Population Pharmacokinetics of Artemether, Lumefantrine, and Their Respective Metabolites in Papua New Guinean Children with Uncomplicated Malaria

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There are sparse published data relating to the pharmacokinetic properties of artemether, lumefantrine, and their active metabolites in children, especially desbutyl-lumefantrine. We studied 13 Papua New Guinean children aged 5 to 10 years with uncomplicated malaria who received the six recommended doses of artemether (1.7 mg/kg of body weight) plus lumefantrine (10 mg/kg), given with fat over 3 days. Intensive blood sampling was carried out over 42 days. Plasma artemether, dihydroartemisinin, lumefantrine, and desbutyl-lumefantrine were assayed using liquid chromatography-mass spectrometry or high-performance liquid chromatography. Multicompartmental pharmacokinetic models for a drug plus its metabolite were developed using a population approach that included plasma artemether and dihydroartemisinin concentrations below the limit of quantitation. Although artemether bioavailability was variable and its clearance increased by 67.8% with each dose, the median areas under the plasma concentration-time curve from 0 h to infinity (AUC0–∞) for artemether and dihydroartemisinin (3,063 and 2,839 µg · h/liter, respectively) were similar to those reported previously in adults with malaria. For lumefantrine, the median AUC0–∞ (459,980 µg · h/liter) was also similar to that in adults with malaria. These data support the higher dose recommended for children weighing 15 to 35 kg (35% higher than that for a 50-kg adult) but question the recommendation for a lower dose in children weighing 12.5 to 15 kg. The median desbutyl-lumefantrine/lumefantrine ratio in the children in our study was 1.13%, within the range reported for adults and higher at later time points because of the longer desbutyl-lumefantrine terminal elimination half-life. A combined desbutyl-lumefantrine and lumefantrine AUC0–∞ weighted on in vitro antimalarial activity was inversely associated with recurrent parasitemia, suggesting that both the parent drug and the metabolite contribute to the treatment outcome of artemether-lumefantrine.

Artemether (ARM)-lumefantrine (LUM) (AL) is a fixed-dose combination therapy used widely for the treatment of malaria (33). ARM is a lipophilic artemisinin derivative that is converted in vivo to dihydroartemisinin (DHA), an active metabolite. Both ARM and DHA have short half-lives (14, 19–21, 24, 25, 31) but a rapid effect on parasitemia. LUM is a highly lipophilic drug with a longer half-life (11, 13, 14, 19, 20, 24, 30) which is combined with ARM primarily to prevent late recruitment. Although the pharmacokinetic (PK) properties of ARM, DHA, and LUM in adults have been well documented (4, 5, 11, 13–15, 19–22, 24, 30), there are scant and inconsistent data relating to the disposition of desbutyl-lumefantrine (DBL), a potent LUM metabolite (26, 28, 29, 32) that may influence AL’s treatment outcome (32). Reported plasma DBL-to-LUM concentration ratios after AL dosing in adults differ >10-fold (15, 24), while the pharmacokinetic properties of DBL in children are unknown. In addition, although several studies have attempted to characterize LUM disposition in children with malaria (1, 16, 25), methodological issues complicate the comparison of child data with adult data. One study involving a limited sampling schedule suggested that AL-treated children with malaria receive an inadequate dose of LUM relative to that for healthy adults (25), while the other studies either used pooled plasma concentrations (1) or used a truncated sampling schedule inadequate to characterize LUM pharmacokinetics (16).

In view of this situation, we have characterized the population pharmacokinetics of ARM, LUM, and their metabolites in pediatric malaria by using a rich sampling schedule to assess potential differences in disposition between children and adults and to add to the limited data on DBL disposition and its role in AL’s treatment outcome.

MATERIALS AND METHODS

Patients. We recruited children aged 5 to 10 years from Alexishafen Health Centre, Madang Province, on the north coast of Papua New Guinea. The clinic serves an area where Plasmodium falciparum and Plasmodium vivax are hyperendemic and Plasmodium ovale and Plasmodium malariae are also transmitted. Children with an axillary temperature of >37.5°C or a history of fever in the previous 24 h were screened with a Giemsa-stained thick blood film read by an on-site, trained microscopist. Those with a mono-infection of P. falciparum (>1000 asexual parasites/microliter) or P. vivax, P. ovale, or P. malariae (>250 microliter) were eligible, provided that the child’s parents gave informed consent, there were no features of severe malaria (34), they had not taken any antimalarial drug in the previous 14 days, there was no evidence of another cause
of fever, and there were no features of malnutrition or other chronic comorbidity. The study was approved by the Medical Research Advisory Committee of the Department of Health, Papua New Guinea.

