. This compound has been available for over two decades and is a first line agent in the treatment of serious opportunistic diseases that include invasive aspergillosis, invasive candidiasis, cryptococcal meningoencephalitis, and mucormycosis. (1-4)

Despite extensive clinical experience, many of the details relating to the underlying pharmacological properties of LAmB remain unclear. A limited number of datasets and population pharmacokinetic (PK) models have been reported for LAmB. (5-7) These analyses were based on data gathered from patients receiving relatively low dosages and exclusively sampled early in the course of therapy. There are very limited data reporting the PK of LAmB in pediatric populations.

A better understanding of the pharmacological properties of LAmB remains a priority and would enable optimal dosing, particularly for special populations such as infants and children. Dosages ranging from 2.5-10 mg kg⁻¹ per day were studied and each patient was intensively sampled. The individual PK profiles for a sub-population of participants (n=25) were compared at the commencement and end of therapy.
Materials & Methods

Patients, Antifungal Regimen

This study was designed as a prospective, multi-center, open-label phase II clinical trial. Study protocol approval was obtained from the Ethics Committees of the National Cancer Institute (Bethesda MD, USA); Children’s National Medical Center (Washington DC, USA) and Georgetown University Medical Center (Washington DC, USA). Informed consent was obtained prior to enrolment in each case. A total of 35 children with a diagnosis of confirmed or suspected IFD were enrolled. Patients received LAmB infused over one hour at dosages of 2.5, 5.0, 7.5, or 10.0 mg kg\(^{-1}\) daily (n = 9, 13, 8, and 8, respectively). Two patients received LAmB as treatment for more than one discrete clinical episode requiring antifungal therapy. Patients undergoing multiple discrete episodes were assigned the same identification number on each occasion and were handled using the dosing reset function in Pmetrics.

LAmB (AmBisome®; Gilead Sciences, Inc., Foster City, California) was supplied as a lyophilized powder and stored at 2-8°C until use. Powder (50 mg) was reconstituted with 12.5 mL of sterile water to a concentration of 4 mg\(^{-1}\) mL, and then further diluted in 5% dextrose. Reconstituted drug was used within 6 hours.

Pharmacokinetic Sampling

PK samples were obtained on the first and last day of therapy. The first day of LAmB administration was defined as day one. Heparinized whole-blood samples (0.6-1 mL) were collected by peripheral intravenous catheter. Samples were obtained prior to administration, and at 0.5-2.0 hourly intervals for 24 hours following the start of each infusion. A total of 7-12 samples were obtained per patient within each sampling period (total sampling blood volumes < 3 mL/kg within 24 hours). Sampling was repeated in sixteen patients on the last
day of therapy (12-41 days) using the same sampling schedule. Plasma fractions were
separated by centrifugation at 1,500 g for 10 min at 4°C and stored at −80°C until analysis.
Concentrations of LAmB in plasma were determined by a high-performance liquid
chromatographic assay. (8) Briefly, total active drug and internal standard, 3-nitrophenol,
were extracted in methanol and separated by reversed-phase chromatography. The separation
was performed isocratically using a Supelcosil ABZ+Plus analytical column (3 µm particle
size, 150 mm x 4.6 mm internal diameter; Supelco, Bellefonte, Pennsylvania), coupled by a
Keysone C18 guard column (3 µm particle size, 7.5 mm x 4.6 mm 7.5 by 4.6 mm; Western
Analytical, Murrieta, California). The mobile phase, consisting of 10 mM sodium acetate
buffer, including 10 mM EDTA (pH 3.6) and acetonitrile (650:350, vol/vol), was delivered at
a flow rate of 1.0 ml/min using a Spectra-Physics Model 250 pump (Thermo Separations, San
Jose, California). UV absorbency peaks were detected at a wavelength of 406 nm using a
Waters Model 440 UV-VIS detector (Waters Corp, Milford, Massachusetts). Two
overlapping standard curves were used: 0.05 to 20 µg/ml and 0.5 to 200 µg/ml. The assay was
linear over a range of 0.05-20 and 0.5 to 200 µg/mL (r² >0.995). Intra- and inter-day
coefficients of variation were 9.5 and 7.0%, and 5.4 and 6.0%, respectively, and the limit of
quantification was 0.05 µg/ml. The average recovery was 90.5% at the concentrations of
quality control samples with a standard deviation of 6.2%.

