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CLINICAL THERAPEUTICS

Modeling Prevention of Malaria and Selection of Drug Resistance with Different Dosing Schedules of Dihydroartemisinin-Piperaquine Preventive Therapy during Pregnancy in Uganda

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ABSTRACT Dihydroartemisinin-piperaquine (DHA-PQ) is under study for intermittent preventive treatment during pregnancy (IPTp), but it may accelerate selection for drug resistance. Understanding the relationships between piperaquine concentration, prevention of parasitemia, and selection for decreased drug sensitivity can inform control policies and optimization of DHA-PQ dosing. Piperaquine concentrations, measures of parasitemia, and *Plasmodium falciparum* genotypes associated with decreased aminoquinoline sensitivity in Africa (*pfmdr1* 86Y, *pfcrt* 76T) were obtained from pregnant Ugandan women randomized to IPTp with sulfadoxine-pyrimethamine (SP) or DHA-PQ. Joint pharmacokinetic/pharmacodynamic models described relationships between piperaquine concentration and the probability of genotypes of interest using nonlinear mixed effects modeling. An increase in the piperaquine plasma concentration was associated with a log-linear decrease in risk of parasitemia. Our models predicted that higher median piperaquine concentrations would be required to provide 99% protection against mutant infections than against wild-type infections (*pfmdr1*: N86, 9.6 ng/ml; 86Y, 19.6 ng/ml; *pfcrt*: K76, 6.5 ng/ml; 76T, 19.6 ng/ml). Comparing monthly, weekly, and daily dosing, daily low-dose DHA-PQ was predicted to result in the fewest infections and the fewest mutant infections per 1,000 pregnancies (predicted mutant infections for *pfmdr1* 86Y: SP monthly, 607; DHA-PQ monthly, 198; DHA-PQ daily, 1; for *pfcrt* 76T: SP monthly, 1,564; DHA-PQ monthly, 283; DHA-PQ daily, 1). Our models predict that higher piperaquine concentrations are needed to prevent infections with the *pfmdr1/pfcrt* mutant compared to those with wild-type parasites and that, despite selection for mutants by DHA-PQ, the overall burden of mutant infections is lower for IPTp with DHA-PQ than for IPTp with SP. (This study has been registered at ClinicalTrials.gov under identifier NCT02282293.)

KEYWORDS PK/PD modeling, antimalarial resistance, dihydroartemisinin-piperaquine, intermittent preventive treatment during pregnancy

Plasmodium falciparum infection during pregnancy, especially during a first pregnancy, places infants at risk for the complications of placental malaria, including intrauterine growth retardation, preterm birth, low birth weight, and death (1). The

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World Health Organization recommends that pregnant women at risk for malaria in Africa use a long-lasting-insecticide-treated bed net and receive at least three doses of sulfadoxine-pyrimethamine (SP) as intermittent preventive treatment during pregnancy (IPTp) (2). However, in much of Africa, including east Africa, the protective efficacy of SP as chemoprevention for pregnant women and children is inadequate (3–5). Compared to three doses of SP during pregnancy, a monthly course of dihydroartemisinin-piperaquine (DHA-PQ), an artemisinin-based combination therapy (ACT) administered once daily for 3 days, dramatically reduced the prevalence of maternal parasitemia and placental malaria in Uganda and Kenya (5, 6). Pharmacokinetic/pharmacodynamic (PK/PD) modeling studies found that plasma piperaquine (PQ) concentrations are excellent predictors of DHA-PQ protective efficacy and that maintaining higher PQ concentrations in the target population, such as with lower dose weekly or daily DHA-PQ, predicts maximal protective efficacy (7–10).

The long half-life of PQ makes DHA-PQ an ideal choice for malaria chemoprevention, but antimalarials with the longest half-lives may be at the greatest risk for resistance selection (11). Although true resistance to DHA-PQ, as observed in southeast Asia (12, 13), has not been confirmed in Africa (14–16), *P. falciparum* infections that emerge following DHA-PQ treatment have had, compared to that of parasites not under drug selection, increased prevalence of mutant genotypes in the putative drug transporters *pfmdr1* (86Y) and *pfcrt* (76T) (14, 16, 17). These mutations are associated with decreased sensitivity to chloroquine and amodiaquine, two aminoquinolines related to piperaquine (14), and these results raise the concern that using DHA-PQ for chemoprevention may provide only a short-term benefit, with eventual loss of efficacy due to the accelerated development of resistance.

We are interested in optimizing DHA-PQ dosing during IPTp to maximize protective efficacy, minimize toxicity, and limit selection for less-drug-sensitive parasites. In this analysis, we used clinical, pharmacokinetic, and molecular data from a trial of pregnant women who were randomized to receive DHA-PQ or SP as IPTp to develop PK/PD models which quantified relationships between PQ exposure, parasitemia, and genetic markers associated with decreased drug sensitivity. We then used the concentration-effect relationships to predict how modifications to DHA-PQ dosing would impact the burden of *P. falciparum* infection, including the risk of infection with parasites with decreased drug sensitivity.

