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PHARMACOKINETICS AND DISPOSITION

Chloroquine pharmacokinetics in pregnant and nonpregnant women with vivax malaria

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Abstract
Purpose We compared the pharmacokinetics of chloroquine in pregnant and nonpregnant women treated for *Plasmodium vivax* malaria.
Methods Twelve pregnant women and 15 nonpregnant women of child-bearing age with acute *P. vivax* malaria were treated with 25 mg chloroquine base/kg over 3 days on the northwestern border of Thailand. Blood concentrations of chloroquine and desethylchloroquine were measured using hydrophilic interaction liquid chromatography coupled with fluorescence detection. Twenty-five women completed the pharmacokinetic study.
Results Although increasing gestational age was associated with reduced chloroquine $AUC_{0\rightarrow\infty}$, there was no significant difference overall in the pharmacokinetics of chloroquine between pregnant and nonpregnant women. Fever was associated with lower chloroquine $AUC_{0\rightarrow\infty}$ values. Desethylchloroquine area under the curve (AUC) values were not significantly affected by pregnancy.
Conclusions Pregnancy did not significantly affect blood concentrations of chloroquine or its metabolite, desethylchloroquine, in women with *P. vivax* malaria.

Keywords Malaria · *Plasmodium vivax* · Chloroquine · Pharmacokinetics · Pregnancy

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Introduction

Chloroquine remains the prophylactic and treatment of choice for *Plasmodium vivax, P. malariae,* and *P. ovale malarias.* Pregnancy affects the pharmacokinetic (PK) properties of the majority of antimalarial drugs [1–7]. Optimal dosing is essential to prevent anemia and low birth weight [8, 9]. There are very few PK data on chloroquine (CQ) in pregnancy; two studies involving four and five women, respectively, gave conflicting results [10, 11]. We therefore assessed CQ PKs in pregnant women with acute vivax malaria in an area where resistance to CQ in *P. vivax* has not yet been documented.

Methods

This study was conducted in an area of low seasonal malaria transmission along the northwestern border of Thailand. In Maela refugee camp, the Shoklo Malaria Research Unit (SMRU) encouraged all pregnant women to attend weekly antenatal clinics (ANC) to detect and treat all parasitemic episodes.

Patients

Pregnant women and nonpregnant women of child-bearing age with microscopically confirmed *P. vivax* infection, fever, or a history of fever, who provided informed consent, were invited to participate. Otherwise healthy pregnant women with a gestational age ≥ 20 weeks and ≤ 32 weeks and a hematocrit of > 25% were enrolled. A full medical history and examination was carried out by a physician and a midwife. Complete blood count, blood glucose, blood group, and parasite count were measured on admission.

Drug regimen

Twenty-five milligrams of CQ base/kg (Chloroquine®, ACDHON CO., Ltd, Bangkok, Thailand) was given over 3 days as 10, 10, and 5 mg/kg doses given strictly, under observation, at 0, 24, and 48 h.

Sampling

Women were admitted to the SMRU inpatient department. A heparinized cannula was inserted into an antecubital vein, and a blood sample (1 ml) was taken immediately before treatment and then at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 h. On days 2, 3, 7, 14, 21, 28, 35, and 42, 1-ml samples were obtained by direct venous sampling. Samples included baseline hematology and biochemistry and follow-up hematology on days 0, 3, 7, 14, 28, and 42.

Ethical review

The study was approved by the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; the Karen Refugee Committee in Mae Sod, Thailand; and the ethics committee of the London School of Hygiene and Tropical Medicine, London, UK.

Follow-up

Malaria smears and axillary temperature measurements were taken daily. After parasite clearance, women were seen weekly and followed up for 6 weeks. If *P. vivax* malaria recurred, the patient was retreated with CQ. All cases of *P. falciparum* malaria were treated with quinine or artesunate [2].

