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Reduced exposure to piperaquine, compared to adults, in young children receiving dihydroartemisinin-piperaquine as malaria chemoprevention

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Abstract

Dihydroartemisinin (DHA)-piperaquine is being evaluated as intermittent preventive therapy for malaria, but dosing has not been optimized for children. We assessed exposure to DHA and piperaquine in Ugandan children at two ages during infancy. Intensive sampling was performed in 32 children at 32 weeks of age, 31 children at 104 weeks, and 30 female adult controls. Compared to adults, DHA area under the concentration time curve ($AUC_{0–8hr}$) was 52% higher at 32 weeks and comparable at 104 weeks. Compared to adults, piperaquine $AUC_{0–21d}$ was 35% lower at 32 weeks and 53% lower at 104 weeks. Terminal piperaquine concentrations on Days 7, 14, and 21 were lower in children compared to adults and lower at 104 compared to 32 weeks. Piperaquine exposure was lower in young children compared to adults, and lower at 104 compared to 32 weeks of age, suggesting a need for age-based DHA-piperaquine dose optimization for chemoprevention.

Keywords

malaria; pharmacokinetics; pediatric; prevention; anti-infective; global health

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AUTHOR CONTRIBUTIONS

M.E.W., P.J.R., and F.T.A. wrote the article; M.K., P.J.R., N.M., and F.T.A. designed the research; M.E.W., R.K., N.C., L.H., F.O., G.D., and N.M. performed the research; M.E.W., N.C., L.H., E.W., P.J., and F.T.A. analyzed the data; and L.H. contributed new reagents/analytical tools.

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INTRODUCTION

Despite the worldwide effort to decrease the incidence of malaria, in 2017 there were an estimated 219 million new cases of malaria and 435,000 deaths, with 93% of these deaths occurring in Africa, primarily due to *Plasmodium falciparum* (1). Children under five years of age are the most vulnerable population, accounting for an estimated 61% of deaths in 2017 (1). Pregnant women are another vulnerable population, with up to 41% of women exhibiting evidence of placental malaria in parts of Africa (2). Placental malaria increases risks for low birth weight and infant mortality (3).

To reduce the burden of malaria, intermittent preventive treatment (IPT), with standard treatment doses of antimalarials administered at regular intervals, has been endorsed for high risk groups. IPT with monthly sulfadoxine-pyrimethamine (SP) is the standard of care in malaria-endemic regions for pregnant women during the second and third trimester, although drug resistance limits the antimalarial efficacy of SP (4). Seasonal malaria chemoprevention, with SP/amodiaquine administered monthly during the transmission season, is endorsed for use in children in regions of West and Central Africa with highly seasonal malaria and relatively low levels of drug resistance (5). However, there is no specific recommendation for chemoprevention in children in most of Africa, where malaria transmission occurs year-round and drug resistance limits the efficacy of SP/amodiaquine. Our group and others are evaluating IPT with dihydroartemisinin (DHA)-piperaquine, an artemisinin-based combination therapy that is well suited for chemoprevention due to the long (~3 weeks) half-life of piperaquine (6, 7). Recent trials showed DHA-piperaquine offered significant reductions in risks of maternal and placental malaria compared to IPT with SP (8, 9). Similarly, in young children (10) and schoolchildren (11) monthly DHA-piperaquine offered potent preventive efficacy.

Despite data demonstrating DHA-piperaquine efficacy for IPT, there is an absence of pharmacokinetic (PK) and pharmacodynamic (PD) information for target populations. In particular, optimized IPT dosing strategies for children are lacking. DHA, which is short-acting, is metabolized by UDP-glucuronyltranferase (UGT)(12). Piperaquine is metabolized by cytochrome p450 (CYP) 3A4/2C8 (13). Physiological changes can alter DHA-piperaquine metabolism and pharmacokinetics. We previously reported that pregnant women exhibit a reduction in exposure to both DHA and piperaquine compared to non-pregnant controls (14), attributable to induction of both UGT and CYP metabolism. These data support refined DHA-piperaquine dosing recommendations for pregnant women (15).

In children, metabolism is also distinct from that in adults, in that maturation of CYP3A4/2C8 and UGT occurs gradually during the first year (16, 17) and first 6 months (18) of life, respectively. Specifically, CYP3A4 levels only approach about 50% of adult levels at 6 months of age (19), but then mature rapidly (20). Owing to these distinctions, we hypothesized that young children exhibit significant differences in DHA-piperaquine disposition compared to adults.

In the context of treatment, limited studies have evaluated the PK of DHA-piperaquine in young children, mostly with a sparse sampling design; it is uncertain if these results,

obtained from ill children, are representative of those in healthy children receiving IPT (21). Therefore, to inform IPT dosing guidelines for young children, we carried out intensive PK studies of DHA-piperaquine administered as IPT at two time points during the first two years of life in Ugandan children.

RESULTS

Study profile

Screening and enrollment of study participants is summarized in Figure 1, and baseline characteristics of study subjects are in Table 1. Adult female controls underwent intensive PK assessments at a median time of 39 weeks post-partum.

