Pharmacokinetics of piperaquine and safety profile of dihydroartemisinin-piperaquine co-administered with antiretroviral therapy in malaria-uninfected HIV-positive Malawian adults.

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ABSTRACT

There are limited data on the pharmacokinetic and safety profiles of dihydroartemisinin-piperaquine (DHA-PQ) among human immunodeficiency virus infected (HIV+) individuals taking antiretroviral therapy (ART). In a two step (parallel-group) pharmacokinetic trial with intensive blood sampling, we compared area under the concentration-time curve ($AUC_{0\text{-}28\ days}$) and safety outcomes of piperaquine among malaria-uninfected HIV+ adults. In step 1, half the adult dose of DHA-PQ was administered for three days as an intitial safety check in four groups (n=6/group) of HIV+ adults (age≥18 years): (i) antiretroviral-naïve, (ii) on nevirapine-based ART, (iii) on efavirenz-based ART, and (iv) on ritonavir-boosted lopinavir-based ART. In step 2, a full adult treatment course of DHA-PQ was administered to a different cohort of participants in three groups: (i) antiretroviral naïve, (ii) on efavirenz-based ART and (iii) on nevirapine-based ART (n=10-15/group). Ritonavir-boosted lopinavir-based ART group was dropped in step 2 due to limited number of participants who were on this second line ART and were eligible for recruitment. Piperaquine's $AUC_{0\text{-}28\ days}$ in both steps was 43% lower among participants on efavirenz-based ART compared to ART naïve participants. There were no significant differences in $AUC_{0\text{-}28\ days}$ between the other ART groups and the ART naïve group in each of the two steps. Furthermore, no differences in treatment-emergent clinical and laboratory adverse events were observed across the groups in steps 1 and 2. Although well tolerated at half and full standard adult treatment courses, efavirenz based antiretroviral regimen was associated with reduced piperaquine exposure which may compromise dihydroartemisinin-piperaquine's effectiveness in programmatic settings.

Key words: piperaquine, antiretroviral therapy, malaria

Antimicrobial Agents and Chemotherapy

INTRODUCTION

Human immunodeficiency virus (HIV) and *Plasmodium falciparum (Pf)* malaria infections are endemic in most areas in sub-Saharan Africa (SSA) and co-infections occur frequently. HIV infection increases susceptibility to malaria (1, 2), severity of *Pf* malaria (3–6) and reduces the efficacy of some antimalarial drugs in current use (7, 8). To combat these dual infections, the World Health Organisation (WHO) recommends initiation of antiretroviral therapy (ART) in HIV-positive (HIV+) individuals and prompt use of artemisinin-based combination therapies (ACTs). Dihydroartemisinin-piperaquine (DHA-PQ), is one of the ACTs being used increasingly in SSA in malaria infected individuals (9) owing to its better safety profile and longer piperaquine half-life of approximately 33 days (10, 11), which makes it an ideal option for treatment of uncomplicated *Pf* malaria (12, 13) and intermittent preventive treatment of malaria in pregnancy (14, 15). Additionally, dihydroartemisinin, which has a half-life of approximately 1 hour, is fast acting and 5-10 times more potent among the artemisinin derivatives (16). Because of the geographical overlap of malaria and HIV, DHA-PQ will likely be commonly co-administered with ART such efavirenz (EFV), nevirapine (NVP) or ritonavir-boosted lopinavir (LPV/r).

It has been postulated that pharmacokinetic interactions between ACTs and non-nucleoside reverse transcriptase inhibitors (NNRTI)- or protease inhibitors (PIs)-containing ART are likely since these classes of drugs affect the activity of cytochrome-P (CYP) 450 liver enzymes. NNRTIs such as NVP and EFV usually induce various CYP450 isoforms but they are also substrates for CYP450 enzymes as are ACTs. Conversely, HIV PIs, particularly ritonavir, are potent inhibitors of CYP3A enzymes (17), which form part of the CYP450 enzyme entity. Administration of ACTs in HIV+ individuals on ART may therefore reduce or increase plasma concentrations of any of the drug components of ACTs. Dihydroartemisinin may have limited pharmacokinetic interactions with ART since it is metabolised through glucuronidation by uridine

diphosphate glucuronosyltransferase (18). However, piperaquine, as a xenobiotic, is metabolised by CYP P450 (CYP3A4 and CYP2C8) for excretion (19). Any induction or inhibition of these enzymes by ART may affect clearance of piperaquine and, therefore, its efficacy and safety.

