Pharmacokinetic properties of artemether, dihydroartemisinin, lumefantrine and quinine in pregnant women with uncomplicated *falciparum* malaria in Uganda

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Running title: Pharmacokinetics of antimalarials in pregnant women

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Pregnancy alters the pharmacokinetic properties of many drugs used in the treatment of malaria, usually resulting in lower drug exposures. This increases the risks of treatment failure, adverse outcomes for the foetus, and the development of resistance. The pharmacokinetic properties of artemether, and its principal metabolite dihydroartemisinin (n=21), quinine (n=21) and lumefantrine (n=26) were studied in pregnant Ugandan women. Lumefantrine pharmacokinetics were also studied in a non-pregnant control group (n=17). Frequently sampled patient data were evaluated with non-compartmental analysis. No significant correlation was observed between estimated gestational age and artemether, dihydroartemisinin, lumefantrine or quinine exposures. Artemether/dihydroartemisinin and quinine exposures were generally lower in these pregnant women compared to values reported previously for non-pregnant patients. Median [range] day 7 lumefantrine concentrations were 488 [30.7-3550] ng/mL in pregnant women compared to 720 [339-2150] ng/mL in non-pregnant women (p=0.128). There was no statistical difference in total lumefantrine exposure or maximum concentration. More studies with appropriate control groups in larger series are needed to characterise the degree to which pregnant women are under-dosed with current antimalarial dosing regimens.

Keywords: Pharmacokinetics; Pregnancy; Malaria; Artemether; Dihydroartemisinin; Lumefantrine; Quinine
INTRODUCTION

Approximately 85 million pregnancies occurred in areas with *P. falciparum* transmission in 2007 (1). Worldwide mortality rates from malaria were estimated at 660,000 (490,000-836,000) in 2010 (2). In the same year an estimated 219 (154-289) million malaria infections occurred (2). Pregnant women are at higher risk of developing severe forms of malaria compared to non-pregnant adults and even asymptomatic infection(s) impair foetal development. Malaria is an important cause of abortion and stillbirth. The first line treatment for uncomplicated *P. falciparum* malaria is artemisinin-based combination therapy (ACT). These comprise an artemisinin-class drug and a more slowly eliminated partner drug (3). Quinine is still used widely especially in the treatment of severe malaria despite the proven superiority of artesunate (4, 5). The ACT's used today commonly provide excellent cure rates above 95% (6-21) but resistance to artemisinin has emerged in South East Asia resulting in slow parasite clearance times and increased treatment failure rates (22, 23). This will also lead to an increased pressure on the partner drugs since a greater number of residual parasites need to be eliminated by the slowly eliminated partner drug.

Pregnancy alters the pharmacokinetic properties of many drugs. Decreased gut motility, increased plasma volume, water and fat content, and/or several changes in CYP enzyme and UGT activities during pregnancy lead to altered absorption, distribution and elimination of antimalarial drugs (24-26). Lower drug exposures have been reported for artemether/dihydroartemisinin (27), artesunate/dihydroartemisinin (28), dihydroartemisinin (29), lumefantrine (30), atovaquone (31) and proguanil (31) in pregnant women. However, some antimalarials show similar (e.g. piperaquine (29, 32-4)
drug exposure in pregnant women compared to the non-pregnant adult patient population. Contradictory results of lower (37, 38), similar (38) and higher (39) exposures have been reported for sulfadoxine and pyrimethamine in pregnant women. Low cure rates (82%) have been reported in pregnant women in Thailand receiving artemether-lumefantrine (40). However, pregnant women in Uganda showed an adequate clinical response after the same treatment (98.2%). This might be explained by differences in pharmacokinetics, resistance patterns or higher levels of background immunity (11). The reported pharmacokinetic properties of intravenous quinine have not shown significant differences between pregnant (n=8) and non-pregnant (n=8) women with uncomplicated \textit{P. falciparum} malaria in a small study from Sudan (41). However, in pregnant women with severe \textit{P. falciparum} malaria (n=10) (42) a shorter quinine elimination half-life (11.3 vs. 16.0 & 18.2 hour) and smaller apparent volume of distribution (0.96 vs. 1.67 & 1.18 L/kg) was reported compared to previously studied patients with uncomplicated \textit{P. falciparum} malaria and patients with cerebral malaria, respectively (43). However, the pharmacokinetic properties of oral quinine in pregnant women have not been reported in the published literature. The aim of this study was to evaluate the pharmacokinetic properties of quinine and artemether-lumefantrine when used for malaria treatment in the second and third trimesters of pregnancy in Uganda.
MATERIALS AND METHODS

Study design

This pharmacokinetic study was nested into a larger efficacy study conducted in the Mbarara National Referral Hospital (MNRH) ante-natal clinic (ANC) in Uganda (11). Full clinical details for the pregnant women in that trial are reported elsewhere (11).

