Diagnosis, prognosis and treatment of severe falciparum malaria in African children
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Chapter 8

The population pharmacokinetic and pharmacodynamic properties of intramuscular quinine in Tanzanian children with severe falciparum malaria

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Submitted
Summary

Although artesunate is superior, parenteral quinine is still widely used for the treatment of severe malaria. A loading dose regimen has been recommended for 30 years but is often not used.

A population pharmacokinetic study was conducted in 75 Tanzanian children aged 4 months to 8 years with severe malaria receiving intramuscular quinine; 69 patients received a loading dose of 20 mg salt/kg. 21 had plasma quinine concentrations detectable at baseline. A zero-order absorption model with one-compartment disposition pharmacokinetics described the data adequately. Body weight was the only significant covariate and was implemented as an allometric function on clearance and volume parameters. Population pharmacokinetic parameter estimates (%RSE) of elimination clearance, central volume of distribution, and duration of zero-order absorption were 0.977 (6.50%) L/h, 16.7 (6.39%) L and 1.42 (21.5%) h, respectively, for a typical patient weighing 11 kg. Quinine exposure was reduced at lower body weights after a standard weight-based dosing; there was 18% less exposure over 24 hours in patients of 5 kg compared with those of 25 kg. Maximum plasma concentrations after the loading dose were unaffected by body weight. There was no evidence of dose related drug toxicity with the loading dosing regimen.

Intramuscular quinine is rapidly and reliably absorbed in children with severe falciparum malaria. Based on the pharmacokinetic data, a loading of 20 mg salt/kg is recommended, provided no loading dose was administered within 24 hours and no routine dose within 12 hours of admission.
Introduction

Malaria kills over 2000 people each day and young children in Africa account for over 85% of the malaria associated mortality worldwide. The case fatality of paediatric severe malaria usually exceeds 10% with the highest mortality within the first 24 hours. Parenteral quinine has been the mainstay of severe malaria treatment since the global spread of chloroquine resistance. Although parenteral artesunate is now firmly established as the treatment of choice, availability is still limited and so quinine remains very widely used.

Quinine dosing regimens were initially based on studies in Asian adults and have been extrapolated to African children, although there is relatively little detailed pharmacokinetic information in this important group. The currently recommended dosing regimen is a loading dose of 20 mg salt/kg followed by 10 mg/kg every eight hours, given by intravenous rate-controlled infusion or intramuscular (i.m.) injection. Peak quinine concentrations are achieved within 4 hours after i.m. injection, which has been postulated to be less expensive, more practicable and safer in resource limited settings where intravenous (i.v.) infusions cannot be established or reliably monitored.

Quinine is metabolized primarily via the cytochrome P450 enzyme CYP3A4 and the more polar metabolites are eliminated mainly by renal excretion. The major metabolite, 3-hydroxyquinine, contributes approximately 10% of the antimalarial activity of the parent compound. The pharmacokinetic properties of quinine are affected by the severity of infection as well as age. The apparent volume of distribution and elimination clearance are significantly reduced in proportion to increased disease severity, partly because of increased quinine binding to plasma-proteins, mainly alpha 1-acid glycoprotein. Quinine clearance is also altered by drug interactions and to some extent by genetic variability affecting CYP3A function, which will influence the conversion of quinine to its major metabolite. The unbound plasma fraction of quinine determines the therapeutic and toxic actions of the drug. In children under 2 years of age with severe malaria, unbound quinine concentrations were found to be slightly higher than in older children and adults. Although it is generally well tolerated, the therapeutic window of unbound quinine is relatively narrow and side effects include hypoglycaemia, cardiotoxicity, ototoxicity and ocular toxicity.

Rapid achievement of therapeutic quinine concentrations while avoiding toxicity is of vital importance in the treatment of severe malaria. Despite its extensive use in millions of critically ill children at risk of death, there are very few detailed assessments of the pharmacokinetic properties in the target population. The primary aim of this study was
to characterize the population pharmacokinetic profile of intramuscular m. quinine after a loading dose in children with severe malaria in Tanzania. A limited pharmacodynamic assessment was included as a secondary aim.