In a two-step (parallel), intensive pharmacokinetic sampling trial, we compared the safety of DHA-PQ and secondary pharmacokinetic parameters ($AUC_{0\text{-}28\ days}$, C_{max}, t_{max}, $t_{1/2}$) of piperaquine between HIV+ adults taking various ART (efavirenz-, nevirapine-, ritonavir-boosted lopinavir-based regimens) and HIV+ adults not on any ART.

MATERIALS AND METHODS

Study Design and population

We conducted an open-label, sequential group, PK trial, from August 2010 to March 2013, at Queen Elizabeth Central Hospital, Malawi. The study was implemented in the following two steps:

In step 1 [PACTR2010030001871293], we administered half adult doses of the DHA-PQ (Euratesim®, Sigma Tau) to the followin groups of malaria-negative research participants (n=6/group):

1) An antiretroviral naive HIV+ (control) group
2) HIV+ individuals on NVP-based ART
3) HIV+ individuals on EFV-based ART
4) HIV+ individuals on LPV/r-based ART

DHA-PQ was administered orally at 0, 24 and 48 hours (once daily for 3 days). One tablet (each containing DHA/PQ 40mg/320mg) was administered orally for study participants weighing

<60kgs and 1.5 tablets to participants weighing >= 60kg. Food intake, including fat containing food, was not restricted. This step served as a safety evaluation step for the drug interaction studies, checking for unexpected clinical toxicities or interactions.

In step 2 [PACTR2010030001971409], after review and consideration of step 1 data by an independent Data Safety Monitoring Board (DSMB), a full standard dose DHA-PQ (3 tablets to study participants weighing < 60kg and 4 tablets to those weighing >= 60kg) was administered to 40 adults in the following groups of malaria-negative research participants (different from those enrolled in step 1):

1) An antiretroviral naive HIV+ (control) group
2) HIV+ individuals on NVP-based ART
3) HIV+ individuals on EFV-based ART

DHA-PQ was administered at 0, 24 and 48 hours (once daily for 3 days). The group of HIV+ individuals on LPV/r-based ART was dropped owing to limited number of participants available for recruitment into the study. Unlike in step 1, DHA-PQ was administered with water only in step 2; no food was given to study participants taking DHA-PQ within a period of 3 hours before and 3 hours after administering the drug; based on a new recommendation from the drug manufacturer, Sigma Tau. In the ART arms, the first dose of DHA-PQ was timed to coincide with the next scheduled dose of the ART.

The study population for step 1 and step 2 were HIV+ male and non-pregnant female participants aged ≥18 years residing in Blantyre, Malawi or neighbouring districts of Thyolo and Chiradzulu. Individuals on ART were eligible to participate if they had been on NVP, EFV or LPV/r-based ART for ≥ 6 months and had CD4 cell count ≥ 250 cells/mm^3. At the beginning of the study, HIV+ antiretroviral naive individuals were eligible for ART if they had a CD4 cell count ≥ 250/mm^3 but this cut-off point was increased to ≥350/mm^3 when the WHO criteria for ART

initiation changed in July 2011. Other inclusion criteria were body weight ≥40kg and willingness to be admitted in the hospital for 3 days, to remain within the study sites and to be contacted at home or by phone during the course of the study.

