Severe falciparum malaria is one of the main causes of death in African children, who account for >85% of the worldwide malaria-related mortality. In children, the course of the disease is more rapid than in adults, with most deaths occurring within the first 24 h after admission despite parenteral antimalarial treatment. Artesunate (ARS), administered parenterally, is currently the drug of choice for the treatment of severe malaria in all age groups and all malaria-endemic settings. A large trial (SEAQUAMAT) carried out in Asia in a study population consisting mostly of adult patients (n = 1,461) with severe falciparum malaria showed a 35% reduction in mortality with ARS treatment as compared with quinine. More recently, a larger trial (AQUAMAT) performed in 5,425 African children with severe malaria showed a 22.5% lower mortality in children treated with parenteral ARS as compared with quinine.

ARS has a broader stage-specificity and more potent parasiticidal effect than quinine. ARS is water-soluble and can be administered either as an i.v. slow bolus or as an i.m. injection. The latter route is more practical in the majority of African hospital and clinic settings with limited facilities. The absorption of i.m. ARS is rapid and reliable, with peak concentrations occurring within 1 h. After being injected, ARS is rapidly and almost completely converted into its active metabolite, dihydroartemisinin (DHA). The elimination of ARS is very rapid, and antimalarial activity is determined mainly by DHA exposure levels. DHA has a terminal elimination half-life of ~45 min and is ~93% plasma protein–bound in patients with falciparum infection. The current dosing recommendation for ARS in the treatment of severe malaria is 2.4 mg/kg on admission, followed by the same dose after 12 h and then a daily dose until the patient is able to take oral antimalarial therapy reliably. This recommendation, for the most part, was derived empirically from studies in adults.

In small children with severe disease, the pharmacokinetic properties of antimalarials may differ from those reported in adults. Parenteral artesunate (ARS) is the drug of choice for the treatment of severe malaria. Pharmacokinetics data on intramuscular ARS are limited with respect to the main treatment group that carries the highest mortality, namely, critically ill children with severe malaria. A population pharmacokinetic study of ARS and dihydroartemisinin (DHA) was conducted from sparse sampling in 70 Tanzanian children of ages 6 months to 11 years. All the children had been admitted with severe falciparum malaria and were treated with intramuscular ARS (2.4 mg/kg at 0, 12, and 24 h). Venous plasma concentration–time profiles were characterized using nonlinear mixed-effects modeling (NONMEM). A one-compartment disposition model accurately described first-dose population pharmacokinetics of ARS and DHA. Body weight significantly affected clearance and apparent volume of distribution (P < 0.001), resulting in lower ARS and DHA exposure levels in smaller children. An adapted dosing regimen including a practical dosing table per weight band is proposed for young children based on the pharmacokinetic model.
results
Clinical details
Seventy patients of ages 7 months to 11 years were included, of whom 59 (84%) were <5 years of age. During the study, nine of the children died (case fatality 13%). Of these, one died within 15 min of admission, and two died within the first 24 h after admission. One patient of age 2.5 years had severe neurologic sequelae 28 days after discharge, comprising spastic hemiparesis, blindness, and hearing impairment. At admission this child had a count of 880,205 parasites/µl and plasma Plasmodium falciparum histidine-rich protein-2 concentration of 1,875 ng/ml, both indicating an extremely heavy parasite burden. At the 3-month follow-up, the neurologic sequelae had resolved completely.

Demographic, clinical, and laboratory characteristics of the study population are described in Table 1. Severe prostration, severe acidosis, convulsions, and severe anemia were the most common severity criteria. Eleven patients (16%) presented with uncompensated or compensated shock. In addition, three patients (4.3%) had blood culture–proven septicemia (unspecified Gram-negative rods, Klebsiella pneumoniae, or Staphylococcus aureus); none of these presented with shock. HIV coinfection was detected in 3/70 (4.3%) of the patients. None of these patients were receiving antiretroviral treatment.

Pretreatment with an oral antimalarial was reported with respect to 31 patients (6 with quinine, 3 with amodiaquine, 13 with sulfadoxine-pyrimethamine, 8 with artemether-lumefantrine, and 1 with amodiaquine followed by artemether-lumefantrine). In addition, 12 patients had received pretreatment with i.m. quinine within the 24 h preceding admission, the median (range) total dose being 16.1 mg/kg (10.1–53.7 mg/kg).

Each of the patients received an i.m. ARS injection of 2.4 mg/kg shortly after admission. Supportive treatments included blood transfusions and fluid resuscitation. Hypoglycemia was corrected with a 10% dextrose bolus at the time of admission and in those who developed hypoglycemia after admission (eight patients). Peripheral blood asexual parasite counts after 24 h were negative in 12/66 (18%) patients (24-h slide was not available for four patients, three of them as a result of death). The geometric mean (95% confidence interval (CI)) parasite count after 24 h was 1,128 (537–2,368) parasites/µl in the rest of the study population (n = 54). The overall geometric mean (95% CI) fractional reduction in parasite counts at 24 h was 96% (94–98%).