The trial was registered at ClinicalTrials.gov (NCT00495508) and ethical approval was obtained from the Uganda National Council for Science and Technology (ethics committee), the Mbarara University Institutional Ethics Committee, Mbarara University Faculty of Medicine Research and Ethics Committee, and the “Comité de Protection des Personnes”, Iles de France XI, France.

Inclusion criteria were residence in the Mbarara Municipality (radius 15 km from MNRH), an estimated gestation age (EGA) of at least 13 weeks, and *P. falciparum* mixed- or mono-infection (detected by microscopy). Exclusion criteria were severe anemia (Hb <7g/dL), known allergy to artemisinin derivatives, lumefantrine or quinine, *P. falciparum* parasitemia above 250,000 parasite/µL, signs or symptoms of severe malaria requiring parental treatment, or inability to comply with the specified follow-up schedule. Patients were enrolled if written informed consent was obtained and if they fulfilled all inclusion criteria and none of the exclusion criteria. Non-pregnant women in the lumefantrine control group were also enrolled from the efficacy study (up to one year during follow-up) and matched to the pregnant women in the lumefantrine arm by history of fever, axillary temperature >37.5, smoking and parasitemia <1000, 1001 – 25000 or 25001-250000.
**Treatment regimen**

Patients in the artemether/lumefantrine arm were given four tablets of the fixed oral combination of artemether and lumefantrine (Coartem® Novartis Pharma AG, Basel, Switzerland; each tablet contained 20 mg artemether and 120 mg lumefantrine) twice daily for 3 days (planned protocol times at 0, 8, 24, 36, 48 and 60 hours). 200 mL of milk tea was given with each dose to optimise the oral bioavailability of lumefantrine (44). Patients in the quinine arm were given 10 mg/kg of oral quinine sulphate (Remedica, Limassol, Cyprus; each tablet contained 300 mg quinine sulphate) three times daily for 7 days (planned protocol times at 0, 8 and 16 hours). Drug treatments were supervised for both treatment arms.

If the dose was vomited within 30 minutes, a full replacement dose was given and if the dose was vomited between 30 minutes and one hour, a half replacement dose was given. The patient was withdrawn from the study and treated with rescue treatment if the replacement dose was vomited again within 30 minutes (i.e. oral quinine for patients in the artemether/lumefantrine arm and oral artemether/lumefantrine for patients in the quinine arm).

**Pharmacokinetic sampling and drug quantification**

Venous blood samples (2 mL) for artemether/dihydroartemisinin measurement were drawn from an indwelling cannula into heparinised tubes at 0, 0.25, 0.5, 0.75, 1, 1.25,
1.5, 1.75, 2, 2.5, 3, 4, 6, 8, and 10 hours after the last dose. Blood samples (2 mL) for lumefantrine measurement were collected similarly at 0, 4, 8, 12, 24, 28, 36, 40, 48, 52, 60, 60.5, 61, 62, 64, 66, 68, 72, 84, 108, 132, 156, 180, 204 and 228 hours after the first dose. Lumefantrine day 7 samples (168 hour) were also drawn from most patients. Blood samples (2 mL) for quinine measurement were collected similarly at 0, 1, 2, 3, 4, 8, 16, 24, 48, 72, 96, 120, 144, 160, 161, 162, 163, 164, 168, 170, 172, 176, and 184 hours after the first dose.

Blood samples were centrifuged for 5 minutes at 1400 g and plasma was stored at -70°C or below until analysis. The artemether/dihydroartemisinin and lumefantrine plasma samples were shipped on dry ice to Department of Clinical Pharmacology, Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand and the quinine plasma samples were shipped on dry ice to the Service de Pharmacologie Clinique, Hôpital St Vincent de Paul in Paris, France for quantification.