We excluded participants who had body mass index ≤18.5kg/m^2, haemoglobin concentration <8.5 g/dL, reported use of any antimalarial drugs within the preceding 4 weeks, reported hypersensitivity to any of the ACTs, were taking other drugs which are known inhibitors or inducers of P450 enzymes or P-glycoprotein (except cotrimoxazole prophylaxis), had a history of regular intake of alcohol (>twice/week), tobacco (>3 times/week) or any use of illicit drugs, had a history or evidence of pre-existing liver, kidney or heart disease, including conductive abnormalities on electrocardiographs (*QTc interval>450ms in men and >470ms in females)*, had clinical and/or laboratory evidence of *Pf* malaria, hepatitis B, pneumonia, tuberculosis, bacteraemia or laboratory evidence of potentially life threatening white blood cell disorders such as absolute neutrophil count <0.500*10^9/L, absolute lymphocyte count <0.35*10^9/L or absolute platelet count <25*10^9/L. Participants with a performance (Karnofsky) score of <80% and who were participating in any other clinical trial were also not included.

In step 1, the sample size was 6 in each of the DHA-PQ/ART and control (ART-naive) groups. This sample size was based on standard practice in early PK studies of antimalarial drugs which aim to safeguard the safety of study subjects and minimize the number of subjects who may be potentially exposed to harmful drug levels. In step 2, a sample size of 15 per group in the DHA-PQ/ART groups and 10 in the ART-naive group was required. This was calculated to detect a two-fold increase in PQ AUC in any of the DHA-PQ/ART groups compared with the ART-naive group, assuming a mean (standard deviation) PQ AUC of 19.4 (15.0) mcg/hr/mL (17) in the ART-naive group, with power set at 90% and level of significance at 5%.

Ethics and data collection procedures

The design and timing of trial procedures was approved by the College of Medicine Research Ethics Committee (COMREC), in Blantyre, Malawi. The study conformed to the principles of the International Conference on Harmonization on Good Clinical Practice. Research nurses and clinicians sought written informed consent from individuals to perform screening procedures including physical medical and anthropometric assessment, electrocardiographs (ECGs) and blood tests to detect blood-borne infections, haematological, renal or hepatic abnormalities. Results from screening procedures were available within 7 days of screening. Based on these results, potential study participants were informed of their eligibility to participate in the study. Thereafter, research nurses or clinicians sought written informed consent from eligible subjects to participate in the study.

Pre-DHA-PQ dosing procedures

Consenting study participants were re-assessed by research nurses or clinicians to determine whether they still met all eligibility criteria, through repeat history taking and physical examination. Eligible participants were admitted in hospital and an indwelling cannula was inserted into a vein before their scheduled dose of ART and the first dose of the ACT. Approximately 1 hour before the scheduled time of ART and ACT dosing, blood samples were collected for haematological, renal and liver function tests and also random glucose test.

Blood sample collection and processing

While the participant was hospitalized, blood samples for pharmacokinetic (PK) assays were collected in heparin vacutainer tubes, pre-treatment and at the following post-treatment times: 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 60 and 72 hours. After discharge, the blood samples were taken at the following times; 4, 5, 6, 7, 14, 21 and 28 days. Immediately after collection, the blood samples were spun in a refrigerated centrifuge and the separated plasma

samples were temporarily frozen in liquid nitrogen before transferred to a -80˚C freezer until HPLC analyses.

Safety Assessments

After the first dose of DHA-PQ, blood samples to detect haematological, renal and liver function abnormalities were collected at the following times; 12, 48 and 72 hrs and at days 7, 14, 21 and 28. In addition, 12-lead ECGs were performed pre-dosing, 5 hours after the first dose and 5 hours after the last dose to assess Fridericia's-corrected QTc interval (20). The study focussed on treatment-emergent adverse events (TEAEs), defined as clinical or subclinical abnormalities which were absent before dosing with DHA-PQ but emerged post dosing or those which were present before dosing with DHA-PQ but worsened post-dosing. Severity of AEs was graded using the DAIDS criteria (21) while seriousness was defined according to the standard definition.

