Artemisinin-Naphthoquine Combination Therapy for Uncomplicated Pediatric Malaria: a Pharmacokinetic Study

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Artemisinin-naphthoquine (ART-NQ) is a coformulated antimalarial therapy marketed as a single-dose treatment in Papua New Guinea and other tropical countries. To build on limited knowledge of the pharmacokinetic properties of the components, especially the tetra-aminoquinoline NQ, we studied ART-NQ disposition in Papua New Guinea children aged 5 to 12 years with uncomplicated malaria, comparing a single dose (15 and 6 mg/kg of body weight) administered with water (group 1; $n = 13$), a single dose (22 and 9 mg/kg) with milk (group 2) ($n = 17$), and two daily doses of 22 and 9 mg/kg with water (group 3; $n = 16$). The plasma NQ concentration was assayed by high-performance liquid chromatography, and the plasma ART concentration was assayed using liquid chromatography-mass spectrometry. Population-based multicompartment pharmacokinetic models for NQ and ART were developed. NQ disposition was best characterized by a three-compartment model with a mean absorption half-life ($t_{1/2}$) of 1.0 h and predicted median maximum plasma concentrations that ranged as high as 57 μg/liter after the second dose in group 3. The mean NQ elimination $t_{1/2}$ was 22.8 days; clearance relative to bioavailability (CL/F) was 1.1 liters/h/kg; and volume at steady state relative to bioavailability (V_{ss}/F) was 710 liters/kg. Administration of NQ with fat (8.5 g; 615 kJ) versus water was associated with 25% increased bioavailability. ART disposition was best characterized by a two-compartment model with a mean CL/F (4.1 liters/h/kg) and V/F (21 liters/kg) similar to those of previous studies. There was a 77% reduction in the bioavailability of the second ART dose (group 3). NQ has pharmacokinetic properties that confirm its potential as an artemisinin partner drug for treatment of uncomplicated pediatric malaria.

Available data relating to the pharmacokinetics of the antimalarial drug naphthoquine phosphate (NQ) are limited and inconsistent. Initial reports suggested that NQ has a high oral bioavailability (>90%) and a half-life ($t_{1/2}$) of 41 to 57 h (50). In a more recent study with healthy Chinese men in which NQ was given alone or coformulated with artemisinin (ART) (41), the elimination $t_{1/2}$ of NQ was substantially longer, at 250 to 300 h. This volunteer study also showed that the area under the concentration-time curve (AUC) for NQ exhibited an unusual relationship between the formulation and coadministered fat. The mean values were similar for the fasted group receiving NQ monotherapy and the fed group receiving combination ART-NQ therapy but more than double this for the fasted volunteers given the fixed combination (41). The fact that the highest bioavailability was in the fasting state appears in contrast to the effect of fat on the absorption of related drugs, such as lumefantrine and piperaquine (3, 11, 24, 46), while the apparently beneficial effects of coformulation on bioavailability were difficult to explain (41).

It has been shown that NQ is a P-glycoprotein substrate and that NQ efflux is saturable (12), suggesting that absorption could be nonlinear at high doses. However, the Chinese volunteer study of ART-NQ found dose-proportional increases in the maximum concentration in plasma (C_{max}) and AUC for NQ at doses between 200 and 600 mg (41). The maximum individual value for C_{max} was just over 100 μg/liter in this study (41), but a C_{max} as high as 245 μg/liter has been reported after a 600-mg dose in adults (50).

The Chinese volunteer study of NQ and ART-NQ reported a $t_{1/2}$ for ART of 3.6 to 4.0 h, a clearance relative to bioavailability (CL/F) of approximately 1.5 liters/h/kg, and a volume of distribution relative to bioavailability (V_z/F) of 8 liters/kg (41). In contrast, a number of previous studies with healthy adult volunteers (4, 16, 18, 26) and patients with uncomplicated *Plasmodium falciparum* malaria (1, 5, 15, 25, 45) have reported lower mean values for $t_{1/2}$ (2.0 to 2.7 h [mean, 2.3 h]), higher mean values for CL/F (5.1 to 9.3 liters/h/kg [mean, 6.7 liters/h/kg]), and a higher mean V/F (16.4 to 35.5 liters/kg [mean, 27 liters/kg]). The reported mean CL/F and V/F for ART in children were even greater, at 14.4 liters/h/kg and 38 liters/kg, respectively (45). The Chinese study did, however, show that the AUC for ART increased with coadministered fat (41), consistent with most past reports (14).

Because of inconsistencies between the few published studies of NQ pharmacokinetics and a lack of pharmacokinetic data for children, we conducted two pharmacokinetic studies of ART-NQ in children from Papua New Guinea with uncomplicated malaria. An initial pilot study (study 1), carried out before the manufacturer had produced a pediatric dosing schedule and utilizing a conservative dose regimen (calculated in milligrams per kilogram of body weight based on the dose for adults), was designed to provide preliminary pharmacokinetic data relating to NQ dispo-

sition in children, while the second study (study 2) aimed to characterize the pharmacokinetics of NQ as well as ART in more detail when these drugs were given at the manufacturer's recommended dose with fat (milk) or as a two-dose regimen.

MATERIALS AND METHODS

Patients and clinical methods. Full details of the studies have been provided in a separate report (7). In brief, children aged 5 to 10 years who presented with an axillary temperature of >37.5°C or a history of fever in the previous 24 h and who were slide positive for malaria (>1,000 asexual *P. falciparum* parasites/μl of whole blood or >250 *Plasmodium vivax* parasites/μl) were eligible provided that they had no complications or concomitant illness, no prior treatment with study drugs, and no history of allergy to ART or aminoquinoline drugs. Each child's parents or guardians gave written informed consent. Approvals were obtained from the Papua New Guinea Institute of Medical Research Institutional Review Board and the Medical Research Advisory Committee of the Papua New Guinea Health Department.

At enrollment, a history was taken and a full physical examination was performed. An intravenous cannula was inserted, and a baseline venous blood sample was drawn. In study 1, all children were administered ARCO tablets (50 mg NQ plus 125 mg ART; Kunming Pharmaceuticals, Kunming, China) orally as a single dose of 2 to 4 whole tablets with water (group 1). The dose was based on body weight according to those recommended by the manufacturer in milligrams per kilogram for adults (33) and represented dose ranges of 5.0 to 7.5 mg/kg for NQ phosphate and 12.5 to 16.8 mg/kg for ART. Subjects were not required to fast prior to, or after, treatment. If the child vomited within 1 h, the same dose was readministered and the time of readministration recorded.

In study 2, children were randomized by a computer-generated sequence to receive ARCO tablets (50 mg NQ plus 125 mg ART) orally based on body weight as recommended by the manufacturer for children (33) as either (i) a single dose of 3 to 6 tablets given with 250 ml full-cream cow's milk (containing 8.5 g fat) with dose ranges of 6.1 to 9.5 mg/kg for NQ and 15.3 to 23.8 mg/kg for ART (group 2) or (ii) the same dose given with water on two occasions 24 h apart (group 3). Each child was kept fasting under observation, and if he/she vomited within 1 h, the same dose was readministered and the time of readministration recorded.

Group 1 patients had additional venous blood samples drawn through the cannula at 1, 2, 4, 8, 12, 18, 24, 48, and 72 h and by venesection at 4, 7, 14, 28, 42, and 56 days. Blood was collected into lithium heparin tubes and was centrifuged at 1,800 $\times$ g for 5 min, and the separated plasma was stored at −80°C until analysis for the NQ concentration within 8 months of collection. These children were reassessed clinically at 4 and 24 h and on days 2, 3, 7, 14, 28, and 56 (7). Group 2 and 3 patients had further 2.5-ml blood samples for drug assay taken at 1, 2, 4, 8, 12, 18, 24, 48, and 72 h through the sampling cannula and by venesection at 4, 7, 14, 28, and 42 days. Group 3 patients received a second ART-NQ dose with water at 24 h. Posttreatment clinical and other monitoring for groups 2 and 3 was similar to that performed for group 1 (7).

Analytical methods. Naphthoquine diphosphate was obtained from ZYF Pharm Chemicals, Shanghai, China; tramadol hydrochloride and artemisinin were from Sigma-Aldrich Chemicals, St. Louis, MO; and artemether was from AApin Chemicals Ltd., Abingdon, Oxon, United Kingdom. All general laboratory chemicals were of analytical grade (Sigma-Aldrich Chemicals, St. Louis, MO; Merck Chemicals, Darmstadt, Germany).

