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MAJOR ARTICLE

Piperaquine-Induced QTc Prolongation Decreases With Repeated Monthly Dihydroartemisinin-Piperaquine Dosing in Pregnant Ugandan Women

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Background. Intermittent preventive treatment with monthly dihydroartemisinin-piperaquine (DHA-PQ) is highly effective at preventing both malaria during pregnancy and placental malaria. Piperaquine prolongs the corrected QT interval (QTc), and it is possible that repeated monthly dosing could lead to progressive QTc prolongation. Intensive characterization of the relationship between piperaquine concentration and QTc interval throughout pregnancy can inform effective, safe prevention guidelines.

Methods. Data were collected from a randomized controlled trial, where pregnant Ugandan women received malaria chemoprevention with monthly DHA-PQ (120/960 mg DHA/PQ; n = 373) or sulfadoxine-pyrimethamine (SP; 1500/75 mg; n = 375) during the second and third trimesters of pregnancy. Monthly trough piperaquine samples were collected throughout pregnancy, and pre- and postdose electrocardiograms were recorded at 20, 28, and 36 weeks' gestation in each woman. The pharmacokinetics–QTc relationship for piperaquine and QTc for SP were assessed using nonlinear mixed-effects modeling.

Results. A positive linear relationship between piperaquine concentration and Fridericia corrected QTc interval was identified. This relationship progressively decreased from a 4.42 to 3.28 to 2.13 millisecond increase per 100 ng/mL increase in piperaquine concentration at 20, 28, and 36 weeks' gestation, respectively. Furthermore, 61% (n = 183) of women had a smaller change in QTc at week 36 than week 20. Nine women given DHA-PQ had grade 3–4 cardiac adverse events. SP was not associated with any change in QTc.

Conclusions. Repeated DHA-PQ dosing did not result in increased risk of QTc prolongation and the postdose QTc intervals progressively decreased. Monthly dosing of DHA-PQ in pregnant women carries minimal risk of QTc prolongation.

Clinical Trials Registration. NCT02793622.

Keywords. intermittent preventive treatment for malaria in pregnancy; dihydroartemisinin-piperaquine; QTc prolongation; pharmacokinetic/pharmacodynamic modeling.

Malaria during pregnancy continues to pose serious health risks to both the mother and developing fetus [1, 2]. Malaria infection can cause maternal anemia, stillbirth, low birth weight, and infant death [2–4]. In Africa, an estimated 822 000 low-birthweight deliveries and 100 000 infant deaths are attributed to malaria each year in regions with moderate to high transmission [5, 6]. Low birth weight has been shown to affect individuals throughout their life and is a risk factor for infant morbidity and mortality as well as cardiovascular disease in adulthood [7–9]. To reduce the burden of malaria and adverse birth outcomes, the World Health Organization recommends the use of long-lasting insecticide-treated nets and intermittent preventive treatment during pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) [5]. Due to the spread of parasite resistance to SP and vector resistance to pyrethroid insecticides, malaria prevention with approved measures has become suboptimal in some areas, including throughout East Africa [10, 11]. A recent study in Uganda reported 50% (n = 49/98) of pregnant women had placental malaria after receiving insecticide-treated nets and IPTp-SP where 78% (n = 154/198) of the parasites detected carried the quintuple mutation (mutations at *pfdhfr* 51I, 59R, and 108N; *pfdhps* 437G and 581G), associated with decreased sensitivity to SP [12, 13]. New safe and effective malaria prevention methods are urgently needed.

Dihydroartemisinin-piperaquine (DHA-PQ), an artemisinin-based combination therapy, has been the focus of recent prevention studies [13–16]. Monthly prevention with DHA-PQ is highly efficacious and is an attractive alternative

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for malaria prevention [13, 14]. However, in clinical trials, piperaquine has been shown to prolong the corrected QT (QTc) interval at peak concentrations, raising safety concerns [17–19]. Additionally, during malaria treatment, higher parasite densities have been shown to lengthen the QTc interval, irrespective of treatment [20, 21]. Most studies have reported mild QTc changes after piperaquine treatment (~4 hours after the last dose), with values returning toward baseline within 7 days [22–25]. Although extremely rare in noncardiovascular drugs, severe QTc prolongation can lead to arrhythmias, including torsades de points, which can, in turn, lead to sudden cardiac death [26]. Piperaquine-associated QTc prolongation is concentration dependent and, given piperaquine's long half-life (~20–30 days), repeated dosing, as required during prevention, could lead to accumulation and elevated drug concentrations, potentially resulting in increased risk of QTc prolongation over time [25, 27]. However, the relationship between repeated dosing of piperaquine and QTc prolongation during long-term use of DHA-PQ has not been defined.

Pregnancy can independently affect both piperaquine pharmacokinetics (PK) and QTc measurements [25, 28, 29]. Previous studies have revealed that pregnancy lowers piperaquine exposure, and hormonal changes during pregnancy decrease QTc intervals [25, 28, 29]. Hence, understanding the longitudinal PK-QTc relationship will be needed to inform optimized DHA-PQ IPTp regimens. While most studies indicate that piperaquine is safe even after multiple doses, QTc measurements following repeated DHA-PQ dosing in pregnant women are scarce [23, 30–32]. In this study, we used repeated QTc measures from a large malaria prevention trial in pregnant women to develop a population PK-QTc model for piperaquine and a separate QTc model for women given SP.

METHODS

Study Design and Participants

Data analyzed originated from a placebo-controlled, double-blind, randomized trial in Busia District, Uganda, which compared monthly SP with DHA-PQ for malaria prevention during pregnancy. Eligible participants were human immunodeficiency virus (HIV)–uninfected pregnant women between 12 and 20 weeks' gestation confirmed by ultrasound with no history of antimalarial use during the current pregnancy. Complete eligibility criteria and main trial findings were previously published [14]. Women received all medical care at a dedicated study clinic and were encouraged to come to the clinic any time they felt ill.

