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Pharmacokinetics of co-formulated mefloquine and artesunate in pregnant and non-pregnant women with uncomplicated *Plasmodium falciparum* infection in Burkina Faso

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Objectives: Mefloquine/artesunate has recently been developed as a fixed-dose combination, providing a promising rescue/alternative treatment for malaria during pregnancy. However, limited data are available on the effect of pregnancy on its pharmacokinetic properties. This study was conducted to assess the pharmacokinetic properties of mefloquine/carboxymefloquine and artesunate/dihydroartemisinin in pregnant and non-pregnant women with uncomplicated malaria.

Methods: Twenty-four women in their second and third trimesters of pregnancy and 24 paired non-pregnant women were enrolled. All patients were treated for uncomplicated *Plasmodium falciparum* malaria with a standard fixed-dose combination of oral mefloquine and artesunate one daily over 3 days. Frequent blood samples were collected before treatment and at scheduled times post-dose for the drug measurements and pharmacokinetic analyses. The study was registered at www.clinicaltrials.gov (identifier: NCT00701961).

Results: The total median exposure to mefloquine and dihydroartemisinin was not significantly different between the pregnant and non-pregnant women ($P > 0.05$). There was a trend of higher exposure to mefloquine in the pregnant women, but this difference did not reach statistical significance (656 700 versus 542 400 h×ng/mL; $P = 0.059$). However, the total exposure to carboxymefloquine was 49% lower during pregnancy (735 600 versus 1 499 000 h×ng/mL; $P < 0.001$) and the total drug exposure to artesunate was 42% higher during pregnancy (89.0 versus 62.9 h×ng/mL; $P = 0.039$) compared with non-pregnant controls.

Conclusions: The plasma levels of mefloquine and dihydroartemisinin appeared to be similar in both pregnant and non-pregnant women, but there were significant differences in carboxymefloquine and artesunate exposure. The data presented here do not warrant a dose adjustment in pregnant patients, but an extensive analysis of the data could provide a better understanding of these findings.

Keywords: PK, antimalarials, pregnancy

Introduction

Malaria during pregnancy is a major public health problem, increasing the risk of low birth weight (LBW; birth weight <2500 g) and infant morbidity/mortality during the first year of life by intrauterine growth retardation, prematurity and anaemia.[1–3] In areas of stable malaria transmission such as Burkina Faso, malaria infections during pregnancy are often asymptomatic and usually persist for long periods at low densities.[3–6] These infections, either peripheral or placental, and their consequences are more frequent in primigravidae and secundigravidae than in multigravidae.[1,3,7] The safety and efficacy

of artemisinin-based antimalarial drug combination treatments (ACTs) has prompted the WHO to recommend them for the treatment of malaria in non-pregnant individuals living in areas with established chloroquine resistance.[4,8–12] In several African countries where ACTs are the recommended first- and/or second-line treatments, these same ACT drug regimens are also used for the treatment of malaria in pregnant women, despite the lack of adequate tolerance and safety and pharmacokinetic data. Several biological processes are altered during pregnancy, producing for example decreased gut motility, increased plasma volume and changes in water and fat content and/or enzyme activities, which might result in altered pharmacokinetic properties of these drugs.[13] This could lead to lower or higher drug exposures[14,15] and a consequently altered efficacy. Mefloquine/artesunate has been developed as a fixed-dose combination. Mefloquine has been used for many years and shown to be safe in pregnant women.[16] Similarly, there is increasing experience with artesunate in pregnancy, with initial clinical trials suggesting that it is well tolerated and efficacious in both pregnant and non-pregnant individuals.[17,18] The accumulated human experience and the convenient dosing afforded by fixed-dose formulations of mefloquine/artesunate make it a promising candidate for rescue/alternative treatment in pregnancy. Preliminary data suggest that the peak concentration of mefloquine is lowered in pregnant women compared with non-pregnant individuals.[19,20] In addition, it has been reported that the combination of mefloquine with artesunate causes significant changes in the pharmacokinetics of mefloquine.[21] Prior to its widespread adoption, there is a need for additional studies on the tolerance, safety and efficacy of mefloquine/artesunate. In particular there is a need for detailed pharmacokinetic studies to be undertaken to assess any requirement for dosage regimen optimization in this patient group.

Methods

Study site

The study was conducted from 7 September 2008 to 15 January 2009 at Nanoro Hospital (maternity). Nanoro is situated in the centre of Burkina Faso, 85 km from Ouagadougou, the capital city. Malaria is holoendemic and transmission is extremely seasonal, overlapping with the rainy season (June–October). The entomological inoculation rate is estimated at 50–60 infective bites/person/year (A. Diabate, Centre Muraz, personal communication). Malaria is the major reason for attending a health facility, with *Plasmodium falciparum* being the predominant malarial parasite. Preventive interventions targeting pregnant women currently consist of intermittent preventive treatment with sulfadoxine/pyrimethamine given twice after quickening and the promotion of insecticide-treated materials. Pregnant women are offered an insecticide-treated net at their prenatal consultations.

