Meningeal Inflammation Increases Artemether Concentrations in Cerebrospinal Fluid in Papua New Guinean Children Treated with Intramuscular Artemether

Laurens Manning,1 Moses Laman,1,2 Madhu Page-Sharp,3 Sam Salman,1 Ilomo Hwaiwhanje,4 Namar Morep,2 Peter Siba,2 Ivo Mueller,5,6 Harin A. Karunajeewa,7 and Timothy M. E. Davis1*

School of Medicine and Pharmacology, University of Western Australia, Fremantle Hospital, Fremantle, Western Australia, Australia1; Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea2; School of Pharmacy, Curtin University of Technology, Bentley, Australia3; Department of Pediatrics, Modilon Hospital, Madang, Papua New Guinea4; Infection and Immunity Division, Walter and Eliza Hall Institute, Parkville, Victoria, Australia5; Centre de Recerca en Salut Internacional de Barcelona (CRESIB), Barcelona, Spain6; and Division of Medicine, Western Hospital, Footscray, Victoria, Australia7

Received 20 March 2011/Returned for modification 17 July 2011/Accepted 13 August 2011

Although the artemisinin-associated neurotoxicity identified in vitro and in animal studies has not been confirmed clinically, only one adult study has measured cerebrospinal fluid (CSF) concentrations after administration of conventional doses. Potential artemisinin neurotoxicity could be serious in children, especially those with meningitis and, consequently, a compromised blood-brain barrier. We measured CSF/plasma artemether and dihydroartemisinin (DHA) concentrations in 32 Papua New Guinean children with a mean age of 39 months with suspected or proven severe falciparum malaria who underwent a single lumbar puncture after intramuscular artemether administration. CSF artemether concentrations were 0 to 43.5 μg/liter and CSF concentration/plasma concentration ratios were 0 to 38.1%. DHA was measurable in CSF in only two children. The seven children with meningeal inflammation (CSF white cell count > 20/mm³) had higher CSF artemether concentration/plasma artemether concentration ratios than those without (median, 6.7% [interquartile ratio, 2.5 to 27.8%] versus 0.0% [interquartile ratio, 0.0 to 2.5%]; P = 0.002). Meningeal inflammation was associated with a 4.6-fold increase in the CSF artemether concentration/plasma artemether concentration ratio in a population pharmacokinetic model. These data suggest that pharmacovigilance should be heightened when intramuscular artemether is given to severely ill children with evidence of meningeal inflammation.

Neurotoxicity associated with artemisinin and its derivatives has been observed in vitro and in animal studies (14, 46). Brain stem neurons are particularly susceptible (8), and the duration of drug exposure is an important determinant (8, 28). On the basis of these data, short-term peak concentrations of artesunate and its active metabolite, dihydroartemisinin (DHA), after intravenous artesunate administration are considered less potentially neurotoxic than the prolonged plasma concentrations of artemether and DHA observed after intramuscular artemether administration, especially with repeated dosing (20, 28).

The relevance of in vitro and animal neurotoxicity data to humans is, however, uncertain. Animal studies have involved allometrically higher doses of artemisinin drugs given for periods longer than those recommended for the treatment of human malaria (8, 20, 46). There are reports of cases of artemisinin-associated neurotoxicity (15, 17, 22, 32, 39), subtle hearing loss in retrospective case-control studies (47), and a suggestion of a longer recovery from coma (48, 49) in artemether-treated patients. In contrast, substantial clinical trial and observational evidence (11, 12, 40), as well as data from oto pathological (25, 31) and neuropathological (10, 21) studies, has not raised safety concerns. Nevertheless, although the mortality benefits of artemisinin drugs over alternative therapies are clear (11, 12), recent reviews of artemisinin-associated neurotoxicity continue to recommend pharmacovigilance, especially in potentially high-risk groups, such as children and pregnant women (14, 18, 44).

For neuronal exposure to occur in vivo, artemisinin drugs must first cross the blood-brain/blood-cerebrospinal fluid (CSF) barrier and enter the CSF compartment (37). Data relating to CSF penetration in humans are confined to a single study of six adults with severe falciparum malaria who were given intravenous artesunate (9). There was a suggestion of CSF accumulation of DHA that could, on the basis of comparative pharmacokinetic properties (19), be greater with intramuscular artemether administration. CSF concentrations of artemisinin derivatives are likely to be increased by meningeal inflammation through compromised blood-brain/blood-CSF barrier integrity, increased CSF outflow resistance, and/or decreased P-glycoprotein activity (37). Therefore, in areas where malaria is endemic, children with meningitis or encephalitis who are given empirical artemether may be at particular risk of neurotoxicity.