Pharmacokinetics of DHA

Comparison of infants and adults—Drug exposure in 32 infants at 32 weeks of age and 31 infants at 104 weeks of age was compared to that in adult controls (Table 2; Figure 2a). Compared to adults, DHA exposure was higher in the 32-week-old infants, with the $AUC_{0–8hr}$ and C_{max} 32% (p=0.016) and 52% (p<0.0001) higher, respectively. Likewise, the DHA C_{8hr} terminal concentration was 97% higher at 32 weeks compared to that in adults (p=0.0001). In contrast, DHA half-life was not significantly different at 32 weeks compared to that in adults. No significant difference in DHA exposure was found between infants at 104 weeks of age and adults for C_{max}, $AUC_{0–8hr}$, half-life or C_{8hr} terminal concentration.

Comparison of infants at 32 and 104 weeks of age—To evaluate the impact of childhood development on DHA pharmacokinetics, data from 32 infants aged 32 weeks and 31 of the same infants aged 104 weeks were compared (Table 2; Figure 2a). By unpaired analysis (permitting a comparison using all of the results), DHA exposure was lower in infants at 104 weeks of age compared to that at 32 weeks of age, with the $AUC_{0–8hr}$ and C_{8hr} terminal concentration lower by 34% (p=0.0011) and 53%, respectively. By paired analysis (comparing the same 31 infants studied at 32 weeks and 104 weeks of age), the impact of age on each PK parameter was nearly identical to that estimated by unpaired analysis (Table 2). When comparing infants receiving DHA-piperaquine every 4 weeks to those receiving treatment every 12 weeks, there was no difference in PK parameters at either 32 or 104 weeks of age (data not shown).

Pharmacokinetics of piperaquine

Capillary and venous concentration correlation—The median capillary plasma piperaquine concentration 24 hours post-3rd dose was 54.5 (range 17.7, 188) ng/mL; the corresponding venous concentration was 58.9 (16.2, 250) ng/mL. For 30 adult controls, the median capillary and venous plasma piperaquine concentrations 24 hours post-3rd dose were 115 (26.4, 292) ng/mL and 104 (24.8, 251) ng/mL, respectively. The correlation equation for simultaneous venous and capillary plasma measurements 24 hours post-3rd dose for infants was $lnC_{cap}=0.773*lnC_{venous}+0.895$ (n=63), $r^2=0.614$, and for adults was $lnC_{cap}=0.975*lnC_{venous}+0.320$ (n=30), $r^2=0.877$.

Comparison of infants and adults—Drug exposure for piperaquine was compared in the same infants studied for DHA (Table 3; Figure 2b). Compared to adults, piperaquine exposure was lower in the 32-week-old infants, with the C_{max} and $AUC_{0–21d}$ 31% (p=0.0034) and 35% (p=0.0002) lower, respectively. Likewise, terminal concentrations of piperaquine on days 7, 14, and 21 were 35% to 42% lower in infants at 32 weeks compared to adults (p=0.0002 for each). Piperaquine exposure was lower still in infants at 104-weeks of age, with the C_{max} and $AUC_{0–21d}$ 38% (p=0.0002) and 53% (p<0.0001) lower than in adults, respectively. Notably, terminal concentrations of piperaquine on days 7, 14, and 21 were 51% to 60% lower in infants at 104 weeks of age compared to those in adults (p<0.0001). As dose varied in children, we utilized weight normalized Dose/AUC (uncorrected for baseline concentration) to estimate changes in drug clearance and found geometric mean values (95% CI) of 1.87 (1.62, 2.16), 2.13 (1.91, 2.38) and 1.39 (1.20, 1.60) L/hr/kg for children at 32 weeks, children at 104 weeks, and adult controls, respectively (p<0.005 comparing 32 and 104 weeks to adults).

Comparison of infants at 32 and 104 weeks of age—To evaluate the impact of childhood development on piperaquine pharmacokinetics, data from 32 infants aged 32 weeks and 31 infants aged 104 weeks were compared (Table 3; Figure 3a). Based on unpaired analysis, piperaquine exposure was lower in infants at 104 weeks of age compared to 32 weeks of age, with $AUC_{0–21d}$ lower by 28% (p=0.003). Terminal concentrations of piperaquine on days 7, 14, and 21 were also significantly lower (25% to 35%) in infants at 104 weeks compared to 32 weeks (Table 3). In contrast, the C_{max} and half-life were not different between infants at 32 and 104 weeks of age. By paired analysis (comparing the same 31 infants studied at 32 weeks and 104 weeks of age), the impact of age on each PK parameter was nearly identical to that estimated by unpaired analysis (Table 3). When comparing infants receiving DHA-piperaquine every 4 weeks to those receiving treatment every 12 weeks, PK parameters were unchanged at both 32 and 104 weeks, except for a 33% lower terminal concentration on day 14 in infants receiving the drug every 12 weeks (at the time of the 32-week evaluation, p=0.012, data not shown). Weight normalized Dose/AUC comparing ages 32 and 104 weeks yielded geometric mean values (95% CI) of 1.87 (1.62, 2.16) and 2.13 (1.91, 2.38) L/hr/kg for the two groups (p=0.20)

Correlation of piperaquine terminal concentrations and AUC—Measurements of piperaquine at 7-, 14-, or 21-days post-treatment may be used to monitor piperaquine concentrations during clinical studies, since intensive PK sampling is often not practical. Therefore, we sought to determine if terminal concentrations were predictive of overall piperaquine exposure. Piperaquine day 7, 14, and 21 concentrations were highly correlated with $AUC_{0–21d}$ (Pearson r = 0.698 (p<0.0001), 0.582 (P<0.0001), and 0.366 (p=0.003) at 7, 14, and 21 days, respectively).