Study design and participants

The pharmacokinetic properties of mefloquine and artesunate, as well as their primary metabolites, were established in 24 pregnant women with uncomplicated *P. falciparum* mono-infection and compared with those of 24 non-pregnant women with *P. falciparum* infection. Uncomplicated malaria was defined as infection with *P. falciparum* <50000 parasites/μL in the absence of danger signs. Women were identified in two health facilities, the Centre de Santé et Promotion Sociale (CSPS) of Nazoanga and of Nanoro, close to the maternity unit of the district hospital. All pregnant women at their first antenatal clinic visit were screened for peripheral *P. falciparum* infection. Non-pregnant women were recruited from patients attending the outpatient department of the hospital and the two CSPSs, provided that they were from the same villages where the pregnant women were residing and they had a positive smear for *P. falciparum*. Each non-pregnant woman was selected to match with a recruited pregnant women by age (either less or more than 20 years old). The study was approved by the National Health Ethics Committee, Ministry of Health, Burkina Faso and by both the Review Board of the Prince Leopold Institute of Tropical Medicine and the Ethics Committee of the University Hospital, Antwerp, Belgium. The study was registered at www.clinicaltrials.gov (identifier: NCT00701961). Informed consent was obtained from each participant before enrolment.

Screening and enrolment

Women attending the two CSPSs were considered for screening and enrolment in the study provided that they fulfilled all the following inclusion criteria: (i) gestational age ≥12 weeks; (ii) *P. falciparum* infection at a density of <50000 parasites/μL; (iii) willingness to sign or thumb-print their written consent; (iv) willingness to be hospitalized for 3 days and to return for scheduled follow-up visits for treatment and observation until delivery; and (v) willingness to deliver in a health facility. Women were excluded when one of the following exclusion criteria was present: (i) a history of known sensitivity to the study drugs or a history of a recent exposure to antimalarials or other medications known to interact with the study drugs; (ii) the presence of danger signs, physical findings of severe illness, severe anaemia or an inability to tolerate oral medicines; or (iii) chronic medical conditions requiring special care beyond what the study could provide. When a *P. falciparum* infection was confirmed the study procedures and objectives were explained in the local language by the study physician before obtaining a signed informed consent form. Gestational age was estimated by last menstrual period and/or assessment of fundal height. Demographic and clinical information was collected by the study clinician, who also assessed that the women met all the inclusion criteria. Women received a unique study number that identified all the forms and blood samples. Non-pregnant women were recruited after the required sample size for pregnant women had been attained and underwent the same study procedures described above. A urine sample was collected for pregnancy testing.

Study medication

The fixed-dose mefloquine/artesunate-containing tablets (100 mg of artesunate and 220 mg of mefloquine per tablet) were provided by Farmanguinhos, Brazil. Each woman was given a dose of 8 mg of mefloquine/kg per day and 3.6 mg of artesunate/kg per day once daily for 3 days. Women weighing <50 kg, 50–60 kg and >60 kg received 1.5, 2 and 2.5 tablets, respectively, per day. Women took their treatment after food. In case of vomiting within 30 min, a full replacement dose was given. If vomiting occurred between 30 and 60 min, a replacement half-dose was given.

Patient follow-up

All the enrolled women were admitted to the hospital for the first 4 days to allow blood collection at specific times. They were then sent home and asked to return for clinical reassessments on Days 5 and 7, and weekly thereafter, until Day 63. If delivery had not occurred by Day 63, the woman was encouraged to attend the clinic when she was sick. Women were encouraged to deliver at the health facility, where a blood sample was collected both from the mother (finger prick) and the baby (heel prick) for malarial blood smear, haemoglobin measurement and filter paper capture for later molecular analysis. Newborns were examined for congenital anomalies. Birth weight was recorded using a digital baby

weighing scale (Seca 354) and neonatal haemoglobin was measured with a HemoCue machine (HemoCue, Basel, Switzerland).

Blood sampling for pharmacokinetic analysis

Blood samples (2 mL) for pharmacokinetic analysis were obtained by venous puncture or via a three-way tap attached to a catheter. For haematology and biochemistry, 2 mL of blood was collected on Day 0, before the first dose of mefloquine/artesunate, and at Day 14. Samples for mefloquine and carboxymefloquine measurement were collected before treatment and at the following timepoints after the first dose: 4, 8, 24, 28, 48, 49, 50, 52, 56, 60 and 72 h, and then 5, 7, 10, 14, 21, 28, 35, 42, 49 and 56 days. Samples for artesunate and dihydroartemisinin measurement were collected before treatment and during the first day of treatment at the following timepoints post-dose: 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12 h. All the samples were placed on ice immediately after blood collection and processed within 30 min. Plasma samples were obtained after centrifugation using a chilled centrifuge and stored at −80°C in liquid nitrogen until shipment on dry ice to Thailand (artesunate/dihydroartemisinin) or the UK (mefloquine/carboxymefloquine) for measurement of the drug concentrations.