Clinical methods. After enrollment, a standardized history was taken and a clinical examination performed. A 3-ml blood sample was taken for blood film microscopy, baseline hemoglobin and blood glucose measurements were taken, and a subsequent drug assay of separated plasma was performed. Urinalysis and audiometric assessment were performed. Each child was given artemether-lumefantrine (Coartem, Novartis Pharma Ltd., Switzerland) at a dose of 1.7 mg/kg body weight, respectively, to the nearest tablet. This dose was repeated at 8, 24, 36, 48, and 60 h, with the exact time of dosing recorded. All doses were given under direct observation with at least 50 ml of cow’s milk (equivalent to 2 g of fat). Further venous blood samples were taken from an indwelling intravenous catheter at 4, 8, 12, 24, 36, 48, 60, 64, 68, and 72 h and then by venesecision on days 4, 5, 7, 14, and 28. All samples were centrifuged promptly, and red cells and separated plasma were stored frozen at −80°C until assayed. A detailed clinical assessment including a symptom questionnaire, a blood film, and hemoglobin and blood glucose measurements was repeated on days 1, 2, 3, and 7, with additional clinical assessment and blood films on days 14, 28, and 42.

Laboratory methods. All blood smears taken at baseline and during follow-up were examined independently by two skilled microscopists in a central laboratory. Each microscopist viewed 100 fields at ×1,000 magnification before a slide was considered negative. Any slide discrepant for positivity/negativity or species was referred to a third microscopist for adjudication.

For drug assays, high-performance liquid chromatography (HPLC)-grade acetonitrile (Merck, Kilsyth, Australia), tert-butyl chloride, ethyl acetate, glacial acetic acid, and tert-butanol (Merck, Darmstadt, Germany), and formic acid (Sigma-Aldrich, Gillingham, United Kingdom) were used. Other solvents and chemicals were of analytical grade. Stock solutions (1 μl/liter in methanol) of ARM (AAPIN Chemicals, Abingdon, United Kingdom), DHA (Sigma, St. Louis, MO), and artemisinin (used as an internal standard; Sigma) were stored and protected from light at −20°C until use and were used to prepare working dilutions (0.1, 1, and 10 μg/ml). Calibration curves (2 to 200 μg/ml) were constructed for DHA and ARM by spiking blank plasma. Quality control (QC) samples were prepared in blank plasma at 10, 20, 50, and 200 μg/ml and also stored at −80°C prior to use.

ARM and DHA were extracted as previously described (7) but with the following modifications. Briefly, solid-phase extraction (SPE) Bond Elut PH columns (Varian Inc., Palo Alto, CA) were preconditioned with 1 ml of methanol followed by 1 ml of 1 M acetic acid. Plasma (0.5 ml) was spiked with an internal standard (artemisinin, 100 μg/ml), loaded onto the SPE column, and drawn through with a medium-suction vacuum. The column was then washed twice with 1 M acetic acid (1 ml), followed by 20% (vol/vol) methanol in 1 M acetic acid (1 ml). The column was dried with a low-suction vacuum for 30 min, and the eluate was then evaporated under vacuum at 35°C, reconstituted with a medium-suction vacuum. The column was then washed twice with 1 M acetic acid (1 ml), followed by 20% (vol/vol) methanol in 1 M acetic acid (1 ml). The chromatographic data (the peak heights of the compounds of interest) were used to quantify the concentration of the analyte. The injection volume was 10 μl.

The liquid chromatography-mass spectrometry (LC-MS) system used was a single-quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) with electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI) systems. Assays were performed with 20 mM ammonium formate (pH 5) and acetonitrile injection volume was 10 μl. The eluate was then evaporated under vacuum at 35°C, reconstituted with 1 M acetic acid (1 ml), followed by 20% (vol/vol) methanol in 1 M acetic acid (1 ml). The chromatographic separation was undertaken at ambient temperature on a Synergi Fusion-RP C18 column (inside diameter [i.d.] 150 mm by 2.0 mm) coupled with a 5-μm-particle C18 guard column (i.d. 4 mm by 3 mm; Phenomenex, Lane Cove, Australia). Retention times were 4.5, 7.5, and 12.7 min for DHA, ARM, and artemisinin, respectively. Optimized mass spectra were acquired with an interface voltage of 4.5 kV, a detector voltage of 1 kV, a heat block temperature of 400°C, and a desolvation gas temperature of 250°C. Nitrogen was used as a nebulizer gas at a flow rate of 1.5 liters/min and as a dry gas at a flow of 10 liters/min. Quantitation was performed by selected ion monitoring using the dual-ionization source mode. The predominant fragmented ions, m/z 221 for ARM and m/z 221 for DHA, were used for artemisinin, m/z 283 was monitored.

