MAJOR ARTICLE

A Simplified Intravenous Artesunate Regimen for Severe Malaria

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Background. We compared a conventional empirically derived regimen with a simplified regimen for parenteral artesunate in severe malaria.

Methods. This was a randomized, double-blind, placebo-controlled comparison to assess the noninferiority of a simplified 3-dose regimen (given at 0, 24, and 48 hours) compared with the conventional 5-dose regimen of intravenous artesunate (given at 0, 12, 24, 48, and 72 hours) in African children with *Plasmodium falciparum* malaria with a prespecified delta of 0.2. The total dose of artesunate in each group was 12 mg/kg. The primary end point was the proportion of children clearing ≥99% of their admission parasitemia at 24 hours. Safety data, secondary efficacy end points, and pharmacokinetics were also analyzed.

Results. In 171 children (per protocol), 78% of the recipients (95% confidence interval [CI], 69%–87%) in the 3-dose group achieved ≥99% parasite clearance 24 hours after the start of treatment, compared with 85% (95% CI, 77%–93%) of those receiving the conventional regimen (treatment difference, −7.2%; 95% CI, −18.9% to 4.4%). Dihydroartemisinin was cleared slightly more slowly in those children receiving the higher 3-dose regimen (7.4 vs 8.8 L/h for a 13-kg child; P 5 .008).

Conclusions. Pharmacodynamic analysis suggests that 3 doses of artesunate were not inferior to 5 doses for the treatment of severe malaria in children.

Clinical Trials Registration. NCT00522132.

Severe malaria kills about a million African children each year. Apart from supportive care that includes glucose for hypoglycemia and blood for anemia, there are no adjunct therapies that reduce mortality from malaria [1]. Antimalarial chemotherapy therefore remains the mainstay of patient management, with artemisinins and cinchona alkaloids the only classes used to treat severe malaria. An early study compared intramuscular artemether with quinine in African children with cerebral malaria but did not demonstrate a survival benefit for artemether [2, 3]. However, artemether is slower to produce clinical improvements than artesunate [4]. Artesunate is a water-soluble artemisinin derivative that can be given intravenously, unlike the more lipid-soluble artemether, making it one of the most rapidly acting antiparasitic agents available [5]. It also has fewer neurotoxic signals than other artemisinins in preclinical studies [6].

For these reasons, artesunate has been considered an alternative to intravenous quinine as the chemotherapy of choice for severe malaria in children. Artesunate was found to be superior to quinine for treating adults with severe malaria [7]. Recently it has also been confirmed to reduce mortality of children with severe malaria in

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a similar comparison with quinine [8]. The dosage regimens in these studies have been empirically derived.

We hypothesized that a simplified once daily 3-day dosing regimen for intravenous artesunate would be as efficacious in clearing peripheral parasitemia as the more complicated 5-dose schedule currently advised for patients with severe malaria. To test this hypothesis, we carried out a randomized, double-blind study in African children using, for the first time, a formulation of artesunate manufactured according to current Good Manufacturing Practice (cGMP) guidelines.

PATIENTS AND METHODS

Study Design

This was a randomized, double-blind, placebo-controlled, dose-finding clinical trial of intravenous artesunate for the treatment of severe malaria in children, conducted according to the principles of Good Clinical Practice and registered at ClinicalTrials.gov (identifier: NCT00522132). Patients were randomly assigned to 1 of 2 study arms: (1) 5 doses of 2.4 mg/kg intravenous artesunate given on admission and 12, 24, 48, and 72 hours later or (2) 3 doses of 4 mg/kg intravenous artesunate on admission and 24 and 48 hours later. Both regimes result in a total dose of 12 mg/kg artesunate. The Regional Ethics Committee in Lambaréné approved the study for Gabon. The Malawi study was approved by committees at Michigan State University and the University of Malawi College of Medicine.