Pharmacokinetic assays

Plasma samples were analysed for PQ levels at Malawi-Liverpool Welcome Trust Clinical Research Programme in Blantyre, Malawi, using a validated HPLC-UV assay adopted and transferred to Malawi from the Liverpool School of Tropical Medicine. The PK laboratory in Blantyre participated in WWARN's External Quality Assurance programme (22). Briefly, PQ and the internal standard (Chloroquine) were recovered from plasma using diethyl/tert-butyl ether. The supernatant was evaporated to dryness in a vacuum concentrator at 25 ^{0}C. The residue was re-dissolved in 200 µl of the reconstitution solvent acetonitrile: phosphate buffer (5:95, pH 2.5) and 75 µL was injected into the chromatograph (Agilent 1100). Quantitation of the drugs was achieved by reverse phase HPLC. The optimum detection wavelength for each drug was 345 nm. The lower limit of quantitation (LLQ) of the piperaquine HPLC-UV assay was 0.025 µg/mL with CV<10%. Reconstituted plasma sample extracts were run in batches comprising all samples

collected from each of any two study participants. Each batch run included a blank plasma extract, two sets of 8-concentration-level calibration standards, and quality controls (QC) at three concentration levels: low, medium and high (0.025, 1.5 and 3.0 µg/mL for PQ. For batch assay to pass the measured concentrations of at least 67% of the QC samples had to be within +/-20% of their nominal value and at least one QC had to be acceptable at the LLQ. The mean inter-assay precision for low, medium and high QCs was 7%, 12% and 10% respectively. In addition, 75% of each calibration curve's concentrations had to lie within +/-20% and +/-15% of the nominal concentration at the LLQ or all other concentrations.

Pharmacokinetic and safety data analyses

Plasma concentrations of piperaquine were analysed using non-compartmental pharmacokinetic analysis (NCA), employing the trapezoidal rule with cubic splines. Observed piperaquine concentrations below the lower limit of quantification (<LLOQ) were treated as missing data except for the pre-dose concentration which was imputed to 0 if below LLOQ. For each study participant, the following PK parameters were computed: $AUC_{0\text{-}28\ days}$, maximum concentration [C_{max}], time to maximum concentration [t_{max}] and terminal elimination half-life [$t_{1/2}$]). We used STATA 15.0 for the NCA and to compare log-transformed PK parameters. Geometric mean ratios with 90% confidence intervals have been presented. To test for significant differences in PK parameters between each ACT/ART group and the ART-naïve group, parametric evaluation of the log-transformed PK parameters was done using analysis of variance (ANOVA) (α=0.1). Fisher's exact test was used to compare proportions of participants across the study groups with day 7 concentrations that were above a value known to predict treatment response by day 28, and of safety parameters across the different ACT/ART groups in comparison to the ART naïve group. Data summaries and graphics were all performed in STATA 15.0.

RESULTS

Characteristics of study participants

In step 1, 24 participants (6 in each group) were enrolled and successfully followed up for 28 days, including 5 who replaced those withdrawn due to protocol violations. In step 2, 40 participants were enrolled (10 in ART naïve and 15 in each of EFV and NVP groups) and completed 28 days of follow-up, including 2 who replaced those withdrawn due to protocol violations. In accordance with the protocol, withdrawn individuals were not included in the PK analyses. As shown in Table 1, participants who completed follow-up in steps 1 and 2 generally had similar baseline characteristics. In step 1, those on ritonavir-boosted lopinavir had longer median duration of ART intake than those on EFV and NVP groups. In addition, baseline alanine aminotransferase was higher in those on EFV based ART.

Pharmacokinetic interactions between piperaquine and ART in step 1

Participants in the EFV-ART group had 43% lower $AUC_{0\text{-}28\ days}$ of piperaquine compared to the ART naïve group (geometric mean ratio [90% CI]: 0.57 [0.38-0.83]; p=0.029). There were no significant differences in $AUC_{0\text{-}28\ days}$ among participants in the other ART groups in comparison to the ART naïve group. Piperaquine's C_{max} was higher in the NVP-ART group than in the ART naïve group (geometric mean ratio [90% CI]: 1.82 [1.13-2.94]; p=0.061), but no significant differences in C_{max} were observed between the rest of the ART groups and the ART naïve group. There were no significant differences in the $t_{1/2}$ of piperaquine in all four study groups (as shown in Table 2a). However, the median t_{max} was higher in the LPV/r-ART group than in the ART naïve group (p=0.049). Figure 1 shows a concentration-time profile between ART groups and the ART naïve group. Compared to the ARV-naïve group, there was a lower piperaquine concentration-time profile in the EFV-ART group.