The standard curves were linear (r2 = 0.999). The chromatographic data (the peak area ratios of DHA to artemisinin and ARM to artemisinin) were processed using LabSolutions software (version 5; Shimadzu, Japan). No matrix effect (ion suppression/enhancement) was observed under methodologies described elsewhere (23), and the performance of both assays, assessed as intra- and interday relative standard deviations across relevant concentration ranges, was similar to that published previously (7, 18). Interday accuracies of QC assays were <15% of nominal values on all occasions. The limits of quantification and detection were, respectively, 2 and 1 μg/liter for DHA and 5 and 2 μg/liter for ARM.

LUM and DBL were quantified in plasma using validated high-performance liquid chromatography with a UV detection assay (HPLC-UV) and a validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay, respectively, as previously described (32). The linear range for LUM was 20 to 20,000 ng/ml; interday variability was 4.94%, 4.95%, 7.16%, and 11.23% and intraday variability was 2.83%, 4.41%, 4.11%, and 9.55% at 20,000, 2,000, 200, and 20 ng/ml, respectively. For DBL, the linear range was 0.5 to 100 ng/ml; interday variability was 3.36%, 3.47%, 9.98%, and 6.74% and intraday variability was 2.47%, 3.46%, 8.16%, and 3.48% at 50, 10, 0.5 ng/ml, respectively. As an LC-MS/MS method was used for DBL, matrix effects were assessed where between-subject variability was 3.37%, 4.47%, and 9.43% at 50, 10, and 1 ng/ml, respectively.

Pharmacokinetic modeling. Log (natural log) plasma concentration-time data sets for LUM with DBL and for ARM with DHA were analyzed by nonlinear mixed-effect modeling using NONMEM (version 6.2.0; Icon Development Solutions, Ellicott City, MD) with an Intel Visual Fortran 10.0 compiler. The first-order conditional-estimation with interaction (FOCE-I) method was used for the LUM-DBL model, and the Laplacian with interaction method was used for ARM-DHA. The minimum objective-function value (OFV) and weighted-residual (WRES) plots were used to choose suitable models during model building. As a FOCE-I estimation was used, additional weighted residuals were constructed in addition to WRESs in the initial stages of model building (17). However, as they were similar, WRESs were considered suitable for further model building. Concentrations were modeled in μg/ml with a conversion factor for all metabolite parameters included in the model to account for the difference in molecular weight between the parent drug and the metabolite. Allometric scaling was used as a priori, with volume terms multiplied by (weight/70)0.33 and clearance terms by (weight/70)0.75 (3). Residual variability (RV) was estimated as an additive error for the log transformed data. Matrix effects were accounted for using the absorption rate constant (ka), central volume of the distribution (Vc/F, where F is bioavailability), clearance (CLF), and peripheral volume of the distribution(s) (Vp/F) and its respective intercompartmental clearance(s) (Q/F).

For the LUM-DBL model, plasma LUM concentrations were initially modeled using inbuilt 2- and 3-compartment model structures with first-order absorption and a fixed lag time of 2 h (22) (Advan 4 and 12). Once a suitable (3-compartment) LUM model had been determined, the DBL data set was added and modeled simultaneously. User-defined linear mammillary models (Advan 5) were constructed by testing 1-, 2-, and 3-compartment models with and without first-pass LUM metabolism. As no data exist regarding the degree of in vivo DBL conversion from LUM, this was set to 100% to allow identifiability. Therefore, all clearance and volume terms for DBL are relative to LUM bioavailability (FLUM) as well as the degree of metabolic conversion from LUM (Fmet-DHA). The term FDBL (representing FLUM times Fmet-DHA) will be used for DBL concentrations. As 45% and 12% of plasma ARM and DHA concentrations, respectively, were below the limit of quantification (BLQ), we used a published method known to produce reliable pharmacokinetic parameters in this situation (9, 10). The method (known as M3) (2) models continuous and categorical data simultaneously. Concentrations above the limit of quantification (LOQ) are included as conventional continuous data, while those BLQ are treated as categorical, and the likelihood (probability) that they are BLQ was maximized with respect to model parameters. This allows BLQ observations to contribute to the determination of the OFV and the finalizing of the model structure.