Study End Points

Primary End Point

The primary end point was the proportion of patients with parasite clearance of ≥99% (PC99) from baseline (determined by quantitative microscopy of Giemsa-stained thick and thin smears) at 24 hours after the initiation of therapy.

Secondary End Points

Efficacy was also measured by time to total clearance of parasites (PCT100) and time to 99% (PC99), 90% (PCT90), and 50% (PCT50) reduction of parasites. Polymerase chain reaction–corrected parasitological response on day 28 and clinical response were also secondary end points. Tabular summaries and descriptive statistics of adverse events (AEs) and serious AEs (SAEs) formed part of the toxicity analysis. A population pharmacokinetic (PK) analysis was also implemented.

Sample Size

We planned to enroll 200 children of either sex, who were admitted to the hospital for severe falciparum malaria (50 each in Lambaréné and Libreville in Gabon and 100 in Blantyre, Malawi). This sample size was calculated to provide an appropriately narrow confidence interval (CI) for the treatment difference in PC99. In a previous study [9], 68% of children receiving artesunate achieved PC99 immediately before the 24-hour dose of study drug. Artesunate in that study was administered as 2.4 mg/kg initially, followed by a smaller dose of 1.2 mg/kg at 12 hours.

Assuming that a proportion of 0.68 of patients achieved PC99 in both treatment groups and 100 patients per treatment group, the 95% CI (based on the normal approximation) for the observed treatment group difference of 0 would be –.13 to .13. The 95% CI for the estimated PC99 for each treatment group would be .59–.77. The proposed sample size provided >80% power to demonstrate noninferiority with a noninferiority margin of 0.20.

Study Drugs and Description of Artesunate

The investigational product, artesunate for injection (WR256283), was kindly provided by the Walter Reed Army Institute of Research (WRAIR) and is manufactured to ICH-cGMP and USP standards by SRI International. Artesunate was a sterile dry filled powder (110 mg/vial) prepared for administration by reconstitution in a sodium phosphate buffer (0.3 mol/L; pH 8.1; 12.2 ml/vial) before infusion. To ensure cure after parenteral artesunate, a therapeutic dose of sulfadoxine-pyrimethamine estimated by the weight of the child was given in Gabon and a dose of artemether-lumefantrine was given in Malawi; both treatments were in accordance with national treatment guidelines.

Determination of Eligibility

Inclusion Criteria

Patients were included in this study if they were children aged 6 months to 10 years (inclusive, with a minimum weight of 5 kg), had a diagnosis of *Plasmodium falciparum* infection (≥5000 parasites/μL on initial smear) with symptoms severe enough to require hospitalization, and had a parent or guardian willing to provide written informed consent and to allow a hospital stay of ≥4 days. Because the subjects presented to the study site with life-threatening symptoms, treatment was implemented promptly after consent by parents.

Exclusion Criteria

Patients with known serious adverse reactions to artemisinins or those who had participated in any investigational drug study within 30 days before screening were excluded from this study. Patients who had any underlying disease that could compromise the evaluation and response to the investigational drug or who had received adequate antimalarial treatment 24 hours before admission were excluded.

Randomization and Subject Assignment

All subjects were randomized to 1 of 2 dosing regimens. Randomization was within blocks of 10 at each study site in a 1:1 ratio of the 2 regimens and was performed by the sponsor according to a written randomization plan. Individual packets of drugs for treating patients were prepared by WRAIR. The study pharmacist received sequentially numbered randomization envelopes containing the treatment allocation. Upon enrollment, the pharmacist opened the envelope with the lowest number and

prepared the study drug. The subject and research team were blinded to treatment.

Study Methods

Blood samples were obtained by venipuncture for hematological and biochemical assessments. Malaria smears (6 hourly until twice negative) and monitoring for glycemia were done using finger-prick sampling if venipuncture was not planned at a time point. Vital signs were recorded at least twice daily, and a physical examination was done during the hospitalization. Smears were prepared by the Lambaréné method and read by 2 microscopists independently [10]. Parasitological and clinical follow-up examinations were performed on days 7, 14, 21, and 28. If patients were parasitemic, samples were collected for genotyping to distinguish between recrudescent parasites and reinfection [11]. Recurrent infection within 28 days was treated with artemether-lumefantrine.