All participants provided written informed consent. All procedures were approved by the ethics committees of the University of California San Francisco, Makerere University School of Biomedical Sciences, and the Ugandan Nation Council for Science and Technology. The clinical trial registration number is NCT02793622.

Routine visits occurred every 4 weeks, at which time participants received study drugs. Chemoprevention began at either 16 or 20 weeks' gestation, with each regimen given monthly: (1) SP was a single dose of 3 tablets (each 500 mg sulfadoxine and 25 mg pyrimethamine; Kamsidar, Kampala Pharmaceutical Industries, Kampala, Uganda) and (2) DHA-PQ was 3 tablets (each 40 mg dihydroartimisinin and 320 mg piperaquine; Duo-Cotexin, Holley-Cotec, Beijing, China) given once daily for 3 consecutive days. Women received placebos to control for regimen duration and number of tablets. For all participants, administration of the first dose of study drug was directly observed in the clinic with the second and third doses (DHA-PQ or placebo) taken at home. At 20, 28, and 36 weeks' gestation, when electrocardiograms (ECGs) were performed, the third daily dose of DHA-PQ or placebo was also directly observed in the clinic.

Laboratory Procedures

At each routine visit, women provided a pre-study drug trough blood sample to measure piperaquine concentrations (Figure 1). Venous samples were collected on weeks 20, 28, and 36. The remaining samples collected were capillary blood from a finger prick. Additionally, at weeks 20, 28, and 36, women received a single-trace 12-lead ECG on day 1, prior to study drug administration, and on day 3, 3–4 hours following the final DHA-PQ or placebo dose. A linear regression of the QTc and RR interval was plotted for the Fridericia and Bazett corrections (Supplementary Figure 1). The QTc interval is reported using the Fridericia formula ($QTcF = \frac{QT}{\sqrt[3]{RR}}$), as this minimized the influence of heart rate. The change in QTc interval (ΔQTcF: postdose QTcF − predose QTcF) was calculated for each of the 3 ECG occasions.

Piperaquine Quantitation

Three routine trough samples from women who received DHA-PQ were randomly selected from each participant for piperaquine concentration quantitation. In half of the women, 2 samples from the second trimester and 1 from the third were selected, and in the remaining half, 1 sample from the second trimester and 2 from the third trimester were selected. In addition, for a separate analysis, piperaquine concentrations were also quantitated when malaria (fever with parasitemia by microscopy) or asymptomatic parasitemia (by quantitative polymerase chain reaction [PCR]) was diagnosed as part of the parent trial and were included in the present analysis [14].

Blood samples for piperaquine quantitation were centrifuged at 2000 *g* for 10 minutes within 60 minutes of being collected. Plasma was stored at −80°C until analysis. Two high-performance liquid chromatography–tandem mass spectrometry methods were used for piperaquine quantitation [33]. The calibration ranges were 10–1000 ng/mL and 0.5–50 ng/mL, with 0.5 ng/mL as the lower limit of quantitation (LLOQ). The

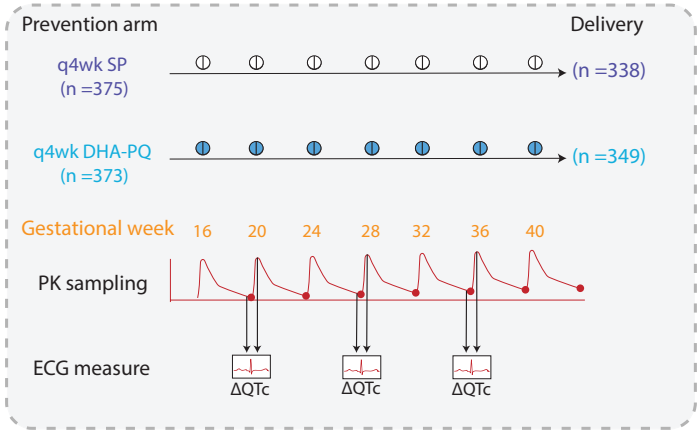

Figure 1. Summary of trial procedures. Tablets indicate when each prevention course of DHA-PQ and SP were provided. The dots indicate when plasma sampling for piperaquine PK occurred relative to the expected PK profile. The arrows indicate when ECGs were recorded relative to the expected PK profile and PK sample collection. The number of participants listed reflects those who received at least 1 course of prevention. Abbreviations: DHA-PQ, dihydroartemisinin-piperaquine; ECG, electrocardiogram; PK, pharmacokinetics; QTc, corrected QT interval; q4wk, every-4-week dosing regimen; SP, sulfadoxine-pyrimethamine.

inter- and intraassay coefficient of variation (CV) was less than 10% for all quality-control samples.

Population PK and QTc Modeling

All data were analyzed by nonlinear mixed-effects modeling using NONMEM VII (Icon Development Solutions, Ellicott City, MD). All parameters were estimated using the first-order conditional estimation with interaction algorithm. Only 6 (0.5%) PK samples fell below the LLOQ and were excluded from the analysis. Given that only trough samples were available, the model structure and parameter estimates from a piperaquine population PK model developed from pregnant Ugandan women (n = 200) who received DHA-PQ for malaria prevention in a nearby district were used as the prior base model [32]. This included an established linear equation ($C_{cap} = 1.35 \times C_{ven} - 0.34$) to account for differences between capillary and venous concentrations.

A stepwise covariate (SCM) search was performed to identify any influence of patient and clinical characteristics on PK parameters, including age, weight, body mass index, gestational weeks, trimester, gravidity, monthly parasite density (both a continuous and binary variable), and body temperature. Linear and nonlinear relationships were investigated, including allometric scaling. A significance cutoff of $P < .05$ was applied for forward inclusion, followed by a cutoff of $P < .01$ for backwards elimination.