Population Pharmacokinetic Modeling

Data were analysed using a non-parametric methodology within the program Pmetrics
(version 1.2.6; University of Southern California, Los Angeles, CA). (9) The observed data
were weighted using the inverse of the estimated assay variance.
Structural models were constructed and used to fit patient data. One-, two- and three-compartment models with zero-order drug input into the central compartment and both first-order and nonlinear (Michaelis-Menten) elimination from the central compartment were explored. A proportion of patients had concentration-time profiles that indicated an intra-individual change in PK during the course of therapy (n=13; 52%). Affected individuals demonstrated a marked increase in excursion of drug concentrations from $C_{\text{max}}$ to $C_{\text{min}}$ and a disproportionate increase in AUC$_{0-24}$ (Figure 1). This change was not associated with rising trough concentrations, suggesting the phenomenon did not result from drug accumulation resulting from conventional nonlinear (Michaelis-Menten) kinetics (AR < 1.2). Inspection of the data suggested the clearance of drug was the same in both sampling periods. Hence, the following structural model that allowed Vd to change with time was explored. In this model, volume contracted with time and was described using an exponential decay function. Clearance (Cl) was scaled according to weight using a standard 0.75 power function. The differential equations describing the final model were as follows:

$$\frac{\delta X(1)}{dt} = R(1) - \left(\frac{\text{wt}}{70}\right)^{0.75} \cdot Vd \cdot X(1) - Kcp \cdot X(1) + Kpe \cdot X(2)$$

$$\frac{\delta X(2)}{dt} = Kcp \cdot X(1) - Kpe \cdot X(2)$$

$$\frac{\delta Vd}{dt} = -Vin \cdot K + Vfin$$

Where: $X(1)$ and $X(2)$ represent the total (bound and free) amount of LAmB (mg) in the central (c) and peripheral (p) compartments, respectively. $R(1)$, $Kcp$ and $Kpc$ represent the rate of infusion into the central compartment (mg h$^{-1}$) and first-order inter-compartmental rate constants, respectively. Clearance (Cl) is normalised according to a 70 kg individual and allometrically scaled. The volume of the central compartment ($V_c$) is described by an
exponential decay function in which initial volume (Vin) reduced over time according to a rate constant (K) to a final volume (Vfin).

The goodness-of-fit of each model to the data was assessed by visual inspection of the observed-predicted values and following linear regression of the observed-predicted values both before after the Bayesian step. The coefficient of determination ($r^2$), slope and intercept of each regression were calculated. Statistical comparison of models was based on likelihood ratio, in which twice the likelihood difference was evaluated against a $\chi^2$ distribution with an appropriate number of degrees of freedom. In addition, predictive performance was assessed according to weighted-mean error (a measure of bias) and bias-adjusted weighted-mean-squared error (a measure of precision).

The final selected model was validated using a nonparametric bootstrap resampling technique. Three hundred bootstrap datasets were constructed based on random sampling with replacement using ADAPT 5. Measures of central tendency and dispersion and the 95% confidence interval (CI) for each parameter value were calculated and compared with estimates from original data. The selected structural model was then implemented within the simulation module of ADAPT 5. (10) Bayesian estimates of the PK parameters for each patient were used to calculate simulated peak plasma concentration ($C_{\text{max}}$), trough plasma concentration ($C_{\text{min}}$), and area under the concentration time curve over 24 hours ($\text{AUC}_{0-24}$) at defined therapeutic time points.

Potential relationships between measures of drug exposure ($C_{\text{max}}$, $C_{\text{min}}$, absolute LAmB dosage, weight adjusted dosage, $\text{AUC}_{0-24}$, and mean $\text{AUC}_{0-24}$) and toxicity were explored. Toxicity was defined as changes from baseline values at commencement of therapy as follows: nephrotoxicity as an increase in serum creatinine (SCR) of $\geq 0.5$ mg/dL or doubling of baseline value, hypokalemia as a fall in potassium of $\leq 3.0$ mmol/L or $\geq 50\%$ from
baseline, anemia as an hemoglobin of ≤8.0 g/dL, and hepatotoxicity as a rise in bilirubin by ≥1.5 mg/dL or AST or ALT ≥3 times above baseline. A conservative definition was used to define change in biological parameters in order to overcome variability in sampling between patients; pre-treatment value was subtracted from the highest measurement observed for each patient during the treatment course.