Population pharmacokinetic-pharmacodynamic analysis
A total of 274 ARS and DHA postdose samples, randomly distributed over the first 12 h of the study, were included in the model. A one-compartment disposition model for both ARS and DHA was adequate to describe the observed plasma concentration–time data (Table 2). All combinations of two-compartment disposition models displayed significant model misspecification despite significantly lower objective function values. A similar finding has been reported previously for DHA.23 Zero-order distribution/absorption from the injection site(s) to the central compartment provided the best description of the data, but too few samples were collected during the absorption phase and the absorption rate was therefore fixed for an accurate estimation. Interindividual variability could be estimated reliably for ARS and DHA clearance and ARS volume of distribution, showing correlation between ARS clearance and volume.

Table 1 Demographic, clinical, and laboratory characteristics of children admitted with severe malaria

| Variable | Value |
|----------|-------|
| Total number of patients | 70 |
| Age (y) (median, range) | 2.5 (0.6 to 11) |
| Weight (kg) | 10.8 (9 to 13.5) |
| Weight-for-age Z-scorea | −1.2 (1.0) |
| Coma (based on GCS/BCS) | 19 (27%) |
| Prostration | 46 (66%) |
| Convulsions | 26 (37%) |
| Shockb | 11 (16%) |
| Respiratory distress | 1 (1%) |
| Acidosis (base excess ≤−8 mmol/l) | 28 (43%) |
| Hypoglycemia (glucose ≤3 mmol/l) | 11 (16%) |
| Anemia (hemoglobin ≤5 g/dl) | 21 (30%) |
| Hemoglobinuria | 1 (1%) |
| Axillary temperature (°C) | 38.3 (1.0) |
| Heart rate (beats/min) | 158 (141 to 176) |
| Respiratory rate (breaths/min) | 49 (40 to 58) |
| Glucose (mg/dl) | 102 (88 to 127) |
| Blood urea nitrogen (mg/dl)c | 12 (8 to 16) |
| Hemoglobin (g/dl) | 7.1 (5.1 to 9.2) |
| pHc | 7.39 (7.33 to 7.43) |
| HCO3 (mmol/l)c | 17.8 (13.2 to 21.5) |
| Base excess (mmol/l)c | −7 (−13 to −3) |
| Aspartate transaminase (U/l)d | 71 (49 to 116) |
| Alanine aminotransferase (U/l)d | 25 (14 to 42) |
| Total bilirubin (μmol/l)d | 31 (24 to 49) |
| HIV-positive | 3/70 (4.3%) |
| Parasitemia (parasites/µl); (geometric mean, 95% CI) | 88,391 (53,547 to 145,909) |
| Plasma P R P2 (ng/ml); (geometric mean, 95% CI) | 1,893 (1,387 to 2,584) |

Data are median (interquartile range), mean (SD), or n (%), unless otherwise stated.

aWeight-for-age Z-scores for children ≤10 years43; data missing for 59 children.
bData missing for 3 children with undetectable histidine-rich protein-2.
cMissing data for 4 children.
dMissing data for 6 children.
eMissing data for 4 children.
fMissing data for 5 children.
gMissing data for 5 children.
hMissing data for 6 children.
iMissing data for 4 children because of missing i-STAT measurements.
jPFP, Plasmodium falciparum histidine-rich protein-2.
kPFP, Plasmodium falciparum histidine-rich protein-2.

Articles 444 VOLUME 93 NUMBER 5 | MAY 2013 | www.nature.com/cpt...
When body weight was used as a fixed allometric function on all elimination clearance (power of 0.75) and apparent volume of distribution (power of 1) parameters, a significantly better model fit was observed (Δobjective function value = −14.6). DHA clearance increased 10.2% per unit (g/dl) of decrease of hemoglobin. Interindividual variability in clearance decreased from 63.6% coefficient of variation to 55.3% coefficient of variation when this covariate was included, suggesting that it accounts for a limited but significant part of the observed variability.

The final model described the observed data well, with adequate goodness-of-fit diagnostics (Figure 1; Eta-shrinkage: elimination clearance for ARS 10.6%, central volume of distribution for ARS 12.2%, elimination clearance for DHA 6.81; Epsilon-shrinkage: 49.4 and 22.5% for ARS and DHA, respectively). A prediction-corrected visual predictive check of the final model resulted in no model misspecification and good simulation properties (Figure 2). The numerical predictive check (n = 2,000) for ARS resulted in 4.35% (95% CI 1.45–9.42) and 5.07% (95% CI 1.45–9.42) of observations above and below the 90% prediction interval, respectively; for DHA, these values were 2.97% (95% CI 1.79–9.52) and 5.95% (95% CI 1.79–8.92), respectively.