**Methods**

**Study design, patients and procedures**
This pharmacokinetic assessment of quinine was part of the “AQUAMAT” trial (registration number ISRCTN 50258054), a large multinational trial comparing quinine and artesunate for the treatment of severe malaria, which results have been published elsewhere. This substudy was conducted in Teule Hospital in Muheza, Tanzania from May 2009 to July 2010. Apart from the additional blood sampling, procedures of the current study were part of the AQUAMAT study protocol. The clinical assessment is reported in full elsewhere. Approval for the study including the pharmacokinetic substudy was obtained from Tanzania Medical Research Coordinating Committee and the Oxford Tropical Research Ethic Committee. A total of 21 patients were co-enrolled in the “FEAST” trial evaluating fluid bolus therapy in children with compensated shock. Children ≤14 years with a clinical diagnosis of severe malaria confirmed by a *Plasmodium* lactate dehydrogenase (pLDH)-based rapid diagnostic test (Optimal, Diamed, Cressier, Switzerland) were recruited, provided written informed consent was given by their parent or carer. Severe malaria was defined as at least one of the following: coma (Glasgow coma scale ≤10 or Blantyre coma scale ≤2 in preverbal children), convulsions (of duration >30 min or ≥2 in 24 h before admission), respiratory distress (nasal alar flaring, costal indrawing/recession or use of accessory muscles, severe tachypnoea) or acidotic breathing (“deep” breathing), shock (capillary refill time ≥3 sec and/or temperature gradient and/or systolic blood pressure <70 mmHg), severe symptomatic anaemia (<5 g/dL with respiratory distress), hypoglycaemia (<3 mmol/L), haemoglobinuria, severe jaundice or a convincing history of anuria or oliguria in older children. Patients who had received full treatment with parenteral quinine or an artemisinin derivative for more than 24 h before admission were excluded.

Physical examination was done at admission and a venous blood sample was taken for peripheral blood parasitaemia, quantitative assessment of plasma *Plasmodium falciparum* histidine-rich protein-2 (a marker of total body parasite burden), HIV serology (SD Bio-Line HIV 1/2 3.0, Standard Diagnostics Inc, Kyonggi-do, Korea /Determine HIV-1/2, Abbott Laboratories, IL, USA), blood culture, liver function tests (AST, ALT, y-GT,
total bilirubin, creatinine and urea, by Reflotron, Roche Diagnostics), Haematocrit (Hct), biochemistry and acid-base parameters (EC8+ cartridge for i-STAT handheld blood analyser). Haematocrit was reported from i-STAT or when not available, derived from haemoglobin (Hb) measured by Haemocue (n=5). At discharge, a neurological examination was performed.

**Antimalarial treatment**
Quinine dihydrochloride (Indus Pharma, Karachi, Pakistan) was given as an i.m. injection. A loading dose (20 mg salt/kg) was given shortly after admission, followed by 10 mg/kg every 8 hours. In case the patient had received pre treatment with a quinine loading dose (20 mg/kg) within 24 hours or a maintenance dose (10 mg/kg) within 12 hours before enrolment, quinine treatment after study enrolment was continued with 10 mg/kg (i.e. no loading dose). Quinine was diluted into normal saline to a concentration of 60 mg salt/ml and injected into the anterior thigh. The dosing was based on body weight and injection volumes over 3 ml were split and divided over both thighs. When the patient was well enough to take oral medication, but after a minimum of 24 hours (3 doses) of i.m. quinine, a full course of oral artemether-lumefantrine (Co-artem, Novartis, Basel, Switzerland) was given to complete the treatment.

**Patient management**
The majority of patients receiving quinine received an intravenous infusion with glucose 5%. Vital signs and glucose were monitored at least 6-hourly and with any deterioration in clinical condition. Hypoglycaemia (defined here as blood glucose <3 mmol/L) was treated with an i.v. 5 ml/kg 10% glucose bolus. A blood transfusion (20 ml/kg) was given to children with haematocrit ≤15% or haemoglobin ≤5 g/dL. All children were empirically treated with i.v. antibiotics (ampicillin and gentamicin or ceftriaxone if clinically suspected of sepsis or meningitis) until blood culture results were known or changed according to antibiotic sensitivity analysis. Convulsions were treated with diazepam or phenobarbitone if they persisted. A peripheral blood smear was repeated after 24 hours.

**Blood sampling**
Blood samples (1.5 mL) were drawn from an indwelling catheter into lithium-heparin tubes before the first dose (at baseline) and 4 subsequent samples taken at pre-set random times in the following time-windows: 1 to 4 hours, 4 to 8 hours 12 to 16 hours and 20 to 24 hours after the injection of the first dose. Randomization of sample times was done by computer-generated randomization in STATA, version 10 (StataCorp, TX, USA).
Quinine blood samples were centrifuged at 2,000×g for 10 minutes to obtain plasma. Duplicate plasma samples (0.5 mL) were stored at -80°C and sent to AMBRELA/NIMR laboratory in Tanga, Tanzania for plasma quinine quantification. Quinine drug content and quality were checked in ampoules taken randomly from the purchase lots (see supplement of 3).