Corresponding author. Mailing address: School of Medicine and Pharmacology, Fremantle Hospital, University of Western Australia, P.O. Box 480, Fremantle, Western Australia 6959, Australia. Phone: 618 9431 3229. Fax: 618 9431 2977. E-mail: tdavis@cyllene.uwa.edu.au.

Published ahead of print on 22 August 2011.
In view of persistent concerns regarding artemisinin-associated neurotoxicity and, in particular, empirical treatment of children with neurological signs with these drugs regardless of malaria parasite infection status, the aims of the present study were (i) to characterize the CSF penetration of artemether/ DHA after administration of conventional intramuscular arte- mether therapy to hospitalized children in Papua New Guinea (PNG) and (ii) to determine whether meningeal inflammation increases CSF concentrations of artemether and DHA in this situation.

Materials and Methods

Patients. The present study was part of a prospective observational study of severe pediatric illness conducted at Modilon Hospital, Madang Province, on the north coast of mainland PNG, between 2007 and 2010. There is local hyperen- demic transmission of Plasmodium falciparum and P. vivax, with approximately 50 infective bites per child per year (36). Modilon Hospital is the only health care facility in the province that offers diagnostic and treatment facilities for severely ill patients. Children aged 2 months to 10 years were considered eligible if (i) written informed consent had been obtained from a parent(s)/guardian(s), (ii) intramuscular artemether treatment was considered appropriate on the basis of the attending clinician’s assessment and the national pediatric guidelines, which recommend empirical treatment of all hospitalized children in areas where malaria is endemic with antimalarial drugs and antibiotics regardless of the initial diagnosis (38), and (iii) a lumbar puncture (LP) was also considered a clinically appropriate part of management.

Clinical methods. The PNG Institute of Medical Research Institutional Re- view Board and the Medical Research Advisory Committee of the PNG Health Department approved the present study. After recruitment, a standardized case report form detailing demographic information, medical history, history of the current illness, and clinical findings was completed. Venous blood was taken for malaria parasite microscopy, plasma biochemistry, and bacterial culture (27). Severe malaria was defined as malaria parasite detection on microscopy of a peripheral blood smear and one or more of the following features: (i) impaired consciousness/coma (Blantyre coma score < 5) (34), (ii) prostration (inability to sit/stand unaided), (iii) multiple seizures, (iv) hyperlactatemia (blood lactate concentration > 5 mmol/liter), (v) severe anemia (hemoglobin level < 50 g/liter), (vi) dark urine, (vii) hypoglycemia (blood glucose level < 2.2 mmol/liter), (viii) jaundice, or (ix) respiratory distress. These criteria are consistent with the World Health Organization (WHO) definition of severe malaria (52). Not all children recruited to the present study were considered severely ill according to these criteria but were given intramuscular artemether and underwent LP for further investigation of neurological symptoms and signs such as febrile convulsion.

Artemether (Kunning Pharmaceuticals, Kunming, China) was given by intra- muscular (gluteal) injection at a minimum initial dose of 3.2 mg/kg of body weight, followed by daily intramuscular doses of at least 1.6 mg/kg, as indicated clinically, from days 2 to 7 (38). Artemether was administered at the time of or soon after hospitalization, and the time and dose were recorded. The timing of LP in relation to the first artemether dose was determined by a number of factors, including the child’s clinical state during regular postadmission monitor- ing of vital signs and consciousness level and the results of initial and subsequent hematological, biochemical, and microbiological tests. The risks and benefits of LP were explained to the parent(s)/guardian(s) by the attending pediatrician who carried out the procedure. When the LP was performed, an initial CSF aliquot was taken for cell count, protein, glucose, and bacteriological studies (27), and a heparinized venous blood sample and an additional CSF aliquot were taken for artemether and DHA assay. The blood sample was centrifuged promptly. The heparinized venous blood sample and an additional CSF aliquot were taken for cell count, protein, glucose, and bacteriological studies (27), and a heparinized venous blood sample and an additional CSF aliquot were taken for artemether and DHA assay. The blood sample was centrifuged promptly. The separated plasma and second CSF aliquot were protected from light and stored at 80°C before assay.

Artemether and dihydroartemisinin assay. High-pressure liquid chromatog- raphy-grade acetonitrile (Merck Pty. Ltd., Kilsyth, Australia), tert-butyl chloride, ethyl acetate, glacial acetic acid, and formic acid (Merck, Darmstadt, Germany), and ammonium formate (Sigma-Aldrich Company Ltd., Gillingham, United Kingdom) were used. Other solvents and chemicals were of analytical grade. Synthetic CSF (Harvard Apparatus, Holliston, MA) was used to assess the CSF matrix effect. Artemether (AAPIN Chemicals Ltd., Abingdon, United Kingdom) and DHA and artemisinin (Sigma Chemical Co, St. Louis) were used as internal standards, and stock solutions (1 μg/μl in methanol) were prepared separately and stored protected from light at −80°C. Working standard dilutions were prepared from the primary stock at 0.1, 1, and 10 μg/ml. Calibration curves (2 to 200 μg/μl) were constructed for DHA and artemether by spiking of blank plasma or blank synthetic CSF. Quality control (QC) samples were prepared in blank plasma or blank artificial CSF at 10, 20, 50, and 200 μg/μl and also stored at −80°C before use.