Drug assays

A liquid/liquid extraction method using N-hexane/isopropyl alcohol and sodium hydroxide (NaOH), previously validated in our lab, was applied to whole-blood samples (100 µl); 4-aminoquinaldine was used as internal standard, and CQ and desethylchloroquine (DEQ) were quantified using fluorescence detection (excitation 335 nm; emission 390 nm). Compound separation was performed on a silica stationary phase (Waters 3.9×390 nm) using a mobile phase composed of acetonitrile/methanol and triethylamine. Linearity, interday, and intraday variability of the method were validated. None of the coefficients of variation for CQ and DEQ was above 10% (data not shown). The lower limits of quantification (LLOQ) for CQ and DEQ were 15 ng/ml whole blood.

Pharmacokinetic analysis

PK parameters of CQ and its main metabolite, DEQ, were determined using noncompartmental analysis. Peak whole-blood concentrations and t_{max} were estimated from the first 24 h data. If levels were below the LLOQ, then the terminal phase was characterized by a log linear fit to the last five time points. A sensitivity analysis was conducted by assessing the area under the curve (AUC) in two other ways: by setting all concentration measurements that were not detected to 0 (i.e., assuming clearance of all CQ) and by comparing AUCs that were calculated from 0 to the maximum value of time available. Results were the same regardless of how the "not detected" measurements were managed. Therefore, only results using interpolated values are reported.

Statistical analyses

Chi-square or Fisher's exact test was used for categorical data. Student's *t* test and the Mann–Whitney *U* test were used for continuous data. Days 28 and 42 cure rates are presented as Kaplan–Meier estimates, and differences between groups were compared using the log rank test or the Wilcoxon-Breslow-Gehan test of equality if the survival lines crossed. Univariate analyses were conducted to check for potential covariates. Variables that had a significant effect on AUC were included in an analysis of variance (ANOVA) that tested for any differences between pregnant and nonpregnant women. All analyses were carried out using STATA, version 9.0 (StataCorp LP, TX, USA).

Results

From 2 March 1998 to 10 August 1998, 12 pregnant women and 15 nonpregnant Karen women of child-bearing age with acute vivax malaria consented to participate. Two nonpregnant women withdrew before completion of the study; their results were not analyzed. Another woman, initially screened as nonpregnant, had a positive pregnancy test on day 35 (i.e., she had been enrolled at 2.4 weeks after her last menstrual period). Her results were analysed in the nonpregnant group as originally assigned. Two women gave birth before 42 days (days 23 and 28). No postpartum samples were analyzed. On admission, there were no significant differences in demographic characteristics between pregnant and nonpregnant women (Table 1).

Pregnant women had lower mean hematocrit, lymphocyte count, and biochemical parameters (i.e., creatinine, blood urea nitrogen (BUN), and albumin) than did nonpregnant women (Table 1), consistent with the physiological changes of pregnancy. The higher bilirubin levels in nonpregnant women may indicate they had more serious malaria infections [12, 13]. Chloroquine was well tolerated and rapidly effective. The cure rate at day 28 (n=25) was 100% (95% confidence interval (CI) 86.7–100). The day 42 cure rate was comparable in pregnant [83.3% (95% CI 48.2–95.6)], and nonpregnant [69.2% (95% CI 37.3–87.2)] women (p=0.52).

Pharmacokinetics of chloroquine

Eight women had late measurements that were below the LLOQ (one pregnant, two values; seven nonpregnant, 12 values). Therefore, these values were extrapolated from the log linear fit to the available concentration measurements from the last five time points. In the univariate and multivariate analyses, for $AUC_{0\rightarrow3}$, there was no difference between pregnant and nonpregnant women (p=0.32) (Fig. 1, Table 2). There was no effect of hematocrit (p=0.11), BMI (p=0.60), or parasitemia (p=0.27) on $AUC_{0\rightarrow3}$. Fever and higher temperatures were associated with lower $AUC_{0\rightarrow3}$ values (p=0.02 and p = 0.001, respectively). Among pregnant women, there was no effect of smoking (p=0.15) or estimated gestational age on $AUC_{0\rightarrow3}$ (Spearman's rho=−0.29, p=0.37). There was no difference in $AUC_{0\rightarrow\infty}$ between pregnant (n=11) and nonpregnant (n=11) women (p=0.99) (Fig. 1); on average, the difference in (log) CQ $AUC_{0\rightarrow\infty}$ between pregnant and nonpregnant women was only 0.2% (95% CI −0.28 to 40). A lower $AUC_{0\rightarrow\infty}$ was associated with higher temperature (p=0.006) in the univariate analysis but not with hematocrit (p=0.91), BMI (p=0.36), or parasitemia (p=0.21). Adjusting the model for hematocrit, platelet count, bilirubin, and temperature made no difference to $AUC_{0\rightarrow\infty}$ between the groups (p=0.26). For pregnant women (n=11), there was