ARS and DHA exposures after the standard 2.4 mg/kg dose were simulated at each body weight level (1,000 simulations each at 1-kg intervals from 6 to 25 kg) using a uniform distribution of hemoglobin concentrations within the observed range (2.72–13.6 g/dl) to account for the observed covariate relationship (Figure 3a, b). Children with body weights between 6 and 10 kg showed a mean reduction of 20.4% (P < 0.0001) in DHA exposure as compared with children with body weights between 21 and 25 kg (median (25th to 75th percentile) exposure: 3,380 (2,130–5,470) ng × h/ml in the 6–10 kg patients, 3,780 (2,430–6,060) ng

![Figure 1](image1.png)  Goodness-of-fit diagnostics of the final population pharmacokinetic model of (a,b,c) artesunate and (d,e,f) dihydroartemisinin in children with severe malaria. The broken line represents a locally weighted least-squares regression; the solid line is the line of identity. The observed concentrations, population predictions, and individual predictions were transformed into their logarithms (base 10).

![Figure 2](image2.png)  Simulation-based diagnostics of the final model describing the population pharmacokinetics of (a,c) artesunate and (b,d) dihydroartemisinin in children with severe malaria. Graphs in a and b display a prediction-corrected visual predictive check with venous plasma concentrations transformed into their logarithms (base 10). Open circles represent observed data points; solid lines represent the 5th, 50th, and 95th percentiles of the observed data; the shaded area represents the 95% confidence interval (CI) of simulated (n = 2,000) 5th, 50th, and 95th percentiles. Graphs in c and d display the observed fraction of data points below the limit of quantification (solid lines) and the 95% CI of the simulated (n = 2,000) fraction below the limit of quantification (shaded area).
articles

Figure 3. Simulated total first-dose exposure levels (AUC_{0–12h}) of (a) ARS and (b) DHA after the standard 2.4 mg/kg dosing in children at different body weights. Simulated total first-dose exposure levels (AUC_{0–12h}) of (c) ARS and (d) DHA after the suggested adjusted dose regimen (Table 3). Open circles represent median values, and bars indicate the 25th to 75th percentiles of simulations (1,000 simulations at each body weight). The broken line represents the median exposure for the largest weight group (i.e., 700 h × ng/ml and 1,230 h × ng/ml for ARS and DHA, respectively). ARS, artesunate; AUC_{0–12h}, area under the concentration–time curve from time point 0 to 12 h; DHA, dihydroartemisinin.

\[ \text{h/ml in the 11–15 kg patients, 4,100 (2,570–6,590) ng × h/ml in the 16–20 kg patients, and 4,240 (2,700–6,840) ng × h/ml in the 21–25 kg patients.} \]

This suggests that smaller children need higher doses of ARS to attain ARS exposures similar to those in children with higher body weights. Using the final model, we evaluated various dosing regimens based on body weight bands. The proposed regimen (Table 3) resulted in similar exposures in all weight bands after the first dose of i.m. ARS (Figure 3c,d). The same simulations were performed assuming a uniform distribution of body weights at different levels of hemoglobin; these resulted in lower DHA exposures with decreasing hemoglobin levels (Figure 4).

There was no statistical difference between survivors and non-survivors with respect to total exposure (ARS \( P = 0.8060 \), DHA \( P = 0.4828 \)) or maximum concentration (ARS \( P = 0.7655 \), DHA \( P = 0.6865 \)) after the first dose. Similarly, an exposure–response relationship could not be established using nonlinear mixed-effects modeling (NONMEM) in a time-to-event approach. However, this might be a consequence of the relatively low number of deaths and the high proportion of pretreatments with different antimalarial drugs, doses, and administration routes.

**DISCUSSION**

Parenteral ARS is currently the drug of choice for the treatment of severe malaria. Optimal treatment strategies depend on detailed knowledge of the pharmacokinetic properties of drugs in the target population in which the drug is used. Age, disease status, and severity are all factors that may affect drug absorption, distribution, metabolism, and elimination.\(^{17}\) The importance of pharmacokinetics in determining the therapeutic response is illustrated by the study of artemether, the first parenteral artemisinin derivative that was compared with quinine for effectiveness in the treatment of severe malaria in large clinical trials.\(^{24,25}\) In a meta-analysis of randomized trials in severe malaria, artemether significantly reduced mortality in Southeast Asian adults but did not do so in African children.\(^{26}\) Subsequent pharmacokinetic studies showed that the oil-based artemether was released slowly and erratically from the i.m. injection site, and that this likely counterbalanced its pharmacodynamic advantages relative to quinine.\(^{19,27}\) Despite having pharmacodynamic properties similar to those of artemether, ARS is superior to artemether as a treatment because of its more favorable pharmacokinetic properties.\(^{28}\)