ECG findings

No adverse events suggestive of cardiotoxicity were observed for infants or adults. Using Bazett's formula, at 32 weeks of age, the QTcB was ≤450 msec for 97% (29/30; range 375–472 msec) of infants pre-treatment, and 73% (22/30; range 386–482 msec) post-treatment. At 104 weeks, all pre- and post-treatment QTcB intervals were ≤450 msec (Figure 3 and

Table S1). Bazett's formula was shown to be less sensitive to heart rate changes than Fridericia's formula (Figure S1). However, results were also analyzed by Fridericia's formula where all pre-treatment and post-treatment QTcF values were ≤450 msec. There were no significant differences in pre- or post-treatment QTcB or QTcF between 32 and 104 weeks of age.

There were mean 21 msec and 14 msec increases in QTcB interval between ECGs pre-dose and 3–4 h following the third dose at 32 weeks ($p<0.001$) and 104 weeks of age($p=0.004$), respectively. The difference between changes in QTcB at 32 and 104 weeks of age was not statistically significant ($p=0.28$), and both changes were similar to that observed in adults (17 msec; $p=0.67$ and $p=0.52$, respectively). Similar differences were seen using the Fridericia's correction. There was no correlation between piperaquine C_{max} or $AUC_{0–21d}$ and QTcB (Figure 3).

DISCUSSION

DHA-piperaquine is a promising regimen for IPT, but optimal dosing in young children is uncertain. We performed an intensive PK analysis of both components of this regimen in infants at 32 and 104 weeks of age receiving DHA-piperaquine as IPT. Compared to adults, infants had either higher or comparable exposure to DHA, but lower exposure to piperaquine, with the difference more pronounced at 104 compared to 32 weeks of age. Since the protective benefits of IPT are largely attributed to piperaquine exposure, these results suggest that young children, especially those about 2 years of age, may have been underdosed in prior IPT studies, leading to inadequate protection.

Our results for DHA-piperaquine exposure in infancy are comparable to prior reports, although most prior studies were in the context of treatment for uncomplicated malaria rather than IPT in healthy children (21). Specifically, in Ugandan children less than 2 years of age treated for malaria with DHA-piperaquine, exposure to piperaquine was ~33% lower compared to that in children 2–10 years of age (22–25). A pooled analysis reported that children less than 5 years of age were at the highest risk of receiving suboptimal DHA-piperaquine doses and at the greatest risk for reinfection after therapy (26). These results informed new guidelines for treatment, but our study evaluated dosing based on older guidelines (27).

The impact of childhood development on drug metabolism has been documented for many drugs (17, 28, 29). Phase I metabolism, including CYP3A4 and CYP2C8 metabolism of piperaquine, is characterized by structural changes of the drug via oxidation, reduction, or hydrolysis. Phase II metabolism, including UGT metabolism of DHA, involves conjugation with a more water-soluble moiety via glucuronidation, acetylation, or sulfation. For DHA, infants had ~30% greater $AUC_{0–8hr}$ and ~50% greater peak concentration compared to adults, at 32 weeks of age, but by 104 weeks of age $AUC_{0–8hr}$ and peak concentrations were similar to those in adults, suggesting a maturation of metabolism over time. DHA is metabolized by UGT1A9 and UGT2B7 (12), with UGTs present in both the gastrointestinal tract and liver (30, 31); thus, metabolism may impact both bioavailability and elimination. In the neonate, glucuronidation is deficient (32), and these conjugative enzymes mature over

the first 6 months of age (16, 18). Our results are consistent with an increase in DHA metabolism as children mature to 104 weeks and UGT function approaches adult levels. Other UGT-metabolized drugs, such as morphine, also metabolized by UGT2B7, have demonstrated a similar pattern (31). Conversion of morphine to its glucuronide metabolites increased ~6 fold from the time of birth to 6 months of age (18). Likewise, two studies investigated lorazepam, a UGT substrate, and found elimination in the newborn is slow compared to adults with a half-life 3–4 fold longer than in adults (33, 34).

In contrast to results for DHA, the $AUC_{0–21d}$ of piperaquine was ~30% and ~50% lower at 32 and 104 weeks of age, compared to levels in adults, respectively, with ~30% lower peak concentrations at both ages. Likewise, terminal concentrations were 35–60% lower at 32 and 104 weeks compared to adult values. Our results suggest that CYP450 metabolism is greater than expected in young children when compared to adults, as shown for other CYP450 substrates (35, 36). CYP450 enzymes mature to adult levels over the first 12 months of life (16). Metabolic maturation over time in infancy has been demonstrated for many CYP3A4 substrates, including alfentanil, sirolimus, and nevirapine(29, 37, 38). However, infants and older children can exhibit higher clearances compared to adults, reducing PK exposure and necessitating higher doses (per kg) of some drugs (39, 40), which may even exceed maximum adult dose recommendations (40). A trend toward increasing weight normalized Dose/AUC for children 32 to 104 weeks of age is consistent with increasing hepatic enzyme maturation.