Pharmacokinetics

Three venous samples (at predetermined times within 8 hours of dosing) per child were collected: 2 samples after the initial dose and 1 sample after the dose at 48 hours. Artesunate and dihydroartemisinin (DHA) concentrations were assayed using liquid chromatography–mass spectrometry [12]. The limit of detection was 1 nmol/L and the limit of quantification was 10 nmol/L. The intraassay (interassay) coefficients of variation were less than 8% (8%) and 15% (10%) for artesunate and DHA, respectively, at 10, 50, 500, 2500 and 3000 nmol/L. A population PK model, assuming 100% conversion of artesunate to DHA, was developed using Monolix software (version 3.1; www.monolix.org). The final population PK models for artesunate and DHA were evaluated using visual predictive checks. Data were simulated from the population PK model (1000 concentrations at each of the sampling times), and at each sampling time the 5th, 50th and 95th percentiles were determined. These values were then superimposed on a scatterplot of the observed concentration data versus time. Allometric scaling was used for clearance (CL) [$CL \times (weight/70)^{0.75}$] and volume of distribution (V) [$V \times (weight/70)$].

Study Population Definitions

Safety Population

All patients who received study drug were included in safety analyses.

Intent-to-Treat Population

The intent-to-treat (ITT) population included all patients from the safety population who had parasitologically confirmed infection with *P. falciparum* prior to treatment, who had an evaluable primary end point, and who remained in the study with no serious protocol violations or withdrawals of consent. This is therefore a modified definition of ITT.

Per-Protocol Population

The per-protocol (PP) population included all patients from the ITT population who received ≥2 doses of randomized study drug and reached the primary efficacy end point. No other antimalarial agent other than the protocol-specified treatment regimen was administered during the period from 72 hours before the start of the randomized study drug until after day 28 unless the subject had recurrent parasitemia on or before day 28.

Statistical Analysis

The PP population was selected for the primary analysis of this noninferiority trial. Two-sided 95% CIs were calculated for the difference between the 3- and 5-dose regimen groups in the proportions of patients with parasite clearance of ≥99% (relative to baseline) at 24 hours after the initiation of therapy. Analyses were done with SAS software, version 9.1 (SAS).

RESULTS

Patients

One hundred and ninety-seven patients were randomized: 50 in Lambaréné, 49 in Libreville, and 98 in Blantyre. Three of these patients did not receive any study treatment (1 because consent was withdrawn after enrollment but before drug administration, 1 because intravenous access could not be achieved, and 1 because parasitemia did not fulfill inclusion criteria).

The safety population therefore included 194 children; 100 were allocated to the 5-dose treatment regimen, and 94 to the 3-dose regimen (Figure 1). Because of serious protocol violations (prior treatment with quinine in 3 patients), withdrawal of consent (1 patient), or aparasitemia on admission (1 patient), 5 additional children were excluded from the safety population. Because data on parasitemia at 24 hours were missing in 7 children, they were also excluded, leaving an ITT population of 182 children; of these, 93 received the 5-dose treatment (51%), and 89 received the 3-dose treatment (49%).

The PP population consisted of 171 children. Parasitemia at admission was <5000 parasites/μL for 4 children, quinine had been given to 3 children, and there was an error in dosing (>10% of dose) for 4 children. These 11 children were classified as major deviations. In the PP population, 86 participants received the 5-dose treatment (50%), and 85 participants received the 3-dose treatment (50%). Of the 197 randomized patients, 156 completed the study to day 28: 76% from the 5-dose group and 82% from the 3-dose group.