Laboratory procedures

Blood smears for parasite counts were obtained from a capillary blood specimen and examined at screening and on Days 0, 3 and 7, and then at each weekly visit thereafter, as well as any other day if the patient spontaneously returned with fever or worsening symptoms.

Blood smears from all pregnant and non-pregnant women were stained with 10% Giemsa and assessed for the presence of malaria parasites. Parasite density was determined by counting the number of asexual parasites against 300 white blood cells (WBCs) in a thick smear. The blood smear was considered negative when the examination of fields containing a total of 500 WBCs did not reveal the presence of asexual parasites. Each smear was read independently by two microscopists. In the case of discordant results for the parasite species or the parasite density (>10% difference), another reading was performed by a third reader. Results from the two closest values among the three readings were used to calculate the mean value. Haemoglobin was measured by a portable spectrophotometer (HemoCue machine) at Days 0, 7, 14, 28 and 63, and then at delivery. In addition, total WBC count, total bilirubin, aspartate amino transferase (AST), alanine aminotransferase (ALT) and creatinine were determined at Day 0 and 14. A filter paper blood spot was collected at each visit until Day 63 post-treatment. All filter papers were subsequently transferred to the Centre Muraz, Burkina Faso, where centralized genotyping according to international recommendations was conducted.[22] Briefly, purification of DNA was conducted as previously described[23] and three polymorphic genetic markers were genotyped sequentially, starting with GluRP, followed by MSP2 and MSP1. Capillary electrophoresis was used for MSP2. Whenever a genetic marker showed a new infection, i.e. no common allele between the day of recurrent infection and Day 0, this was taken as the final result and the analysis was stopped. For samples showing a recurrent infection, i.e. at least one identical allele between Day 0 and the day of recurrent infection, the analysis was carried out up to MSP1. If the latter showed also at least one identical allele between the day of recurrent infection and Day 0, the infection was classified as a recrudescence. All results were double-read and any discrepancies resolved.

Drug assay

Plasma samples for the measurement of artesunate and dihydroartemisinin concentration were transferred to the Department of Clinical Pharmacology, MORU, Bangkok, Thailand. The samples were quantified using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method.[24] Plasma samples for mefloquine and carboxymefloquine measurement were transferred to the Bioanalytical Laboratory of the Liverpool School of Tropical Medicine, Liverpool, UK. The samples were quantified using an LC-MS/MS method validated according to US FDA guidelines (G. R. Davies and S. A. Ward, unpublished results). In short, plasma (100 μL) was precipitated with five volumes of acetonitrile containing the internal standard WR184806. After centrifugation the supernatant was transferred to a microvial prior to evaporation under nitrogen at 40°C and reconstitution in 100 μL prior to injection (20 μL) into the LC-MS/MS system. Tandem mass spectrometry was performed on a Thermo TSQ Quantum Access coupled with a Thermo Scientific Accela pump and autosampler. Electrospray ionization (in positive-ion mode using multiple reaction monitoring) was employed to detect mefloquine and the internal standard, and the system was switched to negative-ion mode for carboxymefloquine determination. The parent and product ions used for analysis were: for mefloquine *m/z* 379.1 and 361.0, for the internal standard *m/z* 308.0 and 264.0 and for carboxymefloquine *m/z* 361.0 and 338.0, respectively. Chromatographic separation was achieved on a Hypersil Gold (50 mm×2.1 mm) column with a mobile phase containing methanol/water (0.1% formic acid) (55:45, v/v). The within-day precision and between-day precision were below 7% for both compounds at all evaluated concentrations. The limit of quantification was 10 and 200 ng/mL for mefloquine and carboxymefloquine, respectively.

Outcome measurements

The primary outcomes were the pharmacokinetic parameters of mefloquine/artesunate in infected pregnant women compared with infected non-pregnant women: C_{max}, $AUC_{0-\infty}$, T_{max}, apparent volume of distribution (*V*/F), oral clearance (CL/F) and the terminal elimination half-life ($t_{1/2}$).

The secondary outcomes included the proportion of women in each treatment group with parasitological cure at 63 days, adjusted by PCR for new infections, and the tolerance and safety profiles, including all adverse events and serious adverse events, abnormal haematological and biochemical values and adverse pregnancy outcomes such as birth defects, miscarriage/abortion, stillbirth and LBW.