Dosing regimens for children are often derived from studies in adults, and this practice has led to substantial underdosing of several antimalarials in children. A pharmacokinetic study of sulfadoxine-pyrimethamine in African children with uncomplicated falciparum malaria showed that, with the usual dose of 25/1.25 mg/kg, the area under the concentration–time curve in children 2–5 years of age was half that in adults. This may have caused failure of antimalarial treatment in small children, thereby contributing to the spread of resistance. This information came decades after the introduction of sulfadoxine-pyrimethamine.\(^{18}\) It has also been shown that piperaquine exposure levels are lower in small children if a standard body weight-based dose regimen is followed.\(^{19}\)

Only limited data are available on the pharmacokinetics of parenteral, rectal, and oral ARS in children. This is the first population pharmacokinetic study of i.m. ARS in African children with severe malaria. A one-compartment model accurately described the distribution of ARS and DHA. ARS was converted rapidly into DHA, the ARS elimination half-life being \~26\ min. This is in agreement with ARS half-life values reported in other
pharmacokinetic studies of i.m. ARS\(^9,10,29\) but is considerably longer than that associated with the i.v. route\(^20,30\). This is because the elimination rate of ARS is limited by the rate of absorption from the i.m. injection site (i.e., “flip-flop” pharmacokinetics).\(^9\) The volume of distribution values, maximum concentration values, and area under the concentration–time curves of ARS and DHA are also comparable with the findings from a previous small and densely sampled pharmacokinetic study of i.m. ARS in children.\(^9\) However, the area under the concentration–time curve of DHA in this study was considerably lower than that reported in adult patients and healthy volunteers after i.v. administration of ARS.\(^30,31\) Despite the lower predicted maximum concentration value after i.m. injection as compared with i.v. administration, this is still far greater than the in vitro–defined DHA concentration value of 2.28 ng/ml that is required for 99% inhibition (IC\(_{99}\)). The excellent bioavailability after i.m. injection (~90%),\(^9,29\) fast absorption, and comparable estimates for ARS and DHA exposure support the use of i.m. ARS as a suitable alternative to i.v. ARS.\(^30\)

Our study had only a relatively small number of patients, thereby limiting its power to detect covariate relationships. A parsimonious

### Table 2 Parameter estimates of the final model describing the population pharmacokinetics of artesunate and dihydroartemisinin in children (n = 70) with severe malaria

| Variable | Population estimate\(^a\) (% RSE\(^b\)) | 95% CI\(^b\) |
|----------|----------------------------------------|-------------|
| CL/F\(_{ARS}\) (l/h) | 45.8 (8.10) | 38.8–53.7 |
| V/F\(_{ARS}\) (l) | 28.2 (11.4) | 22.7–35.2 |
| CL/F\(_{DHA}\) (l/h) | 22.4 (8.40) | 19.2–26.5 |
| V/F\(_{DHA}\) (l) | 13.5 (9.69) | 11.2–16.3 |
| DUR (min) | 1.00 (fixed) | — |

Covariate effect

| Negative effect of hemoglobin on CL/F\(_{DHA}\), (%) | 10.2 (14.9) | 6.84–12.8 |

Random effects

| \(\eta_{CL/F\_ARS}\) | 0.415 (45.3) | 0.0890–0.755 |
| \(\eta_{V/F\_ARS}\) | 0.680 (54.6) | 0.111–1.373 |
| \(\eta_{CL/F\_ARS} – \eta_{V/F\_ARS}\) | 0.497 (52.3) | 0.0732–0.969 |
| \(\eta_{CL/F\_DHA}\) | 0.306 (37.9) | 0.136–0.546 |
| \(\sigma_{ARS}\) | 0.0942 (29.2) | 0.0266–0.249 |
| \(\sigma_{DHA}\) | 0.211 (12.5) | 0.122–0.320 |

Post hoc estimates\(^c\)

| CL/F\(_{ARS}\) (l/kg) | 4.27 | 1.18–11.0 |
| V/F\(_{ARS}\) (l/kg) | 2.58 | 0.479–8.06 |
| \(t_{1/2\_ARS}\) (h) | 0.425 | 0.238–0.727 |
| \(C_{\text{max}\_ARS}\) (ng/ml) | 943 | 329–5,090 |
| AUC\(_{0–12h\_ARS}\) (h x ng/ml) | 570 | 281–2,170 |
| CL/F\(_{DHA}\) (l/kg) | 2.01 | 0.736–5.95 |
| V/F\(_{DHA}\) (l/kg) | 1.24 | — |
| \(t_{1/2\_DHA}\) (h) | 0.427 | 0.145–1.18 |
| \(T_{\text{max}\_DHA}\) (h) | 0.608 | 0.321–1.04 |
| \(C_{\text{max}\_DHA}\) (ng/ml) | 547 | 284–890 |
| AUC\(_{0–12h\_DHA}\) (h x ng/ml) | 890 | 297–2,510 |