Table 1 Admission demographics and laboratory variables of 12 pregnant and 13 nonpregnant Karen women with acute *Plasmodium vivax* malaria

	pregnant	nonpregnant	*P* value
Median age (range), years	25 (15–37)	29 (15–40)	0.43
Median gravidity (range)	3 (1–10)	2 (0–8)	0.39
Median parity (range)	2 (0–6)	1 (0–8)	0.96
Mean weight (SD), kg	49.5 (5.6)	46.0 (4.9)	0.11
Mean BMI (SD), kg/m^2	21.9 (2.5)	20.0 (2.8)	0.10
Median temperature (range), °C	37.2 (36.5–40)	37.3 (36.5–40)	0.11
Percent febrile (*n*)	9.1 (1/11)[a]	38.5 (5/13)	0.17
Percent with headache (*n*)	66.7 (8/12)	100 (13/13)	0.039
Percent with rigors (*n*)	0	38.5 (5/13)	0.039
Percent with anorexia (*n*)	33.3 (4/12)	76.9 (10/13)	0.047
Percent vomiting (*n*)	0	30.8 (4/13)	0.096
Median estimated gestational age (range), weeks	27.4 (23.0–37.1)	-	-
Geometric mean (SD) parasitemia, /μl	244 (13.3)	1776 (15.2)	0.08
Mean platelet count (SD), /μl	160.8 (39.2)	129.5 (40.2)	0.06
Mean hematocrit (SD), %	31.2 (2.9)	37.1 (4.5)	0.0005[b]
Mean white blood cell count (SD), /μl	10.8 (3.0)	9.1 (3.7)	0.23
Mean lymphocytes (SD), %	30 (9.1)	42 (10.4)	0.006
Median bilirubin (range), mg/dl	0.19 (0.08–2.00)	0.53 (0.13–2.20)	0.009
Mean creatinine (SD), mg/dl	0.66 (0.16)	0.78 (0.14)	0.05
Mean BUN (SD), mg%	7.17 (2.4)	10.38 (3.8)	0.02
Mean albumin (SD), g%	3.13 (0.5)	3.69 (0.4)	0.009

BMI body mass index, *BUN* blood urea nitrogen

[a] One pregnant woman was missing temperature, [b] although the distribution did not deviate significantly from normal, the Mann–Whitney *U* test was used because of unequal standard deviations (SD)