Pharmacokinetic and statistical analysis

The sample size calculation was based on the assumption that a 30% difference in the magnitude of the pharmacokinetic parameters between pregnant and non-pregnant patients would be of clinical significance and that *V*/F and CL/F would increase with pregnancy. A minimum of 22 patients/group would provide at least 90% power (alpha=0.05) to detect this difference. To account for a 10% possible loss to follow-up, a sample size of 24 patients/group was chosen. Individual concentration–time data were evaluated using a non-compartmental analysis approach in WinNonlin version 5.0 (Pharsight Corporation, CA, USA). Total exposure up to the last measured concentration (AUC_{0-LAST}) was calculated using the linear trapezoidal method for ascending concentrations and the logarithmic trapezoidal method for descending concentrations. Drug exposure was extrapolated from the last observed concentration to time infinity by C_{LAST}/λ_Z for each individual patient to compute total drug exposure ($AUC_{0-\infty}$). The $t_{1/2}$ was estimated by log-linear regression of the observed concentrations in the terminal elimination phase. C_{max} and T_{max} were taken directly from the observed data. *V*/F and CL/F were computed individually according to standard procedures. Complete *in vivo* conversion of artesunate into dihydroartemisinin and mefloquine into carboxymefloquine was assumed, and the administered dose of dihydroartemisinin and carboxymefloquine was calculated using the relative difference in molecular weights. Estimates of pharmacokinetic parameters were compared between groups (pregnant and non-pregnant) using the non-parametric Mann–Whitney test in STATA v.10.

Results

Patient characteristics

From 7 October 2008 to 15 January 2009, 205 subjects in total were screened, of whom 104 were pregnant women and 101 were non-pregnant women. Twenty-four women were included in each group as initially planned. The reasons for non-inclusion included a negative slide, concurrent diseases and an inability to fulfil the required scheduled visits and/or to deliver at the health facility. One pregnant woman was lost to follow-up on account of moving out of the study area. The two groups were comparable for age, weight and height at enrolment, but the pregnant women had a significantly lower haemoglobin level (Table 1). Nine women were nulliparous in the non-pregnant group.

Pharmacokinetic findings

The C_{max}, T_{max} and *V*/F of mefloquine were similar in the pregnant and non-pregnant women (Table 2). However, the median $t_{1/2}$ was significantly longer in the pregnant (390.2 h) than the non-pregnant (289.2 h) ($P<0.001$) women. The pregnant women also exhibited a trend of a lower CL/F (2.0 versus 2.4 L/h) and a consequently higher $AUC_{0-\infty}$ (656 700 versus 542 400 h×ng/mL), although these differences did not reach statistical significance ($P=0.059$). By contrast, the pharmacokinetics of carboxymefloquine, an inactive metabolite of mefloquine, were markedly different in the pregnant women compared with the non-pregnant women (Table 3). The $AUC_{0-\infty}$ of carboxymefloquine was 49% lower during pregnancy (735 600 versus 1 499 000 h×ng/mL) with proportional increases in *V*/F and CL/F largely accounting for this change ($P<0.001$).

The C_{max}, T_{max} and *V*/F for artesunate were not statistically different ($P>0.05$) in the pregnant compared with non-pregnant women (Table 4). However, a trend of a higher median C_{max} was observed in the pregnant women (94.2 versus 62.9 ng/mL, $P=0.085$). A significantly altered CL/F in pregnant women (2250 L/h) compared with non-pregnant women (3180 L/h) resulted in a 42% increase in total drug exposure during pregnancy ($P=0.039$). Artesunate was rapidly hydrolysed into its active metabolite dihydroartemisinin, in both the pregnant and non-pregnant women, but no differences were observed in the pharmacokinetic properties of dihydroartemisinin between the pregnant and non-pregnant women (Table 5).

Table 1. Baseline characteristics of the study population

Factor	Pregnant women ($n=24$)	Non-pregnant women ($n=24$)
Age (years), mean (SD)	23.6 (6.1)	27.0 (8.9)
Marital status, *n*		
married	19	14
single	5	8
widowed	—	2
Attended school, *n* (%)	4 (16.7)	3 (12.5)
Parity, *n*		
none	—	9
one	10	2
two	8	2
three	6	11
Gestation at enrolment, *n*		
second trimester	12	—
third trimester	12	—
Weight (kg), mean (SD)	54.1 (5.7)	53.5 (5.8)
Height (cm), mean (SD)	160.6 (5.7)	162.8 (5.6)
Body mass index (kg/m^2), mean (SD)	21.0 (1.8)	20.2 (1.9)
Fever in past 48 h, *n*	7	6
Temperature at enrolment (°C), mean (SD)	36.9 (0.4)	36.7 (0.5)
Haemoglobin at enrolment (g/dL), mean (SD)	9.0 (1.1)	11.6 (1.3)
Parasite density (number of parasites/μL), geometric mean (95% CI)	1029 (455–2327)	152 (86–270)
Ownership of one bed net, *n* (%)	3 (12.5)	4 (16.7)
Slept under bed net last night, *n*/*n*	1/3	2/4
At least two antenatal clinic visits attended, *n* (%)	7 (29.2)	—
Previous use of medicine to treat fever during current pregnancy, *n* (%)	7 (29.2)	—
Taking iron, *n* (%)	8 (33.3)	—