ARS, artesunate; AUC\(_{0–12h}\), area under the concentration–time curve from time point 0 to 12 h; CL/F, elimination clearance; DHA, dihydroartemisinin; DUR, duration of zero-order absorption; f, intramuscular bioavailability; \(t_{1/2}\), terminal elimination half-life; \(T_{\text{max}}\), time to maximum concentration; V/F, central volume of distribution; \(\eta\), interindividual variability; \(\eta_{CL/F}\), correlation of random effects on CL/F and V/F; \(\sigma\), additive residual variance.

\(^a\)Computed population mean values from nonlinear mixed-effects modeling are displayed as the 2.5–97.5 percentiles of bootstrap estimates. \(^b\)Post hoc estimates are displayed as median values with 2.5–97.5 percentiles of empirical Bayes estimates.

### Table 3 Proposed body weight–adjusted dosing regimen for i.m. artesunate

| Weight (kg) | Dose i.m. (mg) | Prepared solution (ml)\(^a\) | Dose i.m. (mg/kg) |
|------------|---------------|-----------------------------|-------------------|
| 6–7        | 20            | 2                           | 2.86–3.33         |
| 8–9        | 25            | 2.5\(^b\)                  | 2.78–3.13         |
| 10–11      | 30            | 3\(^b\)                    | 2.73–3.00         |
| 12–13      | 35            | 3.5\(^b\)                  | 2.69–2.92         |
| 14–16      | 40            | 2                           | 2.50–2.86         |
| 17–20      | 50            | 2.5                         | 2.50–2.94         |
| 21–25      | 60            | 3                           | 2.40–2.86         |

\(^a\)For children of body weights ≤14 kg, dilute to 10 mg/ml; for children with body weights ≥14 kg, dilute to 20 mg/ml. \(^b\)Divide the dose equally and administer into both thighs.

---

**Figure 4** (a) Simulated total first-dose exposure levels (AUC\(_{0–12h}\)) of DHA after the standard 2.4 mg/kg dosing in children at different hemoglobin levels. Open circles represent median values, and bars indicate the 25th to 75th percentiles of simulations (1,000 simulations at each hemoglobin level). (b) Simulated total first-dose exposure (AUC\(_{0–12h}\)) of DHA in children at different body weights at very low (open circles: 3 g/dl), low (open squares: 8 g/dl), and normal (open triangles: 13 g/dl) hemoglobin levels. The broken line represents the median exposure for the largest weight group (i.e., 1,230 h x ng/ml). AUC\(_{0–12h}\) area under the concentration–time curve from time point 0 to 12 h; DHA, dihydroartemisinin.
Articles

448

can be avoided by using a dilution of 20 mg/ml. Binning of weight
split and administered in both thighs if injection volumes exceed
study. In children with body weights of <14 kg, doses should be
nation in this group, similar to the dosage regimen in the current
and administered with commonly available types of syringes.

In surviving children was 3 (2–4) doses.6

in 19% of the patients.37 Because oral ARS has a bioavailability of
~80%,38,39 this corresponds to a total parenteral dose of 33.6 mg/
kg, which would be reached only if parenteral treatment were
continued for 14 doses. In the AQUAMAT trial, the median
(interequartile range) number of doses of parenteral treatment in
surviving children was 3 (2–4) doses.6

Underdosing in young children with severe malaria may have
immediate adverse consequences with respect to outcomes.
Parenteral and oral ARS are extremely well tolerated. The only
dose-dependent toxicity identified to date is neutropenia. A
recent study carried out in Cambodia has shown that oral ARS
at a dose of 6 mg/kg/day for 7 days resulted in a reduction in
neutrophil counts (to <1.0 × 10³/μl) and short-term neutropenia
in 19% of the patients.37 Because oral ARS has a bioavailability of

ARS is currently the first-choice treatment for severe malaria,
and a product produced according to Good Manufacturing
Practices, which is WHO prequalified and available. To facilitate
implementation of an optimized dosing regimen in the treatment
of severe malaria in African children, we defined a simplified
weight-band–based dosing regimen based on the current popu-
lation pharmacokinetics model, taking into account accuracy and
practicality issues. We considered 0.5 ml to be the minimum vol-
ume of prepared ARS solution that can be measured accurately
and administered with commonly available types of syringes.