Artemether and DHA were extracted as previously described (5), with the following modifications. Briefly, solid-phase extraction (SPE) Bond Elut PH columns (Varian Inc., Palo Alto, CA) were preconditioned with 1 ml of meth- anol, followed by 1 ml of 1 M acetic acid. Plasma and CSF (0.5 ml) were spiked with internal standard (artemisinin, 100 μg/μl), loaded onto the SPE column, and drawn through under 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 under low vacuum for 30 min, and the retained drugs were eluted using 2 ml r-butyl chloride-ethyl acetate (80:20, vol/vol). The eluate was then evaporated under vacuum at 35°C, reconstituted in 50 μl mobile phase, and kept overnight to equilibrate the α and β anomers of DHA (5). Only the α anomer was used for quantification. The injection volumes for plasma and CSF samples were 10 μl and 20 μl, respectively.

An in-house liquid chromatography-mass spectrometry (LC-MS) assay was developed using a single quadrupole mass spectrometer (Shimadzu, Kyoto, Japan), consisting of a binary pump, vacuum degasser, thermostat autosampler, ther-mostated column compartment, photodiode detector, and mass analyzer with both electrospray ionization (ESI) and positive atmospheric pressure ionization (APCI) systems. Assays were performed in isocratic mode with 20 M ammonium formate (pH 5)-acetonitrile in 0.1% formic acid (40:60) at a flow rate of 0.2 ml/min. Chromatographic separation was undertaken at ambient temperature on a Synergy fusion-RP C18 (150-5 mm inner diameter) column coupled with a 4-μm, 3-mm (inner diameter), 5-μm-particle-size C18 guard column (Dionex, Sunnyvale, CA). Retention time (RT) of artemether was 10.0 min and 12.7 min for DHA, artemisinin, and artemether, 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 dry gas flow rate of 10 liters/min.

All authentic DHA, artemisinin, and artemether standard solutions were first spiking from m/z 100 to 500 ion and APCI positive modes, as well as combined ESI and APCI (dual-ion-source [DUIS]) modes, to identify the abun-dance of ions corresponding to the respective drugs. The base peak intensities of all three modes were compared, and the DUIS mode gave the highest signal intensity. Therefore, quantification was performed by selected ion monitoring (SIM) using the DUIS mode. The predominant fragmented ions m/z 221 for artemether and m/z 221 for DHA were used for quantitation (43). For artemisin- in, m/z 283 was monitored.

Standard curves for both plasma and synthetic CSF were linear (r² ≥ 0.99). Chromatographic data (peak area ratios of DHA/artemisinin and artemether/ artemisinin) were processed using the LAB Solution software package (version 5; Shimadzu, Japan). Using methodologies described elsewhere (30), there was no matrix effect (ion suppression/enhancement) observed in plasma or CSF. Method performance for both assays, assessed as intra- and interday relative standard deviations (RSDs) concentration across the concentration range, was published previously (5, 23), specifically, ±7.9% for plasma artemether from 10 to 200 μg/μl, ±6.7% for plasma DHA from 10 to 200 μg/μl, ±10.7% for CSF artemether from 5 to 200 μg/μl, and ±9.9% for CSF DHA from 5 to 200 μg/μl. Interday accuracies of QC assays were within 15% of nominal values throughout. The limits of quantification and detection for plasma DHA, plasma artemether, CSF DHA, and CSF artemether were 2 and 1 μg/μl, 5 and 2 μg/μl, 1 and 0.5 μg/μl, and 2 and 1 μg/μl, respectively.

Pharmacokinetic modeling. Log concentration-time data sets for artemether in plasma and CSF and DHA in plasma were analyzed by nonlinear mixed effect modeling using the NONMEM program (version 6.2; ICON Development Solutions, Ellicott City, MD) with an Intel Visual Fortran (version 10.0) compiler. A Laplacian method with interaction was used. The minimum value of the objective function (OFV) and weighted residuals (WRES) plots were used to choose suitable models during the model-building process. Allometric scaling was employed, with volume terms multiplied by (WT/70)0.75 (2). Residual variability was estimated as additive error for the log-transformed data.