The relationship between piperaquine concentration and absolute QTcF interval was modeled simultaneously. A separate model without PK data was constructed for the QTcF data from women who received SP. As described above, an SCM analysis was performed for the QTc parameters. In addition to the covariates listed above, the predose QTc, piperaquine concentration, and dose were tested.

Model selection was guided by goodness-of-fit plots, the objective function value (OFV), parameter estimates, and relative standard error values. Simulation-based diagnostics such as visual predictive checks (VPCs; n = 500) and a nonparametric bootstrap (n = 500) were also performed to determine the model's predictive power and the robustness of parameter estimates.

RESULTS

Study Cohort and Data

A total of 373 and 375 women were enrolled and received at least 1 dose of DHA-PQ or SP, respectively (Table 1 and Figure 1). There were 1226 piperaquine trough concentrations available, with an average venous concentration of 11 ng/mL and capillary concentration of 15 ng/mL (Table 2 and Supplementary Figure 2). There was an average 3-ng/mL increase in piperaquine concentration over the course of pregnancy. Each QTcF model was built using 2070 and 1990 QTcF measurements from the DHA-PQ and SP arms, respectively (Table 2 and Figure 2). There were 19 women (QTcF measurements = 22) in the DHA-PQ arm and 12 women (QTcF measurements = 14) in the SP arm with QTcF measurements greater than 450 milliseconds. No woman was reported to have a QTcF value greater than 500 milliseconds. There were 9 women (ΔQTcF measurements = 9), all in the DHA-PQ arm, who had grade 3–4 adverse events due to ΔQTcF >60 milliseconds (maximum, 81 milliseconds), but no arrhythmias or cardiac symptoms were detected. In the DHA-PQ arm, the ΔQTcF was noted to decrease between each evaluation from 18.0 to 12.0 to 10.0 milliseconds at week 20, 28, and 36, respectively. Individual-level trends in the ΔQTcF were investigated and revealed 4 different trajectories (Figure 3). Only 30 (10%) women consistently had an increase in ΔQTcF, with 65 (22%) having a consistent decrease and the majority (n = 204; 68%) showing no consistent trend. Regardless of trajectory, 61% (n = 183) of women had a smaller ΔQTcF at week 36 compared with week 20. No clinically

Table 1. Demographic Characteristics at the Time of First Study Drug Administration

Characteristics	Prevention Arm	
	DHA-PQ	SP
n	373	375
Age, mean (95% percentile), years	23 (17–36)	24 (17–38)
Weight, mean (95% percentile), kg	55.0 (43.2–74.5)	55.8 (44.0–78.6)
Height, mean (95% percentile), cm	158 (147–169)	158 (147–171)
Body mass index, mean (95% percentile), kg/m^2	22.0 (17.6–29.0)	22.0 (18.3–29.8)
Hemoglobin, mean (95% percentile), g/dL	11.4 (8.9–13.8)	11.5 (8.7–13.7)
Anemic (hemoglobin <10 g/dL), n (%)	33 (9)	52 (14)
Gravidity, mean (range), n (%)	3 (1–9)	3 (1–9)
First	84 (22.5)	98 (26.1)
Second	101 (27.1)	81 (21.6)
Third or greater	188 (50.4)	196 (52.3)
Gestational weeks at enrollment, mean (range)	15 (12–20)	15 (12–20)
Study drug started at, n (%)		
16 weeks' gestation	234 (63)	221 (59)
20 weeks' gestation	139 (37)	154 (41)

Abbreviations: DHA-PQ, dihydroartemisinin-piperaquine; SP, sulfadoxine-pyrimethamine.

Table 2. Pharmacokinetic and Toxicity Data

Data	Gestational Weeks	DHA-PQ		SP	
		n	Value	n	Value
Pharmacokinetic					
Piperaquine concentration, mean (95% percentile), ng/mL					
Venous	20	176	9 (2–28)	…	…
Capillary	24	245	13 (3–41)	…	…
Venous	28	247	12 (3–43)	…	…
Capillary	32	236	16 (6–47)	…	…
Venous	36	237	12 (3–38)	…	…
Capillary	40	85	16 (5–35)	…	…
Average					
Venous	…	660	11 (2–38)	…	…
Capillary	…	566	15 (4–43)	…	…
Total samples	…	1232	…	…	…
Samples below the limit of quantification, n (%)	…	6	(0.5)	…	…
Toxicity					
QTcF, mean (95% percentile), milliseconds					
Predose	20	361	394 (354–438)	355	396 (356–436)
Postdose		354	409 (369–454)	343	398 (351–438)
Predose	28	349	396 (356–436)	341	395 (356–446)
Postdose		344	407 (365–446)	333	395 (360–442)
Predose	36	335	393 (355–429)	316	391 (355–442)
Postdose		327	402 (360–442)	302	391 (349–436)
Total samples	…	2070	…	1990	…
QTcF >450 milliseconds, n (%)					
Predose	…	4/1045	(0.4)	7/1012	(0.7)
Postdose	…	18/1025	(1.8)	7/978	(0.7)
ΔQTcF, mean (95% percentile), milliseconds	20	354	18.0 (−33.2 to 56.2)	343	0.0 (−44.5 to 44.8)
	28	344	12.0 (−33.7 to 52.0)	333	0.0 (−44.0 to 44.0)
	36	327	10.0 (−31.9 to 51.0)	302	−1.0 (−46.0 to 45.0)
Total samples	…	1025	…	978	…
ΔQTcF >60 milliseconds, n (%)	…	9/1025	(0.9)	0/978	(0.0)

QTc values are reported using the Fridericia correction. QTc = QT/RR$^{(1/3)}$. ΔQTcF: QTcF (postdose) – QTcF (predose).