In addition to alterations in PK, implementing the former treatment nomogram for chemoprevention, based on weight bands, may have contributed to the differences in drug exposure between 32 and 104 weeks. Children at 32 weeks fell toward the middle of their weight band while children at 104 weeks approached the higher end of their weight band, potentially contributing to lower exposure at 104 weeks. Specifically, for children at 32 weeks, 100% received 20/160 mg DHA-piperaquine, while at 104 weeks, 74% received 20/160 mg DHA-piperaquine and 26% received 30/240 mg DHA-piperaquine. For treatment of malaria, the WHO has recently revised its DHA-piperaquine guidelines for young children (41), based largely on prior PK and PD studies (22–25) (26). These modified treatment guidelines, if utilized for chemoprevention, would compensate in part for the reductions observed in this study. However, to fully compensate, metabolic maturation during infancy must also inform optimized chemoprevention dosing guidelines.

The magnitude of piperaquine exposure required for effective IPT is not well defined. In pregnant Ugandan women receiving IPT with DHA-piperaquine, a plasma concentration of 13.9 ng/mL provided 99% protection from parasitemia. This target concentration is best maintained throughout the IPT dosing interval to minimize risk of placental malaria (15). In Thai adults receiving IPT with DHA-piperaquine, trough piperaquine concentrations exceeding 31 ng/mL were required to protect fully against malaria. For children in the present study, piperaquine concentrations on days 14 and 21 were often below the 13.9 ng/mL target established for pregnant women although the target for children may not be the same and is currently under investigation. Terminal concentrations on day 7, 14, and 21 were strongly correlated with AUCs, underscoring the value of these measurements as surrogates for overall exposure.

The lower AUCs seen in children, compared to adults, and suggest that higher mg/kg dosing of DHA-piperaquine for IPT are appropriate in young children, especially because young children will have decreased antimalarial immunity compared to older children and adults. Recently, an exposure-response evaluation of DHA-piperaquine as seasonal malaria chemoprevention in young children in Burkina Faso revealed that higher doses for DHA-piperaquine than currently used, are necessary to adequately protect against seasonal malaria (42), underscoring the importance of our findings. We plan to use sparse longitudinal sampling in a larger cohort of children over the first 2 years of life to address the impact of covariates, including allometric scaling and age-dependent enzyme maturation on piperaquine exposure and outcomes. These models should permit simulations for optimizing DHA-piperaquine chemoprevention regimens for young children.

As we previously reported in pregnant women (14), we did not observe a significant correlation between piperaquine exposure and QT interval, which may be due to the relatively low peak concentrations seen in these children compared to those reported previously in adults (43, 44). Prior studies demonstrated correlations between peak piperaquine levels and QTcF (43, 44), including one study in Cambodian male adults who received a compressed high dose two-day regimen, with peak piperaquine concentrations of ~ 900 ng/mL and a mean increase in QTcF of 46 msec. In our study of children, standard dosing of DHA-piperaquine was associated with a modest, but insignificant, QTcB prolongation of 21 and 14 msec at 32 and 104 weeks of age, respectively, a change similar to the 17 msec change in QTcF we previously reported for pregnant women (14).

Higher doses for children <20 kg are recommended by the WHO, and adverse cardiac events have not been detected (45). If these guidelines are adopted for chemoprevention, we do not expect the safety profile to be impacted, as peak piperaquine concentrations estimated at 32 and 104 weeks were <350 ng/ml, values much lower than previously associated with clinically relevant QTc prolongation (44).

This study had limitations. Data for infants receiving DHA-piperaquine monthly and every 3 months were merged; this was considered acceptable since the majority of piperaquine is eliminated within 30 days. Our sub-analysis, which compared children receiving the two regimens, showed no difference in PK parameters (data not shown). Our adult controls were postpartum women; they might differ from other adults. The study included venous and capillary measures of DHA-piperaquine and a correlation estimate based on simultaneous venous and capillary sampling at 24 hours post-final dose. Our correlation between venous and capillary measures is imperfect since the correlation may be different for terminal concentrations of DHA-piperaquine than for our 24 hours post-dose correlation. Finally, children received crushed tablets and adults, whole tablets. However, dissolution studies have shown that this variation does not alter the dissolution profiles; as piperaquine absorption is slow (T_{max} ~ 5 h), the rate of dissolution is not expected to influence absorption (46).

In summary, infants at 32 and 104 weeks of age receiving IPT with DHA- piperaquine exhibited lower piperaquine exposure than adults, with this decrease most pronounced at 104 weeks. These differences are attributed to both developmental maturation of piperaquine

metabolism and limitations of current chemoprevention dosing strategies. Studies evaluating higher doses of DHA-piperaquine for IPT are warranted.

METHODS

Study Area and Patients

This study occurred between August, 2015 and May, 2017 in Tororo, Uganda, a region with historically high rates of malaria transmission (47). Eligible participants included a) children born to mothers enrolled in a randomized trial that evaluated DHA-piperaquine as IPT during pregnancy (48) and b) as adult controls, women enrolled in the same study evaluated after delivery, and studied when at least 12 weeks post-partum. All study subjects were HIV uninfected.

The trial was registered at http://ClinicalTrials.gov (). Protocols and procedures were approved by the Uganda National Council of Science and Technology and institutional review boards of Makerere University and the University of California, San Francisco. Written informed consent was obtained from adult study participants, and, for children, their parents or guardians.