Initially, plasma ARM concentrations were assessed using 1- and 2-compartment models with first-order absorption (Advan 2 and 4) to obtain a suitable structure. The ka for ARM was fixed to 1 h−1 (31), as the data did not support its estimation. Once a suitable (2-compartment) ARM model had been determined, the DHA data set was added and modeled simultaneously using a user-defined linear mammillary model (Advan 5). For DHA, 1- and 2-compartment models were assessed and the conversion of ARM to DHA was considered complete for identifiability purposes. Therefore, all clearance and volume terms for DHA are relative to ARM bioavailability (FARM) as well as the degree of metabolic conversion from ARM (Fmet-DHA). The term FDBL (representing ARM times Fmet-DHA) will be used for simplicity.

Once the model structure was established, interindividual variability (IVIV), interoccasion variability (IOV), and their correlations were estimated. Relationships between model parameters and the covariates of age, sex, baseline parasitemia, and baseline hemoglobin were identified using correlation plots and subsequently evaluated within NONMEM. Inclusion of the covariate relationship required a decrease in OFV of ≥6.3 (χ2 distribution with 1 df, P < 0.01), accompanied by a decrease in the IIV of that parameter.
RESULTS

Clinical characteristics and course. The baseline characteristics of the 13 recruited children are summarized in Table 1. Eleven had a monoinfection (9 P. falciparum, 2 P. malariae infections) on confirmatory expert microscopy, while 2 had a mixed P. falciparum/P. vivax infection. AL treatment was well tolerated, and reported symptoms were mild/moderate, short-lived (<3 days), and consistent with clinical features of uncomplicated malaria. Times to initial fever and parasite clearance were <48 h in all cases.

By the 28th day of follow-up, three children had developed slide-positive P. vivax (two had P. vivax at enrollment) and two children had developed P. falciparum (one had P. falciparum at enrollment) parasitemia. By the 42nd day of follow-up, five children had been diagnosed with P. vivax (including the two who had P. vivax at enrollment) and three with P. falciparum (including the one who had P. falciparum at enrollment). These data are consistent with the uncorrected PCR results of a previous, larger comparative treatment trial in younger children performed at the same location (18). The recurrent P. vivax parasitemia could have resulted from (i) a recrudescence infection in those infected with this parasite before treatment, (ii) the acquisition of a new P. vivax infection after treatment, or, since no primaquine therapy was administered, (iii) the appearance of P. vivax from hypnozoites present in the liver at study entry. P. falciparum parasitemia detected during follow-up could have represented recrudescence or reinfec tion.

The mean hemoglobin concentration was significantly higher on day 28 than at enrollment (10.7 versus 8.9 g/liter, P < 0.01). There was no significant change in blood glucose over the first 3 days of enrollment or in audiometric findings over 28 days (data not shown).

Pharmacokinetic modeling. LUM and DBL plasma concentration-time curves are shown in Fig. 1. A 3-compartment model proved superior to a 2-compartment model for LUM, with a lower OFV and reduced bias in the WRES plot. The addition of two compartments and the inclusion of first-pass metabolism provided the best model once the DBL data set had been added. Therefore, the final model comprised 3 compartments for LUM and 2 compartments for DBL. The structural model parameters were $k_a$, $V_{C/F}$, $V_{P1/F}$, $V_{P2/F}$, $V_{FLUM}$, $CL/F_{LUM}$, $Q_{1/F}$, $Q_{2/F}$, and $Q/F_{DBL}$. Interindividual variability was able to be estimated for $k_a$, $CL/F_{LUM}$, $C_{1/F}$, $V_{C}$, $V_{P1}$, $V_{P2}$, $V_{FLUM}$, $F_{LUM}$, and $F_{DBL}$, as was intercompartment variability for $F_{LUM}$ (the population value of $F_{LUM}$ remained fixed to 1). The variability in $F_{LUM}$ values was smaller between individuals than it was between doses in the same individual (20 versus 67%). Once IV and IOV terms were added, inspection of the WRES plot revealed a bias due to the absorption profile of the final dose. Estimation of a separate $k_a$ for the 6th and final dose ($k_{a6}$) improved the bias and reduced the OFV (−7.519, P < 0.01). None of the covariates tested improved the model. Residual variability (20.8% and 20.9% for LUM and DBL, respectively) was low.

The final-model parameter estimates and the bootstrap results are summarized in Table 2. Bias was <10% for structural and random-model parameters. Figures 2 and 3 show good-

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**Table 1. Baseline characteristics of study participants**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>7.7 ± 1.4</td>
</tr>
<tr>
<td>Male</td>
<td>8 (62)</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>19.0 ± 3.5</td>
</tr>
<tr>
<td>Ht (cm)</td>
<td>112 ± 9</td>
</tr>
<tr>
<td>Axillary temp (°C)</td>
<td>36.8 ± 1.0</td>
</tr>
</tbody>
</table>

a Data are numbers (percentages), means ± standard deviations, or medians ± interquartile ranges (n = 13).