RESULTS

Study cohort and data collection. The data were from a randomized controlled trial, in which 300 pregnant women were randomized to one of three IPTp regimens: SP every 8 weeks beginning at gestational week 20, DHA-PQ every 8 weeks beginning at gestational week 20, or DHA-PQ every 4 weeks beginning at gestational week 16 or 20 as previously described (Fig. 1, Table 1) (5). The clinical characteristics were similar between the three study arms (Table 1). Participants returned monthly for routine visits and for any acute illness. At routine visits or when malaria was suspected, an evaluation included a capillary or venous blood draw for the determination of plasma PQ concentration and parasite detection. If the woman was parasitemic, the parasite was genotyped as *pfmdr1* 86 and *pfcrt* 76 (16). A subset of 30 women underwent intensive PQ sampling. A total of 652 venous and 558 capillary PQ concentrations were obtained (Table 1; see also Fig. S1 in the supplemental material). Genotyping for the single nucleotide polymorphisms at *pfmdr1* N86Y and *pfcrt* K76T was successful for >84% of episodes of parasitemia in the SP arm and for >93% in the DHA-PQ arms. Prevalences of mutant genotypes were higher in the DHA-PQ arms than in the SP arm (*pfmdr1* 86Y: SP, 27%; DHA-PQ, 65%; *pfcrt* 76T: SP, 82%; DHA-PQ, 87%), as previously reported (Table 1) (16).

PK/PD model building. Simultaneous continuous categorical PK/PD models were developed using a mixed-effects logistic regression approach. Models were evaluated by using an objective function value (OFV), with a decrease in OFV (ΔOFV) of −3.84 considered a significant improvement if one parameter was added to the model, and

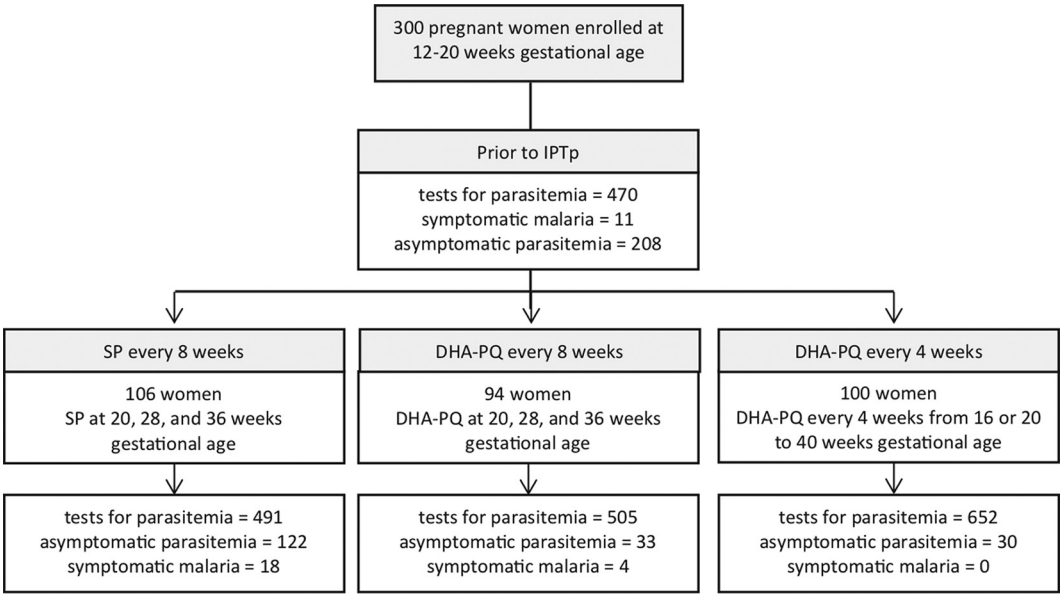

FIG 1 Trial profile. Study subjects were tested for *P. falciparum* parasitemia monthly and when they presented for unscheduled visits due to a febrile illness.

by a visual predictive check (see Fig. S2). Two types of simultaneous PK/PD models were developed for the analysis. PK/PD parasitemia models predicted the risk of parasitemia. PK/PD resistance models predicted the risk of a mutant infection at *pfmdr1* 86 or *pfcrt* 76 when parasitemia was detected.

A two-compartment model for PQ was used to predict plasma concentrations, as previously described (8). For the PK/PD parasitemia model for women who received DHA-PQ, a negative log-linear relationship provided an adequate fit for the association

TABLE 1 Characteristics of study participants

Characteristic	SP every 8 wks (*N* = 106)	DHA-PQ every 8 wks (*N* = 94)	DHA-PQ every 4 wks (*N* = 100)
Age (years) (mean [SD])	21 (3.6)	22 (4.3)	23 (4.0)
Gravidity (*n* [%])			
1	42 (40)	33 (35)	36 (36)
2	32 (30)	28 (30)	28 (28)
≥3	32 (30)	33 (35)	36 (36)
Gestational age (wks) at first study drug treatment (%)			
16			68
20	106	94	32
No. of PQ concn observations			
Venous		300	352
Capillary		278	280
Visits after participant received indoor residual spraying of insecticide (*n*)	101	101	153
First episodes of parasitemia after each administration of study drug (*n*)[a]	140	37	30
Genotypes (*n* [%])			
pfmdr1 N86Y genotype available	117 (84)	37 (100)	28 (93)
pfmdr1 86Y	32 (27)	18 (49)	24 (86)
pfcrt K76T genotype available	122 (87)	37 (100)	28 (93)
pfcrt 76T	92 (82)	31 (84)	26 (93)

[a]Artemeter-lumefantrine (AL) was used to treat malaria during the study. To avoid consideration of the effects of AL on repeated observations of the same parasites, parasitemia detected after treatment with AL and before subsequent receipt of DHA-PQ or parasites detected repeatedly without interval receipt of DHA-PQ were excluded.