The concentration of NQ in plasma was analyzed using a validated high-performance liquid chromatography (HPLC) assay, based on established analytical methods for chloroquine, piperaquine, and mefloquine (13, 30). Briefly, plasma samples (500 μl) were spiked with tramadol as the internal standard (500 ng), alkalinized with a 2% (wt/vol) sodium tetraborate solution (1 ml), and extracted into 8 ml hexane–ethyl acetate (80:20) by shaking for 10 min. The samples were then centrifuged at 1,300 $\times$ g for 10 min. The supernatant (7.5 ml) was back-extracted into

0.1 ml of 0.1 M HCl by shaking for 5 min, followed by centrifugation as described above. The HCl layer was transferred to 1.5-ml microcentrifuge tubes and was recentrifuged at 1,300 $\times$ g for 25 min to evaporate traces of organic solvent, after which 70 μl was injected onto the HPLC. Analytes were separated on a Luna C_{18} HPLC column (length, 100 mm; inner diameter [i.d.], 4.6 mm; particle size, 3 μm; Phenomenex, Australia) in series with an octadecyl C_{18} guard column (length, 4 mm; i.d., 3 mm; Phenomenex, Australia) at 30°C with a mobile phase of 18% (vol/vol) acetonitrile in 50 mM KH_2PO_4 buffer (pH 2.5) pumped at 1 ml/min. The approximate retention times (t_R) for NQ and tramadol were 9.4 min and 6.8 min, respectively, and the analytes were detected by UV absorbance at 222 nm (Fig. 1). The linear calibration range for each assay was 1 to 100 μg/liter, and quality control (QC) samples (5 μg/liter, 20 μg/liter, and 100 μg/liter) were included in each batch. The intraday relative standard deviations (RSDs) of NQ were 8.9, 3.1, and 4.5% at 5 μg/liter, 20 μg/liter, and 100 μg/liter, respectively ($n = 5$), while interday RSDs were 7.7, 5.2, and 3.4% at 5 μg/liter, 20 μg/liter, and 100 μg/liter, respectively ($n = 15$). Intraday and interday accuracy ranges were 92 to 106% and 91 to 98%, respectively. The limit of quantification and limit of detection were 1 μg/liter and 0.5 μg/liter, respectively. Mean levels of recovery of NQ from plasma were 88%, 98%, and 96% at 5 μg/liter, 20 μg/liter, and 100 μg/liter, respectively.

The concentration of ART in plasma was analyzed using liquid chromatography with mass spectrometry detection (LC-MS) based on an established assay for artemether (36). Stock solutions of ART and artemether (the internal standard) were prepared separately (1 g/liter in methanol) and were stored in the dark at −80°C. Working standard solutions were prepared from the primary stock at 1, 10, and 100 mg/liter. Two sets of 5-point calibration curves were constructed (5 to 200 μg/liter for the lower concentrations and 200 to 2,000 μg/liter for the higher concentrations) by spiking into blank plasma. Samples above the standard curve were reanalyzed following appropriate dilution. QC samples were prepared in blank plasma as described above at concentrations of 5, 200, and 1,000 μg/liter and were stored at −80°C prior to use for each batch analyzed.

The extraction procedure used a 1-ml C_{18} solid-phase extraction (SPE) column (Bond Elut PH; Varian Inc., Palo Alto, CA) as described previously (6), with minor modifications. Briefly, the SPE column was preconditioned with 1 ml of methanol followed by 1 ml of 1 M acetic acid. Plasma samples (0.5 ml) were spiked with the internal standard (artemether; 1 μg), loaded onto the preconditioned SPE column, and drawn through by using a medium vacuum. The column was then washed with 1 M acetic acid (1 ml; two washes), followed by 20% (vol/vol) methanol in 1 M acetic acid (1 ml). The column was dried under a low vacuum for 30 min, and retained drugs were eluted with 2 ml of *t*-butyl chloride–ethyl acetate (80:20%, vol/vol). The eluate was evaporated in a vacuum evaporator at 35°C and was reconstituted in 50 μl of the mobile phase, and 5-μl aliquots were injected into the LC-MS system.

The single-quadrupole LC-MS system (model 2020; Shimadzu, Kyoto, Japan) comprised a binary pump (20AD), a vacuum degasser, an autosampler with a thermostat (SIL-20AC HT), a column compartment with a thermostat (CTO 20A), a photodiode detector (SPD M 20A), and a mass analyzer (MS 2020) with both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) systems. Analysis was performed in the isocratic mode with 20 mM ammonium formate (pH 4.8)–methanol (20:80) at a flow rate of 0.2 ml/min. Chromatographic separation was undertaken at 30°C on a Synergy Fusion-RP C_{18} column (length, 150 mm; i.d., 2 mm; particle size, 4 μm) coupled with a C_{18} guard column (length, 4 mm; i.d., 2 mm; particle size, 5 μm; Phenomenex, Australia). The retention times were 4.3 min and 7.9 min for ART and artemether, respectively (Fig. 2). Optimized mass spectra were acquired with an interface voltage of 4.5 kV, a detector voltage of 1 kV, a heat block temperature of 400°C, and a desolvation gas temperature of 250°C. Nitrogen was used as the nebulizer gas at a flow rate of 1.5 liter/min and a dry gas flow of 10 liter/min.
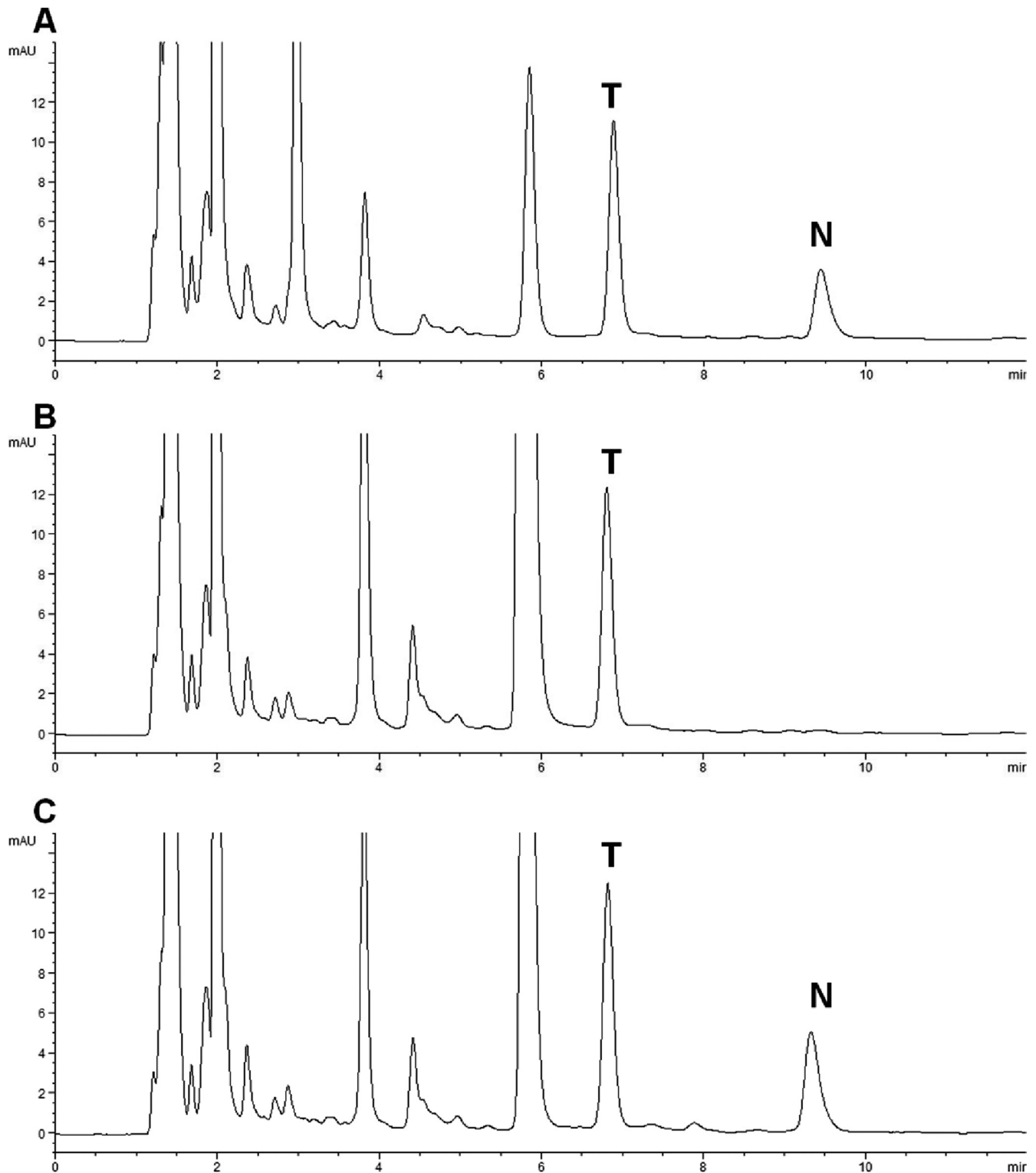
FIG 1 HPLC-UV (222 nm) chromatograms showing naphthoquine (N) (t_R, 9.4 min) and the internal standard, tramadol (T) (t_R, 6.8 min). (A) Spiked plasma used in the calibration curve (20 µg/liter naphthoquine); (B) a patient's blank predose sample (with the internal standard) showing no endogenous interference; (C) a typical sample (25 µg/liter naphthoquine).