Study Design

Infants born to mothers enrolled in the parent trial were separately consented and enrolled prior to or at 32 weeks of age for intensive PK evaluations at both 32 and 104 weeks of age. Mothers had been randomized at time of enrollment in the parent trial to receive IPTp during the second and third trimesters; specifically, SP every 8 weeks, DHA-piperaquine every 8 weeks, or DHA-piperaquine every 4 weeks was initiated at either 16 or 20 weeks gestational age (Figure 1)(48).

Infants born to mothers randomized to SP in the parent study received DHA-piperaquine every 12 weeks; infants born to mothers randomized to either DHA-piperaquine regimen were randomized to DHA-piperaquine every 4 weeks or every 12 weeks. Treatment was initiated in all children at 8 weeks of age. To eliminate a need for unblinding, intensive PK studies were scheduled when all infants (regardless of regimen) received DHA-piperaquine, i.e. at 32 and 104 weeks of age. Adult controls were mothers enrolled in the parent trial who were at least 12 weeks post-partum and consented separately for an intensive PK evaluation around a single treatment of DHA-piperaquine (120/960 mg given daily for 3 consecutive days with or without food, using full strength 40 mg/320 mg tablets, Duo-Cotexin, Holley-Cotec, Beijing, China).

DHA-piperaquine dosing in children consisted of half-strength tablets (20/160 mg DHA-piperaquine) given daily for 3 consecutive days without food. Children ≤5.9 kg received a 1/2 tablet, 6.0–10.9 kg 1 tablet, 11.0–14.9 kg 1 1/2 tablets, 15.0–19.9 kg 2 tablets, 20.0–23.9 kg 2 1/2 tablets, and 24.0–25.9 kg 3 tablets; dosing was based on prior WHO treatment guidelines presently being used for chemoprevention clinical trials. Current treatment guidelines for uncomplicated malaria have been updated (41). All doses for children and adult controls were directly observed in the clinic by study nurses, who administered crushed tablets (in water) to the children or intact tablets to the adults.

For both children and adults, intensive PK sampling occurred before and after the third daily dose of DHA-piperaquine. Venous samples were collected pre-dose, and 0.5, 1, 2, 3, 4, 6, 8, and 24 hours post-dose to determine DHA and piperaquine concentrations. Capillary samples were collected at 24 hours and on days 4, 7, 14, and 21 post-dose to determine piperaquine concentrations. Samples were centrifuged within 60 minutes at 2,000*g* for 10 minutes, and the plasma supernatant was collected and stored at −80°C prior to drug quantitation. Capillary and venous samples collected concurrently 24 hours post-dose were used to determine correlations between capillary and venous piperaquine concentration results.

High performance liquid chromatography tandem mass spectrometry was utilized, as previously described, to determine the concentrations of DHA and piperaquine (49, 50). For DHA, the calibration range was 0.5–200 ng/ml, the lower limit of quantification (LLOQ) was 0.5 ng/ml, and the coefficient of variation (CV%) was <10% for quality control (QC) concentrations. For piperaquine, the original method was modified to expand the calibration range. The overall calibration range was 0.5–1000 ng/ml; the LLOQ was 0.5 ng/mL and the CV was <10% for QC concentrations. The primary outcomes of the study were plasma PK parameters for DHA and piperaquine, which included the maximal concentration (C_{max}), time to C_{max} (T_{max}), elimination half-life ($t_{1/2}$), and area-under-the-plasma concentration versus time curve to 8 hours for DHA ($AUC_{0–8\ hr}$) and to 21 days for piperaquine ($AUC_{0–21d}$). Terminal concentrations were also determined and included the concentration of DHA at 8 hours (C_{8hr}) and piperaquine at days 7, 14 and 21 (C_{7d}, C_{14d}, C_{21d}). Non-compartmental analysis was performed using WinNonlin® 6.4 (Certara L.P., Princeton, NJ, USA) using the linear up-log down trapezoidal rule and first order input. Results that fell below the LLOQ were treated as missing data except for the pre-dose drug concentration before the third dose, which was set at 0 if below the LLOQ.

Linear regression was used to determine the correlation between capillary and venous plasma piperaquine concentration results after natural log transformation of the data using STATA SE 12.1 (StataCorp, College Station, TX, USA). Piperaquine capillary concentration results were converted to predicted venous values using the generated correlation equation. The converted concentrations were used for the estimation of $AUC_{0–21d}$. C_{7d}, C_{14d}, and C_{21d} piperaquine concentrations were presented as non-adjusted capillary concentrations.

Electrocardiogram (ECG) monitoring

For safety assessments, 12 lead ECGs were performed before the first dose and 3 – 4 hours following the third dose in all study participants. Calipers were used to measure QT and RR intervals, and the corrected QT interval (QTc) was calculated using Bazett's formula (QTcB, $/\sqrt{RR}$) and Fridericia's formula (QTcF, $/\sqrt[3]{RR}$).

Statistical Analysis

Data analysis was completed using STATA® version SE12.1 (StataCorp, College Station, TX, USA). Power calculations were based on observed mean AUC and standard deviations from our prior studies. At least 30 subjects for each study group were required to detect a difference in mean AUC between groups of 29.5% for piperaquine and 31% for DHA with a

significance level (alpha) of 0.05 and 80% power using a two-sided two-sample t-test (CV for AUC DHA=38% and piperaquine=35%). To determine PK parameter values, a Wilcoxon signed-rank test was used for paired analysis in children and rank sum tests were used for unpaired analysis between adults and children and between children in the two age groups. Statistical significance was considered a two-sided adjusted p-value <0.017 for comparison between 3 groups. Geometric means (GM) or medians were reported as appropriate. For ECG analyses, pre-treatment and post-treatment comparisons of QTc intervals were performed using paired t-tests, and comparisons between groups were performed using unpaired t-tests. Correlations between changes in the QTcF interval and piperaquine exposure were assessed using Pearson's correlation (Rs).

