Pharmacokinetics of a Novel Sublingual Spray Formulation of the Antimalarial Drug Artemether in Healthy Adults

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The pharmacokinetics of sublingual artemether (ArTiMist) was investigated in two open-label studies. In study 1, 16 healthy males were randomized to each of four single-dose treatments administered in random order: (i) 15.0 mg of sublingual artemether (5×3.0 actuations), (ii) 30.0 mg of sublingual artemether (10×3.0 mg), (iii) 30.0 mg of sublingual artemether (5×6.0 mg), and (iv) 30.0 mg of artemether in tablet form. In study 2, 16 healthy males were randomized to eight 30.0-mg doses of sublingual artemether given over 5 days as either 10 3.0-mg or 5 6.0-mg actuations. Frequent blood samples were drawn postdose. Plasma artemether and dihydroartemisinin levels were measured using liquid chromatography-mass spectrometry. Population compartmental pharmacokinetic models were developed. In study 1, sublingual artemether absorption was biphasic, with both rate constants being greater than that of the artemether tablets (1.46 and 1.66 versus 0.43/h, respectively). Relative to the tablets, sublingual artemether had greater bioavailability (≥ 1.24), with the greatest relative bioavailability occurring in the 30.0-mg dose groups (≥ 1.58). In study 2, there was evidence that the first absorption phase accounted for between 32% and 69% of the total dose and avoided first-pass (FP) metabolism, with an increase in FP metabolism occurring in later versus earlier doses but with no difference in bioavailability between the dose actuations. Sublingual artemether is more rapidly and completely absorbed than are equivalent doses of artemether tablets in healthy adults. Its disposition appears to be complex, with two absorption phases, the first representing pregastrointestinal absorption, as well as dose-dependent bioavailability and autoinduction of metabolism with multiple dosing.

Artemether is a semisynthetic artemisinin derivative with potent *in vitro* activities against chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum* isolates (1). It is utilized clinically as an initial intramuscular monotherapy for severe malaria (2) and as an oral therapy in combination with the longer half-life partner drug lumefantrine for uncomplicated *P. falciparum* (3, 4) and *Plasmodium vivax* (5, 6) infections. Suppository formulations have been developed that can be used when parenteral treatment is not feasible (7), and intravenous administration is theoretically possible, despite its poor water solubility (8), but these routes of delivery are not currently in clinical use (9).

In studies involving healthy adult volunteers, orally administered artemether has been shown to be rapidly absorbed, with a mean time to maximum plasma concentration (T_{max}) of 1.6 to 3.0 h (10–13). It is extensively metabolized, including a significant first-pass (FP) contribution by the intestine and liver, to dihydroartemisinin (DHA), which may have greater antimalarial activity than the parent compound (14). The mean terminal elimination half-life ($t_{1/2}$) of artemether is 2.6 to 3.1 h (10–13). The rapid formation and metabolism of DHA mean that its maximum plasma concentration (C_{max}) and terminal elimination $t_{1/2}$ are similar to those of artemether.

The absolute oral bioavailability of artemether cannot be estimated, given the lack of an approved intravenous formulation. Estimates of the relative bioavailabilities of oral versus intramuscular artemether have varied substantially (15, 16), in part because there is marked interindividual pharmacokinetic variability with both routes of administration. It is likely, given its poor solubility, that the absolute bioavailability of artemether is much less than that of the chemically related but water-soluble derivative artesunate, which may be as high as 80% (17).

Sublingual administration has advantages over oral dosing for drugs that can be given in relatively small doses and which are absorbed through the oral mucosa (18). Pregastrointestinal absorption into the internal jugular vein can both reduce the time to therapeutic plasma concentrations and increase bioavailability by bypassing gut and hepatic FP metabolism. In addition, the nausea and vomiting associated with a febrile illness, such as malaria, which can be exacerbated by oral dosing with fluids or food, are less of a barrier to treatment with sublingual administration. ArTiMist (Essential Nutrition Ltd., Brough, England) is a sublingual formulation of artemether in neutral oil that is administered as a metered sublingual spray and which is being developed as a candidate for prereferral treatment, especially in young children (see the accompanying paper [19]). In the present study, the first of two pharmacokinetic evaluations of this product, the disposition of ArTiMist relative to that of single-dose oral artemether given as tablets, as well as its multidose pharmacokinetics, were examined in healthy adult volunteers.

TABLE 1 Designs and artemether dosing schedules used in studies 1 and 2[a]

Group	Study 1: groups of randomly assigned subjects	Study 2: parallel groups
i	15 mg of ArTiMist (5 × 3.0-mg actuations)	8 × 30 mg (10 × 3.0-mg actuations)
ii	30 mg of ArTiMist (10 × 3.0-mg actuations)	8 × 30 mg (5 × 6-mg actuations)
iii	30 mg of ArTiMist (5 × 6.0-mg actuations)	
iv	30-mg artemether tablets	

[a] The times of drug administration and blood sampling are given in the text. The doses in study 1 were given in random order, and the doses in study 2 were each given over 5 days.

MATERIALS AND METHODS

Study site, approvals, and subjects. Study 1 was conducted in Penang, Malaysia, under approval from the Joint Penang Independent ethics committee (no. 07-0232). Study 2 was carried out in Pretoria, South Africa, after approval by both the Medicines Control Council of South Africa and the Pharma-Ethics Independent Research Ethics Committee (no. 07082267). Both studies enrolled healthy adult males (Malaysians for study 1 and Africans for study 2) between 18 and 45 years of age who had a body mass index of between 18 and 29 kg/m^2, who had not used any systemic or topical prescription or nonprescription medication within the previous 14 days or had been participants in a recent investigational drug study, who were nonsmokers with an alcohol consumption of <14 units/week, and who had no clinically significant dental or oral pathology. All participants underwent a detailed medical assessment to confirm eligibility, and witnessed informed consent was obtained prior to enrollment.

Study procedures. The study design is summarized in Table 1. Study 1 was an open-label, randomized, and crossover study comparing two different doses of an artemether spray (ArTiMist; Essential Nutrition Ltd., Brough, England) given at two different concentrations with a single oral dose of artemether. Sixteen participants were randomized to receive each of four treatments in random order: (i) a single sublingual administration of 15.0 mg of ArTiMist through 5 actuations of 3.0 mg/actuation (treatment I), (ii) a single sublingual administration of 30.0 mg of ArTiMist through 10 actuations of 3.0 mg/actuation (treatment II), (iii) a single sublingual administration of 30.0 mg of ArTiMist through 5 actuations of 6.0 mg/actuation (treatment III), and (iv) a single oral administration of 30.0 mg of artemether in tablet form (Essential Nutrition Ltd.) (10 mg/tablet, administered as 3 tablets with 240 ml of water) (treatment IV).

The subjects were studied after a >10-h fast on each of the four occasions, which were separated by at least a week. An intravenous cannula was inserted for blood sampling (5 ml/sample) at 0.0 (immediately prior to dosing), 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, and 12.0 h. Water intake was restricted from 1 h prior to dosing to 2 h after dosing, with the exception of the specified volume administered with the arte-

mether tablets. A cup of soybean milk was given 3 h after dosing, and the subjects were allowed to eat from 4 h postdose onwards.