Safety assessment in step 1

DHA-PQ was well tolerated in all study groups. However, one participant in the ART-naive group had a 3-day history of headache, heart palpitations, nausea with no vomiting and good appetite following intake of DHA-PQ. These resolved by day 7 of follow up. One participant in the NVP-ART group developed left sided hemiplegia which was not thought to be associated with co-administration with DHA-PQ. There were no clinically-significant treatment-emergent haematological or hepatic abnormalities across the study groups.

Pharmacokinetic interactions between piperaquine and ART in step 2

In step 2, piperaquine's $AUC_{0\text{-}28\ days}$ was 43% lower in the EFV-ART group compared to the ART-naïve group (geometric mean ratio [95% CI]: 0.57 [0.44-0.74]; p=0.002). There was no significant difference in piperaquine's $AUC_{0\text{-}28\ days}$ between the NVP/ART and ART-naïve groups. Furthermore, participants in the EFV-ART group had 43% lower C_{max} of piperaquine compared to the ART naïve group (geometric mean ratio [95% CI]: 0.57 [0.36-0.90]; p=0.065), and piperaquine's $t_{1/2}$ was 64% lower in the EFV-ART group than in the ART naïve group (geometric mean ratio [95% CI]:0.36 [0.15-0.87]; p=0.072). However, there were no significant differences in the C_{max} and $t_{1/2}$ of piperaquine between the NVP-ART and the ART naïve groups as shown in Table 2b. Similarly, no significant differences in the median t_{max} between the two ART-groups and the ART naïve group were observed. Figure 2 illustrates the piperaquine concentration versus time plot in the NVP, EFV and ART-naive groups in step 2. The EFV-ART group had a lower concentration-time profile of piperaquine compared to the ART naïve group and there was a tendency towards higher piperaquine concentration in the NVP-ART group compared to the ART naïve group.

Piperaquine day 7 concentrations

Of the 40 participants in step 2, 22 had piperaquine plasma concentration above the lower limit of quantification (>25ng/mL) at day 7 post-treatment. There was no evidence of a significant difference in day 7 piperaquine concentration across the ART groups (Table 2b). Of the 22 participants with day 7 piperaquine concentration above >25ng/mL (ART naïve=2, EFV-ART=10 and NVP-ART=10), the proportion achieving piperaquine concentrations >30ng/mL was 90% (n=10) in ART-naïve group, 100% (n=2) in EFV-ART and 90% (n=10) in the NVP-ART group. There was no evidence of a difference in these proportions between each of the EFV and NVP-ART groups compared to the ART naïve group (for both comparisons; EFV/NVP-ART vs ART naïve; p= 1.000).

Safety assessment in step 2

DHA-PQ was generally well tolerated in all study groups in step 2. However, one participant in the ART-naïve group reported nausea following intake of DHA-PQ but this resolved within a day. The proportions of study participants who had any grade of treatment emergent transaminitis (elevated ALT and AST levels) after DHA-PQ administration were similar in the ART-naïve and EFV-ART, 50% (5/10) vs 40% (6/15) respectively; p=0.697, and between the ART-naïve and NVP-ART 53% (8/15) groups (p=1.000). None of the elevated AST or ALT levels reached severity levels of grade 3 or 4 or were persistent beyond day 28 of follow up. The proportions of participants who had any grade of treatment-emergent neutropenia after DHA-PQ administration were similar between the ART-naïve, 30% (3/10) and the EFV-ART-group, 33% (5/15) p=1.000, between the ART-naïve group and the NVP-ART, 20% (3/15) groups (p=0.653). There were no cases reaching grade 3 or 4 neutropenia in any of the groups. Additionally, the proportion of participants who had QTc prolongation after DHA-PQ administration (470ms at day 3 of follow up) were 0.0% (0/10), 13.3% (2/15) and 13.3% (2/15) in the ART naïve, EFV-ART and NVP-ART groups respectively, with no evidence of significant difference between the

NVP/EFV-ART groups and the ART-naïve group. All cases of QTc prolongation resolved spontaneously by day 21 of follow up.

