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Optimizing antimalarial treatment and prevention for pregnant women

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Optimizing antimalarial treatment and prevention for pregnant women

by
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Dedication

To my grandfather and inspiration, David Thomas.

Acknowledgments

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Contributions

Chapter 1 was modified from “Malaria PK/PD and the Role Pharmacometrics Can Play in the Global Health Arena: Malaria Treatment Regimens for Vulnerable Populations” as it was originally published in *Clinical Pharmacology and Therapeutics* in 2021. E.H., E.W., A.M.A. and P.J. wrote the manuscript with contributions from R.S.

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Optimizing antimalarial treatment and prevention for pregnant women

Emma Hughes

Abstract

Malaria is a significant contributor to maternal and childhood morbidity and mortality. While precise numbers are lacking, it is estimated that these infections are responsible for 10-20% of maternal deaths in endemic regions or 30,000 mortalities annually. Additionally, malaria during pregnancy is a known risk factor for adverse birth outcomes including stillbirth, pre-term birth and low birthweight deliveries resulting in 100,000 infant mortalities each year. Safe and effective treatment and prevention regimens are needed to reduce the burden of malaria. However, current therapeutic guidelines do not account for the effects of pregnancy, co-morbidities or concomitant medications on drug concentrations and efficacy. The aims of this work were to characterize antimalarial drug exposure, safety, and efficacy to optimize dosing regimens in pregnant women with an emphasis on malaria prevention.

Human immunodeficiency virus (HIV) infection and malaria are endemic in sub-Saharan Africa resulting in a large population of HIV-infected individuals receiving malaria treatment. Both antiretroviral and antimalarial therapies are metabolized by cytochrome P450 enzymes which could lead to drug-drug interactions altering antimalarial exposure and clinical response. In a prospective clinical pharmacokinetic (PK) study, the effects of efavirenz on artemether-lumefantrine (AL), the most commonly prescribed artemisinin-based combination therapy (ACT) for uncomplicated malaria, were investigated. Specifically, the exposure of the artemisinins, artemether

and it's active metabolite dihydroartemisinin (DHA) and long-acting lumefantrine were characterized in HIV-infected and uninfected pregnant Ugandan women. Relative to HIV-uninfected women, concomitant efavirenz therapy significantly lowered peak DHA concentrations as well as the terminal artemether and lumefantrine concentrations. In addition, there were nonsignificant reductions in DHA and lumefantrine area under the concentration–time curve with efavirenz therapy. These results suggest that pregnant women receiving efavirenz may require higher or additional doses of AL to improve drug exposure.

Dihydroartemisinin-piperaquine (DHA-PQ), also considered a first line ACT for uncomplicated malaria, provides highly effective therapy and is being evaluated for malaria chemoprevention in pregnant African women. However, few prevention studies have included a pharmacokinetic component to define the longitudinal PK of PQ during pregnancy. To address this need we pooled data from two large clinical trials to perform a *post hoc* analysis with 274 women and 2,218 PK observations. We included HIV-infected and -uninfected pregnant Ugandan women throughout the second and third trimesters, as well as postpartum women. Pregnancy and efavirenz use resulted in a 72% and 61% increase in PQ clearance, compared to postpartum and HIV-uninfected pregnant women, respectively. Low BMI at 28 weeks gestation was associated with increased clearance (2% increase per unit decrease in BMI). Low-BMI women given DHA-PQ every 8 weeks had a higher prevalence of parasitemia, malaria infection, and placental malaria compared to women with higher BMIs. Simulations indicated women taking efavirenz and/or with a low BMI could benefit from more frequent dosing such as weekly instead of monthly DHA-PQ.

Piperaquine prolongs the corrected QT interval (QTc), and repeated monthly dosing, as required for prevention, could lead to progressive QTc prolongation. Characterization of the relationship between piperaquine concentration and QTc interval throughout pregnancy is needed to inform prevention guidelines. Data from a randomized controlled trial, where pregnant Ugandan women received monthly DHA-PQ or sulfadoxine-pyrimethamine (SP) were used to establish the piperaquine – QTc relationship and trends in longitudinal QTc for the SP arm. A positive linear relationship between piperaquine concentration and Fridericia corrected QTc interval was identified. Interestingly, the slope of this relationship progressively decreased from a 4.42 to 2.13 millisecond increase per 100 ng/mL increase in piperaquine concentration at 20 and 36 weeks gestation, respectively. SP was not associated with any change in QTc. These results indicate that monthly dosing of DHA-PQ in pregnant women carries minimal risk of QTc prolongation and this risk diminishes with repeat doses over the course of pregnancy.

The PK/PD relationship between piperaquine concentrations and malaria parasitemia is not well defined in the context of malaria prevention in pregnant women. A robust PK/PD relationship is required to determine optimal dosing strategies for prevention. This analysis included monthly piperaquine trough concentrations and parasite densities collected from a large prevention trial in Ugandan pregnant women. These data were used to define the PK/PD relationship for piperaquine and parasitemia (binary outcome) in pregnant women. The piperaquine drug effect was best captured by an Emax relationship. Primigravida was identified as a risk factor for being parasite positive. In comparison to a previous study, 10.3 ng/mL was 70 and 80% protective for

primigravida and multigravida women, respectively. To better compare results between the two studies, women with parasite densities below 1,000 parasites/mL were considered negative (this was done to adjust to differences in assay sensitivity) in a sensitivity analysis. This led to 10.3 ng/mL being 95 and 97.5% protective for primigravida and multigravida women, respectively. While increasing piperaquine concentrations led to a decreased probability of parasitemia, the Emax relationship was such that concentrations above 10.3 ng/mL did not lead to a significant reduction in parasitemia. Therefore, simulations were preformed to maximize the number of women maintaining 10.3 ng/mL. These simulations suggested that women, particularly primigravida, could benefit from more frequent dosing.

Collectively, this dissertation research demonstrates that pregnant women are a unique population who warrant dedicated clinical trials with consideration of PK/PD to evaluate treatment and prevention regimens. Our findings provide dosing recommendations for the next generation of antimalarial treatment and prevention studies in pregnant women.

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List of Abbreviations

ACT	artemisinin-based combination therapies
AIDS	acquired immunodeficiency syndrome
AL and AR-LF	artemether-lumefantrine
ALT	alanine aminotransferase
ART	antiretroviral therapy
AST	aspartate aminotransferase
AUC	area under the concentration time curve
BLQ	below the limit of quantitation
BMI	body mass index
CAR	constitutive androstane receptor
CI	confidence interval
CL	drug clearance
C_{max}	maximum concentration
C_{cap}	capillary concentration
C_{ven}	venous concentration
CV	coefficient of variation
CYP	cytochrome P-450 drug metabolizing enzyme
DDI	drug-drug interactions
DP and DHA-PQ	dihydroartemisinin-piperaquine
EC_{50}	half maximal effective concentration
ECG	electrocardiogram
EFV	efavirenz
EGA	estimated gestational age
E_{max}	maximum effect
F	bioavailability
FDA	Food and Drug Administration
hERG	human ether-a-go-go-related gene
HIV	human immunodeficiency virus
IC_{50}	half- maximal inhibitory concentration
IPT	intermittent preventative treatment
IRS	indoor residual spraying of insecticides
LAMP	loop-mediated isothermal amplification
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LLIN	long-lasting insecticide-treated bed nets
LLOQ	lower limit of quantification
LR or LUM	lumefantrine
MIC	minimum inhibitory concentration
MUAC	mid-upper arm circumference
NONMEM	non-linear mixed effects modeling
NR	not reported

NRTI	nucleoside reverse transcriptase inhibitors
OFV	objective function value
PK/PD	pharmacokinetic/pharmacodynamic
PXR	pregnane X receptor
Q4W or q4wk	doses given every 4 weeks
Q8W or q8wk	doses given every 8 weeks
QD	doses given daily
qPCR	quantitative polymerase chain reaction
QSAR	quantitative structure actively relationship
ΔQTcF	change in QTcF (postdose QTcF − predose QTcF)
QTc	corrected QT interval
QTcF	Fridericia corrected QT interval
SAM	severe acute malnutrition
SCM	stepwise covariate modeling
SP	sulfadoxine-pyrimethamine
SXT	trimethoprim-sulfamethoxazole
$t_{1/2}$	terminal elimination half-life
T_{max}	time of maximum concentration
TS	trimethoprim-sulfamethoxazole
VPC	visual predictive check
WHO	World Health Organization
WWARN	WorldWide Antimalarial Resistance Network
λ_z	terminal elimination rate constant

Chapter 1: Malaria PK/PD and the role pharmacometrics can play in the global health arena: malaria treatment regimens for vulnerable populations*

Abstract

Malaria is an infectious disease which disproportionately affects children and pregnant women. These vulnerable populations are often excluded from clinical trials resulting in one-size-fits-all treatment regimens based on those established for a nonpregnant adult population. Pharmacokinetic/pharmacodynamic (PK/PD) models can be used to optimize dose selection as they define the drug exposure-response relationship. Additionally, these models are able to identify patient characteristics that cause alterations in the expected PK/PD profiles and through simulations can recommend changes to dosing which compensate for the differences. In this review, we examine how PK/PD models have been applied to optimize antimalarial dosing recommendations for young children, including those who are malnourished, pregnant women, and individuals receiving concomitant therapies such as those for HIV treatment. The malaria field has had great success in utilizing PK/PD models as a foundation to update treatment guidelines and propose the next generation of dosing regimens to investigate in clinical trials. We propose how the malaria field can continue

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to use modeling to improve therapies by further integrating PK data into clinical studies and including data on drug resistance and host immunity in PK/PD models. Finally, we suggest that other disease areas can achieve similar success in applying pharmacometrics to improve outcomes by implementing three key principals.

Introduction

Infectious diseases with disproportionate burdens in low resource settings create unique challenges in drug development, drug repurposing, and therapeutic evaluation. As an example, malaria, an infectious disease caused by the *Plasmodium* parasite, resulted in an estimated 229 million cases and 409,000 deaths in 2019.[1] Over half of all malaria cases and deaths are in children under 5 years of age in sub-Saharan Africa.[1] Prompt and effective treatment of malaria is a cornerstone of malaria control and, like many infectious diseases, relies on historic drugs developed using empirical methods, initially studied in nonpregnant adults. The spread of drug-resistant parasites and therefore waning efficacy of current therapeutics has created an urgent need to both quickly develop new therapies as well as repurpose approved antimalarials for treatment, prevention, and elimination.

Over the last decade, pharmacometric techniques, which use population models to define drug exposure-response relationships, have been an essential tool for the development of malaria therapeutics. Pharmacometric models incorporate drug dose, drug concentrations, and patient outcome measures to optimize a drug's use by defining what dose(s) are safest and result in the highest efficacy for each patient population. In this review, we describe how pharmacometric techniques, applied as

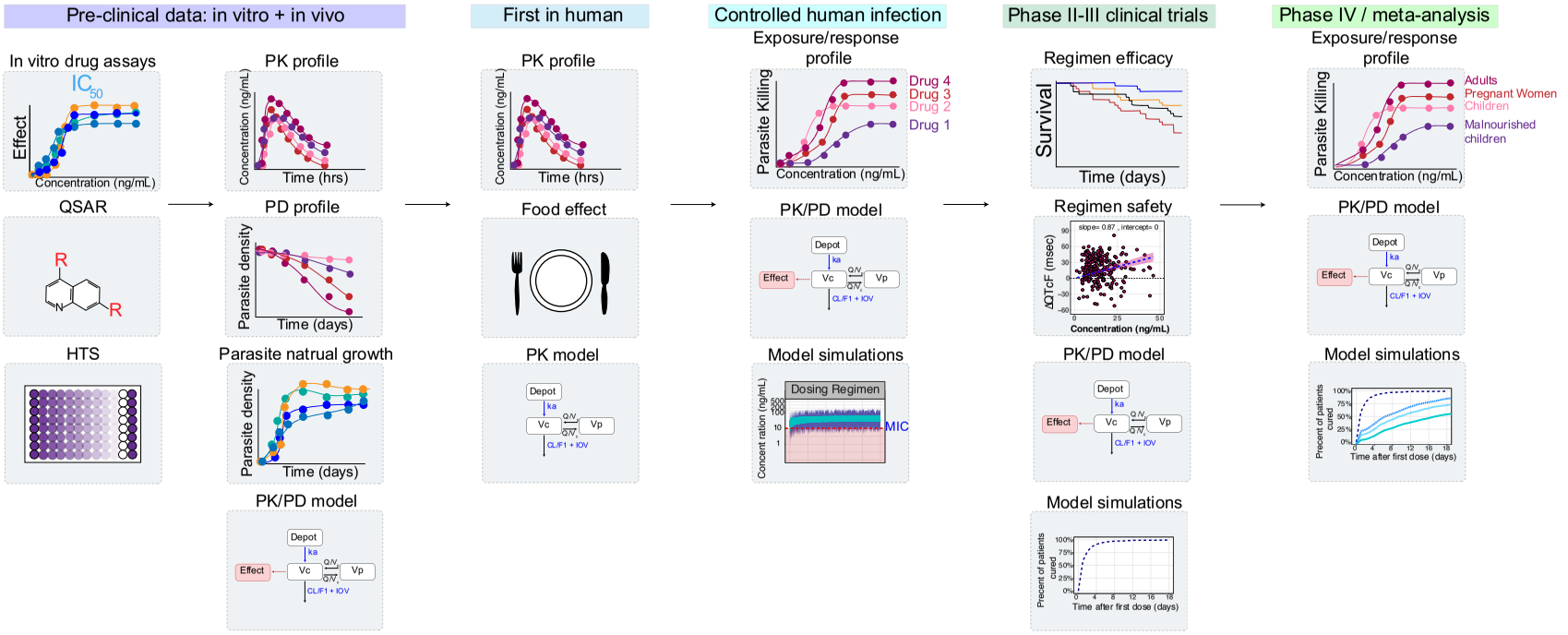
pharmacokinetic/pharmacodynamic (PK/PD) models, have impacted malaria treatment, review future opportunities for the use of PK/PD models to enhance malaria treatment, and share lessons learned that could have applications to other emerging infectious diseases.

Pharmacometric principles

Despite being a relatively new discipline, pharmacometrics changed the drug development paradigm. PK/PD models are mathematical models, which describe a drug's concentration-effect relationship by estimating a drug's PK parameters (e.g., absorption rate, bioavailability, clearance, and volume of distribution) based on drug concentrations and linking this drug exposure to clinical outcomes.

Two main types of models are used for antimalarials: (i) mechanistic and (ii) empirical, both based on preclinical and/or clinical data. Preclinical PK/PD models use *in vitro* drug efficacy data, parasite growth dynamics data, and animal studies to extrapolate drug exposure and efficacy for first-in-human studies (**Figure 1.1**). These preclinical models can incorporate a drug's site of action, assess for synergy in drug combinations, and explore the role of drug resistance prior to entering human studies. Clinical PK/PD models, which have dominated antimalarial research, typically use sparse drug concentrations (1–3 samples per individual) and treatment outcomes (relapse, reinfection, and cure) collected from clinical trials. The PK/PD model framework increases the flexibility of PK sample collection times, maximizes the number of patients who can be sampled, and allows researchers to pool data from multiple studies. Once PK exposure is quantified, it can be directly linked to clinical outcomes.

By understanding the relationship between drug exposure and resultant treatment outcome, one can learn what drug exposure is needed to achieve a positive treatment outcome. After the drug exposure-response relationship is established, a pharmacometrician can explore through simulations (virtual clinical trials) what dosing regimens are needed to achieve the required drug exposure. Similarly, they can also explore the effects of patient characteristics to learn whether all individuals require the same dose or whether certain populations require different doses to be cured. The ability of pharmacometric models to account for variability from multiple sources (patient, concentration, and response) and to perform simulations are two strengths which make these tools so powerful. PK/PD models are particularly advantageous for antimalarials, as malaria impacts children, pregnant women, and patients with comorbidities, all populations which are understudied during drug development and have physiologic characteristics that frequently impact both drug PK and drug exposure-response relationships necessitating precision dosing.


Pre-clinical data: in vitro + in vivo
First in human
Controlled human infection
Phase II-III clinical trials
Phase IV / meta-analysis
In vitro drug assays
IC50
Effect
Concentration (ng/mL)
QSAR
R
R
HTS
PK profile
Concentration (ng/mL)
Time (hrs)
PD profile
Parasite density
Time (days)
Parasite natrual growth
Parasite density
Time (days)
PK/PD model
Depot
ka
Effect
Vc
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Q/V
Q/V
CL/F1 + IOV
PK profile
Concentration (ng/mL)
Time (hrs)
Food effect
PK model
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Exposure/response profile
Parasite Killing
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Model simulations
Concent ration (ng/mL)
Dosing Regimen
MIC
Regimen efficacy
Survival
Time (days)
Regimen safety
slope= 0.87 , intercept= 0
ΔQTcF (msec)
Concentration (ng/mL)
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Q/V
CL/F1 + IOV
Model simulations
Precent of patients cured
Time after first dose (days)
Exposure/response profile
Parasite Killing
Concentration (ng/mL)
Adults
Pregnant Women
Children
Malnourished children
PK/PD model
Depot
ka
Effect
Vc
Vp
Q/V
Q/V
CL/F1 + IOV
Model simulations
Precent of patients cured
Time after first dose (days)
Compoud and dose selection
PK/PD parameters + dose selection for humans
PK parameters in healthy volunteers + dose adjustments
PK/PD parameters with infection + dose adjustments
PD + safety parameters in malaria endemic settings
PK/PD parameters in sub-populations

Figure 1.1 Experimental data collected over drug development that can be used for pharmacometric models. Preclinical data can be used to develop a translational platform for compound and regimen selection. High through screening can be used to select the most potent compounds and computational methods such as quantitative structure actively relationship can be used to design compounds with better PK and potency. Furthermore, preclinical experiments can be used to study development of drug resistance as well as characterize the natural parasite growth dynamics for more mechanistic models. PK/PD mouse studies can be used to develop animal PK/PD models to select clinical candidate compounds and design first in human studies. Clinical PK/PD data used to build human PK/PD models can further be used to select compounds and doses for the next trial whether phase 2,3 or post marketing studies. HTS, high-through screening; IC50, half- maximal inhibitory concentration; PD, pharmacodynamic; PK, pharmacokinetic; QSAR, quantitative structure actively relationship.

Malaria background

Among the five species of *Plasmodium* that cause malaria, *P. falciparum* and *P. vivax* are the most prevalent, and *P. falciparum* is the most deadly.[2-4] Human infection begins with injection of *Plasmodium* sporozoites from the bite of an infected female *Anopheles* mosquito. Typically, there is a 1–2 week incubation period during which individuals are asymptomatic as the parasites in the merozoite stage replicate in hepatocytes. Certain species, including *P. vivax*, can remain dormant in the liver (hypnozoite stage) for weeks to years resulting in relapse infections. Infected hepatocytes rupture after 1–2 weeks, releasing merozoites into the bloodstream where they infect erythrocytes. In the erythrocytes, asexual reproduction continues, with parasite densities in the blood increasing. It is typically in the blood stage of infection when symptoms develop, initially presenting as a febrile or flu-like illness, which, in some cases, can progress to organ dysfunction and death without treatment. Some merozoites will develop into gametocytes, which when ingested by mosquitoes undergo sexual reproduction to complete the lifecycle. [2-4]

After repeated infections, a natural immunity develops, which can control circulating parasite densities and reduces the risk of symptomatic infection, such that older individuals in malaria endemic regions may carry circulating parasites but are unlikely to have clinical disease.[2,3] The presence of asymptomatic carriers of parasites poses additional challenges for eliminating disease as asymptomatic individuals are still infectious and contribute to ongoing malaria transmission.[5] Immunity is lower in travelers from nonmalaria endemic regions, and in young children and pregnant women in malaria endemic settings, making these populations most at risk of malaria complications.[6]

Drugs have several important roles in malaria, including reducing the risk of morbidity and death with prompt and effective treatment, prevention of infections in high-risk populations, such as pregnant women, children, and travelers, and assisting in malaria elimination by clearing a community's parasites with mass drug administration. In addition to diverse purposes, antimalarial drug exposure-response relationships depend on parasite species and infection stage (e.g., liver or blood stages), parasite drug resistance characteristics, and host characteristics, including background immunity.[7,8] *Plasmodium's* complex life cycle and the natural history of malaria have introduced challenges and opportunities for drug development, as many therapeutics are only effective against certain life cycle stages of the parasite. PK/PD models are a valuable tool because they can integrate all these dynamic complexities. To demonstrate the role of PK/PD modeling in antimalarial drug development and postmarketing optimization, we will discuss models developed for malaria treatment.

Malaria treatment

The goal of malaria treatment is to initially prevent disease progression and then provide a cure. Malaria treatment is largely guided by the parasite species and drug susceptibility as well as whether the infection is uncomplicated or severe.[6] Infections are classified as severe if a patient presents with a positive blood smear for Plasmodium, along with one or more of the following: impaired consciousness, acidosis, hypoglycemia, severe anemia, renal impairment, severe hepatic impairment, hypoxia, bleeding, shock, or a parasite density > 10%.[6,9] Any infection not defined as severe is considered uncomplicated. Malaria treatment has largely used a one-size-fits-all dosing regimen where adults, pregnant women, and children receive the same or allometrically equivalent doses, respectively. Pharmacometric models have been used to evaluate the efficacy of the original dose chosen, including testing the assumptions around using the same dose in different populations. In 2015, the World Health Organization (WHO) updated their treatment guidelines for children using a regimen derived from a PK/PD model.[10,11]

In Africa, the current first-line therapies for malaria are artemisinin-based combination therapies (ACTs). There are five approved ACTs with artemether-lumefantrine (AL) being the most widely adopted and dihydroartemisinin-piperaquine (DP) being the newest.[1] The remaining options are artesunate-amodiaquine, artesunate-mefloquine, and artesunate-sulfadoxine-pyrimethamine. The pharmacology behind ACTs is complex and is founded on pairing a potent short-acting artemisinin derivative (artemether, dihydroartemisinin, or artesunate) with a longer acting partner drug (lumefantrine, piperaquine (PQ), amodiaquine, mefloquine, or sulfadoxine-

pyrimethamine; **Figure 1.2**).[6,12] A standard treatment course of ACTs is 3 days with either once or twice daily dosing. Briefly, the artemisinin component, the more potent drug, rapidly clears blood stage parasites resolving clinical symptoms.[13] However, artemisinins have a very short half-life of between 2 and 5 hours and do not remain above the minimum inhibitory concentration (MIC) beyond the standard 3 days of dosing, at which time some parasites may remain untreated.[6,13] The longer acting partner drug is responsible for eliminating these residual parasites to cure a patient and provides a period of post-treatment prophylaxis against new infections (**Figure 1.2**). Combination therapies for malaria treatment are used to minimize treatment duration, improve adherence, and reduce the risk for selection of drug resistance.[6,13]

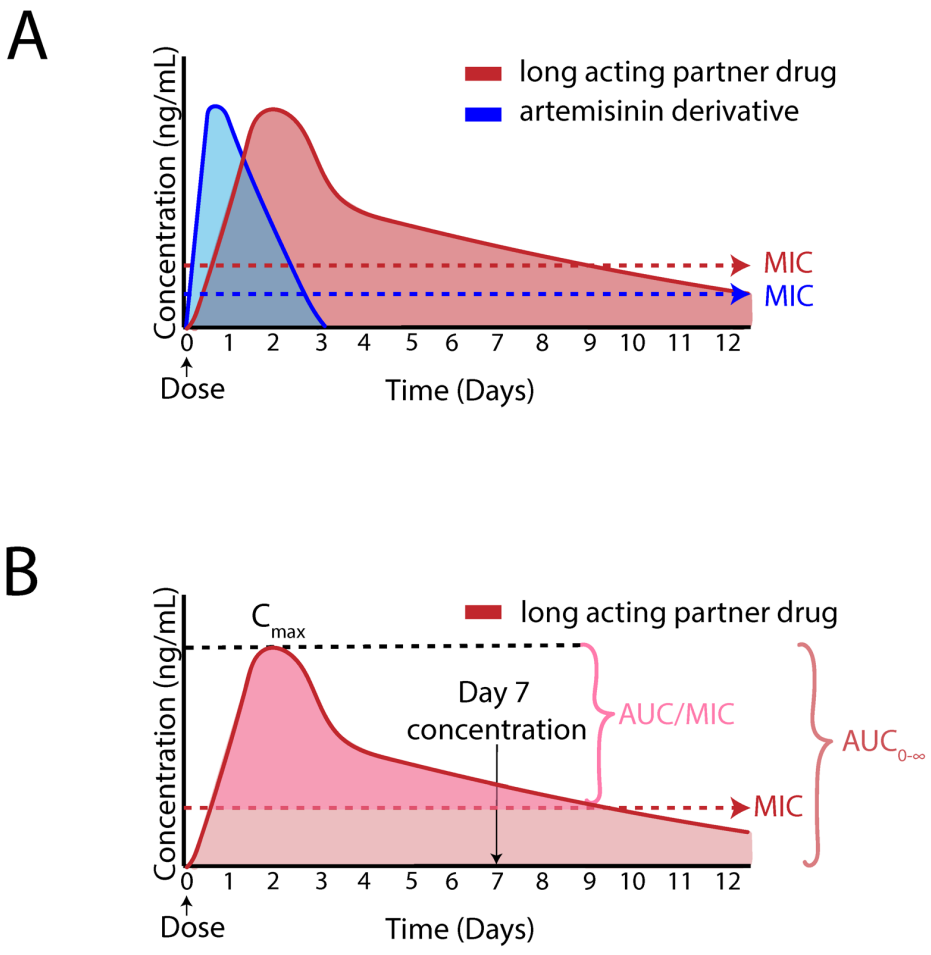

Figure 1.2 Pharmacokinetic profile of ACTs. (a) Schematic representation of the plasma profile of both artemisinin and long- acting partner drug. Although the artemisinin is quickly eliminated, the partner drug has elevated concentrations able to kill parasites for a much longer period of time. (b) Pharmacokinetic markers predictive of treatment outcome. Both day 7 concentration and the area under the concentration time curve (AUC) are associated with malaria treatment outcomes. The C_{max} represents the maximum concentration and the minimum inhibitor concentration able to kill parasites is the MIC. Concentration and MIC are set to arbitrary values.

Since their introduction into sub-Saharan Africa, ACTs have maintained a > 95% treatment efficacy in standard treatment efficacy studies, however, the duration of the post-treatment prophylactic effect varies widely.[14] Despite high treatment efficacy in aggregate, incorporating PK/PD modeling approaches to standard treatment efficacy studies has identified populations at particular risk of malaria treatment failure with current ACT regimens. In this review, we will focus on two of the most commonly prescribed ACTs, AL and DP. Lumefantrine and PQ, in particular, are the most widely studied using PK/PD modeling approaches and these analyses have resulted in changes to dosing guidelines for vulnerable populations or new model informed dosing regimens currently under study.[15,16]

Exposure response biomarker: day 7 concentrations

Some measure of drug exposure is required to understand a drug's concentration-response relationship. Typical measures of drug exposure are area under the concentration time curve (AUC), maximum concentration (C_{max}), or time above the minimum inhibitory concentration (AUC/MIC; **Figure 1.2b**). Day 7 concentrations of the long-lasting partner drugs have been adopted as a surrogate measure of AUC (**Figure 1.2b**). Multiple studies using lumefantrine and PQ have shown this measure is

highly correlated to both AUC and treatment outcomes.[10,17,18] Although this value was not derived from PK/PD models, it has been used extensively for evaluating the efficacy of model-simulated dosing regimens.

Whereas artemisinins are extremely potent with parasite reduction ratios (parasite density pre-treatment/parasite density 48 hours post-treatment initiation) of ~ 10^4-fold, the 3-day treatment regimen only exposes 2 asexual life cycles to artemisinins.[12] This results in the presence of residual parasites after the artemisinins are below effective concentrations. By day 7 post initiation of treatment, only the partner drug is present at concentrations above the MIC and so this concentration reflects the amount of drug present to kill residual parasites. If the concentrations are high enough, a patient will be cured. However, if these concentrations are too low, relapse infections will occur. Studies have proposed day 7 target concentrations of ≥ 57 ng/mL PQ and lumefantrine concentrations of ≥ 200 ng/mL to ensure malaria cure.[10,18,19] The target concentration for lumefantrine is somewhat less certain with values of 175 ng/mL to 500 ng/mL being cited.[20,21] These target concentrations have provided a valuable benchmark and facilitated comparisons between studies. Finally, researchers have argued that in addition to cost effectiveness, this measure is also pragmatic as all patients return to the clinic on day 7 for a blood draw to monitor parasite density. Given there are many possible reasons for why treatment failure can occur, day 7 concentrations can help elucidate whether altered PK may be involved.

Pediatric malaria treatment with dihydroartemisinin piperaquine

As previously described, PK/PD models are well-suited to determine the correct dose of a medication. Because young children (< 5 years) are one of the most vulnerable groups for contracting malaria and also for worse treatment outcomes, this population has been the focus of many recent PK/PD studies.[1] Children are known to have a higher body weight adjusted clearance compared with adults and receive a higher mg/kg dose to counteract these developmental changes.[22] In addition, the PK of infants is particularly difficult to scale due to age-based maturation of metabolizing enzymes such as CYP3A4.[22] DP combines an artemisinin derivative with PQ, a long acting aminoquinoline derivative with the longest half-life (~ 3 weeks) of approved partner drugs. In sub-Saharan Africa, DP provides excellent treatment efficacy > 95% and provides a 1 month post-treatment prophylactic effect. DP, like many ACTs, is dosed according to weight-bands, and day 7 PQ concentrations of 57 ng/mL (capillary) in children < 5 years of age has been associated with a decreased risk of recurrent infection after treatment.[10] In one study, day 7 PQ concentration was the only significant predictor associated with the risk of recurrent malaria. A 5.9% increased risk of new infection for every 1 ng/mL decrease in day 7 PQ concentration was reported.[10] As PQ concentrations have been a key predictor of outcomes, obtaining optimal PQ concentrations in young children has been a major focus of PK/PD modeling efforts.

Although DP is a highly effective therapy, studies which investigated PQ PK revealed that children had reduced PQ exposure compared with adults and older children.[10,23-25] Two studies reported that 40–60% of children did not achieve day 7 PQ concentrations above 57 ng/mL after standard WHO dosing.[10,23] When exploring the

underlying cause for lower PQ exposure, the majority of PK models found that including allometric scaling as a covariate on all clearance and volume parameters was the only significant covariate. One study enrolled both adults and children and reported children had a substantially increased PQ clearance (1.85 vs. 0.9 L/h*kg) compared with adults.[25] However, studies that enrolled infants, 6 months to 2 years of age, detected a relationship between age and PQ clearance $((AGE_i/12)^{0.35})$ where older children had higher clearance than predicted by weight alone.[23] Taken together, the effect of weight and age resulted in 6-month-old children having half the clearance value of a 2-year-old.[23] The authors attributed this finding to represent maturation of the drug metabolizing enzymes (CYPs; CYP3A4 for PQ) responsible for PQ metabolism.[26] By more accurately quantifying the impacts of age and weight on PQ PK, it was found that the approved weight-based dosing for DP was insufficient for low weight children (< 10.5 kg) 1–2 years of age. These findings indicated that the dose children were receiving was not large enough to account for the ontogenetic changes. This was especially true for younger and underweight children.

The two largest PK/PD studies of PQ conducted in African children performed simulations and concluded that children should receive 1.5 to 2 times the current DP dose to achieve day 7 PQ levels of 57 ng/mL.[10,23] The exact percentage of children attaining the target concentration differed (82–86%[10] and 50%[23]) between studies but both indicated that double the number of children would be protected by increasing the dose. In addition to potentially improving treatment outcomes, the authors argued that increasing the dose could have other benefits, such as reducing the selective pressure for drug resistance in parasites and extending the post-treatment prophylaxis period.[10]

The end result of all these studies was that many were combined into individual patient meta-analyses conducted by the WorldWide Antimalarial Resistance Network (WWARN).[11,16] One analysis was focused on defining PQ's PK parameters with population modeling to optimize dosing regimens.[16] This analysis was built from 11 clinical trials and included 8,776 PQ samples from 728 patients. The PK model confirmed the previous findings that young small children were being underdosed. Children < 25 kg were predicted to have a median day 7 PQ concentration of 29.4 ng/mL compared with children and adults > 25 kg who had concentrations of 38.1 ng/mL. Additionally, this model was also able to show in young children enzyme maturation reaches 50% at 6 months of age. By pooling data from multiple studies, this analysis highlighted that underdosing was universal across continents. Due in part to the large dataset size, this analysis was able to refine the previously proposed dosing guidelines and recommend a minimum dose of 64 mg/kg PQ for children 5–15 kg paying close attention to young children and low weight populations. The necessity of dosing changes for DP in young children was confirmed by a second WWARN analysis, which included data from 7,072 patients, with 136 recrudescent infections. This analysis found that after controlling for dose and baseline parasitemia, children 1–5 years of age had a 3.71 increased risk of recrudescence compared with children > 12 years of age and that every 5 mg/kg increase in dose was associated with a 13% decrease in risk of recrudescence. Ultimately, WWARN's work, guided by PK/PD modeling, was the foundation for an update to DP dosing recommendations in young children by the WHO. This is a powerful example of how pharmacometrics and statistical approaches to data integration and analysis led to a high impact policy change.