Both ART and artemether standard solutions were first scanned from m/z 100 to 500 in ESI and APCI positive mode, as well as in the combined ESI-and-APCI (DUIS) mode, to identify the abundance of ions corresponding to the respective drugs. The base peak intensities of

all three modes were compared and showed that the DUIS mode gave the highest signal intensity. Therefore, quantitation was performed by selected ion monitoring (SIM) using the DUIS mode. For ART, the parent molecule $[M + H]^+$ (m/z 283) was used for quantitation, while
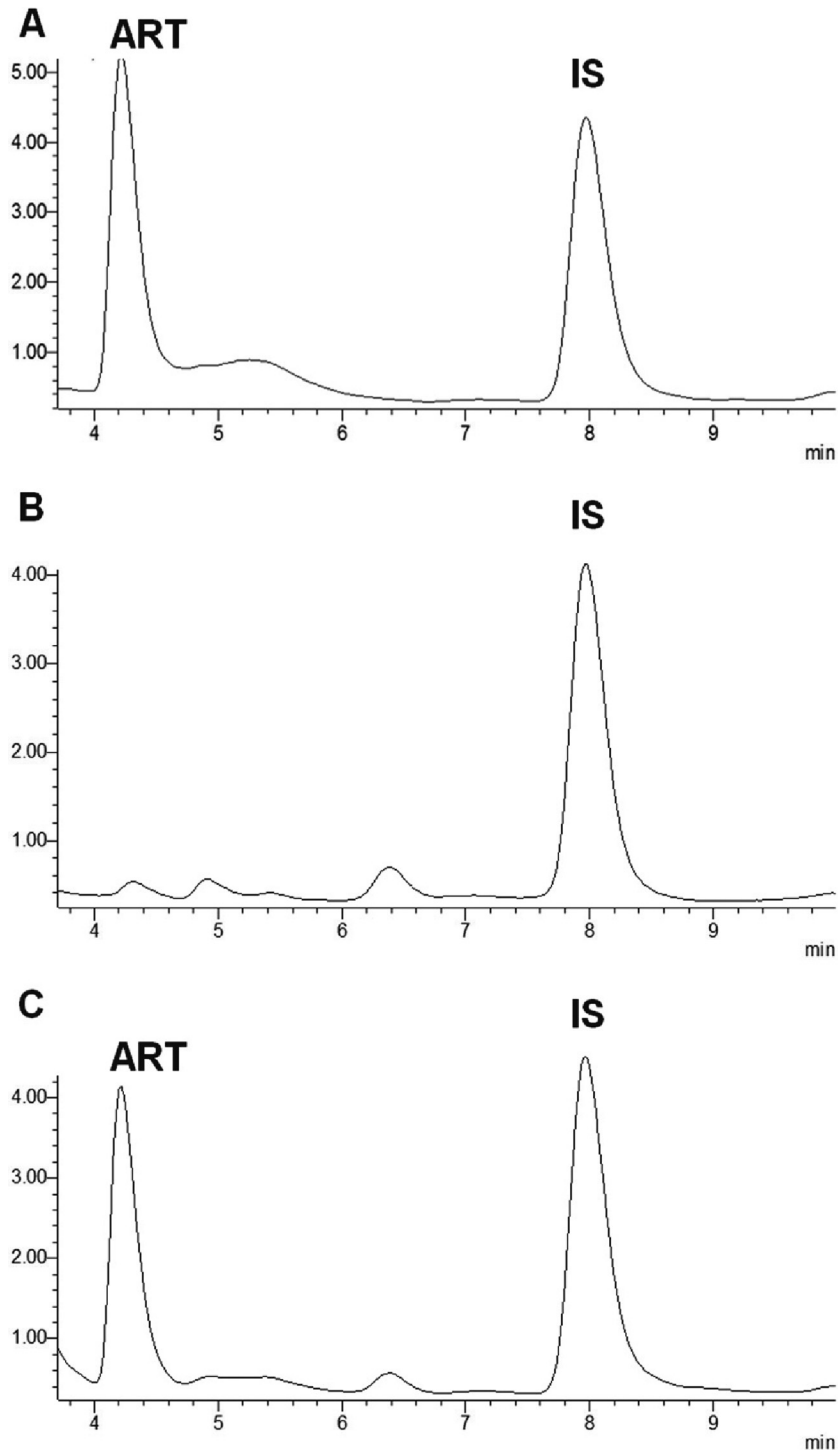
FIG 2 LC-MS chromatograms showing artemisinin (ART) (t_R, 4.3 min) and the internal standard (IS), artemether (t_R, 7.9 min). (A) Spiked plasma used in the calibration curve (200 μg/liter artemisinin); (B) a patient's blank predose sample (with the IS) showing no endogenous interference; (C) a typical sample (136 μg/liter artemisinin).

for artemether, the predominant fragmented ion (*m/z* 221) was monitored (44).

All standard curves were linear (r^2, ≥0.999). Chromatographic data (peak area ratio of ART to artemether) were processed using LAB Solution (version 5; Shimadzu, Japan). Responses from the analysis of three ART concentrations (5, 200, and 2,000 μg/liter) spiked into five separate plasma samples were used to determine matrix effects (ion suppression/ enhancement), absolute recovery, and process efficiency (37, 43). Three sets of matrix solutions were prepared. Set 1 comprised blank plasma spiked first and then extracted; set 2 comprised blank plasma extracted first and then spiked; and set 3 comprised pure solutions of the analyte. The matrix effect, process efficiency, and absolute recovery were ex-

TABLE 1 Demographic data for children given artemisinin-naphthoquine for the treatment of uncomplicated *P. falciparum* malaria

Characteristic[a]	Group 1	Group 2	Group 3
No. of children	13	17	16
Gender	6 male, 7 female	11 male, 6 female	12 male, 4 female
Age (yr)	7.1 ± 1.8	7.7 ± 2.0	6.7 ± 1.6
Wt (kg)	18.0 ± 3.7	18.9 ± 5.2	16.8 ± 3.2
Ht (cm)	110 ± 10	117 ± 12	110 ± 9
Parasitemia (no. of parasites/μl of blood)[b] upon admission	14,757 (5,189–41,966)	6,674 (2,264–19,674)	29,416 (12,290–70,406)
Naphthoquine dose (mg/kg)	6.3 ± 0.9	8.8 ± 1.4	$2 \times (9.5 \pm 0.9)$
Artemisinin dose (mg/kg)	15.7 ± 2.3	22.0 ± 3.6	$2 \times (23.8 \pm 2.2)$

[a] Data are means $\pm$ SD unless otherwise indicated.
[b] Geometric mean (95% confidence interval) for children with parasitemia.

pressed as percentages. The matrix effect was calculated as (set 2 response $\times$ 100)/(set 3 response), the process efficiency as (set 1 response $\times$ 100)/(set 3 response), and the absolute recovery as (set 1 response $\times$ 100)/(set 2 response). The mean matrix effects $\pm$ standard deviations (SD) for ART were 94% $\pm$ 12% (range, 79 to 105%), 90% $\pm$ 13% (range, 75 to 106%), and 91% $\pm$ 2% (range, 88 to 92%) at 5, 200, and 2,000 μg/liter, respectively. The mean process efficiencies $\pm$ SD for ART were 93% $\pm$ 18% (range, 73 to 121%), 84% $\pm$ 11% (range, 75 to 102%), and 82% $\pm$ 4% (range, 80 to 89%) at 5, 200, and 2,000 μg/liter, respectively. The mean absolute recoveries $\pm$ SD for ART were 88% $\pm$ 10% (range, 78 to 101%), 86% $\pm$ 9% (range, 77 to 102%), and 90% $\pm$ 7% (range, 87 to 101%) at 5, 200, and 2,000 μg/liter, respectively. The mean matrix effect, process efficiency, and absolute recovery $\pm$ SD for the internal standard, artemether, were 98% $\pm$ 10% (range, 87 to 113%), 88% $\pm$ 4% (range, 82 to 92%), and 91% $\pm$ 6% (range, 81 to 96%) at 1,000 μg/liter. The intraday RSDs for the assay were 9.3, 7.2, and 3.7% at 5, 200, and 2,000 μg/liter, respectively ($n = 5$), while the interday RSDs were 9.5, 7.1, and 6.5% at 5, 200, and 2,000 μg/liter, respectively ($n = 15$). Interday accuracies determined from the QC samples for each assay batch at 5, 200, and 1,000 μg/liter were 108% $\pm$ 7% (range, 86 to 114%), 103% $\pm$ 6% (range, 93 to 109%), and 107% $\pm$ 8% (range, 86 to 115%), respectively ($n = 16$). The limits of quantification and detection for ART were 2.5 and 1 μg/liter, respectively.

Pharmacokinetic and statistical analyses. The pharmacokinetic properties of NQ were assessed using noncompartmental analysis (Kinetica, version 4.4.1; Thermo LabSystems Inc., Philadelphia, PA) for group 1 subjects, and the data (not shown) were used to refine the study design for groups 2 and 3. All NQ data were subsequently pooled and analyzed by population pharmacokinetic methods, as were ART data, which were available for groups 2 and 3.