TABLE 2 Pharmacokinetic/pharmacodynamic model parameters

Parameter	Parameter estimate		Between-subject variability	
	Value	RSE[a] (%)	CV[b] (%)	RSE (%)
SP PD model				
Baseline logit	−0.441	39	115	14
Primigravida baseline logit[c]	0.511	78		
Indoor residual spraying	−0.72	60		
Dry season	−1.13	28		
DHA-PQ PK/PD model for parasitemia				
Baseline logit	−0.508	72	73	17
Primigravida baseline logit[c]	0.582	64		
Slope of concentration-dependent effect (ml/ng)	−0.204	16		
Indoor residual spraying	−10 fixed			
Third trimester	−1.45	45		
DHA-PQ PK/PD model for *pfmdr1* N86Y				
Baseline logit	−1.16	11	3.8	53
Slope of concentration-dependent effect	0.317	21		
DHA-PQ PK/PD for *pfcrt* K76T				
Baseline logit	1.06	11	2.2	22
Slope of concentration-dependent effect	0.218	22		

[a]RSE, relative standard error.
[b]CV, coefficient of variation.
[c]Baseline logit used for all gravidities after start of IPTp, as gravidity was not a significant predictor of parasitemia after the start of chemoprevention.

between plasma PQ concentration and risk of parasitemia (ΔOFV −230) (Fig. S2B). Being primigravida was associated with a significant 26.6% increased risk of parasitemia prior to IPTp compared to that for multigravida participants (ΔOFV −24). However, after the initiation of DHA-PQ, gravidity was not a significant predictor of parasitemia in the model. Significant covariates after the initiation of IPTp included being in the second or third trimester and household receipt of indoor residual spraying of insecticide (IRS). Compared to the second trimester, the third trimester was associated with a 19.0% reduction in risk of parasitemia while receiving IPTp (ΔOFV −41). Finally, receipt of IRS, which in the clinical trial only occurred after the start of chemoprevention, was associated with complete protection from parasitemia, eliminating the concentration effect of PQ when present (ΔOFV −36) (Table 2). The additional covariates tested included body mass index (BMI) at enrollment, change in BMI compared to that at enrollment, and the presence of a dry season, and these were not significantly associated with the risk of parasitemia for women who received DHA-PQ. Gravidity, trimester, and BMI were also tested as covariates on the relationship between PQ and risk of parasitemia; these did not significantly improve the PK/PD parasitemia model for DHA-PQ. The final model for the probability of parasitemia is described in equation 1, where P is the probability of parasitemia, B is the baseline risk of parasitemia, sl is the slope of the concentration-dependent change in probability, [PQ] is the PQ concentration in nanograms per microliter, θ represents covariates that were estimated in the model, and ε and η indicate residual error.

$$\text{Logit}\,(\text{P}) = \text{B} + \text{sl} \times [\text{PQ}] + \theta_{\text{IRS}} + \theta_{\text{trimester}} + \varepsilon + \eta \quad (1)$$

For women who were not exposed to IRS, even low PQ concentrations were associated with a decreased risk of parasitemia compared to that at baseline, and PQ was a predictor of parasitemia risk regardless of the trimester (Fig. 2).

For SP, pharmacokinetic data were not available, and a PD model for parasitemia was developed. In a stepwise manner, binary covariates were added to the baseline probability of parasitemia for the SP PD parasitemia model as seen in equation 2, where P is the probability of parasitemia, B is the baseline risk of parasitemia, θ represents covariates that were estimated in the model, and ε and η indicate residual error.

$$\text{Logit}(\text{P}) = \text{B} + \theta_{\text{IRS}} + \theta_{\text{season}} + \varepsilon + \eta \quad (2)$$