Abbreviations: DHA-PQ, dihydroartemisinin-piperaquine; QTc, corrected QT interval; SP, sulfadoxine-pyrimethamine; …, not applicable.

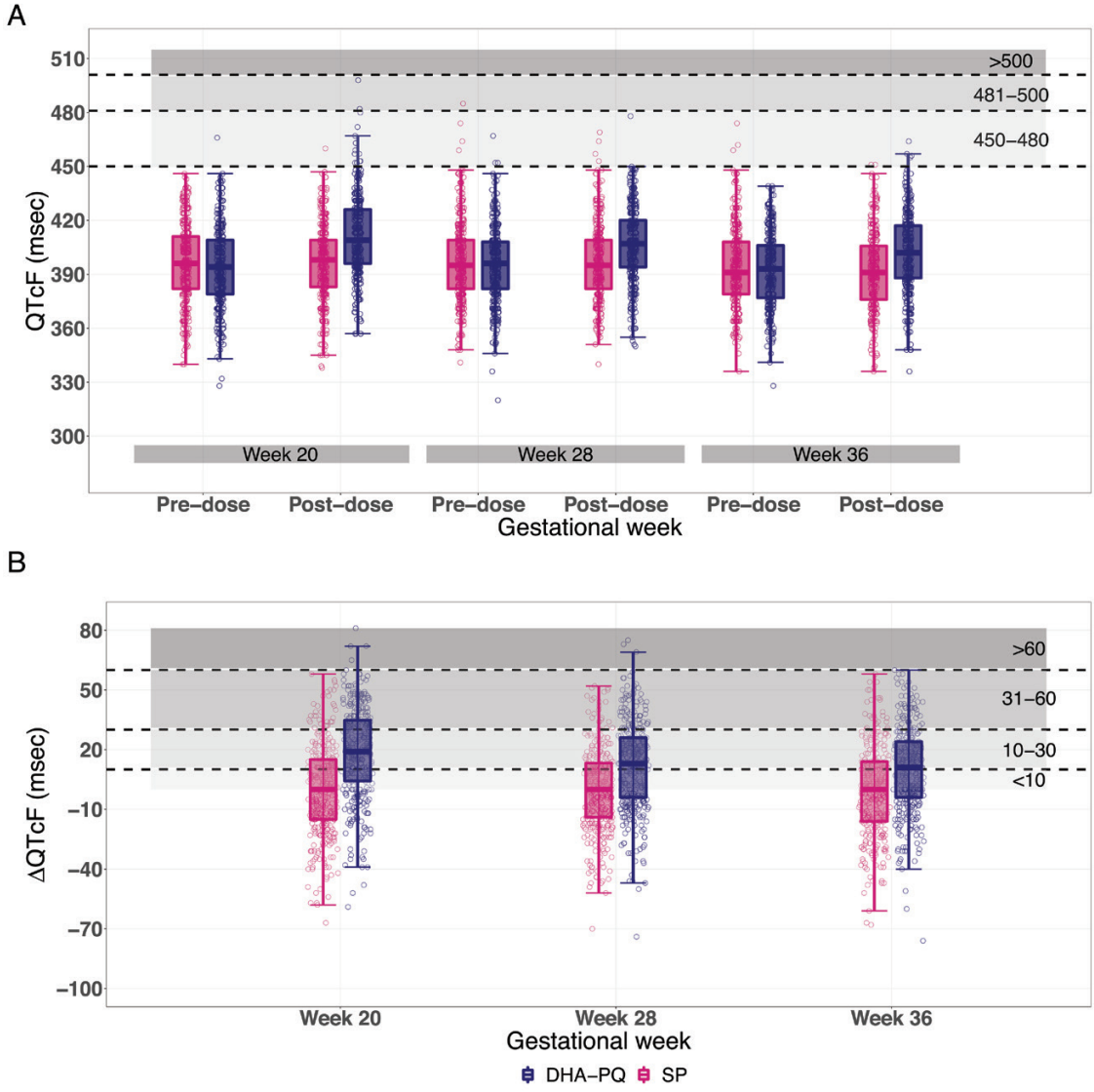

Figure 2. Absolute and change in Fridericia corrected QT interval (QTcF) stratified by prevention regimen and gestational week. *A*, QTcF pre- and postdose measurements for SP (left) and DHA-PQ (right), respectively. *B*, Change in QTcF interval. Points represent the observed data, boxes indicate 75% of the data, and bars indicate 95% of the data. Shaded regions indicate different cutoffs set by the FDA for grading the absolute and delta QTcF values [40]. Abbreviations: DHA-PQ, dihydroartemisinin-piperaquine; ECG, electrocardiogram; FDA, Food and Drug Administration; ΔQTcF, QTcF (postdose) – QTcF (predose); SP, sulfadoxine-pyrimethamine.

significant ΔQTcF was noted in the SP arm and values (−1.0 to 0 milliseconds) were consistent over pregnancy.

Population PK Model

A 2-compartment model provided an adequate fit of the PK data. Pharmacokinetic parameters were fixed to their respective values from the prior analysis to improve model stability [32], but additive and proportional errors (separate for venous and capillary samples), bioavailability, and inter-occasion variability on clearance were estimated (Table 3). Interindividual variability was included as a fixed value on the central volume compartment and the absorption rate. No covariates were identified from the SCM analysis. A VPC of the final model demonstrated satisfactory predictive performance (Figure 4A) and a bootstrap indicated good precision in parameter estimates (Table 3).