In the population pharmacokinetic analysis, log$_e$ concentration-time data sets for NQ and ART were analyzed by nonlinear mixed-effect modeling using NONMEM (version 6.2.0; Icon Development Solutions, Ellicott City, MD) with an Intel Visual Fortran (version 10.0) compiler. NQ data were available for all three groups, while ART data were available only for groups 2 and 3. Linear mammillary model subroutines within NONMEM, first-order conditional estimation (FOCE) with η-ε interaction, and the objective function value (OFV; a NONMEM-calculated global goodness-of-fit indicator equal to -2 times the log of the likelihood) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of $\geq$3.84 (χ^2 distribution with 1 df; $P < 0.05$) was considered statistically significant. Secondary pharmacokinetic parameters, including the volume of distribution at steady-state (V_{ss}; calculated as the sum of volumes of individual compartments), the area under the curve from 0 h to infinity (AUC$_{0-\infty}$), and the elimination $t_{1/2}$ for the participants, were obtained from *post hoc* Bayesian prediction in NONMEM using the final model parameters. Macro constants for the three-compartment model were calculated from the modeled parameters using previously published equations (49). C_{max} and the time to C_{max} (T_{max}) were estimated by predicting the concentrations of NQ and ART for each individual at 6-min intervals to capture the postdose peak.

Allometric scaling to a standard adult body weight (WT) was used *a priori* with all volume terms scaled using $\times(WT/70)^{1.0}$ and all clearance terms scaled using $\times(WT/70)^{0.75}$ (2). Between-subject variability (BSV) was added to parameters for which it could be estimated from the available data. An additive error model was used for residual unexplained variability (RUV), approximating proportional error as log$_e$ concentration data were used. In the development of the final models, the influence of the following covariates on the various model parameters was investigated: dose group, dose occasion, relative dose (in milligrams per kilogram), gender, spleen grade, malaria status (by slide positivity), baseline log$_{10}$ parasitemia, age, fever, and initial hemoglobin concentration. Covariate relationships identified using the generalized additive modeling procedure within Xpose (29) and by inspection of correlation plots of η versus a covariate were evaluated within NONMEM. The potential effect of these covariates, particularly the dose group and occasion, on bioavailability was also considered in cases where similar relationships was identified for all volume and clearance terms, given that these were relative to bioavailability. The effect size (expressed as a percentage) of categorical data was assessed, while both linear and power relationships were evaluated for continuous covariates. Linear relationships were calculated as follows: individual parameter value = population parameter value $\times$ {1 + effect parameter $\times$ [(covariate value for individual) $-$ (median value of covariate)]}. Power relationships were calculated as follows: individual parameter value = population parameter value $\times$ [(covariate value for individual)/ (median value of covariate)$^{effect\ parameter}$]. A stepwise forward inclusion and backward elimination method with a significance level (P value) of $<$0.05, accompanied by a decrease in the BSV of the parameter, was required for the inclusion of a covariate relationship, and a P value of $<$0.01 was required to retain a covariate relationship. For relationships involving bioavailability, a decrease in the BSV of any volume or clearance was required. Correlations among BSV terms were also investigated, and conditional weighted residuals (CWRES) plots were assessed in arriving at a final model. Two- and three-compartment models for NQ and one- and two-compartment models for ART were compared with first-order absorption, with and without a lag time.

A bootstrap procedure in Perl-speaks-NONMEM (PsN), stratified according to dose group and weight, was used to sample individuals from the original data set and to generate 1,000 new data sets that were subsequently analyzed using NONMEM. The resulting parameters were then summarized as the median and 2.5th and 97.5th percentiles (95% empirical confidence interval [CI]) to facilitate evaluation of the final model parameter estimates. In addition, prediction-corrected visual predictive checks (pcVPCs) (9) were performed using PsN with 1,000 replicate data sets simulated from the original data set. The observed 10th, 50th, and 90th percentiles were plotted with their respective simulated 95% confidence intervals to assess the predictive performance of the model (9) Because a number of covariate effects were found in the model-building process for NQ, numerical predictive checks (NPCs), stratified according to those covariates, were performed and were assessed by comparing the actual with the expected number of data points within the 20, 40, 60, 80, 90, and 95% prediction intervals (PI).

Data analysis and representation were performed using SigmaPlot, version 11 (Systat Software Inc., San Jose, CA). Data are means ± SD unless otherwise indicated. Student's *t* test (for parametric data) or the Mann-Whitney U test (for nonparametric data) was used for two-sample comparisons as appropriate with a significance level (*P* value) of <0.05.

RESULTS

Thirteen of 15 group 1 children completed all essential requirements for the pharmacokinetic component of the study. All of these children had *P. falciparum* infections at baseline, and one had a mixed infection with *P. vivax* at low density (160 parasites/μl of blood). Four children in group 2 and four in group 3 were considered to have low-grade parasitemia on screening microscopy at the study site but were subsequently found to be slide negative on confirmatory expert microscopy. All group 2 and 3 children recruited were included in the pharmacokinetic study. Demographic data are summarized in Table 1.

The content of NQ in the ARCO tablets was determined by dissolving each tablet (*n* = 5) in 500 ml water by using sonication (twice, for 5 min each time) and measuring the concentrations in 8 aliquots. The ART content was determined after dissolving each tablet (*n* = 6) in 250 ml methanol and following the same procedure. The mean NQ and ART contents of the ARCO tablets used in the study were 49 ± 5 mg (nominal potency, 50 mg NQ) and 129 ± 3 mg (nominal potency, 125 mg ART), respectively.

Naphthoquine pharmacokinetics and pharmacodynamics. The plasma concentration-time profiles for NQ are shown in Fig. 3. For pooled data from the three groups, a three-compartment model proved superior to a two-compartment model, with a lower OFV (−404.855 versus −388.736; *P*, <0.01) and no bias in the CWRES plot in the initial stages of modeling. Because there was no evidence of model misspecification by use of a three-compartment model with first order-absorption with a lag time, more-complex models were not tested. The structural model parameters (where C refers to the central compartment and P1 and P2 to the two peripheral compartments) were the absorption rate constant (k_a), lag time, CL/F, V_C/F, V_{P1}/F, V_{P2}/F, and Q_1/F and Q_2/F (intercompartment clearances between V_{P1}/F and V_C/F and between V_{P2}/F and V_C/F, respectively). Estimates for the BSV of k_a, V_C, V_{P2}, CL, and Q_2 and the correlation between some BSV pairs (k_a and V_C/F, V_C/F and CL/F, CL/F and V_{P2}/F, and V_{P2}/F and Q_2/F) could be obtained (Table 2). Significant covariate relationships were added in the following order [given as covariate–parameter (relationship type)]: fever–predicted *F* (negative categorical), first dose for group 3–predicted *F* (negative categorical), and hemoglobin–V_C/F (positive linear). Although children in group 2 were estimated to have an approximately 50% lower k_a, this relationship did not satisfy the significance requirements for inclusion in the final model (0.01 < *P* < 0.05). Fever (axillary temperature, >37.3°C) and the first NQ dose in group 3 were associated with 32% and 26% decreases in bioavailability, respectively. Every 1-g/dl increase in the hemoglobin level increased V_C/F by 16%. Slide positivity at baseline and $\log_{10}$ parasitemia were not significant covariates in the model. The residual error for the model was 24% (Table 2).