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**Fig. 1.** Time-concentration plots showing concentrations of LUM (○) and DBL (□) in µg/liter on a log_{10} scale. Curves of the median concentrations for LUM (solid black line) and DBL (dashed black line) are also shown.
neous-of-fit plots and VPCs, respectively. The half-lives and AUCs of LUM and DBL are shown in Table 3. The first distribution, second distribution, and terminal elimination half-lives for LUM had median values of 10.4, 46.6, and 126 h, respectively, while DBL had a median distribution half-life of 19.7 h and a median terminal elimination half-life of 141 h. Overall, the metabolite-to-parent-drug ratio was 1.13% (obtained from AUC_{0–19.7 h} for DBL), but there was a higher ratio at later time points. Day 7 LUM concentrations obtained from younger children were consistent with predictions based on the final model, which resulted in the expected numbers of observations above and below the 20, 40, 60, 80, 90, and 95% simulated PIs. When the same data for DBL were compared, there was an excess of points above the 20, 40, 60, 80, and 90% PIs and a lack of points below the 20 and 40% PIs, especially at a younger age, demonstrating that the day 7 DBL levels in the younger children were higher than expected from the model.

Initial modeling of ARM-DHA data sets proved difficult, given the large proportion of BLQ data (45% and 12% for ARM and DHA, respectively). Once these data were incorporated into the model using the M3 method from Ahn et al. (2), more-acceptable models were obtained. The dispositions of ARM and DHA were best described by a 2-compartment model for ARM and a 1-compartment model for DHA. The structural-model parameters were $k_{a,\text{ARM}}$, $V_{p,\text{ARM}}$, $V_{e,\text{ARM}}$, $CL/ F_{\text{ARM}}$, $Q/ F_{\text{ARM}}$, $V_{c,\text{DHA}}$, and $CL/ F_{\text{DHA}}$. As with LUM, the IV and IOV of $F_{\text{ARM}}$ were estimated, and the variability between doses was larger than between individuals (84.1 versus 38.1%). The IV of $CL_{\text{ARM}}$ was also estimated. A relationship between $CL_{\text{ARM}}$ and dose number was included and demonstrated that for each subsequent dose of ARM, $CL_{\text{ARM}}$ increased by 67.8% relative to its value after the first dose. This relationship was accompanied by a decrease in the OFV ($\chi^2 = 82.774, P < 0.001$) and a reduction in the RVs of both ARM and DHA. No other covariate relationship improved the model. After the inclusion of IV/IOV terms and the covariate relationship, the RVs were still high, at 51.6% and 53.3% for ARM and DHA, respectively.

The final-model parameter estimates and the bootstrap results are summarized in Table 4. As the covariance step was not successful, NONMEM-derived relative standard errors could not be obtained. Bias was <11% for structural and random parameters, except the IV for $F_{\text{ARM}}$, which had a negative 48% bias. Figures 4 and 5 show goodness-of-fit plots and VPCs, respectively. The VPCs show all observed 10th, 50th, and 90th percentiles within their simulated 95% CIs and the fraction of BLQ data at each time point within its 95% CI for both ARM and DHA. Secondary parameters for study participants are shown in Table 3. The $AUC_{0–19.7\text{h}}$ and half-lives of ARM decreased with each dose, while the median DHA-to-ARM ratio increased.

Relationship between drug exposure and treatment outcome. The LUM $AUC_{0–19.7\text{h}}$ in children with recurrent parasitemia on days 28 ($n = 5$) and 42 ($n = 8$) tended to be lower than that in children who remained aparasitemic at these times ($P = 0.057$ and 0.086, respectively). There were no differences in the $AUC_{0–19.7\text{h}}$ for DBL ($P = 0.46$ and 0.89, respectively). However, a combined $AUC_{0–19.7\text{h}}$ with DBL weighted four times more than LUM (con-