Study 2 was an open-label, parallel group, and multidose study. Sixteen participants were randomized by a computer-generated schedule to one of two equal groups. The group A participants received eight doses of 30.0 mg of ArTiMist through 10 actuations of 3.0 mg/actuation over 5 days (at 0, 24, 36, 48, 60, 72, 84, and 96 h), and those in group B received 30.0 mg of ArTiMist through 5 actuations of 6.0 mg/actuation on the same schedule as that of group A. An intravenous cannula was inserted for blood sampling (5 ml) on (i) day 1 (dose 1) at 0.0 (predose), 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, and 12.0 h, (ii) days 2, 3, and 4 (doses 2 to 7) at 0.0 (premorning dose), 0.5, 1.0, 2.0, and 4.0 h after the morning dose and 0.0 (preevening dose) and 1 h after the evening dose, and (iii) day 5 (dose 8) at 0.0 (predose), 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, and 24.0 h. The subjects fasted overnight before doses 1 and 8, with water restricted from dosing to 2 h afterwards and food restricted until 4 h after each dose. On days 2, 3, and 4, the subjects were given a single dose following breakfast and another dose 12 h later after dinner.

In both studies 1 and 2, the weight of the delivery device was measured before and after each dose so that the actual dose delivered could be calculated.

Drug assays. The plasma artemether and DHA concentrations were measured using a high-performance liquid chromatography-tandem mass spectrometric (LC-MS/MS) method based on that of Shi et al. (20), with modifications. All chemicals used were of high-performance liquid chromatography (HPLC) or analytical grade. Briefly, 200 μl of human plasma was mixed with 50 μl of internal standard (artemisinin) solution and extracted with 2 ml of methyl *tert*-butyl ether. After vortexing and centrifugation, the upper organic layer was collected and evaporated to dryness at 40°C under a gentle stream of nitrogen. The sample was then reconstituted in 200 μl of 0.1% formic acid-methanol (50/50 [vol/vol]), and 5 μl of the resulting solution was injected onto a Zorbax Eclipse XDB C$_{18}$ column (Agilent, Waldbronn, Germany) maintained at 30°C. The mobile phase was 0.1% formic acid-acetonitrile (30/70 [vol/vol]), with a

TABLE 2 Baseline characteristics of participants in studies 1 and 2

Characteristic[a]	Study 1	Study 2			P value[b]
		Total	Group A	Group B	
n	16	16	8	8	
Racial group (no. [%])					
Malay	10 (63)	0 (0)	0 (0)	0 (0)	
Chinese	5 (31)	0 (0)	0 (0)	0 (0)	
Indian	1 (6)	0 (0)	0 (0)	0 (0)	
African	0 (0)	16 (100)	8 (100)	8 (100)	
Age (median [IQR]) (yr)	22 (18–31)	22 (19–37)	22 (19–37)	22 (20–28)	0.87
Wt (median [IQR]) (kg)	63.6 (45.6–79)	64.8 (52.1–74.5)	67.1 (52.1–74.5)	62.2 (54–73.1)	0.28
Ht (median [IQR]) (cm)	170 (157–181)	171 (161–180)	173 (162–180)	170 (161–180)	0.56
BMI (median [IQR]) (kg/m^2)	21.3 (17.9–26.1)	22.3 (18.7–25.6)	22.9 (18.7–25.6)	22 (18.9–22.5)	0.09

[a] IQR, interquartile range; BMI, body mass index.
[b] P values relate to comparisons between subjects in groups A and B in study 2.
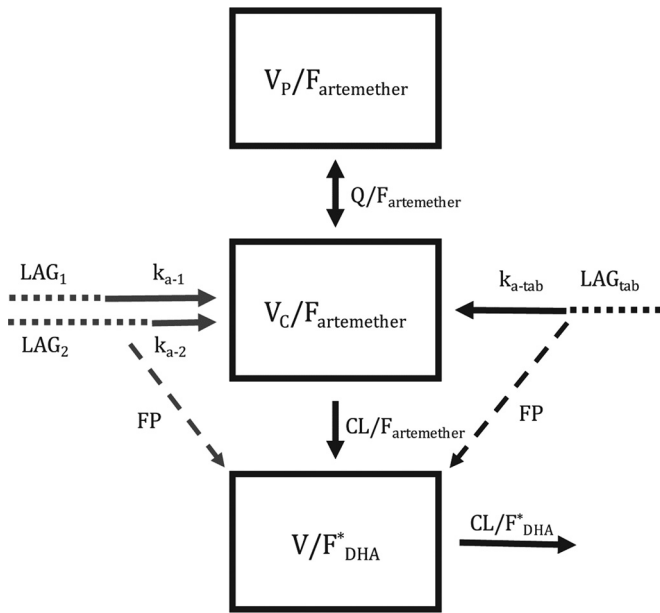
FIG 1 Schematic representation of the final model demonstrating input from ArTiMist in gray on the left and artemether tablet formulation in black on the right, where k_{a1} and k_{a2} are the absorption rates for the first and second absorption phases of ArTiMist, LAG_1 and LAG_2 are the lag times for the first and second absorption phases of ArTiMist, CL/F_{ARM} is artemether clearance relative to bioavailability, V_c/F_{ARM} is the artemether central volume of distribution relative to bioavailability, Q/F_{ARM} is artemether intercompartmental clearance relative to bioavailability, V_p/F_{ARM} is artemether first peripheral volume of distribution relative to bioavailability, CL/F^*_{DHA} is dihydroartemisinin (DHA) clearance relative to bioavailability, V_c/F^*_{DHA} is the central volume of distribution for DHA relative to bioavailability, FP is first-pass metabolism, F_{ARM} is the relative bioavailability of artemether, and F^*_{DHA} is $F_{ARM} \times$ metabolic conversion of artemether to DHA.

gradient flow rate. Positive ion mode was used to identify artemether, DHA, and artemisinin, with *m/z* transitions of 221.2 to 163.2, 221.2 to 163.2, and 283.2 to 219.2 and retention times of 5.1, 2.9, and 3.3 min, respectively, using the API 3000 LC-MS/MS system (Applied Biosystems, Foster City, CA, USA).

The calibration curves were linear for both artemether and DHA from

2 to 200 ng/ml ($r > 0.995$, $P < 0.0001$), with between-day and within-day precision rates of <11% for both analytes at the limit of quantification (LOQ), as well as at low, medium, and high plasma concentrations. The LOQ was 2 ng/ml (signal-to-noise ratio, >5), and the limit of detection was 1 ng/ml (signal-to-noise ratio, >3) for both artemether and DHA.