To evaluate whether maternal DHA-piperaquine exposure (via IPTp) and/or or infant sex were associated with piperaquine exposure at 32 or 104 weeks, generalized estimating equations with robust standard errors were used and marginal estimates reported. In these analyses, evidence for effect modification was assessed between maternal IPTp, infant sex, and piperaquine exposure in infancy, and results are reported from stratified analysis where significant interaction (P<0.20) was noted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STUDY HIGHLIGHTS

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

DHA-piperaquine is being studied for malaria chemoprevention in young children, but the differences between adults and children, and the impact of age on DHA-piperaquine exposure is not fully understood.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study is first to evaluate the intensive pharmacokinetics of DHA-piperaquine in young children when used as intermittent preventative therapy (IPT). This study also explored the impact of enzyme maturation on DHA-piperaquine exposure.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

By comparing DHA and piperaquine exposure between children and adults, children at 104 weeks of age have significantly reduced DHA exposure compared to those at 32 weeks of age, and that both ages have significantly reduced piperaquine exposure compared to adults.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Our results suggest that the study of modified dosing strategies of DHA-piperaquine in children, including using the updated weight-based treatment dosing for DHA-piperaquine when given as IPT, is urgently needed. Further research is also needed to better characterize the impact of enzyme maturation on DHA-piperaquine pharmacokinetics.

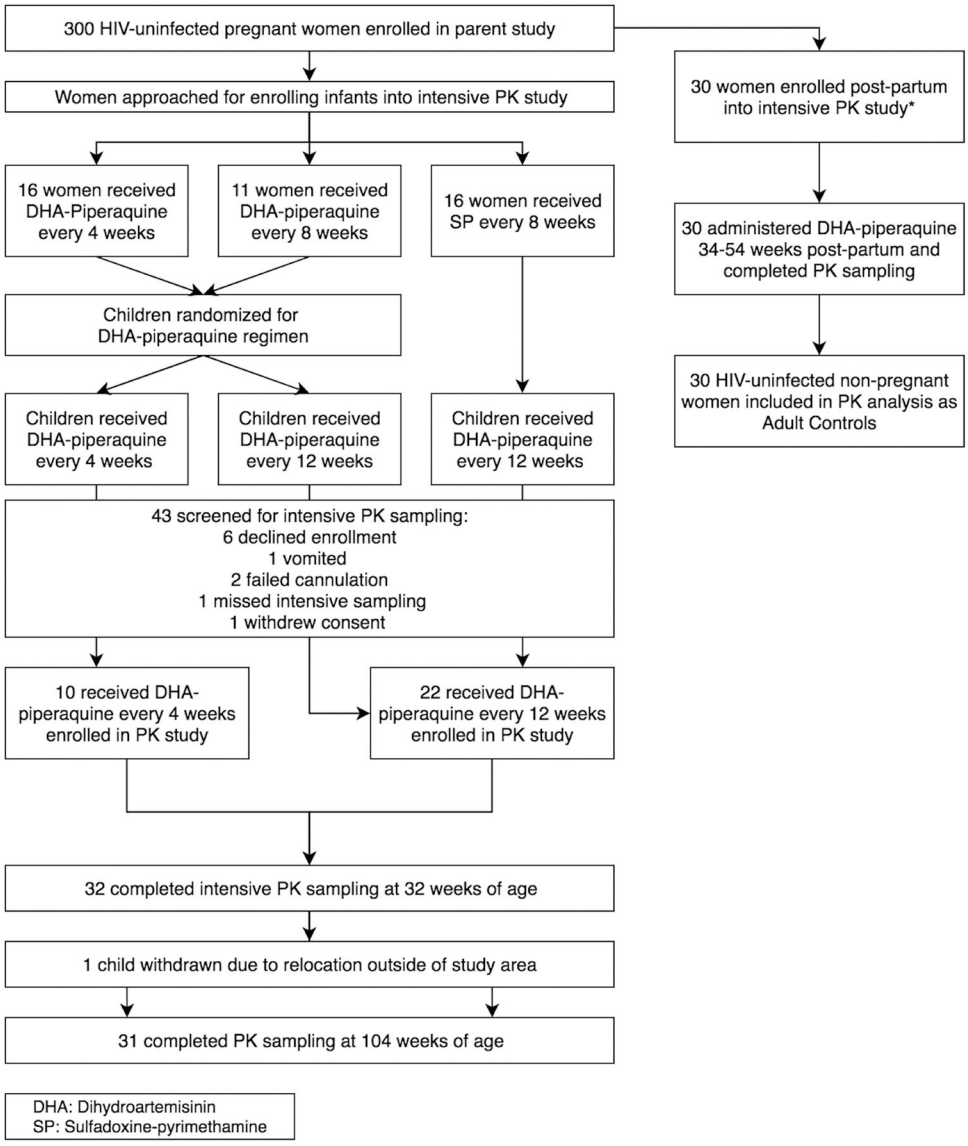

Figure 1.