Goodness-of-fit and CWRES plots for NQ are shown in Fig. 4. The results of the parameter estimates and the bootstrap results are summarized in Table 2, and *post hoc* Bayesian parameter estimates with derived secondary pharmacokinetic parameters are given in Table 3. The bootstrap demonstrated reasonable esti-
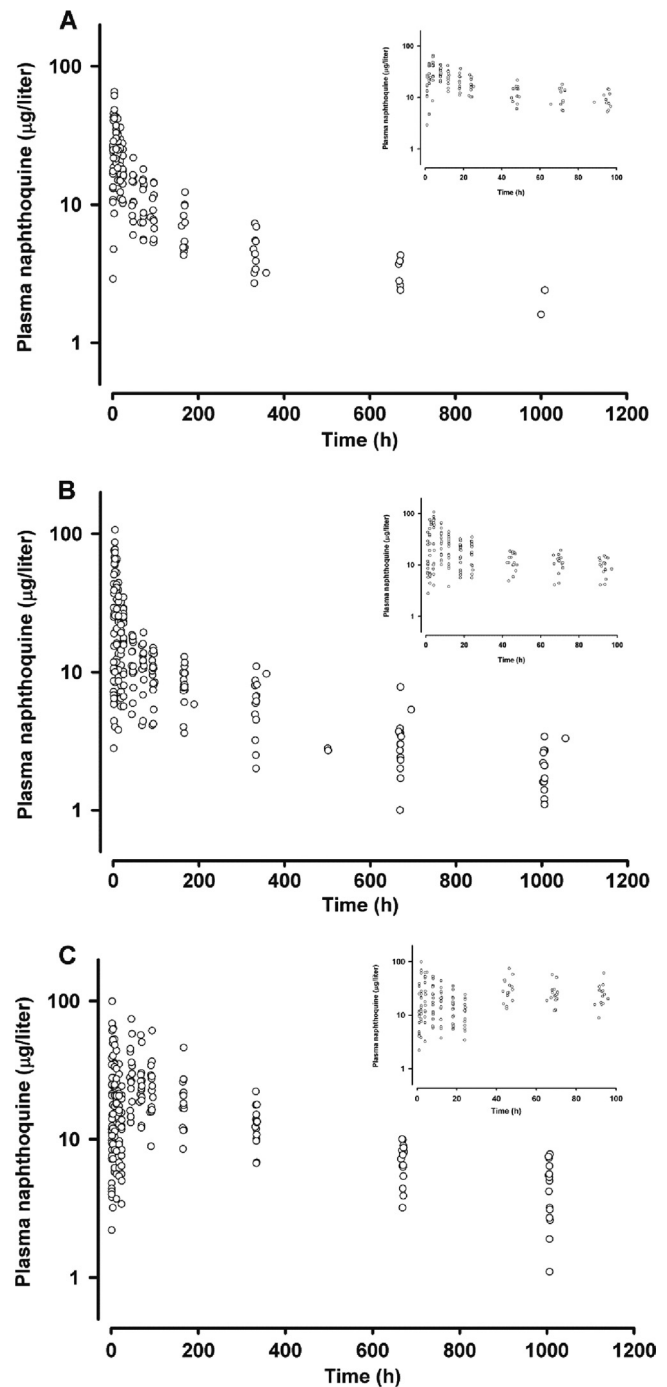
FIG 3 Concentration-time plots for NQ in plasma for group 1 (A), group 2 (milk) (B), and group 3 (water and double dose) (C) patients. (Insets) Plasma concentration-time data from 0 to 100 h after the dose.

mates of structural and covariate effect parameters with a bias of <10% for all parameters except V_{P1}, for which the bias was 19%. Random parameters had a bias of <7%. The AUC was significantly higher in group 3 (two doses) than in groups 1 and 2 (*P* < 0.001) and was higher in group 2 than in group 1. The predicted C_{max} was <200 μg/liter for all children, apart from one group 3 child with a value of 270 μg/liter after the second dose. When the AUC was normalized for the total relative dose (in milligrams per

TABLE 2 Population pharmacokinetic parameters and bootstrap results for NQ in children with uncomplicated *P. falciparum* malaria

Parameter	Mean (RSE[a] [%]) in the final model	Bootstrap median (95% CI)
Objective function value	−687.786	−712.006 (−817.316 to −615.107)
Structural model parameters		
k_a (h^{-1})	1.1 (22)	1.0 (0.7 to 1.6)
Lag time (h)	0.7 (7)	0.7 (0.6 to 0.8)
V_C/F (liters/70 kg)	12,500 (15)	12,200 (9,503 to 14,958)
V_{P1}/F (liters/70 kg)	15,500 (19)	17,000 (11,843 to 83,415)
V_{P2}/F (liters/70 kg)	17,200 (8)	16,000 (10,343 to 21,600)
CL/F (liters/h/70 kg)	51.9 (6)	51.5 (30.1 to 58.7)
Q_1/F (liters/h/70 kg)	40.6 (9)	48.2 (24.2 to 113.0)
Q_2/F (liters/h/70 kg)	398 (13)	407 (318 to 536)
Covariate effect parameters (%)		
Decrease in predicted F with fever	31.8 (21)	31.8 (18.3 to 47.1)
Decrease in predicted F with 1st dose in group 3	26.3 (33)	27.7 (9.3 to 40.9)
Increase in V_C/F per g/dl hemoglobin	16.4 (66)	14.9 (3.2 to 19.1)
Random model parameters		
BSV (%)		
k_a	104 (14)	103 (80 to 131)
V_C/F	77 (9)	77 (63 to 90)
CL/F	32 (13)	31 (23 to 57)
V_{P2}/F	37 (17)	40 (25 to 59)
Q_2/F	52 (41)	50 (6 to 84)
Correlation coefficient		
k_a, V_C/F	0.20	0.25 (−0.08 to 0.58)
V_C/F, CL/F	0.47	0.46 (0.04 to 0.72)
CL/F, V_{P2}/F	0.50	0.51 (0.09 to 0.86)
V_{P2}/F, Q_2/F	0.20	0.18 (−0.62 to 0.91)
RUV (%)	24 (7)	24 (21 to 26)

[a] RSE, relative standard error.

kilogram), there was no longer any significant difference between the groups. The pcVPC for NQ, shown in Fig. 5, demonstrate the reasonable predictive performance of the model. NPCs stratified according to dose group (three strata), hemoglobin (three strata), and fever (two strata) showed good predictive performance, with the expected number of data points above and below most prediction intervals (data not shown).

Since the dose-corrected pharmacokinetic parameters were consistent across the three groups (Table 3), data were pooled to provide estimates for the total of 46 patients. Overall, the mean ± SD CL/F, V_{ss}/F, $t_{1/2\alpha}$, $t_{1/2\beta}$, and $t_{1/2\gamma}$ for NQ were 1.30 ± 0.45 liters/h/kg, 805 ± 256 liters/kg, 8.2 ± 3.8 h, 98 ± 16 h, and 518 ± 94 h, respectively.

Of the 13 (of 15) group 1 patients included in the pharmacokinetic analysis, 7 developed recurrent parasitemia during the 42-day follow-up period (7). One had a PCR-confirmed recrudescence of *P. falciparum*; four had reinfections with *P. falciparum*; and two had an emergence of *P. vivax*. In group 2, there was only one emergent *P. vivax* recurrence and no *P. falciparum* recurrence, while there were no episodes of slide positivity during follow-up in group 3. The $AUC_{0-\infty}$ and day 7 concentrations of NQ were significantly lower for the children with any parasitemia during follow-up than for those who remained free of malaria infection (*P*, 0.001 and 0.005, respectively). However, the NQ dose in milligrams per kilogram was also significantly lower (*P*, 0.001), and the difference in $AUC_{0-\infty}$ was no longer significant when corrected for dose (*P*, 0.97), indicating that the lower dose, rather than individ-

ual pharmacokinetic differences, was responsible. Day 7 NQ concentrations correlated significantly with $AUC_{0-\infty}$ overall (*r*, 0.91; *P*, <0.001) and in each of the three groups (*r*, >0.79; *P*, <0.001).

Artemisinin pharmacokinetics. Raw plasma ART concentration-time data are presented in Fig. 6. A two-compartment model was superior to a one-compartment model for ART, with a lower OFV (255.146 versus 122.637; *P*, <0.01) and an improved CWRES. Since there was no evidence of model misspecification by use of a two-compartment model with first-order absorption and a lag time, more-complex models were not tested. The structural model parameters for ART were k_a, lag time, CL/F, V_C/F, V_p/F, and Q/F (intercompartmental clearance for V_p/F). Estimates of the BSV of CL, V_C/F, k_a, and lag time could be made, and a full covariance matrix was obtained. The correlation between CL/F and V/F was >0.99 and was fixed at 1. Since the CWRES plot revealed that plasma ART concentrations after the second dose were lower than expected, the effect of dose occasion on observed F was tested as a negative categorical relationship. The addition of this relationship reduced the OFV by 46.626 (*P*, <0.001) and reduced the residual error of the model by 7%. The second ART dose had 77% lower bioavailability than the first. No other covariate relationships were identified. The residual error in the final model was 51% (Table 4).

Goodness-of-fit and CWRES plots for ART are shown in Fig. 7. The results of the final parameter estimates and the bootstrap results are summarized in Table 4, and *post hoc* Bayesian parameter estimates with derived secondary pharmacokinetic parame-
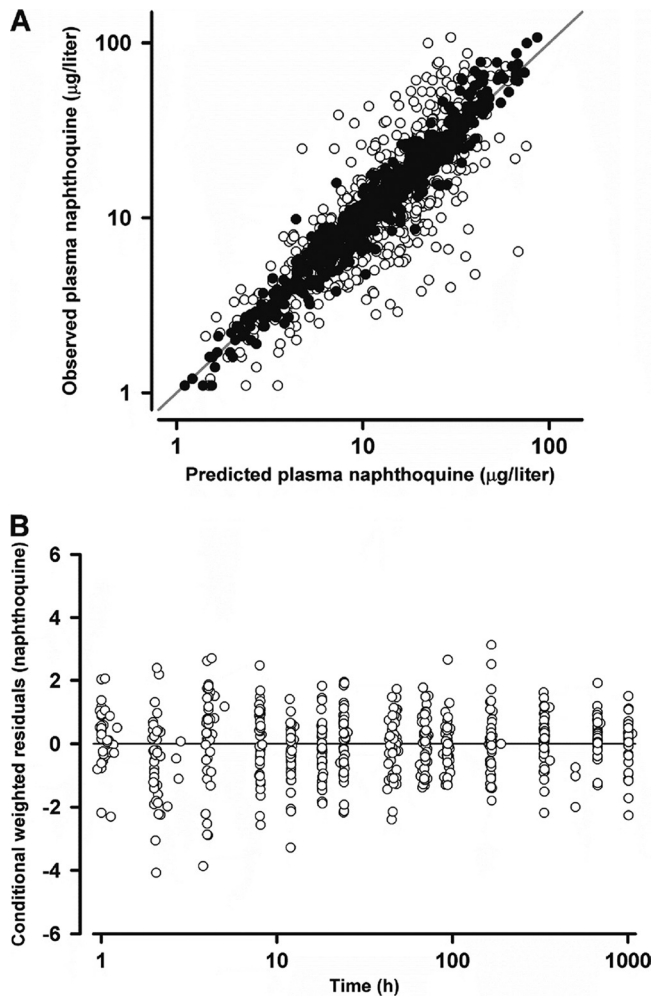
FIG 4 (A) Population predicted (○) and individual predicted (●) versus observed plasma NQ concentrations (μg/liter; log scale) for the final model. The line of identity is shown. (B) Conditional weight residuals versus time (log scale) for the final NQ model.