Quantification of artemether and dihydroartemisinin was performed by a previously published method using liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) (45). Triplicates of quality control samples at three concentrations (3.46 ng/ml, 36 ng/ml and 375 ng/ml) for both artemether and dihydroartemisinin were analysed within every batch to ensure precision and accuracy during quantification. The overall relative standard variation (i.e. RSD) was less than 5.4% and the lower limit of quantification (LLOQ) was set to 1.43 ng/mL for both compounds.
Quantification of lumefantrine was performed by a previously published method using LC with UV-detection (46). Triplicates of quality control samples were analysed at three concentrations (200 ng/mL, 2,000 ng/mL and 15,000 ng/ml for pregnant patients and 74.3 ng/mL, 1,056 ng/mL and 15,000 ng/mL for non-pregnant patients). The overall RSD was less than 9.99% for all quality control samples and the LLOQ was set to 26 ng/mL.

Quinine drug analysis was performed using LC with fluorimetric detection (unpublished method). Fifty µL of NaOH 0.1 M and 50 µL of the internal standard (hydroquinidine 7.5 µg/L) were added to 50 µL of plasma. Liquid/liquid extraction was performed with 4 mL of dichloromethane:isopropyl alcohol (80:20). After 10 minutes of mixing, the samples were centrifuged and the supernatant was separated and evaporated under a stream of nitrogen. The dry residue was reconstituted with 100 µL of the mobile phase and 30 µL were injected in the chromatographic system. Chromatographic separation was performed on a Cluzeau C8+ satisfaction column (250x3 mm; 3 µm; Sainte Foy la Grande, France) with a mobile phase consisting of dihydrogen potassium phosphate 0.1 M:acetonitrile:acetic acid (695:300:5). The retention times of quinine and the internal standard were 4.9 minutes and 6.1 minutes, respectively. Excitation and emission wavelengths were 350 and 440 nm, respectively. The recovery was between 76% and 80% within the calibration range of 1-10 µg/mL. Duplicates of quality control samples were analysed at three concentrations at 2 µg/mL, 6 µg/mL and 8 µg/mL. Overall accuracy (bias) and precision (RSD) was less than 5.0% and 9.9%, respectively, and the LLOQ was set to 1 µg/mL. Both bioanalytical laboratories are participating in the

Pharmacokinetic analysis

Individual plasma concentration-time data were evaluated using a non-compartmental approach in WinNonlin version 5.3 (Pharsight Corporation, California, USA). Total exposure up to the last measured concentration ($AUC_{0-\text{LAST}}$) was calculated using the linear trapezoidal method for ascending concentrations and the logarithmic trapezoidal method for declining concentrations. The terminal elimination half-life ($t_{1/2}$) was estimated by the slope ($\lambda_z$) of the best-fit log-linear regression of the observed concentrations in the terminal elimination phase. Drug exposure was extrapolated from the last observed concentration to infinity by $C_{\text{LAST}}/\lambda_z$ for each individual subject to compute total drug exposure ($AUC_{0-\infty}$). $C_{\text{MAX}}$, $T_{\text{MAX}}$ and $T_{\text{LAG}}$ were taken directly from the observed data. Apparent volume of distribution ($V_z/F$) and oral clearance ($CL/F$) were computed individually using equation 1 and 2.

\[
\frac{V_z}{F} = \frac{DOSE}{\ln 2} \times \frac{1}{t_{1/2}} \times AUC \quad \text{[Eq. 1]}
\]

\[
\frac{CL}{F} = \frac{DOSE}{AUC} \quad \text{[Eq. 2]}
\]

Patients who did not provide a sufficient number of samples for a full pharmacokinetic evaluation were excluded from the analysis but included in the summary statistics for
C\textsubscript{MAX}, T\textsubscript{MAX} and T\textsubscript{LAG} if data allowed. Complete \textit{in vivo} conversion of artemether into dihydroartemisinin was assumed and the administered dose of dihydroartemisinin was calculated using the relative difference in molecular weights. Lumefantrine samples were collected frequently for all doses and could therefore capture the accumulation of drug over time. Residual lumefantrine exposure from the three days of dosing could not be accurately subtracted from the lumefantrine exposure of the last dose because of its multi-compartment pharmacokinetics and the long terminal elimination half-life. Therefore, the total dose of lumefantrine (i.e. the sum of the six doses) was used as input dose together with all observed concentration-time data in the non-compartmental analysis of lumefantrine. Quinine plasma samples taken after the first dose (samples taken up to 8 hours after the first dose) were used for analysis since subsequent samples were too sparse (i.e. only one trough value per day) to compensate fully for the accumulation of the drug over time.

Individual pharmacokinetic parameter estimates for lumefantrine were compared between pregnant women and non-pregnant women using the Mann-Whitney test in STATA v.11. Artemether/dihydroartemisinin and quinine pharmacokinetics were compared to literature values.