Results

The patient demographics of the study cohort are summarized in table 1. The mean ± SD weight was 26.9 ± 14.0 kg with a range of 8.8-67.5 kg. There was wide variability in the duration of therapy: the mean ± SD was 11.9 ± 9.41 days of therapy with a range of 1-41 days. The most common underlying diagnosis was hematological malignancy (n =21). Nine patients had undergone allogeneic hematopoietic stem-cell transplantation (HSCT) and 23 received concomitant antineoplastic chemotherapy. The majority of patients received LAmB as empirical therapy for suspected IFI (n=31). Seven patients received treatment for confirmed IFI. There were two cases of invasive aspergillosis due to A. fumigatus, and a further case that developed during treatment with LAmB that was classified as a breakthrough infection. Three patients had invasive candidiasis: one central-line infection and one severe oesophagitis due to C. albicans, and one case of candidaemia caused by C. parapsilosis. There was a single case of cryptococcal meningoencephalitis complicating HIV infection. Clinical success was defined according to clinical, radiological, and mycological response during the study period plus relapse-free survival at 2 months after the end of therapy. Clinical success was reported in 76% of probable (n=29) and 43% (n=3) of proven fungal infections.
The Bayesian estimates for clearance (Cl) obtained from standard two-compartment models for each patient were plotted against weight. A relationship between the log10-transformed estimates was apparent. The performance of models incorporating an allometric power function was therefore investigated using a scaling exponent fixed at 0.75. No significant relationship was found between Bayesian estimates for volume (Vd) and weight. Differences in clinical factors that might be predicted to alter the PK of LAmB were explored. No significant differences were identified in liver function, serum albumin, white blood cell (WBC) count and total protein concentrations, use of parenteral nutrition and concomitant steroids. A relatively poor fit of standard model structures was apparent (see, for example performance of a standard two-compartment model, figure 2). Conventional compartmental model structures failed to account for the widening excursion of drug concentrations observed in a portion of patients. The parameter estimates for the base and final model are summarized in table 2. The fit of the selected model incorporating a function describing contraction in Vd was satisfactory ($r^2 = 0.90$), and compared favourably to a standard 2-compartment model. The final model consisted of eight support points. Measures of bias and precision were acceptable (see figure 2). The bootstrap mean and 95% CI values for parameters closely approximated the estimates obtained from the final model (table 3), indicating that the parameter estimates from the final model were robust. Both the mean and median parameter values resulted in comparable intercept, slopes and overall $r^2$ values. The log-likelihood value for the final model was significantly better (more positive) than for the standard 2-compartment model ($\chi^2 = 48.95$, $p = <0.001$). Figure 3 shows the simulated concentration-time profiles and raw data for two examples of patients that exhibited time-dependent and time-independent changes in PK profiles.

Dose-exposure relationships were further explored. No correlation between absolute dose and exposure ($C_{\text{max}}$, $C_{\text{min}}$ or $AUC_{0-24}$) was observed, an expected finding given the
significant variability in weight within the study population. Significant relationships between dose per-unit-weight and exposure were observed. Plots of dose-normalized $C_{\text{eq}}$ and $\text{AUC}_{0-24}$ suggest nonlinearity (figure 4), although a dosing threshold associated with a discrete change in exposure was not observed.

Transient renal impairment and hypokalemia were common, occurring in 46% (n=16) and 23% (n=8) of patients, respectively. A significant correlation between steady state exposure ($\text{AUC}_{0-24}$) and change in serum creatinine ($\Delta$SCR) was observed (Figure 5, r=0.594, p=0.015). A statistically significant relationship between mean $\text{AUC}_{0-24}$ and probability of developing nephrotoxicity (OR 2.37; 95% CI 1.84-3.22, p=0.004). There was insufficient clinical information to explore the impact of other potential determinants of renal impairment (for example disease severity and concomitant nephrotoxic drugs) in this study cohort. No significant correlations were found between LAmB exposure (in terms of absolute dose, weight adjusted dose, $\text{AUC}_{0-24}$ or mean $\text{AUC}_{0-24}$) and other toxicity including hypokalemia, anemia, and hepatotoxicity.

Discussion

Liposomal amphotericin B is used extensively for the treatment of IFD. Dosages of 3-6 mg kg$^{-1}$ are approved in the U.S.A and the E.U. in both adults and children. These dosages are not based on an in-depth knowledge of the pharmacology of the drug, but rather results from preclinical in vivo studies and clinical trials that have attempted to identify regimens that appear safe and effective. There continues to be considerable uncertainty regarding the lowest effective dosage of LAmB that achieves adequate antifungal effect. As a result, dosages of 1-15 mg kg$^{-1}$ have been studied in a range of clinical settings including...
empirical therapy, invasive aspergillosis, invasive candidiasis, and cryptococcal meningoencephalitis. (11-14)