ters are given in Table 5. The bootstrap demonstrated reasonable 12/$12.00 Antimicrobial Agents and Chemotherapy p. 2472–2484estimates of structural and random parameters with biases of <8% and <10%, respectively, except for V_p/F, where there was a positive bias of 27%. No significant differences in secondary parameters were found between the groups, although there was substantial variability within each group. The pcVPC for ART is shown in Fig. 8 and demonstrates the reasonable predictive performance of the model.

The dose-corrected, first-dose data indicated that the median AUC for ART was 5% higher in group 3 than group 2. However, these and other pharmacokinetic parameters for the two groups were not significantly different (Table 5); hence, the data were pooled for total patient group estimates. Overall, mean (±SD) CL/F, V_{ss}/F, and $t_{1/2\alpha}$ for ART were 4.1 ± 2.0 liters/h/kg, 21 ± 10 liters/kg, and 2.7 ± 0.3 h, respectively. Although the best pharmacokinetic model was a two-compartment model with a $t_{1/2\beta}$ of 6.7 ± 0.5 h, this may be a spurious finding, due to the limited concentration-time data in the present study design and the 27% bias in the bootstrap for V_p/F.

DISCUSSION

The present study has provided the first pediatric pharmacokinetic data for NQ and additional ART disposition data to complement the few available for this age group. NQ given in the form of ART-NQ fixed combination therapy was promptly absorbed (mean absorption $t_{1/2}$, 1.0 h) and reached a predicted C_{max} of <200 μg/liter in all but one child even after the second dose in group 3. The mean elimination $t_{1/2}$ of NQ (524 h) was longer than estimates in early reports (41 to 57 h) (50) and in the recent Chinese adult volunteer study (156 to 299 h) (41). There was some evidence of a modest increase in NQ bioavailability when it was administered with a small amount of fat, in contrast to the substantial food-associated reduction in NQ bioavailability in Chinese adults (41). The CL/F (1.1 liters/h/kg) and V/F (71 liters/kg) for NQ in our study were lower than the results reported for healthy Chinese adults (7.0 liters/h/kg and 2,277 liters/kg) (41), but no other data are presently available for direct comparison. In the case of ART, the mean CL/F and V/F (4.1 liters/h/kg and 21 liters/kg, respectively) were comparable to those in most previous studies (means, 6.7 liters/h/kg and 27 liters/kg, respectively) (1, 4, 5, 15, 16, 18, 25, 45).

The long elimination $t_{1/2}$ and high V/F of NQ in our children were consistent with those for most other quinolines and related drugs in clinical use (22, 30, 40). Pharmacokinetic modeling indicated that a three-compartment model best described the disposition of NQ in the present study. This finding is consistent with similar pharmacokinetic studies involving chloroquine (21, 23, 32, 52) and piperaquine (48). A number of studies of quinoline and related antimalarial drugs have shown biphasic drug concentration-time profiles that can be analyzed using a two-compartment model (10, 19, 27, 30, 39, 40, 46, 47, 53). Improved pharmacokinetic study design, including more-frequent sampling of longer duration, as well as lower limits of quantification for the analytical techniques, may explain why recent studies such as ours reveal more-complex elimination kinetics. Indeed, the relatively short NQ elimination $t_{1/2}$ in the Chinese volunteer study (41) could be explained by a short sampling period (216 h) as well as by the use of noncompartmental methods. In relation to the latter point, we found an elimination $t_{1/2}$ of 298 h by noncompartmental methods in group 1 patients versus 547 h in compartmental population analyses of pooled NQ data.

The effect of fat on NQ bioavailability in the present study needs interpretation in light of the study design. In the preliminary pharmacokinetic study in group 1 children, there was no requirement for fasting before or after drug administration. It is likely that these children consumed some fat around the time ART-NQ was given, even though the dose was administered with water. Group 3 children, who were required to fast throughout and were given the dose with water, had a 26% lower relative bioavailability than children in group 1 and also group 2, in which ART-NQ was administered with milk. This evidence of a modest positive effect of fat on bioavailability contrasts with the observation that the AUC and $t_{1/2}$ of NQ were approximately 50% lower after food (60% lipid; 2,400 kJ) in healthy Chinese adults (41), suggesting an increased CL and/or reduced oral bioavailability. Studies with quinolines and related drugs have shown increased absorption with high-fat meals (3, 11, 46), but a standard Vietnamese meal (17 g fat; 2,000 kJ) had little effect on the pharmacokinetic properties of piperaquine (24). It is possible that relatively

TABLE 3 *Post hoc* Bayesian parameter estimates and derived secondary pharmacokinetic parameters for NQ in children with uncomplicated *P. falciparum* malaria

Parameter[a]	Group 1 ($n = 13$)	Group 2 ($n = 17$)	Group 3 ($n = 16$)
k_a (h^{-1})[b]	1.3 (0.9–1.6)	0.7 (0.4–1.0)	1.7 (0.6–2.2)
CL/F (liters/h)	17.3 (15.3–21.8)	19.5 (16.2–25.1)	16.6 (14.9–19.2)
V_C/F (liters)	2,115 (1,735–2,753)	3,494 (1,817–7,818)	3,610 (1,383–7,030)
V_{P1}/F (liters)	3,986 (3,432–4,318)	3,986 (3,321–4,871)	3,543 (3,183–3,903)
V_{P2}/F (liters)	4,392 (3,602–5,208)	4,662 (3,816–5,347)	4,370 (3,633–4,760)
V_{ss}/F (liters)	10,464 (9,366–13,888)	13,161 (10,485–14,053)	12,001 (9,390–14,882)
$t_{1/2\alpha}$ (h)[c]	6.8 (4.4–9.2)	8.2 (5.7–9.7)	7.3 (5.5–12.0)
$t_{1/2\beta}$ (h)[c]	109 (92–121)	115 (103–126)	118 (104–130)
$t_{1/2\gamma}$ (h)[c]	525 (490–544)	500 (455–629)	595 (525–624)
AUC$_{0-\infty}$ (μg · h/liter)[d]	5,935 (4,776–6,551)	7,104 (5,954–7,914)	15,385 (13,200–18,486)
AUC$_1$/dose (μg · h/liter per mg/kg)	917 (822–1,158)	728 (611–1,004)	813 (629–999)
Relative bioavailability[e]	1.00 (1.00–1.00)	1.00 (0.68–1.00)	0.75 (0.75–0.87)
Observed day 7 level (μg/liter)[d]	7.0 (4.9–8.3)	8.1 (7.3–9.8)	17.9 (12.0–22.9)
Dose 1			
Predicted C_{max1} (μg/liter)	40.6 (32.6–45.5)	33.9 (14.7–52.7)	22.9 (14.1–49.1)
Predicted T_{max1} (h)	3.1 (2.7–3.7)	4.6 (3.7–7.1)	3.3 (2.4–4.8)
Dose 2			
Predicted C_{max2} (μg/liter)			57.0 (42.2–138)
Predicted T_{max2} (h) (h)			27.3 (26.7–28.3)

[a] Data are medians (interquartile ranges).
[b] P, 0.053 and 0.094 for the comparison between groups 2 and 1 and groups 2 and 3, respectively.
[c] $t_{1/2\alpha}$, $t_{1/2\beta}$, and $t_{1/2\gamma}$ are the first distribution, second distribution, and terminal elimination half-lives, respectively.
[d] P, <0.01 for comparisons between groups 2 and 1 and groups 3 and 1.
[e] P, <0.01 for comparison between groups 3 and 1.

high fat meals such as that used by Qu et al. (41) might interfere with the absorption of NQ from the gastrointestinal tract, but our experience is that amounts of fat given in milk greater than that used in the present study (>8.5 g; >615 kJ) have a high likelihood of inducing significant nausea in an unwell child with malaria. This observation and our NQ pharmacokinetic data do not suggest that food-associated underdosing will be problematic in chil-

FIG 5 Prediction-corrected VPC plots for NQ in children with uncomplicated *P. falciparum* malaria, showing the observed 50th percentile (●) and the 10th and 90th percentiles (○) with the simulated 95% CIs for the 50th percentile (solid black line) and the 10th and 90th percentiles (dashed gray lines). (Inset) Plasma concentration-time data from 0 to 100 h after the dose.

dren. The improved NQ bioavailability after the second dose relative to that after the first dose in group 3 may relate to clinical improvement reflecting parasite clearance, as has been seen with lumefantrine (20).