**RESULTS**

**Pharmacodynamics**

Between October 2006 and May 2009, 304 women were recruited in an efficacy trial (152 in the quinine arm and 152 in the artemether-lumefantrine arm). The study
participants originated from a cohort of 1,197 pregnant women who were screened for malaria on a weekly basis. The Day 42 PCR-adjusted cure rate (95% confidence interval) among analysable patients was high in both arms; 97.6% (93.1-99.5%) in the quinine arm and 99.3% (96.0-99.9%) in the artemether-lumefantrine arm. Details have been published elsewhere (11) and admission demographics for the patients included in the pharmacokinetic study are summarised in Table 1.

Artemether and dihydroartemisinin pharmacokinetics

Artemether and dihydroartemisinin pharmacokinetics were well described in pregnant women (N=21) with *P. falciparum* malaria and pharmacokinetic parameters reported elsewhere (47)(Figure 1). Several patients showed a clear distribution phase with multi-compartment pharmacokinetics whereas other patients did not. A double absorption peak for both artemether and dihydroartemisinin was observed in 3 patients. One patient had a double absorption peak for artemether only and one patient had a double peak for dihydroartemisinin only. The second peaks occurred between 2 and 4 hours after dosing. No cases of vomiting or additional dosing were recorded. Total median [range] artemether maximum concentration (35.4 [5.69-143] ng/mL) and exposure (104 [10.8-351] hr×ng/mL) and dihydroartemisinin maximum concentration (83.0 [18.8-153] ng/mL) and exposure (200 [55.9-456] hr×ng/mL) displayed substantial between-patient variability (Figure 1). A regression analysis of total exposure and maximum concentration versus estimated gestational age did not deviate from zero for artemether (p=0.487 and p=0.671, respectively) or dihydroartemisinin (p=0.773 and p=0.866, respectively).
respectively) which suggests no significant correlation between gestational age and drug exposure (data not shown). Similarly, there was no significant difference between trimesters in total artemether exposure ($p=0.972$), dihydroartemisinin exposure ($p=0.972$), maximum artemether concentration ($p=0.751$) or maximum dihydroartemisinin concentration ($p=0.503$). The same was seen when combining the total exposures and maximum concentrations of artemether and dihydroartemisinin for total malaria activity ($p=0.517$ and $p=0.682$, respectively). Similarly, there was no significant difference between trimesters in combined total exposure ($p=0.976$) or combined maximum plasma concentration ($p=0.689$).

**Lumefantrine pharmacokinetics**

Lumefantrine pharmacokinetics were well described in pregnant ($N=26$) and non-pregnant ($N=17$) women with *P. falciparum* malaria (Table 2; Figure 2). Times to maximum concentration and the terminal elimination half-life estimates were shorter in pregnant compared with non-pregnant patients (Table 2). However, there was no statistical difference in total lumefantrine exposure, apparent volume of distribution or elimination clearance between the two groups. Therefore, a compartmental analysis is needed to evaluate and understand potential differences in the pharmacokinetics between pregnant and non-pregnant women. Total lumefantrine exposures from 72 hours (i.e. 12 hours after last dose) until the last sample were similar in pregnant and non-pregnant women ($p=0.691$). Day 7 concentrations were generally higher in non-pregnant women (median [range]: 720 [339-2,150] ng/mL) compared to pregnant
women (488 [30.7-3,550] ng/mL) but this difference did not reach statistical significance (p=0.128). Overall 5% and 15% of the pregnant women had day 7 lumefantrine plasma concentrations below the suggested cut-off values of 175 ng/mL (48) and 280 ng/mL (49, 50), respectively, for therapeutic efficacy. However, none of the women in the non-pregnant control group had day 7 lumefantrine plasma concentrations below 280 ng/mL. A regression analysis of total exposure and maximum concentrations versus estimated gestational age did not deviate from zero (p=0.334 and p=0.245, respectively) and suggests no significant correlation between week of gestational age and drug exposure (data not shown). Similarly, there was no significant difference in total exposure (p=0.281) or maximum concentration (p=0.359) between trimesters.