Pediatric malaria treatment with artemether lumefantrine

Lumefantrine concentrations and other measures of drug exposure are quite variable.[15,27,28] Similar to PQ, lumefantrine exposure is predictive of treatment outcomes.[21,29,30] Multiple studies have documented lower lumefantrine exposure in children and pregnant women compared with adults and, in some cases, these lower lumefantrine exposures have been linked to higher risks of recurrent infection after treatment with AL (**Table 1.1**).[15,29-31] The most comprehensive study on this topic, an individual patient data meta-analysis conducted by WWARN, which included PK data from 4,122 participants including pregnant women, children, and nonpregnant adults from across multiple continents, reported that pregnant women and children < 25 kg had lower day 7 lumefantrine levels compared with nonpregnant adults.[15] After allometrically scaling clearance and volume for weight, children < 15 kg had 24% lower day 7 lumefantrine levels compared with adults, and children 15–24 kg had 13% lower day 7 levels. As the investigators considered optimized AL regimens to improve lumefantrine exposure in children, it was noted that lumefantrine was associated with dose limited absorption, such that longer treatment courses rather than increased daily doses were predicted to improve lumefantrine exposure. This robust PK meta-analysis led to recommendations to extend twice daily dosing for 5 days, which was predicted to better match the distribution of adult and pediatric day 7 concentrations. It also predicted that 75% of children would maintain lumefantrine concentrations over the 200 ng/mL previously recommended threshold.

Table 1.1 Population PK and PK/PD models of partners drugs in vulnerable populations after malaria treatment with ACTs

Country, Year	Population	No. of participants	PK Covariate(s) found	Drug exposure target used for dose optimization	PK/PD relationship from study data	Dosing recommendations
Lumefantrine						
Multi-Country (meta-analysis), 2018[15]	Adults and children including pregnant women	4122	Lumefantrine bioavailability decreased when parasitemia was detected and there was dose-limiting absorption	Day 7 LUM concentrations from non-pregnant adults for dose optimization.	Insufficient power for recrudescent infection to identify population specific associations	Twice daily dosing for 5 days would improve day 7 LUM levels for children
Uganda, 2016[30]	Children 6 months to 2 years	105	CL allometrically increased with weight, lower age had lower relative bioavailability	A day 7 LUM capillary concentration <200 ng/mL	A day 7 LUM concentration <200 increased hazard of 28-day recurrent parasitemia by 3-fold	Children <2 had suboptimal concentrations (median 7-day of 216) but new regimens were not recommended
Tanzania, 2014[65]	Pregnant and non-pregnant women	55	34% lower bioavailability and 78% higher clearance during pregnancy	Literature derived LUM targets used (50, 175, 280, 600 ng/mL) for day 7 simulations.	Four-fold increased odds of recurrent malaria in pregnant women after AL	6 doses over 5 days predicted to decrease the number of individuals below the LUM targets
Tanzania, 2013[27]	Adults, pregnant women, and children (1-78 yrs)	143	CL allometrically increased with weight	Literature derived LUM targets used (50, 175, 280, 600 ng/mL) for day 7	Not evaluated	6 doses over 5 days predicted to decrease the number of individuals below the LUM targets
Papua New Guinean, 2011[29]	Children 5 to 10 years	13	Weight effect on CL, lower LUM exposure was observed in small children (15 - 35 kg)	Not evaluated	Lower average LUM AUC among children with recurrent infection children	Not evaluated
Mali & Niger, 2019[74]	Children age 5-59 months with and without severe acute malnutrition	397	Malnutrition measured by MUAC lowered LUM bioavailability by 25.4% per decrease in MUAC Age (maturation effect) and weight (allometric scaling) increased CL	LUM AUC, day 7 concentration and Cmax values reported in non-SAM children	SAM children had reduced LUM exposure and increased risk of new infections	6 doses over 5 days or 9 doses over three days predicted to result in equivalent exposure in non-SAM and SAM children
Multi-Country (meta-analysis) 2007[55]	Non-pregnant adults HIV-malaria co-infected, malaria-infected and HIV-infected	793	CL allometrically increased with weight Lopinavir/ritonavir: 50.1% slower clearance 67.2% increased bioavailability 47.6% reduction in absorption rate Efavirenz: 89.9% increased clearance	Literature derived LUM target of 200 ng/mL for day 7 simulations.	Not evaluated	Extending treatment over 5 or 6 days was predicted to increase lumefantrine exposure for patients on efavirenz No changed need for lopinavir/ritonavir

Table 1.1 (Continued)

Country, Year	Population	No. of participants	PK Covariate(s) found	Drug exposure target used for dose optimization	PK/PD relationship from study data	Dosing recommendations
Tanzania, 2015[54]	HIV-malaria co-infected adults	269	Efavirenz 58% lower bioavailability Nevirapine: 32% increased bioavailability	Literature derived LUM target of 280 ng/mL for day 7 simulations.	Not evaluated	6 doses over 5 days predicted to decrease the number of individuals below the LUM targets for efavirenz patients
Uganda, 2015[52]	HIV-infected adults	89	Efavirenz 72.6% increased clearance Lopinavir/ritonavir: 62.1% decreased clearance Nevirapine: 24.8% decreased clearance	Literature derived LUM targets used (175, 280, ng/mL) for day 7	Not evaluated	Extending treatment over 7 days was recommended to increase lumefantrine exposure for patients on efavirenz
Piperaquine						
Multi-Country (Meta-analysis) 2017[16]	Adults and children including pregnant women	728	CL increased by age (maturation effect) and allometrically by weight. Dose-occasion impacted bioavailability.	Non-pregnant adult median day 7 PQ concentrations	Not evaluated	Increased dose and increased bands for weight-band dosing of children <25 kg
Uganda, 2015[23]	Children 6 months to 2 years	107	CL increase by age (maturation effect) and allometrically by weight. There was lower exposure in low weight for age children.	Literature derived 57 ng/mL capillary PQ concentration on day 7	Not evaluated	1.5-2 times dosing for each weight band from 6 mo – 2 years, but recommended further QT evaluation of these regimens
Cambodia, 2013[27]	Adults and children (7 - 53 years)	60	CL allometrically increased with weight	Not evaluated	Not evaluated	Not evaluated
Burkina Faso, 2012[10]	Children 2 to 10 years	236	CL allometrically increased with weight, despite increased weight-normalized PQ dose, young children had lower day 7 PQ concentrations.	Literature derived 30 ng/mL venous transformed to 57 ng/mL capillary PQ concentration on day 7	5.9% increased risk of recurrent malaria for each 1 ng/m decrease in day 7 capillary drug concentrations.	30 mg/kg/day dose in children <34 kg decreased percentage of children with day 7 PQ concentration <57 ng/mL from up to 45% to <20%
Papua New Guinean, 2012[24]	Children 5 to 10 years	34	CL allometrically increased with weight	Not evaluated	Lower PQ average AUC among children with recurrent infection (n=8 children)	No model-based changes recommended
Cambodia, 2004[25]	Children 2 to 10 years and adults > 16 years	85	Separate models for adults and children, clearance 2 times higher for children compared to adults.	Not evaluated	Underpowered (high cure rates) to detect associations.	No model-based changes recommended.

ACT, artemisinin-based combination therapy; AL, artemether-lumefantrine; AUC, area under the curve; CL, clearance; Cmax, peak concentration; LUM, lumefantrine; MUAC, mid-upper arm circumference; PD, pharmacodynamic; PK, pharmacokinetic; PQ, piperaquine; SAM, severe acute malnutrition.

Multiple smaller studies have also investigated lumefantrine PK in pediatric populations (**Table 1.1**). One challenge with these studies and the WWARN meta-analysis is identification of a consistent lumefantrine target associated with treatment response. Despite the large patient population from the WWARN study, the low rate of treatment failure prevented identification of population specific day 7 lumefantrine targets associated with efficacy, so a conservative 596 ng/mL nonpregnant adult level was used. Other proposed day 7 targets have included 50 ng/mL, 175 ng/mL, and 200 ng/mL, which were associated with lower risks of recurrent malaria (i.e., combined recrudescence and new infections after treatment). When using these targets for dose optimization, different AL regimens were identified, including spreading the standard 6 doses of AL over 5 days. Currently, twice daily AL for 5 days is being explored for malaria treatment in pediatric populations (NCT03453840).[15] A consensus on which outcomes and PK targets are most relevant for antimalarial dosing, is still needed.

PK/PD modeling has also played an important role in understanding the impact of food on AL absorption. Many studies have investigated lumefantrine's PK to identify the sources of variability in hopes of defining strategies to improve drug exposure. As a lipophilic hydrophobic compound, poor bioavailability was quickly identified as one area limiting drug exposure.[28,32,33] Researchers noted increased bioavailability when lumefantrine was given with food and performed different modeling studies to understand the food effects.[34,35] Dedicated studies indicated that regardless of the food type, lumefantrine bioavailability increased and, for milk, increased by an estimated 57% and 65% for crushed tablets and dispersible tablets compared with no food, respectively.[34] It has also been shown that only 36 mL of milk or 1.2 g of fat is needed to

achieve 90% bioavailability.[35] These findings were significant as they indicated that AL should be administered with food and increasing lumefantrine exposure may not be as simple as increasing the dose.

ACT drug-drug interactions

Drug-drug interactions (DDIs), if clinically significant, can put a patient at risk for malaria treatment failure if concentrations are reduced, or, for adverse events if concentrations are elevated. Malaria endemic regions in sub-Saharan Africa have a large population of HIV-infected individuals receiving antiretroviral therapy (ART).[1,36] Many ACTs have common mechanisms of metabolism with ARTs, namely CYP450 isoenzymes, including CYP3A4/5, CYP2B6, and CYP2C9. [20,37,38] The CYP450 enzymes responsible for ACT metabolism are also those which are induced or inhibited by ARTs.[39-41] Artemisinin components, such as artemether and artesunate, active against *P. falciparum*, are also pro-drugs which undergo metabolic activation by CYP3A4 and CYP2B6 to form dihydroartemisinin.[42] Lumefantrine is also metabolized by CYP3A4 to form desbutyl-lumefantrine.[43] Although studies report desbutyl-lumefantrine is more potent than the parent, it has relatively low exposure making its contribution to treatment outcomes unclear.[44] Both dihydroartemisinin (DHA) and PQ are metabolized by UDP-glucuronosyltransferases and CYP3A4, respectively, to inactive metabolites.[26,45] The impact of co-administration of AL with several commonly used ARTs in malaria endemic regions, lopinavir/ritonavir, efavirenz, and nevirapine, have been studied.[46,47-50] A clear understanding of the extent of these interactions and benefits of dose adjustments have been explored with PK/PD models.

Three studies utilized a population approach to identify interactions between efavirenz and nevirapine and two of these further documented changes by lopinavir/ritonavir.[51-53] These studies used data from clinical trials conducted in various populations ranging from healthy volunteers, HIV-infected but not malaria infected individuals, and HIV-malaria co-infected patients from Africa and the United States. Only one of these studies included artemether and dihydroartemisinin in their analysis.[51] Across all three studies, efavirenz reduced lumefantrine exposure (AUC) by 47–70% in comparison to patients not receiving ART. In two of the studies, this effect was due to 72.6–89.9% increased lumefantrine clearance with the remaining study indicating a 58% reduced bioavailability. All studies attributed these findings to induction of intestinal and/or hepatic CYP3A4 by efavirenz.[54] Interestingly, each study reported a different effect of concomitant nevirapine use, which is a weak CYP3A4 and moderate CYP2B6 inducer. The effect ranged from a 25% reduction to a 32% increase in lumefantrine bioavailability in comparison with patients who did not receive ART. The reason(s) for these conflicting results are unclear but may be due to differences in study design or factors not measured. Ritonavir is a CYP3A4 inhibitor and is responsible for any ACT PK differences noted for the lopinavir/ritonavir combination .[55] Lopinavir/ritonavir was found to increase lumefantrine exposure; both studies reported a 50–62% reduced clearance and the larger study of the two (a pooled analysis of 62 individuals) also identified a 67% increase in bioavailability and a 47.6% slower absorption rate in comparison with patients not receiving ART. The pooled analysis, also tested for a disease effect on PK, confirming that neither HIV nor malaria alone altered lumefantrine

concentrations. An understanding of what parameters are most effected by covariates can help explain possible mechanisms underlying these DDIs.

A single study assessed artemether and DHA PK among individuals with HIV receiving different ARTs (efavirenz, nevirapine, and lopinavir/ritonavir).[51] Lopinavir/ritonavir increased artemether clearance by 32% and DHA clearance by 143% compared with patients not receiving ARTs. Both efavirenz and nevirapine were found to reduce artemether bioavailability by 71% and 66%, respectively, compared with patients not receiving ARTs. Nevirapine was additionally found to decrease DHA clearance by 44%. However, the lower clearance was not sufficient to compensate for lower artemether levels, and there was a net decreased DHA exposure ($AUC_{0–894hrs}$). These interactions all led to reduced artemether exposure and are of concern as these may result in reduced parasite clearance potentially putting patients at risk for malaria treatment failure.

These PK/PD studies concluded that artemether-lumefantrine treatment with lopinavir/ritonavir should be monitored for any toxicities due to increased exposure but appeared safe and effective.[51,52] However, researchers agree that the reduced AL exposure seen with concomitant efavirenz is concerning and warrants trials to investigate dose adjustments.[51-53] Clinical trial simulations based on the final PK/PD models found that extending treatment to 5 or 7 days would equalize lumefantrine exposure between those receiving an interacting ART regimen, and those who do not take these drugs. Although the studies discussed above enrolled adult patients, similar results have been seen in children and pregnant women indicating the universal benefit of dosing changes for all patient populations.[50-56] In addition to testing these novel

regimens, the importance of designing the next set of clinical trials to collect and integrate treatment outcome data should not be overlooked. Along with understanding how DDIs impact antimalarial PK exposure, it is equally important to understand their effects on parasite clearance rates and treatment outcomes.

Pregnancy

Malaria during pregnancy poses a serious risk to the health of both the mother and developing fetus.[57,58] Pregnant women have reduced immunity against malaria and immunity is acquired over subsequent pregnancies.[59] Pregnant women require both effective malaria treatment and prevention options. However, the physiological changes, including increased plasma and body water volume, reduced plasma protein concentrations, altered expression of drug metabolizing enzymes, and increased gastric transit time that occur during pregnancy can alter the PK/PD of therapeutics, including antimalarials.[60,61] Pharmacometric models have been used to quantify the effects of pregnancy on treatment and prevention regimens.

Artemether-lumefantrine PK have been evaluated extensively in pregnant women. Although the results are somewhat conflicting,[62,63] the majority of studies found pregnancy reduced lumefantrine exposure (**Table 1.1**).[17,64-67] Only three studies, however, recorded treatment outcomes. One study conducted in pregnant women reported a 12% increased odds of treatment failure for women who were enrolled later in pregnancy measured by estimated gestational age (EGA; in weeks).[67] This difference in response was attributed largely to altered PK. Pregnancy was found to increase the volume of the central compartment by 7.2% per increase in EGA. The second study

reported a 4.04 increased odds of treatment failure in pregnant women (categorical covariate) compared with nonpregnant controls.[64] The parameter covariate relationships identified were pregnancy decreased bioavailability by 34% and increased intercompartmental clearance by 78%. The final study only enrolled pregnant women.[65] The population PK model identified that pregnancy decreased the rate of absorption (EGA/median EGA$^{)-0.715}$) and linearly decreased the intercompartmental clearance (EGA-(median EGA)*-2.71). A time to event model was built to capture therapeutic outcomes and identified a maximum effect (E_{max}) relationship between lumefantrine concentrations and the hazard of relapse infection (E_{max} fixed to 1 half-maximal effective concentration; 169 ng/mL). The differences in parameters, which pregnancy was reported to effect, may speak to the many and complex changes that pregnancy can create. Pregnancy can change body composition, gastrointestinal motility, and CYP expression, all plausible explanations for the results detailed.[64,65,67] The differences in study design, namely the enrollment of comparator arms, may also have influenced these findings. Only Mosha *et al*. included nonpregnant women as the comparator arm in their study and may be the best positioned to comment on pregnancy's effect on PK parameters.[64] However, this study reported a very low number of new and relapse infections (6 in pregnant women and 1 in nonpregnant women). The remaining two studies were better powered to investigate outcomes (38[67] and 39[65] new and relapse infections). All three analyses suggested that elongating the dosing interval over 5 or more days would increase lumefantrine concentrations and provide equivalent exposure to that seen in nonpregnant controls (**Figure 1.3**). A strength of these studies was that

they indicated reduced exposure in both African and Asian women suggesting these findings have broad applications.

Figure 1.3 Artemether-lumefantrine dosing in different populations. The World Health Organization (WHO) endorsed treatment guidelines are based solely on a patient's weight with adults receiving 4 tablets (80 mg artemether (AR) and 480 mg lumefantrine (LF). Young children < 15 kg receive 1 tablet of 20 mg artemether and 120 mg lumefantrine. Pharmacokinetic/pharmacodynamic (PK/PD) models have proposed that artemether-lumefantrine dosing be extended over 5 instead of 3 days in special populations, including pregnant women, young and underweight children, as well as HIV co-infected patients receiving efavirenz based antiretroviral therapy or other CYP450 inducers.

Based on the findings from PK/PD models, one group has studied the extended 5-day regimen in pregnant women in the Democratic Republic of Congo with other trials planned.[68] The investigators found for both the standard 3 day and extended 5 day regimen minimal differences in outcome measures (100% polymerase chain reaction-

corrected clinical and parasitological response in both populations) and lumefantrine exposure (AUC: 3 day regimen 531 h*µg/mL nonpregnant, 586 pregnant; 5 day regimen 933 h*µg/mL nonpregnant, 853 pregnant) between pregnant and nonpregnant controls. It is unexpected that this study did not detect any effect of pregnancy on lumefantrine PK as this differs from the majority of studies reported in the literature. However, artemether and DHA exposure was reduced by 1.2% per increase in each gestational week, due to lower artemether bioavailability compared with nonpregnant adults. The study also identified that pregnant women had longer parasite clearance rates (3.3 hours vs. 2.43 hours) compared with nonpregnant controls. This finding was not associated with artemether or DHA exposure and the authors suggest this may be due to reduced splenic clearance instead.[68] Most importantly, this study confirmed that extending AL dosing over 5 days increased exposure to artemether, DHA, and lumefantrine in both pregnant and nonpregnant populations. The extended regimen was safe for both mother and fetus and well-tolerated. This proof-of-concept clinical trial confirmed that extending AL dosing over 5 days is a promising alternative for areas where AL treatment efficacy is waning due to low immunity or high levels of drug resistance.

Malnutrition

While multiple studies have evaluated malnutrition as a risk factor for malaria,[69-72] few dedicated studies have been conducted to investigate malnutrition's effects on ACT PK/PD.[73] Only one study to date was specifically designed to analyze the interplay between severe acute malnutrition and lumefantrine PK/PD in African children.[73] After

controlling for ontogenetic changes with allometric scaling and enzyme maturation, mid-upper arm circumference was found to alter bioavailability whereby for every 1 cm decrease in mid-upper arm circumference, there was a corresponding 25.4% decrease in bioavailability. Decreased absorption was predicted to reduce lumefantrine exposure when measured as both $AUC_{0\text{-}28days}$ and day 7 concentrations. Malnutrition was not independently associated with increased risk of malaria when tested as a covariate on the PD model parameters. It is due to reduced exposure that malnourished children had an increased risk of reinfection compared with nourished children. Similar to the lumefantrine studies discussed above, simulations for this study population supported extending AL treatment to 5 days (**Figure 1.3**). This study highlights the importance of conducting dedicated PK/PD analyses in subgroups, such as malnourished children who are at particular risk of modified PK exposure. PK/PD models, by quantifying drug exposure-response relationships, have increased power to detect PK differences in understudied populations.

Lessons learned and future directions

Evaluation of drug exposure-response relationships in large, diverse populations receiving antimalarials has led to important dosing and guidance changes for ACTs, such as DP, and has been instrumental in identifying optimized regimens for clinical trials in vulnerable populations, such as pregnant women and childrend.[1] However, we argue that with adjustments to future antimalarial trials and studies, PK/PD models could be leveraged to have an even more rapid and broader impact on antimalarial policy. We recommend: (i) studies of new and established antimalarials enrich

enrollment for populations at high risk of altered PK exposure (e.g., pregnant women, young children, and those with comorbidities such as severe malnutrition, HIV, and tuberculosis), (ii) PK/PD studies be designed to assess for a broader range of drug exposure-response relationships (e.g., high sensitivity assays for parasitemia, rigorous assessment of nutritional status, and birth outcomes), and (iii) PK/PD modeling studies expand their analyses to include biomarkers of immunity and drug resistance.

1. Quantifying antimalarial PK in high-risk subpopulations

PK/PD models increase the power to detect drug exposure-response relationships by quantifying variability in drug exposure and treatment response in the population. This is particularly helpful for small clinical studies focused on under-represented subgroups. Malaria endemic regions have significant burdens of malnutrition, HIV, and tuberculosis.[74] As a result, antimalarials are frequently used in individuals at especially high risk of malaria in combination with physiologic conditions (e.g., chronic inflammation or low protein binding) or DDIs known to reduce antimalarial exposure.[75] However, these populations are often excluded from standard clinical trials or are difficult to recruit. As a result, there have been delays in quantifying PK/PD relationships for antimalarials in malnourished individuals and those with DDIs. Recent data in these populations, nearly 20 years after the introduction of ACTs, suggests that longer treatment durations (5 days for AL) or higher daily doses of antimalarials (DP) may be needed for these groups to achieve target treatment outcomes.[15,49] Early inclusion of high-risk subgroups into clinical trials designed to incorporate PK/PD modeling techniques can help us more rapidly identify and characterize the needs of

high-risk subpopulations and devise precision dosing regimens that are acceptable for low resource settings.

2. Optimizing clinical study designs for PK/PD modeling approaches

Another challenge faced by clinical PK/PD models for antimalarials has been accurate measurement of covariates, which impact PK exposure and outcomes during the study. The mismatch between self-reported and actual adherence is an example of this limitation. Lower than reported adherence can limit the generalizability of PK/PD models and can bias dose optimization recommendations.[76] Antimalarials pose a particular challenge as drug absorption for ACTs can be variable between individuals and dosing occasions[20] even when all doses are directly observed. This confounding factor limits our ability to differentiate between physiologic and behavioral effects impacting pharmacology and identification of safe and effective antimalarial regimens. Incorporation of more rigorous adherence measures into clinical studies can improve the generalizability of PK/PD models and inform novel dosing regimens which maintain effectiveness despite imperfect adherence.[76,77] We encourage clinical studies to more robustly collect data on adherence and other covariates (e.g., nutritional status, concomitant drug concentrations, and markers of parasite drug resistance) of importance for PK and PD.

In addition to measuring clinical covariates that could impact drug exposure and treatment response, we can improve our understanding of drug efficacy by utilizing the more sensitive and quantitative measures of malaria outcomes which are now being developed in malaria research. These include use of ultrasensitive quantitative

polymerase chain reaction to quantify parasite densities, including submicroscopic parasitemia, or measurement of placental malaria using a severity grading metric for histopathology.[78] In conjunction with measuring biomarkers of immunity and drug resistance, as described in the section below, these more sensitive and quantitative measures of malaria outcomes in PK/PD studies can enhance our mechanistic understanding of drug response and will allow us to consider goals for malaria control beyond preventing symptomatic disease, such as malaria elimination. It can also allow us to develop population-specific drug exposure targets for children, pregnant women, and those with comorbid conditions.

Finally, PK/PD models can be utilized to explore the impact of pharmacologic interventions beyond antimalarial efficacy. To achieve this valuable goal, measurement of select nonmalarial outcomes must be included in clinical studies. As an example, despite widespread antifolate resistance in east Africa, monthly intermittent preventative treatment in pregnant women (IPTp) with the antifolate sulfadoxine-pyrimethamine increased birthweight at delivery.[79] Antibacterial benefits may mediate this effect,[79] but bacterial outcomes have been poorly characterized in malaria IPT studies. In addition, in longitudinal malaria chemoprevention studies, although birth outcomes or progression of malnutrition are often measured in clinical studies, PK data is rarely available in a sufficient number of study participants to detect associations between drug exposure and these outcomes. Highly sensitive drug quantification methods using low volume plasma or blood spots are likely to improve our ability to concurrently quantify PK and outcomes, including rare events, in large trials.

However, birth outcomes and child morbidity are drivers of malaria control policy. In fact, the WHO has made clear that any new IPTp regimens should not only prevent malaria but must also decrease adverse birth outcomes.[80] PK/PD modeling is perfectly positioned to clarify relationships between drug exposures and these key outcomes and to leverage these relationships for dose optimization. PK data and comprehensive outcomes data must be collected concurrently in large study populations and dedicated trials must be carefully designed.

3. Quantifying the impacts of drug resistance and immunity drug efficacy and drug resistance

An important goal of antimalarial drug development and policy is to select antimalarial combinations with high barriers of drug resistance and to identify and react to a failure of a treatment regimen due to drug resistance early. Antimalarial drug resistance develops stepwise, with mutations accumulating, which decrease but do not fully eliminate activity against parasites. Decreased sensitivity to artemisinins and PQ is spreading in southeast Asia,[81] and AL treatment failure rates > 10% have been reported in sub-Saharan Africa.[82] Ideal antimalarial treatment regimens would achieve high efficacy while minimizing dosing frequency, toxicity, and selection for drug resistance. Unfortunately, these goals can be at odds when long acting antimalarials prolong efficacy but can also result in long subtherapeutic tails that can select for more resistant parasites.[83,84] Capturing the dynamics of how drug exposure selects for drug resistance with PK/PD models is a valuable contribution to antimalarial regimen selection and dose optimization. Drug resistance in PK/PD models has been influential in simulation

studies, guiding selection of the triple ACT regimens,[85] and predicting failure of malaria chemoprevention regimens.[86] However, relationships between antimalarial exposure and biomarkers of drug resistance have rarely been quantified and validated with clinical data. Higher drug concentrations can overcome decreased antimalarial sensitivity and with PD targets, drug regimens can be optimized to minimize malaria recrudescence after treatment or malaria infection after chemoprevention.[8,87] It will be important for PK/PD models to incorporate drug resistance biomarkers as they become available, and that these biomarkers be incorporated into clinical trials that include PK data.

Malaria immunity

Naturally acquired immunity to malaria develops with increasing age and following repeated exposure to malaria parasites. This immunity is characterized by a decreasing likelihood that blood-stage parasite infections are associated with symptoms, and thought to be comprised of two distinct but complementary processes: (i) anti-parasite immunity, which helps control blood-stage infection such that in highly endemic settings older (more immune) individuals carry lower parasite densities than younger individuals; and (ii) anti-disease immunity, which allows individuals to tolerate high parasite densities without developing a fever.[88] Importantly, pre-existing antimalarial immunity can influence malaria treatment outcomes and PK/PD relationships by accelerating parasite clearance and reducing the risk of recrudescence following treatment.[89] Antibodies specific for blood-stage malaria antigens have been associated with a reduced risk of treatment failure.[7,90-92] A recent paper further found

that functional characteristics of the Ig subclass of antimalarial antibodies—the ability to both fix complement and mediate opsonic phagocytosis—were also associated with faster parasite clearance.[93] These data show the importance for future malaria PK/PD models to include biomarkers of antimalarial immunity, particularly as malaria vaccines are studied in conjunction with pharmacological-based malaria control interventions.

Model-informed drug development

With variability in drug response predicted due to drug resistance, immunity, and potentially other comorbidities, it is not surprising that predicting dose, response, and the best combinations of antimalarial drugs from preclinical data using PK/PD models has been challenging.

Translational PK/PD

Developing a translational mechanistic model for malaria to accelerate drug combination and dose selection has been challenging (**Figure 1.1**). Standard 48 hour *in vitro* parasite sensitivity experiments with synchronized parasite cultures are likely a poor surrogate for drug efficacy over a 1–2 week treatment duration with drug combinations. *In vitro* systems to assess *P. falciparum* drug sensitivity and a mouse model with *P. berghei* (rodent specific parasite species) allowed for a pipeline of new antimalarial drug candidates, but mechanistic PK/PD models based on preclinical data have overpredicted clinical benefits.[94-96] Recent developments have focused on creating a mouse model able to sustain *P. falciparum* infections and now a humanized mouse model engrafted with human erythrocytes exists.[97] Unfortunately, this advanced model

still overpredicts the clinical benefit of drug candidates.[98] Modeling parasite dynamics with and without drugs from preclinical data requires the reliable transformation of *in vitro* drug efficacy to *in vivo* drug activity. Furthermore, our understanding of human parasite burden and dynamics without drug pressure continues to evolve to incorporate polyclonal infections, immunity, infection timing, age, and size. We expect that by incorporating longitudinal parasite density measurements from murine and human infection volunteer data, we may improve predictions of mechanistic PK/PD models for drug efficacy and/or develop a superior method to rank compounds allowing for more rapid translation of drugs from discovery to development.

Controlled human malaria infection models

PK/PD models are an indispensable tool for drug development and drug repurposing. Achieving safe single dose malaria treatment and prevention regimens would transform the malaria therapeutic landscape. To fill this gap, a controlled human *P. falciparum* infection model has been developed for human studies, and is being used to quantify the initial PK/PD relationships. These studies are conducted in a hospital settling which allows for the collection of intensive PK and PD data. Non-immune healthy volunteers are infected with malaria either by mosquito bites (sporozoite-induced) or by direct injection of blood-stage parasites.[99] If the sporozoite-induced infection is used, researchers can study if the candidate drugs have any effect on liver stage parasites. Typically, studies will define a set parasite density threshold at which treatment will begin. Parasite densities are closely monitored before treatment is provided and this data can be used to establish the natural growth dynamics of

P. falciparum. Controlled human malaria infection studies provide the malaria community with a unique opportunity to understand the PK/PD of new therapeutics early in develop and make informed decisions on the next dose to be used.

Lessons learned

Antimalarial PK/PD relationships have been some of the best quantified among anti-infectives in the global health arena. Pharmacometric methods, including PK/PD modeling, led directly to dosing changes for ACTs, have facilitated selection of repurposed drug regimens for malaria treatment and prevention, and are becoming an integral component of mathematical models which guide malaria control policy. Other global health disease areas can improve treatment, prevention, and elimination efforts by following the example set and lessons learned within the malaria field.

1. **Standardized clinical trial design, biomarker and outcomes measurement, and PK data collection enhances the quality of clinical studies**

Malaria clinical trials are conducted in many countries, with diverse populations, drugs, and sample sizes. However, the research community has been able to maximize the reach and scientific conclusions from these trials owing to the standardized manner in which they are conducted. As an example, malaria treatment efficacy studies use directly observed therapy, conduct follow-up for clinical or parasitological relapse at standard intervals through 28 to 42 days after treatment, and when PK data is collected, usually obtain day 7 PK concentrations. Outcome measures have standard definitions, including adequate clinical and parasitological response, recrudescence infection

(treatment failure), or reinfection. Both designing and conducting trials in a similar manner has facilitated comparison of results across studies, including the ability to pool data to identifying subpopulations at risk and in indicating to regulators how pervasive dosing issues are globally. Infectious disease clinical trials could greatly benefit from standardization to facilitate *post hoc* data analyses especially as it pertains to PK/PD measures.

2. Strong systems to share clinical, molecular, and PK data enhances our ability to identify optimal antimalarial regimens for vulnerable populations

WWARN has been an instrumental organization in collecting, standardizing, and generating PK/PD databases for malaria research. They have demonstrated the power of pooled individual patient data meta-analyses by aggregating historic data and leveraging the large number of patients and observations to answer important questions about understudied populations. WWARN has encouraged investigators and set a precedent that clinical data be shared. As described above, these studies have helped to identify high-risk subpopulations and used PK/PD modeling to recommend new dosing regimens. Although not all of these studies have ultimately resulted in changes to dosing guidelines, they have helped indicate the next steps in dosing regimens, which are currently being explored in clinical trials.

3. **Clear translation of findings from PK/PD analyses into predicted improvements in treatment outcomes has led to policy changes in antimalarial dosing guidelines**

PK/PD model-informed dosing of antimalarials has become a valued tool for antimalarial research and policy. Pharmacometricians have presented the results of their PK/PD modeling work in terms of clinical impact and have identified dosing regimens that consider safety, efficacy, and implementation. Some of these changes have been enacted directly (e.g., DP dosing in pediatric populations) whereas others are already in clinical trials (AL in young children and pregnancy, triple ACT regimens in South East Asia). Although we note that PK/PD studies for antimalarials could be improved by diversifying population specific outcomes to include those that are highest priority for regulators, such as birth outcomes among pregnancy, pharmacometricians in antimalarial research have made significant progress in translating scientific findings into action. As the value of PK/PD modeling has been demonstrated, it has opened the door to more advanced applications, and a more rapid translation of scientific discovery to policy.

Conclusions

Pharmacometric modeling has played an instrumental role in improving malaria treatment by generating dosing regimens and new drug candidates. Malaria investigators have used PK/PD modeling to extensively study dosing in high-risk groups and identified that pregnant women, young and underweight children, as well as individuals receiving concomitant therapy with CYP450 inducers could all benefit from

dose adjustments. As PK/PD modeling becomes more widespread in clinical studies, we expect to see more updates to malaria treatment and prevention guidelines. By understanding how the malaria field has experienced success in applying pharmacometrics to improve outcomes, we suggest these successes can be achieved in other disease areas.