Enrollment and completion of intensive PK studies from trials evaluating DHA- piperaquine as IPTi for malaria; DP denotes DHA-piperaquine; SP, sulfadoxine-pyrimethamine; PK, pharmacokinetics; DHA, dihydroartemisinin; PQ, piperaquine. *Adult data previously reported (14).

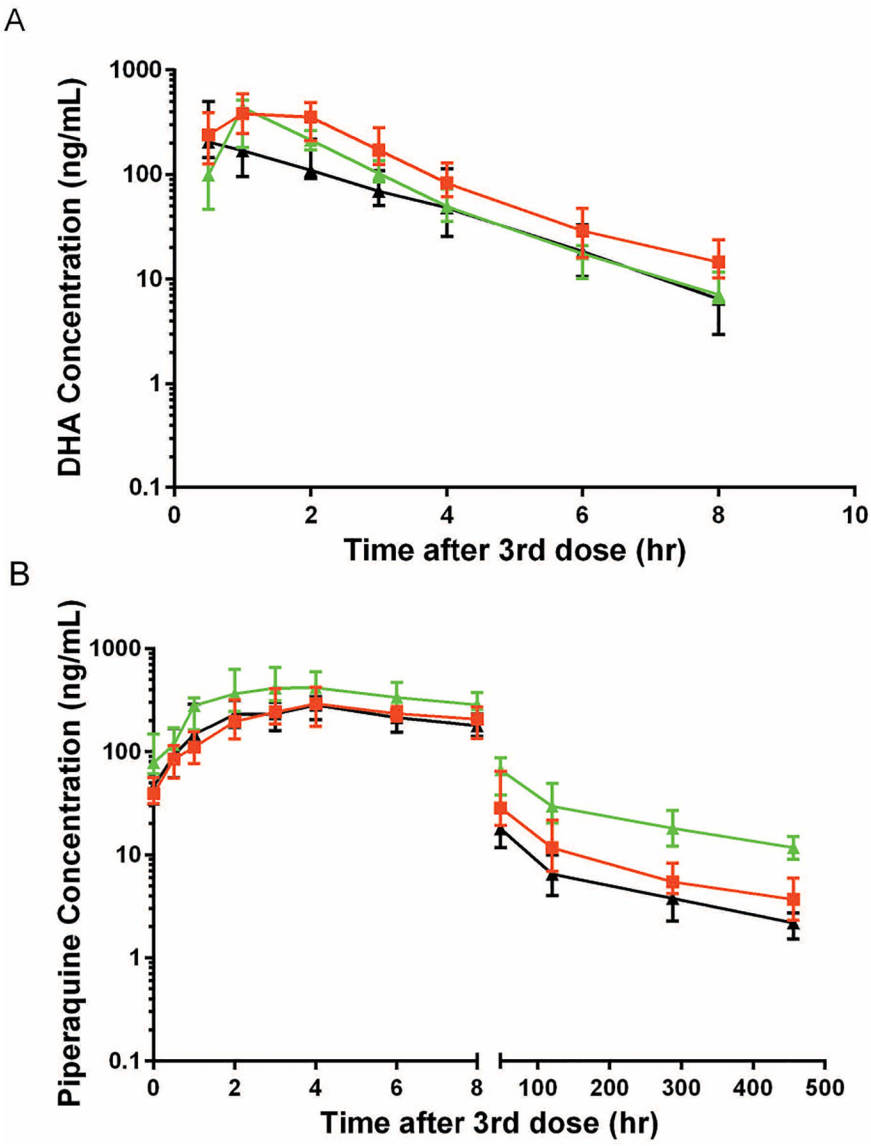

Figure 2.

Plasma concentration-time profile of DHA (A) and piperaquine (B) in children aged 104 weeks (black line) and 32 weeks (red line) and postpartum women (green line). Data is reported as median (IQR).

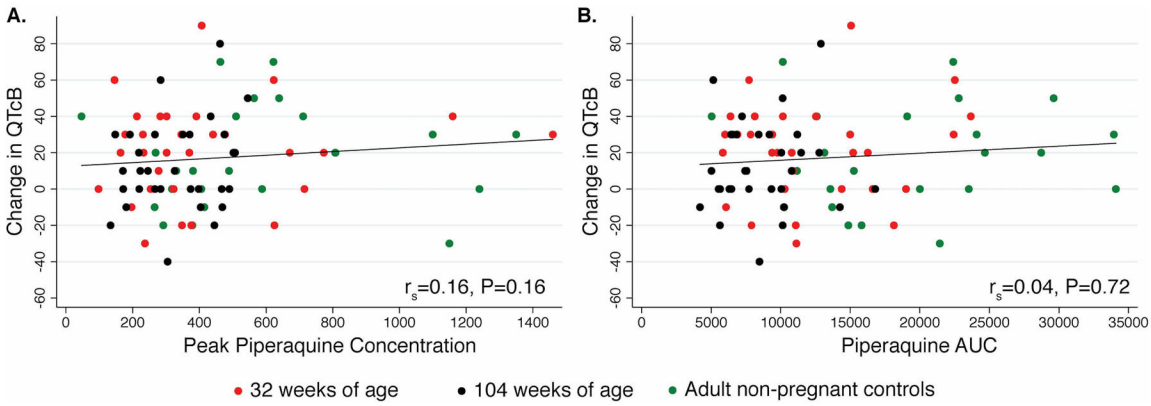

Figure 3.