Dose proportionality between ART naïve participants in steps 1 and 2

Assuming linear disposition of piperaquine, increasing the dose in step 2 should result in increased $AUC_{0\text{-}28\ days}$ in this step compared to step 1. As part of an exploratory analysis, not determined a priori, we assessed dose proportionality between the ART naïve groups in steps 1 and 2 using a linear quadratic regression approach by regressing dose normalised $AUC_{0\text{-}28\ days}$ ($AUC_{0\text{-}28\ days}$/ Dose) with total dose received by each participant (23). The fitted linear regression equation was:

$$AUC_{0\text{-}28\ days}/Dose = alpha + beta1*Dose + beta2*Dose^2 \quad (a)$$

The null hypothesis was that beta2 and alpha are equal to zero. Dose proportionality was declared if alpha and beta2 were not significantly different from zero. The above equation could be further simplified to the equation below when beta2 is not significantly different from zero:

$$AUC_{0\text{-}28\ days}/Dose = alpha + beta*Dose \quad (b)$$

Both equations showed no evidence against the null hypothesis as illustrated below in the result of the equation (a), which was derived from ART naïve participants in steps 1 and 2, showing that beta2 and alpha were not very significantly different from zero:

$$AUC_{0\text{-}28\ days}/Dose = 0.116 - 0.00011*Dose + 3.37e\text{-}08*Dose^2$$

DISCUSSION

The aim of this study was to compare secondary pharmacokinetic parameters of piperaquine and safety of dihydroartemisinin-piperaquine between HIV infected adults taking various antiretroviral therapy (efavirenz, nevirapine, ritonavir-boosted lopinavir based regimens) and HIV infected adults not on any antiretroviral therapy. We found that co-administration of

piperaquine and efavirenz based ART regimen significantly lowered piperaquine's exposure ($AUC_{0\text{-}28days}$) at half and full standard adult courses, and reduced piperaquine's half-life and achieved maximum concentration at full standard adult course than when administered alone among non-malaria-HIV infected adults. Additionally, the day 7 piperaquine concentration was not significantly different between the ART-groups following intake of a full standard adult course. Furthermore, DHA-PQ was well tolerated at both half and full adult courses across all ART groups with no evidence of significant differences in treatment emergent clinical and laboratory adverse events across all ART-groups.

The finding of a significantly lower piperaquine concentration in the EFV group in both steps is consistent with known metabolism of EFV, which is a potent inducer of CYP3A4 (17) and is one of the major CYP450 isoforms responsible for metabolic clearance of piperaquine (24). There is paucity of published evidence on the interaction between piperaquine and ART among non-pregnant individuals. However, our findings are consistent with previous findings among pregnant women receiving DHA-PQ for intermittent preventive treatment of malaria in Uganda, where piperaquine exposure was shown to be 38% lower among pregnant women receiving EFV based ART compared to HIV uninfected pregnant women (25). Thus, in the present study, EFV induction of CYP3A4 in the EFV-treated group might have led to enhanced clearance and shorter half-life of piperaquine seen in step 2.

Unexpectedly, we found non-significantly higher concentration of piperaquine in the NVP based ART group in steps 1 and 2 than in the ART naïve group. While there is some evidence that NVP induces CYP3A4 (26, 27), other studies have suggested that it may act as an inhibitor of other drugs metabolised by the CYP3A4 as shown with increased C_{max} and AUC of darunavir (28) and maraviroc (29), when co-administered with NVP. The non-significantly increased $AUC_{0\text{-}28\ days}$ and C_{max} of piperaquine in our study could suggest increased bioavailability or reduced

metabolism. As this study was not designed to elucidate the mechanism of interaction between piperaquine and nevirapine, studies in future should aim to explore and define these mechanisms which could include competitive inhibition of metabolic enzymes (30) or variations in availability of proteins to transport drugs (31).