Thesis aims

As discussed above, in the context of malaria, one size fits all dosing guidelines leave vulnerable populations such as pregnant women at risk for underdosing and poor treatment outcomes.[17,64-67] This is particularly important for pregnant women with comorbidities such as HIV which require taking concomitant medications.[51-53] Through analyzing data from dedicated treatment trials in pregnant women, we are beginning to understand the effects of pregnancy and propose changes to improve efficacy.[67] However, more work is still required to change treatment guidelines. In addition, the use of ACTs for malaria prevention in pregnant women is a relatively new area of research. As such, optimal ACT dosing is still under study with many knowledge gaps.

The aims of this thesis were to inform on malaria treatment and prevention guidelines for pregnant women by addressing some of the gaps in our knowledge. Two of the most important antimalarials, artemether-lumefantrine and dihydroartemisinin-piperaquine were investigated. This work utilized recent clinical trials which collected PK, efficacy and toxicity data in pregnant women. Chapters 2 and 3 investigate how pregnancy and other comorbidities alter ACT PK and importantly propose dosing changes to compensate for lower drug exposure. Chapter 4 focuses on understanding the longitudinal PK-QTc relationship for piperaquine in pregnant women to ensure the experimental prevention regimen is safe. Lastly, Chapter 5 addresses the piperaquine-parasitemia relationship to validate the previously proposed exposure targets in pregnant women and again suggest dosing regimens to ensure all women achieve these protective PK levels.

Collectively these chapters show the importance of conducting dedicated clinical trials which include PK and PD measures in pregnant women and how this data can be used to optimize drug exposure, efficacy and minimize toxicity.

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Chapter 2: Efavirenz-based antiretroviral therapy reduces artemether-lumefantrine exposure for malaria treatment in HIV-infected pregnant women*

Abstract

Background: The choice of malaria treatment for HIV-infected pregnant women receiving efavirenz-based antiretroviral therapy must consider the potential impact of drug interactions on antimalarial exposure and clinical response. The aim of this study was to investigate the effects of efavirenz on artemether–lumefantrine (AL) because no studies have isolated the impact of efavirenz for HIV-infected pregnant women.

Methods: A prospective clinical pharmacokinetic (PK) study compared HIV-infected, efavirenz-treated pregnant women with HIV-uninfected pregnant women in Tororo, Uganda. All women received the standard 6-dose AL treatment regimen for *Plasmodium falciparum* malaria with intensive PK samples collected over 21 days and 42-days of clinical follow-up. PK exposure parameters were calculated for artemether, its active metabolite dihydroartemisinin (DHA), and lumefantrine to determine the impact of efavirenz.

Results: Nine HIV-infected and 30 HIV-uninfected pregnant women completed intensive PK evaluations. Relative to controls, concomitant efavirenz therapy lowered the 8-hour artemether concentration by 76% (P = 0.013), DHA peak concentration by

* Modified from the publication: Hughes E, Mwebaza N, Huang L, et al. Efavirenz-Based Antiretroviral Therapy Reduces Artemether Lumefantrine Exposure for Malaria Treatment in HIV-Infected Pregnant Women. J Acquir Immune Defic Syndr **2020**; 83(2): 140-7.

46% (P = 0.033), and day 7 and 14 lumefantrine concentration by 61% and 81% (P = 0.046 and 0.023), respectively. In addition, there were nonsignificant reductions in DHA area under the concentration–time curve$_{0-8hr}$ (35%, P = 0.057) and lumefantrine area under the concentration–time curve$_{0-\infty}$ (34%, P = 0.063) with efavirenz therapy.

Conclusions: Pregnant HIV-infected women receiving efavirenz-based antiretroviral therapy during malaria treatment with AL showed reduced exposure to both the artemisinin and lumefantrine. These data suggest that malaria and HIV coinfected pregnant women may require adjustments in AL dosage or treatment duration to achieve exposure comparable with HIV-uninfected pregnant women.

Introduction

Malaria and human immunodeficiency virus (HIV) infection are endemic in sub-Saharan Africa imposing an extensive burden of morbidity and mortality on vulnerable populations such as pregnant women.[1,2] In 2017, there were an estimated 940,000 HIV-infected pregnant women in eastern and southern Africa.[3] Approximately 28 million pregnancies occurred in malaria endemic African regions and, without intervention, 11.4 million are estimated to have placental infection with *Plasmodium falciparum*.[4,5] Pregnant women are at an increased risk for malaria compared to nonpregnant populations and HIV-infected pregnant women have an even greater risk for malaria and experience higher rates of adverse birth outcomes. [5-8]

Malaria infection during pregnancy poses a risk to both mother and fetus as the parasite will concentrate in the placenta leading to many adverse birth outcomes.[6,9-11] Recent estimates report 41% of all live births have evidence of placental malaria while others have attributed 75,000–200,000 infant deaths to placental infection.[4,6,7,9] The artemisinin-based combination therapy (ACT), artemether-lumefantrine (AL), is the most widely prescribed first-line treatment for malaria.[12] Artemether is converted to dihydroartemisinin (DHA) and both compounds actively reduce parasite density, while the long-acting partner drug lumefantrine clears residual parasites, and the combination of the two drugs reduces the spread of drug resistance.[13] Due to the risks associated with clinical malaria in pregnancy, it is imperative to establish optimized dosing guidelines for pregnant women.

All pregnant HIV-infected women require antiretroviral therapy (ART).[14] Dolutegravir-based ART is now considered safe for pregnant women and the WHO has

recently recommended it as the first line regimen.[15] However, millions of women remain on efavirenz (EFV)-based ART which they will continue until countries transition to dolutegravir or if adverse reactions to dolutegravir occur.[15] Multiple studies in nonpregnant populations, including children and adults, have shown that ART choice influences AL pharmacokinetics (PK) as well as malaria treatment outcomes due to pronounced drug-drug interactions.[16-18] This paper details the AL-EFV interaction specifically in pregnant women, a previously unstudied population. Both artemether and lumefantrine are metabolized by cytochrome p450 3A4 (CYP3A4), leaving them susceptible to either metabolic inhibition or induction depending on the concomitant ART.[19-21] Efavirenz, in particular, is a strong CYP450 inducer.[22-24] Studies in efavirenz-treated HIV-infected children and nonpregnant adults, compared to a control group not on ART, revealed highly significant reductions in the PK exposure of both artemether and lumefantrine leading to reduced clinical response.[16,18,22,25]

We therefore hypothesize that the drug-drug interaction between efavirenz and AL in HIV-infected pregnant women undergoing malaria treatment will lead to reductions in AL exposure which may put this particular population of women at risk for inadequate treatment, treatment failure, or a reduction in the post-treatment prophylactic period.[22-27] Despite the wide-spread use of AL, no reports to our knowledge have addressed the effects of efavirenz-based ART on AL pharmacokinetics in HIV-infected pregnant women; previous studies have only investigated the effects of pregnancy on this treatment combination.[28] Our goal is to inform specific artemether-lumefantrine dosing guidelines for efavirenz-treated HIV-infected pregnant women.

Methods

Study participants and ethical approval

This prospective, single center study was carried out in the high malaria transmission district of Tororo, Uganda from February 2012 to November 2014. HIV-infected and HIV-uninfected pregnant women with uncomplicated *P. falciparum* malaria (presenting with a fever or history of fever within the last 24 hours, tympanic temperature of ≥ 38°C and a positive thick blood smear) or asymptomatic parasitemia (confirmed by thick blood smear) were enrolled from the Tororo District Hospital or a local referral center. Six HIV-infected women were co-enrolled from a parent trial which investigated whether ARTs confer malaria protection in pregnant women (NCT00993031).[29] Eligible women were ≥16 years of age; between 12–38 weeks gestational age confirmed by ultrasound; lived within 60 km of the study clinic; had not taken an antimalarial within two weeks prior to enrollment; did not have severe malaria or other significant co-morbidities; had hemoglobin levels >7.0 g/dL; and had not taken medications (other than the study drugs) known to affect CYP3A4 metabolism such as antituberculosis (i.e. rifampin) and antifugals (i.e itraconazole and ketoconazole).[30] HIV status was confirmed with 2 assays and HIV-infected individuals must have initiated EFV-based ART for at least 10 days prior to enrollment.

Approval for this study was independently granted by all ethical review boards involved: the Makerere University School of Medicine Research and Ethics Committee (Kampala, Uganda), the Uganda National Council for Science and Technology (Kampala, Uganda), the Yale University Human Investigations Committee (New Haven, CT), and the University of California, San Francisco Committee on Human Research

(San Francisco, CA). Written informed consent from all women was received prior to beginning the study. The trial was funded by the National Institutes of Health (R01HD068174; Clinicaltrials.gov number, NCT01717885).

Study design

At enrollment, a routine medical examination was performed which included a detailed medical history and obstetric ultrasound. A blood sample was obtained for thick and thin blood smears, complete blood count, liver function (AST and ALT) and PK analysis. Active follow-up was conducted on days 1, 2, 3, 4, 8, 14, 21, 28 and 42, and participants were advised to come to the study clinic if they were sick in between visits (**Figure 2.1**).

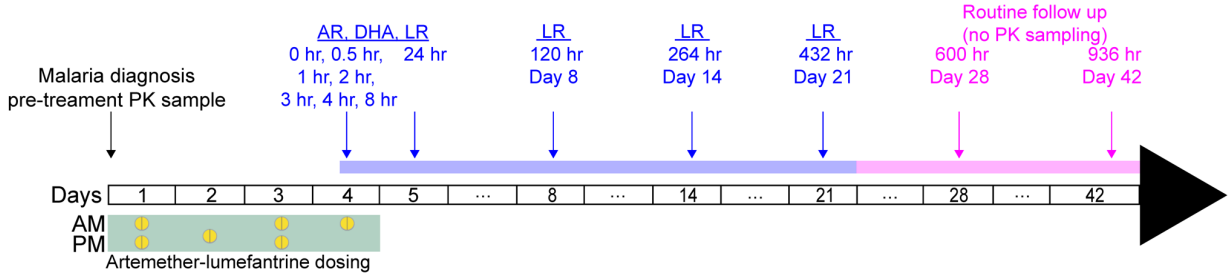

Figure 2.1 Treatment and PK sampling schedule. Following malaria diagnosis on study day 0, six doses of AL were administered from study days 0 to 3 (green box). Plasma PK samples were collected on day 0 prior to treatment, before (0hr) and at 0.5, 1, 2, 3, 4, 8, 24, 120 (day 8*), 264 (day 14), and 432 (day 21) hr post sixth dose (blue arrows). Active follow up for malaria was performed on days 28 and 42 (pink arrows). The 120 hr sample occurred on day 8 in this study due to the elongated dosing schedule. Previously studies using the standard three day dosing report the 120 hr sampling point as day 7 values. Given that sampling occurred at the same post-dose time we will refer to day 8 as day 7 throughout. AR, artemether; DHA, dihydroartemisinin; LR, lumefantrine.

All pregnant women received six doses of artemether-lumefantrine (Coartem®; Novartis Pharma AG, Basel, Switzerland; four tablets each 20 mg of artemether and 120 mg of lumefantrine) with 200 mL of whole milk, a high fat content drink, to increase lumefantrine absorption.[31] The first, third, fourth and sixth doses (all scheduled for daytime administration) were observed in the clinic with the second and fifth doses taken at home. The dosing schedule was slightly extended so that the last dose was administered in the morning to facilitate intensive PK sampling during the daytime (**Figure 2.1**).

HIV-infected women received standard dosing of efavirenz and two nucleoside reverse transcriptase inhibitors (NRTIs; either tenofovir plus lamivudine, tenofovir plus emtricitabine, or zidovudine plus lamivudine) each morning within 3 hours of their artemether-lumefantrine. HIV-infected women also received daily trimethoprim-sulfamethoxazole (TS) per WHO treatment guidelines for opportunistic infection prophylaxis.[12,32] Based on Ugandan national guidelines, HIV-uninfected women received two doses of sulfadoxine-pyrimethamine between 16–24 and 28–36 weeks gestation for malaria prevention.[33]

PK study design and sample collection

Blood samples were collected as displayed in **Figure 2.1**. Due to the dosing schedule, the 120 hour PK sample which typically falls on day 7 occurred on day 8 instead. As was done in previous publications, we will refer to day 8 as day 7 (since both refer to the 120 hour sample) in the remainder of the publication for easier comparisons.[34] Venous samples were collected for PK analysis before the start of treatment on study day 0, and prior to and following the last dose at 0, 0.5, 1, 2, 3, 4, 8,

12 and 24 hours and 7, 14, and 21 days. Only participants who took all six doses proceeded with PK procedures. Blood samples (200–500 µL) were collected in K3EDTA tubes and immediately placed on ice. Plasma was obtained by centrifugation at 2000 X g for 10 minutes at 4°C and then stored at −70°C.

Parasitological follow up

Parasite densities from Giemsa-stained thick smears were calculated as the number of asexual parasites per 200 leukocytes assuming there were 8,000 leukocytes per µL. If no asexual parasites were seen under 100 high-power fields, the smear was declared negative.

Drug assay

Plasma concentrations of artemether, DHA, and lumefantrine were quantified using an accurate and sensitive validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method as previously described.[35,36] The calibration range for artemether and DHA was 0.5 – 200 ng/mL and for lumefantrine was 50 – 20,000 ng/mL. The coefficient of variation was <5% CV for lumefantrine and <10% CV for the artemisinins. The lower limit of quantification (LLOQ) was 0.5 ng/mL for artemether and DHA and 50 ng/mL for lumefantrine.

Data analysis

The primary objective of this study was to evaluate the plasma PK parameters for artemether, DHA, and lumefantrine. Parameters included the area under the concentration-time curve (AUC_{0-8hr} for artemether and DHA; $AUC_{0-\infty}$ for lumefantrine), maximal concentration (C_{max}), time to C_{max} (T_{max}), terminal elimination half-life ($t_{1/2}$), and plasma concentrations at 8 (C_{8hr}) and 24 (C_{24hr}) hours for artemether and DHA and on

days 7 (C_{7d}), 14 (C_{14d}), and 21 (C_{21d}) for lumefantrine. Secondary safety and tolerability endpoints, including adverse events, were measured using the grading criteria developed by the National Institutes of Allergy and Infectious Diseases Division of AIDS.[37] Treatment outcomes including early treatment failure, late clinical failure, late parasitological failure, and adequate clinical and parasitological response were assessed on day-28 and −42 using standard WHO criteria.[38]

Noncompartmental analysis was performed using WinNonlin (version 6.4; Certara, Princeton, NJ). The C_{max}, T_{max}, and terminal concentrations (C_{8hr} and C_{24hr} for artemether and DHA, and C_{7d}, C_{14d} and C_{21d} for lumefantrine) were reported as observed. The linear-up/log-down trapezoidal method with first-order input was used to calculate the AUC_{0-8hr}. The $AUC_{0-\infty}$ was determined by dividing the last measured concentration by the terminal elimination rate constant (λ_z) where λ_z was measured using WinNonlin's best fit feature. Plasma samples below the lower limit of quantification (LLOQ) were generally treated as missing values. Exceptions to this rule were the pre-dose samples which were set to zero and, during the terminal phase, when the first value to fall below the LLOQ was essential to determining the AUC, in which case the sample was assigned a value of half LLOQ.

Statistical analysis was performed using STATA version SE 12.1 (StataCorp, College Station, TX, USA). Pairwise PK parameters were compared using a Wilcoxon rank sum test with a p-value < 0.05 considered significant. Data are presented as the geometric mean or median as appropriate. The relationship between AL exposure (AUC, C_{max} and terminal concentrations) and treatment outcome (late clinical failure and late parasitological failure were both considered treatment failure and handled as binary

data) was explored using logistic regression (R Studio version 1.1.423 with package stats version 3.4.3).

Results

Study profile

From February 9, 2012 to November 17, 2014, 69 pregnant women were screened of whom 49 (35 HIV-uninfected; 10 HIV-infected) were enrolled (**Figure 2.2**). Ten HIV-infected participants and 31 HIV-uninfected participants completed the study. Four HIV-uninfected women were withdrawn due to lack of study drug adherence (n=2), lost to follow up (n=1) and use of other antimalarials during study period (n=1). One HIV-uninfected woman gave birth on day 11 and one HIV-infected woman had greater than half her blood samples missing so both were excluded from the final analysis. In total, 30 HIV-uninfected and 9 HIV-infected women were included in this PK analysis. Data from the HIV-uninfected women has been previously reported.[34] Baseline characteristics for all women are listed in **Table 2.1**. All characteristics were comparable in these two groups.

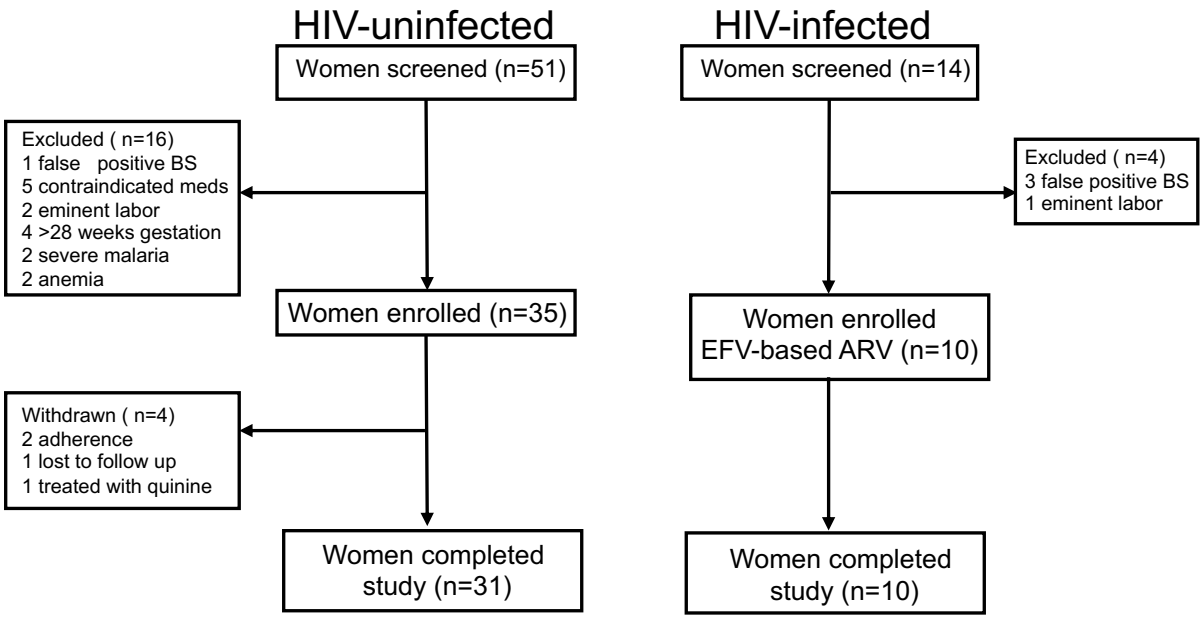

Figure 2.2 Enrollment and completion for PK-trial profile.

Table 2.1 Baseline characteristics of enrolled study participants

Characteristic[a]	HIV-uninfected Pregnant women (n =30)	HIV-infected Pregnant women (n =9)	p-value
Age (yr)	25 (18-39)	26 (18-34)	0.97
Body Weight (kg)	59.4 (44.5-81.1)	64.2 (56-93)	0.07
BMI (kg/m^2)	21.9 (17.4-28.9)	19.2 (21-35)	0.07
Gestational age (wk)	28 (14-34)	25 (14-31)	0.21
Gravidity	2 (1-8)	2.5 (1-6)[b]	0.60
Parasite density (parasites/uL) (geometric mean [95% CI])	13,227 (7,728-22,639)	3,078 (846-11,193)	0.02
Temperature (˚C)	37 (36-37.6)	37 (36.2-38.5)	0.43
Alanine aminotransferase (IU)	12 (7-43)	11 (1-23)	0.74
Aspartate aminotransferase (IU)	23 (12-57)	21 (17-42)	0.91
Serum creatinine (mg/mL)	0.64 (0.17-1.27)	0.69 (0.29-0.99)[b]	0.96
Platelet count (10^3/mL)	142 (36-309)	163 (90-243)[b]	0.75
Hemoglobin level (g/dL)	10.5 (7.6-13.1)	9.6 (7.9-12.3)[b]	0.71

[a] All values are the median (range), unless otherwise specified. CI, confidence interval

[b] Only four participants had baseline creatinine values available

Pharmacokinetic parameters

Pharmacokinetic parameters for artemether and DHA are summarized in **Table 2.2** and **Figure 2.3**. The exposure parameters of interest were both the AUC and terminal concentrations. No significant difference was detected in artemether AUC_{0-8hr}. However, compared to HIV-uninfected pregnant women, HIV-infected pregnant women on efavirenz-based ART had a 76% lower artemether C_{8hr} concentration (p = 0.013). Although changes were expected in the C_{24hr}, too many samples in the efavirenz-based group were below the limit of quantitation to measure statistical significance (BLQ-1.34 and BLQ for the HIV-uninfected and infected women, respectively). Both artemether C_{max} and $t_{1/2}$ were comparable between groups. The AUC_{0-8hr} for DHA was 35% lower in efavirenz-treated women but this difference was not statistically significant

(p = 0.057). Additionally, there was no difference between groups for DHA C_{8hr} or t1/2 values. DHA C_{max} was 46% lower in HIV-infected than HIV-uninfected pregnant women (p= 0.033).

Table 2.2 Artemisinin PK parameters after administration of artemether-lumefantrine in HIV-uninfected and infected adults

	HIV-Uninfected No ART (n=30)[a]	HIV-Infected EFV-based ART (n=9)[b]	EFV/ no ART Ratio (p-value)
Artemether			
C_{max} (ng/mL)	33.2 (24.3-45.4)	18.8 (8.9-39.5)	0.566 (p=0.19)
T_{max} (hr)	2.00 (1, 2.25)	2.00 (1.01, 2.03)	1.00 (p=0.91)
$t_{1/2}$, hr	4.24 (3.43, 5.24)	2.51 (1.54, 4.1)	0.592 (p=0.08)
AUC_{0-8hr} (hr•ng/mL)	95.7 (74-124)	52.7 (29.8-93.4)	0.551 (p=0.10)
C_{8hr} (ng/mL)	4.00 (1.81, 5.04)	0.955 (0.82, 2.75)	0.239 (p= 0.013)
C_{24hr} (ng/mL)	0.877 (BLQ, 1.34)	BLQ (BLQ, BLQ)	NR
Dihydroartemisinin			
C_{max} (ng/mL)	69.1 (57.6, 82.9)	37.6 (21.5, 66)	0.544 (p=0.033)
T_{max} (hr)	2.00 (2.00, 3.00)	2.00 (1.02, 2.54)	1.00 (p=0.90)
$t_{1/2}$, hr	1.34 (1.21, 1.48)	1.47 (1.04, 2.08)	1.10 (p=0.97)
AUC_{0-8hr} (hr•ng/mL)	173 (145-206)	113 (72.5-175)	0.653 (p=0.057)
C_{8hr} (ng/mL)	3.2 (2.35, 4.6)	1.53 (1.16, 3.29)	0.478 (p=0.14)

Data are presented as geometric mean (90% confidence interval). T_{max}, and C_{24hr} were reported as median with the 25th and 75th percentile.

Abbreviations: ART, antiretroviral therapy; AUC, area under the concentration-time curve; BLQ, below the limit of quantitation; C_{max}, maximal concentration; EFV, efavirenz; NR = not reported because samples were BLQ; T_{max}, time to maximal concentration.

Significance level: alpha=0.05, Wilcoxon rank sum test was used

[a] n = 28 for artemether $t_{1/2}$, n = 29 for artemether AUC and and C_{8hr}, dihydroartemisinin $t_{1/2}$ and C_{8hr}

[b] n = 8 for artemether $t_{1/2}$, n = 7 dihydroartemisinin $t_{1/2}$

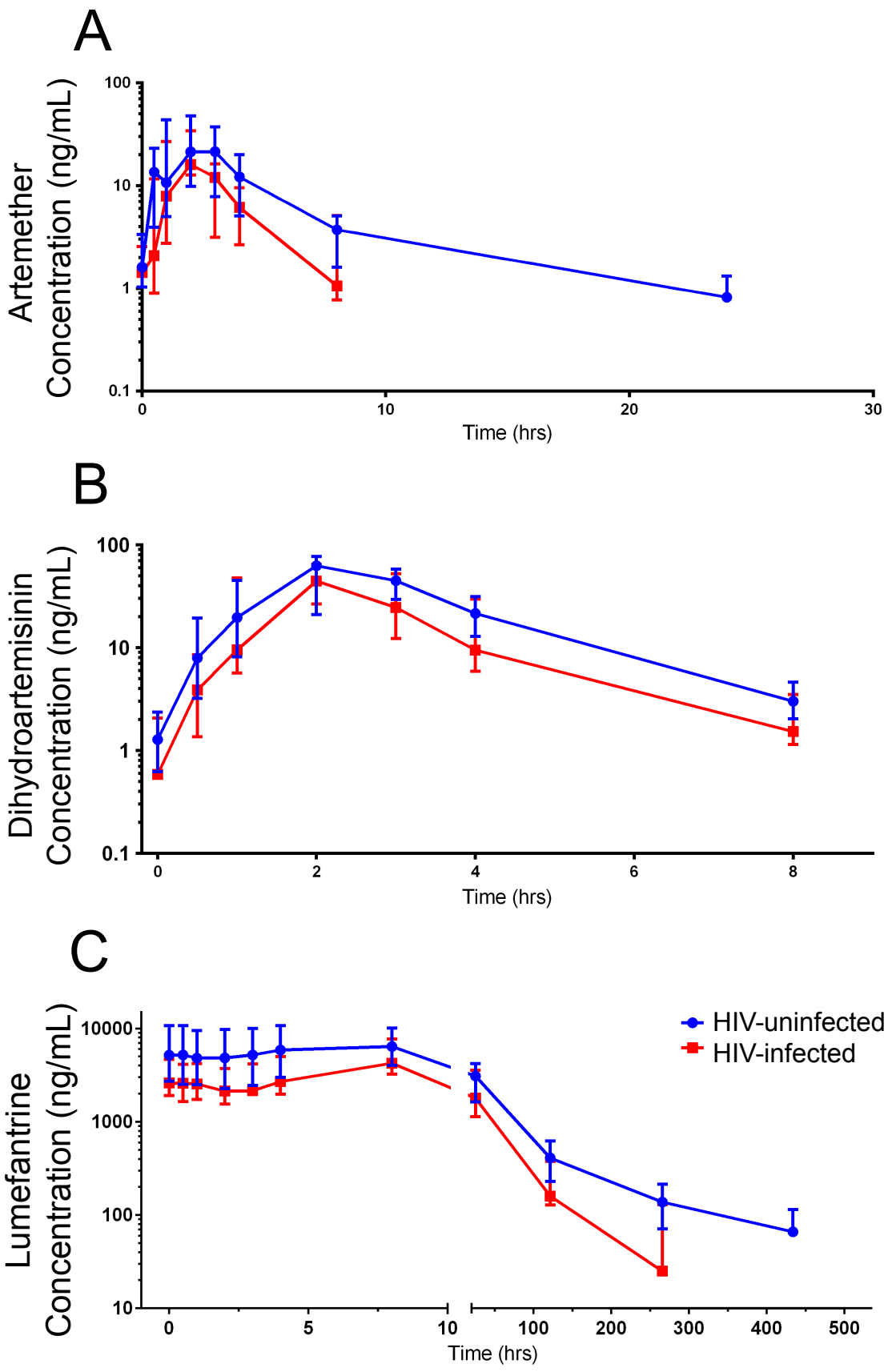
A
Artemether Concentration (ng/mL)
100
10
1
0.1
0
10
20
30
Time (hrs)
B
Dihydroartemisinin Concentration (ng/mL)
100
10
1
0.1
0
2
4
6
8
Time (hrs)
C
Lumefantrine Concentration (ng/mL)
10000
1000
100
10
0
5
10
100
200
300
400
500
Time (hrs)
HIV-uninfected
HIV-infected

Figure 2.3 Artemether (A), dihydroartemisinin (B) and lumefantrine (C) plasma concentration-time profiles in pregnant HIV-uninfected and infected women with malaria. The median concentrations are reported with the error bars indicating interquartile ranges.

Lumefantrine pharmacokinetic parameters are summarized in **Table 2.3** and **Figure 2.3**. The $AUC_{0-\infty}$ was 34% lower in HIV-infected women, though this did not meet statistical significance (p =0.063). Plasma lumefantrine concentrations on day 7 and 14 were 61% and 80% lower, respectively, in HIV-infected women (p= 0.046 and p=0.023, respectively). Changes in day 21 concentrations were also evident as the majority of samples in the efavirenz group fell below the limit of quantitation (with values ranging from BLQ to 68 ng/mL and BLQ to 232 ng/mL in HIV-infected and HIV-uninfected pregnant women, respectively). Compared to HIV-uninfected pregnant women, HIV-infected women had a 34% shorter $t_{1/2}$ (p = 0.033). No significant difference was seen in lumefantrine C_{max} between the two groups.

Table 2.3 Lumefantrine PK parameters after administration of artemether-lumefantrine in HIV-uninfected and infected pregnant women

	HIV-Uninfected No ART (n=30)[a]	HIV-Infected EFV-based ART (n=9)[b]	EFV/ no ART Ratio (p-value)
Lumefantrine			
C_{max} (ng/mL)	6785 (5633, 8172)	4943 (3513-6954)	0.729 (p=0.15)
T_{max} (hr)	8 (0.58, 8.00)	7.9 (7.61, 8.04)	0.988 (p=0.40)
$t_{1/2}$, hr	89.5 (75.3, 106.3)	59.2 (46.7, 75.1)	0.661 (p=0.033)
$AUC_{0-\infty}$ (hr•ug/mL)	287 (237, 349)	188 (125-281)	0.655 (p=0.063)
C_{7d} (ng/mL)	409 (231, 617)	160 (134, 309)	0.391 (p=0.046)
C_{14d} (ng/mL)	138 (72.1, 210)	BLQ (BLQ, 130)	<1 (p=0.023)
C_{21d} (ng/mL)	63.7 (BLQ, 105)	BLQ (BLQ, 31.9)[c]	NR

Data are presented as geometric mean (90% confidence interval). T_{max}, and $C_{7d, 14d, 21d}$ were reported as median with the 25th and 75th percentile.
Abbreviations: ART, antiretroviral therapy; AUC, area under the concentration-time curve; BLQ, below the limit of quantitation; C_{max}, maximal concentration; EFV, efavirenz; NR = not reported because samples were BLQ; T_{max}, time to maximal concentration.
Significance level: alpha=0.05, Wilcoxon rank sum test was used
[a] n = 29 for C_{21d}
[b] n = 8 for $t_{1/2}$, AUC and C_{14d}
[c] n = 5 for C_{21d}

Adverse events and treatment outcomes

No significant adverse events occurred in this trial and treatment was well tolerated. Three HIV-uninfected women had grade 3 thrombocytopenia on day 0, which quickly resolved on its own. A total of 3 late parasitological failures occurred over follow-up (2 of 30 HIV-uninfected and 1 of 9 HIV-infected women), and 4 late clinical failures (2 of 30 HIV-uninfected and 2 of 9 HIV-infected women) by day 42. Associations between day 42 treatment outcomes and AL exposure were explored using logistic regression when controlling for covariates such as HIV status. No relationship was observed between artemether, DHA, and lumefantrine C_{max}, AUC, and terminal concentrations and outcomes (all p-values >0.4).

Discussion

This intensive pharmacokinetic study evaluated the drug-drug interaction between efavirenz-based ART and artemether-lumefantrine for malaria treatment in pregnant women. For the short-acting artemisinins, we observed a significant reduction in the artemether terminal concentrations and DHA C_{max}, and an additional trend toward lower DHA $AUC_{0–8hr}$. Compared to HIV-uninfected pregnant women, HIV-infected pregnant women had significant changes which lowered the terminal lumefantrine concentrations with a trend toward lower $AUC_{0-\infty}$. Lower exposure, particularly for terminal lumefantrine concentrations, has been shown to increase the risk for recrudescence and to shorten the post-treatment prophylactic period.[18,39-42] The lower exposures observed in this study indicate that HIV-infected pregnant women on efavirenz may be receiving subtherapeutic doses.

Globally, ninety percent of HIV-infected pregnant women reside in sub-Saharan Africa where artemether-lumefantrine and efavirenz are the most widely prescribed therapies.[12,14,43] Indeed, AL is the most widely used ACT, and efavirenz-based ART was, until July 2019, the preferred treatment for HIV in 86% of WHO priority countries.[12,14,44] While dolutegravir is now the new first line regimen, it is unclear how long it will take countries to transition patients to dolutegravir ensuring that many will continue to use efavirenz.[15] In addition, EFV-based ART continues to be an alternative first-line ART to dolutegravir and would also be chosen in the setting of dolutegravir adverse events.[15] HIV-infected pregnant women are a particularly complex and vulnerable population when addressing dosage optimization and guidelines. Pregnancy alone can affect drug disposition resulting in either an increase or decrease in drug

exposure.[45,46] Previous studies addressing the impact of pregnancy alone on AL are conflicting and report either no effect or more commonly a decrease in exposure.[34,47-49] The additional consideration of ART's effect on exposure further complicates the situation. Hence, the extent to which drug-drug interactions potentially alter the efficacy of malaria treatment in this understudied population must be fully addressed as these interactions will affect a substantial percentage of high-risk populations for malaria.