Because there was only one plasma concentration-time coordinate per patient, it was not possible to estimate simultaneously the between-subject variability (BSV) and the residual variability (RV) (16). Therefore, to facilitate model building, RV was initially set to 10% for plasma compartments, while no BSV was estimated for the CSF compartment. Initially, plasma artemether profiles were analyzed with one- and two-compartment models combined with zero- and first-order absorption and first-order elimination. Once a suitable model for
plasma arteether alone was found, a CSF compartment was added and CSF concentrations were modeled by estimating the ratio of the CSF arteether concentration/plasma arteether concentration. A large percentage (61%) of observations in the CSF compartment were below the limit of quantification (BLQ). Therefore, a previously described method (1) was used to enable parameter estimation in this situation. Finally, an additional compartment was added for DHA in plasma. A CSF compartment for DHA was not added, as only two patients had measurable levels. Whenever possible, BSV and correlations between BSV terms were estimated. A covariate-versus-parameter plot was used to explore possible covariate relationships in plasma compartments for age, sex, malarial parasitemia, and meningeal inflammation. As no BSV was estimated in the CSF compartment, covariate effects were evidenced by a pattern in covariate-versus-weighted residuals plots.

Body weights were not recorded for nine children. The mean weight for age and gender from WHO reference values was used instead (50). Of the children with known WTs, the majority were between the 15th and 85th percentiles of the respective WHO reference ranges. The impact of each of these missing data on parameter estimates was assessed by simulating 1,000 new data sets with different WTs on the basis of the distribution of WT by age and gender from the WHO reference ranges for these children and then rerunning the final models. As the samples for the pharmacokinetic analysis were not based on predefined sampling times, a simulation study was also performed to ensure that estimates of parameter estimates (in particular, CSF arteether concentration/plasma arteether concentration ratios) could be reasonably obtained given the nature of the data. One thousand simulated data sets with properties similar to our own data (single point, same number of BLQ observations) were produced and analyzed.

A bootstrap procedure using 1,000 new data sets was used to facilitate validation of the final model parameter estimates. A visual predictive check (VPC) was used to assess the performance of the model for the plasma compartments, while a numerical predictive check (NPC) was used for CSF, both with 1,000 simulated replicate data sets. The NPC was assessed by comparing the number of BLQ observations in the original data set with the simulated data. Finally, a sensitivity analysis was performed for RV in both plasma compartments, as they were fixed during the modeling procedure.

Statistical analysis. Nonparametric tests were used for statistical analysis, and data are summarized as median and interquartile ranges (IQRs) unless otherwise indicated.

### RESULTS

**Baseline characteristics and clinical course.** Thirty-two children were eligible and had CSF and plasma available for arteether/DHA assay. Sixteen (50%) had clinical/laboratory features of severe malaria (52), and all but one had suffered a convulsion before or soon after presentation. Baseline clinical and laboratory data are summarized in Table 1.

The median time between initial arteether administration and LP was 7 h (range, 0.3 to 98 h). Twenty-six (81%) children underwent LP between the times of administration of the first and second doses of intramuscular arteether, while the other six children had at least one additional dose after the first dose before they underwent LP. There were no adverse outcomes associated with LP, and CSF samples from only 3 patients showed the presence of erythrocytes, all at low densities (≤45/mm³). Seven children (22%) had ≥20 white cells/mm³ in the CSF (which also contained no detectable erythrocytes), a threshold for meningeal inflammation used previously in children (6, 27). Three of these had Haemophilus influenzae and one had Streptococcus pneumoniae isolated from bacterial CSF culture. The remaining three children with evidence of meningeal inflammation had negative CSF bacterial culture and antigen testing results and were therefore assumed to have probable acute bacterial meningitis (27).

Two children died: one from H. influenzae meningitis and the other from probable acute bacterial meningitis and late complications from a colostomy for Hirschsprung’s disease. Neither child had malaria parasites on blood film microscopy. Another three survived but with chronic disability from non-malarial neurological disease. Of these five children, three had plasma arteether concentrations above the mean predicted from pharmacokinetic modeling (Fig. 1).