Baseline Characteristics in Study Populations

In both ITT and PP populations, all patients were African; demographic features, prehospital clinical features, malaria signs and symptoms on admission, or initial laboratory features, including parasitemia, were similar (Table 1).

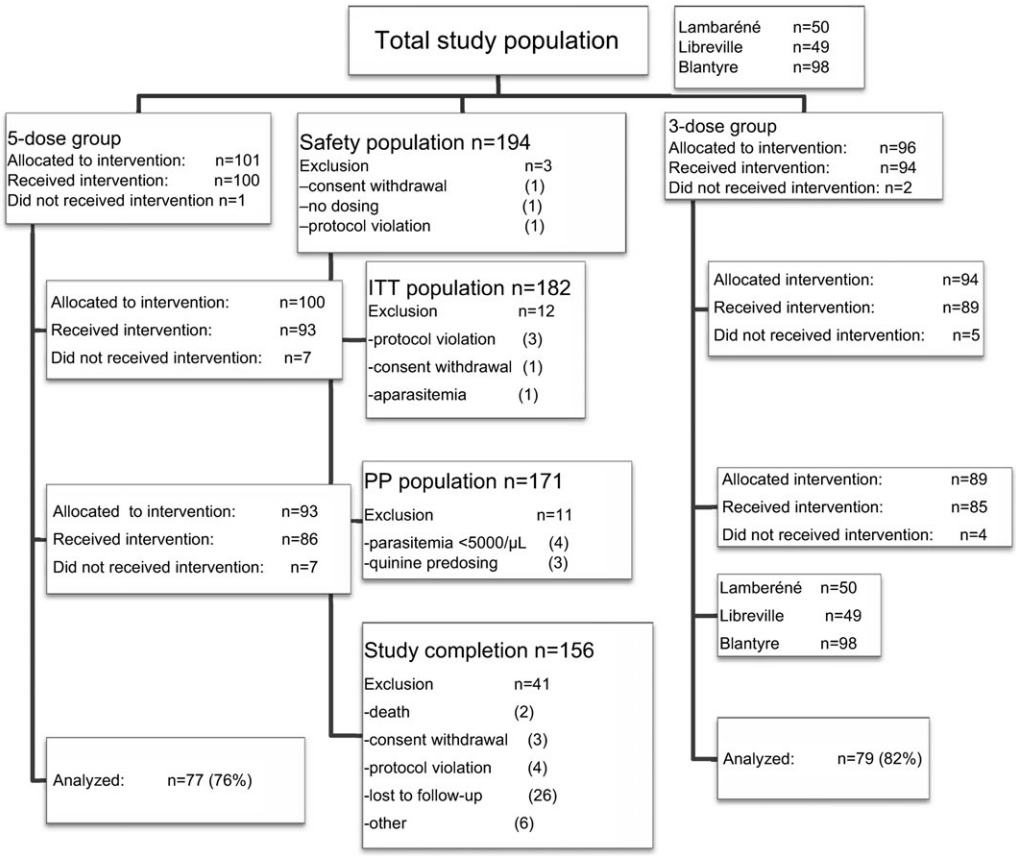

Figure 1. Patient flow. ITT, intent-to-treat; PP, per-protocol.

Efficacy

Rapid parasite clearance occurred in all subjects. Two patients died (overall mortality, 1.1% in PP population), one on day 1 and the other on day 2 after the start of treatment. Both were receiving the 3-dose regimen.

Primary End Point

Seventy-eight percent of the 3-dose recipients in the PP population (95% CI, 69%–87%) achieved ≥99% parasite clearance 24 hours after the start of treatment. Among those in the PP population allocated to receive 5 doses, 85% (95% CI, 77%–93%) achieved ≥99% parasite clearance 24 hours after the start of treatment. This gave a treatment difference of −7.2% (95% CI, −18.9% to 4.4%). Because the confidence interval does not include −20% (prespecified noninferiority margin), the null hypothesis of inferiority was rejected. In the ITT population, 76% (95% CI, 69%–86%) of those randomized to receive 3 doses reached ≥99% parasite clearance at 24 hours, compared with 86% (95% CI, 79%–93%) of the 5-dose group, giving a treatment difference of −9.6% (95% CI, −20.9% to 1.7%).