HIV-infected pregnant women displayed altered PK indicating a downward trend in artemether, DHA and lumefantrine exposure. Artemether and lumefantrine are both metabolized by CYP3A4 and DHA primarily by UGT1A9 and UGT2B7.[19-21] Efavirenz induces CYP3A4 and various UGTs through activation of the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR), likely accounting for the concentration reductions seen in this study.[23,24,26,27] Since both groups of women in this study were pregnant, we were able to control for any effects pregnancy alone may have on either drug. Overall these findings reveal that HIV-infected pregnant women on efavirenz-based ART may require specific dosing guidelines.[22,47-49]

We have reported that efavirenz co-administration in children receiving AL results in a 2.8-fold reduction in DHA C_{max}, a 61% shorter lumefantrine half-life, and a 3.1-fold lower day-7 lumefantrine concentration compared to HIV-uninfected children.[18] In nonpregnant HIV-infected adults, similar results were reported whereby EFV lowered the C_{max}, AUC and/or terminal concentration values for all three (artemether-DHA-lumefantrine) drugs.[16,22,25] These reductions were clinically significant resulting in up to a 19-fold higher risk of recurrent parasitemia in the EFV arm compared to controls (no-ART).[39] While the magnitude of reduction seen in each population differs, the

overall trend of efavirenz reducing artemether, DHA and lumefantrine exposure is consistent among groups.

The effects of pregnancy on the pharmacokinetics of lumefantrine in HIV-infected pregnant and non-pregnant women already stabilized on EFV-based ART have been detailed by Adegbola *et al.*[28] Their work showed a paradoxical increase in lumefantrine $AUC_{0-\infty}$ in pregnant women, a change the investigators attributed to lower EFV exposure, and thus less CYP3A4 induction. Lumefantrine exposure in EFV treated pregnant women was modestly higher in the former study than in our study, which may be explained by variation in EFV exposure (e.g due to CYP2B65 genotype) in the two populations.[50]

Lumefantrine day 7 concentration and $AUC_{0-\infty}$ have both been used as predictive measures of AL treatment efficacy.[21,25,39-42,51] The 4.1-fold $AUC_{0-\infty}$ reduction of lumefantrine we reported in HIV-infected children on efavirenz led to a significant 3.7-fold increase in 28-day odds of malaria recurrence in comparison to children on LPV/r based ART.[18] In pregnant Tanzanian women, day 7 concentrations below 280 ng/mL were associated with a 4.8-fold higher recurrent parasitemia risk.[40] While the HIV-infected pregnant Ugandan women in this study are a unique treatment population, it is worth noting that they had a 61% lower day 7 lumefantrine concentration with a median value of 160 ng/mL. While we did not detect an association between lumefantrine concentrations and outcomes, these data suggest pregnant women on efavirenz-based ART may be at risk for recrudescent infections as the concentrations seen are associated with reduced efficacy.[39,40,47,48]

We and others have suggested that HIV and malaria co-infected individuals, particularly on efavirenz, should receive a longer duration of AL treatment.[18,22,25] Given lumefantrine displays dose limited absorption, extending treatment or increasing dosing frequency rather than increasing the actual dose are more effective at achieving day 7 lumefantrine concentrations comparable to groups not on ART.[52] Similar dosing recommendations have been made for pregnant women being treated for malaria where extending dosing over five days achieved simulated day 7 concentrations above 280 ng/mL.[47-49]

This study had a few limitations. First, the targeted enrollment number of HIV-infected women was 30 in order to have 80% power to detect a 35% difference in exposure. However, only 9 HIV-infected women completed the study increasing the change we were powered to detect to a 45% difference. Given the lower than anticipated enrollment, it is possible that clinically important changes were not captured in this trial and we may have underestimated the effects of efavirenz on artemether-lumefantrine PK. Similarly, we investigated the associations between AL exposure and treatment outcome but the low enrollment hindered our ability to detect any trends in pharmacodynamic outcomes. Lastly, desbutyl-lumefantrine, the primary metabolite of lumefantrine, and efavirenz could not be quantified due to the small plasma sample volumes collected.

Conclusion

In summary, efavirenz-based ART reduced terminal concentrations of artemether and lumefantrine and decreased the C_{max} value for DHA in pregnant Ugandan women co-infected with HIV and malaria. These findings further support the need to study extended dosing regimens for patients receiving efavirenz or other CYP3A4 inducers.

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Chapter 3: Piperaquine exposure is altered by pregnancy, HIV, and nutritional status in Ugandan women*

Abstract

Dihydroartemisinin-piperaquine (DHA-PQ) provides highly effective therapy and chemoprevention for malaria in pregnant African women. PQ concentrations of >10.3 ng/mL have been associated with reduced maternal parasitemia, placental malaria, and improved birth outcomes. We characterized the population pharmacokinetics (PK) of PQ in a *post hoc* analysis of human immunodeficiency virus (HIV)-infected and -uninfected pregnant women receiving DHA-PQ as chemoprevention every 4 or 8 weeks. The effects of covariates such as pregnancy, nutritional status (body mass index [BMI]), and efavirenz (EFV)-based antiretroviral therapy were investigated. PQ concentrations from two chemoprevention trials were pooled to create a population PK database from 274 women and 2,218 PK observations. A three-compartment model with an absorption lag best fit the data. Consistent with our prior intensive PK evaluation, pregnancy and EFV use resulted in a 72% and 61% increased PQ clearance, compared to postpartum and HIV-uninfected pregnant women, respectively. Low BMI at 28 weeks of gestation was associated with increased clearance (2% increase per unit decrease in BMI). Low-BMI women given DHA-PQ

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every 8 weeks had a higher prevalence of parasitemia, malaria infection, and placental malaria compared to women with higher BMIs. The reduced piperaquine exposure in women with low BMI as well as during EFV coadministration, compared to pregnant women with higher BMIs and not taking EFV, suggests that these populations could benefit from weekly instead of monthly dosing for prevention of malaria parasitemia. Simulations indicated that because of the BMI-clearance relationship, weight-based regimens would not improve protection compared to a 2,880 mg fixed-dose regimen when provided monthly. (The clinical trials described in this paper have been registered at ClinicalTrials.gov under identifiers NCT02163447 and NCT02282293.)

Introduction

An estimated 40 to 50 million African women are at risk of malaria infection during pregnancy each year.[1,2] Without intervention, up to 41% of all pregnant African women living in malaria regions of endemicity are estimated to have placental malaria.[3] Malaria during pregnancy can lead to an array of adverse outcomes for both the mother and developing fetus and is estimated to cause 900,000 low birthweight deliveries and 19.7% of all stillbirths in Africa annually.[4-6] This situation is further complicated by common comorbidities such as malnutrition and human immunodeficiency virus (HIV) infection. Malnutrition is reported in up to 20% of African women of reproductive age.[7-9] When combined with malaria infection, maternal malnutrition leads to a 17.8% increased risk of a low birthweight delivery compared to HIV-uninfected women without malnutrition.[10] HIV-infected pregnant women are also at an increased risk for both contracting malaria and for worse birth outcomes compared to HIV-uninfected pregnant women.[11-13] Given the geographic overlap of HIV infection and malnutrition in malaria regions of endemicity, there is a large pregnant population with comorbidities at risk for malaria.[10,14,15]

The World Health Organization (WHO) recommends the use of long-lasting insecticide-treated bed nets (LLIN) and intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) during pregnancy in malaria regions of endemicity of Africa.[16,17] However, concerns regarding the efficacy of these prevention measures have arisen as a result of increased resistance of anopheline mosquitoes to pyrethroid insecticides used in LLINs and of malaria parasites to SP.[18-20] In addition, HIV-infected women taking trimethoprim-sulfamethoxazole (SXT) as part of their HIV

care to prevent opportunistic infections are not advised to use SP, as this might lead to increased risk of severe cutaneous reactions.[13] A promising alternative for IPTp-SP is the artemisinin-based combination therapy (ACT) dihydroartemisinin-piperaquine (DHA-PQ).[21] DHA-PQ is an appealing option for IPTp, as the DHA component rapidly kills circulating parasites and PQ has a slow clearance rate, maintaining protective concentrations against subsequent infections for about a month.[22,23] Previous studies have shown DHA-PQ to be safe and as effective as IPTp in both HIV-infected and -uninfected pregnant women, significantly lowering the malaria burden compared to IPTp-SP.[24-26]

Few prevention studies have included a pharmacokinetic (PK) component to define the PK of PQ during pregnancy.[27-30] We previously demonstrated in a focused intensive PK analysis that both pregnancy and efavirenz (EFV)-based antiretroviral therapy (ART) independently reduced PQ exposure at 28 weeks gestation.[31] In a group of HIV-uninfected women, we evaluated the pharmacodynamics (PD) for DHA-PQ used as IPTp and established that 10.3 ng/mL PQ was 95% protective against parasitemia during pregnancy when parasitemia was measured with a highly sensitive molecular assay. [27,32] Other studies, including PK assessments with IPTp, were small and recorded PQ PK after only a single course of study drug. [28,29] To gain more comprehensive insights into sources of variability and optimal IPTp dosing regimens for women receiving DHA-PQ, we pooled data from two large clinical trials to perform a *post hoc* analysis. We included HIV-infected and -uninfected pregnant Ugandan women throughout the second and third trimesters, as well as postpartum women. Our

goal was to provide a comprehensive understanding of PQ PK in both HIV-infected and -uninfected women during pregnancy.

Methods

Study population.

Data were pooled from two clinical trials conducted in Tororo, Uganda between December 2014 and March 2016 investigating the efficacy of DHA-PQ given as an IPTp. [25,26] For the first parent study, HIV-uninfected pregnant women were randomized to receive either standard treatment doses of SP given every 8 weeks or DHA-PQ given every 4 or every 8 weeks during the 2nd and 3rd trimesters of pregnancy (note that "pregnancy" in this report refers to the second and third trimesters). Additionally, a subset of the HIV-uninfected pregnant women underwent intensive PK sampling postpartum, providing nonpregnant control samples. In the second parent study, HIV-infected pregnant women receiving EFV-based ART were randomized to receive either monthly DHA-PQ in combination with daily SXT (standard of care for HIV-infected populations to prevent opportunistic infections) or SXT alone. Eligible participants were pregnant women between 12 and 28 weeks gestation confirmed by ultrasound, ≥16 years of age, living within 30 km of the study clinic, and having known HIV status. Only women randomized to DHA-PQ were included in our PK analyses.
Written informed consent was obtained from all study participants. Study protocols were approved by the ethics committees at Makerere University, the Ugandan National Council of Science and Technology, and the University of California, San Francisco. The clinical trial registration numbers are NCT02163447 and NCT02282293.

Study design.

At enrollment, each subject was given a long-lasting insecticide-treated net and underwent a routine medical examination, including height and weight measurements and a blood smear to detect parasitemia. Women received all their medical care at the study clinic and were encouraged to come to the clinic any time they felt ill. Routine visits occurred every month, at which placebo or study drug was administered and finger-stick or venous blood was taken for blood, a PK sample collection, and detection of submicroscopic parasitemia by loop-mediated isothermal amplification (LAMP). Symptomatic malaria was diagnosed when a woman presented to the clinic with a fever or history of fever (tympanic temperature ≥38°C) and a positive blood smear. At delivery, presence of placental malaria was detected by histopathology.[32,33]

Women randomized to DHA-PQ every 8 weeks received the study drug at 20, 28, and 36 weeks gestation, while those randomized to DHA-PQ every 4 weeks received the study drug beginning at enrollment (16 to 28 weeks gestation) (**Figure 3.1**). A standard dose of 3 tablets (40 mg DHA/320 mg PQ; Duo-Cotecxin, Holley-Cotec) was given once a day for 3 consecutive days, with the first dose observed in the clinic and the remaining two taken at home. A subset of 30 HIV-uninfected women (28 enrolled from the DHA-PQ arms, 2 enrolled from the SP arm) were reenrolled at 34 to 54 weeks postpartum to provide nonpregnant control data. Twenty seven of these 30 women were those who contributed intensive sampling at 28 weeks of gestation. HIV-infected women received efavirenz/tenofovir/lamivudine, which was initiated at least 4 weeks prior to PK sampling; they were instructed to take it every morning.

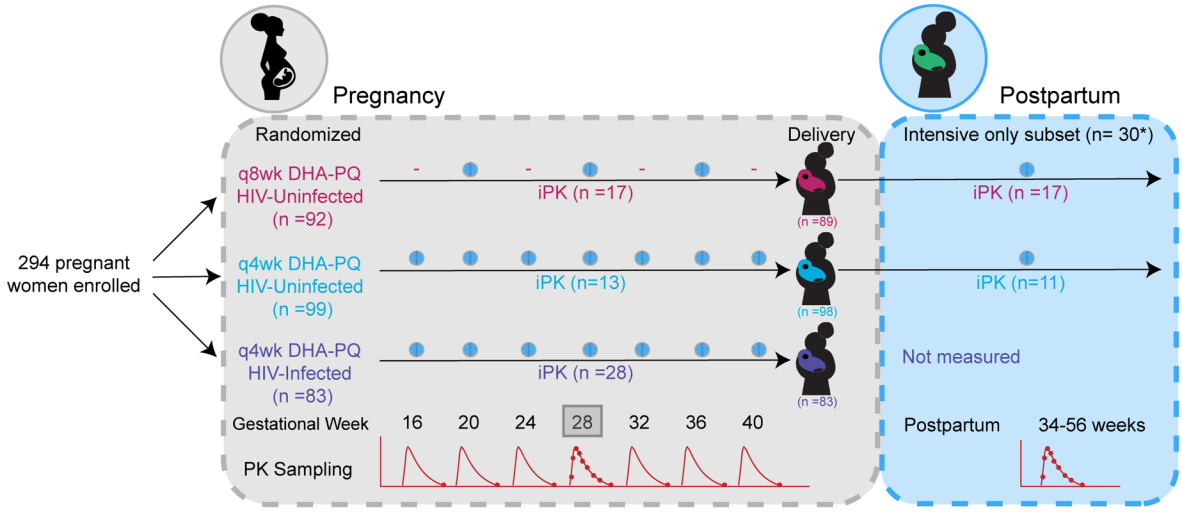


Figure 3.1 Trial diagram. Women were enrolled at 16 to 28 weeks gestation. PK sampling began at 20 weeks gestation and continued until delivery. iPK (box) indicates intensive PK sampling at 28 weeks gestation. The asterisk indicates two of the women included in the postpartum sampling group received SP during pregnancy. The number of women enrolled and randomized reported here reflects only those who went on to initiate study drug. q4wk, doses given every 4 weeks; q8wk, doses given every 8 weeks.

Estimation of nutritional status.

The weight of each woman was recorded at monthly visits during pregnancy and postpartum (for those providing control data). Weight was used to calculate body mass index (BMI), the rate of weight gain during pregnancy, as well as to group women into weight and BMI tertiles. For plotting purposes only, a week-28 BMI of 20.5 kg/m^2 or less was used to classify pregnant women as malnourished. This value was derived using the enrollment weight from a woman in our trial with a BMI of 18.3 kg/m^2 (a value considered to define a woman as malnourished pre-pregnancy) and weight gain guidelines during the second and third trimesters from the Institute of Medicine (see the supplemental material for further explanation of this calculation).[34] This threshold was used as there are no weight-based guidelines for nutritional status during pregnancy.

Initially, BMI at enrollment was identified as a significant covariate on PQ clearance in the covariate search. However, women were enrolled at various points throughout the second trimester. Women who were enrolled later would have had more time over which to gain weight, potentially biasing the results. In order to standardize this measure, BMI as a continuous variable at 28 weeks gestation (the earliest time point at which all women were enrolled) was tested and found to be significant.

To explore how weight gain could change BMI for a hypothetical malnourished woman over the second and third trimesters of pregnancy we used weight gain guidelines from the Institute of Medicine.[34] We needed to establish if it is therefore reasonable to believe the low BMI women in our trial were malnourished at 28 weeks gestation and to determine what range of continuous BMIs could be expected from a malnourished woman if she gained the ideal amount of weight (**Figure 3.2**).[34] Weight gain guidelines do not exist for the first trimester so to overcome this knowledge gap while exploring realistic scenarios we selected a woman from our trial who was enrolled early in the second trimester (at 14 weeks gestation) with a BMI of 18.3 kg/m^2 as she would be considered malnourished based on pre-pregnancy guidelines (BMI of <18.5 kg/m^2 is considered malnourished). Our derived BMI trajectories, assuming the minimal (0.45 kg/week) and maximal (0.6 kg/week) recommended weight gain, resulted in a week 28 BMI of 20.5 and 21.5 kg/m^2, respectively. The more conservative cutoff of 20.5 kg/m^2 was used when plotting our data but likely underestimates the number of women malnourished given that recent studies report up to 62% of Ugandan women gain inadequate weight during pregnancy.[35]

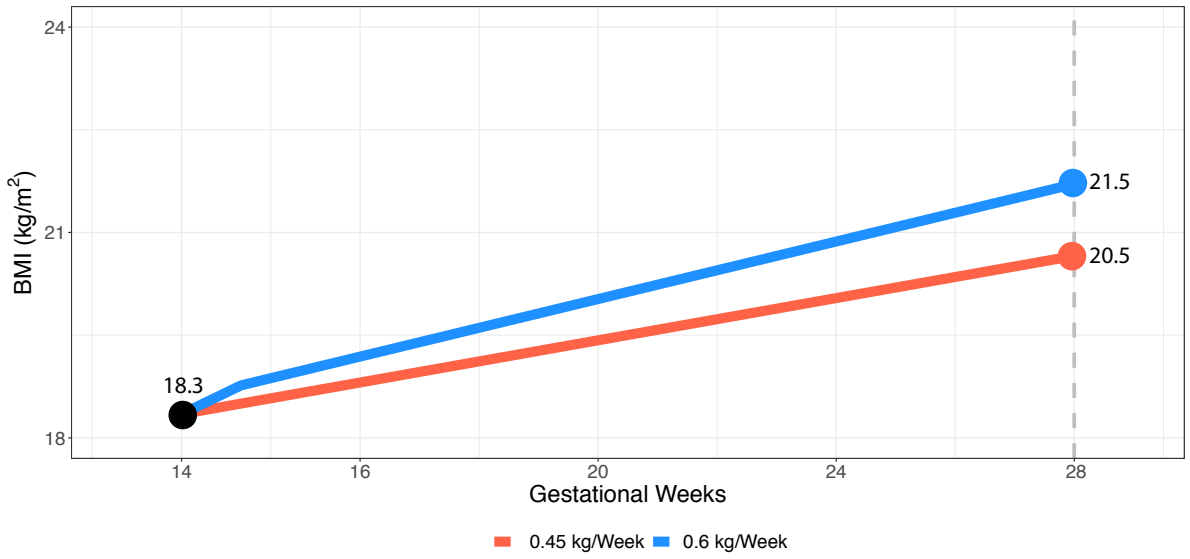


Figure 3.2 Second trimester BMI. Anticipated changes in BMI over the second trimester as a function of recommended weight gain guidelines during the second trimester from the Institute of Medicine [34]. The minimal (0.45 kg/week) and maximal (0.6 kg/week) recommended weight gain are plotted. The week 14 BMI value (18.3 kg/m^2) was chosen based on a study participant's value.

Pharmacokinetic sample collection and analysis.

All 191 HIV-uninfected women provided monthly samples. Venous samples were collected at 20, 28, and 36 weeks and finger-stick samples at 24, 32, and 40 gestational weeks. A subset of 30 women (n = 17 every 8 weeks; n = 13 every 4 weeks) were enrolled in an intensive PK sub-study: between 27 and 28 weeks gestation, these women had venous plasma samples taken before and after their last dose at times pre-dose, 0.5, 1, 2, 3, 4, 6, 8, and 24 h post-last-dose. Finger-stick samples were collected at 24 h post-last-dose, and days 4, 7, 14, and 21 post-dose. This intensive sampling schedule was also followed during the postpartum visit. Venous and finger-stick samples (24 h time point) were collected simultaneously in order to establish a relationship between these two sample types, allowing for simultaneous fitting of all

data. Identical intensive sampling procedures were followed for 28 HIV-infected women. Monthly samples were quantified in a convenience sample of 83 HIV-infected women.

Plasma PQ concentrations were determined using high-performance liquid chromatography tandem mass spectrometry, as previously described.[31,36] Two different methods were used and the calibration ranges were 10 to 1,000 ng/mL and 0.5 to 50 ng/mL, with 0.5 ng/mL as the lower limit of quantification (LLOQ). The inter- and intrarun coefficient of variation (CV) was below 10% for all quality control samples for both assays.

Population modeling.

Piperaquine PK data were analyzed using nonlinear mixed-effects modeling in the software NONMEM VII (Icon Development Solutions, Ellicott City, MD). All parameters were estimated using the first order conditional estimation with interaction algorithm. Both exclusion and inclusion of samples below the LLOQ were tested.[37] One, two-, and three-compartment models with first order absorption were explored. An absorption lag time and transit compartments were also tested. Venous and finger-stick samples were modeled simultaneously using a linear relationship to describe any concentration differences. Between-subject variability was evaluated on structural model parameters assuming a log-normal distribution. A combined error model with both additive and proportional terms was used to describe the residual unexplained variability.

A stepwise covariate (SCM) search was performed to identify characteristics that influenced PQ PK. Characteristics tested were pregnancy status, gravidity, gestational weeks, trimester, weight, weight tertile, weight gained, rate of weight gain during

pregnancy, BMI, BMI tertile, age, HIV status, and treatment arm. Gravidity, weight gained, age, HIV status, and treatment arm were treated as time independent. All other characteristics were tested as time-dependent variables and as time independent using the respective enrollment values. Linear and nonlinear relationships between parameters and covariates were investigated, including allometric scaling. Covariate-parameter relationships were sequentially tested with a significance cutoff of $P < 0.05$ for forward inclusion, followed by backward elimination with a cutoff of $P < 0.01$, in order to account for multiple hypothesis testing.

Model development and selection was guided by goodness of fit plots, the objective function value, parameter estimates, and their respective relative standard error values. Simulation-based diagnostics such as visual predictive checks ($n = 500$) and a nonparametric bootstrap ($n = 1000$) were also performed to determine the model's predictive power and the precision of parameter estimates.

Optimal dosing assessment.

The final PK model was used to perform simulations, adjusting for the dose frequency and amount. Monthly (2,880 mg once per month), weekly (960 mg every 7 days), and low daily (160 mg) doses were evaluated. PQ PK was simulated over 1,000 times for pregnant HIV-uninfected and -infected women with week-28 BMIs ranging from 16 to 27 kg/m^2. Weight-based dosing simulations were performed by simulating the 274 women from our database over 50 times. To assess the relationship between PK and PQ's known QTc prolongation, we utilized two previously developed PK-QTc models (one for HIV-uninfected and one for HIV-infected women) which described the linear relationship between PQ concentration and change in QT interval.[27,30] The

maximum PQ concentrations predicted from each regimen were input into the QTc models to assess if any clinically significant prolongation (>60 msec) was predicted to occur.

Each dosing schedule was evaluated based on the number of women who maintained 10.3 ng/mL PQ, how quickly this threshold was achieved, and if the maximum concentrations were predicted to result in QT prolongation greater than 60 msec. Adequate protection for this analysis was considered as maintaining 10.3 ng/mL PQ for 95% of the time on prevention. These criteria were based upon a prior study that concluded that maintaining 10.3 ng/mL PQ provided 95% protection against parasitemia in HIV-uninfected pregnant women, and the FDA's safety guidelines regarding QT prolongation.[27,38]

Results

Study cohort

A total of 274 (191 HIV-uninfected and 83 HIV-infected) pregnant women contributed 797 intensive and 1,001 monthly plasma samples used to build the population PK model (**Figure 3.1**). Twenty-eight HIV-uninfected women given DHA-PQ and two given SP during pregnancy were re-enrolled a minimum of 34 weeks postpartum and contributed an additional 420 intensive samples (**Figure 3.3A and B**). Three HIV-uninfected women in the q8wk arm who did not deliver were withdrawn from the study because they could not be located for more than 60 days. One HIV-uninfected woman in the q4wk arm was withdrawn because she moved out of the study area. The demographic characteristics of these participants are detailed in **Table 3.1** and

Table 3.2 in the supplemental material. At enrollment, 34 women had a BMI of less than 18.5 kg/m^2, and at 28 weeks gestation, 70 women had a BMI less than or equal to 20.5 kg/m^2 (**Table 3.1** and **Figure 3.4**).

Table 3.1 Study participant characteristics

Characteristics	HIV-Uninfected Pregnant		HIV-Infected Pregnant	HIV-Uninfected Postpartum
	DHA-PQ[e] every 8 weeks (n = 92)	DHA-PQ every 4 weeks (n=99)	DHA-PQ every 4 weeks (n=83)	Single course DHA-PQ (n=30)[a,e]
Age in years, [median (2.5-97.5% percentile)]	21.5 (16.3-32.0)	22.1 (17.1-32.0)	30.3 (17.9-41.5)	23.0 (19.6-29.9)
Intensive PK sample no.[b]	237	182	378	420
Monthly PK sample no.	421	453	127	-
Gestational age in weeks, no.(%)				
16 wk	63 (68.5)	67 (67.7)	19 (23.0)	-
>16 to 20 wk	29 (31.5)	32 (32.3)	25 (30.0)	-
> 20 to 24 wk	0 (0)	0 (0)	20 (24.0)	-
>24 to 28 wk	0 (0)	0 (0)	19 (23.0)	
Gravidity, no.(%)				
1	32 (34.8)	36 (36.4)	12 (14.5)	-
2	28 (30.4)	28 (28.3)	10 (12.0)	-
≥ 3	32 (34.8)	35 (35.3)	61 (73.5)	-
Weight in kg, [median (2.5-97.5% percentile)]	56.4 (43.7-69.5)	55.0 (46.3-70.8)	56.2 (44.4-73.1)	52.9 (41.1-66.8)
Height in cm, [median (2.5-97.5% percentile)]	162 (150-177)	162 (153-175)	163 (150-173)	162 (152-173)
BMI in kg/m^2, [median (2.5-97.5% percentile)]	21.1 (17.4-26.0)	21.3 (17.0-26.6)	21.4 (18.0-28.2)	20.1 (16.1-23.5)
Low BMI at enrollment, no.(%)[c]	11 (12.0)	17 (17.0)	6 (7.2)	6 (20)
Low BMI at 28 weeks gestation, no.(%) [c]	15 (16.3)	26 (26.3)	29 (34.9)	-
Weight gained in kg, [median (2.5-97.5% percentile)][d]	1.6 (-1.3-6.3)	1.6 (-2.1-4.6)	1.1 (-1.4-4.3)	-

[a]Two of the women enrolled in the postpartum cohort received sulfadoxine-pyrimethamine in the parent trial.
[b]30 HIV-uninfected women and 28 HIV-infected women contributed intensive PK samples.
[c]Low BMI at enrollment was defined as a BMI of less than 18.5 kg/m^2. At 28 weeks, low BMI was defined as a BMI of 20.5 kg/m^2 or less to account for weight gained during pregnancy.
[d]Weight gained was calculated from 28 gestational weeks through delivery in order to standardize the measurement.
[e]DHA-PQ, dihydroartemisinin-piperaquine; PK, pharmacokinetic; BMI, body mass index; -, not applicable

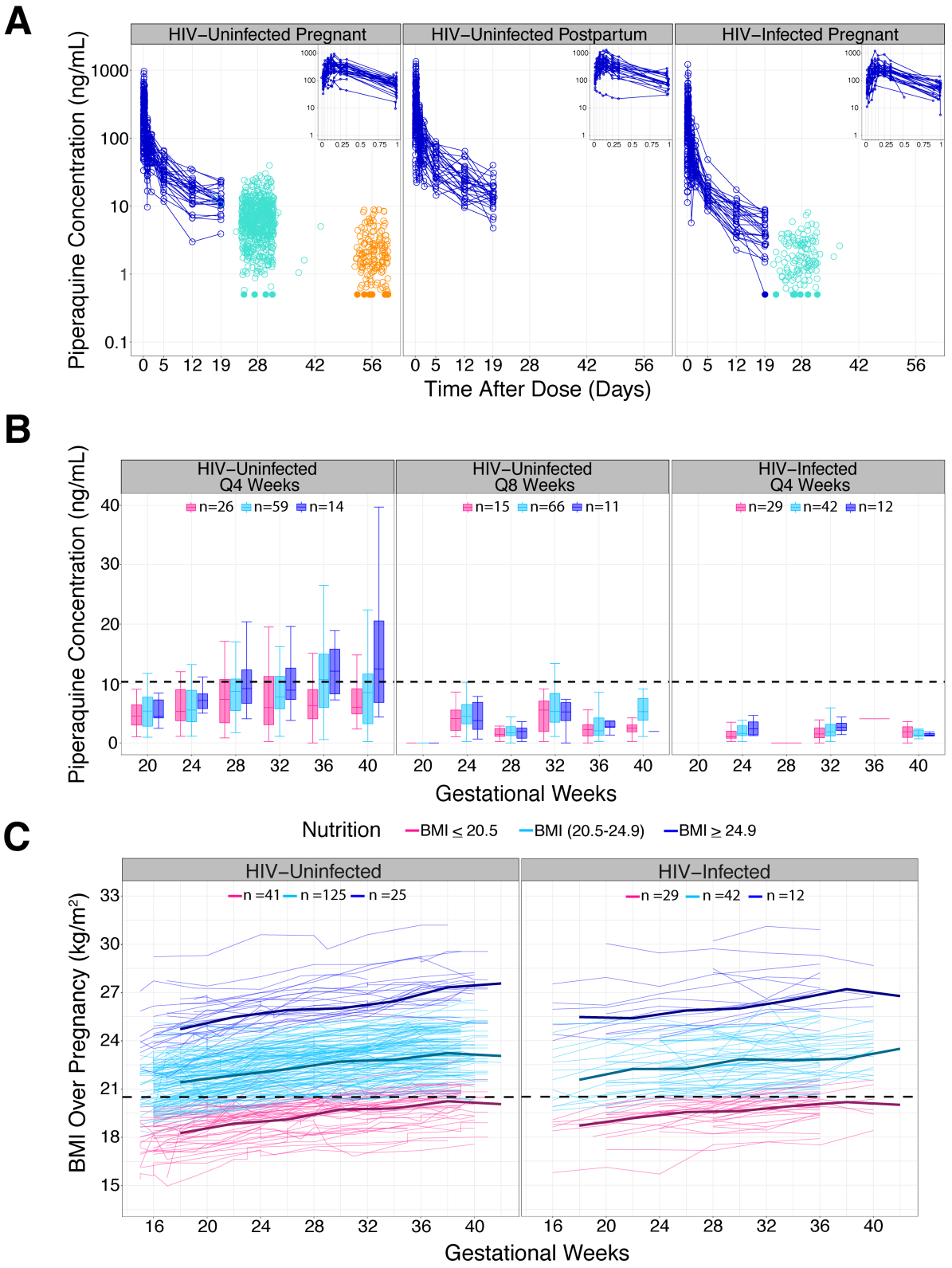

A
HIV−Uninfected Pregnant
HIV−Uninfected Postpartum
HIV−Infected Pregnant
Piperaquine Concentration (ng/mL)
Time After Dose (Days)
B
HIV−Uninfected Q4 Weeks
HIV−Uninfected Q8 Weeks
HIV−Infected Q4 Weeks
n=26 n=59 n=14
n=15 n=66 n=11
n=29 n=42 n=12
Piperaquine Concentration (ng/mL)
Gestational Weeks
C
Nutrition
BMI ≤ 20.5
BMI (20.5-24.9)
BMI ≥ 24.9
HIV−Uninfected
HIV−Infected
n =41 n =125 n =25
n =29 n =42 n =12
BMI Over Pregnancy (kg/m²)
Gestational Weeks

Figure 3.3 Time profiles. (A) Piperaquine concentrations over time used to build the population PK model. The profiles in blue represent intensive PK sampling. Each line represents one individual. Monthly (28-day) concentrations are in green and56-day trough concentrations are in orange. Insets in the upper right corner show the intensive PK profiles for the first day post-dose. To avoid overlap of monthly points, random noise was added about the x-axis to separate the data. (B)Piperaquine monthly concentrations stratified by treatment arm, HIV status, and BMI. Women were grouped based on gestational week-28 BMIs. The number of women in each group is displayed. The dashed line at 10.3 ng/mL marks the previously defined threshold for malaria protection in HIV-uninfected pregnant women. (C) BMI over time profile. Women were grouped based on week-28 BMIs. Each line represents one individual. The dashed line at 20.5 kg/m2marksthe plotting cutoff for defining a woman as malnourished during the third trimester (see the supplemental material).