Table 2. Pharmacokinetic properties of mefloquine in pregnant women and non-pregnant women

	Pregnant women ($n=24$)	Non-pregnant women ($n=24$)	P
Body weight (kg)	52.0 (46–69.5)	52.8 (46.5–70.0)	0.973
Total dose (mg/kg)	25.4 (18.99–28.70)	25.1 (18.86–29.33)	0.693
No. points lambda	11.0 (3–15)	11.0 (3–14)	0.991
C_{max} (ng/mL)	1558 (625–2475)	1392 (660–1864)	0.283
C_{max}/dose [(ng/mL)/(mg/kg)]	59.3 (24.38–105.90)	57.5 (25.25–78.00)	0.336
T_{max} (h)	56.0 (50–240)	60.0 (49–240)	0.306
CL/F (L/h)	2.0 (0.93–3.94)	2.4 (1.24–5.27)	0.059
CL/F (L/h/kg)	0.04 (0.016–0.076)	0.04 (0.024–0.108)	0.083
V/F (L)	1036.0 (489.9–2,201.0)	948.7 (674.1–2195.0)	0.534
V/F (L/kg)	21.0 (8.67–42.73)	17.3 (12.49–38.98)	0.655
$t_{1/2}$ (h)	390.2 (177.6–570.6)	289.2 (200.0–394.7)	<0.001
AUC_{0-LAST} (h×ng/mL)	544 500 (211 100–1 301 000)	501 200 (118 400–960 000)	0.258
$AUC_{0-\infty}$ (h×ng/mL)	656 700 (334 700–1 418 000)	542 400 (250 600–1 066 000)	0.059
$AUC_{0-\infty}$/dose [(h×ng/mL)/(mg/kg)]	25 690 (13 190–60 700)	22 810 (9281–41 990)	0.083
Ext. AUC (%)	12.5 (5.4–42.5)	5.7 (1.2–56.5)	<0.001

No. points lambda, number of observations used in the log-linear regression in the terminal elimination phase; F, oral bioavailability; Ext. AUC, percentage of $AUC_{0-\infty}$ extrapolated from the last observation to infinity.
Values are presented as median (range).

Table 3. Pharmacokinetic properties of carboxymefloquine in pregnant women and non-pregnant women

	Pregnant women ($n=24$)	Non-pregnant women ($n=24$)	P
Body weight (kg)	52.0 (46–69.5)	52.8 (46.5–70.0)	0.973
Dose (mg/kg)	25.4 (19.0–28.7)	25.0 (18.8–28.5)	0.972
No. points lambda	6.0 (3–10)	6.0 (3–9)	0.791
C_{max} (ng/mL)	806 (432–1619)	1616 (914–2912)	<0.001
C_{max}/dose [(ng/mL)/(mg/kg)]	34.4 (18.3–65.0)	64.3 (34.9–103.7)	<0.001
T_{max} (h)	240 (120–504)	240 (120–504)	0.080
CL/F (L/h)	1.8 (0.7–3.9)	0.9 (0.4–1.3)	<0.001
CL/F (L/h/kg)	0.04 (0.02–0.07)	0.02 (0.01–0.03)	<0.001
V/F (L)	1316.0 (502.4–2179.0)	537.3 (348.1–1438.0)	<0.001
V/F (L/kg)	24.2 (9.3–38.6)	10.3 (5.5–28.5)	<0.001
$t_{1/2}$ (h)	466.5 (355.8–765.6)	486.2 (276.3–1360.0)	0.645
AUC_{0-LAST} (h×ng/mL)	561 000 (220 900–1 513 000)	1 329 000 (658 000–2 163 000)	<0.001
$AUC_{0-\infty}$ (h×ng/mL)	735 600 (337 300–1 979 000)	1 499 000 (997 300–3 387 000)	<0.001
$AUC_{0-\infty}$/dose [(h×ng/mL)/(mg/kg)]	28 420 (14 310–68 980)	63 050 (39 020–141 100)	<0.001
Ext. AUC (%)	25.4 (12.14–40.96)	19.3 (7.30–55.84)	0.245

No. points lambda, number of observations used in the log-linear regression in the terminal elimination phase; F, oral bioavailability; Ext. AUC, percentage of $AUC_{0-\infty}$ extrapolated from the last observation to infinity.
Values are presented as median (range).