Pharmacokinetic modeling. Log_e plasma concentration-time data sets for artemether and DHA were analyzed by nonlinear mixed-effects modeling using NONMEM (version 7.2.0; Icon Development Solutions, Ellicott City, MD, USA) with an Intel Visual Fortran 10.0 compiler. The Laplacian with interaction estimation method was used, as required for the M3 method of handling below the limit of quantification (BLQ) data (21). The minimum value of the objective function (OFV) and visual predictive checks were used during the model-building process to choose suitable models. A P value of <0.05 was set as the level of significance for a comparison of the nested models. Allometric scaling for body weight (BW) was employed *a priori*, with volume terms multiplied by $(BW/70)^{1.0}$ and clearance terms by $(BW/70)^{0.75}$ (22). Residual variability (RV) was estimated as the additive error for the log-transformed data. Secondary pharmacokinetic parameters, including the area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) and elimination half-life ($t_{1/2}$) for the participants, were obtained from *post hoc* Bayesian predictions in NONMEM using the final model parameters. The base models were parameterized using k_a (absorption rate constant), V_c (central volume of distribution), CL (clearance), and V_p and Q (peripheral volumes of distribution and their respective intercompartmental clearances, respectively). The data from the two studies were modeled separately.

The initial modeling was carried out on the artemether data set alone, and one-, two-, and three-compartment models (ADVAN2, -4, and -12, respectively) were assessed. As a double peak was present in many of the individual plasma concentration-time curves after ArTiMist administration, a number of absorption models were tested, including single- and double-phase absorption with bolus, zero-, and first-order rates with and without an initial lag time. Once a suitable structural model for artemether was established, the DHA plasma concentration-time data were added, and custom general linear disposition models were constructed using ADVAN5. Models with FP metabolism during both phases of absorption, as well with the second phase only, were tested. The modeling of artemether and DHA was performed simultaneously.

To allow identifiability in the parent drug-metabolite model, the complete conversion of artemether to DHA was assumed (14). Therefore, all artemether parameters were relative to bioavailability (F), while all DHA parameters were relative to F × metabolic conversion (F*). One and two
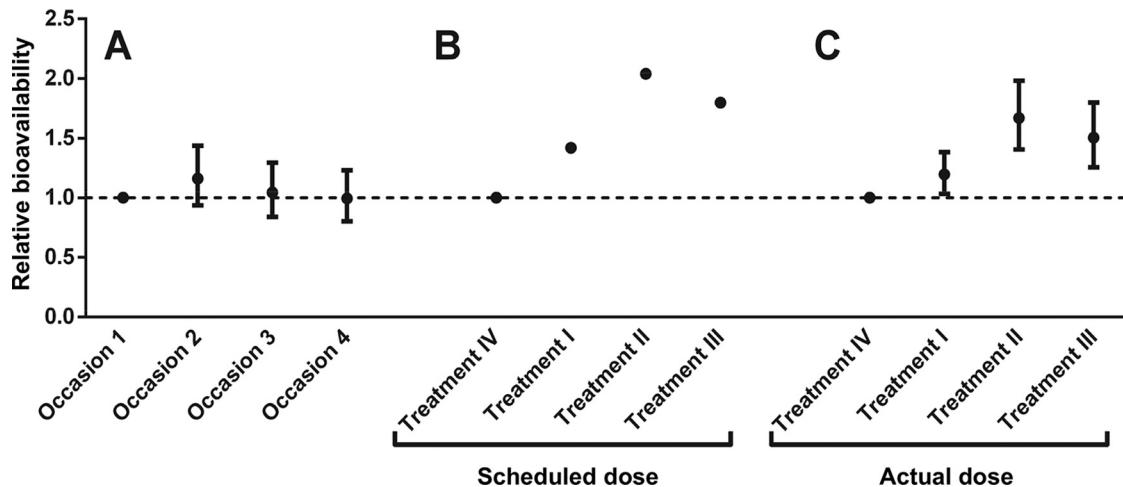
FIG 2 Estimates and 95% confidence interval (CI) for relative bioavailability in study 1 of different study occasions (A), different treatments using scheduled doses (B), and different treatments using actual doses (C).

TABLE 3 Final population pharmacokinetic variable estimates and bootstrap results of artemether and DHA for study 1

Parameter[a]	Mean	RSE%[b]	Bootstrap median (95% CI)
Objective function value	−5.85721		−37.090 (−326.511–189.691)
Structural model parameters			
k_{a1} (/h)	1.46	10	1.50 (1.26–1.76)
k_{a2} (/h)	1.66	51	1.62 (1.08–4.38)
$k_{a\text{-tab}}$ (/h)	0.43	12	0.431 (0.347–0.533)
LAG_1 (h)	0.21	3	0.211 (0.196–0.226)
LAG_2 (h)	1.37	7	1.38 (1.26–1.48)
LAG_{tab} (h)	0.13	19	0.134 (0.077–0.167)
RATIO	0.99	46	1.02 (0.681–1.74)
CL/F_{ARM} (liters/h/70 kg)	862	19	890 (609–1,140)
V_c/F_{ARM} (liters/70 kg)	571	14	590 (471–759)
Q/F_{ARM} (liters/h/70 kg)	124	67	110 (46–350)
V_p/F_{ARM} (liters/70 kg)	1,695	80	1,210 (387–10,096)
$CL/F^{\star}_{DHA}$ (liters/h/70 kg)	768	12	786 (633–981)
$V/F^{\star}_{DHA}$ (liters/70 kg)	302	12	306 (251–376)
FP_{max} (%)	41.7	32	38.2 (20.0–59.9)
FP_{min} (%)	6.9	76	9.0 (0.1–28.0)
Relative bioavailability of treatment			
A	1.36	10	1.36 (1.17–1.65)
B	1.97	11	2.02 (1.58–2.39)
C	1.74	12	1.73 (1.44–2.14)
Variability model parameters (shrinkage [%])			
IIV in:			
CL/F_{ARM}	61 (1)	23	58 (28–77)
$CL/F^{\star}_{DHA}$	17 (16)	27	16 (7–23)
k_{a1}	38 (12)	40	35 (15–59)
k_{a2}	105 (3)	42	100 (47–191)
$k_{a\text{-tab}}$	48 (8)	20	46 (26–63)
IOV in F	25 (30, 5, 7, 7)	12	25 (19–29)
IOV in RATIO	167 (2, 15, 16)	15	166 (136–211)
RV for artemether	44 (10)	8	44 (39–51)
RV for DHA	41 (10)	7	40 (37–46)

[a] k_{a1}, absorption rate constant for first absorption phase of ArTiMist; k_{a2}, absorption rate constant for second absorption phase of ArTiMist; $k_{a\text{-tab}}$, absorption rate constant for tablet form of artemether; LAG_1, lag time for first absorption phase of ArTiMist; LAG_2, lag time for second absorption phase of ArTiMist; LAG_{tab}, lag time for tablet form of artemether; RATIO, ratio between the first and second absorption phases of ArTiMist; CL/F_{ARM}, ARM clearance; V_c/F_{ARM}, ARM central volume of distribution; Q/F_{ARM}, ARM intercompartmental clearance; V_p/F_{ARM}, ARM first peripheral volume of distribution; $CL/F^{\star}_{DHA}$, DHA clearance; $V_c/F^{\star}_{DHA}$, DHA central volume of distribution; FP_{max}, maximum percent first-pass metabolism in the study population using a logit distribution; FP_{min}, minimum percent first-pass metabolism in the study population using a logit distribution; F_{ARM}, relative bioavailability of ARM; $F^{\star}_{DHA}$, F_{ARM} × the metabolic conversion of ARM to DHA; IOV, interoccasion variability; IIV, interindividual variability; RV, residual variability. IOV and IIV are presented as 100% × the square root of the variability estimate.
[b] RSE, relative standard error.

additional compartments were tested for DHA, as well as models estimating the degree of FP metabolism of artemether to DHA. Once the structures of the models were established, interindividual variability (IIV), interoccasion variability (IOV), and correlations between the IIV terms were evaluated for each suitable parameter and included where supported by the data. IIV was exponentially modeled for all parameters, with the exception of FP metabolism, for which a logit distribution (with variability set to 1) was utilized to ensure the values were between 0 and 1.