Table 3.2 Study participant characteristics for intensive PK sampling cohort

Characteristics	HIV-Uninfected Pregnant		HIV-Infected Pregnant
	DHA-PQ every 8 weeks (n = 17)	DHA-PQ every 4 weeks (n=13)	DHA-PQ every 4 weeks (n=28)
Age in years, [median (2.5-97.5% percentile)]	21 (18-29)	23 (20-30)	30.3 (18.2-40.4)
Gestational age in weeks, no.(%)			
16 wk	15 (88.2)	12 (92.3)	8 (28.6)
>16 to 20 wk	2 (11.8)	1 (7.7)	10 (35.7)
> 20 to 24 wk	0 (0)	0 (0)	8 (28.6)
>24 to 28 wk	0 (0)	0 (0)	2 (7.1)
Gravidity, no.(%)			
1	4 (23.5)	2 (15.4)	5 (17.9)
2	6 (35.3)	5 (38.5)	3 (10.7)
≥ 3	7 (41.2)	6 (46.1)	20 (71.4)
Weight in kg, [median (2.5-97.5% percentile)]	58.3 (49.1-76.8)	57.0 (47.3-79.2)	57.6 (43.3-71.8)
Height in cm, [median (2.5-97.5% percentile)]	162 (152-172)	165 (157-177)	163 (146-174)
BMI in kg/m^2, [median (2.5-97.5% percentile)]	22.3 (19.0-26.4)	21.7 (17.3-25.3)	22.0 (18.5-26.4)
Low BMI at enrollment, no.(%)***	1 (5.9)	4 (30.8)	3 (10.7)
Low BMI at 28 weeks gestation, no.(%)***	2 (11.8)	4(30.8)	10 (35.7)

DP; Dihydroartemisinin-piperaquine;
*** Low BMI at enrollment is defined as a BMI of less than 18.5 kg/m^2. At 28 weeks low BMI is defined as a BMI of 20.5 kg/m^2 or less to account for weight gained during pregnancy.

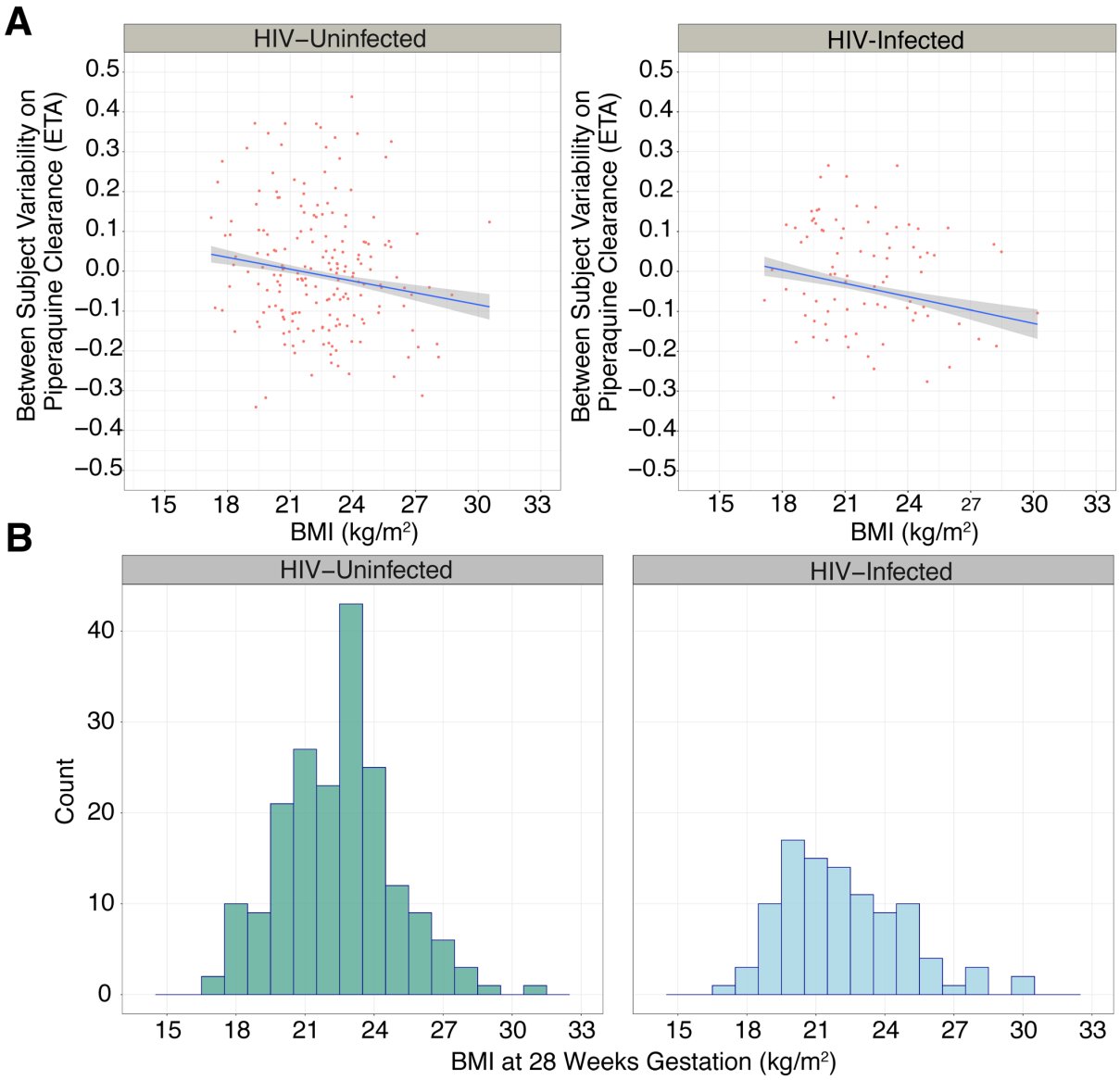


Figure 3.4 BMI at 28 weeks gestation. A. The linear relationship between PQ clearance variability and BMI at 28 weeks gestation stratified based on HIV status. Each red circle represents one woman in the study. B. The distribution of BMI values at 28 weeks gestation. The median and interquartile range are 21.5 (19.4 – 22.7) kg/m^2 and 22.4 (20.1 – 24.1) kg/m^2 for HIV-uninfected and -infected women, respectively.

Population PK model

A three-compartment disposition model with an absorption lag best fit the observed data (**Figure 3.5**). Samples below the lower limit of quantification (LLOQ) made up only 4% ($n = 94$; 11 from HIV-infected women, 83 from HIV-uninfected women) of the data and were well captured when imputing the first sample to fall below the limit as half the LLOQ. Additionally, two samples with results that differed more than 10-fold from the patient's previous and subsequent sample concentrations were deemed to be outliers and excluded from the analysis. Specifically, a day 28 trough of 306 ng/mL was excluded given the average trough value for this patient was 7 ng/mL. The second sample excluded was a 6 hr intensive sample below the LLOQ for which every other sample in the participant's intensive profile was above the LLOQ. Residual error was well described by a combined error model. A linear relationship with a slope of 0.80 and an intercept estimated as 0.54 for HIV-uninfected women and intercept fixed to zero for HIV-infected women was used to describe the difference between venous and finger-stick PQ concentrations. Parameter estimates from the final model are listed in **Table 3.3**. Time profiles for intensive and monthly PQ concentrations are shown in **Figure 3.3A and B**. Goodness of fit plots (**Figure 3.6**) and visual predictive checks confirmed our model accurately fit and predicted the data in all populations (**Figure 3.7**).

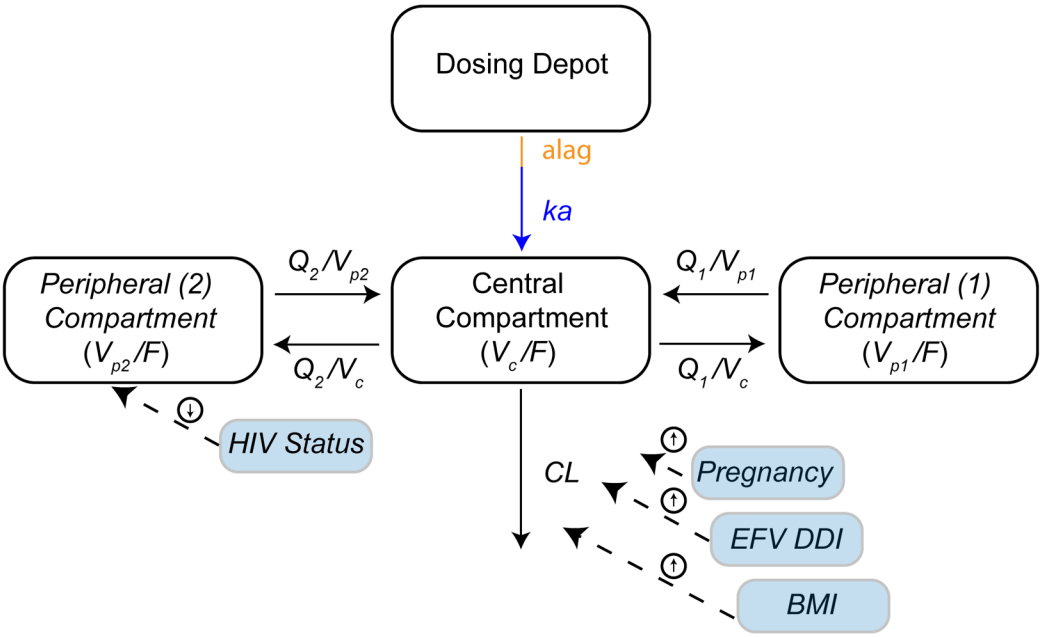


Figure 3.5 Final piperaquine population pharmacokinetic model. A three-compartment model with an absorption lag. Four significant parameter-covariate relations were included in the final model. Covariates are shown in blue boxes, with dashed arrows indicating which parameter is influenced and the direction of the effect indicated by the arrows enclosed in circles. Clearance (CL) in this model is the oral clearance (CL/F).

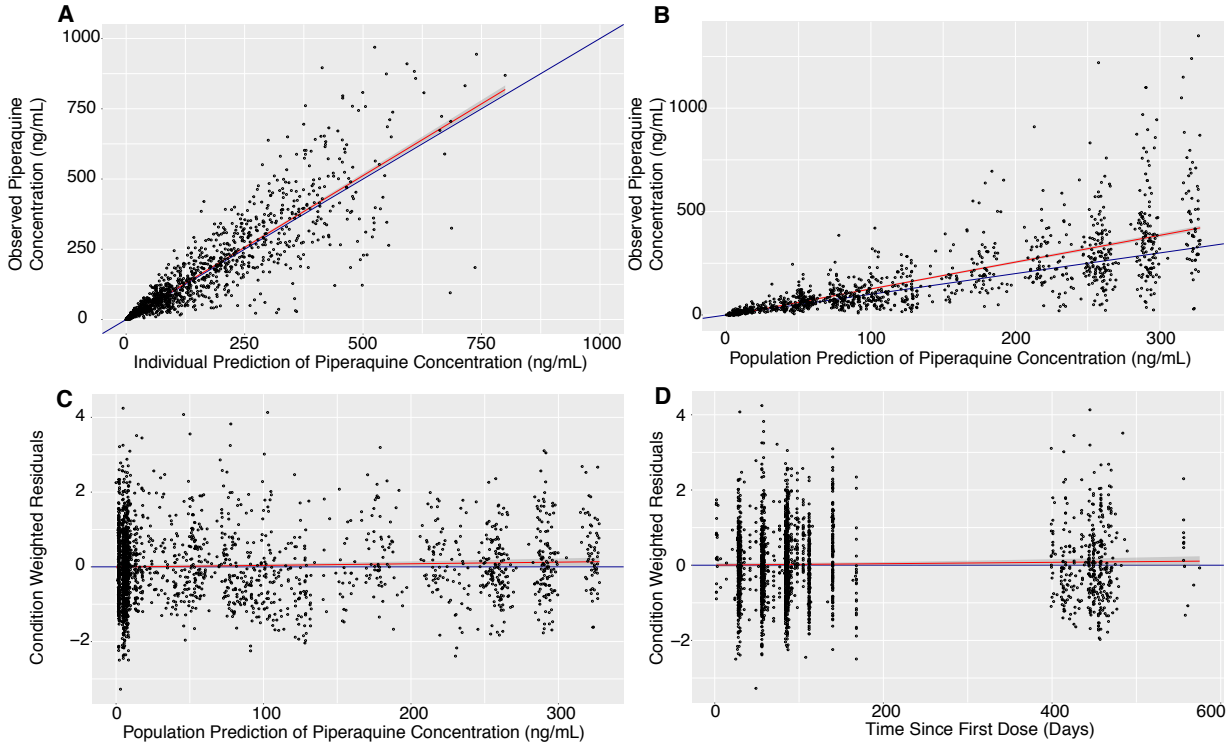


Figure 3.6 Goodness-of-fit plots for the final PQ model. A. Individual predictions versus observations. B. Population model predictions versus observations. C. Population model predictions versus conditional weight residuals D. Time after the first dose versus conditional weight residuals. Each black circle represents one observation. Each black line is the line of unity, and each red regression line is the model based locally weighted least-squares.

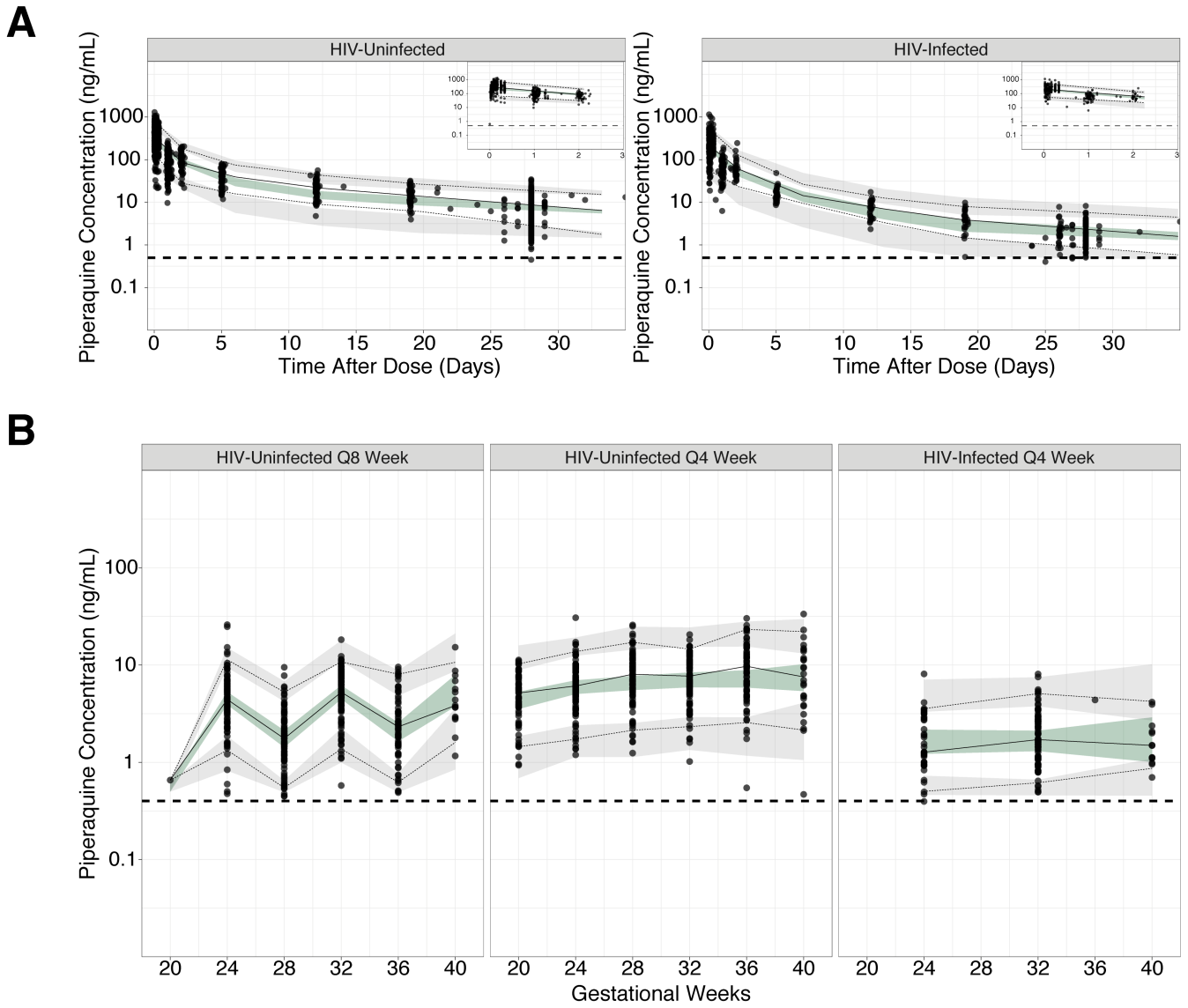

A
HIV-Uninfected
HIV-Infected
Piperaquine Concentration (ng/mL)
Time After Dose (Days)
B
HIV-Uninfected Q8 Week
HIV-Uninfected Q4 Week
HIV-Infected Q4 Week
Piperaquine Concentration (ng/mL)
Gestational Weeks

Figure 3.7 Prediction-corrected visual predictive check of the final pharmacokinetic model. (A) Intensive profiles at 28 weeks gestation. Plot of intensive data, including day-28 levels stratified based on HIV status. Insets in the upper right corner show the intensive profiles for 3 days post-dose. (B) Monthly concentrations plotted over pregnancy. The observed data for each subject are plotted as black circles. The solid and dashed lines are the observed median and 5thand 95thpercentiles of the observed data. The shaded areas represent the 95% confidence intervals of the model simulated data. Q8, DHA-PQ dosing given every 8 weeks; Q4, DHA-PQ dosing given every 4 weeks.

Covariate effects

Pregnancy, BMI, and EFV use in HIV-infected women were all found to independently increase PQ clearance (**Table 3.3**). Pregnancy was treated as a dichotomous variable and was found to increase PQ clearance by 72% compared to postpartum controls. Trimester was also explored and included during the forward covariate selection, indicating that PQ clearance increased during the third trimester, but this relationship was dropped during backward elimination (see the Materials and Methods). BMI at 28 weeks gestation was treated as a continuous variable and influenced PQ clearance in a linear fashion; low BMI pregnant women had higher elimination rates (2% increase for every unit drop in BMI) (**Figure 3.4**). Similarly, HIV-infected women taking EFV had clearance increased by 61% in comparison to HIV-uninfected pregnant women. HIV-infected women also had a 51% smaller volume for the second peripheral compartment in comparison to HIV-uninfected women. No other covariate effects were identified.

Table 3.3 Final pharmacokinetic parameter estimates for piperaquine

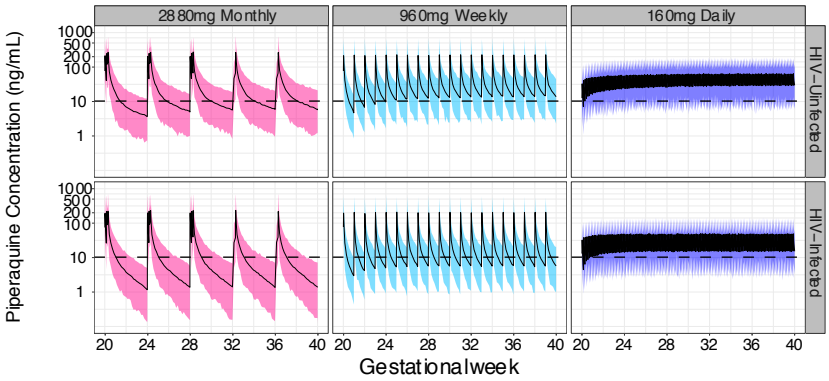
PK Parameter[a]	Population estimate (bootstrap 95% CI)[a]	CV (%) of BSV (bootstrap 95% CI)[a]
CL/F HIV-uninfected (L/day)	3126 (2712 - 3397)	21.8 (22 - 70)
Ka (day^{-1})	20 (3.0 - 34)	
V_c/F (L)	3155 (369 - 3659)	34.7 (5.4 - 91)
V_{p1}/F (L)	4449 (3772 - 7952)	36.3 (3.2 - 24)[b]
V_{P2}/F (L)	31820 (23707 - 39216)	36.3 (3.2 - 24)[b]
Q/F(L/day)	3428 (2418 - 5874)	
Q_2/F (L/day)	1664 (974 - 39216)	
Absorption lag time (day)	0.026 (0.016 - 0.035)	
Proportional error (%)	42 (36 - 44)	
Additive error HIV-infected (ng/mL)	0.0001 (NA)[d]	
Additive error HIV-uninfected (ng/mL)	0.29 (0.085 - 0.41)	
Intercept of venous/capillary ratio HIV-uninfected	0.54 (0.075 - 1.4)	
Intercept of venous/capillary ratio HIV-infected	0 (NA)[d]	
Ratio, venous/capillary	0.80 (0.73 - 0.93)	
F (%)	1 (NA)[d]	34.7 (0.67 – 17)
θ_{HIV} ; CL/F= (1+ θ_{HIV})	0.61 (0.48 - 0.85)	
$\theta_{postpartum}$; CL/F= (1+ $\theta_{postpartum}$)	-0.42 (-0.54 to -0.31)	
θ_{BMI} ; CL/F= (1+θ_{BMI})*(*BMI*-22.3)[c]	-0.020 (-0.032 to -0.0044)	
$\theta_{HIV\text{-}infected}$; V_{P2}/F = (1 + $\theta_{HIV\text{-}infected}$)[e]	-0.49 (-0.59 to -0.33)	

[a]CV, coefficient of variation; BSV, between subject variability; CI, confidence interval; CL, clearance; Ka, absorption rate constant; Vc, volume of the central compartment; Vp1, volume of the first peripheral compartment; Vp2, volume of the second peripheral compartment; F, bioavailability; Q and Q2, intercompartmental clearance.
[b]The same term for variability was used for both peripheral volume compartments.
[c]BMI effect on CL is the BMI at 28 weeks gestation.
[d]Indicates term was fixed to reported value.
[e]All HIV-infected women received efavirenz-based antiretroviral therapy. The HIV effect is thought to be a drug-drug interaction due to efavirenz.

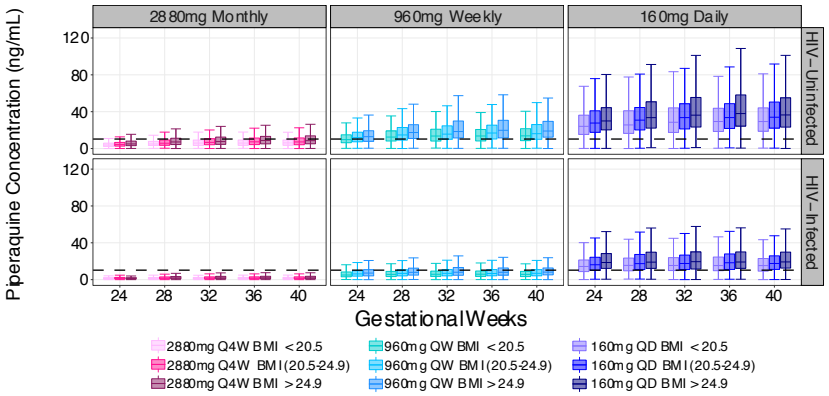
Simulations

Simulations were performed to investigate whether alternative IPTp DHA-PQ regimens adjusting for dosage and frequency would provide higher PQ exposure and therefore improved protection against parasitemia (**Figure 3.8A to D** and **Figure 3.9A to C**). Monthly dosing of 2,880 mg (3 tabs × 3 days), regardless of HIV or nutritional status, provided inadequate protection against parasitemia throughout the second and third trimesters, as less than 20% of women stayed above the protective concentration (**Figure 3.8C and D).** Weekly dosing of 960 mg (3 tabs × 1 day) resulted in protection for >45% (49 to 74.2%) and >10% (11.7 to 1.6%) of HIV-uninfected and -infected women, respectively. A daily dose of 160 mg (1 tab) provided the best protection for all women, with 75% protection reached before 24 and 32 weeks gestation in HIV-uninfected and -infected women, respectively. No regimen was predicted to result in QTc prolongation greater than 30 msec (**Table 3.4**). Monthly dosing resulted in the greatest prolongation and daily dosing resulted in the least, with HIV-infected women showing greater prolongation across all regimens compared to HIV-uninfected women. Low BMI and HIV-infected women consistently had the lowest protection regardless of dosing regimen and benefitted the most from increasing dosing frequency.

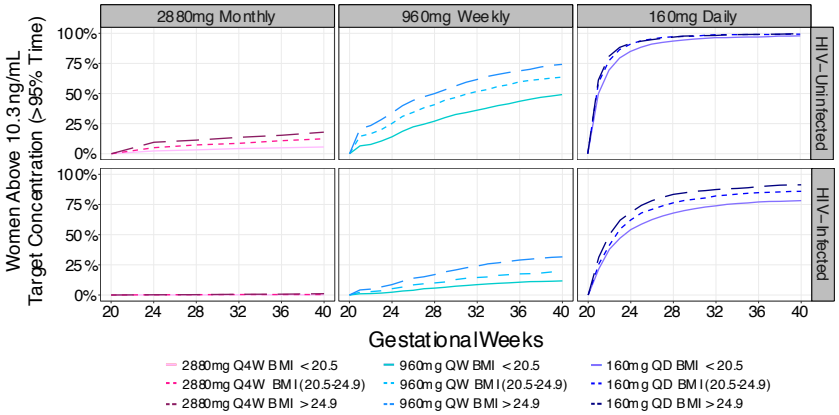
A


2880mg Monthly
960mg Weekly
160mg Daily
HIV−Uninfected
HIV−Infected
Piperaquine Concentration (ng/mL)
1000
500
200
100
10
1
20 24 28 32 36 40
Gestational week

B


2880mg Monthly
960mg Weekly
160mg Daily
HIV−Uninfected
HIV−Infected
Piperaquine Concentration (ng/mL)
120
80
40
0
24 28 32 36 40
Gestational Weeks
2880mg Q4W BMI <20.5
2880mg Q4W BMI(20.5-24.9)
2880mg Q4W BMI >24.9
960mg QW BMI <20.5
960mg QW BMI(20.5-24.9)
960mg QW BMI >24.9
160mg QD BMI <20.5
160mg QD BMI(20.5-24.9)
160mg QD BMI >24.9

C


2880mg Monthly
960mg Weekly
160mg Daily
HIV−Uninfected
HIV−Infected
Women Above 10.3 ng/mL
Target Concentration (>95% Time)
100%
75%
50%
25%
0%
20 24 28 32 36 40
Gestational Weeks
2880mg Q4W BMI <20.5
2880mg Q4W BMI(20.5-24.9)
2880mg Q4W BMI >24.9
960mg QW BMI <20.5
960mg QW BMI(20.5-24.9)
960mg QW BMI >24.9
160mg QD BMI <20.5
160mg QD BMI(20.5-24.9)
160mg QD BMI >24.9

D

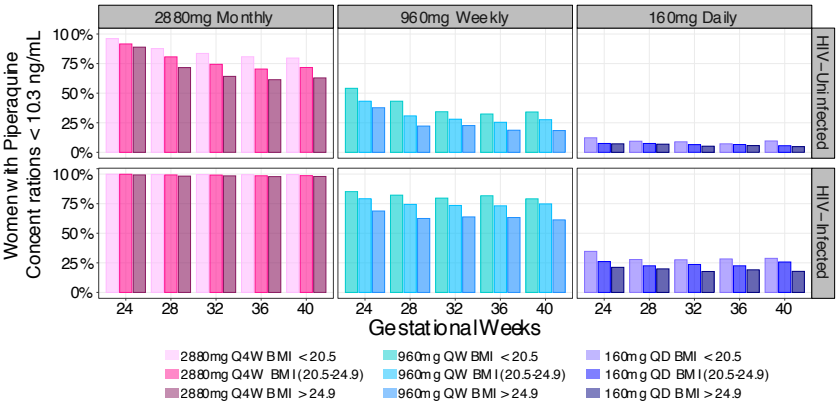

2880mg Monthly
960mg Weekly
160mg Daily
HIV−Uninfected
HIV−Infected
Women with Piperaquine
Concentrations < 10.3 ng/mL
100%
75%
50%
25%
0%
24 28 32 36 40
Gestational Weeks
2880mg Q4W BMI <20.5
2880mg Q4W BMI(20.5-24.9)
2880mg Q4W BMI >24.9
960mg QW BMI <20.5
960mg QW BMI(20.5-24.9)
960mg QW BMI >24.9
160mg QD BMI <20.5
160mg QD BMI(20.5-24.9)
160mg QD BMI >24.9

Figure 3.8 Alternative IPTp regimen simulations. (A) Full PK profiles. Simulated PQ concentrations over pregnancy stratified based on HIV status for three different dosing regimens. The dashed line at10.3 ng/mL marks the previously defined threshold for malaria protection in HIV-uninfected pregnant women. (B) Day-28 concentrations. Simulated PQ day-28 concentrations over pregnancy stratified based on HIV status and week-28 BMI. The dashed line at 10.3 ng/mL marks the previously defined threshold for malaria protection in pregnant women. (C) Percentage of women protected. Percentage of women achieving protection based on HIV status and week-28 BMI for different prevention regimens over pregnancy. Protection was defined as sustaining a PQ concentration of 10.3 ng/mL or greater for 95% of their pregnancy. (D) Percentage of women with day-28 concentrations below 10.3 ng/mL. Based on simulated PQ concentrations, the percentage of women not protected at the end of the month is stratified based on HIV status and week-28 BMI. Q4W, doses given every 4 weeks; QW, doses given every week; QD, doses given daily.

Outcomes

The prevalence of malaria parasitemia, placental malaria, and number of women with ≥1 episode of symptomatic malaria is reported in **Table 3.5.** HIV-uninfected women with low BMI who received DHA-PQ every 8 weeks had a higher percentage of outcomes for all measures (22.1% parasitemia; 40% placental malaria; 26.7% symptomatic malaria) compared to women in the highest BMI group receiving 8-week dosing (17% parasitemia; 20% placental malaria; 10% symptomatic malaria), as well as those in the highest BMI group receiving monthly dosing (6% parasitemia; 28.6% placental malaria; 0% symptomatic malaria). No difference in outcomes was detected between BMI groups in the monthly dosing group. HIV-infected women receiving concomitant indoor residual spraying of insecticides (IRS) had the lowest number of outcomes among the dosing groups.

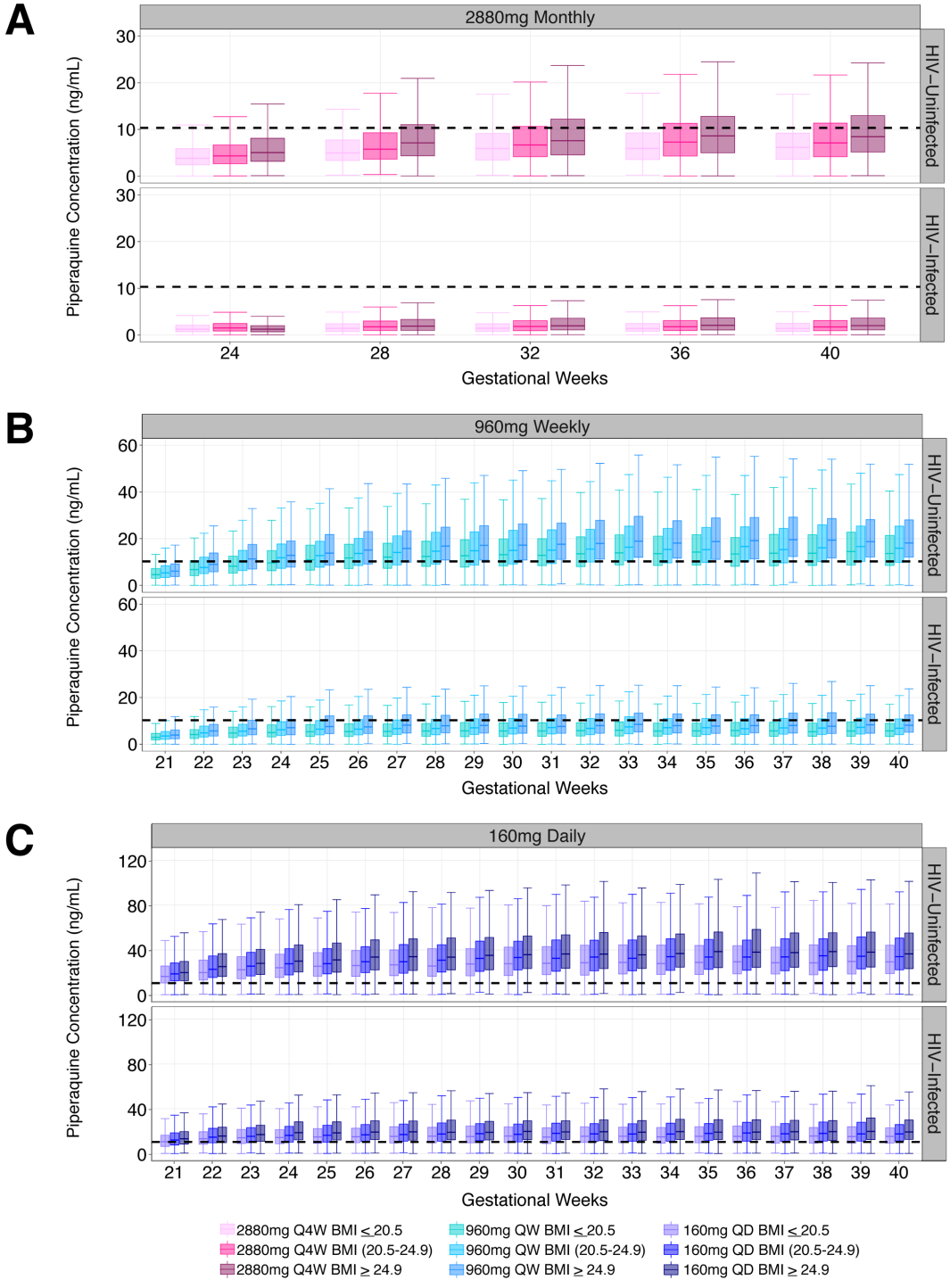

A
2880mg Monthly
HIV−Uninfected
HIV−Infected
Piperaquine Concentration (ng/mL)
Gestational Weeks
B
960mg Weekly
C
160mg Daily
2880mg Q4W BMI ≤ 20.5
2880mg Q4W BMI (20.5-24.9)
2880mg Q4W BMI ≥ 24.9
960mg QW BMI ≤ 20.5
960mg QW BMI (20.5-24.9)
960mg QW BMI ≥ 24.9
160mg QD BMI ≤ 20.5
160mg QD BMI (20.5-24.9)
160mg QD BMI ≥ 24.9

Figure 3.9 Simulated concentrations for alternative IPTp dosing regimens. Simulated PQ concentrations over pregnancy stratified based on HIV status and week 28 BMI. Weekly and daily regimens are displayed with day 7 levels while the monthly regimen is displayed with day 28 levels. The dashed line at 10.3 ng/mL marks the previously defined threshold for malaria protection in pregnant women [27]. Q4W: doses given every four weeks; QW: doses given every week; QD: doses given daily.