Quinine pharmacokinetics

Quinine pharmacokinetics after the first dose were well described in pregnant women (N=21) with *P. falciparum* malaria (Table 3; Figure 3). Quinine elimination clearance was approximately 20% higher in pregnant women in this study compared to non-pregnant Thai patients (0.11 L/hr/kg vs 0.091 L/hr/kg) (51). This would suggest a lower total exposure in pregnant compared to non-pregnant patients. A regression analysis of total exposure and maximum concentration versus estimated gestational age did not deviate from zero (p=0.945 and p=0.375, respectively) which suggests no significant correlation between gestational age and drug exposure (data not shown). Similarly, there was no significant difference in total exposure (p=0.970) or maximum concentration (p=0.433) between trimesters.
DISCUSSION

Artemether and dihydroartemisinin pharmacokinetics

Pharmacokinetic parameter estimates in this study were generally comparable to those reported previously in pregnant Thai patients (27), which is the only available comparator group in the literature. Median (range) maximum artemether concentrations and total artemether exposures reported in this study were 35.4 (5.69-143) ng/mL and 104 (10.8-351) hr×ng/mL compared with 35 (14-104) ng/mL and 65.6 (10.5-280) hr×ng/mL, respectively, reported previously in pregnant Thai patients (27). Maximum dihydroartemisinin concentrations and total dihydroartemisinin exposures reported in this study were also in a similar range compared to that in pregnant Thai patients ($C_{\text{MAX}}$: 83.0 (18.8-153) vs 165 (72-224) ng/mL; AUC: 200 (55.9-456) vs 357 (29.8-585) hr×ng/mL) (27). However, pharmacokinetic parameter estimates vary substantially between different studies which complicate the interpretation of these data as no non-pregnant contemporaneous control group was available. Total exposure of artemether and dihydroartemisinin was substantially lower compared to that reported in two non-pregnant patient studies in Thailand (52, 53). Artemether is metabolised by the cytochrome P450 (CYP) enzyme 3A4 into its active metabolite, dihydroartemisinin (54), which is then glucuronidated by UDP-glucuronosyltransferase (UGT) 1A9 and 2B7 (55). Both these enzyme systems have been reported to be induced during pregnancy (56, 57) and might explain the lower exposures in pregnant women compared to literature values. An expansion of the volume of distribution seen in pregnant women could lead...
to a reduction in peak levels. Although this should not result in a difference in total drug exposure, it might reduce the exposure to concentrations providing maximum effects (i.e. exceeding the minimum parasiticidal concentration). However, only limited data were available in the literature and larger studies are urgently needed to assess the impact of pregnancy on the pharmacokinetics of artemether and dihydroartemisinin. A more extensive pharmacometric modelling approach of these data are published elsewhere (47).

**Lumefantrine pharmacokinetics**

Lumefantrine is metabolised predominantly by CYP3A4 (58, 59) and lumefantrine exposure would be expected to be lower in pregnant women compared with non-pregnant women. However, there were no statistical differences in total exposure or maximum concentration in pregnant women compared to non-pregnant women in this study. Pharmacokinetic parameter estimates for pregnant and non-pregnant women in this study were also similar to that reported for non-pregnant and pregnant women in the literature (13, 27, 30, 50, 60, 61). Interestingly, the terminal elimination half-life was shorter in pregnant women compared to non-pregnant women which resulted in a substantial, but non-significant difference in measured day 7 concentrations. This might have clinical implications in the duration of post-treatment prophylactic effect and for intermittent preventive treatment in pregnant women. Indeed, 5% and 15% of pregnant women and none of the non-pregnant women in this study had day 7 lumefantrine plasma concentrations below the previously defined therapeutic cut-offs of 175 ng/mL.
(48) and 280 ng/mL (49, 50), respectively. Furthermore, 31% of the pregnant women in the efficacy study had plasma lumefantrine concentrations below 280 ng/ml at day 7 supporting the suggestion that pregnant women are under dosed (11). The difference between studies (15% vs 31%) might reflect a difference in study size. The relatively low patient numbers in this study and the large inter-individual differences might mask potential pregnancy-related differences. A pharmacometric approach could be more informative as it would have greater statistical power to detect true differences.