For study 1, the relative bioavailability of artemether in the three ArTiMist arms was estimated in comparison with that of the tablet artemether arm, in which bioavailability was set at unity. This same process was performed for the calculated actual dose received, as assessed by weighing the delivery device before and after dosing. In addition, the relative bioavailabilities of occasions 2, 3, and 4 were estimated, with bioavailability in the first study occasion set at 1.0. A log-likelihood procedure was then used to determine the 95% confidence intervals of these estimates.

For study 2, the time-dependent pharmacokinetics of artemether were assessed using two different approaches. The first approach assumed that the clearance of artemether increased with subsequent doses, as used previously for children with malaria treated with artemether-lumefantrine (23, 24). The second approach considered the time-dependent kinetics to be attributable to an increase in the FP metabolism of artemether. As previously described for Chinese patients with malaria (25), this may reflect the induction of CYP 3A4 activity in either the gut wall or liver. For each approach, the data for individual subjects for each dose as well as for grouped doses (i.e., doses 1 to 4 and doses 5 to 8) were used. The two approaches were compared before selecting the most appropriate model to describe the time-dependent pharmacokinetics.

For the model evaluation, plots of observed versus individual- and population-predicted values and time versus weighted residuals (WRES) were first assessed. A bootstrap using Perl speaks NONMEM (PSN) with 200 samples was performed, and the parameters derived from this analysis were summarized as the median and 2.5th to 97.5th percentiles (95% empirical confidence interval [CI]) to facilitate an evaluation of the final model parameter estimates. In addition, visual predictive checks (VPCs) and numerical predictive checks (NPCs) were performed with 1,000 data sets simulated from the final models. The observed 10th, 50th, and 90th
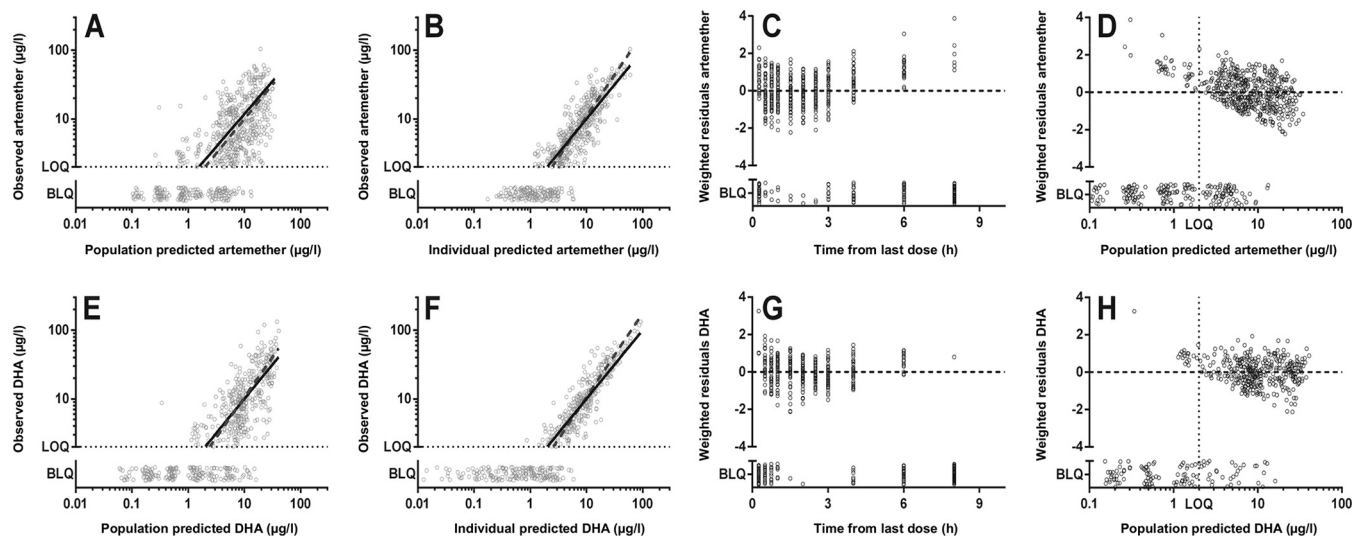
FIG 3 Goodness-of-fit plots for artemether (A to D) and dihydroartemisinin (DHA) (E to H) for study 1. The observed plasma concentration has been plotted against population-predicted (A and E) and individual-predicted (B and F) plasma concentrations, weighted residuals against time (C and G), and population-predicted plasma concentrations (D and H). Data below the limit of quantitation (BLQ) have been separated to assist with visual interpretation.

percentiles and the fraction of BLQ data observed were plotted with their respective simulated 95% CIs to assess the predictive performance of the model and to evaluate any major bias. VPCs were plotted against the time from the last dose and against the time from the first dose. The shrinkage of the population variability parameters and residual variability were assessed to help determine whether the models were overparameterized and to determine the reliability of the diagnostic plots (26).

Statistical analysis. Statistical analysis was performed using R version 2.14.2 (R Foundation for Statistical Computing, Vienna, Austria). Two-sample comparisons for nonnormally distributed variables were done using the Mann-Whitney U test. Unless otherwise stated, all P values are two-tailed and unadjusted for multiple comparisons.

RESULTS

Patient characteristics and study course. The baseline characteristics of the participants by study number and randomized therapy are summarized in Table 2. In study 2, the two randomized groups were well matched.

The ArTiMist spray formulation was well tolerated, and there were no adverse events during either study. In study 1, one participant was absent on occasion 3 and therefore did not receive the single sublingual administration of 30.0 mg of ArTiMist via 3.0-mg actuations. All other participants received all doses as scheduled.

Pharmacokinetic modeling. (i) Study 1. In study 1, there were 681 individual plasma artemether and DHA concentrations available for analysis, and 181 (27%) and 217 (32%), respectively, were below the limit of quantification (BLQ). As only a single plasma DHA concentration out of 64 observations was above the LOQ at 12 h postdose, the observations at this time point were not included in the analysis.