Secondary Efficacy Analysis

Secondary efficacy analysis was calculated using the ITT population, and results are shown in Table 2. In subjects receiving the 5-dose regimen, the mean parasite reduction at 24 hours was 99%; among those in the 3-dose group, the mean parasite reduction at 24 hours was 98%. These values are not significantly different. Similarly, there were no significant differences between the 5- and 3-dose treatment groups in mean parasite reductions at 48 hours (100% and 99%, respectively), or in any other measure of parasite clearance (Table 2). A Kaplan-Meier analysis confirmed there were no differences in secondary parasite clearance end points between treatment groups. The median time to fever clearance was 12 hours in both groups, with interquartile ranges of 6–24 hours in the 5-dose group and 0–24 hours in the 3-dose group. Sixteen patients had recurrence of parasitemia at day 28 (7 of these had received the 5-dose regimen), and polymerase chain reaction analysis determined that 5 patients, all of whom received the 3-day dose, had recrudescent infections.

Safety

During the study, 133 subjects (69%) had ≥1 AE: 68 (68%) of the subjects were in the 5-dose group and 65 (69%) in the 3-dose group. These 133 subjects experienced a total of 339 AEs: 165 occurred in subjects allocated to the 5-dose group and 174 in those receiving 3 doses. Thirty-one AEs were considered SAEs, and they occurred in 23 subjects (12%). Ten subjects (10%) were in the 5-dose group, and 13 (14%) were in the 3-dose group. All

Table 1. Admission Clinical Features and Laboratory Findings in the Intent-to-Treat Populations, Including Variables Used to Define Severe Malaria According to World Health Organization Criteria

Variable	5-Dose Group (n = 93)	3-Dose Group (n = 89)
Pulse rate, mean (SD), beats/min	149 (26)	144 (24)
Systolic blood pressure, mean (SD), mm Hg	100 (13)	100 (12)
Diastolic blood pressure, mean (SD), mm Hg	59 (15)	60 (11)
Respiratory rate, mean (SD), respirations/min	44 (13)	42 (13)
Body temperature, mean (SD), °C	38.3 (1.1)	37.8 (1.2)
Laboratory findings and symptoms, % of patients		
Severe anemia	17	15
Hyperlactatemia (>5 mmol/L)	19	19
Hyperparasitemia (≥10%)	40	40
Hypoglycemia (<2.2 mmol/L)	2	2
Jaundice (visible or serum bilirubin ≥3 mg/dL)	20	11
Hemoglobinuria (dipstick-positive dark urine)	3	7
Respiratory distress	8	7
Severe vomiting	18	23
Prostration	51	35
Initial parasitemia, median (range), parasites/μL	195 200 (869–1 870 264)	150 500 (1127–1 800 860)

Abbreviation: SD, standard deviation.

of these SAEs were either definitely not or unlikely to be related to study treatment. No significant difference was found in the number of subjects with AEs or SAEs between the treatment groups. The mean changes between day 0 and day 2 or day 7 or day 28 for hematological and biochemical parameters were similar in both groups (not shown).

Fatal Cases

Fatal Case 1. A 4-year-old girl was admitted to the trial in Lambaréné with vomiting, prostration, severe malarial anemia, and parasitemia of 95 400 parasites/μL. One day later she developed a urinary tract infection and unrousable coma (Blantyre coma score, 3/5), with respiratory distress, tachypnea, and hemoglobinuria. She was treated for urinary tract infection. Her condition deteriorated with 3 tonic-clonic convulsions, for which she received diazepam. Despite supportive treatment, her neurological symptoms progressed to deep coma and fixed dilated pupils. She died 2 days after admission. Her parasitemia had declined to 0.3% of her admission value within 24 hours and had cleared by the time of her death.