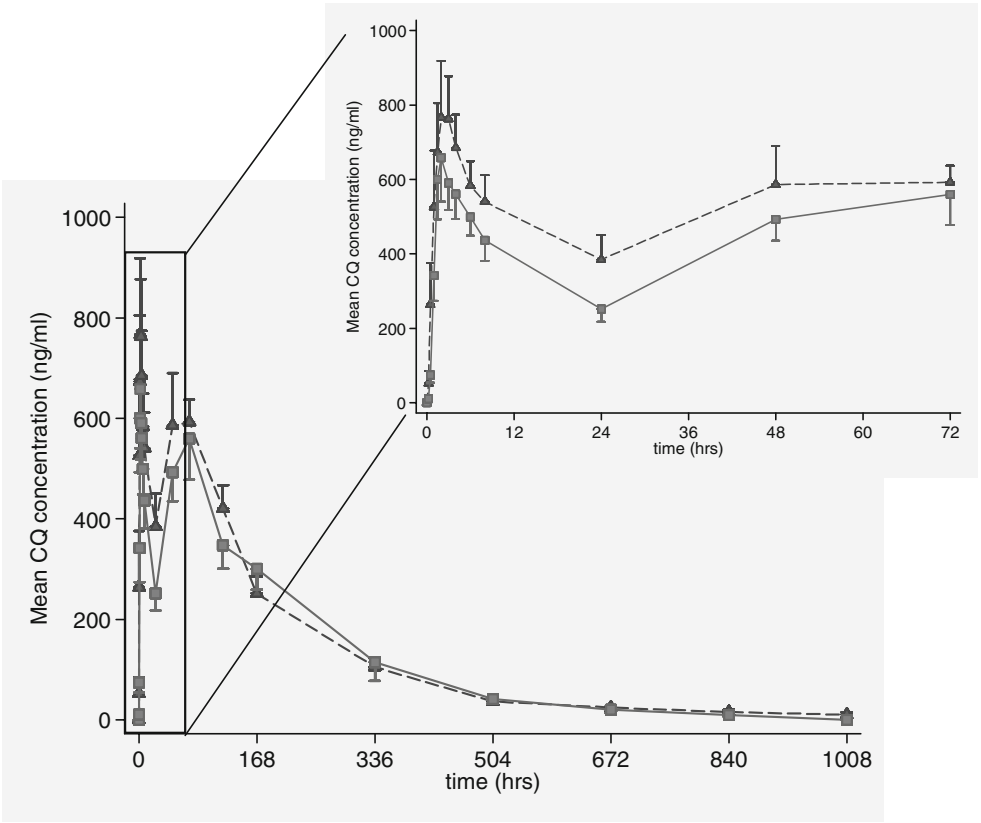

Fig. 1 Comparison of mean [standard error (SE)] whole-blood chloroquine concentrations in pregnant (*dashed line, triangle markers*) and nonpregnant (*solid line, square markers*) women with acute vivax malaria from day 0 to day 3 (*inset*) to day 42

no effect of smoking (p=1.0) on $AUC_{0\rightarrow\infty}$, but a higher gestational age at entry to the study was associated with a lower $AUC_{0\rightarrow\infty}$ (Spearman's rho=−0.67 p=0.02).

Pharmacokinetics of desethylchloroquine

Two women (both pregnant) had no DEQ measurements, which left ten pregnant and 13 nonpregnant women for comparison. C_{max} and t_{max} did not differ between pregnant and nonpregnant women (p=0.29 and p=0.33, respectively). The ratio of CQ to DEQ $AUC_{0\rightarrow3}$ was also not different [median 4.89 (range 3.21–6.28) and 4.39 (2.34–8.77), respectively; p=0.66]. In the univariate analysis, the CQ to DEQ $AUC_{0\rightarrow3}$ ratio was significantly correlated with parasitemia on admission (p= 0.003) but not with temperature (p=0.22), fever (p=0.14), or BMI (p=0.50). Inclusion of parasitemia in the model made no difference in the CQ to DEQ $AUC_{0\rightarrow3}$ ratio between pregnant and nonpregnant women (p=0.70).

Table 2 Comparison of noncompartmental pharmacokinetic data for chloroquine from day 0 to day 42 in 12 pregnant women and 13 nonpregnant women of child-bearing age with acute vivax malaria. Values are medians (range)

	pregnant	nonpregnant	*P* value
$t_{1/2\ elim}$ (h)*	180.4 (139.4, 926.1)	209.0 (116.5, 1794.0)	0.82
k (h^{-1})*	0.004 (0.0007, 0.0050)	0.003 (0.0004, 0.006)	0.82
C_{max} (ng/ml)**	960.5 (297, 1835)	700 (403, 1625)	0.55
t_{max} (h)**	3 (1.5, 8)	2 (1.5, 6)	0.17
$AUC_{0\rightarrow\infty}$ (ng/ml · h)*	122 216 (74 145, 269 600)	134 087 (62 940, 229 695)	0.78
CL/F (ml/h/kg)*	209.4 (95.0, 349.1)	189.1 (110.4, 406.6)	0.77
$AUC_{0\rightarrow3}$ (ng/ml · h)	33 086 (16 806, 89 285)	27 036 (13 197, 68 040)	0.28