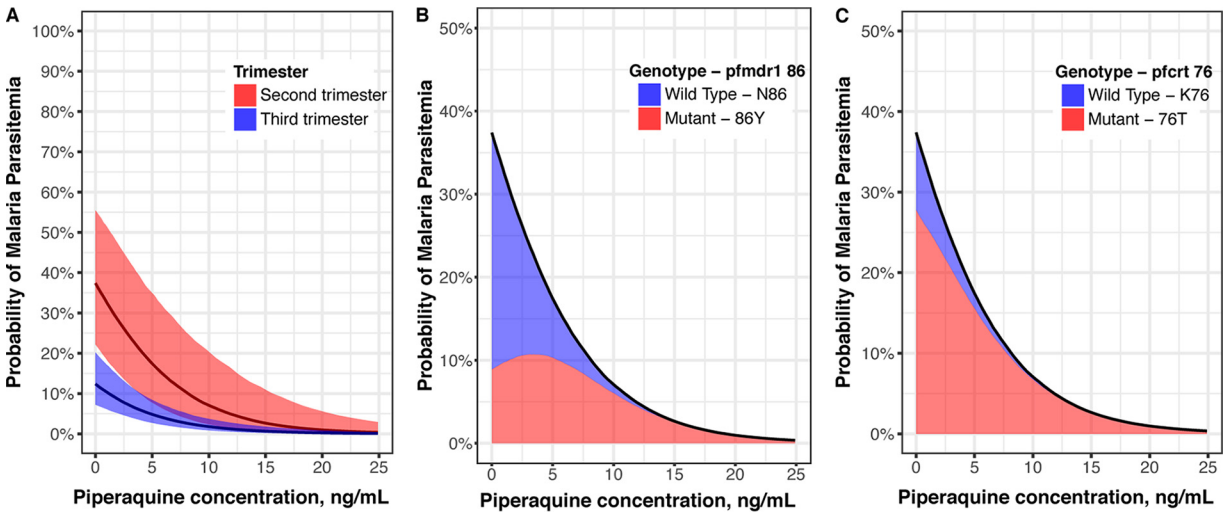

FIG 2 (A) Predicted probability of parasitemia with increasing piperaquine concentration in the absence of indoor residual spraying of insecticide for women receiving DHA-PQ stratified by trimester. The solid lines show the median probabilities and shading encompasses probabilities for 95% of the population. The median probability of parasitemia while receiving SP as IPTp was 39%. Contributions of mutant and wild-type genotypes to overall parasitemia probability during the second trimester for *pfmdr1* 86 (B) and *pfcrt* 76 (C). The black lines represent the median probabilities of all parasitemia, and shaded areas indicate the proportions of the probabilities attributed to wild-type and mutant parasites. Results for the third trimester are shown in Fig. S3 in the supplemental material.

Similar to that for DHA-PQ, being primigravida significantly increased the risk of parasitemia prior to IPTp by 23.3% (ΔOFV −8.0). Receipt of IRS was associated with a reduced risk of parasitemia (32.7%, ΔOFV −27) (Table 2). In addition, for the SP arm, the dry season was independently associated with a decreased risk of parasitemia (24.4%, ΔOFV −17). After adjusting for significant covariates, the model did not support the addition of an SP effect (added as time varying, treatment arm effect, or binary covariate). In addition, enrollment BMI, change in BMI, and trimester were not associated with significant changes in risk of parasitemia.

PK/PD resistance models were then developed to estimate the probability of a mutant infection with *pfmdr1* N86Y or *pfcrt* K76T. A log-linear relationship between PQ concentration and the probability of a mutant infection provided the best fit for both *pfmdr1* 86Y (ΔOFV −11) and *pfcrt* 76T (ΔOFV −9.6) (Fig. S2C and D). Increasing PQ concentrations were associated with increasing probabilities of a mutant infection at both loci (Fig. 3). As expected, there was no significant relationship between IPTp with SP and detection of a mutant *pfmdr1* 86Y or *pfcrt* 76T allele. Compared to that in the SP group, the odds of detecting *pfmdr1* 86Y increased with increasing PQ concentration, with a maximum median odds of 4.3 occurring at 17.9 ng/ml PQ (Fig. 3C). In the setting of a high baseline risk of *pfcrt* 76T, PQ exposure was associated with a slight increase in the odds of detecting a mutant compared to that in the SP arm, peaking at a maximum median odds of 1.3 at 10.1 ng/ml PQ (Fig. 3C).

Derivation of PQ concentration targets. The PQ concentrations required to prevent 99% of parasitemia episodes varied by trimester. Women in the second trimester were predicted to require 19.6 ng/ml PQ (95% confidence interval [CI], 13.2 to 31.6 ng/ml) to achieve 99% protection from parasitemia, while in the third trimester 12.8 ng/ml (95% CI, 9.2 to 19.2 ng/ml) was required.

The PQ concentrations required to prevent 99% of parasitemia episodes stratified by wild-type and mutant genotypes were derived from a joint model of the final PK/PD models for predicting parasitemia and genotype. Since women in the second trimester were predicted to require the highest PQ concentrations for protection (see Table S1), this population was used to estimate the target protective concentrations, as shown in Fig. 4. For *pfmdr1* 86, an increased risk of mutant parasites was predicted compared to that at baseline at subprotective plasma concentrations of PQ, peaking at 3.3 ng/ml (Fig. 2). PQ concentrations required to prevent 99% of parasitemia episodes were