Fatal Case 2. A 34-month-old boy was admitted in Libreville with a diagnosis of cerebral malaria associated with severe anemia and parasitemia of 224 000 parasites/μL. Ten hours after the first dose of artesunate, he had prolonged seizures associated with respiratory distress and a Blantyre coma score of 1. Despite intensive care support, his symptoms worsened and he died later that day.

Table 2. Secondary Efficacy Analyses: Time in Hours to Parasite Clearance

	Time to Clearance, Median (IQR), h	
Degree of Clearance	5-Dose Group	3-Dose Group
PCT100	36 (30–48)	36 (30–48)
PC99	24 (18–24)	18 (18–30)
PCT90	18 (12–18)	12 (12–18)
PCT50	12 (6–12)	12 (6–12)

Abbreviations: IQR, interquartile range, PCT100, 100% parasite clearance; PC99, 99% parasite clearance; PCT90, 90% parasite clearance; PCT50, 50% parasite clearance.

Pharmacokinetics

A total of 262 artesunate and 359 DHA concentrations (within 8 hours of the initial dose) from 184 children (95 in Blantyre, Malawi; 47 in Libreville, Gabon, 42 in Lambaréné, Gabon) were available for population PK modeling. Figure 2 presents observed artesunate and DHA concentrations by time (hours) separately for the 2 doses. Population PK parameter values and their respective interpatient variability are presented in Table 3. A visual predictive check of population PK models for both artesunate and DHA showed that variation in the observed concentrations was adequately captured with the models (not shown). The distributions of clearance and volume of distribution for artesunate were not altered by dose (2.4 vs 4 mg/kg) or study site. For DHA, a slightly slower clearance (0.83 fold; 95% CI, .73–.95; $P = .008$) was observed for children receiving the higher dose of 4 mg/kg (population mean clearance for 4 vs 2.4 mg/kg, 7.4 vs 8.8 L/h for a 13-kg child) (see Figure 2*C* and 2*D*). Of the 184 children, 175 had data available for parasite clearance. No associations between the primary outcome