Phase I/II clinical studies of LAmB in children and adults have highlighted variable, dose-dependent PK. Children and adults receiving LAmB at conservative daily doses of 1-3 mg kg\(^{-1}\) exhibit linear PK that are described by standard two- or three-compartment models with first-order elimination. (6, 7)(5) Limited data suggest nonlinearity at higher dosages. Walsh et al. observed time-dependent nonlinear PK and an apparent paradoxical dose-dependent exposure plateau in adults receiving daily dosages of 7.5-15 mg kg\(^{-1}\). (3) The data from paediatric patients in this study similarly suggests that a proportion of patients exhibit time-dependent nonlinear PK. When the concentration-time profiles of patients exhibiting nonlinear PK are examined a significant excursion in \(C_{\text{min}}-C_{\text{min}}\) is observed, a change not associated with a proportional increase in half-life that would be expected with classical nonlinear (Michaelis-Menten) clearance, but rather appears to reflect a contraction in the volume of distribution during the course of therapy. Whereas the limited data from adults has suggested a paradoxical dose-dependent reduction in exposure at doses >7.5 mg kg\(^{-1}\), in children higher doses appear to be associated with an increased probability of nonlinearity. The reason for this difference is unclear and warrants further study.

High-density lipoproteins (HDL) mediated opsonization of lipid formulations of amphotericin B within plasma has been shown to drive uptake into mononuclear phagocytes and deposition within the liver and spleen. (15-18) Hong et al. reported a negative correlation between Bayesian estimates volume of distribution and the fraction of HDL-associated LAmB in 21 children and adolescence receiving LAmB at daily doses of 0.8-6 mg kg\(^{-1}\). We hypothesize that variable HDL saturation and/or phagocyte uptake may be the pathophysiological processes driving the inter-individual variability observed in this study. However, many patients in this small clinical cohort exhibited significant fluctuations in
hematological parameters such as WBC count over the course of antifungal therapy, primarily due to underlying hemato-oncological diagnoses, and we were not able to further characterise relationships between specific hematological parameters and volume contraction. Other significant data such as plasma HDL concentrations were not quantified in this study. This is an interesting hypothesis that warrants further study in experimental models and/or as part of larger clinical trials. LAmB is generally well tolerated with a significantly improved toxicity profile when compared to conventional amphotericin B deoxycholate. (14) Dosages of LAmB as high as 15 mg kg\(^{-1}\) daily have been reportedly well tolerated in adults. (3) A number of studies including one large RCT have, however, described dose-dependent toxicity with significantly higher rates of renal impairment and hypokalemia at dosages at or above 10 mg kg\(^{-1}\) daily. (1) In this study, a significant proportion of patients developed transient renal impairment and/or hypokalemia during the course of treatment. In view of the limited data available, significant inter-individual variability and lack of obvious inflection point in this relationship further analysis to define exposure thresholds was not possible. The correlation between drug exposure and ΔScr observed here suggests, however, that clinical vigilance and assiduous monitoring of renal function is required to minimize the probability of toxicity associated with LAmB.

Taken together these data suggest that a significant proportion of pediatric patients receiving LAmB at daily doses > 5.0 mg kg\(^{-1}\) exhibit nonlinear PK with significantly higher peak concentrations and overall drug exposure. This phenomenon was not predicted by clinical covariates quantified in this study. Therapeutic drug monitoring (TDM) is thus likely to be of value in identifying this subpopulation in order to prevent toxicity. Effective implementation of TDM would require a more detailed understand of exposure-toxicity relationships and data describing disease severity in children with proven or probably IFD in order to define target exposure thresholds.
Acknowledgements

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References


Table 1 Patient demographics of cohorts undergoing sampling on day one of therapy and at steady state