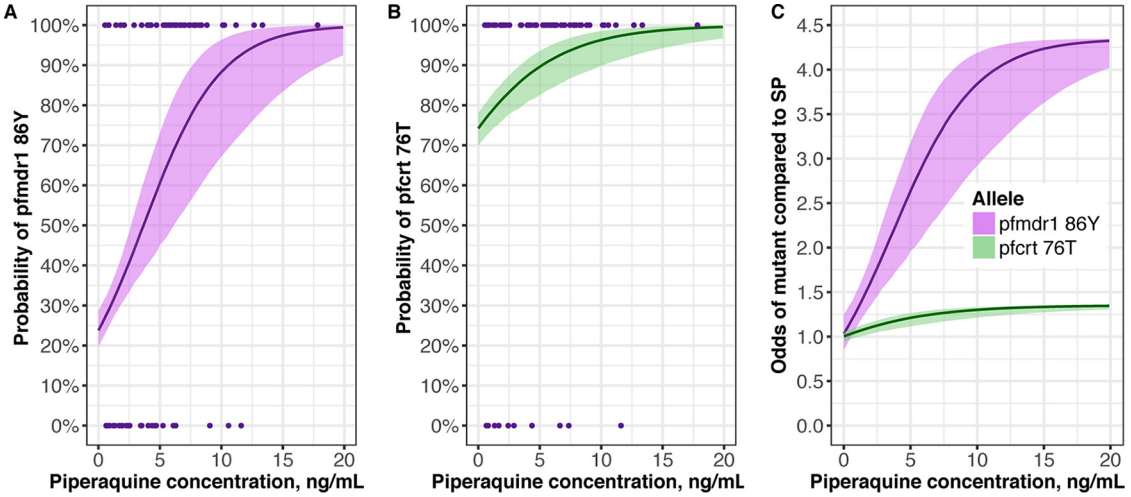

FIG 3 Predicted probability of detecting mutant *pfmdr1* 86Y (A) or *pfcrt* 76T (B) parasites with increasing piperaquine concentrations for women receiving DHA-PQ with parasitemia. Points are the raw data, showing isolates with mutant (100%) or wild-type (0%) genotypes. (C) Odds of detecting mutant genotypes in the DHA-PQ treatment arms compared to that in the SP arm. The solid lines are the median probabilities or increased odds of detecting a mutant parasite during an episode of parasitemia, and the shading encompasses the probability or increased odds of detecting a mutant parasite for 95% of the population.

predicted to be higher for parasites with mutant *pfmdr1* 86Y (19.6 ng/ml [95% CI, 12.9 to 32.2]) than with wild-type *pfmdr1* N86 (9.6 ng/ml [7.0 to 12.4]) and for mutant *pfcrt* 76T (19.6 ng/ml [13.1 to 32.2]) than for wild-type *pfcrt* K76 (6.5 ng/ml [4.1 to 9.3]) (Fig. 4).

Simulations to predict the optimal DHA-PQ dosing schedule. Simulations were conducted of 1,000 women who received SP every 8 weeks or DHA-PQ monthly, weekly, or daily using the joint PK/PD models to estimate the percentage of time above protective concentrations during pregnancy and the predicted number of mutant *pfmdr1* 86Y and *pfcrt* 76T infections for each regimen (Table 3, Fig. 5). All simulations assumed no exposure to IRS or seasonal variation. Both the number of parasitemia episodes and the number of mutant parasitemia episodes were predicted to be lower with any of the considered DHA-PQ regimens than with SP. Low-dose (320 mg PQ) daily DHA-PQ was predicted to result in the lowest median number of infections and mutant infections, with an estimated reduction in mutant infections >99%.

DISCUSSION

A monthly treatment course of DHA-PQ markedly reduced the burden of parasitemia during pregnancy in Uganda and Kenya, but there is concern that IPTp with DHA-PQ will accelerate selection for drug resistance. With simultaneous PK/PD modeling, we used PQ concentrations and clinical covariates to predict the probability of detecting malaria parasitemia and the probability of detecting parasites with relevant genotypes associated with drug resistance in women receiving DHA-PQ or SP as IPTp in Uganda. Higher concentrations of PQ were needed to reduce the probability of mutant than of wild-type infections at *pfmdr1* 86 and *pfcrt* 76, but these concentrations were achievable with practical DHA-PQ dosing regimens, including a novel low-dose daily regimen that should minimize toxicity concerns (8, 18). Despite selection for mutants by DHA-PQ, the overall burden of mutant infections was predicted to be lower for IPTp with DHA-PQ than with SP. Thus, a low daily dose of DHA-PQ for chemoprevention during pregnancy is predicted to maximize protective efficacy, with limited burden of mutant parasites with decreased aminoquinoline sensitivity, and to decrease the risk of cardiotoxicity compared to that with monthly dosing (8, 18).

In our model, we were unable to predict a malaria protective benefit attributable to IPTp with SP after controlling for covariates. *P. falciparum* polymorphisms associated with antifolate resistance were at high prevalence at the study site (16), and there was