Table 3.4 Maximum concentration and change in QTc interval for simulated dosing regimens

Regimen[a]	HIV-Uninfected		HIV-Infected	
	C_{max}[b] (ng/mL)	ΔQTc[c] (msec)	C_{max}[b] (ng/mL)	ΔQTc[c] (msec)
2880 mg Monthly	289 ± 126	14.4 ± 6.31	243 ± 113	18.7 ± 8.67
960 mg Weekly	231 ± 107	11.5 ± 5.35	204 ± 89	15.7 ± 6.87
160 mg Daily	71 ± 34	3.53 ± 1.69	48 ± 25	3.67 ± 1.91

[a]All values are the median (SD), unless otherwise specified.
[b] C_{max}, maximum concentration
[c]ΔQTcF, QTcF (postdose) – QTcF (predose)

Table 3.5 Malaria and parasitological outcomes in HIV-infected and -uninfected women

Malaria and parasitological outcomes[c]	HIV-Uninfected Pregnant (no. of positive samples/ total samples [%])		HIV-Infected Pregnant[b] (no. of positive samples/ total samples [%])
	DHA-PQ every 8 weeks (n =88)[a,c]	DHA-PQ every 4 weeks (n=96)[a]	DHA-PQ every 4 weeks (n=81)[a]
Parasite prevalence by monthly LAMP detection			
BMI at 28 weeks gestation $\leq$ 20.5 kg/m^2	17/77 (22.1)	6/129 (4.7)	0/113 (0)
BMI at 28 weeks gestation > 20.5 & $\leq$ 24.9 kg/m^2	49/321 (15.3)	16/300 (5.3)	5/227 (2.2)
BMI at 28 weeks gestation > 24.9 & kg/m^2	8/47 (17.0)	4/67 (6.0)	0/59 (0)
Prevalence of placental malaria by histopathological assessment			
BMI at 28 weeks gestation $\leq$ 20.5 kg/m^2	6/15 (40.0)	4/25 (16)	3/28 (10.7)
BMI at 28 weeks gestation > 20.5 & $\leq$ 24.9kg/m^2	22/63 (35.0)	18/57 (31.6)	2/41 (4.9)
BMI at 28 weeks gestation > 24.9 & kg/m^2	2/10 (20.0)	4/14 (28.6)	0/12 (0)
Women with at least one episode of malaria on chemoprevention			
BMI at 28 weeks gestation $\leq$ 20.5 kg/m^2	4/15 (26.7)	0/25 (0)	0/28 (0)
BMI at 28 weeks gestation > 20.5 & $\leq$ 24.9 kg/m^2	7/63 (11.1)	0/57 (0)	0/41 (0)
BMI at 28 weeks gestation > 24.9 & kg/m^2	1/10 (10.0)	0/14 (0)	0/12 (0)

[a]Two HIV-infected women, one woman in the every 8 week and 2 women in the every 4 week DHA-PQ arm were excluded from this analysis because they did not have a placental sample collected for histopathological assessment.
[b]These women also received indoor residual spraying of insecticides.
[c]DHA-PQ; dihydroartemisinin-piperaquine; LAMP; loop-mediated isothermal amplification; BMI, body mass index

Discussion

We evaluated the population PK of PQ in a cohort of 274 pregnant women receiving DHA-PQ for malaria prevention. We employed a population approach to identify and quantify the effects of important covariates which might affect drug exposure in a *post hoc* analysis. A three-compartment model with an absorption lag time best described our data, using pregnancy, 28-week gestational BMI, and EFV use as significant covariates. Pregnancy increased clearance by 72% compared to postpartum controls. Interestingly, we identified a trend in which for every 1 unit decrease in 28 -week gestational BMI, there was a 2% increase in clearance, revealing that low-BMI pregnant women have lower PQ exposures. HIV-infected women who were receiving EFV based-ART had a 61% increased clearance and a 51% smaller volume for the second peripheral compartment compared to HIV-uninfected pregnant women. Simulations suggested that increasing PQ dosing frequency may improve efficacy, with low daily dosing of DHA-PQ resulting in the highest number of women maintaining protective concentrations. Furthermore, due to the association between low BMI with higher clearance, weight-based dosing was associated with an increased disparity between PQ levels (**Figure 3.10**). These findings suggest that weight-based dosing for pregnant women may not be needed, as heavier women are able to achieve adequate exposure when given fixed-dose (non-weight-based) regimens. Given the pragmatic benefits of fixed-dose regimens, we recommend this option. By building a PK model which simultaneously fits three different populations, we have created a novel integrated model which is also a tool that others can use when designing future clinical trials and evaluating PQ levels.

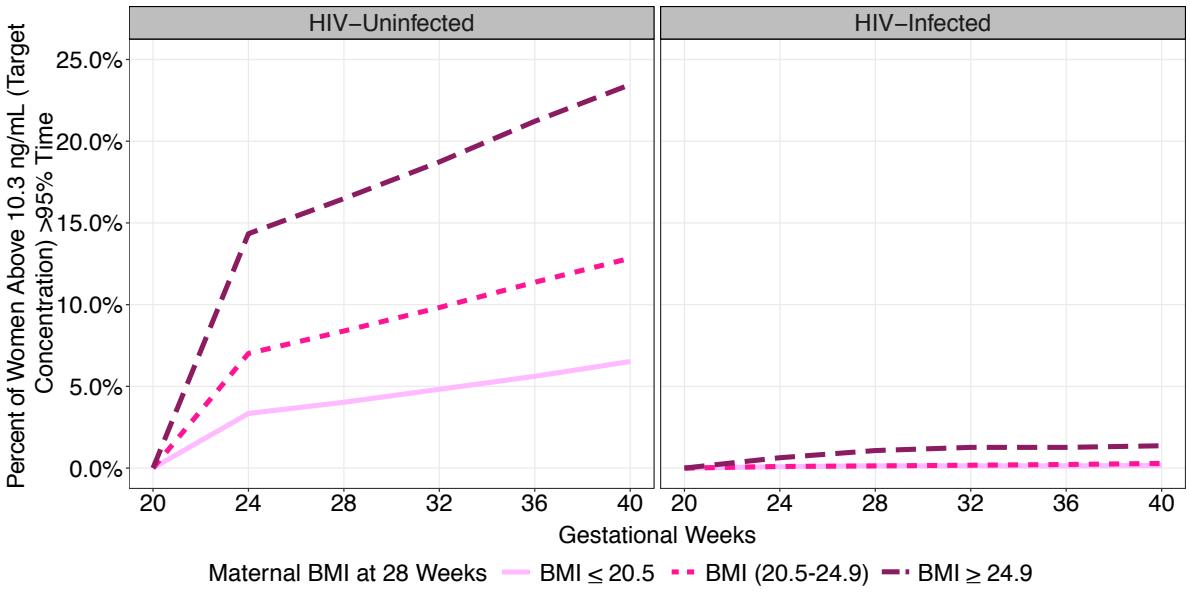


Figure 3.10 Weight based dosing simulations. Percent of women achieving protection based on HIV status for a monthly prevention regimen using weight-based dosing guidelines [21]. Protection was defined as sustaining a PQ concentration of 10.3 ng/mL or greater for 95% of their pregnancy.

Piperaquine metabolism is primarily hepatic and mediated by cytochrome P450 (CYP) 3A4 enzymes.[39] The physiological changes that occur during pregnancy are known to alter CYP activity, including that of CYP3A4, likely leading to the increased clearance compared to nonpregnant adults that was noted in this study.[40,41] By including longitudinal samples throughout the second and third trimester, our model was able to confirm our previous findings (from just the intensive cohort) that pregnancy increases PQ clearance. Additionally, we explored whether this pregnancy effect changed over trimesters, as some studies have shown the effect of pregnancy is greater or is only clinically relevant during the third trimester.[42-44] Our analysis indicates that pregnancy's effect is consistent over the second and third trimesters.[31] Previous trials investigating DHA-PQ for malaria treatment and prevention in Thai and Papua New Guinean women

found similar increases of 42 to 45% in PQ clearance compared to nonpregnant control women.[28,45] In contrast, a treatment trial in Sudanese women did not find a significant pregnancy effect, possibly due to a small trial size, although we cannot exclude impacts of ethnicity or genetics on PQ PK.[46]

An inverse trend was identified between PQ clearance and maternal 28-week gestational BMI in which low-BMI women displayed increased clearance after controlling for the effects of HIV and pregnancy (**Figure 3.4**). When comparing the clearance values for women with the lowest and highest recorded BMIs at 28 weeks gestation (17.1 and 30.5 kg/m^{2,} respectively), low-BMI women had a 24.1% higher clearance. We predict that following fixed monthly dosing of 2,880 mg PQ, women with a 28-week BMI of ≤20.5 kg/m^2 will have 3- and 6-fold less time above protective concentrations compared to HIV-uninfected and -infected women with a BMI of ≥25 kg/m^2, respectively (**Figure 3.8C**). Our findings suggest the use of weight-bands for PQ dosing, as per current World Health Organization guidelines, may not provide the intended benefit over fixed-dose regimens.[21] For example, when using the current weight-based treatment guidelines, women with a BMI of ≥25 kg/m^2 (average weight: 70.2 kg) will receive higher (160/1,280 mg DHA/PQ total dose) DHA-PQ doses compared to women with a BMI of ≤20.5 kg/m^2 at 28 weeks (average weight: 52 kg; 120/960 mg DHA/PQ total dose) resulting in 4- and 8-fold less time protected for HIV-uninfected and -infected women of lower BMIs, respectively (**Figure 3.10**). Our findings indicate that malnourished HIV-infected pregnant women are consistently the least protected population. This finding is concerning, as previous studies have reported up to

14.6% of HIV-infected pregnant women lose weight during pregnancy and no study to date has directly investigated DHA-PQ dosing in this population.[15]

Due to lower than expected parasitological outcomes observed in these studies as a result of concurrent IRS, we were not able to investigate associations between PK covariates and malaria outcomes. However, we observed in the raw data that low-BMI women who received DHA-PQ every 8 weeks had the highest prevalence of parasitemia, the highest percentage of women with a malaria infection, and the highest prevalence of placental malaria (**Table 3.5**) compared to women with higher BMIs and those given DHA-PQ monthly. HIV-uninfected women given DHA-PQ monthly had fewer outcomes, likely indicating the benefit of more frequent dosing. It is likely that malnourished HIV-infected women would have even higher outcome rates; however, these women were also protected by IRS. Given IRS's efficacy, only 5 women had parasitemia and placental malaria detected.

Previous studies have reported increased phenylbutazone clearance and shorter antipyrine half-life in malnourished men compared to well-nourished men.[47,48] In a similar cohort of HIV-infected Ugandan pregnant women, food insecurity was found to significantly reduce the bioavailability of different ART combinations compared to healthy nourished controls.[49] While BMI did not appear to have any significant effects on bioavailability in our model, clearance is measured as CL/F and it is possible that the signal we detected could be due in part to reduced bioavailability. Alternative explanations for increased clearance of PQ are altered absorption, decreased protein binding or an array of other physiological changes induced by malnutrition.[48,50-52] Multiple studies have shown malnutrition alone or in combination with other diseases

such as HIV and malaria is associated with adverse birth outcomes.[10,15] Given that malnutrition is a modifiable, albeit difficult, risk factor, prevention regimens which include nutritional supplementation could potentially lead to improved maternal and birth outcomes and warrant investigation. However, by increasing the dosing frequency, optimal chemoprevention can readily be achieved in this special population.

It is possible that this inverse BMI-clearance relationship is a result of physiological changes due to maternal malnutrition, given that recent studies report inadequate weight gain in up to 62% of Ugandan women during pregnancy.[15,35] Indeed, in the present study, 21 women (16 HIV-infected; 5 HIV-uninfected) lost weight during pregnancy. We have investigated both weight and BMI as potential covariates for clearance as often the two are correlated (in our case $r^2 = 0.65$). We found that BMI was superior and the only significant predictor of clearance ($P = 0.027$ versus $P = 0.32$). Furthermore, it is difficult to classify a pregnant woman as malnourished, given that no guidelines using weight-based measures exist. Instead, measures of maternal nutrition are defined by weight gained during pregnancy, and only criteria regarding pre-pregnancy BMI are used to classify a woman as malnourished.[34] While weight gained during pregnancy was tested as a covariate, this measure was highly variable, and was potentially confounded by gains due to the growing fetus, possibly explaining a lack of relationship. Additionally, pre-pregnancy BMI and weight gained during pregnancy are not clinically useful measures for determining dosage guidelines, as many women do not know their pre-pregnancy BMI, and weight gained during pregnancy can only be determined retrospectively. In contrast, BMI at 28 weeks of gestation could be used clinically to guide dosing recommendations.

The HIV-infected women in this trial received concomitant EFV-based ART; EFV is a known CYP3A4 inducer.[53,54] After controlling for the effect of pregnancy, there was an additional 61% increase in PQ clearance in women receiving EFV, which we attributed to EFV-mediated induction of CYP3A4. This extends our previous work by confirming the effects of EFV and indicating this effect lasts throughout the second and third trimesters.[31] In the only other study that investigated administration of EFV and PQ in HIV-positive nonpregnant adults, the PQ area under the curve from 0 to 28 days ($AUC_{0–28days}$) was 43% lower than that in patients not receiving EFV, in agreement with our findings.[55] Our model identified a difference in peripheral volume, whereby HIV-infected pregnant women had a 51% reduction in the second peripheral compartment. This finding is likely an artifact due to differences in terminal PK sampling between trials, where some HIV-uninfected women had PK samples obtained up to 56 days postdose. There is evidence to suggest that if sampling does not sufficiently capture the elimination phase for drugs with long terminal half-lives, such as PQ, the true terminal phase will not be not defined, and models will under predict the volume and/or compartment number.[56]

Pregnancy, low BMI, and EFV use all decreased PQ exposure, potentially reducing the efficacy of DHA-PQ for IPTp. Given that both pregnant women and HIV-infected individuals are at an increased risk for contracting malaria, it is essential to optimize prevention measures to protect these high risk groups.[11,12] When administered monthly, less than 20% of HIV-uninfected women and less than 2% of HIV-infected women were predicted to maintain PQ exposure above the protective level

(**Figure 3.8** and **Figure 3.9**). Regardless of HIV status, malnourished women had the smallest amount of time above the protective concentration. Simulations showed that increasing the frequency of dosing improved protection, with low daily dosing achieving the best protective coverage of >75% of both HIV-infected and malnourished women protected. Animal toxicity studies have documented that prolonged exposure to artemisinins can cause neurological and auditory toxicity.[57-59] Unfortunately, limited clinical data exist.[60,61] Clinical trials which explore more frequent dosing, including daily, will need to include neurological and auditory toxicity assessments to ensure these regimens are, in fact, safe. Regimens with more frequent lower doses showed less QTc prolongation, indicating they are less cardiotoxic (**Table 3.4**).

As alternative dosing regimens are explored, clinical trials which employ fixed dosing should be conducted. The trials which provided data for this analysis used a fixed dose of 3 tablets per dosing day. The results indicate that heavier women did not disproportionately contract malaria or parasitemia and therefore may not need a higher dose (**Table 3.5**). Instead, low-BMI women given DHA-PQ every 8 weeks had the highest prevalence of parasitological outcomes compared to all other groups. Additionally, fixed dosing is more pragmatic, especially in resource-limited settings.

This study had some limitations. Information regarding pre-pregnancy weight, mid-upper-arm circumference, plasma protein, free drug, and nutrient levels was unavailable. To decrease bias associated with variable enrollment times, we used weight and BMI measures at 28 weeks gestation as a baseline measurement. It is possible that by using BMI at 28 weeks gestation we underestimated the effects of malnutrition on clearance. The studies that enrolled our subjects did not record food

intake; thus, we cannot account for food effects on drug absorption. Further, only the first of three daily DHA-PQ doses each month was directly observed, and so limited adherence to prevention may have affected results. Lastly, the parasite prevalence and malaria outcomes among these women were low (**Table 3.5**) due to DHA-PQ's efficacy and the effects of other prevention measures such as IRS. As a result, we were unable to fully explore the effects of malnutrition as well as HIV/EFV use on parasitological outcomes. Given that we could not establish a PK/PD relationship for these two groups, the protective concentration based on HIV-uninfected women was used instead.

DHA-PQ is a safe and effective regimen which shows promise as an alternative for IPTp-SP. In order to best protect all women from malaria and parasitemia, it is important to carefully consider dosing strategies in vulnerable populations. Our findings indicate that pregnant women, especially those who are low BMI and/or receiving concomitant CYP3A4 inducers such as EFV, have reduced PQ exposure, increasing their risk for malaria. It is these malnourished/HIV-infected pregnant women who may benefit from weekly or low daily dosing using fixed-dose regimens. Trials exploring alternative DHA-PQ regimens in high-risk populations, such as malnourished women, are needed to confirm our recommendations for IPTp.

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Chapter 4: Piperaquine induced QTc prolongation decreases with repeated monthly dihydroartemisinin-piperaquine dosing in pregnant Ugandan women*

Abstract

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

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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 adverse events due to ΔQTcF >60 milliseconds, but no arrhythmias or cardiac symptoms were detected. 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.

Introduction

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 birthweight, 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 birthweight 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 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 immuno-deficiency 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 tab-lets (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 4.1**). 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 (**Figure 4.2**). 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 (∆QTcF: postdose QTcF − predose QTcF) was calculated for each of the 3 ECG occasions.

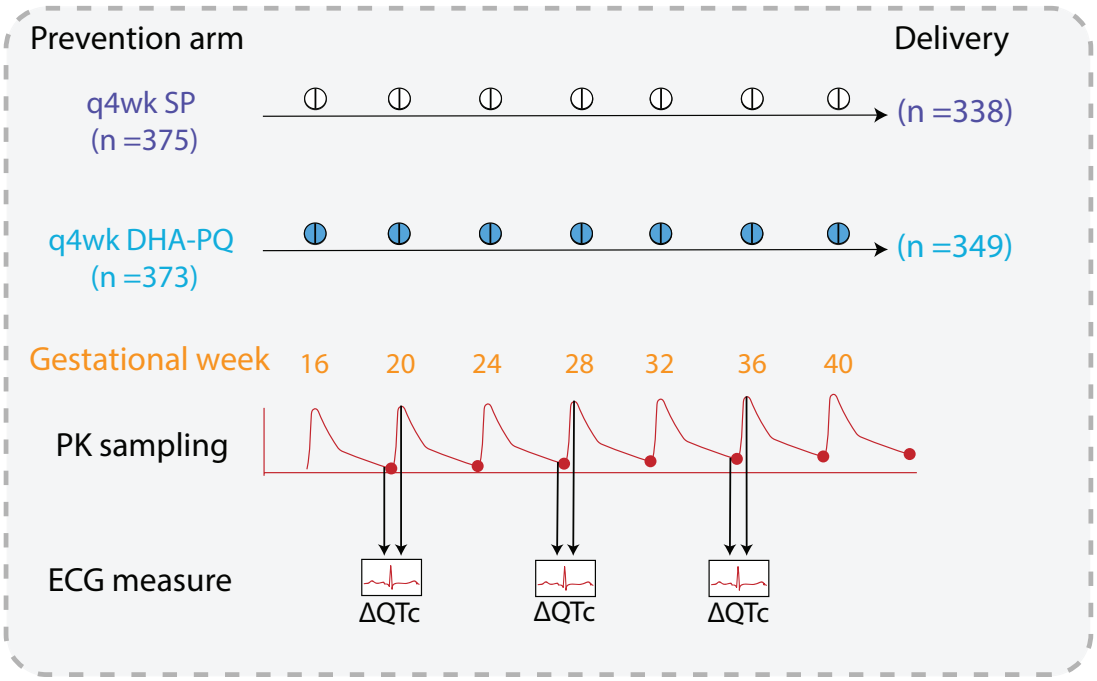


Figure 4.1 Summary of trial procedures. Tablets indicate when each prevention course of DHA-PQ and SP were provided. The dots indicate when plasma sampling for piperaquine PK occurred relative to the expected PK profile. The arrows indicate when ECGs were recorded relative to the expected PK profile 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.

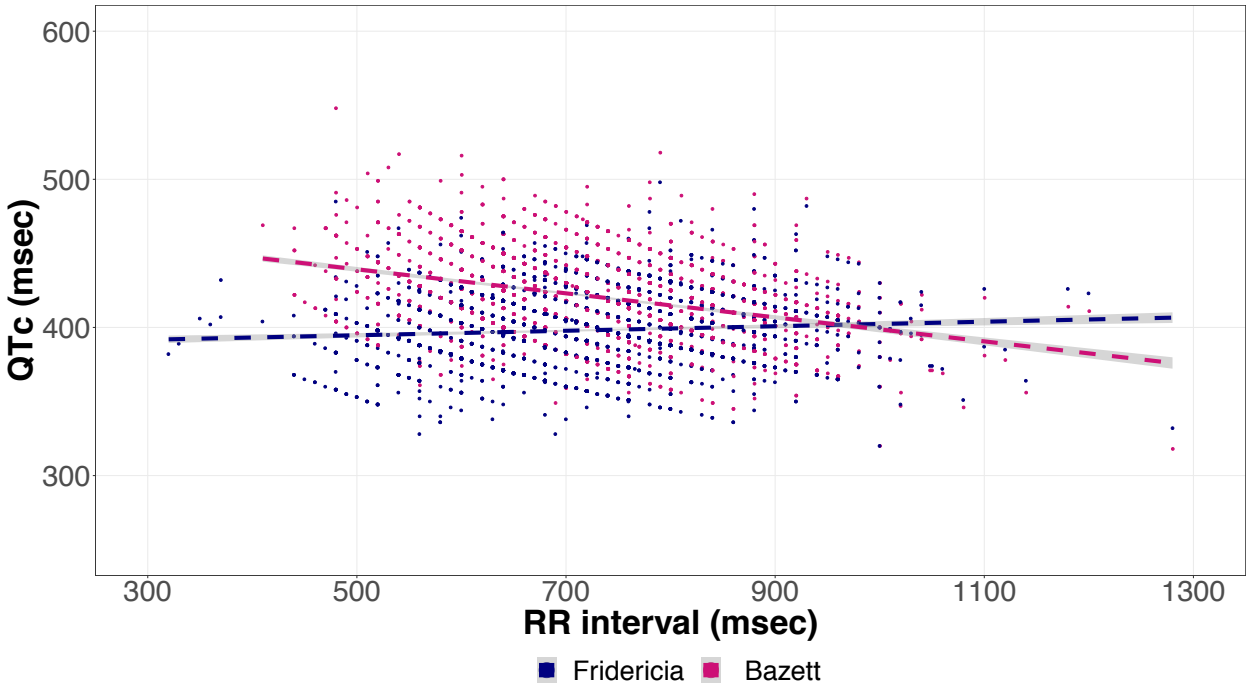


Figure 4.2 Correlation between RR interval and corrected QT interval using Fridericia's or Bazett's correction. Each point represents the observed data and all QTc measurements for both prevention arms are included (all occasions and both pre- and post-dose). The slope is displayed as the mean regression line (dashed line) and 95% confidence interval (shaded region). $Fridericia$: $QTcF = \frac{QT}{\sqrt[3]{RR}}$ $Bazett$: $QTcB = \frac{QT}{\sqrt[2]{RR}}$

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 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 $P < 0.05$ 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 4.1 and Figure 4.1**). There were 1226 piperaquine trough concentrations available, with an average venous concentration of 11 ng/mL and capillary concentration of 15 ng/mL (**Table 4.2 and Figure 4.3**). 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 4.2 and Figure 4.4**). 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 (∆QTcF measurements = 9), all in the DHA-PQ arm, who had grade 3–4 adverse events due to ∆QTcF >60 milliseconds (maximum, 81 milliseconds), but no arrhythmias or cardiac symptoms were detected. In the DHA-PQ arm, the ∆QTcF 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 ∆QTcF were investigated and revealed 4 different trajectories (**Figure 4.5**). Only 30 (10%) women consistently had an increase in ∆QTcF, 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 ∆QTcF at week 36 compared with week 20. No clinically significant ∆QTcF was noted in the SP arm and values (−1.0 to 0 milliseconds) were consistent over pregnancy.

Table 4.1 Demographic characteristics at the time of first study drug administration

Characteristic	Prevention Arm	
	DHA-PQ	SP
n	373	375
Age (years) [mean (95% percentile)]	23 (17 - 36)	24 (17 - 38)
Weight (kg) [mean (95% percentile)]	55.0 (43.2 - 74.5)	55.8 (44.0 - 78.6)
Height (cm) [mean (95% percentile)]	158 (147 - 169)	158 (147 - 171)
Body mass index (kg/m^2) [mean (95% percentile)]	22.0 (17.6 – 29.0)	22.0 (18.3 - 29.8)
Hemoglobin (g/dL) [mean (95% percentile)]	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)]	3 (1 - 9)	3 (1 - 9)
First [n (%)]	84 (22.5)	98 (26.1)
Second [n (%)]	101 (27.1)	81 (21.6)
Third and greater [n (%)]	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 4.2 Pharmacokinetic and toxicity data

Data	Gestational weeks	DHA-PQ		SP	
		n	Value	n	Value
Pharmacokinetic					
PQ concentration (ng/mL) [mean (95% percentile)]					
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)	-	-

Table 4.2 (Continued)

Data	Gestational weeks	DHA-PQ n	DHA-PQ Value	SP n	SP Value
Toxicity					
QTcF (msec) [mean (95% percentile)]					
Pre-dose	20	361	394 (354-438)	355	396 (356-436)
Post-dose		354	409 (369-454)	343	398 (351-438)
Pre-dose	28	349	396 (356-436)	341	395 (356-446)
Post-dose		344	407 (365-446)	333	395 (360-442)
Pre-dose	36	335	393 (355-429)	316	391 (355-442)
Post-dose		327	402 (360-442)	302	391 (349-436)
Total samples		2070		1990	
QTcF > 450 msec [n (%)]					
Pre-dose		4/1045	(0.4)	7/1012	(0.7)
Post-dose		18/1025	(1.8)	7/978	(0.7)
ΔQTcF (msec) [mean (95% percentile)]					
	20	354	18.0 (-33.2 - 56.2)	343	0.0 (-44.5 - 44.8)
	28	344	12.0 (-33.7 - 52.0)	333	0.0 (-44.0 - 44.0)
	36	327	10.0 (-31.9 - 51.0)	302	-1.0 (-46.0 - 45.0)
Total samples		1025		978	
ΔQTcF >60 msec [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

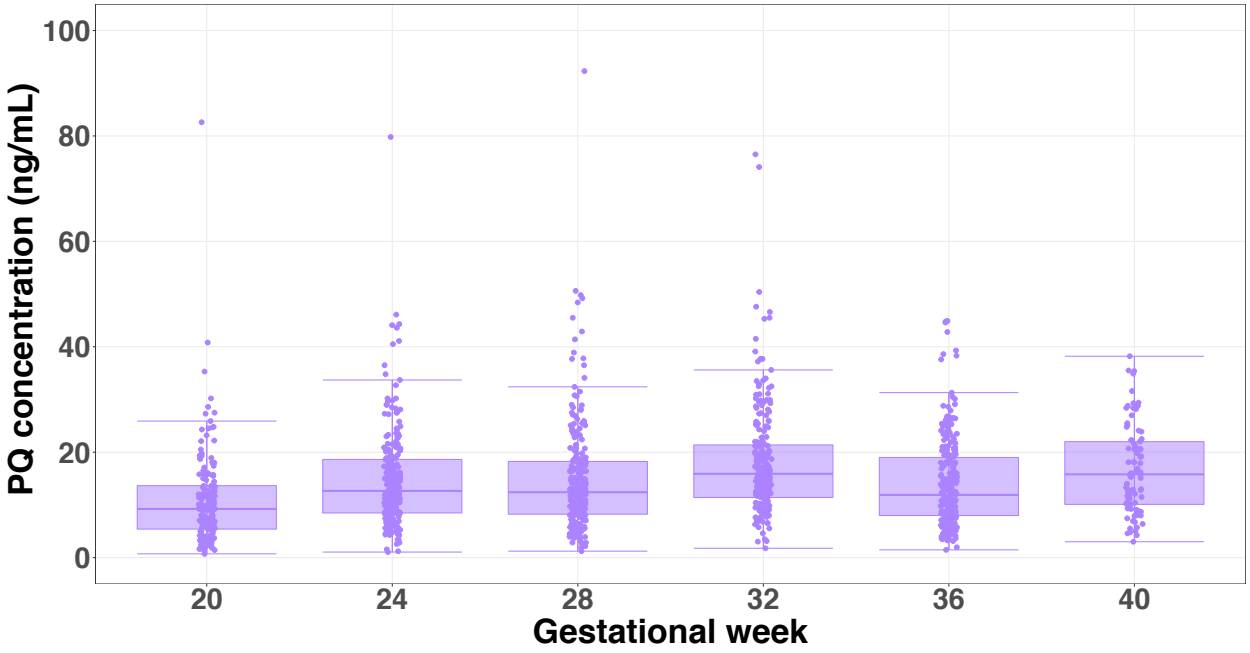


Figure 4.3 Observed trough concentrations by gestational week. Each point represents the observed data. Three trough concentrations (>100 ng/mL) were included in the PK model but omitted from this plot as they obscured visualizing the data's central tendencies. At week 20, 28 and 36 venous samples were collected and at the remaining weeks capillary samples were collected. PQ; piperaquine.

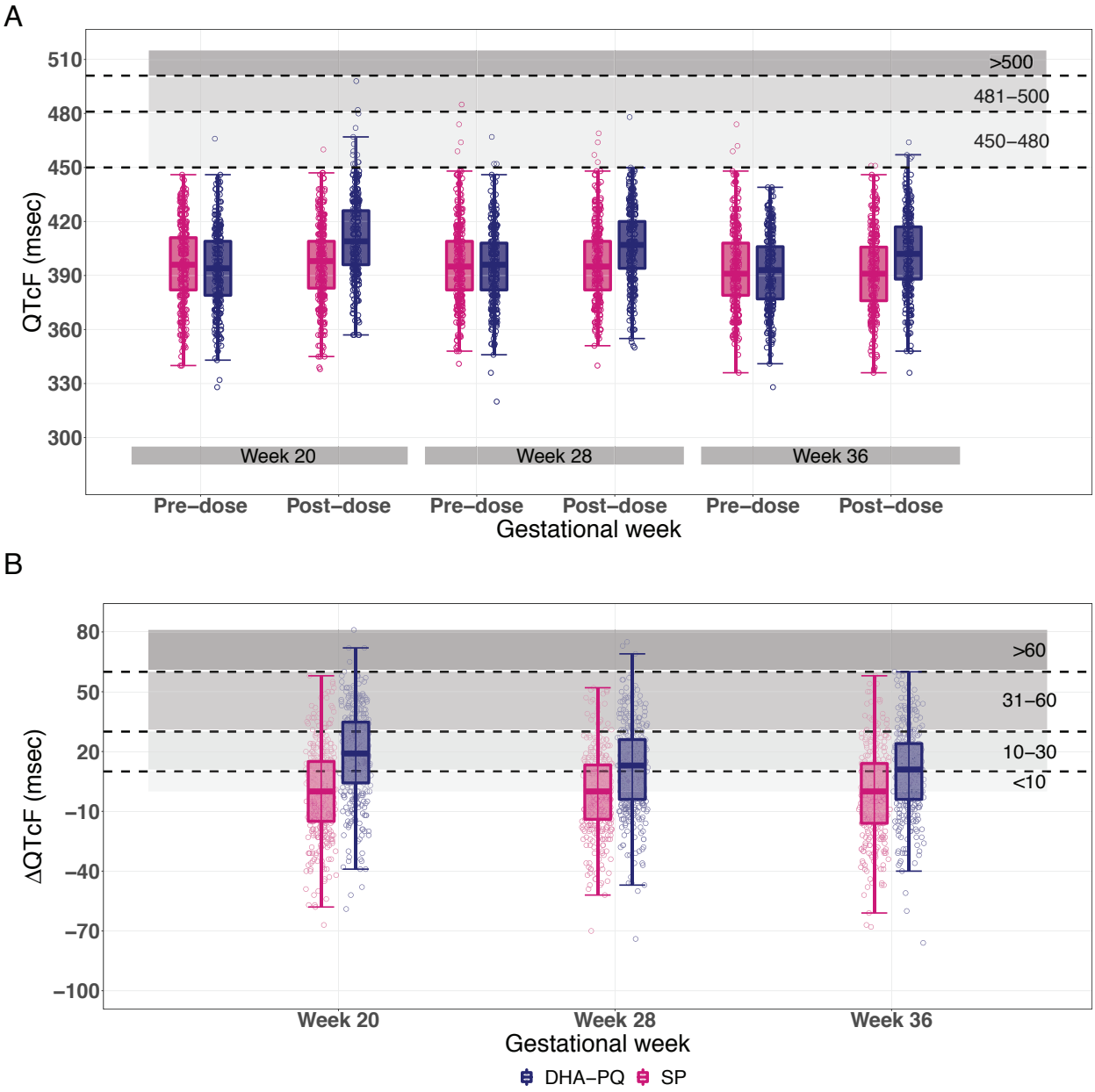


Figure 4.4 Absolute and change in Fridericia corrected QT interval (QTcF) stratified by prevention regimen and gestational week. A, QTcF pre- and postdose measurements for SP (left) and DHA-PQ (right), respectively. B, Change in QTcF interval. Points represent the observed data, boxes indicate 75% of 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.[34] Abbreviations: DHA-PQ, dihydroartemisinin-piperaquine; ECG, electrocardiogram; FDA, Food and Drug Administration; ΔQTcF, QTcF (postdose) – QTcF (predose); SP, sulfadoxine-pyrimethamine.