A two-compartment model for artemether was most appropriate, with no benefit being achieved with additional compartments ($P > 0.05$). For ArTiMist, biphasic absorption was superior to a single phase (ΔOFV, 30.474; $P < 0.001$). This was represented by two separate lag times (LAG$_1$ and LAG$_2$), followed by first-order absorption with two separate absorption rates (k_{a1} and k_{a2}) (see Fig. 1). The ratio of the fraction of the dose absorbed in the first phase versus the second phase (RATIO) was estimated within

NONMEM. For the tablet formulation, absorption was modeled with a single first-order absorption ($k_{a\text{-tab}}$) with lag time (LAG$_{\text{tab}}$) (see Fig. 1), which was slower than both k_{a1} and k_{a2} (0.43/h versus 1.46/h and 1.66/h, respectively). The second phase of ArTiMist absorption occurred approximately 1 h after the drug administration. A model estimating a bolus input was also tested but was <1% of the total dose and did not improve the overall fit. A single additional compartment was adequate to describe the disposition of DHA. The inclusion of FP metabolism in the second absorption peak for ArTiMist and in the absorption of the tablet form of artemether significantly improved the fit of the model (ΔOFV, 55.613; $P < 0.001$) and was accompanied by an improvement in ArTiMist VPC plots.

The IIV was estimable for the clearance of artemether (ARM) (CL/F$_{\text{ARM}}$), DHA clearance (CL/F*$_{\text{DHA}}$), k_{a1}, k_{a2}, and $k_{a\text{-tab}}$, with values of 61%, 17%, 38%, 105%, and 48%, respectively. There was no significant correlation between the IIV terms. IIV and IOV could not be estimated simultaneously for RATIO, and the estimate for IOV was much higher (167%), indicating that most of the variability was between occasions in the same individual rather than between different individuals. The IOV of the relative bioavailability was relatively small, at 25%.

The relative bioavailabilities for the ArTiMist treatments were significantly higher than that of the tablet formulation (estimate [95% CI from bootstrap], 1.36 [1.17 to 1.65], 1.97 [1.58 to 2.39], and 1.73 [1.44 to 2.14] for treatments I, II, and III, respectively). When the actual dose values were used, the bioavailabilities were lower but remained statistically significantly higher (estimate [95% CI from log-likelihood profiling], 1.24 [1.01 to 1.53], 1.75 [1.42 to 2.16], and 1.58 [1.28 to 1.95] for treatments I to III, respectively). There was no significant effect of study occasion on relative bioavailability, with the 95% CI for estimates encompassing unity (i.e., having no effect) (estimate [95% CI from log-likelihood profiling], 1.18 [0.97 to 1.44], 1.01 [0.83 to 1.23], and 1.00 [0.82 to 1.21] for occasions 2, 3, and 4, respectively, compared to occasion 1 (see Fig. 2).
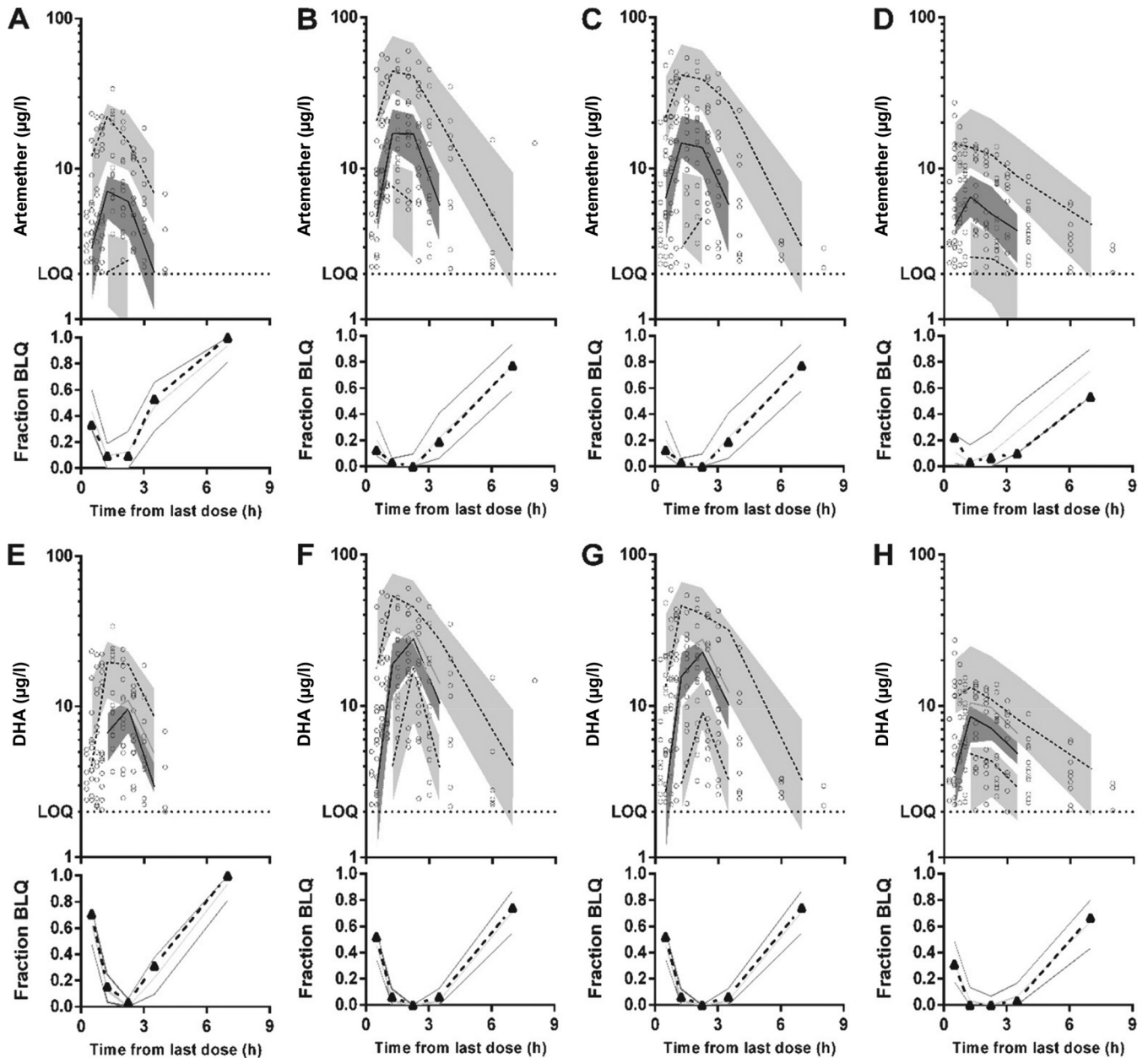
FIG 4 Prediction-corrected visual predictive check for artemether (A to D) and dihydroartemisinin (DHA) (E to H) (in micrograms per liter on a log$_{10}$ scale) separated by treatment (I to IV, from left to right) for study 1, with observed 50th (solid line) and 10th and 90th (dotted lines) percentiles within their simulated 95% CI (gray shaded areas), with overlying data points (○). The fractions of BLQ observations from the data (▲, dashed line) with the simulated 95% prediction interval are also shown.