Correlation of changes in the QTcB interval and pharmacokinetic exposure of piperaquine. AUC denotes area under the concentration vs. time curve to 21 days.

Table 1.

Baseline characteristics of participants at time of PK study enrollment. Data represent median (range).

	Children 32 Weeks, n=32	Children 104 Weeks, n=31	HIV-Uninfected Adults, n=30
Age (yr)	0.61	2	24 (19, 32)
Female	14	14	30
Male	18	17	
Weight (kg)	7.53 (6.01, 9.03)	10.55 (8.37, 12.86)	52.9 (38.5, 72.9)
Height (cm)	68 (62, 71)	83 (77, 89)	162 (148, 174)
Weeks post-partum			38.5 (34, 54)

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Table 2.

Impact of infancy on the pharmacokinetics of DHA

	Children			Ratio			
	32 weeks	104 weeks	Adults	104 weeks / 32 weeks			
	n=32	n=31	n=29	Paired (n=31)	All subjects	32wk/adults	104wk/adults
C_{max}, ng/ML	480 (402, 574)	355 (264, 478)	363 (304, 432)	0.737 (0.24)	0.740 (0.12)	1.32 (0.016)	0.978 (0.89)
T_{max}, hr	1.07 (0.99, 2.03)	0.57 (0.53, 2.00)	1.03 (1.00, 1.98)	0.533 (0.052)	0.533 (0.037)	1.04 (0.72)	0.553 (0.015)
$t_{1/2}$, hr *	1.72 (1.48, 2.00)	1.49 (1.24, 1.79)	1.49 (1.37, 1.62)	0.855 (0.098)	0.866 (0.078)	1.15 (0.20)	1.00 (0.35)
AUC_{0-8hr}, hr·ng/mL	1150 (978, 1344)	762 (623, 932)	754 (662, 860)	0.662 (0.0045)	0.663 (0.0011)	1.52 (<0.0001)	1.01 (0.71)
C_{8hr}, ng/mL	14.7 (11.6, 18.6)	6.85 (4.99, 9.39)	7.45 (5.96, 9.31)	0.460 (0.0004)	0.466 (0.0001)	1.97 (0.0001)	0.919 (0.50)

C_{max}, maximal concentration; T_{max}, time to reach maximal concentration; $t_{1/2}$, drug elimination half-life; AUC, area under concentration-time curve; C_{8hr}, DHA concentrations at 8hr post 3rd dose. Data are presented as geometric means (95% confidence interval) except for T_{max}, which is reported as median (interquartile range). P-values are calculated using the signed rank test for paired analysis and rank sum test for unpaired. Significance level: alpha = 0.05.

* n=30 for 32 weeks, 29 for paired analysis, and 28 for adults.

Table 3.

Impact of infancy on the pharmacokinetics of piperaquine

	Children			Ratio			
	32 weeks	104 weeks	Adults	104 weeks /32 weeks			
	n=32	n=31	n=30	Paired (n=31)	All subjects	32wk/adults	104wk/adults
C_{max}, ng/mL	345 (279, 427)	309 (266, 359)	499 (393, 633)	0.939 (0.46)	0.896 (0.69)	0.691 (0.0034)	0.619 (0.0002)
T_{max}, hr	4.03 (3.06, 6.01)	4.02 (2.03, 6.05)	3.06 (2.07, 4.03)	0.998 (0.38)	0.998 (0.22)	1.32 (0.0063)	1.31 (0.40)
$t_{1/2}$, hr *	174 (149, 203)	177 (148, 212)	208 (187, 232)	1.01 (0.47)	1.02 (0.96)	0.837 (0.090)	0.851 (0.11)
AUC_{0-21d}, hr·µg/mL	11.4 (9.77, 13.2)	8.24 (7.28, 9.33)	17.6 (15.1, 20.7)	0.741 (0.0020)	0.723 (0.0033)	0.648 (0.0002)	0.468 (<0.0001)
C_{7d}, ng/mL	22.6 (18.8, 27.2)	16.3 (13.6, 19.6)	39.0 (32.3, 47.2)	0.732 (0.013)	0.721 (0.013)	0.579 (0.0002)	0.418 (<0.0001)
C_{14d}, ng/mL	13.9 (12.0, 16.0)	9.10 (7.68, 10.8)	22.6 (18.7, 27.3)	0.654 (0.0005)	0.655 (0.0006)	0.615 (0.0002)	0.403 (<0.0001)
C_{21d}, ng/mL	9.42 (8.18, 10.8)	7.09 (6.03, 8.34)	14.5 (12.2, 17.1)	0.754 (0.011)	0.753 (0.0046)	0.650 (0.0002)	0.489 (<0.0001)

Abbreviations as in Table 2. AUC was calculated using piperaquine concentrations from venous plasma, including conversion of capillary to venous concentrations; C_{7d}, C_{14d}, and C_{21d} are actual capillary plasma concentrations at day 7, 14, and 21 post 3rd dose. Data are presented as geometric means (95% confidence interval) except for T_{max}, which is reported as median (interquartile range). P-values are calculated using the signed rank test for paired analysis and the rank sum test for unpaired. Significance level: alpha = 0.05.

* n=24 for 32 weeks, 22 for 104 weeks, 16 for paired analysis, 29 for adults.