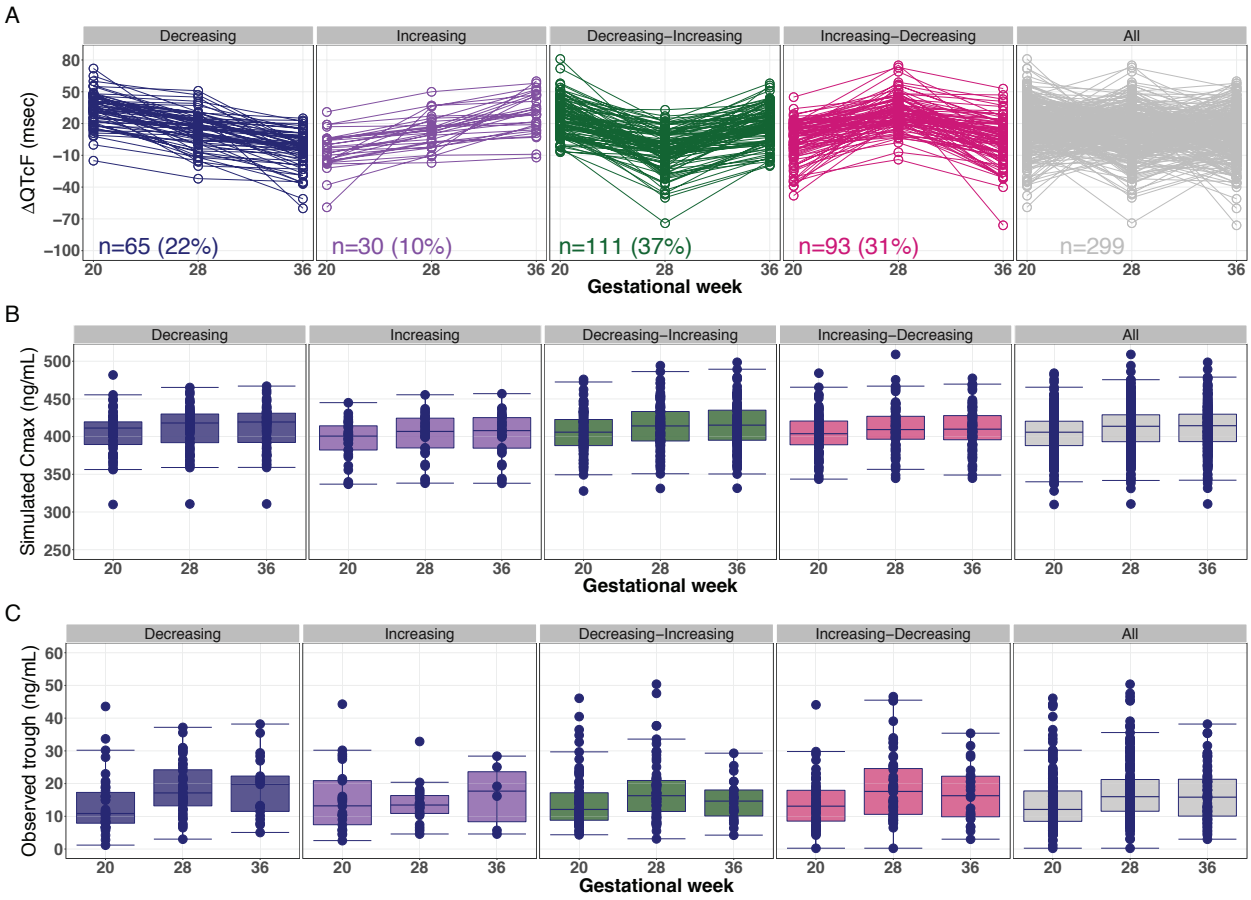


Figure 4.5 Individual-level data for women randomized to DHA-PQ. A, Individual-level trends in ΔQTcF measurements. Simulated C_{max} (B) and observed trough (C) piperaquine concentrations. Data arestratified according to the 4 predominant ΔQTcF patterns. Each point represents the observed orsimulated 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: C_{max}, maximum concentration; DHA-PQ, dihydroartemisinin-piperaquine; PK, pharmacokinetics; QTc, corrected QT interval.

Table 4.3 Final model parameter estimates

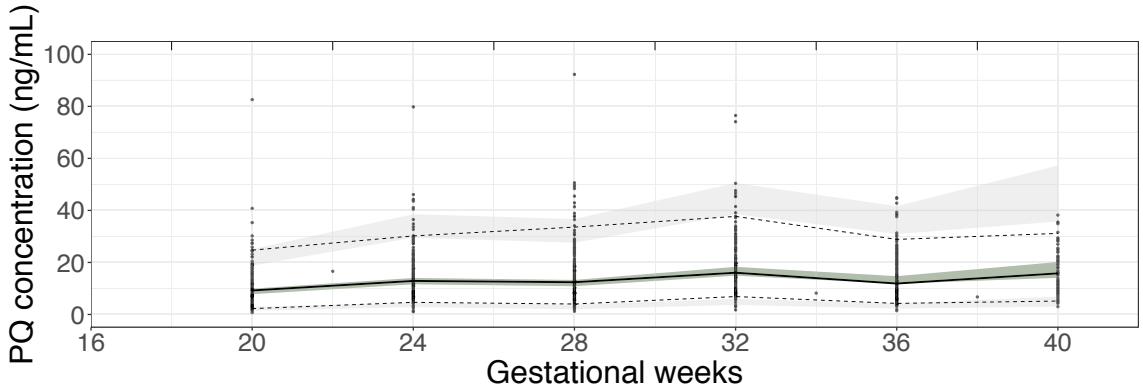
	DHA-PQ, Median (95% CI)		SP, Median (95% CI)	
Parameter estimates	Population estimate	Interindividual variability/ Interoccasion variability	Population estimate	Interindividual variability
Piperaquine pharmacokinetic parameters				
Clearance (L/days)	3204[a]	15.8% (14.3%-17.5%)[b]	-	-
Volume central compartment (L)	5302[a]	56.6%	-	-
Volume peripheral compartment (L)	33584[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				
Pre-dose QTcF (msec)	393 (391 - 395)	3.18% (2.9% -3.42%)	-	-
Slope of Concentration dependent effect of QTcF (msec*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 (msec)	-	-	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).
[a]Parameter value fixed
[b]Inter-occasion variability
Abbreviations: CI, confidence interval; DHA-PQ, dihydroartemisinin-piperaquine; QTcF, Fridericia corrected QT interval; SP, sulfadoxine-pyrimethamine

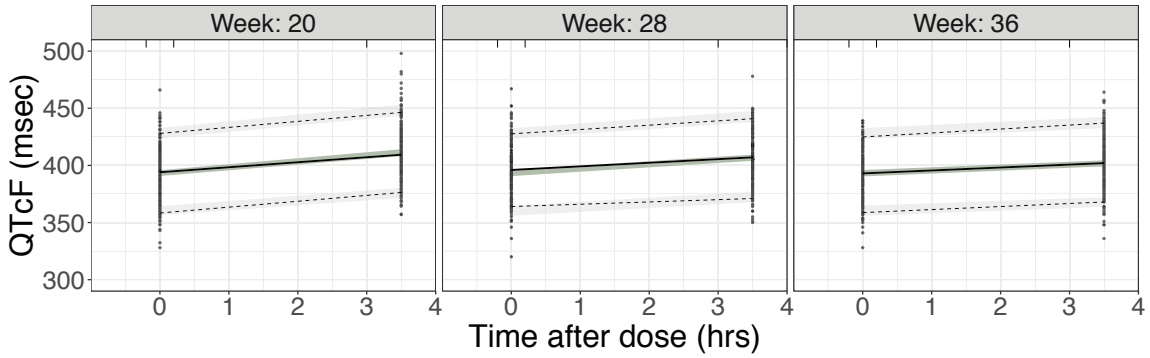
Population PK model

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 4.3**). 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 4.6A**) and a bootstrap indicated good precision in parameter estimates (**Table 4.3**).

A


PQ concentration (ng/mL)
100
80
60
40
20
0
16
20
24
28
32
36
40
Gestational weeks

B


Week: 20
Week: 28
Week: 36
QTcF (msec)
500
450
400
350
300
0
1
2
3
4
Time after dose (hrs)

C

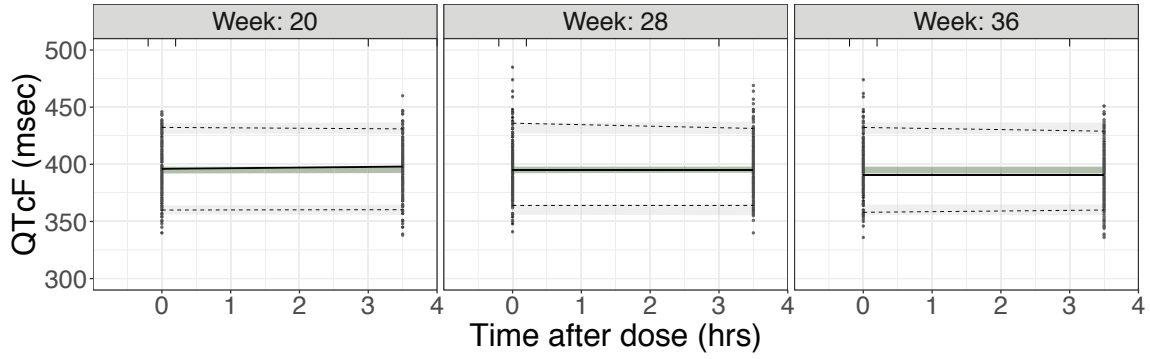

Week: 20
Week: 28
Week: 36
QTcF (msec)
500
450
400
350
300
0
1
2
3
4
Time after dose (hrs)

Figure 4.6 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.

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 4.7**). Interestingly, the extent of QTc prolongation decreased over time despite an increase in observed piperaquine trough and simulated maximum concentrations (C_{max}) (**Figure 4.5**). The decreasing slope of the PK-QTc relationship was best captured by estimating 3 separate slope terms (OFV, −353; $P < .001$), 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 4.3**).

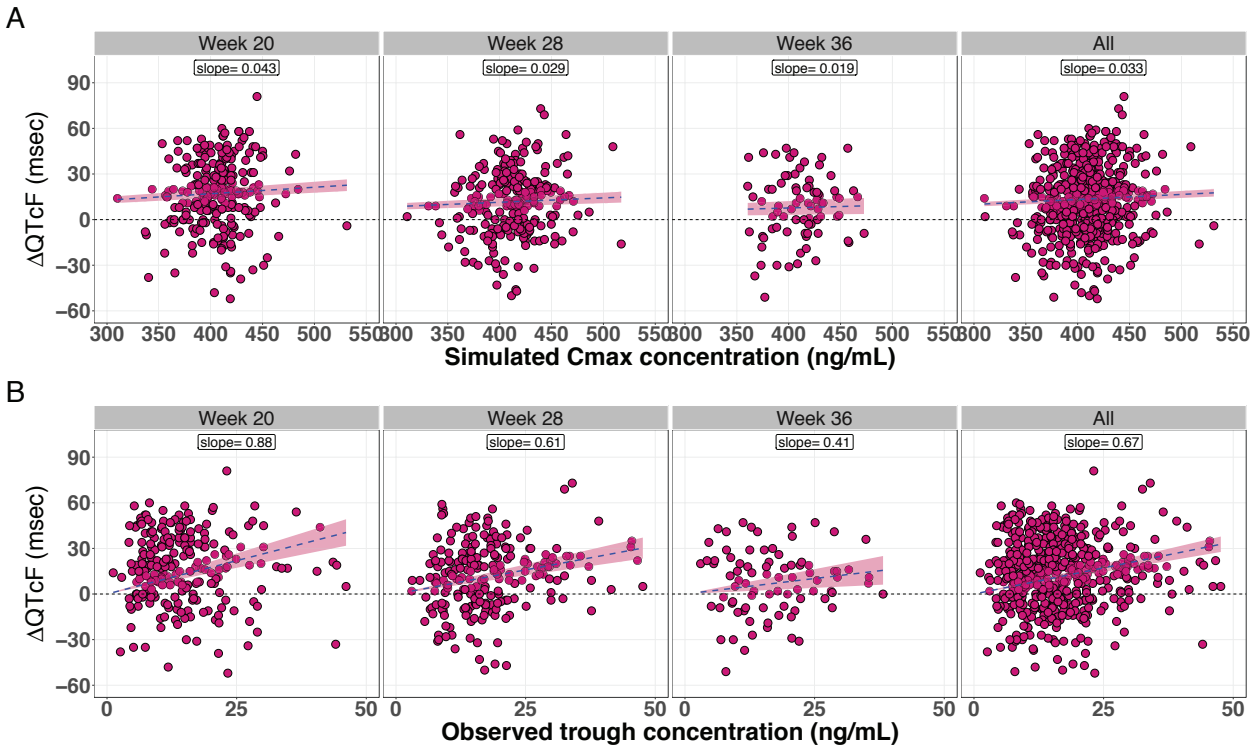


Figure 4.7 Linear regression of ΔQTcF and piperaquine concentration showing decreasing regression slope with increasing gestation age. Model-estimated piperaquine concentrations at the time of ECG recording (Cmax) (A) and observed trough concentrations (B). The piperaquine troughs shown in this figure 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.

A separate model was built from the QTc measurements re-corded 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 4.6B and 4.6C**, respectively) and a bootstrap indicated good precision in parameter estimates (**Table 4.3**).

Discussion

In the setting of treatment and prevention, piperaquine has consistently been shown to prolong the QTc interval.[32,35] 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 C_{max} 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]

Perhaps our most interesting finding was the population de-crease in the PK-QTcF relationship after repeated DHA-PQ courses without a decrease in observed trough or simulated C_{max} piperaquine concentrations (**Figure 4.7**). 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 4.5**). A similar observation was reported in healthy volunteers where sotalol concentrations and QTc measurements were recorded following a single and 7 doses.[36] 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.[36] 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. [29,37,38] 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. [29,37,38] 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 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 C_{max} 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 fac-tors altering the PK profile or QTc profile for DHA-PQ or SP. Additionally, most piperaquine studies conducted in adults have not reported covariate effects.[16,27,32,39,40] Only the 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 re-peated 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.

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Chapter 5: Identifying protective piperaquine concentrations for malaria prevention in pregnant Ugandan women

Introduction

In 2020, the number of malaria cases and deaths increased by 14 million and 69,000 compared to 2019, respectively.[1] These increases have largely been attributed to healthcare disruptions during the COVID-19 pandemic and mark a concerning trend. An additional effect of decreased access to healthcare is that these numbers likely underestimate the extent of malaria.[1] While resources are allocated to fighting COVID-19, efforts to combat the malaria epidemic cannot be overlooked.

Pregnant women are particularly susceptible to malaria due to reduced immunity.[2-4] Malaria during pregnancy can result in severe maternal anemia and death as well as adverse birth outcomes such as stillbirth, low birthweight and pre-term birth.[5-8] It was estimated that in 2020 in African countries with high and moderate transmission, 11.6 million pregnant women were infected with malaria leading to 819,000 low birthweight deliveries.[9] These pregnancy estimates are strikingly similar to those reported for 2010, (12.4 million infections and 900,000 low birthweight deliveries) further indicating a recent lack of progress in reducing the burden of malaria.[10]

To protect pregnant women, the World Health Organization (WHO) recommends monthly doses of sulfadoxine-pyrimethamine (SP) beginning in the second trimester.[11] This practice is known as intermittent preventative treatment in pregnancy (IPTp). It has a twofold goal of i) curing patients with existing parasitemia and ii) providing a period of post treatment prophylaxis to protect women from new infections. While SP can improve

birth outcomes through non-malaria meditated pathways, it's efficacy toward reducing malaria has been compromised by widespread parasite drug resistance.[12,13] Both replacing SP or combining it with an effective antimalarial are appealing alternatives which could better protect pregnant women. Recent clinical trials have shown the artemisinin-based combination therapy dihydroartemisinin-piperaquine (DHA-PQ) to be safe and highly effective against malaria and is therefore an appealing option for IPTp.[14-16] However, monthly doses of DHA-PQ have not consistently been shown to improve birth outcomes compared to SP alone or eliminate all malaria risk suggesting a different DHA-PQ dose or frequency may be required to maximize efficacy.[14,15]

To date, only one publication has established a pharmacokinetic/ pharmacodynamic (PK/PD) relationship for piperaquine in the context of malaria prevention in pregnant women.[17] This study found that a minimum concentration of 10.3 ng/mL piperaquine was required to prevent parasitemia in 95% of women and lower more frequent (weekly or daily) dosing should maintain these levels throughout the dosing interval. While pivotal, this study had a few important limitations: i) it enrolled a relatively small number of women, ii) parasite density was measured using a semi-quantitative assay which can only measure densities >1,000 parasites/mL and, iiI) community-wide indoor residual spraying of insecticides was implemented over the course of the study, greatly reducing malaria transmission intensity. Additional analyses are required to validate or revise the proposed PQ target concentration to ensure the correct dosing regimen is utilized. To address this goal, PQ concentrations and parasite densities (measured by a highly sensitive quantitative PCR (qPCR) assay) from a large

prevention trial conducted in a high transmission setting were used to build a PK/PD model.

Methods

Study design and participants

Data analyzed originated from a large 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 immuno-deficiency 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 and continued until delivery, 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.

Laboratory procedures

At each routine visit, women provided a pre-study drug trough blood sample to measure piperaquine concentrations (**Figure 5.1**). Venous samples were collected on weeks 20, 28, and 36. The remaining samples collected were capillary blood from a finger prick. Additionally, at each monthly visit or if a patient was febrile, blood was collected to measure parasite densities.

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, piperaquine concentrations were also quantitated when malaria (fever with parasitemia by microscopy) or asymptomatic parasitemia (detected by microscopy or quantitative polymerase chain reaction [qPCR]) 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.[18] 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 inter- and intraassay coefficient of variation (CV) was less than 10% for all quality-control samples.

Parasite quantitation

Parasite densities were initially measured by microscopy. Blood smears were prepared with 2% Giemsa stain and measured by two microscopists. Any discrepant readings were resolved by a third microscopist. Negative samples were defined as smears with no detectable asexual parasites when read at 100 high power fields. All microscopy negative samples were then re-analyzed using a qPCR assay.[19] This assay targeted the *var* gene acidic terminal sequence (*var*ATS, 59 copies/genome) and had a lower limit of quantification of 1 parasite/mL blood.[19] Samples which were positive by microscopy or qPCR were included in this analysis. Blood samples which were parasite negative when measured by microscopy without a corresponding qPCR measurement were excluded from the analysis.

Population PK/PD modeling

All data were analyzed by nonlinear mixed-effects modeling using NONMEM VII (Icon Development Solutions, Ellicott City, MD). The PK model used in this study has previously been published.[20] Briefly, a two compartment 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.[17] This model includes a linear relationship to capture the differences between capillary and venous concentrations with all model estimated concentrations reported as venous values. Pharmacokinetic parameters were fixed to

their respective values from the prior analysis to improve model stability[17], but additive and proportional errors (separate for venous and capillary samples), bioavailability, and inter-occasion variability on clearance were estimated.

Sequential modeling was used for the piperaquine PK/PD model. The PK model was used to generate concentration, cumulative AUC and C_{max} estimates which were tested to define the exposure-response relationship. The parasite densities were converted to binary measures indicating the presence or absence of parasites. This was modeled using Equation 1,

$$p_{parasites} = \frac{\exp(b)}{1+\exp(b)} \qquad \text{Equation 1}$$

Where parameter *b* is the baseline probability. Both linear and non-linear relationships were tested to define the effect of piperaquine on parasitemia using a logistic regression model described by Equation 2,

$$p_{parasites} = \frac{\exp(b+EFF\times Covaraites)}{1+\exp(b+EFF\times Covaraites)} \qquad \text{Equation 2}$$

where *EFF* is the effect from piperaquine and covariates were any demographic or clinical factor as described below.

A stepwise covariate (SCM) search was performed to identify any influence of patient and clinical characteristics on parasite model parameters. The covariates tested were age, weight, body mass index, gestational weeks, trimester, gravidity including primigravida, and body temperature. Linear and nonlinear relationships were

investigated. A significance cutoff of P <0.05 was applied for forward inclusion, followed by a cutoff of P <0.01 for backwards elimination.

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.

The previous PK/PD model used parasite density data generated from a different assay (loop-mediated isothermal amplification; LAMP) which had a lower limit of quantification of 1,000 parasites/mL. To facilitate comparisons between models, a sensitively analysis was performed where parasite densities less than 1,000 parasites/mL were considered negative. In addition to being the LLOQ of the LAMP assay at this density, both assays have similar sensitivity and specificity.[21]

Simulations

The final model was used to perform simulations (n=500), adjusting for the dose frequency and amount. Monthly (2,880 mg or 3,840 once per month), weekly (960 mg every 7 days), and low daily (160 mg) doses were tested. Each dosing schedule was evaluated based on the number of women who maintained 10.3 ng/mL PQ and how quickly this threshold was achieved.

Results

Study cohort and data

A total of 373 women were enrolled and received at least 1 dose of DHA-PQ (**Table 5.1 and Figure 5.1**). There were 1226 piperaquine trough concentrations available, with an average venous concentration of 11 ng/mL and capillary concentration of 15 ng/mL (**Table 5.2**). There was an average 3 ng/mL increase in piperaquine concentration over the course of pregnancy. At enrollment 81% of women in the DHA-PQ arm were positive for parasites measured by microscopy or qPCR (**Figure 5.2**). Patients enrolled before 16 weeks gestation had multiple parasite density measures before beginning study drugs and 82.7% of all pre-drug samples were positive for parasites. By 24 weeks gestation, the proportion of women that were parasitemic by microscopy or qPCR decreased to 23% and plateaued around 15% at week 28 until delivery. Women who were parasitemic after initiation of DHA-PQ had low densities with a mean of 472 and median of 0.1 parasites/μL. The proportion of women positive was much lower when using the LAMP LLOQ of 1,000 parasites/mL and leveled off at 3.5% positivity (**Figure 5.3**). In total, there were 956 positive samples out of 2456 (39%) using the qPCR LLOQ compared to 574 (23%) using the LAMP LLOQ.

Table 5.1 Demographic characteristics at the time of first study drug administration

Characteristic	Prevention arm
	DHA-PQ
n	373
Age (years) [mean (95% percentile)]	23 (17 - 36)
Weight (kg) [mean (95% percentile)]	55.0 (43.2 - 74.5)
Height (cm) [mean (95% percentile)]	158 (147 - 169)
Body mass index (kg/m^2) [mean (95% percentile)]	22.0 (17.6 – 29.0)
Hemoglobin (g/dL) [mean (95% percentile)]	11.4 (8.9 - 13.8)
Anemic (hemoglobin<10 g/dL) [n (%)]	33 (9)
Gravidity [mean (range)]	3 (1 - 9)
First [n (%)]	84 (22.5)
Second [n (%)]	101 (27.1)
Third and greater [n (%)]	188 (50.4)
Gestational weeks at enrollment [mean (range)]	15 (12 - 20)
Study drug started at [n (%)]	
16 weeks gestation	234 (63)
20 weeks gestation	139 (37)

Abbreviations: DHA-PQ, dihydroartemisinin-piperaquine

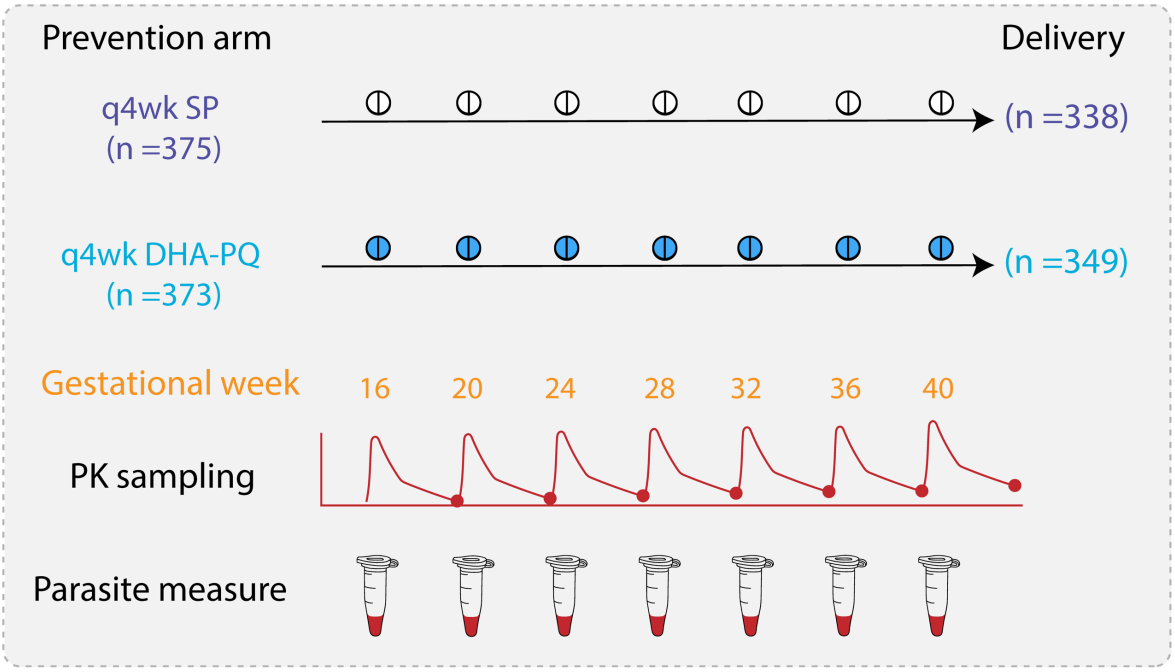

Figure 5.1 Trial summary. Blue and white tablets indicate when each prevention course of DHA-PQ and SP were provided relative to gestational week. The red dots indicate when plasma sampling for piperaquine occurred relative to the expected PK profile. The tubes indicate when additional blood samples were collected for routine parasite density measures. The initial number of participants listed reflects those who received at least 1 course of prevention followed by the number of women who completed the trial. Abbreviations: DHA-PQ, dihydroartemisinin-piperaquine; PK, pharmacokinetics; q4wk, every-4-week dosing regimen; SP, sulfadoxine-pyrimethamine.

Table 5.2 Pharmacokinetic and pharmacodynamic data

Data	Gestational weeks	DHA-PQ n	DHA-PQ Value
Pharmacokinetic			
PQ concentration (ng/mL) [mean (95% percentile)]			
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)
Pharmacodynamic		n*/Total	
Parasite density (parasite/μL) [mean (95% percentile)]			
Pre-study drug	-	579/710	4797 (0.03-29019)
On prevention			
	20	110/225^	1863 (0.01-1591)
	24	81/355	591 (0.01-2147)
	28	56/350	175 (0.01-335)
	32	50/342	421 (0.01-3124)
	36	52/335	77 (0.01-737)
	40	19/139^	2061 (0.01-21529)

^The sample size for week 20 is lower than subsequent weeks because it only includes women who began prevention at 16 weeks gestation. Similarly, the sample size for week 40 is reduced because it only includes women who had not yet given birth.

*Parasite densities are reported as qPCR values. These values reflect the mean parasitemia for women who were parasitemic only. Women who were parasite negative were removed from this calculation. The n value indicates how many women were positive relative to the total number of samples measured for each week.

Abbreviations: DHA-PQ, dihydroartemisinin-piperaquine

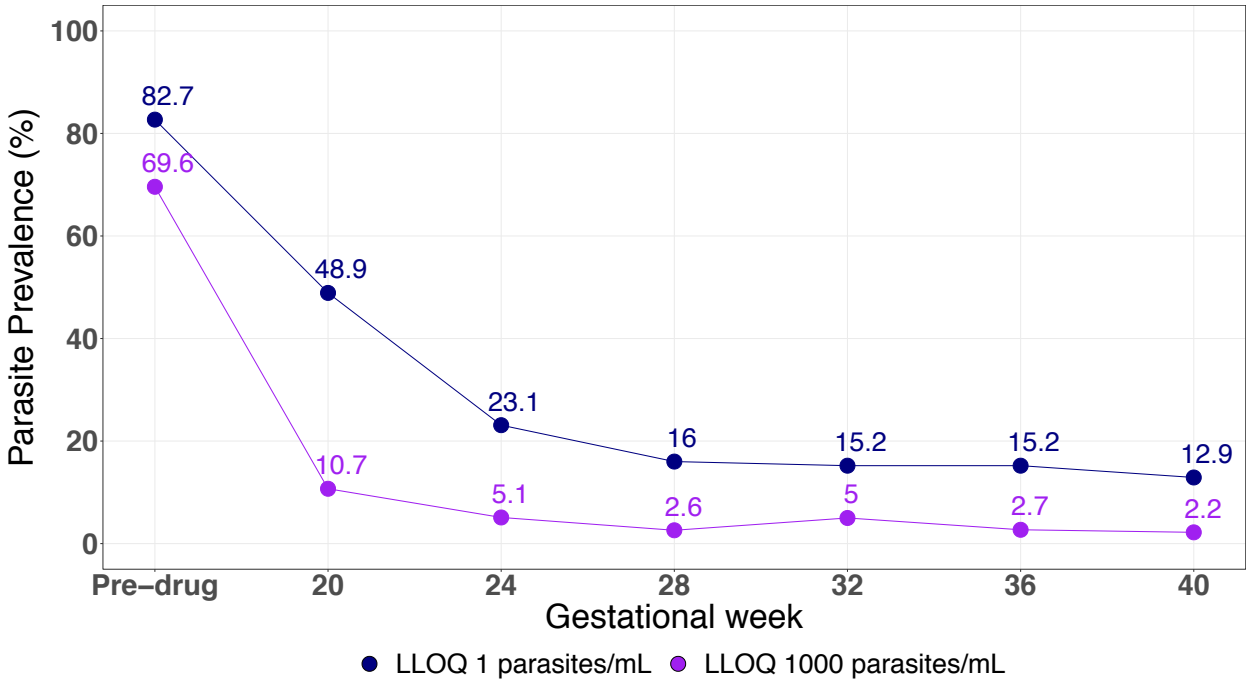

Figure 5.2 Percentage of women positive for parasitemia. Parasite prevalence in women who received DHA-PQ over pregnancy. The blue values were calculated using the qPCR assay's LLOQ of 1 parasite/mL and the purple values using the LAMP assay's LLOQ of 1000 parasites/mL. DHA-PQ, dihydroartemisinin-piperaquine; LAMP, Loop-mediated isothermal amplification, LLOQ, lower-limit of quantification, qPCR, quantitative polymerase chain reaction.