**Plasma arteether and dihydroartemisinin concentrations.** Thirty children (94%) had measurable plasma arteether concentrations at the time of LP. Of the two with undetectable concentrations, one underwent LP at 6 h after the first arteether dose and the other underwent LP 2 h after the fifth dose, which was 98 h after the first arteether dose. Twenty-five children (78%) had detectable plasma DHA concentra-

### TABLE 1. Baseline clinical and laboratory data

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (no. of male/no. of females)</td>
<td>19/13</td>
</tr>
<tr>
<td>Age (mo)</td>
<td>39 (17–42)*</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>13 (9–16)*</td>
</tr>
<tr>
<td>Blantyre coma score</td>
<td>4 (2–5)*</td>
</tr>
<tr>
<td>At least one convulsion (no. yes/no. no)</td>
<td>31/1</td>
</tr>
<tr>
<td>Severe malaria (no. yes/no. no)</td>
<td>16/16</td>
</tr>
<tr>
<td>Cerebrospinal fluid white cell count ≥20/mm³ (no. yes/no. no)</td>
<td>7/25</td>
</tr>
<tr>
<td>Culture-positive bacterial meningitis (no. yes/no. no)</td>
<td>4/28</td>
</tr>
</tbody>
</table>

* Data are medians (interquartile ranges).
artemether and DHA concentrations were 43.8 μg/liter 24 h after the first dose. In all 32 patients, the median plasma concentrations, and all but one had detectable concentrations during the 24 h after the first dose. In all 32 patients, the median plasma artemether and DHA concentrations were 43.8 μg/liter (IQR, 21.6 to 97.0 μg/liter) and 6.3 μg/liter (IQR, 1.9 to 13.7 μg/liter), respectively, and they correlated significantly (Spearman correlation coefficient \( r_s = 0.87, P < 0.001 \)).

**Cerebrospinal fluid artemether and dihydroartemisinin concentrations.** There were measurable CSF artemether and DHA concentrations in 12 (38%) and 2 (6%) children, respectively. In those with detectable CSF artemether, concentrations ranged from 2.0 to 43.5 μg/liter (Fig. 2). The two children with detectable CSF DHA (4.2 and 6.0 μg/liter) were those with the highest CSF artemether concentrations (20.9 and 43.5 μg/liter, respectively). Both these children underwent LP within 24 h of the first artemether dose. CSF and plasma artemether correlated significantly (\( r_s = 0.75, P < 0.001 \)). In the group of 32 children as a whole, the median CSF artemether concentration/plasma artemether concentration ratio was 0.0% (IQR, 0.0 to 3.3%) (Fig. 2). In those with measurable CSF artemether, the CSF artemether concentration/plasma artemether concentration ratios ranged from 1.1 to 38.1%.

Excluding the patient in this group with undetectable plasma and CSF artemether/DHA, the children with meningeal inflammation had higher CSF artemether concentration/plasma artemether concentration ratios than those without (6.7% [IQR, 2.5 to 27.8%] versus 0.0% [IQR, 0.0 to 2.5%], \( P = 0.002 \); Fig. 3). Five of the children with meningeal inflammation underwent LP within the first 4 h of artemether administration, at a time before peak plasma concentrations (Fig. 1). In children without meningeal inflammation, CSF artemether was detected only in those who underwent LP >6 h after artemether dosing.

**Pharmacokinetic modeling.** The modeling process was designed to allow an understanding of the population kinetics of CSF artemether/DHA penetration and, in particular, the effect of meningeal inflammation using individual patient concentration-time coordinates determined by clinical indication rather than a prespecified sampling protocol. Data from the two children with no detectable plasma or CSF artemether or DHA (one of whom had CSF with >20 white cells/mm³) were excluded from modeling, primarily because their CSF artemether concentration/plasma artemether concentration ratios were uninformative.

Initial modeling of plasma artemether demonstrated that a two-compartment model was not superior to a one-compartment model and that absorption (absorption rate constant \( k_a \)) was best described by a first-order process. Once an additional CSF compartment was added, the apparent volume of distribution of artemether in plasma relative to bioavailability (\( V_{artemether}/F \)) could no longer be estimated reliably, and it was therefore fixed at its estimated value from plasma concentration modeling (245 liters/70 kg). It was then possible to estimate BSV for absorption (\( k_a \)) and clearance (CL\(_{artemether}\)). The only significant covariate was a raised CSF white cell count (>20/mm³), which increased the CSF artemether concentration/plasma artemether concentration ratio (\( P < 0.001 \), \( \chi^2 \) distribution, 1 degree of freedom).

After the addition of a plasma DHA compartment, the population estimates of the parameters related to artemether were fixed to allow the modeling process to minimize successfully. This strategy has been shown to be superior to other sequential analysis methods (53). Final parameter estimates along with results from the bootstrap procedure are summarized in Table 3. Results from the bootstrap procedure showed parameter estimates with biases of <10%. Visual predictive checks for the modeled plasma artemether and DHA are shown in Fig. 1. These do not suggest model misspecification. The NPC for the CSF compartment revealed that the number of BLQ concentrations in the observed data was similar to the number in the simulated data (61% versus 65% [IQR, 52 to 77%]). Sensitivity analysis of the residual variability of the plasma compartments (value range, 1% to 30%) demonstrated minimal changes in the fixed parameter estimates (<15%). The impact of missing WT data was also minimal, with no difference in CSF concentration/plasma concentration ratios over the 1,000 simulations and little difference in other parameters (<10% relative standard deviation). The simulation study revealed that, despite the lack