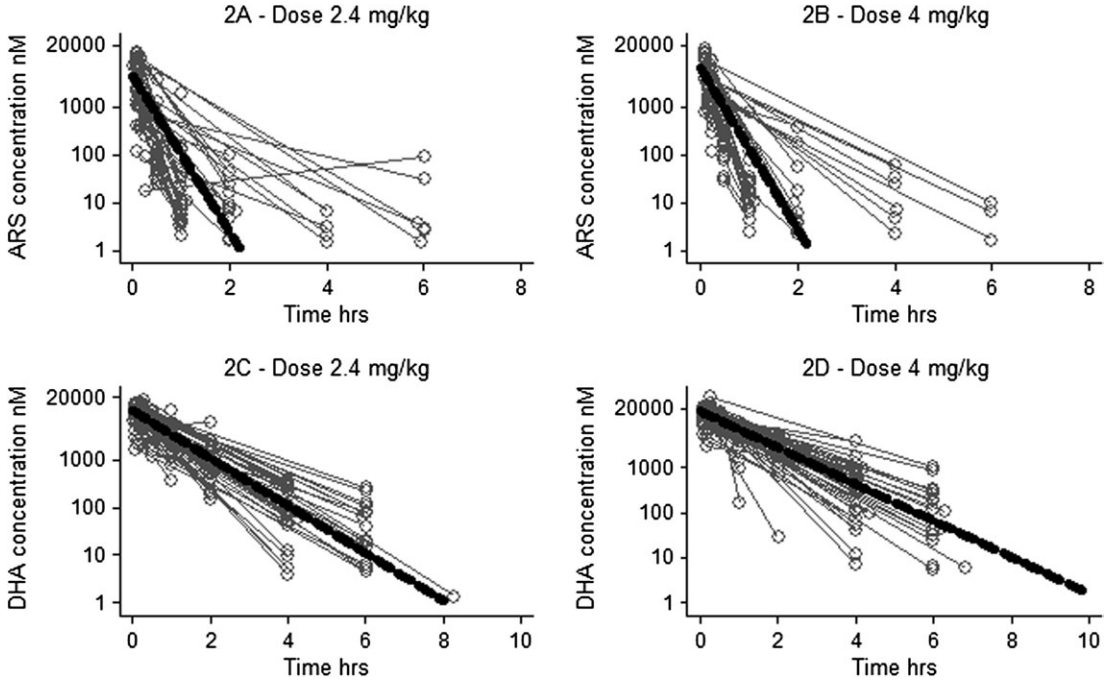

Figure 2. Observed individual artesunate (ARS) and dihydroartemisinin (DHA) concentrations (gray circles) and estimated population mean pharmacokinetic profiles (dashed black lines).

(achieved ≥99% parasite clearance 24 hours after the start of treatment) and empirical Bayes estimates of the PK parameters of artesunate and DHA were observed.

DISCUSSION

The availability of a cGMP-quality formulation for parenteral artesunate and an optimized dosing regimen should accelerate its use as a first line treatment for severe malaria in African children. Surprisingly, neither of these 2 requirements has been met until recently. A cGMP-quality product developed by WRAIR overcame some of the manufacturing hurdles associated with artesunate. This product has undergone extensive preclinical and clinical testing in adults, but this study is the largest experience to date in children. This artesunate seems to be very well tolerated in both regimens and has been made available for individual patient use under a treatment investigational new drug application (IND), pending registration. Recently a World Health Organization (WHO)

Table 3. Parameter Estimates of the 1-Compartment Model Describing Population Pharmacokinetics of Artesunate (ARS) and Dihydroartemisinin (DHA)

Parameter	ARS Estimate (RSE, %)	DHA Estimate (RSE, %)
Clearance, L/h		
70-kg patient	265 (7)	29 (4)
13-kg patient[a]	75	8
Between-subject variability[b]	67 (9)	43 (7)
Volume of distribution, L		
70-kg patient	105 (9)	43 (3)
13-kg patient[a]	19.5	8
Between-subject variability[b]	70 (12)	24 (16)
Residual standard deviation[c]	51 (12)	33 (7)
Elimination half-life, median (5th–95th percentile), h[d]	0.13 (0.06–0.60)	0.63 (0.35–1.03)

Abbreviation: RSE, relative standard error.

[a] Note that 13 kg was the mean body weight of children in this study.

[b] Log-normal error model.

[c] Proportional error model.

[d] Individual values were calculated from empirical Bayes estimates of clearance and volume of distribution.

prequalified version of parenteral artesunate (Guilin Pharmaceutical) also became available for malaria. Results from this study are comparable to those obtained in a classic PK study of parenteral artesunate in African children with severe malaria that used the Guilin formulation [9], supporting the idea that results obtained here can apply to children with a range of disease severities.

The recommended dosing regimen for intravenous artesunate in adults with severe malaria was derived empirically. Initially, WHO recommended a dose of 2.4 mg/kg artesunate followed by 1.2 mg/kg subsequently. Based on the SEAQUAMAT trial [7], this recommendation was changed to 2.4 mg/kg artesunate administered at the following times: 0 hours, 12 hours, 24 hours, and then daily thereafter for 7 days, with a switch to oral dosing as soon as patients can take sustenance by mouth. This regimen has also been used to reduce mortality in children with malaria [8, 13].