**Drug analysis**

Total quinine was quantified in plasma samples using High-Performance Liquid Chromatography (HPLC) with UV-detection. Quinine was extracted from plasma samples by liquid-liquid extraction using ethyl acetate/hexane (1:1 v/v). Separation was performed by isocratic elution from a reverse phase Synergi MAX-RP (250 x 4.6 mm; 4 μ) column (Phenomenex, USA) with an acidic (adjusted to pH 2.8 with o-phosphoric acid) mobile phase (25mM KH₂PO₄:methanol; 80:20 % (v/v) + 1% (v/v) triethylamine) at a flow rate of 1.2 mL/min. Quinidine internal standard (25 μl aliquot of 100 μg/ml quinidine working standard) and quinine were detected at 254 nm and resolved to baseline with retention times of 9.3 min and 11.8 min respectively. The lower limit of quantification was 100 ng/ml. Quality control samples at 1, 10 and 20 mg/L were prepared by spiking drug free plasma. Intra-assay and inter-assay coefficients of variation ranged from 5.4% to 12.7%.

**Population pharmacokinetic-pharmacodynamic analysis**

Quinine concentrations were transformed into their natural logarithms and modelled using NONMEM version 7 (ICON Development Solutions, Hanover MD). Automation and model diagnostics were performed using Pearl-speaks-NONMEM (PsN) and Xpose. The first-order conditional estimation method with interactions was used throughout modelling. The difference in objective function value (ΔOFV) computed by NONMEM as -2×Loglikelihood was used as statistical criteria for hierarchical models (ΔOFV>3.84 was considered statistically significant at p<0.05 with one degree of freedom difference). Goodness-of-fit plots and simulation-based diagnostics were used for model evaluation.

Population pharmacokinetic models were parameterized as a first-order rate constant (ka) or a duration of a zero-order absorption (DUR), elimination clearance (CL/F), inter-compartment clearance (Q/F) and apparent volume of distribution(s) (V/F). The injection sites were considered to be a single depot compartment and bioavailability (F) was assumed to be 100%. Pre-dose concentrations were handled by flagging patients with pre-dose concentrations to allow a baseline value to be estimated for these individuals.
Between-subject variability (BSV) and between-dose occasion variability (BOV) were modelled exponentially. One- and two-compartment disposition models were evaluated. First-order and Michaelis-Menten elimination was evaluated. A Box-Cox transformation was tried on individual population parameters to assess formally the assumption that pharmacokinetic parameters are log-normally distributed.\(^{34}\) The residual random variability was assumed to be additive since data were transformed into their natural logarithms (i.e. essentially equivalent to an exponential error model on an arithmetic scale).

The implementation of body weight as a covariate on clearance and volume of distribution in the final structural model was assessed using an allometric function (individual clearance value = typical clearance value × [individual body weight/median body weight in the population])\(^{0.75}\) and individual volume value = typical volume value × [individual body weight/median body weight in the population]). An age-related enzyme-maturation effect was also investigated on clearance.\(^{35}\)

Demographic, clinical and laboratory data on admission were considered as potential covariates and 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. The following admission covariates were investigated using the stepwise approach: age (y), weight-for-age \(z\) scores,\(^ {36,37}\) temperature (°C), heart rate (beats/min), coma (continuous variable based on GCS/BCS coma score), cerebral malaria (coma and/or convulsions), blood urea nitrogen (mg/dL), haemoglobin (g/dL), base excess (mg/dL), parasitaemia, (parasites/µL), plasma \textit{Plasmodium falciparum} histidine-rich protein-2 (ng/mL) as a marker of total parasite burden,\(^ {28}\) total bilirubin (µmol/L), creatinine (high: age <1 yr ≥44.2 µmol/L, age≥1 year≥62 µmol/L, low: age<1 yr<44.2 µmol/L, age≥1 year>62 µmol/L), HIV coinfection, shock (compensated or decompensated), fluid bolus and/or blood transfusion.