Quinine pharmacokinetics

Quinine is metabolised mainly to its major metabolite, 3-hydroxquinine, by CYP3A4 (62). Pregnancy could theoretically have an impact on the pharmacokinetics of quinine. However, previous studies have reported similar pharmacokinetic properties of quinine in pregnant and non-pregnant patients after parenteral administration of quinine (41, 63). Only sparse literature data are available after oral administration of quinine in non-pregnant patients (51) and no published information is available in pregnant women. Total exposure was not reported by Supanaranond et al, but oral clearance (n=15) was somewhat lower in those non-pregnant women compared to that estimated in the pregnant women in this study (0.091 L/hr/kg vs 0.11 L/hr/kg, respectively). This suggests a decreased exposure in pregnant women compared to non-pregnant adult patients. However, the regression analysis showed no significant correlation between estimated gestational age and exposure parameters, which at least supports a lack of a pregnancy-related effect on quinine pharmacokinetics from the second to the third
trimester. A non-compartmental analysis could only be performed on data after the first
dose to avoid the accumulation of drug over time and a pharmacometric approach might
therefore be more appropriate in order to utilize all the available data. This methodology
could give more insight about the impact of gestational age, disease and other relevant
biological covariates. Studies in pregnant and non-pregnant women with uncomplicated
malaria are needed.

The impact of pharmacokinetic changes on therapeutic responses will be greatest in
non-immune mothers. In many parts of Uganda malaria transmission is intense and
host immune responses can eliminate partially treated infections. Failure rates with
artemether-lumefantrine in pregnant women studied in Thailand were ten times higher
than in Uganda, despite similar dose regimens and relatively similar drug exposures.

In conclusion, pharmacokinetics of artemether/dihydroartemisinin, lumefantrine and
quinine were well characterised in pregnant patients with uncomplicated *P. falciparum*
malaria. Lumefantrine pharmacokinetics was also evaluated in a non-pregnant control
group and resulted in no statistical difference in total exposure between the groups.
However, the terminal elimination half-life was shorter in pregnant women compared to
non-pregnant women, which will affect cure-rates and post-prophylactic effects,
particularly in women with little background immunity. Artemether/dihydroartemisinin
and quinine exposures were generally lower in pregnant women compared to literature
data but more data are needed to evaluate the potential impact of pregnancy on
therapeutic responses.
ACKNOWLEDGMENTS

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TABLE 1 Admission demographics of patients included in the pharmacokinetic study

<table>
<thead>
<tr>
<th></th>
<th>Artemether/dihydroartemisinin</th>
<th>Lumefantrine</th>
<th>Quinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant women (n=21)</td>
<td>Pregnant women (n=26)</td>
<td>Non-pregnant women (n=17)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21 (16-35)</td>
<td>20 (18-38)</td>
<td>21 (18-29)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>55 (49-88)</td>
<td>56 (44-74)</td>
<td>49 (40-63)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>27 (13-36)</td>
<td>22.5 (16-38)</td>
<td>-</td>
</tr>
<tr>
<td>2nd trimester (%)</td>
<td>47.6</td>
<td>69.2</td>
<td>-</td>
</tr>
<tr>
<td>3rd trimester (%)</td>
<td>52.4</td>
<td>30.8</td>
<td>-</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>36.7 (36.0-38.5)</td>
<td>36.7 (36.0-39.3)</td>
<td>36.7 (36.1-38.2)</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
<td>Lower Limit</td>
<td>Upper Limit</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>---------------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td><strong>P. falciparum</strong> (parasites/uL)</td>
<td>1,570 (88.0-148,000)</td>
<td>638 (32-11,800)</td>
<td>751 (48-152,190)</td>
</tr>
<tr>
<td>Platelets (x 10^9/L)</td>
<td>167 (64-285)</td>
<td>185 (83-255)</td>
<td>153 (78-247)</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.91 (0.56-5.53)</td>
<td>0.75 (0.25-2.27)</td>
<td>1.41 (0.39-2.80)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>34.0 (23.2-44.5)</td>
<td>29.5 (20.3-35.0)</td>
<td>37.9 (35.0-43.3)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>60.0 (46.0-75.0)</td>
<td>60.5 (44.0-73.0)</td>
<td>65.0 (49.0-81.0)</td>
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<tr>
<td>Haemoglobin (g/dL)</td>
<td>11.3 (7.6-14.6)</td>
<td>10.0 (6.9-12.4)</td>
<td>12.8 (11.5-14.5)</td>
</tr>
<tr>
<td>Red blood cell (x10^12/L)</td>
<td>3.71 (2.37-4.79)</td>
<td>3.39 (2.23-4.51)</td>
<td>4.28 (3.89-4.81)</td>
</tr>
<tr>
<td>Neutrophils (x10^9/L)</td>
<td>2.75 (1.14-4.13)</td>
<td>3.30 (1.89-6.03)</td>
<td>2.58 (0.74-4.86)</td>
</tr>
<tr>
<td>Eosinophils (x10^9/L)</td>
<td>70 (20-570)</td>
<td>230 (40-810)</td>
<td>280 (110-640)</td>
</tr>
<tr>
<td>Basophils (x10^9/L)</td>
<td>20 (10-60)</td>
<td>20 (10-50)</td>
<td>40 (20-160)</td>
</tr>
<tr>
<td>Lymphocytes (x10^9/L)</td>
<td>1.98 (1.12-3.51)</td>
<td>1.82 (0.77-3.75)</td>
<td>1.34 (0.62-2.99)</td>
</tr>
<tr>
<td></td>
<td>Median (Range)</td>
<td>Median (Range)</td>
<td>Median (Range)</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Monocytes (x10^9/L)</td>
<td>0.55 (0.26-1.00)</td>
<td>0.31 (0.01-3.02)a</td>
<td>0.26 (0.02-0.44)d</td>
</tr>
<tr>
<td>ALAT results (IU/L)</td>
<td>14.0 (5.0-35.0)</td>
<td>16.0 (8.0-86.7)b</td>
<td>23.0 (7.0-109)</td>
</tr>
<tr>
<td>Creatinine results (mg/dL)</td>
<td>0.47 (0.33-0.66)</td>
<td>0.54 (0.38-0.93)g</td>
<td>0.71 (0.40-0.96)</td>
</tr>
</tbody>
</table>