Our rationale for evaluating a simplified regimen was to reduce the number of doses of artesunate required to achieve adequate parasite clearance kinetics without incurring additional toxicity. To do this, we increased the amount in each dose of artesunate (aiming to reduce the risks of undertreatment) but maintained the same total dose of artesunate (12 mg/kg) as regimens that were safe and effective. A higher dose of artesunate (6 mg/kg/day for 7 days) in uncomplicated malaria has been associated with neutropenia, highlighting the need for careful dose ranging studies in children with severe malaria [14]. In resource-poor settings, an additional advantage of the 3-dose regimen is a reduction in the number of vials of drug, ancillary equipment, and staff required to administer the drug, providing a cost reduction of ~40% in this respect.

Pharmacodynamic aspects of artesunate use are still poorly understood, because the main PK variables are inconsistently related to efficacy end points [15]. Nevertheless, a single dose of 10 mg/kg artesunate administered by the rectal route reduced children's mortality significantly compared with placebo in a large community trial [15, 16]. This dosage is likely to result in a similar summed bioavailability of artesunate and DHA to the 4 mg/kg dose of intravenous artesunate, lending further support to the once-daily dosing regimen proposed here. Results from PK analyses will allow comparisons to be made between the different routes of administration.

A pharmacodynamic end point based on parasite clearance kinetics was chosen for this comparison because it is a visible measure of the effectiveness of this class of antimalarial. Despite the relatively short elimination half-life of DHA (~45 minutes [9]), twice-daily dosing in the first day does not confer any advantage over a higher single daily dose when parasitemia is the end point in this study. This suggests that when parasite clearance rates are a measure of assessment, the dosing interval is not a critical determinant of antiparasitic efficacy as long as the first dose is adequate. This may be yet another distinction between the mechanism of action of artemisinins and other classes of antimalarials. More detailed modeling of relationships between exposure to artesunate and DHA and parasite clearance measures can also now be examined. For example, modeling of the response to artesunate of a ring stage "resistance phenotype" described from Cambodia suggests that twice-daily dosing should be preferable to a single higher daily dose [17]. Because there are no significant differences in the clearance parameters between the 2 dosing regimens studied here, these results, taken with the modeling exercise, may provide some reassurance that there is no evidence for emerging artesunate resistance in our study population. Larger numbers of patients may need to be studied to establish this with confidence.

This study forms part of a series aimed at optimizing the use of artesunate, using cGMP material and guided by appropriate PK and pharmacodynamic assessments in pediatric patients hospitalized with malaria. This study also provides detailed PK information on the conventional WHO-recommended regimen for children with severe malaria. It shows that a simplified, 3-dose artesunate regimen leads to rapid parasite clearance and clinical recovery of African children with severe malaria. This regimen compared well with the currently recommended but more complicated 5-dose regimen. Thus, a simple 24-hour, 3-dose regimen should be further studied and developed to licensure for treating severe malaria in children. Such studies should also include the intramuscular route of administration.

Notes

Acknowledgments. P.G.K. and S.K. developed the idea for an intravenous artesunate study, with I.M., S.D., T.T., R.S.M., T.P., P.W., and C.K. making substantial contributions to the concept and design of the study. S.I., M.K., M.K.A., M.M., E.N., A.H., K. Kösters, K. Kawaza, Y.C., J.F.J.K., M.D., and T.T. were involved in the acquisition of data. P.G.K. and S.K. wrote the first draft of the paper and together with J.M., S.D., J.F.J.K., C.K., T.T., T.P., M.K., B.M., J.S., and P.T. contributed to the analysis and interpretation of data. All authors critically reviewed the paper and approved the final version.

Disclaimer. The opinions expressed herein are those of the authors and do not necessarily reflect the opinions of their institutions or official policy or opinions of the US Department of the Army.

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Potential conflicts of interest. J.M. and S.D. are employed by the Medicines for Malaria Venture. P.W. and R.S.M. are employed by the US Army, which holds an IND with the US Food and Drug Administration for this product. S.K. has been a consultant to GlaxoSmithKline and Sanofi Aventis. All other authors: no conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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