### Table 2. Final population pharmacokinetic estimates and bootstrap results for LUM and DBL

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (RSE %)</th>
<th>Bootstrap median (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective-function value</td>
<td>$-586.510$ (668.678 to $-559.564$)</td>
<td></td>
</tr>
<tr>
<td>Structural-model parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{a}$ (h)</td>
<td>$0.461$ (20)</td>
<td>$0.442$ (0.285 to 0.644)</td>
</tr>
<tr>
<td>$V_{c,\text{LUM}}$ (liters/h/70 kg)</td>
<td>$227$ (12)</td>
<td>$225$ (147 to 284)</td>
</tr>
<tr>
<td>$V_{e,\text{LUM}}$ (liters/h/70 kg)</td>
<td>$1.52$ (19)</td>
<td>$1.57$ (0.96 to 2.32)</td>
</tr>
<tr>
<td>$Q_{\text{LUM}}$ (liters/h/70 kg)</td>
<td>$115$ (16)</td>
<td>$109$ (57 to 214)</td>
</tr>
<tr>
<td>$Q_{\text{LUM}}$ (liters/h/70 kg)</td>
<td>$0.743$ (13)</td>
<td>$0.805$ (0.208 to 1.27)</td>
</tr>
<tr>
<td>$k_{e,\text{DBL}}$ (h)</td>
<td>$164$ (8)</td>
<td>$168$ (97 to 240)</td>
</tr>
<tr>
<td>$V_{c,\text{DBL}}$ (liters/h/70 kg)</td>
<td>$1.20$ (52)</td>
<td>$1.14$ (0.50 to 3.68)</td>
</tr>
</tbody>
</table>

* RSE percentages are the NONMEM-produced values from the covariance step.
sistent with its greater antimalarial potency in vitro [26, 28, 29, 32], was significantly lower in children with recurrent parasitemia on day 28 than in aparasitemic children (P/H11005 0.028) and was of borderline significance on day 42 (P/H11005 0.063).

**DISCUSSION**

In the present study of Papua New Guinean children with uncomplicated malaria treated with a conventional AL regimen, rich data sets of plasma concentrations of LUM, ARM, and their active metabolites measured during an extended follow-up period were successfully analyzed using population pharmacokinetic modeling that allowed for a high proportion of BLQ plasma ARM and DHA concentrations. Our analyses included the first compartmental PK analysis of plasma DBL levels. We found that current dose recommendations for AL in children result in a LUM AUC similar to that achieved in adults despite children receiving a higher average mg/kg dose than a 50-kg adult. However, the subgroup of children weighing 12.5 to 15 kg receives the lowest mg/kg dose and may be at risk of being underdosed.

Three studies, all from Africa, have examined LUM pharmacokinetics after AL treatment in children. The first and simplest compared crushed tablets and a dispersible formulation by using a pooled analysis of single blood samples taken at one of six time points during a 14-day period from 726 children.

**FIG. 3.** Visual predictive check showing the observed 50th (○), 10th (197), and 90th (C) percentiles with the simulated 95% CIs for LUM (A) and DBL (B) concentrations (μg/liter on a log_{10} scale) from the final model.

**TABLE 3.** Secondary pharmacokinetic parameters derived from post hoc Bayesian estimates for study participants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LUM</th>
<th>DBL</th>
<th>ARM</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose 1</td>
<td>Dose 6</td>
<td>All doses</td>
<td></td>
</tr>
<tr>
<td>t_{1/2A} (h)</td>
<td>10.4 (10.3–11.8)</td>
<td>19.7 (18.4–22.5)</td>
<td>0.62 (0.60–0.64)</td>
<td>0.16 (0.12–0.33)</td>
</tr>
<tr>
<td>t_{1/2B} (h)</td>
<td>46. (44.8–48.2)</td>
<td>141 (135–150)</td>
<td>16.4 (15.7–16.8)</td>
<td>11.9 (11.2–13.2)</td>
</tr>
<tr>
<td>t_{1/2C} (h)</td>
<td>123 (120–127)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0–24} (μg·h/liter)</td>
<td>459,980 (391,330–632,730)</td>
<td>5,434 (4,394–8,542)</td>
<td>983 (371–1,770)</td>
<td>164 (145–254)</td>
</tr>
<tr>
<td>AUC_{0–24} (μg·h/liter)</td>
<td>1.13 (0.93–1.55)</td>
<td>36.8 (36.8–36.8)</td>
<td>186 (91.8–268)</td>
<td>92.7 (59.2–94.3)</td>
</tr>
</tbody>
</table>

\[a\] Data are medians (interquartile ranges).

\[b\] For LUM, t_{1/2A}, t_{1/2B}, and t_{1/2C} are the first-distribution, second-distribution, and terminal-elimination half-lives, respectively, while for DBL and ARM, t_{1/2A} and t_{1/2B} represent the distribution and terminal-elimination half-lives, respectively, and for DHA, t_{1/2B} represents the terminal-elimination half-life.

\[c\] Represents either the AUC_{0–24} for all six doses together or the AUC_{0–24} for individual doses as if they were given alone.
<12 years of age (1). The LUM AUC for both formulations was higher than in the present study (574,000 and 636,000, respectively, versus 459,980 μg·h liter⁻¹). In the second study (25), six blood samples were taken from children aged 5 to 13 years, starting when the last AL dose was given, and the LUM AUC₆₀⁻ was calculated using noncompartmental analysis. When we used our final models to generate an AUC₆₀⁻, it was higher (257,010 versus 210,000 μg·h liter⁻¹). Based on their data, the authors reported that children have lower levels of exposure to LUM than adults using recommended AL dose schedules (25). A third study of children aged 1 to 10 years utilized a population approach (16), but there was no sampling beyond 72 h and no secondary pharmacokinetic parameters were provided. A comparison with LUM disposition in the present study was, therefore, not possible.