Population QTc Model

The final PK model was used to estimate piperaquine concentrations at the time of ECG recording. A significant positive linear relationship was identified between piperaquine concentration and absolute QTcF measurements (Figure 5). Interestingly, the extent of QTc prolongation decreased over time despite an increase in observed piperaquine trough and simulated maximum concentrations (Cmax) (Figure 3). The decreasing slope of the PK-QTc relationship was best captured by estimating 3 separate slope terms (OFV, −353; $P < .001$),

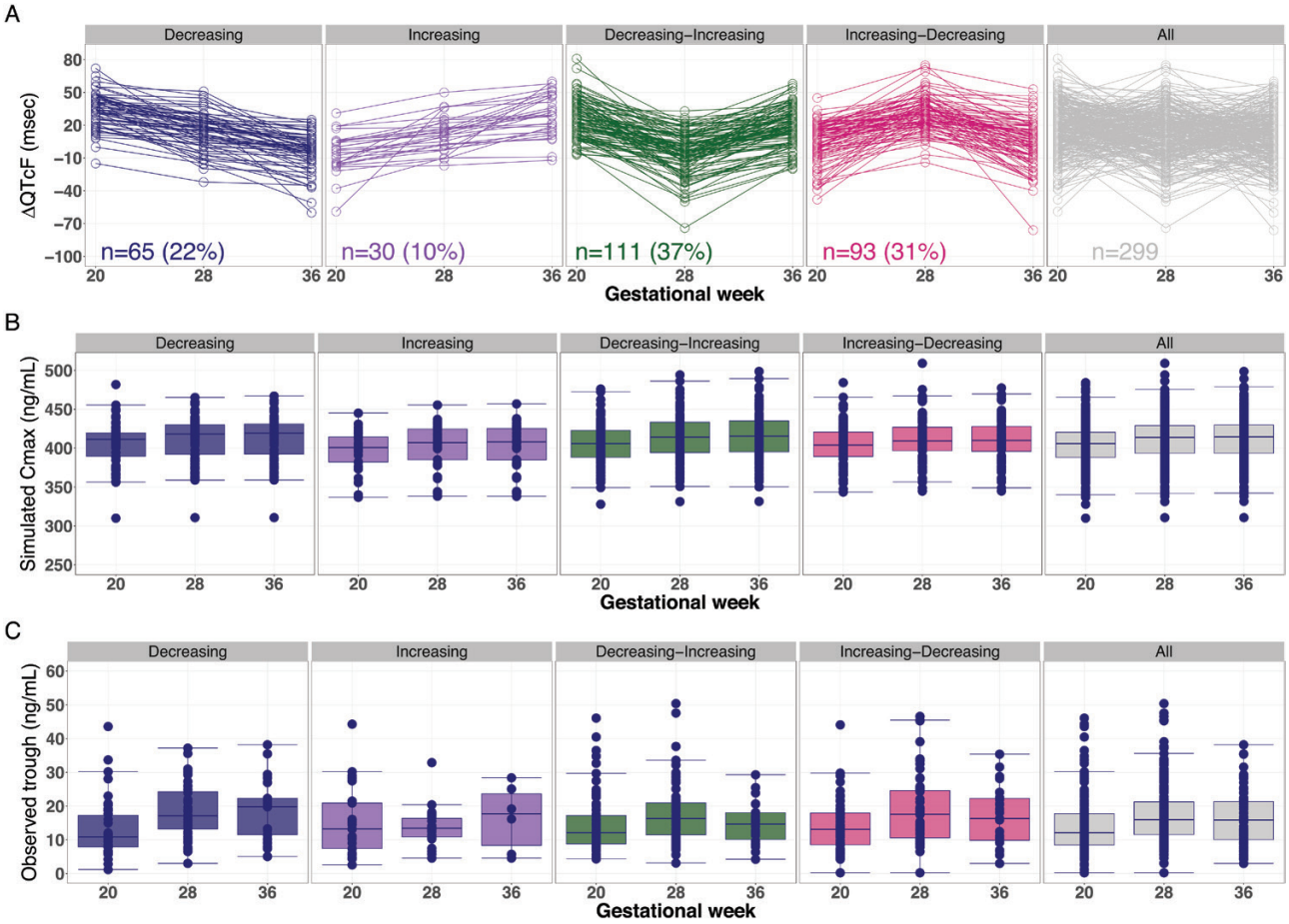

Figure 3. Individual-level data for women randomized to DHA-PQ. *A*, Individual-level trends in ΔQTcF measurements. Simulated Cmax (*B*) and observed trough (*C*) piperaquine concentrations. Data are stratified according to the 4 predominant ΔQTcF patterns. Each point represents the observed or simulated data. The piperaquine troughs shown in this figure are those from the preceding week (week 24, 32, 40) as they best reflect the concentrations for the dosing interval when the QTcF was recorded. Only women with all 6 QTcF measurements available were included in this plot. Nine women had different trends from the 4 main ones displayed and were excluded in the plot but included in the QTc model. Eight trough concentrations (>70 ng/mL) were included in the PK model but omitted from this plot as they obscured visualizing the data's central tendencies. QTc values were corrected using the Fridericia formula (QTcF). Abbreviations: Cmax, maximum concentration; DHA-PQ, dihydroartemisinin-piperaquine; PK, pharmacokinetics; QTc, corrected QT interval.

and no other covariate tested was able to explain this observation. At 20, 28, and 36 weeks' gestation, the model predicted a 4.42-, 3.28-, and 2.13-millisecond increase in QTcF per 100-ng/mL increase in piperaquine concentration, respectively (Table 3).

A separate model was built from the QTc measurements recorded in women given SP. No significant QTcF prolongation was detected and only 1 term was used to estimate pre- and postdose QTcF. We did find that 36 weeks' gestation was associated with a 4.0-millisecond shorter QTcF compared with 20 and 28 weeks; however, 4.0 milliseconds was not deemed clinically significant, and this relationship was not included in the final model. No other covariates were identified. Visual predictive check plots for both DHA-PQ and SP models demonstrated satisfactory predictive performance (Figure 4B and 4C, respectively) and a bootstrap indicated good precision in parameter estimates (Table 3).