Numerical- and visual predictive checks were used to assess the predictive performance of the final model. The final model with included variability was used to simulate 2000 concentrations at each sampling time-point and the 95% confidence interval around the simulated 5\(^{\text{th}}\), 50\(^{\text{th}}\) and 95\(^{\text{th}}\) percentiles were overlaid with the same percentiles of observed data to evaluate the predictive power of the model (visual predictive check). The percentages of observations below and above the simulated 5\(^{\text{th}}\) and 95\(^{\text{th}}\) percentile were also calculated for a numerical predictive check. Non-parametric bootstrap diagnostics (n=2000), stratified on body weight (above or below 10 kg), were performed for accurate relative standard errors and non-parametric confidence intervals of population parameter
estimates. The final model was also used for Monte-Carlo simulations evaluating the quinine exposure in children at different body weights with or without a loading dose. Survival data were modelled in NONMEM using a time-to-event analysis. Patients were censored at 12 hours after the last intramuscular quinine administration. The survival data were modelled using a constant hazard function, Weibull-distribution hazard function, or an exponential hazard function. OFV and simulation based diagnostics were used to compare models. There were only 13 deaths out of 75 patients in this study which was too few for a formal covariate analysis on the time-to-event. Drug concentration-response relationships were evaluated by a direct effect driven by plasma concentrations or a delayed effect by cumulative quinine exposure.

Results

Clinical details
Seventy five (75) children were included, of whom 28 (37%) were under 2 years. Demographic, clinical and laboratory characteristics are described in Table 1. Severe prostration, convulsions, severe acidosis, severe anaemia and coma were the most common severity criteria. None of the 18 patients who presented with hypoglycaemia at admission and only 4 out of 7 patients with haemoglobinuria had a history of quinine pre treatment. Seven (9.3%) patients had blood culture confirmed bacteraemia (12.5% in shocked patients versus 8.5% in non-shocked patients, p=0.623). The identified organisms were non-typhi Salmonella, Enterobacter cloaceae, Klebsiella spp., Escherichia coli, Burkholderia cepacia, Streptococcus Group A. HIV coinfection was found in 5/75 (6.7%) of patients. None of these patients was receiving antiretroviral treatment. Out of 75 patients 13 (17%) died, of whom 10 (77%) within 24 hours. Children who survived had no neurological sequelae at discharge. Sixty nine (92%) patients received a quinine loading dose at the start of the study, and the remainder started with 10 mg/kg. During the first 24 hours of admission, 37 patients received a blood transfusion and 19 patients received a fluid bolus. Eleven patients (15%) developed hypoglycaemia after admission, including 5/18 (27.7%) of those who presented with hypoglycaemia at admission. Hypoglycaemia occurred in 6/11 children despite intravenous 5% dextrose infusion, five of whom subsequently died.
Table 1. Demographic, clinical and laboratory characteristics of children admitted with severe malaria

<table>
<thead>
<tr>
<th>Variable</th>
<th>Children with severe malaria, n=75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>2.4 (0.33-8.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>11 (5.5-27)</td>
</tr>
<tr>
<td>Weight-for-age Z-score</td>
<td>-1.0 (-4.1-1.0)</td>
</tr>
<tr>
<td>Coma</td>
<td>27 (36%)</td>
</tr>
<tr>
<td>Prostration</td>
<td>46 (61%)</td>
</tr>
<tr>
<td>Convulsions</td>
<td>34 (45%)</td>
</tr>
<tr>
<td>Shocka</td>
<td>16 (21%)</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td>7 (9%)</td>
</tr>
<tr>
<td>Acidosis (base excess &lt; -8 mmol/L)</td>
<td>32 (46%)</td>
</tr>
<tr>
<td>Hypoglycaemia (glucose &lt; 3 mmol/L)</td>
<td>18 (24%)</td>
</tr>
<tr>
<td>Anaemia</td>
<td>27 (36%)</td>
</tr>
<tr>
<td>Haemoglobinuria</td>
<td>7 (9%)</td>
</tr>
<tr>
<td>Axillary temperature (°C)</td>
<td>38.2 (35.4-41.8)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>154 (98-202)</td>
</tr>
<tr>
<td>Respiratory rate (breath/min)</td>
<td>50 (24-98)</td>
</tr>
</tbody>
</table>

**Laboratory variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>95 (15-240)</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>13 (4-97)</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>7.1 (2.6-13.2)</td>
</tr>
<tr>
<td>pH</td>
<td>7.36 (7.28-7.42)</td>
</tr>
<tr>
<td>HCO₃ (mmol/L)</td>
<td>17.8 (3.5-25.6)</td>
</tr>
<tr>
<td>Base excess (mmol/L)</td>
<td>-8 (-28-2)</td>
</tr>
<tr>
<td>ASAT (U/L)</td>
<td>85 (7-3180)</td>
</tr>
<tr>
<td>ALAT (U/L)</td>
<td>22 (3-1490)</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>38 (5-250)</td>
</tr>
<tr>
<td>HIV-positive</td>
<td>5 (6.7%)</td>
</tr>
<tr>
<td>Parasitaemia (parasites/µL)</td>
<td>31 900 (17 300-58 900)</td>
</tr>
<tr>
<td>Plasma PfHRP2 (ng/mL)</td>
<td>2070 (1470 to 2900)</td>
</tr>
</tbody>
</table>

Data are No. (%) of patients, median (range), unless otherwise stated.