Because the weight bands are smaller for children with body
weights <14 kg, we propose an incremental ARS dose increase of
5 instead of 10 mg and a dilution of 10 mg/ml for i.m. administra-
tion in this group, similar to the dosage regimen in the current
study. In children with body weights of <14 kg, doses should be
split and administered in both thighs if injection volumes exceed
2 ml. In children of body weights ≥14 kg, larger injection volumes
can be avoided by using a dilution of 20 mg/ml. Binning of weight
bands was also chosen in accordance with the currently available
vial, which contains a dose of 60 mg, demarcating the upper limits of
weight bands at 25 kg.

The dosing recommendations we propose do not extend
beyond the weight ranges of the children included in this study.
No children with body weight <6.5 kg were included; therefore
more studies of ARS population pharmacokinetics are needed to
evaluate dosing in these very small children and to support the
current dosing recommendations. More extensive sampling in
the first 15 min and during the first 12 h after the dose could give
more information than the current study provided.

In conclusion, ARS and DHA exposures were lower in small
children after i.m. administration of ARS in severe malaria,
 warranting dose adaptation in this group. Independently, hemolytic
anemia may aggravate the lower exposures in young children. We
propose a body weight–adjusted and convenient dosing regimen
for i.m. ARS in children with body weights between 6 and 25 kg.

METHODS

Study design. This pharmacokinetic assessment of ARS was part of
the AQUAMAT trial (registration number ISRCTN 50258054), a large
multinational trial for which the results have been published else-
where.5 This substudy was conducted at Teule Hospital in Maheza,
Tanzania, from May 2009 to July 2010. Except for the additional blood
sampling, the procedures for this substudy were part of the AQUAMAT
study protocol.8 Ethical approval was obtained from the Tanzania
Medical Research Coordinating Committee and the Oxford Tropical
Research Ethics Committee. A total of 18 patients were co-enrolled in
the “FEAST” trial evaluating fluid bolus therapy in children with com-
penased shock.10

Children ≤14 years with a clinical diagnosis of severe malaria con-
firmed by Plasmodium lactate dehydrogenase (pLDH)-based rapid
diagnostic test (OptiMAL, DiaMed, Cressier, Switzerland) were recruited,
and written informed consent was obtained from the respective parents
or caregivers. Severe malaria was defined as the presence of at least one
of the following: coma (Glasgow Coma Score ≤10 or Blantyre Coma
Score ≤2 in preverbal children), convulsions (duration >30 min or ≥2
episodes in the 24 h preceding admission), respiratory distress (nasal alar
flaring, costal indrawing/recession or use of accessory muscles, severe
tachypnea) or acidic breathing (“deep” breathing), shock (capillary refill
time ≥3 sec and/or temperature gradient and/or systolic blood pressure
<70 mm Hg), severe symptomatic anemia (<5 g/dl with respiratory dis-
tress), hypoglycemia (<3 mmol/l), hemoglobinuria, severe jaundice, or,
in older children, a convincing history of anuria or oliguria. Patients who
had received full treatment with parenteral quinine or a parenteral
artemisinin derivative >24 h before admission were excluded.

Physical examination was carried out at admission, and a venous blood
sample was taken for peripheral blood parasite count, quantitative assess-
ment of plasma Plasmodium falciparum histidine-rich protein-2 (a meas-
ture of total body parasite burden),41 HIV serology (SD Bio-Line HIV
1/2 3.0; Standard Diagnostics, Kyonggi-do, Korea/Determine HIV-1/2,
Abbott Laboratories, IL), blood culture, liver function tests (aspartate
aminotransferase, alanine transaminase, γ-glutamyltransferase, total bilirubin,
creatinine, and urea, by Reflotron, Roche Diagnostics, Basel, Switzerland),
hematocrit, biochemistry, and acid–base parameters (ECG + cartridge for
i-STAT handheld blood analyzer, Abbott Laboratories, Abbott Park, IL).
Hematocrit was reported from the i-STAT reading or, when not available,
measured by HemoCue (HemoCue AB, Ängelholm, Sweden) (n = 5).
A neurologic examination was conducted at discharge, and repeated at day
28 in children who had not made a full neurologic recovery at discharge.

Antimalarial treatment. ARS (Guilin Pharmaceutical Factory, Guangxi, China) was administered as an i.m. injection (2.4 mg/kg)
shortly after admission, again at 12 h and 24 h, and daily thereafter. The

www.nature.com/cpt

448 VOLUME 93 NUMBER 5 | MAY 2013
contents of each 60 mg vial of ARS were dissolved in 1 ml 5% sodium bicarbonate (provided with the drug) and further diluted with 5 ml 5% dextrose (final concentration of 10 mg/ml) before administration as a deep i.m. injection into the anterolateral thigh. Dosing was based on the measured body weight of the patient, and injection volumes of >2–3 ml were split and divided into two injections, one in each thigh. When the patient was well enough to take oral medication, but after a minimum of 24 h (two doses of i.m. ARS), a full 3-day course of oral arteether-lumefantrine (Co-artem; Novartis, Basel, Switzerland) was given to complete the treatment.