DISCUSSION

In the setting of treatment and prevention, piperaquine has consistently been shown to prolong the QTc interval [32, 34]. Both linear and Emax (maximum effect) PK-QTc relationships have been observed for piperaquine [17, 32]. We previously showed among 30 Ugandan women at 28 weeks' gestational age that there was a linear PK-QTc relationship for piperaquine (5-millisecond increase in QTcF per 100-ng/mL increase in piperaquine concentration). In the current trial, at 28 weeks' gestation, our model estimated an increase of 3.28 milliseconds per 100-ng/mL increase in piperaquine concentration. While we noted a lesser extent of prolongation, both models identified a modest linear PK-QTc relationship. While it is possible that an Emax relationship exists for piperaquine during pregnancy, in this study, the predicted Cmax concentrations (308–526 ng/mL) were likely within the linear range of the function (EC50 [half maximal effective concentration] of 209 ng/mL and Emax of 35 milliseconds) [17].

Table 3. Final Model Parameter Estimates

Parameter Estimates	DHA-PQ, Median (95% CI)		SP, Median (95% CI)	
	Population Estimate	Interindividual Variability/ Inter-Occasion Variability	Population Estimate	Interindividual Variability
Piperaquine pharmacokinetic parameters				
Clearance, L/days	3204[a]	15.8% (14.3–17.5%)[b]	…	…
Volume central compartment, L	5302[a]	56.6%	…	…
Volume peripheral compartment, L	33 584[a]	…	…	…
Absorption rate, $days^{-1}$	17.5[a]	40.2%	…	…
Intercompartmental clearance, L/days	2023[a]	…	…	…
Bioavailability, %	1.67 (1.58–1.75)	…	…	…
Ratio, venous/capillary	1.35[a]	…	…	…
Intercept, venous/capillary	-0.34[a]	…	…	…
Proportional error				
Venous samples	37.3% (32.4–43.8%)	…	…	…
Capillary samples	38.2% (35.2–41.7%)	…	…	…
Additive error, ng/mL	2.86 (0.33–4.0)	…	…	…
Piperaquine pharmacodynamic parameters				
Predose QTcF, milliseconds	393 (391–395)	3.18% (2.9–3.42%)	…	…
Slope of concentration-dependent effect of QTcF, milliseconds × mL/ng				
Gestational week 20	0.0442 (0.0387–0.0491)	…	…	…
Gestational week 28	0.0328 (0.0279–0.0376)	…	…	…
Gestational week 36	0.0213 (0.0159–0.0264)	…	…	…
Proportional error	4.2% (4.1–4.4%)	…	…	…
SP pharmacodynamic parameters				
QTcF, milliseconds	…	…	395 (394–397)	3.3% (3.0–3.6%)
Proportional error	…	…	4.4% (4.2–4.6%)	…

All parameters estimated are reported as the oral parameter estimates (ie, CL/F; V/F, etc).

Abbreviations: CI, confidence interval; DHA-PQ, dihydroartemisinin-piperaquine; QTcF, Fridericia corrected QT interval; SP, sulfadoxine-pyrimethamine.

[a]Parameter value fixed.

[b]Inter-occasion variability.

Perhaps our most interesting finding was the population decrease in the PK-QTcF relationship after repeated DHA-PQ courses without a decrease in observed trough or simulated Cmax piperaquine concentrations (Figure 5). Individual ΔQTcF profiles showed that 22% of women had a consistent decrease in prolongation over time and 61% of women had a smaller ΔQTcF at week 36 compared with week 20 (Figure 3). A similar observation was reported in healthy volunteers where sotalol concentrations and QTc measurements were recorded following a single and 7 doses [35]. Repeated doses lead to an increase in sotalol concentration but a decrease in QTc interval in comparison to concentration and QTc values after a single dose. One other study in healthy volunteers from Papua New Guinea also measured monthly ECGs among participants receiving malaria prevention with DHA-PQ [23]. While no information on piperaquine pharmacokinetics was available, the ΔQTcF values for the first and last months decreased from a median of 19.6 to 17.1 milliseconds, and similar to our study, the predose QTc values for the second and third ECGs did not differ significantly from the initial predose values. Together, these data support the conclusion that repeated DHA-PQ doses in healthy participants including pregnant women do not increase the risk of QTc prolongation.

The mechanisms underlying the decreasing PK-QTc relationship we observed are unknown. One hypothesis is that, with inhibition of hERG (human ether-a-go-go-related gene) channels, other cardiac potassium channels are upregulated [35]. Although piperaquine is known to inhibit hERG potassium channels [18], no studies have investigated piperaquine's effect on cardiac ion channel expression. In vivo studies to evaluate the underlying mechanisms behind ΔQTcF shortening are warranted.

Another potential contributor to the shortened PK-QTc relationship is that hormonal changes during pregnancy decreased the QTcF [29, 36, 37]. A study investigated QTc prolongation in pregnant women on the Thailand-Myanmar border given DHA-PQ, artesunate-mefloquine, artemether-lumefantrine, or chloroquine for malaria treatment [21] found that higher gestational age was associated with a shorter QTc (−0.40 milliseconds/gestational week) [21]. It is believed that this pregnancy effect may be the result of increasing progesterone levels/ratios, a hormone reported to shorten the QTc interval during pregnancy [29, 36, 37]. However, if a hormone effect was the only factor, we would expect the effect of progesterone to shift the absolute QTcF values both pre- and postdose in the DHA-PQ arm and for women who received SP, rather than alter the slope