The final model parameter estimates and the bootstrap results for study 1 are summarized in Table 3. The bias was <10% for all fixed- and random-model parameters, with the exception of V_p/F$_{ARM}$ and the minimum percent first-pass metabolism in the study population using a logit distribution (FP$_{min}$), for which it was <30%. Figures 3 and 4 show goodness-of-fit plots and VPCs, respectively. The apparent bias in the weighted residuals is due to the increasing number of BLQ observations with time and decreasing population-predicted values. The actual 10th, 50th, and 90th percentiles fell within their respective 95% CIs for both artemether and DHA for all doses. The half-lives and AUC$_{0-\infty}$s derived post hoc from individual parameters are shown in Table 4. The distribution and terminal elimination $t_{1/2}$ for artemether were 0.39 and 10.9 h, respectively, and the elimination $t_{1/2}$ for DHA was 0.27 h. The AUC$_{0-\infty}$/dose (a surrogate for relative bioavailability) was significantly different between the ArTiMist and tablet formulations ($P < 0.001$ for all comparisons) and for the 15.0-mg versus 30.0-mg ArTiMist treatments ($P < 0.01$ for both comparisons), but there was no significant difference between the 30.0-mg ArTiMist treatments ($P > 0.05$). The median simulated concentrations of artemether and dihydroartemisinin from the final model fitted to the study 1 data are shown in Fig. 5.

TABLE 4 Derived secondary pharmacokinetic parameters from *post hoc* individual parameters for studies 1 and 2

Parameter by study[a]	Artemether	Dihydroartemisinin	Relative bioavailability	DHA:ART ratio
Study 1				
$t_{1/2\alpha}$ (h)	0.39 (0.31–0.55)	0.27 (0.25–0.29)		
$t_{1/2\beta}$ (h)	10.9 (10.1–11.5)			
AUC$_{0-\infty}$ (µg · h/liter) for treatment:				
I	21.5 (15.0–36.1)	28.6 (23.3–33.2)	1.48 1.10–1.84)	
II	63.6 (42.5–102)	79.3 (69.0–97.9)	2.01 1.72–2.68)	
III	63.4 (39.9–97.0)	74.4 (58.1–87.9)	1.81 1.62–2.06)	
IV	29.1 (20.9–51.5)	47.2 (34.4–52.5)	1 (FIXED)	
Study 2				
$t_{1/2\alpha}$ (h)	0.39 (0.35–0.43)	0.31 (0.21–0.39)		
$t_{1/2\beta}$ (h)	42.3 (41–44.8)			
AUC$_{0-\infty}$ (µg · h/liter)				
Doses 1–4 (avg)	72.3 (56.3–93.7)	74.8 (53.2–101)		1.22 (0.924–1.49)
Doses 5–8 (avg)	55.5 (46.3–71.8)	67.6 (51.7–99.7)		1.41 (1.22–1.92)
Total	507 (422–665)	569 (417–806)		1.28 (1.04–1.63)

[a] $t_{1/2\alpha}$, first half-life; $t_{1/2\beta}$, second half-life; AUC$_{0-\infty}$, area under the concentration-time curve from time 0 to infinity.

(ii) Study 2. In study 2, there were 720 individual plasma artemether and DHA concentrations available for analysis, and 182 (25%) and 246 (34%), respectively, were BLQ. Given that a significant majority of the plasma concentration data for artemether and DHA were below the LOQ from 12 h postdose, these observations were not included in the analysis.

The initial modeling of the study 2 data generated a structural model similar to that derived in study 1. This was a two-compartment model for artemether, with a single additional compartment for DHA. Absorption was characterized by two phases with two separate lag times (LAG$_1$ and LAG$_2$), followed by first-order absorption with two separate absorption rates (k_{a1} and k_{a2}). A RATIO term was also included. The values for these parameters were comparable between the two studies (see Tables 3 and 5). As with study 1, a model estimating bolus input was also tested, but it was <1% of the total dose and did not improve the overall fit.

The models with increasing FP metabolism for subsequent doses were better than those with increasing clearance for subsequent doses in describing the time-dependent kinetics of artemether. A better fit of the data was obtained when FP metabolism was applied to the second absorption phase only. When FP metabolism was considered for individual doses, it was poorly estimated; therefore, early (doses 1 to 4) and later (doses 5 to 8) doses were grouped to allow a simple binary comparison. The estimate for doses 1 to 4 was also poorly estimated, and it was therefore

fixed to the results obtained from study 1. The data did not support a robust estimation of the parameters when linear, hyperbolic maximum effect (E_{max}), and sigmoid E_{max} models were tested. FP metabolism was estimated to be doubled for doses 5 to 8 compared to that with doses 1 to 4, with population maximum values of 82.7% versus 41.7%, respectively.

The IIV was estimable for CL/F$_{ARM}$, CL/F$^*_{DHA}$, and k_{a1}, with values of 52%, 49%, and 51%, respectively. As for study 1, the IIV and IOV could not be estimated simultaneously for RATIO, with a much higher estimate for IOV (146%). The IOV of relative bioavailability was small, at 30%. These results were consistent with those of study 1.

The final model parameter estimates and bootstrap results for study 2 are summarized in Table 5. The bias was <10% and 11% for all fixed- and random-model parameters, respectively, with the exception of Q/F$_{ARM}$ and V_p/F$_{ARM}$, where it was 20% and 30%, respectively. Figures 6 and 7 show goodness-of-fit plots and VPCs, respectively. The 10th, 50th, and 90th percentiles fell within their respective 95% CIs for all but one and three time points for artemether and DHA, respectively. The half-lives and AUC$_{0-\infty}$ values derived from post hoc individual parameters are shown in Table 4. The distribution and terminal elimination $t_{1/2}$ for artemether were 0.39 and 42.3 h, respectively, and the elimination $t_{1/2}$ for DHA was 0.27 h. The significantly longer terminal elimination $t_{1/2}$ relative to that of study 1 reflects the longer sampling time in
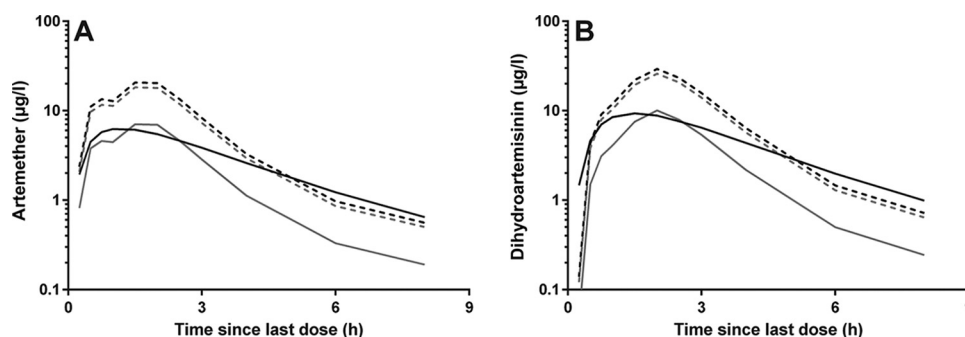

FIG 5 Median simulated concentrations of artemether (A) and dihydroartemisinin (B) (micrograms per liter on a log$_{10}$ scale) from the final model for study 1. Treatment I is represented by a solid gray line, treatment II by a dashed black line, treatment III by a dashed gray line, and treatment IV by a solid black line.