*a Compensated and decompensated shock combined

Coma after admission occurred in 7 (9%) patients. Peripheral parasite densities after 24 hours treatment were (geometric mean, 95% CI) 3681 (1422–10 790) parasites/µL with a
median (IQR) fractional reduction of 78% (38%–99%) in 60 patients (1 and 14 patients with missing baseline or 24 hours parasitaemia, respectively, including 10 due to death). A history of oral antimalarial treatment before admission was given for 41 (55%) patients (8 with quinine, 5 with amodiaquine, 17 with artemether-lumefantrine, 10 with sulfadoxine-pyrimethamine and 1 with sulfadoxine-pyrimethamine followed by artemether-lumefantrine). Parenteral quinine pre treatment within 24 hours prior to admission was reported for 15 patients, with a maximum of 3 doses and a mean (SD) of 10 (1.8) mg/kg. Of these patients, 12 had detectable baseline plasma quinine concentrations ranging from 1.56 to 17.38 mg/L. Three patients treated with oral quinine had baseline drug concentrations of 3.34, 5.10 and 8.26 mg/L. Another 6 patients without history of quinine treatment before admission had detectable plasma quinine concentrations ranging from 0.85 to 14.88 mg/L (of whom 5 had baseline concentrations <4.0 mg/L).

Population pharmacokinetic-pharmacodynamic analysis
Each patient contributed 1 to 5 plasma samples resulting in a total of 341 concentration-time samples distributed randomly over the first 24 hours of the study. All patients were included in the population pharmacokinetic analysis and pre-dose quinine concentrations were accommodated by estimating a baseline concentration in these patients (median [range] concentrations of 6.90 [0.976-14.9] mg/L). All post-dose plasma concentrations were determined to be above the lower limit of quantification, ranging from 0.850 to 33.8 mg/L. Four patients had very high plasma quinine concentrations (>25 mg/L) but no serious adverse events or deaths could be attributed to these high plasma quinine concentrations.

A one-compartment disposition model with zero-order absorption resulted in adequate fit to the observed data. No additional benefit was seen with an additional peripheral disposition compartment (∆OFV=-4.12, two degrees of freedom difference). There was no substantial between-dose occasion variability in any population parameters (∆OFV<-0.150) and this was therefore not included in the final model. Michaelis-Menten elimination did not significantly improve the model diagnostics (∆OFV=-2.75) compared to a first-order elimination model. A Box-Cox transformation of population parameters did not significantly improve the model fit (∆OFV<-1.88) compared to the usual assumed log-normal distribution. Incorporation of an off-diagonal element in the covariance-matrix of elimination clearance and apparent volume of distribution resulted in a significant correlation (99.9%) and an improvement in model fit. Between-subject variability could not be reliably estimated for the duration of zero-order absorption (RSE=171%) and was therefore not retained in the final model.
Body weight as a fixed allometric function on elimination clearance and apparent volume of distribution resulted in a significant improvement in model fit (ΔOFV=-39.0) and decreased the between-subject variability (%CV) from 47.4% to 35.9% and from 61.2% to 51.3%, respectively. The following covariate relationships were selected in the forward step-wise addition (p<0.05): base excess on CL/F, base excess on V/F, haemoglobin on V/F and age on CL/F as a maturation model. However, none of these covariates could be retained in the backward step with a more stringent statistical criterion (p<0.001). The final population parameter estimates, variability estimates and post-hoc estimates are summarized in Table 2.