**Patient management.** Vital signs and glucose were monitored at least every 6 h and also at any sign of deterioration in clinical condition. A majority of the patients (i.e., other than those who were able to be orally fed) received an infusion with dextrose 5%. Hypoglycemia (defined here as blood glucose <3 mmol/l) was treated with an i.v. bolus of 5 ml/kg of 10% dextrose. Blood transfusion (20 ml/kg) was given to children with hemoglobin concentrations of <5 g/dl. Fluid bolus was given to children with signs of shock.40 All the children were treated empirically with i.v. antibiotics. Convulsions were treated with i.v. diazepam or phenobarbital if persistent. Peripheral blood smears were repeated after 24 h.

**Blood sampling.** Blood samples (1.5 ml) were drawn from an indwelling catheter into prechilled fluoride oxalate tubes42 for ARS and DHA quantification before the first dose (at baseline). Four subsequent samples were taken from each patient at preset random time points within the following time windows: 0–1, 1–4, 4–12, and 12–24 h after the first dose. Randomization of sampling times was done by computer-generated randomization (STATA version 12 (Stata, College Station, TX)). Immediately after blood collection, the blood samples for drug measurements were centrifuged at 4 °C at 2,000g for 7 min. Plasma samples (0.5 ml) were stored at −80 °C and shipped on dry ice to the MORU Department of Clinical Pharmacology, Bangkok, Thailand, for drug quantification. ARS drug content and quality were checked in vials taken randomly from the purchase lots (see Supplementary Data online).6

**Drug analysis.** ARS and DHA plasma concentrations were measured using liquid chromatography–tandem mass-spectrometry.43 Quality control samples at low, middle, and high concentrations were analyzed in triplicate within each analytical batch to ensure accuracy and precision during the analysis. The total coefficients of variation were <8% for all quality control samples. The lower limit of quantification was set at 1.2 ng/ml for ARS and 2.0 ng/ml for DHA.

**Pharmacokinetic modeling.** Venous plasma concentrations were transformed into molar units and modeled as natural logarithms, using NONMEM v.7 (ICON Development Solutions, Ellicott City, MD). ARS and DHA were modeled simultaneously, using a drug–metabolite model with complete in vivo conversion of ARS into DHA (for details, see Supplementary Data online). The first-order conditional estimation method with interaction was used throughout the modeling. Model selection was based on the objective function values computed by NONMEM, goodness-of-fit graphical analysis, and physiologically plausibility. Potential covariates were investigated using a stepwise forward addition and backward elimination approach. A P value of 0.05 was used in the forward step and a P value of 0.001 in the backward step to compensate for the relatively small population studied. Simulation-based diagnostics (visual and numerical predictive checks) and bootstrap diagnostics were used to evaluate the performance of the final model.44 Monte Carlo simulations using the final model with the observed variability were performed for different body weights to obtain representative population estimates of the exposure levels during the first day of dosing (area under the concentration–time curve from time point 0 to 12 h) after prospective dose regimens. No drug exposure target is defined for parental ARS; therefore, for the purpose of arriving at a practical parenteral dosing regimen, different body weight “bins” were evaluated to ensure similar target exposures in all weight bands in agreement with the exposure in children of body weight 25 kg. The same simulations were used to evaluate the effect of other significant covariates on drug exposure.

**Pharmacodynamics.** Peripheral blood smears were taken at admission and after 24 h. Reduction in parasite load over 24 h, survival, and severe neurologic sequelae were evaluated as part of the pharmacodynamic analysis. The effects of ARS and DHA exposures on outcomes were investigated using a time-to-event analysis in NONMEM, with predicted drug concentrations being used to modulate the hazard function in a traditional maximum effect (Emax) relationship. Group comparisons were performed using the nonparametric Mann–Whitney U-test in STATA.

**SUPPLEMENTARY MATERIAL** is linked to the online version of the paper at http://www.nature.com/cpt

**ACKNOWLEDGMENTS**

We are grateful to the patients and their caregivers. We thank Ben Amos from Teule Hospital in Muheza for microbiology and laboratory management. Permission to publish this work was given by the director general, National Institute for Medical Research, Tanzania. This work was supported by The Wellcome Trust of Great Britain (grants 076908 and 082541) and was coordinated as part of the Mahidol–Oxford Tropical Medicine Research Programme funded by the Wellcome Trust of Great Britain.