Fever was independently associated with reduced NQ bioavailability, consistent with pharmacokinetic studies in other contexts (8, 35). There was an independent association between hemoglobin and V_C that might suggest NQ accumulation in red blood cells, but assessment of partitioning was beyond the scope of the present study. As is the case for a range of other drugs, including anti-infectives (51), coadministration of milk reduced the rate of NQ absorption.

Qu et al. (41) reported CL/F values (mean, 2.7 liters/h/kg) for adults given ART-NQ that were lower than those obtained in previous studies of ART pharmacokinetics (1, 4, 5, 15, 16, 18, 25, 45) and with ART monotherapy, while Sidhu et al. (45) found a significantly higher value for this parameter (14.4 liter/h/kg) when ART was given to children with uncomplicated *P. falciparum* malaria. While the former observation is difficult to explain, an apparently high CL/F may relate to underestimation of the ART AUC. Almost all previous studies have used noncompartmental analysis or one-compartment models to determine the pharmacokinetic parameters for ART. A two-compartment model was, however, the best fit for the ART concentration-time data in the present study, probably reflecting the fact that our limits of quantification (2.5 μg/liter) and detection (1 μg/liter) were considerably lower than those in previous studies (4 to 20 μg/liter) (4, 15, 16, 25, 45). A prolonged elimination phase may have been undetected in past studies, thus truncating the AUC. Our assay sensitivity led, in part, to an unexpected limitation of the present study, namely, a lack of sampling >24 h postdose. Based on the established pharmacokinetic properties of ART, we anticipated that
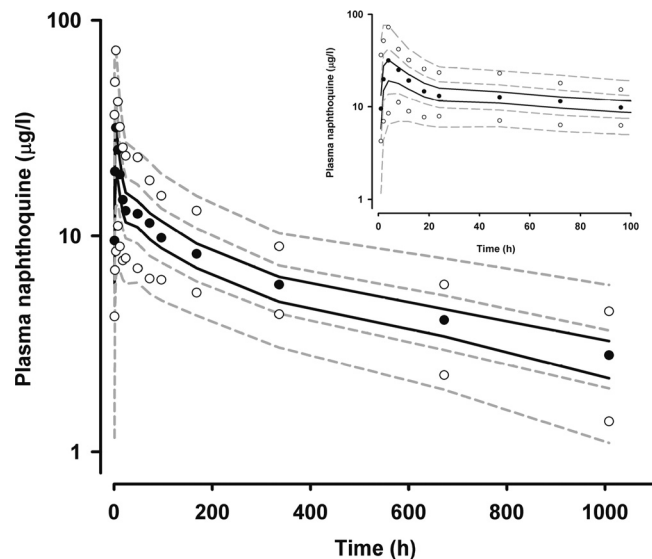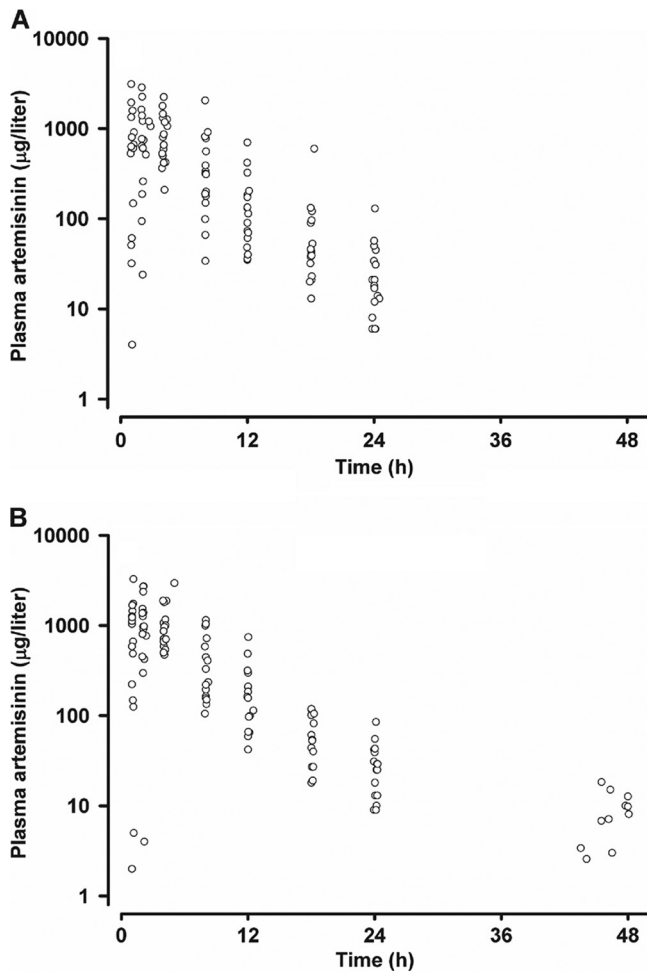
FIG 6 Plasma ART concentration-time plots for group 2 (milk) (A) and group 3 (water and double dose) (B) patients.

plasma ART concentrations would not be detectable beyond 24 h. If prolonged sampling had been performed, it would have allowed a more definitive multicompartment pharmacokinetic characterization.

In a Vietnamese study, coingestion of food was reported to be associated with a nonsignificant 20% reduction in the ART AUC after oral ART administration to healthy adults (16). In contrast, Qu et al. (41) reported that the AUC and $t_{1/2}$ of ART were approximately 75% higher after coadministration of food with ART-NQ combination therapy, suggesting increased bioavailability and a possible reduction in CL. Our data are consistent with the earlier study of Dien et al. (16), in that we also found a nonsignificant 5% lower AUC for ART after ingestion of food (milk) compared with administration with water. There were no significant differences when the dose group was added as a covariate in the population pharmacokinetic model, further evidence that fat has no clinically meaningful effect on the pharmacokinetics of ART.

Although the importance of developing pediatric formulations of antimalarial drugs has been emphasized (34), it is not clear how the manufacturer's pediatric ART-NQ dose recommendations have been developed. Using either a weight-based equation (28) (dose for a child [mg] = dose for an adult [mg] × [weight of a child/weight of an adult]$^{0.75}$) or a body surface area (BSA) equa-

tion (28, 42) (dose for a child [mg] = dose for an adult [mg] × [BSA of a child/BSA of an adult]), where the regular adult dose of NQ is 400 mg, adult weight is assumed to be 50 kg, and adult BSA is 1.73 m^2, the adult dose of 8 mg/kg would scale up to ≥10 mg/kg in children. Our initial, conservative mean dose of 6.3 mg/kg NQ for group 1 as part of ART-NQ was associated with a relatively high late-treatment failure rate (7). The regimens used for groups 2 and 3 (means, 9.0 and 9.5 mg/kg NQ per dose) were based on the manufacturer's recommendations of 6.5 to 9.5 mg/kg for children weighing as much as 40 kg (33, 38), doses that still fall short of the allometrically scaled dose of ≥10 mg/kg.

Efficacy against asexual parasite forms over 42 days of follow-up for groups 2 and 3 was 100%, but prolonged gametocyte carriage was observed in some patients (7). The latter observation, together with concerns regarding the emergence of artemisinin resistance in areas of endemicity with a history of subtherapeutic drug use (17), the implication that higher individual pediatric doses than those recommended by the manufacturer can be used, and the safety of the two-dose ART-NQ regimen employed for group 3 (7), supports an argument for a 3-day ART-NQ regimen in line with WHO recommendations for all artemisinin combination therapies (54). We have used the final NQ model to simulate C_{max} after three ART-NQ doses given with milk to 1,000 children with characteristics similar to those of the present subjects. The median C_{max} values (95% prediction intervals) after three consecutive daily doses were 36 (19 to 76), 69 (44 to 128), and 89 (61 to 152) μg/liter, respectively, with an absolute range up to 350 μg/liter after the third simulated dose. A predicted C_{max} of >300

TABLE 4 Population pharmacokinetic parameters and bootstrap results for ART in children with uncomplicated *P. falciparum* malaria

Parameter	Mean (RSE^a [%]) in the final model	Bootstrap median (95% CI)
Objective function value	85.171	73.924 (−60.386–178.368)
Structural model parameters		
k_a (h^{-1})	1.8 (110)	1.8 (0.6–6.5)
Lag time (h)	0.7 (31)	0.7 (0.4–0.9)
V_C/F (liters/70 kg)	1,160 (31)	1,140 (625–1,520)
V_p/F (liters/70 kg)	166 (37)	211 (96.1–1,270)
CL/F (liters/h/70 kg)	178 (12)	176 (141–216)
Q/F (liters/h/70 kg)	14.2 (103)	15.3 (6.6–52.3)
Covariate effect parameter: % decrease in predicted F with 2nd dose	77.0 (9)	78.6 (63.3–89.9)
Random model parameters		
BSV		
CL/F (%)	57 (25)	56 (43–67)
Lag time (%)	23 (169)	21 (5–57)
k_a (%)	139 (81)	141 (72–230)
V_C/F to CL/F (ratio)	0.995 (28)	0.989 (0.760–1.671)
Correlation coefficient		
CL/F, lag time	0.571	0.565 (0.201–0.997)
CL/F, k_a	0.0225	0.011 (−0.550–0.628)
Lag time, k_a	−0.340	−0.343 (−0.956–0.319)
CL/F, V_C/F	1	Fixed
RUV (%)	51 (31)	50 (37–65)

^{a} RSE, relative standard error.