![FIG. 2. Cerebrospinal fluid concentrations of artemether (squares) and dihydroartemisinin (circles) following intramuscular (i.m.) artemether treatment in the presence (closed symbols) or absence (open symbols) of meningeal inflammation. The dashed line denotes the lower limit of quantification for CSF artemether (1 μg/liter).](http://aac.asm.org/)

![FIG. 3. Cerebrospinal fluid artemether concentration/plasma artemether concentration ratios after intramuscular artemether grouped by presence or absence of meningeal inflammation.](http://aac.asm.org/)
TABLE 2. Final parameter estimates and nonparametric bootstrap results of the pharmacokinetic model for artemether and dihydroartemisinin

<table>
<thead>
<tr>
<th>Drug and parameter</th>
<th>Final mean (Median) (95% CI)</th>
<th>Bootstrap median (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemether</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Objective function value</td>
<td>37.768 (30.808 (11.225–46.513)</td>
<td>30.000 (0.000–60.000)</td>
</tr>
<tr>
<td>$k_d$ (h$^{-1}$)</td>
<td>0.0321 (0.0341 (0.0121–0.0597)</td>
<td>0.0341 (0.0121–0.0597)</td>
</tr>
<tr>
<td>$V_{artemether/F}$ (liters/70 kg)</td>
<td>245 (110–295)</td>
<td>245 (110–295)</td>
</tr>
<tr>
<td>$CL_{artemether/F}$ (liters/h/70 kg)</td>
<td>71.8 (31.0)</td>
<td>70.5 (26.9–105.0)</td>
</tr>
<tr>
<td>CSF concentration/plasma concentration ratio</td>
<td>CSF white cells &lt; 20 white cells/mm$^3$</td>
<td>0.0222 (0.0225 (0.0144–0.0307)</td>
</tr>
<tr>
<td>CSF white cells &gt; 20 white cells/mm$^3$</td>
<td>0.102 (0.103 (0.047–0.182)</td>
<td>0.103 (0.047–0.182)</td>
</tr>
<tr>
<td>% BSV ($k_d/F$)</td>
<td>101 (12)</td>
<td>99 (76–124)</td>
</tr>
<tr>
<td>% BSV ($CL_{artemether/F}$)</td>
<td>65 (30)</td>
<td>61 (20–91)</td>
</tr>
<tr>
<td>% RV (plasma)</td>
<td>10</td>
<td>Fixed</td>
</tr>
<tr>
<td>% RV (CSF)</td>
<td>81 (17)</td>
<td>79 (61–90)</td>
</tr>
<tr>
<td>Dihydroartemisinin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Objective function value</td>
<td>48.650 (48.766 (21.215–73.763)</td>
<td>47.916 (21.685–73.763)</td>
</tr>
<tr>
<td>$V_{DHA/F}$ (liters/70 kg)</td>
<td>144 (5)</td>
<td>154 (37–1,290)</td>
</tr>
<tr>
<td>$CL_{DHA/F}$ (liters/h/70 kg)</td>
<td>501 (16)</td>
<td>478 (354–617)</td>
</tr>
<tr>
<td>% BSV ($CL_{DHA/F}$)</td>
<td>68 (24)</td>
<td>67 (20–94)</td>
</tr>
<tr>
<td>% RV (plasma)</td>
<td>10</td>
<td>Fixed</td>
</tr>
<tr>
<td>Abbreviations: $k_d$, absorption rate constant; $V_{artemether/F}$, apparent volume of distribution of artemether in plasma; $CL_{artemether/F}$, apparent clearance of artemether in plasma; $V_{DHA/F}$, apparent volume of distribution of DHA in plasma; $CL_{DHA/F}$, apparent clearance of DHA in plasma; RSE, residual standard error.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3. Post hoc Bayesian modeled estimates of primary and secondary pharmacokinetic parameters for individual study subjects given intramuscular artemether