Comparisons of LUM AUCs between studies in adults are also difficult, as some report the AUC from the first dose, while others use the AUC₆₀⁻. Table 5 summarizes the available data for both measures of drug exposure. There is a difference between LUM exposure in healthy adults and that in subjects with malaria, but the AUCs for nonpregnant adults, pregnant adults, and children with malaria are similar. Current AL dose recommendations for children ensure that those weighing 15 to 35 kg receive a 35%-higher average mg/kg dose than a 50-kg adult, but those weighing 12.5 to 15 kg receive a lower mg/kg dose. The AUC data support the higher average mg/kg dose for children and suggest that those weighing 12.5 to 15 kg should receive 2 tablets rather than 1 to avoid underdosing while not exceeding the highest recommended mg/kg dose (Fig. 6). As LUM exposure, measured as either the AUC or day 7 levels, has previously been shown to be a prime determinant of efficacy (12, 27), it is important that underdosing is avoided.

The three studies of AL in children also measured plasma ARM-DHA concentrations (1, 16, 25). The first was not able to calculate AUCs from pooled concentration data due to a sparse sampling schedule (1). The second employed a limited sampling schedule starting from the last AL dose (25), and the
AUCs were therefore lower than those of the present study (168 versus 217 μg·h·liter⁻¹ for ARM and 382 versus 402 μg·h·liter⁻¹ for DHA). The population approach used in the third study (16) produced models of the dispositions of ARM (two compartments) and DHA (one compartment) that were similar to those of the present study. Those authors reported a similar increase in CL/F₁ARM with each dose (57% versus 67.8% in the children in our study) and higher RVs (61% versus 51.6% and 82% versus 53.3% for ARM and DHA, respectively), the latter observation likely a reflection of the fact that many plasma concentrations were close to or below the LOQ. As no secondary PK parameters were provided, a comparison of AUCs could not be performed. However, the half-lives of ARM, estimated from the pharmacokinetic parameters provided, were longer than those in the children in our study (0.89 versus 0.62 h and 32.0 versus 16.4 h for distribution and elimination, respectively, of the first dose), while the elimination half-life of DHA was shorter (0.38 versus 0.80 h).

The AUCs for ARM and DHA in the present study were similar to those reported previously in adults with malaria (21, 24) but higher than those in healthy adults (14, 19, 20). Our terminal elimination half-life for ARM was longer than those reported in these studies (16.4 versus 1.5 to 3.9 h), while for DHA, it was shorter (0.80 versus 1.2 to 2.1 h). The adult studies used noncompartmental methods to determine these half-lives, and this may account for the differences. Nevertheless, based on these comparisons, exposure to ARM and DHA in children is adequate with current AL dose recommendations.

Few studies have evaluated the disposition of DBL, an active metabolite of LUM. Our DBL/LUM ratio (1.13%) falls between values reported in previous treatment studies (0.33% and 5.2%) (15, 24). The lower value (0.33%) was from a study of nonimmune Colombian adults with malaria that sampled to 168 h and reported the AUC₀–₁₆₈. The higher value (5.2%) was from a study of pregnant Thai women with malaria in which sampling started after the last dose and the AUC₀–₁₆₈ was reported. The difference between these values can, at least in part, be explained by the study designs, as the metabolite-to-parent-drug percentage calculated from the AUC₀–₁₆₈ in the present study is more than double that for the AUC₀–₁₆₈ (1.96 versus 0.76%). However, it is likely that ethnicity and pregnancy contribute to the difference. Age may also influence the metabolic conversion of LUM to DBL, as our PK model was effectively able to predict concentrations of LUM, but not DBL, in young children. It is uncertain whether malaria itself also influences the ratio, since it was 0.45%, within the range of values from studies of malaria after a single dose of AL in 22 healthy adults (G. Lefevre, personal communication).