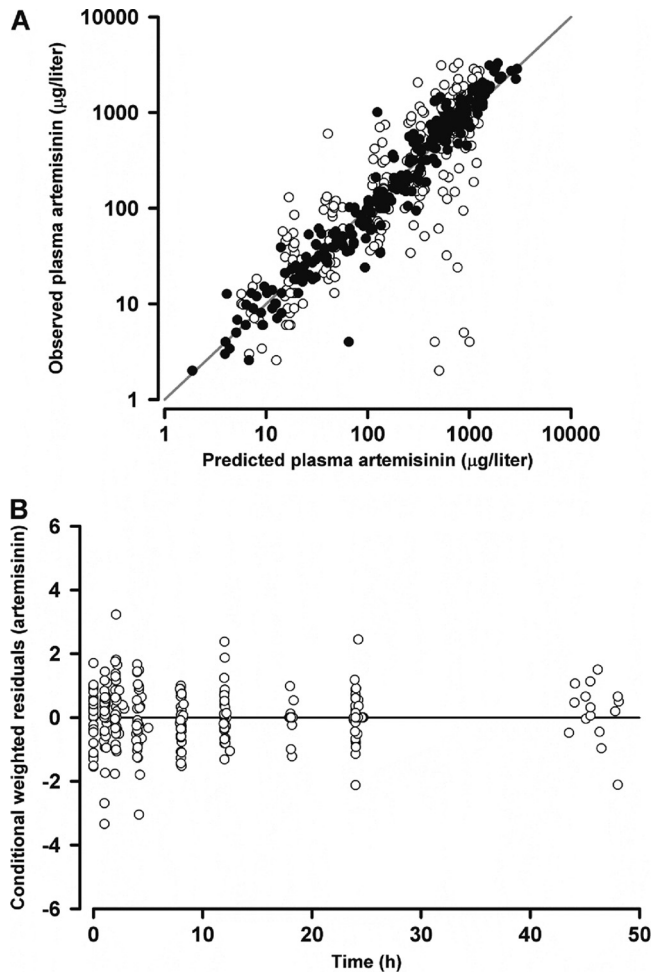**FIG 7** (A) Population predicted (○) and individual predicted (●) versus observed plasma ART concentrations (μg/liter; log scale) for the final model. The line of identity is shown. (B) Conditional weight residuals versus time (log scale) for the final ART model.

TABLE 5 *Post hoc* Bayesian parameter estimates and derived secondary pharmacokinetic parameters for artemisinin in children with uncomplicated *P. falciparum* malaria[a]

Parameter	Group 2 (*n* = 17)	Group 3 (*n* = 16)
k_a (h^{-1})	2.0 (0.7–3.5)	1.1 (0.8–4.1)
CL/F (liters/h)	82.1 (76.2–74.8)	66.9 (61.5–62.4)
V_C/F (liters)	348 (246–449)	279 (165–324)
Q/F (liters/h)	5.13 (4.47–5.96)	4.69 (4.33–5.05)
V_P/F (liters)	42.7 (35.6–52.2)	37.9 (34.1–41.8)
V_{ss}/F (liters)	388 (289–482)	315 (202–362)
$t_{1/2\alpha}$ (h)	2.8 (2.7–3.0)	2.7 (2.5–2.8)
$t_{1/2\beta}$ (h)	6.8 (6.2–7.0)	6.6 (6.5–6.9)
AUC$_1$ (μg · h/liter) (dose 1)	5,127 (3,631–8,237)	6,770 (5,249-10,235)
AUC$_1$/dose (μg · h/liter per mg/kg)	267 (170–340)	281 (202–417)
AUC$_2$ (μg · h/liter) (dose 2)		1,557 (1,207–2,354)
AUC$_{0-\infty}$ (μg · h/liter)		8,327 (6,457–12,590)
Dose 1		
Predicted C_{max1} (μg/liter)	843 (522–1,353)	1,105 (736–1,398)
Predicted T_{max1} (h)	2.1 (1.6–2.9)	2.5 (1.5–3.0)
Dose 2		
Predicted C_{max2} (μg/liter)		269 (179–345)
Predicted T_{max2} (h)		26.6 (26.0–27.4)

[a] Data are medians (IQR). All between-group comparisons were statistically nonsignificant.

second dose in group 3, but relatively robust estimates for model parameters could still be derived. It was unfortunate that no pure *P. vivax* malaria cases were recruited, but the fact that there was only one late *P. vivax* infection in groups 2 and 3 suggests that the long NQ $t_{1/2}$ helps prevent the emergence of this infection, which is seen after other therapies for *P. falciparum* malaria in this area, including artemether-lumefantrine (31).

In conclusion, when normalized by body weight, the pharmacokinetic parameters for ART in children are comparable to those
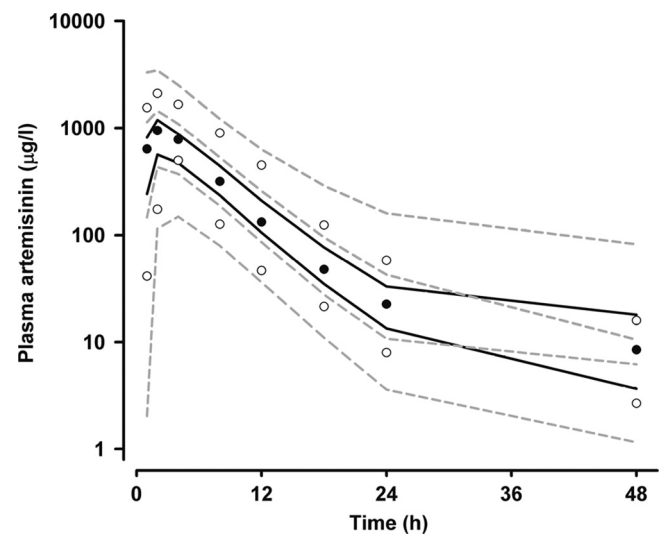
μg/liter occurred in a small minority of subjects in the simulation. The group 3 child with a predicted C_{max} of 270 μg/liter had an uncomplicated clinical course in the present study, and a C_{max} of 245 μg/liter in an adult was not reported to be associated with toxicity (50), but careful tolerability and safety monitoring would need to be carried out if a three dose regimen were implemented.

A further argument for multiple-dose ART-NQ relates to the disposition of the ART component. The conventional dosage regimen for orally administered ART of 10 to 20 mg/kg on the first day, followed by 500 mg daily for 4 days (14), has been questioned due to the autoinduction of ART metabolism that, as in the present study, progressively and substantially reduces the bioavailability of subsequent doses but does not increase CL (25). The 15- to 24-mg/kg dose of ART used in the present study could, therefore, be an appropriate part of a 3-day ART-NQ regimen based on the single dose now recommended by the manufacturer.

The present study had limitations, in part because of the present paucity of pharmacokinetic and other data relating to ART-NQ (especially when group 1 was recruited) but also because of the context of a pediatric study in the rural tropics. The sampling schedule could have included more time points after the

FIG 8 Prediction-corrected VPC plots for ART in children with uncomplicated *P. falciparum* malaria, showing the observed 50th percentile (●) and the 10th and 90th percentiles (○) with the simulated 95% CIs for the 50th percentile (solid black line) and the 10th and 90th percentiles (dashed gray lines).

obtained in most previous studies with adults, but CL/*F* was higher than that in data recently reported when ART-NQ was coadministered to healthy adults (41). In contrast, CL/*F* and *V*/*F* for NQ were lower in the present study, and the terminal elimination $t_{1/2}$ was longer, at a mean of 21.8 days. Although the predicted bioavailability of the first dose of NQ was lower in a fasted state, this is unlikely to translate into clinically meaningful effects. The present pharmacokinetic characterization, as well as associated tolerability, safety, and preliminary efficacy data (7), may justify using the currently recommended single dose of ART-NQ for 3 days for children with uncomplicated malaria.

ACKNOWLEDGMENTS

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