$t_{1/2\ elim}$ elimination half-life, k elimination rate constant, C_{max} peak whole blood concentration, t_{max} time to reach C_{max}, AUC area under the whole-blood concentration-time curve, CL/F apparent clearance

* n=11 for pregnant women because one patient had a higher chloroquine concentration at day 42 than day 35, therefore an AUC based on a log linear fit of the terminal values could not be calculated; n = 11 for nonpregnant women because 2 had levels of chloroquine below the lower limits of quantification (LLOQ) for the last 4 time points

**Calculated using measurements from the first 24 h only

DEQ levels were reliably detected for 14 days. There was no difference in median $AUC_{0\rightarrow14}$ or the median ratio of CQ to DEQ $AUC_{0\rightarrow14}$ between pregnant and nonpregnant women [for median $AUC_{0\rightarrow14}$: 29,841 (range 17,533–46,849) vs. 29,556 (10,145–64,102), $p=0.65$ and 3.78 (2.42–5.14) vs. 3.50 (2.47–5.48), $p=0.41$ for median CQ:DEQ ratio]. The CQ:DEQ $AUC_{0\rightarrow14}$ ratio was not associated with temperature ($p=0.72$), parasitemia ($p=0.56$), fever ($p=0.42$), or BMI ($p=0.86$) at admission. In pregnant women ($n=10$), the CQ:DEQ $AUC_{0\rightarrow14}$ ratio did not correlate significantly with smoking ($p=0.09$) or estimated gestational age ($p=0.49$) and did not correlate with the CQ $AUC_{0\rightarrow14}$ (Spearman's rho=0.08, $p=0.75$).

Discussion

This study found no significant differences in the pharmacokinetic parameters of CQ and its main metabolite DEQ in pregnant women and nonpregnant women treated for acute vivax malaria. Lower CQ $AUC_{0\rightarrow\infty}$ and $AUC_{0\rightarrow3}$ values were associated with fever on admission, and a lower $AUC_{0\rightarrow\infty}$ was significantly associated with greater estimated gestational age. There was also no difference in the DEQ $AUC_{0\rightarrow3}$, $AUC_{0\rightarrow14}$, or CQ:DEQ $AUC_{0\rightarrow14}$ ratio between pregnant and nonpregnant women, although there was wide interindividual variation in the concentration measurements between women in both groups.

Previous studies of antimalarial drug pharmacokinetics have reported lower plasma concentrations in pregnant women for mefloquine, sulfadoxine, dihydroartemisinin, artemether, lumefantrine, atovaquone, proguanil, and cycloguanil [1—5]. CQ appears different in that its pharmacokinetic properties are not significantly affected by pregnancy, although there was evidence that AUC values decreased as gestation increased, compatible with a pregnancy-related expansion in the apparent volume of distribution (Vd). CQ has unusual pharmacokinetic properties. It has very long terminal half-life and an enormous Vd [14, 10, 11]. Previous pharmacokinetic studies in pregnancy measured plasma concentrations, which has limitations, as CQ is concentrated in platelets and granulocytes and then variably released from these cells in blood samples [15]. On the other hand, because large within-group variations can result in an apparent small between-groups variation, the wide interindividual variation in CQ concentrations can make it more difficult to detect moderate to small differences between groups.

A previous study reported higher plasma DEQ concentrations in pregnant women compared with nonpregnant Nigerian women [11]. Our study found no significant differences in DEQ for median peak whole-blood concentration, time-to-peak concentration, $AUC_{0\rightarrow3}$, or the CQ:DEQ $AUC_{0\rightarrow14}$ ratio among pregnant and nonpregnant Karen women. The CQ:DEQ $AUC_{0\rightarrow14}$ was also not correlated with CQ $AUC_{0\rightarrow14}$ values, which suggests that metabolic biotransformation was not a major determinant of CQ clearance.

In conclusion, in this study of Karen women with *P. vivax* malaria, pregnancy did not significantly affect blood concentrations of CQ or its metabolite, DEQ, when compared with nonpregnant women. Weight-based standard doses are appropriate for treating malaria in pregnancy.

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