As reported previously (24), DBL had a longer terminal elimination half-life than LUM in the present study (141 versus 123 h), and therefore, the DBL/LUM ratio will increase with time. Although the ratios found in available studies are low, the in vitro potency of DBL is between 2.2 and 7.2 times that of LUM (26, 28, 29, 32) and it may therefore contribute to the therapeutic outcome. We found that a combined weighted LUM-DBL AUC was likely to be lower than the AUC of either LUM or DBL alone in subjects with recurrent parasitemia at days 28 and 42. This supports the suggestion that DBL may influence AL’s treatment outcome (32).

Although the variable bioavailabilities of ARM and LUM have been previously reported (13), they have not been previously quantified in children. Given the significant increase in the number of fed versus fasted healthy volunteers (22), it is recommended that AL be administered with fat in order to improve absorption. Based on a study in healthy adults who received a single dose of AL, 1.2 g of fat (equivalent to 35 ml of full-cream milk) is required to achieve 90% of the maximal LUM bioavailability (4). These results may not be directly applicable to the children with malaria in our study, as they ingested 2 g of fat with each dose, but there was still significant between-dose variability in the bioavailabilities of both LUM (67.0%) and ARM (84.1%). We were unable to identify factors that may be responsible for these observations.

In the analysis of the ARM-DHA data set, there was a significant number of BLQ plasma concentrations. This is an issue encountered in pharmacokinetic analyses of a variety of other antimalarial drugs (6, 8, 16). Traditional approaches to this problem, such as excluding BLQ data from the analysis or setting them to a specific value (such as 0 or 50% of the LOQ), have been shown to bias the pharmacokinetic parameters, even

![FIG. 6. Doses of lumefantrine and artemether in mg/kg given to children weighing 5 to 35 kg under current (solid black line) and suggested (dashed gray line) dosing regimens. The horizontal dotted black line represents the dose in mg/kg recommended for a 50-kg adult.](http://aac.asm.org/)

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**TABLE 5. Summary of studies reporting the AUC for lumefantrine**

<table>
<thead>
<tr>
<th>Population sample</th>
<th>AUC₀–₁₆₈ or AUC₁₆₈–₄₂₈ (μg · h/liter) (reference[s])</th>
<th>AUC₀–₆₀ or AUC₁₆₈–₄₂₈ (μg · h/liter) (reference[s])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy adults</td>
<td>383,000–456,000 (14, 19) 1,242,000–2,730,000 (11, 14)</td>
<td>335,000–755,000 (5, 13, 15, 22)</td>
</tr>
<tr>
<td>Nonpregnant adults</td>
<td>252,000 (24)</td>
<td>472,000 (30)</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>210,000 (16)</td>
<td>572,000–636,000 (17)</td>
</tr>
<tr>
<td>Children with malaria</td>
<td>257,000</td>
<td>459,980</td>
</tr>
</tbody>
</table>

a AUCs are either medians or means and are reported either to the last data point (t) or to infinity.

b As subjects in the study by Bindschedler et al. (11) received only a single dose, the reported AUC has been multiplied by 6.

c This study used a pooled approach from single observations of each subject to calculate the AUC.
when only 10% of the data are BLQ (2, 9, 10, 35). Our approach was to use a method within NONMEM shown to have little bias in situations with up to 40% of data BLQ in a population analysis (10). This method treats BLQ data points as categorical data and maximizes the likelihood that their values are truly below the LOQ (2). Although the implementation of this method has previously been published and time-consuming, changes to NONMEM and more-efficient data processing have increased its accessibility. The benefits of this method when demonstrated in relatively simple models are likely to apply to more-complex models with parent drugs and metabolites. We were unable to obtain relative standard errors (RSEs) for our parameters in this model, as the covariance step was unsuccessful, a common problem when this method is used (9, 10). However, this does not impact the reliability of the results obtained, and other methods of model evaluation (such as bootstrap and VPC analyses) can still be used.

Our novel data relating to DBL pharmacokinetics and DBL’s favorable pharmacodynamic effects suggest that future efficacy and pharmacokinetic studies of LUM should include DBL assays to further elucidate its role. We have also shown that analytical techniques that utilize BLQ data to refine pharmacokinetic parameter estimates can be applied in this situation. Extended sampling and a population pharmacokinetic parameter estimates can be applied in this situation. Extended sampling and a population pharmacokinetic approach allow flexibility in deriving secondary parameters, an important consideration when comparisons with published nonstandard measures, such as time-limited AUCs, are of interest. Our data confirm that current AL dose recommendations produce ARM, DHA, and LUM exposures in children that are similar to those in adults with malaria. However, small children weighing 12.5 to 15 kg are at risk of being underdosed, and AL doses could be doubled without exceeding the current, weight-based, maximum mg/kg dose in this patient group.

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