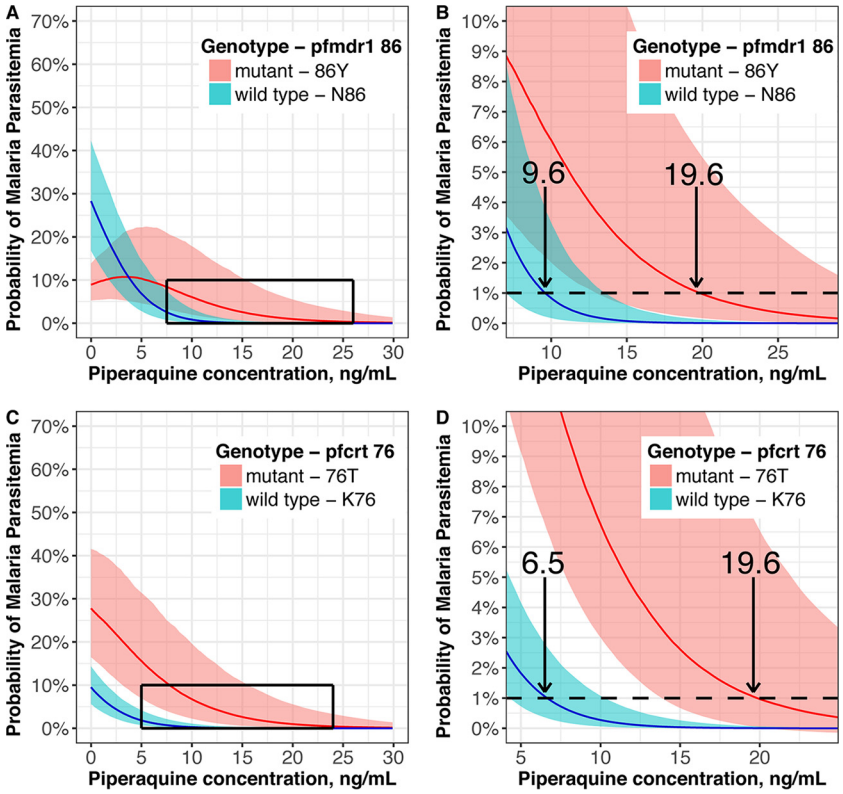

FIG 4 Association between piperaquine concentration and probability of wild-type or mutant genotype among women in the second trimester receiving DHA-PQ. Probabilities of detecting *pfmdr1* 86 (A) or *pfcrt* 76 (B) genotypes are shown, with closer visualization of the curves enclosed in boxes shown for *pfmdr1* 86 (C) and *pfcrt* 76 (D). Arrows indicate the median concentrations (ng/ml) providing 99% protection against parasitemia. Lines indicate the median probabilities, and the shading indicates the probability of detecting mutant parasites for 95% of the population.

a high burden of parasitemia and malarial illness in the SP arm of the study (5). Considering protective efficacy, monthly DHA-PQ was effective for adult males in Thailand (19) and was superior to SP for pregnant women in Uganda and Kenya (5, 6) and for children in Uganda (20). But, as with SP, might regular use of DHA-PQ for IPTp increase the burden of parasites that are no longer inhibited by the regimen? Importantly, in this setting, it does not appear to be the case, as the overall reduction in episodes of parasitemia is predicted to lead to a lower burden of infections with mutant parasites with DHA-PQ as IPTp.

The risk of selecting for *P. falciparum* with decreased susceptibility to antimalarials will be dependent on the prevalence of these mutants in the circulating parasite population, as selection appears to be due primarily to the amplification of existing clones, rather than *de novo* selection of new mutants (11). Since our trial was conducted, there have been significant increases in the prevalence of wild-type infections at *pfmdr1* 86 and *pfcrt* 76 in the region, likely selected by the use of artemether-

TABLE 3 Predicted number of mutant infections after starting chemoprevention per 1,000 pregnancies by dosing regimen[a]

Piperaquine dose	No. of infections per 1,000 pregnancies (95% CI)	*pfmdr1* 86Y mutant infections			*pfcrt* 76T mutant infections		
		No. (95% CI)	Ratio (DHA-PQ/SP)	*P* value	No. (95% CI)	Ratio (DHA-PQ/SP)	*P* value
0 mg (SP)	2066 (1988–2162)	607 (570–650)			1564 (1495–1564)		
2,880 mg monthly	317 (280–358)	198 (165–232)	0.32	<0.001	283 (248–315)	0.18	<0.001
960 mg weekly	105 (85–122)	87 (71.0–104)	0.14	<0.001	99 (80.4–115)	0.06	<0.001
160 mg daily	8.0 (4.0–14.0)	8.0 (3.5–13.5)	0.01	<0.001	8.0 (4.0–14.0)	0.005	<0.001
320 mg daily	1.0 (1.0–2.1)	1.0 (0.96–2.1)	0.002	<0.001	1 (1.0–2.1)	0.001	<0.001

[a]Estimated based on monthly surveillance for parasitemia in the absence of indoor residual spraying of insecticide or seasonal variation in transmission.