Treatment efficacy

At enrolment, the geometric mean parasite density (number of parasites/μL) was higher in the pregnant [1029 (95% CI 455–2327)] than in the non-pregnant [152 (95% CI 86–270)] women. In both groups, all the parasites were cleared by Day 3. Only one recurrent infection (classified as a new infection by genotyping) was observed in a pregnant woman at Day 49. Therefore, the PCR-unadjusted adequate clinical and parasitological response (ACPR) at Day 63 was 95.8% in the pregnant women and 100% in the non-pregnant women while the PCR-adjusted ACPR was 100% in both the pregnant and non-pregnant women. The mean birth weight was 2797 g (95% CI 2624–2971 g), with four (16.7%) LBW babies.

Adverse events and safety

No adverse reactions related to the study treatment were observed. The frequency of events was similar in both groups.

Table 4. Pharmacokinetic properties of artesunate in pregnant women and non-pregnant women

	Pregnant women ($n=24$)	Non-pregnant women ($n=23$)	*P*
Body weight (kg)	52.0 (46.0–69.5)	53.0 (46.5–70.0)	0.8312
Total dose (mg/kg)	3.9 (2.9–4.4)	3.8 (2.9–4.3)	0.8312
No. points lambda	2.5 (2.0–4.0)	2.0 (2.0–3.0)	0.7796
C_{max} (ng/mL)	94.2 (8.6–455)	62.9 (12.9–151)	0.0847
C_{max}/dose [(ng/mL)/(mg/kg)]	25.9 (2.24–125)	16.6 (3.42–35.1)	0.0738
T_{max} (h)	1.0 (0.3–4.0)	1.00 (0.5–3.0)	0.7217
CL/F (L/h)	2250 (390–6180)	3180 (1860–6290)	0.0390
CL/F (L/h/kg)	39.5 (7.1–119)	60.0 (34.5–131)	0.0300
V/F (L)	1010 (252–20200)	1410 (700–13800)	0.0773
V/F (L/kg)	19.3 (4.58–389)	26.0 (12.5–259)	0.1013
$t_{1/2}$ (h)	0.3 (0.2–2.3)	0.4 (0.2–1.7)	0.9322
AUC_{0-LAST} (h×ng/mL)	83.7 (11.4–512)	62.3 (14.6–106)	0.0705
$AUC_{0-\infty}$ (h×ng/mL)	89.0 (32.4–513)	62.9 (31.8–107)	0.0390
$AUC_{0-\infty}$/dose [(h×ng/mL)/(mg/kg)]	25.3 (8.4–141)	16.7 (7.6–29.0)	0.0300
Ext. AUC (%)	2.3 (0.1–64.8)	2.3 (0.6–58.7)	0.5513

No. points lambda, number of observations used in the log-linear regression in the terminal elimination phase; *F*, oral bioavailability; Ext. AUC, percentage of $AUC_{0-\infty}$ extrapolated from the last observation to infinity.
Values are presented as median (range).
One subject was excluded from the non-pregnant group because of a lack of descending concentrations.

Table 5. Pharmacokinetic properties of dihydroartemisinin in pregnant women and non-pregnant women

	Pregnant women ($n=24$)	Non-pregnant women ($n=23$)	
Body weight (kg)	52.0 (46.0–69.5)	52.8 (45.8–70.8)	0.6495
Total dose (mg/kg)	2.9 (2.1–3.2)	2.8 (2.1–3.3)	0.6495
No. points lambda	4.0 (4.0–8.0)	5.0 (4.0–7.0)	0.0136
C_{max} (ng/mL)	756 (237–1680)	696 (292–1070)	0.3376
C_{max}/dose [(ng/mL)/(mg/kg)]	271 (80.7–584)	239 (113–435)	0.2238
T_{max} (h)	2.0 (0.5–4.0)	2.0 (1.0–3.0)	0.2114
CL/F (L/h)	111 (50.0–276)	109 (62.8–209)	0.6062
CL/F (L/h/kg)	2.1 (1.0–5.0)	2.0 (1.0–4.1)	0.6801
V/F (L)	229 (83.2–756)	192 (117–356)	0.6952
V/F (L/kg)	4.0 (1.4–13.7)	3.7 (2.2–6.4)	0.7887
$t_{1/2}$ (h)	1.2 (0.6–2.0)	1.3 (0.9–2.0)	0.8528
AUC_{0-LAST} (h×ng/mL)	1330 (533–2960)	1360 (705–2360)	0.6062
$AUC_{0-\infty}$ (h×ng/mL)	1330 (538–2970)	1360 (709–2360)	0.6062
$AUC_{0-\infty}$/dose [(h×ng/mL)/(mg/kg)]	476 (200–981)	491 (241–1010)	0.6801
Ext. AUC (%)	0.4 (0.1–2.2)	0.4 (0.1–1.1)	0.7887

No. points lambda, number of observations used in the log-linear regression in the terminal elimination phase; *F*, oral bioavailability; Ext. AUC, percentage of $AUC_{0-\infty}$ extrapolated from the last observation to infinity.
Values are presented as median (range).
One subject was excluded from the non-pregnant group because of a lack of descending concentrations.