Table 2. Parameter estimates of the final model describing quinine population pharmacokinetics in children (n=75) with severe malaria

<table>
<thead>
<tr>
<th>Variable</th>
<th>Population estimate(^{a}) (% RSE(^{b}))</th>
<th>95% CI(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixed effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>0.792 (6.50)</td>
<td>0.692–0.895</td>
</tr>
<tr>
<td>V/F (L)</td>
<td>13.7 (6.39)</td>
<td>12.2–15.5</td>
</tr>
<tr>
<td>DUR (h)</td>
<td>1.42 (20.3)</td>
<td>0.527–1.74</td>
</tr>
<tr>
<td><strong>Random effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>η(_{CL/F})</td>
<td>0.128 (28.7)</td>
<td>0.0622–0.206</td>
</tr>
<tr>
<td>η(_{V/F})</td>
<td>0.176 (24.6)</td>
<td>0.101–0.273</td>
</tr>
<tr>
<td>η(<em>{CL/F} - \eta</em>{V/F})</td>
<td>0.15 (23.7)</td>
<td>0.0763–0.206</td>
</tr>
<tr>
<td>σ</td>
<td>0.0942 (8.00)</td>
<td>0.0652–0.120</td>
</tr>
<tr>
<td><strong>Post-hoc estimates(^{c})</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F (L/h/kg)</td>
<td>0.0741</td>
<td>0.0455–0.144</td>
</tr>
<tr>
<td>V/F (L/kg)</td>
<td>1.24</td>
<td>0.645–2.89</td>
</tr>
<tr>
<td>t(_{1/2}) (hours)</td>
<td>12.1</td>
<td>9.63–14.3</td>
</tr>
<tr>
<td>C(_{MAX}) (mg/L)</td>
<td>13.4</td>
<td>7.20–24.8</td>
</tr>
<tr>
<td>AUC(_{0-7.5h}) (h×mg/L)</td>
<td>78.9</td>
<td>42.3–148</td>
</tr>
</tbody>
</table>

\(^{a}\) Computed population mean values from NONMEM are calculated for a typical patient with a body weight of 11 kg.

\(^{b}\) Assessed by non-parametric bootstrap method (n=1,413 successful iterations out of 2000) of the final pharmacokinetic model. Relative standard error (% RSE) is calculated as 100×(standard deviation/mean value). 95% confidence interval (95% CI) is displayed as the 2.5 to 97.5 percentile of bootstrap estimates.

\(^{c}\) Post-hoc estimates are displayed as median values with 2.5 to 97.5 percentiles of empirical Bayes estimates.

CL/F=elimination clearance; V/F=central volume of distribution; F= intramuscular bioavailability; DUR=duration of zero-order absorption; η= inter-individual variability; η\(_{CL/F} - \eta_{V/F}\) = correlation of random effects on CL/F and V/F; σ =additive residual variance error; t\(_{1/2}\) =terminal elimination half-life; AUC\(_{0-7.5h}\) =area under the concentration-time curve from time-point 0 to 7.5 hours; C\(_{MAX}\) =predicted peak concentration after first dose.
The final model described the observed data well with adequate goodness-of-fit diagnostics (Figure 1) and calculated shrinkages below 15% (CL/F: 11.3%, V/F: 11.3%, Epsilon-shrinkage: 14.0%). A prediction-corrected visual predictive check of the final model resulted in no model misspecification with good simulation properties (Figure 2). The numerical predictive check resulted in 6.92% (95% CI 2.08–9.00) and 2.08 (95% CI 1.73–9.00) of observations above and below the 90% prediction interval.

**Figure 1**

![Figure 1](image)

Goodness-of-fit diagnostics of the final population pharmacokinetic model of quinine in children with severe malaria. Broken line, a locally weighted least-squares regression; solid line, line of identity. The observed concentrations, population predictions, and individual predictions were transformed into their logarithms (base 10).

Body weight was the only significant covariate in the final model with lower body weights being associated with slightly reduced exposure during the first 24 hours after the standard weight-based dose. There was a mean reduction of 7.19% in simulated quinine exposure during the first dose (0 to 8 hours) when comparing patients weighing 5 kg with patients...
of 20 kg body weight after a 20 mg salt/kg loading dose (data not shown). This reduction in exposure accumulated with the repeated maintenance dosing of 10 mg/kg over the first 24 hours to a total mean difference of 15.4% (data not shown). Peak concentration after the first dose was unaffected by body weight but accumulated to a mean difference of 15.9% lower peak concentrations for patients weighing 5 kg compared to patients of 20 kg after the third dose (Figure 3A). This difference (<20%) in total exposure and maximum concentration is not likely to have a significant clinical impact since more than 85% of patients irrespectively of body weight reached plasma quinine concentrations over the therapeutic margin of 8 mg/L after a loading dose of 20 mg/kg and more than 95% of patients reached the target during the first 24 hours with the subsequent maintenance dose of 10 mg/kg. In absence of a loading dose, the therapeutic range would only be reached in less than 30% of patients after the first dose and in 89% of patients during the first 24 hours (Figure 3B).