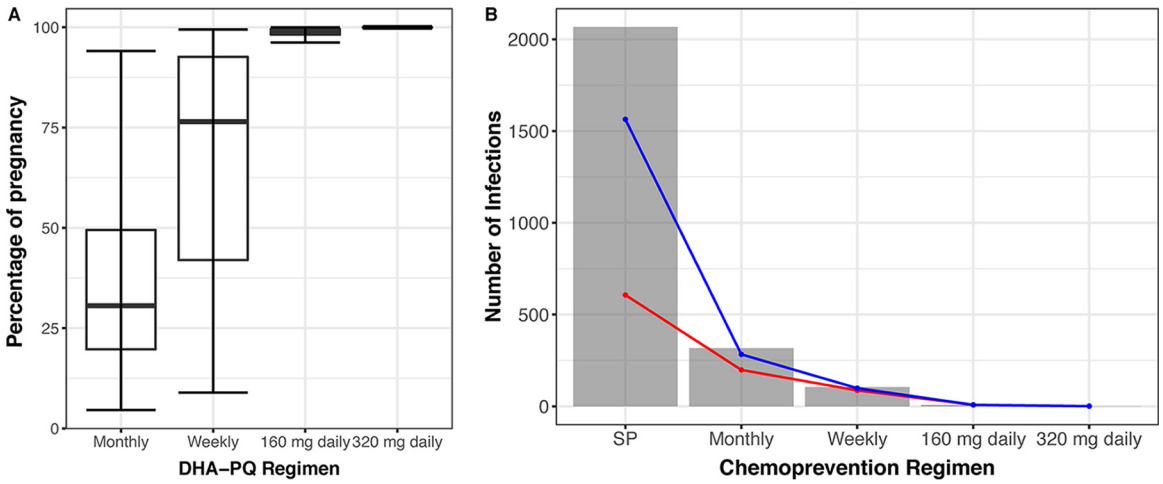

FIG 5 (A) Predicted percentage of time above piperaquine concentrations protective against 99% of parasitemia episodes during pregnancy by DHA-PQ regimen. Boxes indicate the interquartile ranges and error bars represent 95% of the population. (B) Predicted numbers of new episodes of parasitemia (gray bars) and episodes of parasitemia with a mutant infection at *pfmdr1* 86 (red) and *pfcrt* 76 (blue) during pregnancy for each chemoprevention regimen. Monthly, weekly, and daily indicate DHA-PQ regimens.

lumefantrine (AL) to treat malaria in Uganda (14, 21). An additional wild-type polymorphism, *pfmdr1* D1246, also increased in prevalence with AL pressure (14, 21). A haplotype analysis found that mutant *pfmdr1* 1246Y may be required to select for *pfmdr1* 86Y under PQ pressure, further reducing the risk of selecting for *pfmdr1* 86Y under DHA-PQ pressure with current circulating parasites (22). In this setting, a recent Ugandan treatment efficacy study found that, in contrast to results from earlier studies, DHA-PQ did not select for *pfmdr1* and *pfcrt* mutations in recurrent infections (23). Considering our modeling results for this population, it is unlikely that IPTp with DHA-PQ will increase the burden of mutant parasites with decreased sensitivity to the regimen in Uganda. However, risks of resistance selection could change over time based on ACT usage or other factors. Longitudinal surveillance of drug resistance markers and reevaluation of PK/PD models will remain important as we consider using DHA-PQ for IPTp.

Our analysis identified important covariates which modified the risk of parasitemia among women receiving DHA-PQ chemoprevention, including gravidity in the pre-IPTp period and trimester and IRS during IPTp. Remarkably, the combination of monthly DHA-PQ and receipt of IRS eliminated the risk of parasitemia. The benefits of IRS were not as large for the SP arm, likely due to persistent parasitemia despite treatment with SP (3). Recent studies from Uganda found that receipt of IRS is associated with improvements in birth outcomes (24). Taken together, the available results suggest enormous potential for the joint use of highly effective intermittent preventive treatment and IRS for the control and potential elimination of malaria.

Our study had some limitations. First, parasitemia was assessed at 28-day intervals. We could not determine the exact time when an individual became parasitemic and thus the exact concentration required to prevent parasitemia. However, monthly PQ concentrations offered a practical sampling strategy with good predictive power in our models. Second, PK data were not available to assist in detecting a concentration-effect relationship between SP and the prevention of malaria. We found that, after controlling for covariates which are associated with reduced risk of malaria infection, a model without an SP effect predicted the data adequately. The absence of a protective benefit for SP was further supported by a placebo-controlled chemoprevention trial in Uganda that did not demonstrate a significant protective effect of SP in children (4). Third, treatment failure due to DHA-PQ resistance and associated genetic markers has not been identified in Africa and thus could not be used in this analysis. The markers associated with DHA-PQ resistance in Southeast Asia (*pfkelch*, *plasmepsin2* copy num-

ber, and *exo-E415G* [13, 25, 26]) were assessed for this population and were either not present or, in the case of *plasmepsin2* copy number, only present in a minority of isolates (16). *Pfmdr1* 86Y and *pfcrt* 76T have been consistently associated with PQ exposure in Uganda (17, 27, 28) and have recently been associated with a modest increase in *ex vivo* 50% inhibitory concentration (IC_{50}) for PQ (14). As a result, these markers of antimalarial sensitivity were the most relevant for this population.

By taking a PK/PD modeling approach, we found that higher PQ concentrations are needed to prevent mutant malaria infections than to prevent wild-type malaria infections, but that safe and achievable PQ concentrations can provide >99% protection from parasitemia. In addition, a low-dose daily DHA-PQ regimen was predicted to maximally reduce parasitemia. Our findings support the use of DHA-PQ for chemoprevention and the optimization of DHA-PQ dosing to maximize protective efficacy while minimizing toxicity and potential selection of drug resistance. Future clinical trials of DHA-PQ as chemoprevention during pregnancy should consider alternative dosing strategies, including low-dose daily DHA-PQ.

MATERIALS AND METHODS

Study population. Pregnant women were enrolled in the clinical trial that provided samples for our analyses in Tororo, Uganda, from June through October 2014 (5). Eligible women were ≥16 years of age, HIV uninfected, and pregnant at 12 to 20 weeks gestation. Written informed consent was obtained from all study participants. The study protocol was approved by the Makerere University School of Biomedical Sciences Research and Ethics Committee, the Uganda National Council for Science and Technology, and the University of California, San Francisco Committee on Human Research. The clinical trial registration number is NCT02282293.