Cough [21.8% (12/55) in the pregnant versus 37.8% (14/37) in the non-pregnant women], dizziness [14.5% (8/55) in the pregnant versus 21.6% (8/37) in the non-pregnant women], headache [14.5% (8/55) in the pregnant versus 10.8% (4/37) in the non-pregnant women] and vomiting [9.1% (5/55) in the pregnant versus 0% in the non-pregnant women] were the main complaints, but there was no difference between the two study groups. All vomiting cases occurred within the first 30 min following the treatment administration and therefore a full dose of treatment was readministered. Vital signs were comparable between the two groups at Days 0, 7 and 14 except for heart rate, which was higher in the pregnant women, with an increasing significance from Day 0 to Day 14 (data not shown). No differences in haematology and biochemistry parameters were reported between the two groups, with the exception of haemoglobin level, which was significantly lower in pregnant women from Day 0 and throughout the follow-up period. The mean platelet count at Day 14 was also significantly lower in pregnant women (Table 6). Three serious

Table 6. Laboratory parameters of study participants at Days 0, 7 and 14

	Day 0			Day 7			Day 14		
	pregnant women ($n=24$)	non-pregnant women ($n=24$)	P	pregnant women ($n=24$)	non-pregnant women ($n=24$)	P	pregnant women ($n=24$)	non-pregnant women ($n=24$)	P
Haemoglobin (g/dL)	9.0 (8.59–9.48)	11.6 (11.02–12.15)	<0.001	8.8 (8.33–9.17)	12.0 (11.46–12.62)	<0.001	9.2 (8.80–9.60)	11.74 (11.30–12.17)	<0.001
WBCs (cells/μL)	6553 (5716–7390)	5362 (4893–5831)	0.013	—	—	—	6755 (6082–7428)	6200 (5498–6900)	0.07
AST (IU/L)	26.6 (17.68–35.54)	29.3 (24.38–34.16)	0.58	—	—	—	33.2 (24.06–42.36)	26.5 (23.6–29.40)	0.77
ALT (IU/L)	22.8 (17.25–28.37)	24.3 (19.34–29.23)	0.68	—	—	—	20.4 (13.31–27.58)	21.35 (17.97–24.74)	0.76
Bilirubin (g/dL)	0.6 (0.41–0.77)	0.6 (0.40–0.71)	0.77	—	—	—	0.5 (0.39–0.63)	0.43 (0.32–0.54)	0.35
Platelets (cells/μL)	21925 (175979–263270)	214916 (183521–246312)	0.86	—	—	—	286304 (206997–365610)	328818 (291747–365888)	0.005

Values are presented as mean (range).

adverse events were reported (two cases of arthralgia and one infectious syndrome), all registered in the group comprising pregnant women, but none of them related to the study drugs. One case of polydactyly was observed.

Discussion

We observed pregnancy-related differences in the drug exposures to carboxymefloquine and artesunate, but not to mefloquine and dihydroartemisinin.

The $t_{1/2}$ of mefloquine was significantly longer in the pregnant women, but with no statistical difference in total drug exposure. However, the pharmacokinetic parameters of carboxymefloquine were markedly different in the pregnant women. A number of previous studies have reported significant differences in mefloquine pharmacokinetics in pregnant women. A review by Wilby and Ensom[25] described the results of two studies conducted in Thailand; one study included nine pregnant and eight non-pregnant women, all infected with *P. falciparum*,[20] and the other study included 20 healthy pregnant women, all near the beginning of the third trimester of pregnancy.[19] In the first study, the pregnant women received mefloquine at a dose of 15 mg/kg as a single oral dose while in the second study the subjects were randomized to receive either 250 mg or 125 mg of weekly mefloquine. Both studies suggested that plasma concentrations of mefloquine may be altered during pregnancy, most likely because of the increased volume of distribution. The results of our study indicated that although the exposure to the parent mefloquine was somewhat higher, exposure to carboxymefloquine was substantially reduced in the pregnant women, with concomitant increases in both the *V*/F and clearance of the metabolite. These findings raise the possibility of reduced carboxylation as an explanation for the increased exposure to the parent compound in pregnant women. However, this may not be the only explanation since the increase in exposure to the parent compound was not fully proportional to the decrease in metabolite. This hypothesis could only be resolved with a more extensive investigation of metabolite profiles and a compartmental analysis. Changes may also occur in the pharmacokinetics of mefloquine when it is co-administered with artesunate, with higher mefloquine concentrations in patients receiving only mefloquine than in those treated with mefloquine/artesunate.[21]