Values are given as median (range) unless otherwise specified.

- `a` Based on 17 patients
- `b` Based on 21 patients
- `c` Based on 16 patients
- `d` Based on 13 patients
- `e` Based on 15 patients
- `f` Based on 14 patients
- `g` Based on 10 patients
- `h` Based on 22 patients
TABLE 2 Non-compartmental analysis of lumefantrine in pregnant and non-pregnant patients with uncomplicated *P. falciparum* malaria

<table>
<thead>
<tr>
<th></th>
<th>Pregnant women</th>
<th>Non-pregnant women</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=25)</td>
<td>(n=17)</td>
<td></td>
</tr>
<tr>
<td>Total dose (mg/kg)</td>
<td>51.4 (38.9-65.5)</td>
<td>58.8 (45.7-72.0)</td>
<td>0.010</td>
</tr>
<tr>
<td>TMAX (hr)</td>
<td>4.00 (0.0833-12.1)</td>
<td>6.00 (1.00-14.0)</td>
<td>0.032</td>
</tr>
<tr>
<td>CMAX (μg/mL)</td>
<td>9.19 (0.485-22.4)</td>
<td>8.88 (4.50-17.0)</td>
<td>0.747</td>
</tr>
<tr>
<td>CL/F (L/hr)</td>
<td>4.40 (1.54-36.3)</td>
<td>4.63 (2.46-9.87)</td>
<td>0.828</td>
</tr>
<tr>
<td>CL/F (L/hr/kg)</td>
<td>0.0829 (0.0288-0.825)</td>
<td>0.0942 (0.0503-0.224)</td>
<td>0.377</td>
</tr>
<tr>
<td>V/F (L)</td>
<td>414 (63.4-2,510)</td>
<td>421 (227-1,330)</td>
<td>0.450</td>
</tr>
<tr>
<td>V/F (L/kg)</td>
<td>6.90 (1.22-57.1)</td>
<td>7.65 (4.63-30.3)</td>
<td>0.148</td>
</tr>
<tr>
<td>T1/2 (hr)</td>
<td>53.5 (28.5-79.4)</td>
<td>65.7 (48.2-93.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>AUC_{72-LAST} (hr×μg/mL)</td>
<td>177 (63.0-1,130)</td>
<td>163 (86.1-4400)</td>
<td>0.691</td>
</tr>
<tr>
<td>AUC_{72-∞} (hr×μg/mL)</td>
<td>189 (64.7-1,170)</td>
<td>197 (99.0-544)</td>
<td>0.949</td>
</tr>
<tr>
<td>AUC_{0-LAST} (hr×μg/mL)</td>
<td>632 (77.7-1,840)</td>
<td>591 (270-1,080)</td>
<td>0.729</td>
</tr>
<tr>
<td>AUC_{0-∞} (hr×μg/mL)</td>
<td>654 (79.4-1,870)</td>
<td>621 (292-1,170)</td>
<td>0.828</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AUC&lt;sub&gt;0-∞&lt;/sub&gt;/dose</strong></td>
<td>12.1 (1.21-34.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(hr×μg/mL/(mg/kg))</td>
<td>10.6 (4.46-19.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.377</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 7 concentration</strong></td>
<td>488 (30.7-3,550)&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/mL)</td>
<td>720 (339-2,150)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.128</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given as median (range) unless otherwise specified.