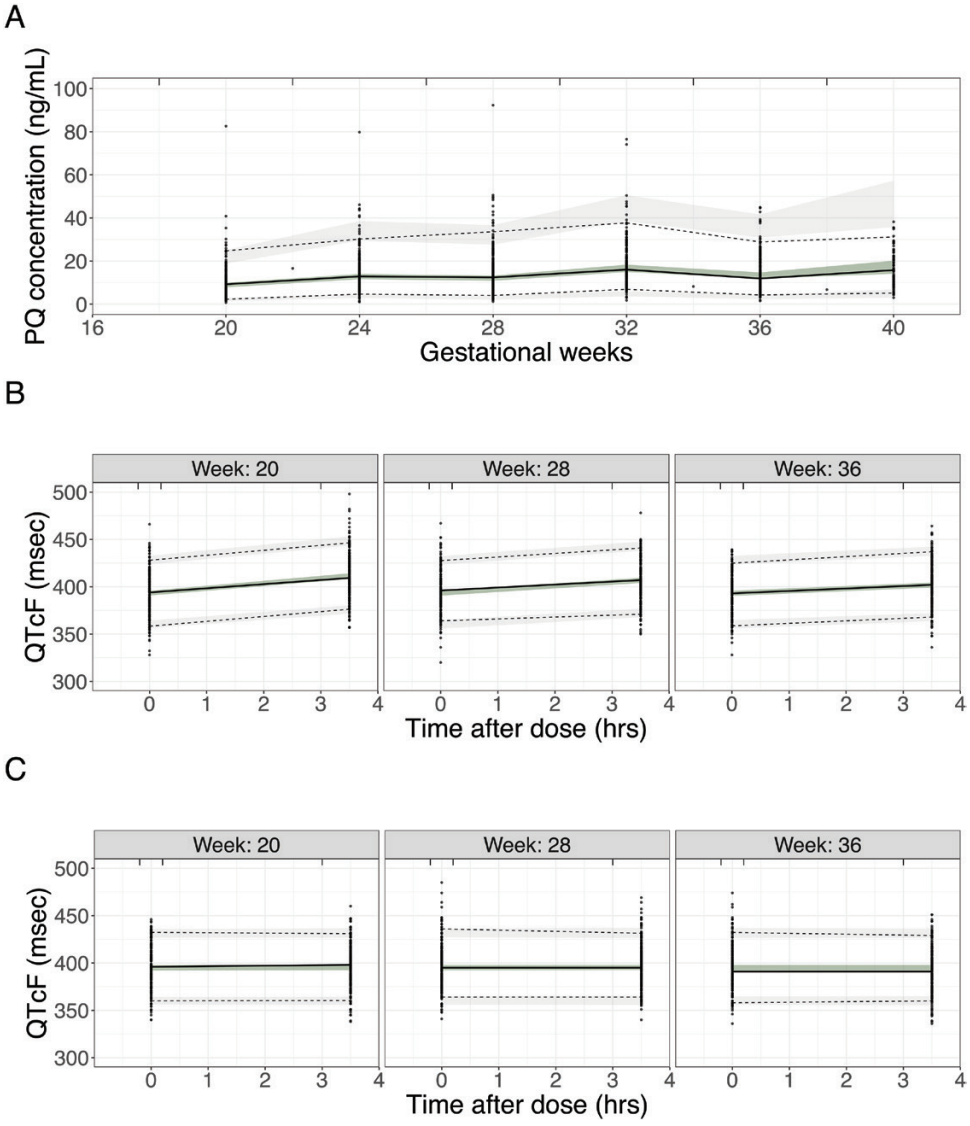

Figure 4. Visual predictive check (VPC) of the DHA-PQ final PK (*A*), PK-QTcF (*B*), and SP QTcF model (*C*). The black circles represent the observed data. The solid line indicates the median of the observed data, and the dashed lines indicate the 5% and 95% confidence intervals of the observed data. The shaded areas indicate 5%, 50%, and 95% of the simulated data. QTc values were corrected using the Fridericia formula (QTcF). Panel *B* is a prediction-corrected VPC plot. Three trough concentrations (>100 ng/mL) were included in the PK model but omitted from panel *A* as they obscured visualizing the data's central tendencies. Abbreviations: DHA-PQ, dihydroartemisinin-piperaquine; PK, pharmacokinetics; QTc, corrected QT interval; SP, sulfadoxine-pyrimethamine.

of the PK-QTc relationship. Hence, it is likely that other mechanisms are also involved. In the QTcF model for women given SP there was a 4-millisecond decrease in QTcF at 36 weeks' gestation in comparison to weeks 20 and 28. This relationship was not included in the final model. However, this small change may reflect the differences due to progesterone that others have noted.

A limitation of this study is that only pregnant women were enrolled with ECGs recorded beginning at 20 weeks' gestation. It is possible that the lack of a nonpregnant comparator group prevented us from identifying gestational weeks as a covariate. While understanding if pregnancy effects the QTc interval is important, given that pregnancy is associated with a shortening of the QTc interval, it is unlikely to change the conclusion that repeated dosing of DHA-PQ is safe during pregnancy. However, caution should be taken when extrapolating these findings prior to 20 weeks' gestation. Additionally, all ECG values were measured by a single trace. While this could add variability to our data it is unlikely to fully explain the trends we detected. Piperaquine concentrations were not available at the time the peak ECG was recorded. It is possible that there were trends in the Cmax concentrations not captured by modeling trough piperaquine concentrations. To account for any possible changes to the PK profile, we performed a comprehensive covariate search. The covariate search did not identify any clinically significant factors altering the PK profile or QTc profile for DHA-PQ or SP. Additionally, most piperaquine studies conducted in adults have not reported covariate effects [16, 27, 32, 38, 39]. Only the