Artesunate was rapidly hydrolysed into its active metabolite, with $t_{1/2}$ values in the pregnant and non-pregnant women of 0.3 h and 0.4 h, respectively, a finding that is consistent with previous published data.[26–28] The total drug exposure to artesunate was significantly higher in the pregnant women compared with the non-pregnant women, a consequence of a reduced elimination clearance. This was unexpected since pregnancy was assumed to induce hepatic metabolism, resulting in an increased clearance. This finding is still unexplained and needs further investigation. The pregnant women had a similar dihydroartemisinin drug exposure and CL/F compared with the non-pregnant controls. This is also unexpected and different from previous reports that suggested a 42% and 38% reduction in total drug exposure in pregnant women as a consequence of increased elimination clearance[28] and reduced bioavailability,[29] respectively. Similarly, a study conducted in the Democratic Republic of Congo concluded that there was a higher volume of distribution in pregnant women

compared with non-pregnant women, resulting in a lower drug exposure and lower plasma peak concentrations.[26]

The lack of a pregnancy-related effect on the pharmacokinetic properties of dihydroartemisinin in this study might result from the difference in parasite density between the two groups. McGready *et al*.[30] suggested a disease-related effect on the pharmacokinetics of artemisinins with a higher drug exposure during the acute phase of malaria. The higher parasite densities seen in pregnant patients in this study might therefore have masked a true difference in exposure.[30]

Cure rates were high and did not differ between the pregnant and non-pregnant women. All parasites were cleared at Day 3 and no recurrence was observed until Day 28. However, one woman had a recurrent parasitaemia that was classified as a new infection. Nonetheless, these results should be interpreted with caution as the study was not powered to assess the drug efficacy. The combination of mefloquine and artesunate exploits the capacity of artesunate to clear parasitaemia quickly and hence rapidly improve clinical symptoms, while retaining the full schizontocidal effect of the slower action of mefloquine.[31] This prevents recrudescence and thus improves the cure rate. A Cochrane review by Bukirwa and Orton[32] provided evidence that mefloquine/artesunate is more efficacious than mefloquine alone for treating uncomplicated falciparum malaria in areas of low malaria transmission.

The tolerance and safety profile of the combination was good, as no major adverse events were observed. Vomiting occurred only in the pregnant women, indicating that it might be related to the pregnancy itself. However, the number of women included in this study was small and it would not have been possible to identify less frequent adverse events. In a trial carried out in Benin investigating the safety and efficacy of mefloquine given as intermittent preventive treatment to pregnant women, more adverse events were recorded in the mefloquine group than in the sulfadoxine/pyrimethamine group,[33] indicating a lower tolerance to mefloquine. In addition, one woman in the mefloquine group had a severe neurological adverse event consisting of confusion, anxiety, dizziness and insomnia that resolved spontaneously within 3 days.[33] Therefore, mefloquine tolerability may be a problem when deployed on a large scale and this is one of the reasons for considering mefloquine/artesunate as a possible rescue/alternative antimalarial treatment. In terms of pregnancy outcome, a recent drug tolerance and safety database analysis of mefloquine exposure in pregnancy showed that the prevalence of birth defects and fetal loss in maternal, prospectively monitored cases were comparable to background rates.[34] In 2011, the safety of mefloquine was reviewed by the US FDA, which concluded that pregnant women who took mefloquine at various doses for both the prevention and treatment of malaria did not have an increased risk of teratogenic effects or adverse pregnancy outcomes compared with the background rate in the general population. Therefore, mefloquine has been recategorized from a pregnancy Category C to a Category B drug, with Categories A and B demonstrating the least risk to the pregnancy. Furthermore, the CDC has changed its guidelines and now recommends mefloquine for pregnant women both as a malaria treatment option and as an option for all trimesters to prevent malaria infection.

The mefloquine/artesunate fixed-dose combination appeared to be safe, well tolerated and highly effective for treating malaria infections. The plasma levels of mefloquine appeared to be similar in the pregnant women and non-pregnant women, although the exposure to its inactive metabolite, carboxymefloquine, was significantly higher in the pregnant women. Artesunate exposure was significantly higher in the pregnant women, but its active metabolite, dihydroartemisinin, showed a similar drug exposure in the pregnant women compared with the non-pregnant controls. These findings do not warrant a dose adjustment. However, more extensive investigation of the pharmacokinetic properties of this combination is needed for a better understanding of these findings.

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Transparency declarations

None to declare.

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