Evidence on the interaction between piperaquine and LPV/r-based ART is sparse. In step 1, we found an expected but non-significant tendency towards higher piperaquine exposure ($AUC_{0\text{-}28\ days}$) in the LPV/r ART group compared to the ART naïve group and were unable to further evaluate this finding with a larger sample size in step 2 due to a limited number of study participants on this second line ART regimen during the study period. Since LPV/r is increasingly being used in malaria-HIV endemic settings as second line antiretroviral therapy, its impact on piperaquine's PK profile needs to be further studied.

Previous studies found that lower day 7 plasma piperaquine concentrations are associated with recurrent malaria (32, 33). The lack of significant evidence of a difference in day 7 piperaquine concentrations between the EFV or NVP-ART groups and ART-naïve group could be due to the small number of participants that had day 7 piperaquine concentrations that were above the lower limit of quantification of our assay, which may not have been able to detect low piperaquine concentrations. As efavirenz has been shown to also lower day 7 piperaquine concentrations in pregnancy (25), future studies should further explore this in HIV infected, non-pregnant adults.

We found no major differences in the incidence of neutropenia and transaminitis and QTc prolongation across the various ART groups, which is reassuring. However, these results need to be interpreted with caution, since this study was not powered to detect differences in safety endpoints.

Concomitant intake of piperaquine with food has previously been shown to increase bioavailability of piperaquine (34). Lack of food restriction in step 1, including intake of fat containing food, may have resulted in increased absorption of piperaquine in this step, with subsequent higher $AUC_{0\text{-}28days}$ in step 1 than in step 2. Although assessing dose proportionality was not the primary aim of this study, dose normalisation of the $AUC_{0\text{-}28days}$ (adjusting for the effect of the total administered dose) showed that there was evidence of dose proportionality between the two steps. The inability to detect significant differences in PK parameters, including dose proportionality between steps1 and 2, may be due to the use of the parallel-group design which is more prone to effects of inter-individual anthropometric and genetic variations than a cross-over design. Thus, other covariates such as genetic polymorphisms in CYP450 iso-enzymes may have contributed to very wide interquartile ranges of PQ PK parameters observed within each study group and between the two steps. However, our study sample size is unlikely to have missed large (>2-fold), clinically important differences in AUC across the study arms. Nevertheless, future studies need to assess the effect of genetic polymorphisms in CYP450 iso-enzymes on the pharmacokinetics of piperaquine and quantify any changes in plasma ART levels when co-administered with antimalarial drugs.

In our study, we did not assess the impact of ART on the PK profile of the faster acting and potent partner drug of piperaquine, dihydroartemisinin. In future, studies should aim to examine any potential impact of ART on the PK profile of dihydroartemisinin and evaluate its association with parasite clearance rates among malaria-HIV co-infected individuals.

In conclusion, this study found that although generally well tolerated, co-administration of piperaquine and efavirenz based ART regimen significantly lowered piperaquine's exposure among non-malaria HIV infected adults compared to an ART -naïve subgroup. There were no major variations in piperaquine's exposure among the ART naïve and participants on nevirapine

and ritonavir-boosted lopinavir based ART. The pharmacodynamic implications of these findings need to be evaluated in programmatic settings especially in malaria-infected individuals.

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CONFLICT OF INTEREST

The authors do not have any association that might pose a conflict of interest (e.g. pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding).

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LEGENDS

Figure 1. Piperaquine concentration-time profile (semi-logarithmic scale) following administration of half of the standard dihydroartemisinin-piperaquine adult dose in step 1, *n=23* (one participant excluded. ART-naïve, n=6; efavirenz (EFV), n=6; ritonavir-boosted lopinavir lopinavir-boosted (LPV/r), n=6; nevirapine (NVP), n=5. Below lower limit of quantification concentrations are excluded resulting in plotted observation time up to 336 hours in the efavirenz group and 672 hours in the rest of the study groups. Data are represented as mean (95% confidence interval)

Figure 2. Piperaquine concentration-time profile (semi-logarithmic scale) following administration of full standard adult dose of dihydroartemisinin-piperaquine in step 2, n=40. (ART naïve, n=10; efavirenz (EFV), n=15; nevirapine (NVP), n=15). Below lower limit of quantification points are excluded resulting in plotted observation time up to 336 hours. Data are represented as mean (95% confidence interval)