**Figure 2**

Visual predictive check of the final model describing the population pharmacokinetics of quinine in children with severe malaria. Open circles, observed data points; solid lines, 5th, 50th, and 95th percentiles of the observed data. Shaded area, 95% confidence interval of simulated (n=2000) 5th, 50th, and 95th percentiles. Venous plasma quinine concentrations were transformed into their logarithms (base 10).
Predicted population pharmacokinetic profiles of quinine A) at different body weights (gray solid profiles): 5 kg, (− ∙ −), 10 kg (− −) and 20 kg (−) are highlighted in black; and B) after a loading dose of 20 mg/kg (—) versus a routine dose of 10 mg/kg (− −): 10 kg are highlighted in black.

A constant hazard function model with cumulative exposure implemented as an Emax function modulating the hazard described the survival over time well in this study (data not shown). A simulation-based visual predictive check resulted in the observed survival curve to be contained within the 95% confidence interval of the simulated survival. Median time to reach a 50% reduction in hazard was approximately 6 hours. However, no concentration-effect relationship could be established and there was no significant difference in post-hoc estimates of total exposure (p=0.1358) or maximum concentrations (p=0.1786) after the first dose in children who died compared to children who survived.
(Figure 4). The exposure-effect relationship is likely to describe the delayed antimalarial effect of quinine but this approach is biased since many patients had a pre treatment history of quinine and a larger data set would be necessary for a formal concentration-effect analysis.

**Figure 4**

Total quinine exposure (AUC\(_{0-8hrs}\)) and maximum concentration (C\(_{MAX}\)) after the first dose stratified for outcome. Error bars indicate median and interquartile range.

**Discussion**

The therapeutic range for quinine in severe malaria has been estimated between 8 and 15 mg/L based on observations in uncomplicated malaria suggesting reduced therapeutic responses when serum or plasma concentrations fall below 5 mg/L and taking into
account variation in parasite susceptibility and the reduced free fraction observed in severe malaria. Plasma concentrations up to 20 mg/L have not been associated with significant toxicity. In the present study i.m. quinine was rapidly and reliably absorbed and the current loading dose dosing regimen resulted in plasma concentrations that rapidly reached the therapeutic range in African children with severe malaria. A wide range of patient covariates did not significantly affect the pharmacokinetic parameters suggesting that this applied to children of all ages and with all forms of severe malaria. Body weight was the only significant covariate affecting quinine exposure. Monte-Carlo simulations resulted in a modest mean reduction of 7.19% in total quinine exposure after the loading dose (0 to 8 hours) in children weighing 5 kg compared to that of children weighing 20 kg. This accumulated to a larger difference of 15.4% over 3 doses (0 to 24 hours). This is unlikely to have a significant clinical impact since therapeutic levels of 8 mg/L were reached rapidly in all weight groups (Figure 2). Dose adjustment is therefore not recommended in younger children based on this pharmacokinetic difference. Simulations resulted in median (95% CI) maximum concentrations of 12.6 (5.60–28.4) mg/L after a loading dose of 20 mg/kg compared to 6.32 (2.80–14.2) mg/L after a non-loading dose of 10 mg/kg. This supports that a loading dose should be used to achieve target concentrations within the first dose interval.

An i.m. loading dose of quinine was rapidly and reliably absorbed and patients in this study reached estimated peak median (95% CI) plasma quinine concentrations of 13.4 (7.20–24.8) mg/L within 1.50 hours. This is in accordance with previous studies showing similar peak plasma quinine concentration compared to the intravenous route with similar efficacy. Dilution of the quinine solution to 60 mg/mL has been reported to accelerate the absorption from the i.m. injection site.