<table>
<thead>
<tr>
<th>Parameter$^a$</th>
<th>Median</th>
<th>Interquartile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_d$ (h$^{-1}$)</td>
<td>0.031</td>
<td>0.017–0.043</td>
</tr>
<tr>
<td>$t_{1/2}$, absorption (h)</td>
<td>22.2</td>
<td>16.2–42.1</td>
</tr>
<tr>
<td>$V_{artemether/F}$ (liters)</td>
<td>42.0</td>
<td>24.9–51.0</td>
</tr>
<tr>
<td>$CL_{artemether/F}$ (liters/h)</td>
<td>17.9</td>
<td>12.4–23.0</td>
</tr>
<tr>
<td>$t_{1/2}$, artemether (h)</td>
<td>1.5</td>
<td>1.3–1.7</td>
</tr>
<tr>
<td>$V_{DHA/F}$ (liters)</td>
<td>24.7</td>
<td>14.6–30.0</td>
</tr>
<tr>
<td>$CL_{DHA/F}$ (liters/h)</td>
<td>124</td>
<td>87–189</td>
</tr>
<tr>
<td>$t_{1/2}$, DHA (min)</td>
<td>7.2</td>
<td>6.0–9.0</td>
</tr>
</tbody>
</table>

$^a$ Abbreviations: $k_d$, absorption rate constant; $V_{artemether/F}$, apparent volume of distribution of artemether in plasma; $CL_{artemether/F}$, apparent clearance of artemether in plasma; $V_{DHA/F}$, apparent volume of distribution of DHA in plasma; $CL_{DHA/F}$, apparent clearance of DHA in plasma.

DISCUSSION

The present study is the first to examine the CSF penetration of artemether and its metabolite, DHA, after treatment with recommended doses of intramuscular artemether. In most of our young PNG children, CSF artemether concentrations were <6% of those in plasma at the same time, while no CSF DHA could be detected by sensitive liquid chromatography-mass spectrometry assay. However, the CSF penetration of both artemether and DHA was increased by meningeal inflammation. Population pharmacokinetic modeling showed that CSF artemether concentration/plasma artemether concentration ratios were 5-fold higher in children with >20 white cells/mm$^3$ in the CSF. In addition, the two patients with the highest CSF artemether concentrations (one with culture-positive bacterial meningitis and the other with probable bacterial meningitis) were the only two to have measurable CSF DHA concentrations.

It is difficult to relate these CSF data to the findings of in vitro studies that have demonstrated artemisinin-associated neurotoxicity. Drug exposure at concentrations in vitro of >1 μg/liter causes ATP depletion from and oxidative stress in brain stem neurons after 24 h and both inhibition of the mitochondrial membrane potential and degradation of cytoskeletal neurofilaments after longer periods (7 days) (42). However, more serious cytotoxicity and cell death occur with prolonged exposure at much higher in vitro concentrations (>100 μg/liter) (46). Animal studies, almost without exception, have used higher mg/kg doses (up to 600 mg/kg) given for periods (up to 30 days) usually longer than those recommended in humans (46), and so their clinical relevance is debated. Available in vitro and animal data, the present findings, and a lack of convincing neurotoxicity in humans (10, 21, 25, 31, 40) suggest that CSF artemether and DHA concentrations after conventional intramuscular artemether treatment fall short of those that could produce clinically evident and lasting neurotoxic effects in young children with well-defined severe malaria and no other neurological illness.

One situation in which there may be persistent concern is, however, when meningeal inflammation is present. In areas where malaria is endemic, such as coastal PNG, and especially where there are limited basic diagnostic facilities for bacterial infections and even malaria itself, empirical antimalarial therapy is commonly recommended for children who are hospitalized and have neurological features such as convulsions and altered consciousness (38). Bacterial, cryptococcal, and tuberculous meningitis and viral encephalitis commonly occur in PNG children in this situation (3, 26, 27). In addition, African studies reveal that approximately one-quarter of children diagnosed with cerebral malaria have such alternative diagnoses (45), and the proportion of adults with such alternative diagnoses appears to be substantially higher (29). Although artemether may improve the outcome from sepsis in the absence of malaria (35), our predictive modeling suggests that repeated recommended daily doses of intramuscular artemether will, in the presence of meningeal inflammation, result in accumulation to levels approaching and in some cases exceeding those

of a predefined sampling, pharmacokinetic parameters could be estimated with biases of <11%. In particular, the median of the difference in the CSF concentration/plasma concentration ratio for children with and without meningeal inflammation, set at 5.0 in the true model, was 5.3 (empirical 95% confidence interval [CI], 2.8 to 10.5) in simulation models.

Using the final model, we simulated CSF artemether concentrations in children receiving an initial dose of 3.2 mg/kg followed by six daily doses of 1.6 mg/kg (Fig. 4). Because the absorption half-life of plasma artemether is close to the time between doses, there will be initial CSF artemether accumulation. On day 7, the peak predicted CSF artemether concentration for children without meningeal inflammation has a 95% prediction interval (PI) of 0.4 to 37 μg/liter. A doubling of the dose at each time point increases this to 0.8 to 74 μg/liter. For children with >20 white cells/mm$^3$, conventional dosing gives a peak predicted CSF artemether concentration 95% PI of 2 to 171 μg/liter (7% of children with >100 μg/liter), and double dosing gives one of 4 to 343 μg/liter (15% of children with >100 μg/liter).