TABLE 5 Final population pharmacokinetic variable estimates and bootstrap results of ARM and DHA for study 2

Parameter[a]	Mean	RSE%[b]	Bootstrap median (95% CI)
Objective function value	774.197		802.853 (663.142–926.848)
Structural model parameters			
k_{a1} (/h)	1.81	22	1.72 (1.39–2.24)
k_{a2} (/h)	0.75	16	0.72 (0.59–1.94)
LAG_1 (h)	0.17	9	0.18 (0.14–0.19)
LAG_2 (h)	1.32	10	1.32 (0.99–1.40)
RATIO doses 1, 8	2.23	64	1.97 (0.79–12.5)
RATIO doses 2–7	0.48	28	0.42 (0.21–0.90)
CL/F_{ARM} (liters/h/70 kg)	410	21	423 (294–633)
V_c/F_{ARM} (liters/70 kg)	323	29	308 (179–431)
Q/F_{ARM} (liters/h/70 kg)	164	17	130 (92–161)
V_p/F_{ARM} (liters/70 kg)	7,270	30	5,075 (3,516–8,476)
$CL/F^\star_{DHA}$ (liters/h/70 kg)	455	20	475 (357–675)
$V/F^\star_{DHA}$ (liters/70 kg)	196	27	197 (137–298)
$FP_{max,doses\ 1-4}$ (%)	41.7		Fixed
$FP_{max,doses\ 5-8}$ (%)	82.7	11	78.0 (15.6–92.2)
FP_{max}-to-FP_{min} ratio	6.07		Fixed
Variability model parameters (shrinkage [%])			
IIV in CL/F_{ARM}	52 (4)	24	52 (16–71)
IIV in $CL/F^\star_{DHA}$	49 (4)	34	47 (24–72)
IIV in k_{a1}	51 (15)	28	47 (27–78)
IOV in F	30 (18, 24, 27, 30, 49)	24	30 (18–40)
IOV in RATIO	146 (1, 19, 17, 36, 7)	13	145 (105–193)
RV for ART	60	5	59 (54–63)
RV for DHA	78	4	77 (71–82)

[a] k_{a1}, absorption rate constant for first absorption phase of ArTiMist; k_{a2}, absorption rate constant for second absorption phase of ArTiMist; LAG_1, lag time for first absorption phase of ArTiMist; LAG_2, lag time for second absorption phase of ArTiMist; RATIO, ratio between the first and second absorption phases of ArTiMist; CL/F_{ARM}, ARM clearance; V_c/F_{ARM}, ARM central volume of distribution; Q/F_{ARM}, ARM intercompartmental clearance; V_p/F_{ARM}, ARM first peripheral volume of distribution; $CL/F^\star_{DHA}$, DHA clearance; $V_c/F^\star_{DHA}$, DHA central volume of distribution; FP_{max}, doses 1–4 and FP_{max}, doses 5–8, degree of first-pass metabolism for doses 1 to 4 and 5 to 8, respectively; FP_{max}-to-FP_{min} ratio, ratio of the population maximum of FP compared to the population minimum; F_{ARM}, relative bioavailability of ARM; $F^\star_{DHA}$, F_{ARM} × the metabolic conversion of ARM to DHA; IOV, interoccasion variability; IIV, interindividual variability; RV, residual variability. IOV and IIV are presented as 100% × the square root of the variability estimate.
[b] RSE, relative standard error.

study 2, which facilitated the further characterization of this phase of elimination. The median artemether T_{max} values for the first peak of ArTiMist were 0.7 h in study 1 and 0.8 h in study 2 compared to 1.4 h for the tablet formulation in study 1. For the second peak, the median artemether T_{max} values were 1.7 h and 1.5 h for studies 1 and 2, respectively.

DISCUSSION

The present study shows that artemether administered as the ArTiMist sublingual spray has ≥20% greater bioavailability than do artemether tablets given in an equivalent dose to healthy adult volunteers, and it was also more rapidly absorbed. Pharmacokinetic modeling showed that the disposition of ArTiMist is relatively complex. It has two absorption phases, with plasma concentration peaks that both occur earlier than that of the single absorption profile associated with the ingestion of artemether tablets. In addition, the first peak does not involve FP metabolism. Drug absorption during the first phase accounted for between 32% and 69% (RATIO, 0.48 and 2.23, respectively) of the total dose and was consistent with that seen in the case of other sublingually absorbed drugs (27). The lower relative bioavailability of the 15.0-mg dose versus the 30.0-mg ArTiMist doses, together with a comparison of the present $AUC_{0-\infty}$/dose data and those of previously published studies, suggest that artemether has dose-dependent pharmacokinetics.

The biphasic absorption profile of ArTiMist and the indication

from the present kinetic modeling that FP metabolism occurs only during the second phase of absorption suggest that there is pre-gastrointestinal absorption of artemether when it is administered sublingually to healthy adults. We postulate that during the first absorption phase, some administered ArTiMist is absorbed from within the oral cavity (sublingually and perhaps via other parts of the oral mucosa where the drug is dispersed during or after administration), with a plasma concentration peak at around 45 min, and the remainder is swallowed, with a slow transfer into the upper gastrointestinal tract, resulting in a later plasma concentration peak approximately 1.5 h after dosing. Given that the rate and extent of absorption of the tablet formulation will depend on dispersion, dissolution in the stomach, and transfer into the upper small intestine, it is not surprising that the ArTiMist solution is more rapidly and completely absorbed.

In the multidose study, there was a modest increase in FP artemether metabolism over time. This phenomenon has been observed in previous studies of healthy volunteers (28) and may reflect the autoinduction of the hepatic enzymes CYP3A4, CYP3A5, and/or CYP2B6 (29). The magnitude of this effect was less in the present study than that reported previously (28), with only a doubling of the extent of FP metabolism. This may reflect the fact that the plasma concentrations achieved in the present study were, consistent with the dose administered, less than those in previous studies (25, 28), with the possibility that higher con-
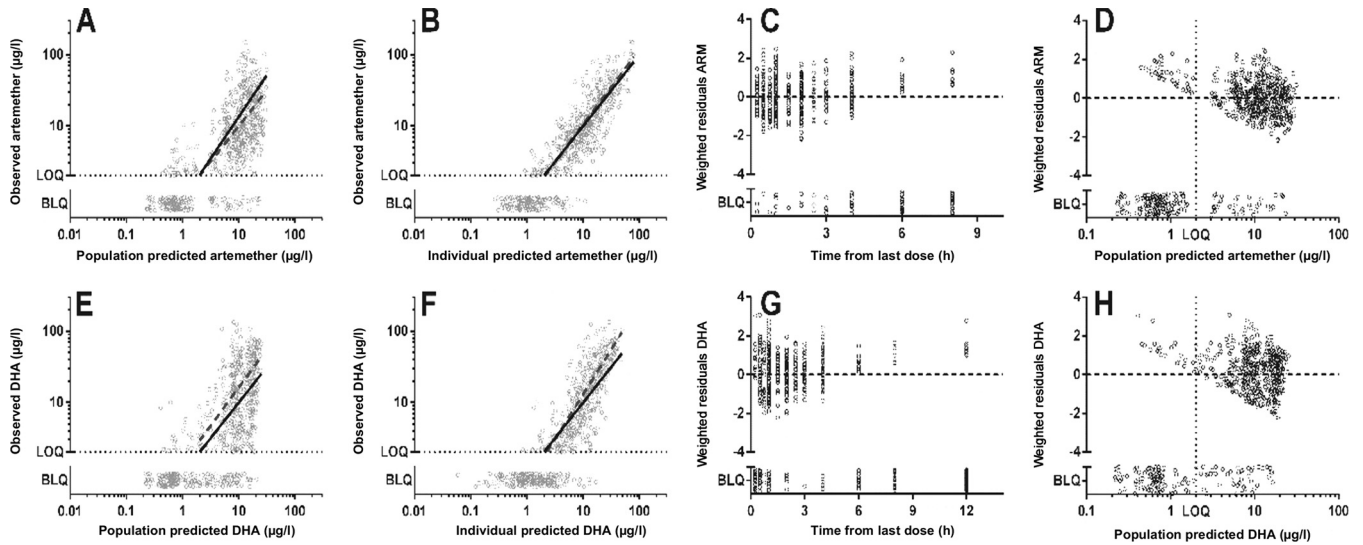
FIG 6 Goodness-of-fit plots for artemether (A to D) and dihydroartemisinin (DHA) (E to H) for study 2. The observed plasma concentration has been plotted against population-predicted (A and E) and individual-predicted (B and F) plasma concentrations, weighted residuals against time (C and G), and population-predicted plasma concentrations (D and H). Data below the limit of quantitation (BLQ) have been separated to assist with visual interpretation.