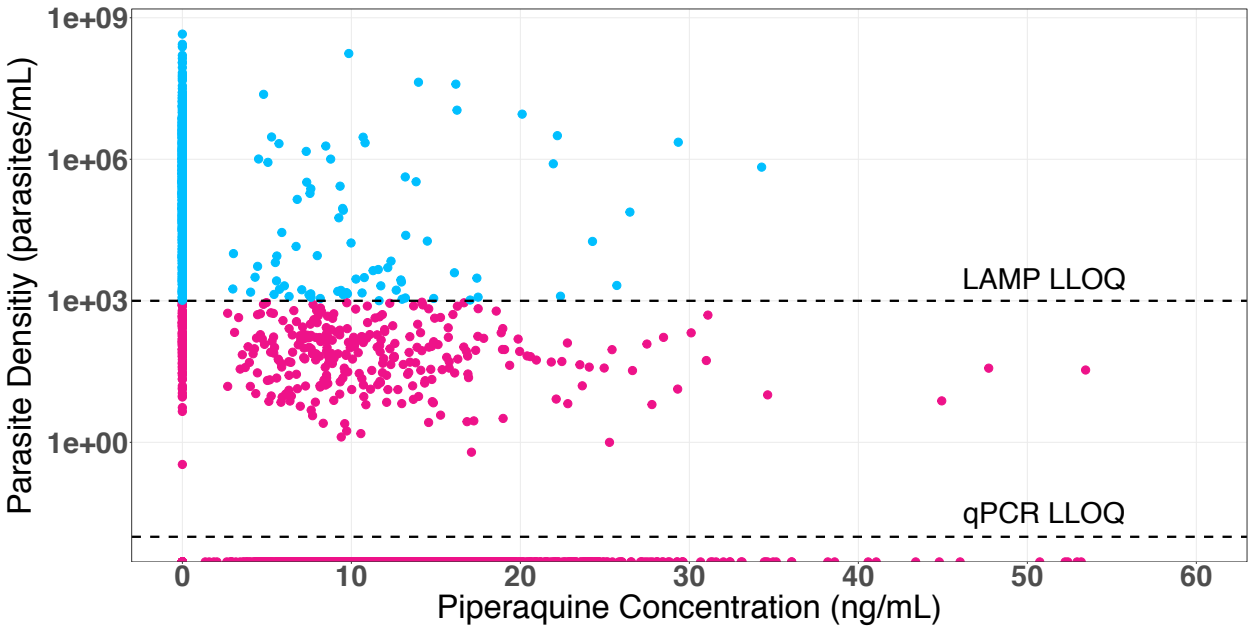

Figure 5.3 Observed parasite density over simulated piperaquine concertation. The model simulated piperaquine concentrations are displayed because not all women had paired PK and parasite density samples measured. Blue points represent those which both qPCR and LAMP would detect as positive whereas the pink dots between dashed lines represent those only qPCR would detect as positive. The dashed lines indicate the LLOQ of both assays. DHA-PQ, dihydroartemisinin-piperaquine; LAMP, Loop-mediated isothermal amplification, LLOQ, lower-limit of quantification, qPCR, quantitative polymerase chain reaction.

Population PK model

The results of the PK model have been reported previously.[20] Briefly, the 2-compartment model provided an adequate fit of the PK data. Model parameters were fixed to their respective values from the prior analysis to improve model stability, [17] but additive and proportional errors, bioavailability, and inter-occasion variability on clearance were re-estimated (**Table 5.3**). No covariates were identified from the SCM analysis. A VPC of the final model demonstrated satisfactory predictive performance (**Figure 5.4**).

Table 5.3 Final PK model parameter estimates

	DHA-PQ, Median (95% CI)	
Parameter estimates	Population estimate	Interindividual variability/ Interoccasion 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)	33584[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)	-

All parameters estimated are reported as the oral parameter estimates (ie, CL/F; V/F, etc).
[a]Parameter value fixed
[b]Inter-occasion variability
Abbreviations: CI, confidence interval; DHA-PQ, dihydroartemisinin-piperaquine

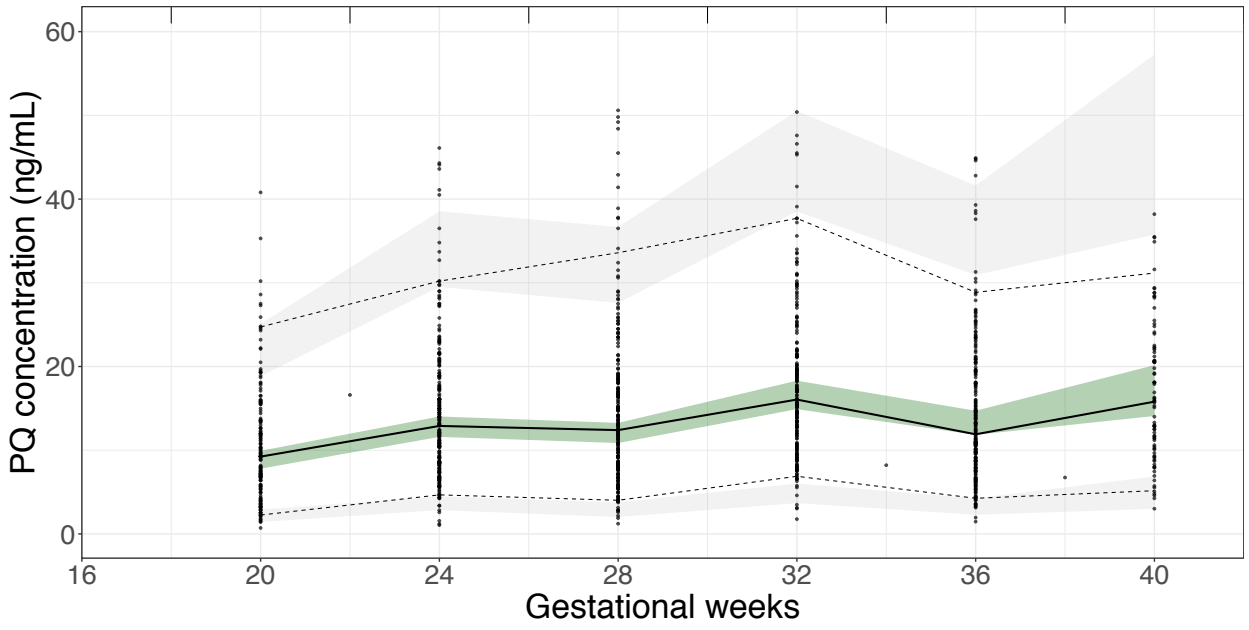

Figure 5.4 Visual predictive check (VPC) of the final DHA-PQ PK model. The black circles represent the observed data. The solid line indicates the median of the observed data, and the dashed lines indicate the 5% and 95% confidence intervals of the observed data. The shaded areas indicate 5%, 50%, and 95% of the simulated data. 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.

Population PK/PD model

An Emax relationship best described the piperaquine effect on parasitemia using estimated piperaquine concentrations from the final PK model (**Table 5.4**). The instantaneous piperaquine concentration was a better predictor compared to AUC and C_{max}. The SCM analysis identified primigravida as a significant covariate. This relationship revealed that primigravida (women who are pregnant for the first time) women have a higher probability of being parasite positive compared to multigravida women (**Table 5.4**). Parameter estimates indicated that PQ is more effective using the

LAMP LLOQ compared to qPCR. A VPC of the final models demonstrated satisfactory predictive performance (**Figure 5.5**).

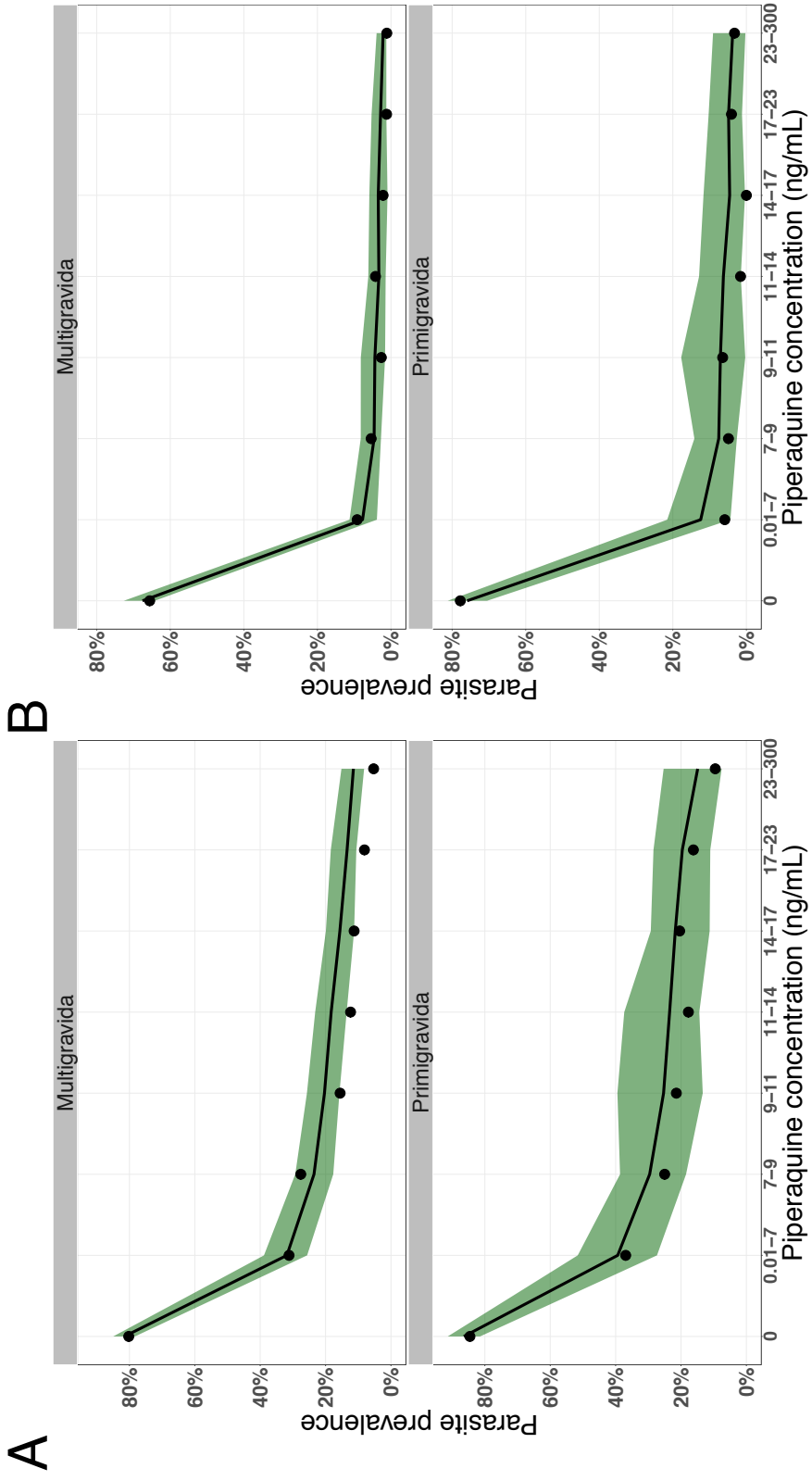

Figure 5.5 Visual predictive check (VPC) of the final qPCR DHA-PQ PK/PD model stratified by gravidity. Panel A represents the model using an LLOQ of 1 parasite/mL and panel B represents the model using an LLOQ of 1,000 parasite/mL. The black circles represent the observed data over the specified concentration bins. The solid line indicates the median of the observed data. The shaded areas indicate 5% and 95% confidence intervals of the simulated data. Multigravida women are those who have had at least one previous pregnancy and primigravida women are those whose current pregnancy is their first. DHA-PQ, dihydroartemisinin-piperaquine; PKPD, pharmacokinetic/pharmacodynamic; LLOQ, lower-limit of quantification.

Simulations were run using the final PK/PD models to identify the target piperaquine concentration protective against parasitemia (**Figure 5.6**). When qPCR negativity is the efficacy measure, our model indicated that 10.3 ng/mL is 76 and 82.5% effective and that 13.9 ng/mL is 80 and 86% effective for the typical primigravida and multigravida women, respectively. However, when using the upper bound of the 95% confidence interval, 10.3 ng/mL is 70% and 80% protective and 13.9 ng/mL is 74.5% and 83.5% effective for primigravida and multigravida women, respectively. Ninety five percent of observed trough concentrations in the trial were <50 ng/mL and over this range PQ is not predicted to be 95% efficacious. In contrast, when LAMP negativity is the efficacy measure, when using the upper bound of the confidence interval, 10.3 ng/mL was 95% protective in primigravida and 97% protective in multigravida women. A lower concentration of 6.5 ng/mL was 95% protective in multigravida women. The cut off of 13.9 ng/mL was 96% and 97.6% protective for primigravida and multigravida women.

Table 5.4 Final PD model parameter estimates

	DHA-PQ, Median (95% CI)	
Parameter estimates	Population estimate	Interindividual variability
Piperaquine pharmacodynamic parameters LLOQ 1 parasite/mL		
Baseline	1.68 (1.44-1.94)	78% (31-101)[c]
Emax	4.45 (3.87-5.39)	
EC50	3.86 (2.37-6.2)	
Primigravida covariate	1.25 (1.06-1.47)	
LLOQ 1,000 parasite/mL		
Baseline	1.03 (0.73-1.37)	118% (123-332)[c]
Emax	5.9 (5.12-7.62)	
EC50	2.07 (0.60-4.19)	
Primigravida covariate	1.56 (1.11-2.18)	

All parameters are reported on the logit scale.
[a]Parameter value fixed
[b]Inter-occasion variability
[c]Varability was put on the logit function
Abbreviations: CI, confidence interval; DHA-PQ, dihydroartemisinin-piperaquine

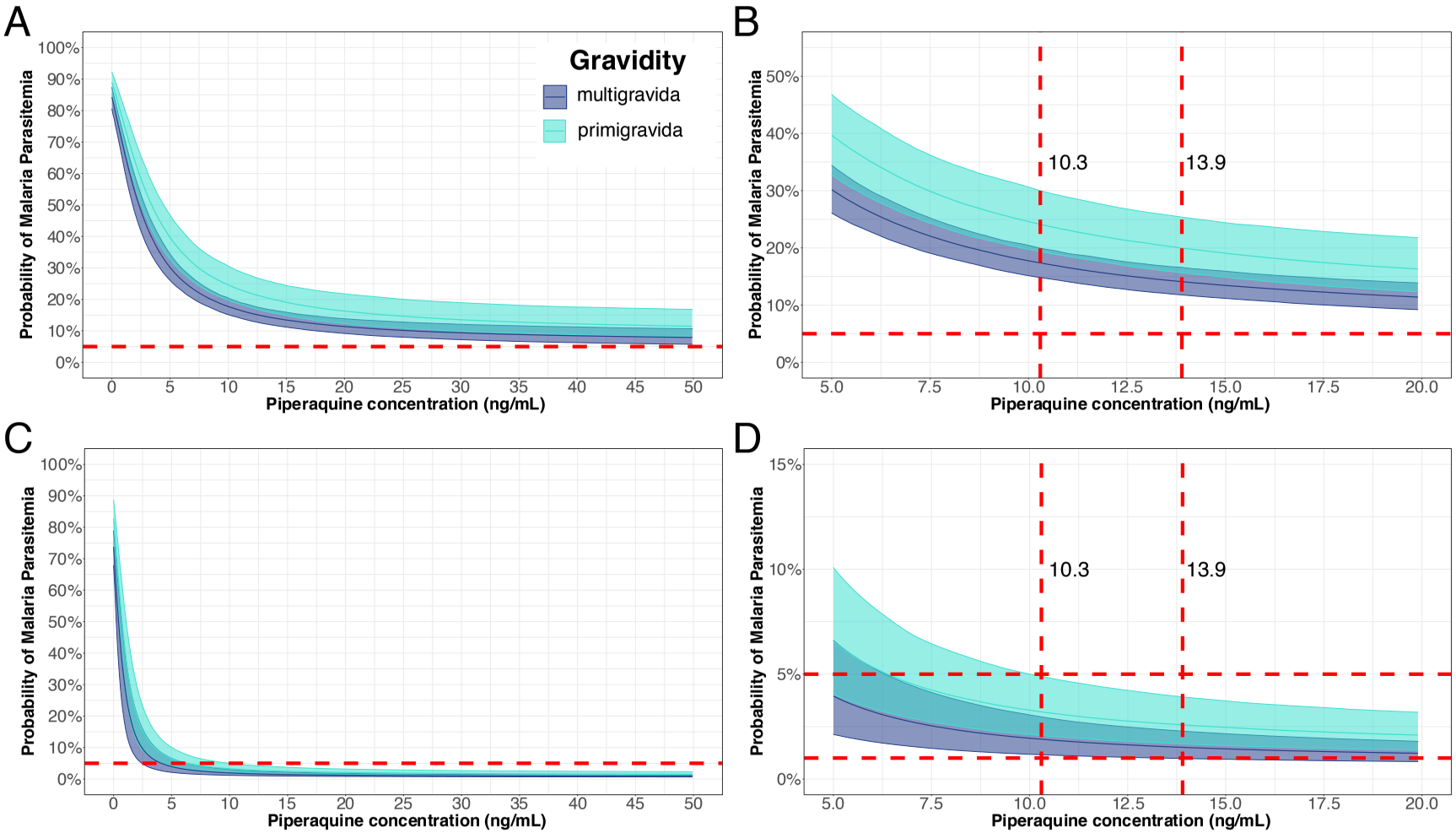
A
B
C
D
Gravidity
multigravida
primigravida
Probability of Malaria Parasitemia
Piperaquine concentration (ng/mL)
10.3
13.9

Figure 5.6 Simulations of the final DHA-PQ PK/PD models stratified by gravidity. Panels A and B represent the model using an LLOQ of 1 parasite/mL and panels C and D represent the model using an LLOQ of 1,000 parasite/mL. Simulations used monthly dosing from 16 to 40 weeks gestation. The dashed horizontal line marks a 5% probability of being positive for parasites. The dashed vertical lines in panels B and D at 10.3 and 13.9 ng/mL mark the previously defined 95% and 99% thresholds for malaria protection in pregnant women. The shaded areas indicate 5% and 95% confidence intervals of the simulated data. Multigravida women are those who have had at least one previous pregnancy and primigravida women are those whose current pregnancy is their first. DHA-PQ, dihydroartemisinin-piperaquine; PKPD, pharmacokinetic /pharmacodynamic; LLOQ, lower-limit of quantification.

Simulations

While 10.3 and 13.9 ng/mL piperaquine are not predicted to protect 95% of pregnant women against qPCR negativity they were predicted to protect 95% of the population from LAMP positive parasitemia. Additionally, due to the nature of the Emax relationship, higher piperaquine concentrations were not predicted to significantly decrease the probability of qPCR parasitemia. Therefore, we explored different dosing regimens with the goal of finding one where women maintain 10.3 ng/mL throughout pregnancy (**Figure 5.7**). The current trial regimen of monthly prevention had the lowest piperaquine exposure and only 51% of women maintain protective concentrations. Additionally, this regimen had the slowest monthly increase in women protected. Adding a fourth day of dosing to the standard monthly regimen led to over 75% of women being protected and importantly most women achieved protective concentrations after the first dose of DHA-PQ. These results were very similar to weekly dosing; however, women took longer to achieve protective concentrations with weekly therapy. One tablet daily lead to all women maintaining 10.3 ng/mL after 1 week on prevention.

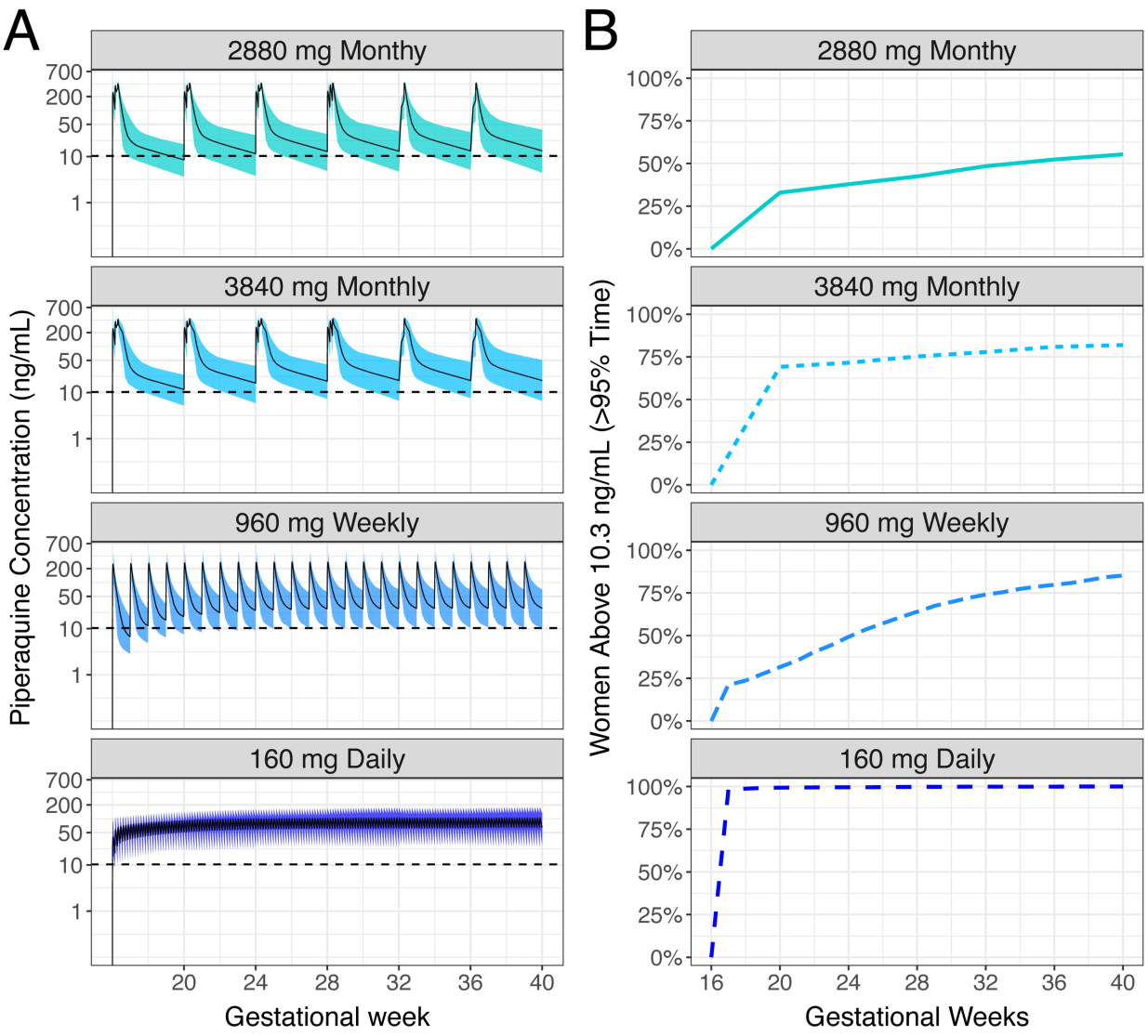

Figure 5.7 Alternative IPTp regimen simulations. (A) Full PK profiles. Simulated PQ concentrations over pregnancy for four different dosing regimens. The dashed line at 10.3 ng/mL marks the previously defined threshold for malaria protection. (B) Percentage of women protected. Protection was defined as sustaining a PQ concentration of 10.3 ng/mL or greater for 95% of their pregnancy. DHA-PQ, dihydroartemisinin-piperaquine; PKPD, pharmacokinetic/pharmacodynamic; LLOQ, lower-limit of quantification.

Discussion

Multiple clinical trials have found that IPTp with DHA-PQ is more efficacious at preventing malaria and parasitemia compared with SP.[14-16] Interestingly, this added protection has not led to improved birth outcomes with DHA-PQ over SP. A recent analysis found that sub-microscopic parasitemia can still lead to placental malaria which is an important risk factor for birth outcomes.[22] As detection measures for parasitemia improve, IPTp dosing regimens should be re-evaluated to ensure they can eliminate these low levels. This PK/PD analysis found that 15% of women remained qPCR positive even at high (>30 ng/mL) piperaquine trough concentrations. When low level parasitemia (<1,000 parasites/mL) was reclassified as being negative, the previously defined target concentration of 10.3 ng/mL was 95% protective. Simulations suggest that additional doses could improve efficacy. These changes are particularly important for primigravida women as they had a higher probability of remaining positive for parasites.

One previous study has defined 10.3 ng/mL as the target 95% protective concentration in the context of malaria prevention in pregnant women.[17] When the current data was modified to match the sensitively of the LAMP assay used in the previous study, we found this target concentration protected 95% of women as well. Additionally, we found that a lower concentration of 6.5 ng/mL was protective in multigravida women. The previous study did not include gravidity in their model. It is likely, that we were able to detect this relationship given the larger trial size. Nonetheless, these findings support that 10.3 ng/mL is an appropriate target concentration for LAMP negativity in pregnant women.

When all samples with detectable levels of parasites by microscopy or qPCR were considered as positive (≥1 parasite/mL blood), 10.3 ng/mL was only 70-80% protective. Although parasite densities as low as 1,000 parasites/mL are confirmed to increase a women's probability of placental malaria, an important risk for adverse birth outcomes,[22] it is unclear at what point densities below 1,000 parasites/mL remain harmful. Additionally, while the qPCR assay used to detect parasites is specific to *P.falciparum* DNA, it does not distinguish between live and dead or asexual and sexual stage parasites.[19] Unlike asexual parasites, gametocytes, the sexual-stage, are not known to cause adverse birth outcomes.[23] DHA-PQ has minimal efficacy against gametocytes which could explain why women remained qPCR positive despite having PQ concentrations predicted to confer protection.[24] Therefore, qPCR negativity may not be an appropriate efficacy target. Studies which can inform on what levels of asexual parasitemia lead to adverse outcomes are needed as these will help refine prevention dosing regimens.

Our model found that primigravida women (women who are pregnant for the first time) have a higher probability of parasitemia compared to multigravida women. This difference may reflect primigravida women's reduced immunity to malaria. [2-4] Infected red blood cells express different antigens during pregnancy compared to those in the non-pregnant population and the immunity a woman has built through childhood does not fully protect her during pregnancy. Successive exposures to malaria during pregnancy establish new immunity which is why primigravida women likely have a higher risk of parasitemia.

Higher piperaquine concentrations are predicted to improve efficacy regardless of the density of infection. Additionally, attaining protective piperaquine concentrations as early as possible is important because it reduces the risk of parasitemia. Also, the risk of adverse birth outcomes such as miscarriage due to malaria are higher early in pregnancy.[25,26] Therefore, dosing regimens were evaluated by how many women were protected and how quickly. Monthly dosing protected the least number of women and importantly led to the most gradual increase in women maintaining protective concentrations (**Figure 5.6**). A single daily tablet resulted in all women maintaining concentrations of 10.3 ng/mL or greater for at least 95% of the time on prevention after one week of dosing. Due to the frequency of dosing, this regimen is more forgiving when a dose is missed. However, daily dosing may not be pragmatic. A weekly dose of 960 mg is one alternative as this increased the number of women protected. Similar to monthly dosing weekly regimens may not protect a sufficient number of women fast enough. To minimize the time it takes for women to achieve protective concentrations, one alternative is to extend monthly dosing over four days instead of three. After one prevention course, over 50% of women maintain 10.3 ng/mL for at least 95% of the time on prevention. This option is appealing as it involves minimal adjustments. These alternative regimens are particularly important for primigravida women as they are at an increased risk of parasitemia.

Measuring parasite densities using qPCR can detect very low levels (1 parasite/mL) of parasitemia.[19] A limitation of this study is that qPCR does not distinguish live and dead or asexual and sexual stage parasites. This could lead to over predicting the number of women positive for parasites and therefore the trough

concentration needed to afford protection. This study collected monthly samples which should reduce the number of women considered positive due to dead parasites. However, gametocytes have been detected a month post malaria treatment in non-pregnant adults.[27,28] Gametocytes do not sequester in the placenta and have not been associated with adverse pregnancy outcomes.[23,29] While sub microscopic infections can still result in placental malaria and therefore need to be prevented, the lowest density which we must prevent remains unclear. qPCR negativity may underestimate DHA-PQ's efficacy by measuring gametocytes and dead parasites, again resulting in higher piperaquine concentrations being recommended. Further studies are needed to establish a target asexual parasite density or range to improve dosing recommendations. Lastly, the current study collected monthly piperaquine and parasite density levels. Because piperaquine is highly effective even after the first cycle of prevention, most samples occurred when piperaquine was at or close to its maximum effect. In fact, 80% of qPCR samples were parasite negative. To better define the full PK/PD relationship, more frequent paired samples should be collected after the first prevention dose in future trials.

In conclusion, 10.3 ng/mL was 95% protective against parasitemia for infections greater than 1,000 parasites/mL but only 70 and 80% protective against qPCR negativity for primigravida and multigravida women, respectively. Simulations indicated that extending dosing or increasing the dosing frequency may improve piperaquine exposure and improve efficacy. Clinical trials exploring alternative DHA-PQ regimens are needed especially in primigravida women, given their increased risk, to confirm our recommendations for IPTp.

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Chapter 6. Conclusions

The research presented in this dissertation improves our understanding of malaria treatment and prevention in pregnant women. Quantitative models were applied to identify patient characteristics which reduced drug exposure, investigated safety concerns for longitudinal dosing and evaluated preventative efficacy using advanced parasite detection methods. This work primarily focused on malaria prevention, where the models discussed below can inform both policy and the design of future clinical trials.

HIV-infected pregnant women are a known high-risk population for malaria infection.[1-3] In addition to the potential effects of HIV disease itself, antiretrovirals may alter antimalaria drug exposure.[4-7] However, few clinical studies have enrolled this patient population and even less have collected PK/PD data to establish any effects HIV/ARTs have on malaria outcomes. Therefore, the current malaria treatment and prevention guidelines do not include specific dosing recommendations for HIV-infected pregnant women. In Chapters 2 and 3, the drug-drug interaction between efavirenz, an antiretroviral, and two different antimalarial combinations (artemether-lumefantrine and dihydroartemisinin-piperaquine) were described in HIV-infected pregnant Ugandan women. These analyses found that efavirenz reduced dihydroartemisinin, lumefantrine and piperaquine exposure compared to HIV-uninfected pregnant women. Of particular concern was the reduction in lumefantrine and piperaquine concentrations because day 7 levels for these drugs are predictive of therapeutic efficacy suggesting that current regimens may under-dose these women.[8,9] Piperaquine dosing simulations found that

one tab daily would achieve protective concentrations in the greatest number of women. Daily dosing may not be pragmatic in which case a weekly course is an appealing alternative. A limitation of both studies was that few women had recrudesce or new infections which prevented us from including outcomes in the analyses. This work underscores the need for larger trials powered to detect differences in parasite prevenance and rate of new infections to define the true PK/PD relationship for this population. While our findings indicates that current treatment and prevention guidelines may not adequately protect HIV-infected pregnant women receiving efavirenz, without dedicated PK/PD studies, it is likely that the dosing guidelines will remain unchanged leaving this population at risk.

In Chapter 3 we continued to explore piperaquine exposure in HIV-uninfected pregnant women for malaria prevention. Our analysis found that pregnancy and lower BMI both increased piperaquine clearance compared to postpartum women and women with higher BMIs, respectively. These findings have important policy implications as they suggest that flat dosing may result in better piperaquine exposure for women with low BMIs compared to the current weight-based regimens. Furthermore, this work highlights that (i) future studies should include a large and diverse patient population to continue identifying populations at risk for subtherapeutic drug exposure, (ii) more frequent PK sampling is needed to confirm the effects of BMI on drug clearance and (iii) because weight-based measurements of nutrition are susceptible to bias during pregnancy, studies should record the mid-upper arm circumference. In addition to providing recommendations, the model built in this analysis can be used to design these future clinical studies.

Currently, the WHO does not recommend using dihydroartemisinin-piperaquine for malaria prevention during pregnancy.[10] In 2015, the WHO Malaria Policy Advisory Committee evaluated DHA-PQ for use as prevention and highlighted that more research is needed to establish the safety of repeated DHA-PQ doses before changes can be made to prevention policy.[11] In Chapter 4, we address these safety concerns by describing the relationship between PQ concentration and QTc prolongation over the second and third trimesters in pregnancy. In contrast to our hypothesis that PQ concentrations would increase leading to greater QTc prolongation, we found that the concentration-prolongation slope decreased after repeated doses. However, the underlying cause of this decrease could not be identified and requires further investigation. Two likely causes for this trend are that pregnancy reduced PQ's C_{max} or that changes in hormones independently decreased the QTc interval. [5,12-14] Future clinical studies can help clarify our findings by collecting an additional blood sample to measure PQ and/or select hormones at the time of ECG measurement. Despite this uncertainly, these findings indicated that repeated DHA-PQ doses do not increase the risk of QTc prolongation and can safety be used for longitudinal prevention.

In Chapter 5, we evaluated piperaquine's protective efficacy in pregnant women using qPCR parasite densities. Given the added sensitivity, the previously proposed protective concentrations of 10.3 ng/mL and 13.9 ng/mL were less effective at preventing qPCR detectable parasitemia.[15] A sensitively analysis indicated that after reclassifying parasite densities below 1,000 parasites/mL as parasite negative, 10.3 ng/mL was 95% and 97% effective for primigravida and multigravida women,

respectively. While qPCR's added sensitively to detect low levels of parasitemia is valuable, it also raises the question of whether very low parasite densities (<10 parasites/mL) still increase a pregnant women's risk of adverse outcomes as this will affect the dosing regimen. Our analysis identified that primigravida women are at an increased risk for parasitemia and future clinical trials should focus on this population. Simulations suggested that more frequent dosing could lead to improved efficacy and should be explored in future clinical trials, particularly for primigravida women.

In summary, we used quantitative, model-based approaches to identify and quantify the effects of patient characteristics on drug exposure and to evaluate PQ's efficacy and longitudinal safety in pregnant women. As discussed in Chapter 1, the malaria field is on the forefront of using PK/PD models to inform policy guidelines. However, in the case of malaria prevention in pregnant women, more PK/PD studies need to be conducted to further inform policy. The presented work contributed both models and recommendations to inform the next generation of clinical trials. Together with further research we hope this work can inform policy recommendations that will help protect pregnant women against malaria.

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