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Day one (n=35)</th>
<th>Steady state (n=25)</th>
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</thead>
<tbody>
<tr>
<td>Age (μ ± SD, range; years)</td>
<td>8.7 ± 4.6 (1 - 17)</td>
<td>10.5 ± 6.6 (1 - 17)</td>
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<tr>
<td>Gender (M:F)</td>
<td>22:13</td>
<td>15:10</td>
</tr>
<tr>
<td>Weight (μ ± SD, range; kg)</td>
<td>26.9 ± 14.0 (8.8 - 67.5)</td>
<td>25.4 ± 16.2 (11.2 – 67.5)</td>
</tr>
<tr>
<td>Duration of therapy (μ ± SD, range; days)</td>
<td>11.9 ± 19.4 (1 - 41)</td>
<td>15.5 ± 11.3 (9.5 - 41)</td>
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<td>Underlying diagnosis (no. patients)</td>
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<td>Lymphoma</td>
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<td>Established infection</td>
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<tr>
<td>Breakthrough</td>
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| Solid tumor                      | 7        |
| HIV                             | 4        |
| Chronic granulomatous disease   | 1        |
Table 2. The parameter estimates for the final 2-compartment pharmacokinetic model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vin (L)</th>
<th>Vfin (L)</th>
<th>Kcp (h⁻¹)</th>
<th>Kpc (h⁻¹)</th>
<th>K (h⁻¹)</th>
<th>Cl (L h⁻¹ 70 kg⁻¹)</th>
</tr>
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<tbody>
<tr>
<td><strong>Base model</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean</td>
<td>4.543</td>
<td>n/a</td>
<td>0.28</td>
<td>0.888</td>
<td>n/a</td>
<td>0.488</td>
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<td>Median</td>
<td>4.095</td>
<td>n/a</td>
<td>0.184</td>
<td>0.254</td>
<td>n/a</td>
<td>0.545</td>
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<tr>
<td>Standard Deviation</td>
<td>3.44</td>
<td>n/a</td>
<td>0.252</td>
<td>0.387</td>
<td>n/a</td>
<td>0.29</td>
</tr>
<tr>
<td>Error (CV%)</td>
<td>75.72</td>
<td>n/a</td>
<td>90.025</td>
<td>43.581</td>
<td>n/a</td>
<td>59.426</td>
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<tr>
<td><strong>Selected model</strong></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Mean</td>
<td>10.654</td>
<td>2.326</td>
<td>0.21</td>
<td>0.057</td>
<td>0.303</td>
<td>0.67</td>
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<tr>
<td>Median</td>
<td>7.998</td>
<td>2.986</td>
<td>0.178</td>
<td>0.033</td>
<td>0.027</td>
<td>0.665</td>
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<tr>
<td>Standard Deviation</td>
<td>1.523</td>
<td>0.978</td>
<td>0.130</td>
<td>0.01</td>
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<tr>
<td>Error (CV%)</td>
<td>14.295</td>
<td>42.064</td>
<td>61.905</td>
<td>17.544</td>
<td>31.023</td>
<td>35.672</td>
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</table>

CV%, coefficient of variation; Vin, initial volume of distribution; Vfin, final volume of distribution; K, first-order inter-volume rate constant; Kcp/Kpc, first-order inter-compartmental rate constants; Cl, clearance.
Table 3. Bootstrap estimates of the selected pharmacokinetic model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bootstrap</th>
<th>Final model</th>
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<tbody>
<tr>
<td></td>
<td>Mean estimate</td>
<td>95% CI</td>
</tr>
<tr>
<td>V&lt;sub&gt;i&lt;/sub&gt; (L)</td>
<td>10.677</td>
<td>10.646 – 10.87</td>
</tr>
<tr>
<td>V&lt;sub&gt;f&lt;/sub&gt; (L)</td>
<td>2.345</td>
<td>2.181 – 3.023</td>
</tr>
<tr>
<td>K&lt;sub&gt;e&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.311</td>
<td>0.127 – 0.42</td>
</tr>
<tr>
<td>K&lt;sub&gt;pc&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.057</td>
<td>0.043 – 0.061</td>
</tr>
<tr>
<td>K (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.303</td>
<td>0.21 – 0.355</td>
</tr>
<tr>
<td>Cl (L h&lt;sup&gt;-1&lt;/sup&gt; 70 kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.675</td>
<td>0.555 – 0.781</td>
</tr>
</tbody>
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Figure 1. Concentration-time profiles for each patient on day one of therapy (n=35) and at completion of therapy (n=25). Closed circles are the raw pharmacokinetic data from each patient.

Figure 2. Scatter plots showing observed-versus-predicted values for population pharmacokinetic models after the Bayesian step with a standard 2-compartment model (A) and selected model (B). Open circles, dashed lines and solid lines represent individual
observed-predicted data points, line of identity, and the linear regression of observed-predicted values, respectively.

Figure 3. Concentration-time profiles for two patients receiving LAmB (10 mg kg\(^{-1}\)). Initial (Vin) and final (Vfin) estimates for volume of distribution (Vd) are shown. Open circles and solid lines represent the raw data and simulated concentration-time profiles for each patient, respectively. Patient A exhibits evolving PK with a contraction in the Vd while patient B exhibits stable Vd.

Figure 4. Comparisons of dose-normalised Cmax (A) and AUC\(_{0-24}\) (B) at steady state with respect to dose per unit weight. Solid and dashed lines represent linear regression and 95% confidence intervals, respectively.

Figure 5. Relationship between Bayesian estimates of AUC\(_{0-24}\) at steady state with respect to change in serum creatinine. Solid and dashed lines represent linear regression and 95% confidence intervals, respectively.