**AUTHOR CONTRIBUTIONS**

J.T., I.C.E.H., and A.M.D. wrote the manuscript. J.T., I.C.E.H., N.L., N.P.J.D., L.v.S., N.J.W., and A.M.D. designed research. I.C.E.H., G.M., and A.K. performed research. J.T., I.C.E.H., and N.L. analyzed data. S.G., H.R., and M.M.L. contributed new reagents/analytical tools.

**CONFLICT OF INTEREST**

The authors declared no conflict of interest.

**Study Highlights**

**WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC?**

- Parenteral ARS is currently the drug of choice for the treatment of severe malaria in all age groups. The current recommended dosing regimen was, for the most part, derived empirically from the results of studies in adults. Most deaths from severe malaria occur in children, but pharmacokinetic data on i.m. ARS in this age group are scarce.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

- We studied the pharmacokinetic properties of i.m. ARS and its active metabolite, DHA, in the treatment of severe malaria in African children. From our findings, we derived a practical dosing regimen that avoids underdosing in smaller children.

**WHAT THIS STUDY ADDS TO OUR KNOWLEDGE**

- Body weight significantly affected elimination clearance and apparent volume of distribution, resulting in lower exposure levels of ARS and DHA in smaller children.

**HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS**

- We propose an adapted ARS dosing regimen for small children, including a practical dosing table per weight band.
1. World Health Organization. World Malaria Report 2008. <http://www.who.int/malaria/publications/atoz/9789241563697/en/index.html> (2008). Accessed 10 October 2011.

2. Marsh, K. et al. Indicators of life-threatening malaria in African children. N. Engl. J. Med. 322, 1399–1404 (1995).

3. Waller, D. et al. Clinical features and outcome of severe malaria in Gambian children. Clin. Infect. Dis. 21, 577–587 (1995).

4. World Health Organization. Guidelines for the treatment of malaria 2010, second edition–Rev.1. <http://www.who.int/malaria/publications/atoz/9789241547925/en/index.html> (2011). Accessed 4 May 2011.

5. Dondorp, A., Nosten, F., Stepneywiska, K., Day, N. & White, N. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. Lancet 366, 717–725 (2005).

6. Dondorp, A.M. et al. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT); an open-label, randomised trial. Lancet 376, 1647–1657 (2010).

7. ter Kuile, F., White, N.J., Holloway, P., Plass, G. & Krishna, S. Plasmodium falciparum: in vitro studies of the pharmacodynamic properties of drugs used for the treatment of severe malaria. Exp. Parasitol. 76, 85–95 (1993).

8. White, N.J. Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. Antimicrob. Agents Chemother. 41, 1413–1422 (1997).

9. Nealon, C. et al. Intramuscular bioavailability and clinical efficacy of artesunate in gabonese children with severe malaria. Antimicrob. Agents Chemother. 46, 3933–3939 (2002).

10. Hien, T.T. et al. Comparative pharmacokinetics of intramuscular artesunate and artemether in patients with severe falciparum malaria. Antimicrob. Agents Chemother. 48, 4224–4239 (2004).

11. Navaratnam, V., Mansor, S.M., St, N.W., Grace, J., Li, Q. & Olliaro, P. Pharmacokinetics of artemisinin-type compounds. Clin. Pharmacokinet. 39, 255–270 (2000).

12. Davis, T.M. et al. Pharmacokinetics and pharmacodynamics of intravenous artesunate in severe falciparum malaria. Antimicrob. Agents Chemother. 45, 181–186 (2001).

13. Simpson, J.A. et al. Population pharmacokinetics of artesunate and dihydroartemisinin following intra-rectal dosing of artesunate in malaria patients. PLoS Med. 3, e444 (2006).

14. Battty, K.T., Ilett, K.F. & Davis, T.M. Protein binding and alpha : beta anomer ratio of dihydroartemisinin in vivo. Br. J. Clin. Pharmacol. 57, 529–533 (2004).

15. Newton, P.N. et al. The pharmacokinetics of intravenous artesunate in adults with severe cerebral malaria. Trans. R. Soc. Trop. Med. Hyg. 91, 195–198 (1997).

16. Bethell, D.B. et al. Pharmacokinetics of oral artesunate in children with moderately severe Plasmodium falciparum malaria. Trans. R. Soc. Trop. Med. Hyg. 95, 263–270 (2001).

17. Tarning, J. et al. Population pharmacokinetics of dihydroartemisinin and piperaquine in pregnant and non-pregnant women with uncomplicated malaria. Antimicrob Agents Chemother 56:1997–2007 (2012).

18. Tran, T.H. et al. A controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. N. Engl. J. Med. 335, 76–83 (1996).

19. van Hensbroek, M.B. et al. A trial of artemether or quinine in children with cerebral malaria. N. Engl. J. Med. 335, 69–75 (1996).