<sup>a</sup> based on 26 patients,  <sup>b</sup> based on 20 patients

*CMAX* maximum observed plasma concentration after the last dose, *T<sub>MAX LAST</sub>* observed time after last dose to reach *CMAX*, *CL* elimination clearance, *V* apparent volume of distribution, *T<sub>1/2</sub>* terminal elimination half-life, *AUC<sub>72-LAST</sub>* observed area under the plasma concentration-time curve from 72 hours to last observed concentration, *AUC<sub>72-∞</sub>* predicted area under the plasma concentration time curve from 72 hours to infinity, *AUC<sub>0-LAST</sub>* observed area under the plasma concentration-time curve from zero time to last observed concentration, *AUC<sub>0-∞</sub>* predicted area under the plasma concentration time curve from zero time to infinity, *Day 7 concentration* observed day 7 concentration after repeated drug administration, and *F* oral bioavailability.
TABLE 3 Non-compartmental analysis of quinine in pregnant patients with uncomplicated *P. falciparum* malaria

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Quinine (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total dose (mg base /kg)</td>
<td>7.10 (6.66-7.93)</td>
</tr>
<tr>
<td>C(_{\text{MAX}}) (µg/mL)</td>
<td>4.52 (2.58-8.05)</td>
</tr>
<tr>
<td>C(_{\text{MAX}})/dose (µg/mL/(mg/kg))</td>
<td>0.640 (0.370-1.20)</td>
</tr>
<tr>
<td>T(_{\text{MAX}}) (hr)</td>
<td>2.03 (1.07-4.00)</td>
</tr>
<tr>
<td>CL/F (L/hr)</td>
<td>6.07 (1.88-11.3)</td>
</tr>
<tr>
<td>CL/F (L/hr/kg)</td>
<td>0.110 (0.0300-0.210)</td>
</tr>
<tr>
<td>V/F (L)</td>
<td>74.2 (51.3-161)</td>
</tr>
<tr>
<td>V/F (L/kg)</td>
<td>1.45 (0.820-2.59)</td>
</tr>
<tr>
<td>T(_{1/2}) (hr)</td>
<td>9.28 (3.24-21.9)</td>
</tr>
<tr>
<td>AUC(_{0-\text{LAST}}) (hr×µg/mL)</td>
<td>26.5 (15.2-53.3)</td>
</tr>
<tr>
<td>AUC(_{0-\infty}) (hr×µg/mL)</td>
<td>61.4 (33.0-231)</td>
</tr>
<tr>
<td>AUC(_{0-\infty})/dose (hr×µg/mL/(mg/kg))</td>
<td>9.06 (4.65-34.6)</td>
</tr>
<tr>
<td>Day 7 concentration (µg/mL) (^a)</td>
<td>3.93 (1.02-7.77)</td>
</tr>
</tbody>
</table>

Values are given as median (range) unless otherwise specified.
Based on 23 individuals, day 7 concentration from individual 199 and 251 were also included.

$C_{MAX}$ maximum observed plasma concentration after the first dose, $T_{MAX}$ observed time to reach $C_{MAX}$, $CL$ elimination clearance, $V$ apparent volume of distribution, $T_1/2$ terminal elimination half-life, $AUC_{0-LAST}$ observed area under the plasma concentration-time curve after the first dose from zero time to last observed concentration, $AUC_{0-\infty}$ predicted area under the plasma concentration-time curve after the first dose from zero time to infinity, Day 7 concentration observed day 7 concentration after repeated drug administration, and $F$ oral bioavailability.
FIGURES

Figure 1. Mean artemether and dihydroartemisinin venous plasma concentration-time curves after the last dose in pregnant women with uncomplicated *P. falciparum* malaria. Error bars indicate standard deviation. Insert show concentration-time profiles up to 3 hours after the last dose.

Figure 2. Mean lumefantrine venous plasma concentration-time curves in pregnant and non-pregnant women with uncomplicated *P. falciparum* malaria. Error bars indicate standard deviation. Insert show concentration-time profiles up to 3 days after dose initiation.

Figure 3. Mean quinine venous plasma concentration-time curve after the first dose in pregnant women with uncomplicated *P. falciparum* malaria. Error bars indicate standard deviation.