The reported median (95% CI) estimates of a quinine terminal half-life of 12.1 (9.63–14.3) hours and elimination clearance of 0.0741 (0.0455–0.144) L/hr/kg are in agreement with previously published estimates from small conventional densely sampled pharmacokinetic studies in children with severe falciparum malaria: median half-lives ranging from 8.4 to 23.5 hours and clearance from 0.027 to 0.0816 L/h/kg have been reported (reviewed in ). Minor differences between our findings with that reported in a population pharmacokinetic analysis by Krishna et al (mean (SD) half-life of 19.9 (4.4) hours and elimination clearance of 0.05 L/h/kg) may be explained by using different structural models (a one-compartment model versus a two-compartment model). We only sampled patients for 24 hours in this study which could also contribute to the difference in structural models. Thus, a two-compartment model could prove to
be a more appropriate structural model when enough data are collected to support a
differentiation between a distribution and a terminal phase. However the terminal
elimination half-life estimate reported by Krishna et al is similar to that in adults with
severe malaria, whereas the majority of published data point to more rapid elimination
in children compared with adults. Body weight has not been described as a covariate for
quinine pharmacokinetics before but it was significant in this analysis.\textsuperscript{10,11} Physiological
processes do not scale linearly with body weight and consequently children with a lower
body weight will have a higher body weight-normalised elimination clearance, which has
been reported previously for other antimalarials.\textsuperscript{44,45}
In accordance with the only previous population pharmacokinetic study, we did not find
any other covariates explaining the between-subject variability in children with severe
malaria despite the different clinical presentations.\textsuperscript{11} Compared with uncomplicated
malaria, patients with severe disease have a smaller distribution volume and a slower
clearance due in part to a higher fraction of plasma-protein bound quinine.\textsuperscript{5}
It is reassuring that intramuscular quinine was reliably absorbed in children with
impaired perfusion, shock and severe anaemia, although these were largely corrected for
in this study with supportive treatments. In addition, none of the clinical or laboratory
parameters with strong prognostic value such as coma, impaired renal function (elevated
BUN) or acidosis affected the pharmacokinetics of quinine.\textsuperscript{46} Therefore, the quinine
dosing does not need to be adapted according to the presentation of the disease in
children with severe malaria.
Intramuscular quinine is painful, but local toxicity is rare when a sterile injection technique
is used and the quinine is diluted to 60 mg/ml.\textsuperscript{10} In our study site, all concomitant
medications including routinely administered antibiotics were given by i.m. injection in
the anterior thigh. However, no mobility problems were noted and all surviving children
were well at discharge. The neurological examination at discharge did not disclose any
evidence of systemic quinine toxicity such as blindness or hearing problems, even though
4/75 (5\%) of children reached quinine concentrations above 25 mg/L within the first
24 hours of treatment. Peak total plasma concentrations tend to increase during the
treatment of severe malaria,\textsuperscript{17} so a higher proportion of patients might have experienced
potentially toxic quinine concentrations later in their treatment course. The levels of
quinine associated with toxicity in severe malaria are not clear-cut, since toxicity derives
from free quinine concentrations, which depend on the levels of plasma-proteins,
predominantly AGP that vary substantially.\textsuperscript{18,19} The pharmacokinetic study of Hensbroek
et al showed that young children could be more prone to quinine toxicity as evidenced
by prolongation of QRS interval on the electrocardiogram (depolarization), although this
was not related to plasma quinine concentrations.\textsuperscript{10} The main adverse effect of quinine in severe malaria is hypoglycaemia resulting from quinine stimulated insulin release. Otherwise given the extensive use of quinine, the widespread and often unreported pre-treatment, its use in severe malaria is otherwise remarkably free from serious toxicity. In our study, children who died did not have higher or lower plasma quinine concentrations than children who survived (Figure 4) and 12/13 of fatal cases had received a loading dose at admission. The one child who died and did not receive a loading dose at admission had a reported pre-treatment with 2 quinine injections; however the baseline quinine concentration was undetectable suggesting that the history was incorrect. Unreliability of the history is commonplace in severe malaria. Administering a loading dose of 20 mg/kg when the history is uncertain may be safest as undertreating severe malaria risks death. The therapeutic benefit of the loading dose has been widely accepted, but unsubstantiated toxicity concerns have long hindered its implementation in the field.\textsuperscript{5,47} Although there is no large randomized controlled trial evidence that the loading dose is life-saving, the faster fever and parasite clearance times and an understanding of the basic pathobiology of severe malaria suggest that it is beneficial in the treatment of severe malaria (reviewed in \textsuperscript{48}). Importantly, the loading dose does not alter the risk of hypoglycaemia due to quinine induced hyperinsulinemia.\textsuperscript{48,49} One fifth of the children in our study had already received routine dosing parenteral quinine prior to admission, none of whom presented hypoglycemic at admission. Hypoglycaemia was also an indicator of severe disease in this series, associated with an increased case fatality.\textsuperscript{49-52} The high incidence of shock and positive blood cultures suggests that concomitant sepsis might also have contributed to the high mortality in our study population. If artemisinin derivatives are unavailable and quinine is used then a loading dose should be given, unless there is convincing evidence of adequate pre-treatment, since the risk of death of severe malaria is highest and the risk of systemic toxicity is lowest during the first 24 hours. Starting with the routine dose is justified in children that have already received a loading dose within 24 hours prior to admission and those that have received a routine dose within 12 hours of admission, but if in doubt, a loading dose should be given.
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