centrations have a greater effect on autoinduction. However, given that artemether autoinduction is slower and less marked than that associated with artemisinin itself (30) and that ArTiMist as monotherapy should not be given for more than a few doses with a likely role in prereferral treatment, this phenomenon appears to be of limited clinical relevance.

The doses used in the present study were the lowest in all published pharmacokinetic studies of artemether in healthy volunteers, specifically, ≤0.6 mg/kg of body weight versus 1.6 to 9.6 mg/kg of body weight, respectively (10, 11, 13, 15, 25). The $AUC_{0-\infty}$ (in microgram-hours per liter) corrected for dose (in milligrams per kilogram) was also generally lower in our adult volunteers, including in those who were allocated artemether tablets (range, 51 to 100 $\mu g \cdot$ h/liter versus 70 to 275 $\mu g \cdot$ h/liter [10, 11, 13, 15, 25]), suggesting that artemether bioavailability was attenuated at lower doses. This is in accord with the significantly lower relative bioavailability of the 15.0-mg dose versus 30.0-mg

dose of ArTiMist in the present study and suggests that oral artemether exhibits dose-dependent kinetics.

A possible explanation for this phenomenon relates to the intestinal mucosal metabolism of artemether. Studies involving grapefruit juice administration (31) show that the inhibition of intestinal CYP3A4 substantially increases plasma artemether concentrations. This implies that the normal activity of this enzyme attenuates bioavailability. This effect may be most pronounced at low artemether doses but is saturable at higher doses (32). A formal evaluation of this effect was beyond the scope of the present study, but it may have clinical significance. Inadvertent repeated underdosing might carry a disproportionate risk of subtherapeutic plasma concentrations of artemether and DHA and increase the risk of treatment failure if artemether were used as monotherapy.

An alternative explanation for the relatively low dose-corrected $AUC_{0-\infty}$ values in the present study compared with those of
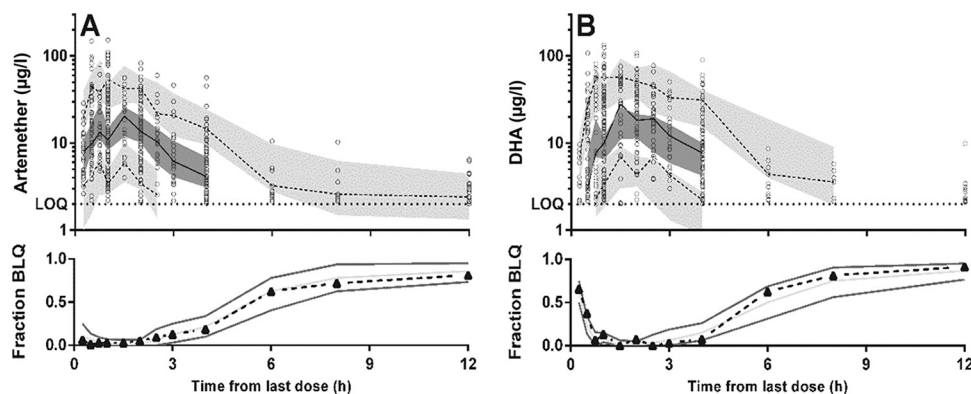
FIG 7 Prediction-corrected visual predictive check for artemether (A) and dihydroartemisinin (DHA) (B) (micrograms per liter on a $\log_{10}$ scale) for study 2, with observed 50th (solid line) and 10th and 90th (dotted lines) percentiles within their simulated 95% CI (gray shaded areas), with overlying data points (○). The fractions of BLQ observations from the data (▲, dashed line) with the simulated 95% prediction interval are also shown.

other published data might be our strict requirement for the subjects to fast. Fat coadministered with the drug increases the bioavailability of artemether (14). Food was either provided or allowed before or during sampling in some studies (10, 11), and whether subjects were kept fasting was not specified in others (12, 13). Nevertheless, the one study in which fasting throughout the 8-h sampling period was specified (28) appeared to show greater bioavailability than that in our subjects.

The terminal elimination $t_{1/2}$ of artemether in our volunteers was long relative to that in other studies ($\geq$10.4 h in our study versus 2.6 to 3.1 h in others [10–13]). However, our elimination $t_{1/2}$ was derived from a two-compartment model rather than the one-compartment or noncompartmental analyses used in other healthy adult studies. In a pharmacokinetic study of uncomplicated malaria in children in which a two-compartment model was used, the artemether elimination $t_{1/2}$ was similarly long ($\geq$23 h) (23). This second (terminal) elimination phase contributes <20% to the AUC in the present studies and may reflect the combination of a long duration of sampling postdose and a relatively sensitive assay, as we have found with artemisinin itself (30).

In conclusion, ArTiMist is a novel sublingual spray formulation of the well-established antimalarial drug artemether. The present studies in healthy adults show that it has greater bioavailability and is more rapidly absorbed than is an equivalent dose of artemether in tablet form. Given that the antimalarial activity of artemether results from the actions of both artemether and its active metabolite DHA, ArTiMist dosed sublingually should ensure a clinically relevant initial increase in plasma artemether with an increasing contribution from DHA as artemether is metabolized to the more potent DHA metabolite. The present and previous studies of artemether disposition provide some evidence that lower doses are associated with reduced bioavailability, but higher oral doses are more potent in promoting autoinduction, suggesting dose-dependent pharmacokinetics. However, giving the same dose of ArTiMist in a more concentrated solution does not alter its absorption. These pharmacokinetic data justify studies of ArTiMist as an initial therapy for use in patients with uncomplicated malaria.

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