Study design and randomization. After enrollment, women randomized to SP (1,500 mg sulfadoxine/75 mg pyrimethamine) every 8 weeks or DHA-PQ (120 mg DHA/960 mg PQ daily for 3 days) every 8 weeks began chemoprevention at 20 weeks gestational age, and those randomized to DHA-PQ every 4 weeks began chemoprevention at either 16 or 20 weeks gestational age. The administration of the first dose of DHA-PQ was observed in the clinic, and the remaining two doses were taken at home. At enrollment, study participants received a long-lasting-insecticide-treated bed net, underwent a physical exam, had height and weight determination, and had blood collected. All women attended routine visits at 4-week intervals and were asked to return to the clinic for all of their medical needs. The date of IRS in the household was collected for each subject (24).

Pharmacokinetic sampling. Women randomized to receive DHA-PQ underwent sparse venous (gestational weeks 20, 28, and 36) and capillary (gestational weeks 24, 32, and 40) sampling to determine plasma PQ concentrations (8). Sparse PQ concentrations were determined either 28 days after receiving the drug in the 4-week DHA-PQ arm or every 28 days and every 56 days after receiving the drug in the 8-week DHA-PQ arm (8). Venous or capillary specimens were also collected at the time of any malaria diagnosis. A subset of individuals were enrolled in an intensive PK substudy. For this study, as previously reported (29), venous plasma samples were obtained predose and at 0.5, 1, 2, 3, 4, 6, 8, and 24 h postdose, and capillary plasma samples were collected at 24 h and at 4, 7, 14, and 21 days postdose. PQ base concentrations were determined using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS) (30). Modification and partial validation of the original method for PQ quantitation was performed to cover a concentration range of 0.50 to 1,000 ng/ml, with a coefficient of variation <10% for quality control samples (30).

***P. falciparum* detection and genotyping.** A blood spot was collected and stored on filter paper at all routine visits and if malaria was diagnosed at an unscheduled visit. DNA was extracted from dried blood spots using Chelex-100 and tested for the presence of *P. falciparum* DNA by loop-mediated isothermal amplification (LAMP) for all microscopy negative samples, as previously described (5, 31). Genotyping for *pfmdr1* N86Y and *pfcrt* K76T was conducted using a ligase detection reaction-fluorescent microsphere assay as previously described (28, 32). Isolates were classified as mutant for either pure mutant or mixed mutant and wild-type genotypes.

PK/PD models. To estimate the concentration effect relationship between PQ PK and probability of parasitemia and between PQ PK and the probability of detecting particular alleles at the loci of interest, simultaneous PK/PD models were developed using nonlinear mixed-effects modeling and LAPLACE methods (33). All available PQ concentration data above the limit of quantitation were used in the development of a two-compartment PQ PK model, as previously described (8). The population PQ PK model was then used as part of a simultaneous continuous categorical PK/PD model with logit transformation to determine the probability of parasitemia or a mutant genotype. To avoid repeated sampling of persistent circulating parasites, testing for parasitemia was censored after the first episode of parasitemia identified following each administration of study drug. Model appropriateness was evaluated by a likelihood ratio test, inspection of the diagnostic plots, and internal model validation techniques, including visual and numerical predictive checks.

We first developed a simultaneous continuous categorical PK/PD parasitemia model to predict the probability of parasitemia among women who received DHA-PQ. Dose response, linear, and maximum effect (E_{max}) models were tested for the relationship between PQ concentration and the probability of

parasitemia. Gravidity, trimester (defined as <28 weeks for the second trimester and ≥28 weeks for the third trimester), enrollment BMI, change in BMI compared to that at enrollment, dry season (defined as December to February), and receipt of IRS were then tested as covariates in the model. We then developed a PD model for the probability of parasitemia for women who received SP. We estimated that SP had a 28-day effect based on prior modeling studies (34). The same covariates were tested for SP as for DHA-PQ.

PK/PD resistance models were developed to estimate the relationship between PQ concentration and parasite genotype at *pfmdr1* N86Y or *pfcrt* K76T, also using simultaneous PK/PD modeling with logit transformation. All PQ PK data and available genotype data from episodes of parasitemia were used to develop models to predict sequences at the *pmfdr1* N86Y and *pfcrt* K76T alleles when parasitemia was detected. Baseline, dose response, linear, and E_{max} relationships between PQ concentration and genotype were tested for those who received DHA-PQ. Since PK data were not available for SP, a PD resistance model was used to evaluate a study arm effect of SP chemoprevention on selection for mutant infections compared to the prechemoprevention baseline.

The final PK/PD-parasitemia models, with epidemiologic covariates, and PK/PD resistance models for PQ were utilized sequentially, and the concentration of PQ needed to prevent parasitemia with mutant or wild-type infections at each locus was defined as the median value needed to provide 99% protection against parasitemia. One hundred simulations of 1,000 pregnancies were conducted using the final PK/PD models to determine the median number of parasitemia episodes and mutant parasitemia episodes with 95% confidence intervals for 1,000 pregnancies. Dosing strategies were selected to maximize protective efficacy. Simulated regimens included monthly dosing (2,880 mg PQ and 360 mg DHA divided into three consecutive daily oral doses), once weekly dosing (960 mg PQ and 120 mg DHA), and two once daily dosing options (160 mg PQ with 20 mg DHA and 320 mg PQ with 40 mg DHA). All statistical analyses were conducted in R (version 3.3.2) and STATA (version 14.2).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01393-18.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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The authors declare no conflicts of interest.

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