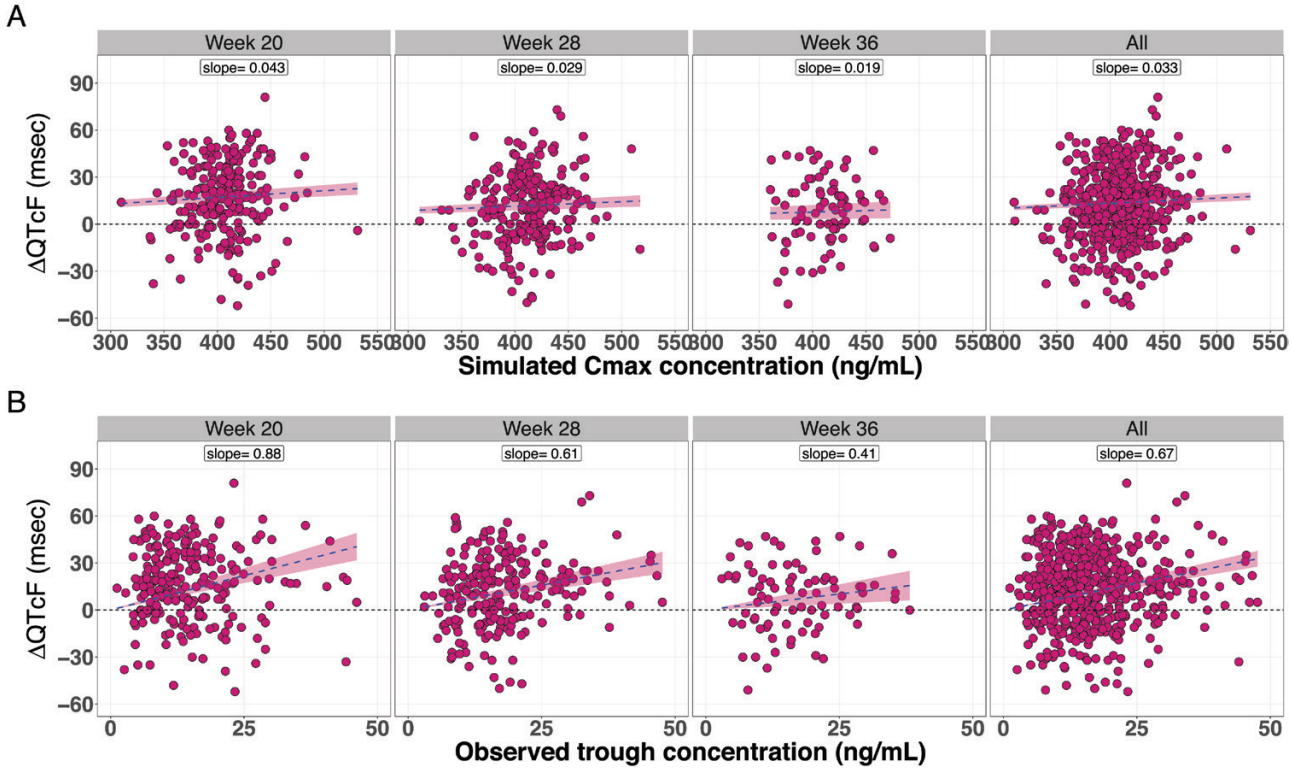

Figure 5. Linear regression of ΔQTcF and piperaquine concentration showing decreasing regression slope with increasing gestation age. Model-estimated piperaquine concentrations at the time of ECG recording (Cmax) (*A*) and observed trough concentrations (*B*). The piperaquine troughs shown in this figure are those from the preceding week (week 24, 32, 40) as they best reflect the concentrations for the dosing interval when the QTcF was recorded. Only the subset of women with PK samples available for the respective gestational weeks were included in this plot. Each point is an individual's observed (*B*) or simulated (*A*) data. The slope is displayed as the mean regression line (dashed line) and 95% confidence interval (shaded region). Eight trough concentrations (>70 ng/mL) were included in the PK model but omitted from this plot as they obscured visualizing the data's central tendencies. QTc values were corrected using the Fridericia formula (QTcF). Abbreviations: Cmax, maximum concentration; DHA-PQ, dihydroartemisinin-piperaquine; ECG, electrocardiogram; PK, pharmacokinetics; QTc, corrected QT interval.

first dose of DHA-PQ was directly observed each month and it is possible that some women were not fully adherent. While nonadherence may have occurred, we found that piperaquine trough concentrations increased over pregnancy and the concentrations we report are consistent if not higher than previous studies, suggesting that adherence likely does not explain our findings [16, 32]. Last, while the peak ECG for each woman was consistently recorded 3–4 hours after the last dose for each of the 3 occasions, the exact time of day differed between women. It is possible that time of measurement affected the QTc interval. However, given the range of ECG timing, any influence likely added variability rather than consistent bias.

In conclusion, using a population approach to model repeated ECG and PK data from a large clinical trial, a positive linear relationship between piperaquine concentration and QTcF prolongation was identified. We showed that clinically, and by the PK-QTc relationship, QTcF prolongation was modest and unlikely to be a safety concern. Interestingly, the extent of piperaquine-induced QTc prolongation decreased throughout pregnancy. This finding could not be explained by any covariate or by the SP QTc model. Further studies are needed to investigate the underlying mechanisms behind this observation. Nevertheless, monthly DHA-PQ dosing for IPTp carries minimal risk of QTc prolongation and our findings suggest that DHA-PQ is a safe alternative to SP.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. E. H., E. W., P. J., P. J. R., G. D., F. A. and R. M. S. contributed to the analysis plan. R. K., T. O., A. K., and M. R. K. conducted the clinical trial and collected the data. E. H., P. J., and T. D. C. prepared the data for analysis. E. H. performed the modeling and simulation with support from E. W. and R. M. S. E. H. drafted the figures, tables, and manuscript with critical editorial support from E. W., R. K., P. J., P. J. R., G. D., F. A., and R. M. S.

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