Abbreviations: $k_d$, absorption rate constant; $V_{artemether/F}$, apparent volume of distribution of artemether in plasma; $CL_{artemether/F}$, apparent clearance of artemether in plasma; $V_{DHA/F}$, apparent volume of distribution of DHA in plasma; $CL_{DHA/F}$, apparent clearance of DHA in plasma; RSE, residual standard error.
resulting in significant neuronal toxicity and death in vitro (>100 µg/liter) (46).

There have been recent reports of artemisinin resistance in southeast Asia (13, 41). Although there are no supportive data (13) and evidence of potential harm (7) in the case of the artemisinin derivatives, the usual response to emerging parasite resistance is to increase the doses of the drugs involved (4). Our predictive modeling suggests that this would further increase the risk of potential neurotoxicity in children with meningeal inflammation. This applies even if only a few doses are given (Fig. 4). It should, however, be borne in mind that this aspect of our predictive modeling is a worst-case scenario. If a child responds to antibiotic therapy and the integrity of the blood-brain barrier is progressively restored as a result, this should mean that CSF artemether concentrations will be lower than predicted. In addition, a change from intramuscular to oral dosing (which may comprise a 3-day course of artemisinin combination therapy) (51) and thus a change from prolonged to time-limited artemether exposure would also attenuate potential neurotoxicity.

Our data show considerable between-patient variability in the disposition of intramuscular artemether, with some children having the minimal absorption that has been reported in other studies (24, 33). Nevertheless, the present modeling generated pharmacokinetic parameters that were similar to those in a previous study in severely ill PNG children (24). In the only other human study of CSF penetration of an artemisinin derivative, CSF DHA concentrations in Vietnamese adults after intravenous artesunate were considerably higher than those in our children (9). Although both artesunate and artemether are DHA prodrugs, the plasma DHA concentrations were also much higher in the adult study, consistent with the comparative pharmacokinetics of intravenous artesunate and intramuscular artemether (19). Although there was concern that CSF DHA may accumulate with repeated intravenous artesunate doses in adults (9), sampling was restricted to within 2 h of the first dose. Our modeling, based on a greater number of samples and doses, does not suggest that this occurs with artemisinin drugs.

The present analyses had limitations. Although WT was not available for nine of the children, different values were simulated for these missing covariate data, and there was no significant effect on CSF artemether concentration/plasma artemether concentration ratios, the primary parameters of interest. In addition, a number of CSF artemether concentrations were BLQ, and consistent with conventional clinical management, only one CSF sample was available for each patient. Using a simulation study, we were able to show that reasonable estimates for the CSF artemether concentration/plasma artemether concentration ratios could still be obtained despite these limitations.

Given that nonmalarial conditions causing meningeal inflammation in tropical countries such as PNG can themselves lead to neuropathology (3, 26, 27, 45), it would be difficult to identify the contribution of empirical artemether/other artemisinin therapy to chronic disability and death in this situation. For example, the two children in our series with the highest CSF artemether and DHA concentrations died or developed significant neurological disability. In both cases, these outcomes could have reasonably been ascribed to bacterial meningitis. Prominent brain stem involvement might suggest at least a contribution from artemisinin treatment in such cases, but the relevant signs can be difficult to elicit in a severely ill child, and there may be cultural and logistic difficulties in obtaining postmortem neurohistopathology. Since comparative treatment trials are likely to exclude patients with nonmalarial infections causing meningeal inflammation (12, 49), enhanced pharmacovigilance in individual cases and analysis of pooled observational data may offer the best hope of identifying whether children with meningeal inflammation given artemisinin derivatives develop drug-related neurological complications. In the meantime, our data suggest that possible neurotoxicity should be borne in mind in children with bacterial meningitis treated with long courses of parenteral artemether.

**ACKNOWLEDGMENTS**

We thank the children and their parents/guardians for their participation. We are also grateful to the medical/nursing staff of the Pediatric Ward and Outpatient Department at Modilon Hospital and the...
research nurses, microscopists, data management team, and support staff of the Papua New Guinea Institute of Medical Research for clinical and logistic assistance.

This study was funded by a National Health and Medical Research Council (NHMRC) of Australia grant (grant 513782). The authors also acknowledge infrastructure support from the MalariaGen Genomic Epidemiology Network. M.L. was supported by a Fogarty Foundation scholarship. I.M. was supported by a Basser scholarship from the Royal Australasian College of Physicians and an NHMRC scholarship, and T.M.E.D. was supported by an NHMRC prisoners fellowship.

REFERENCES