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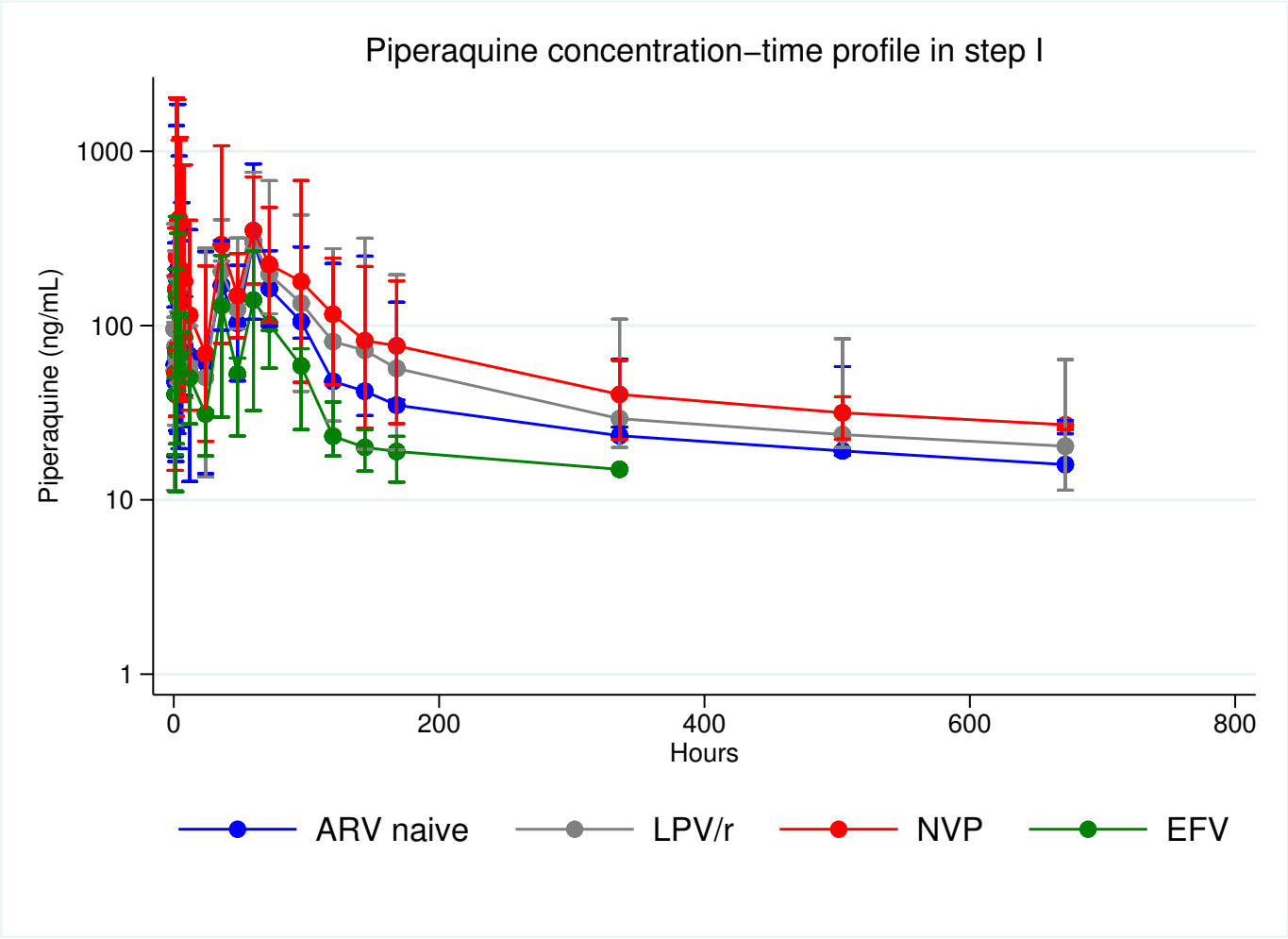

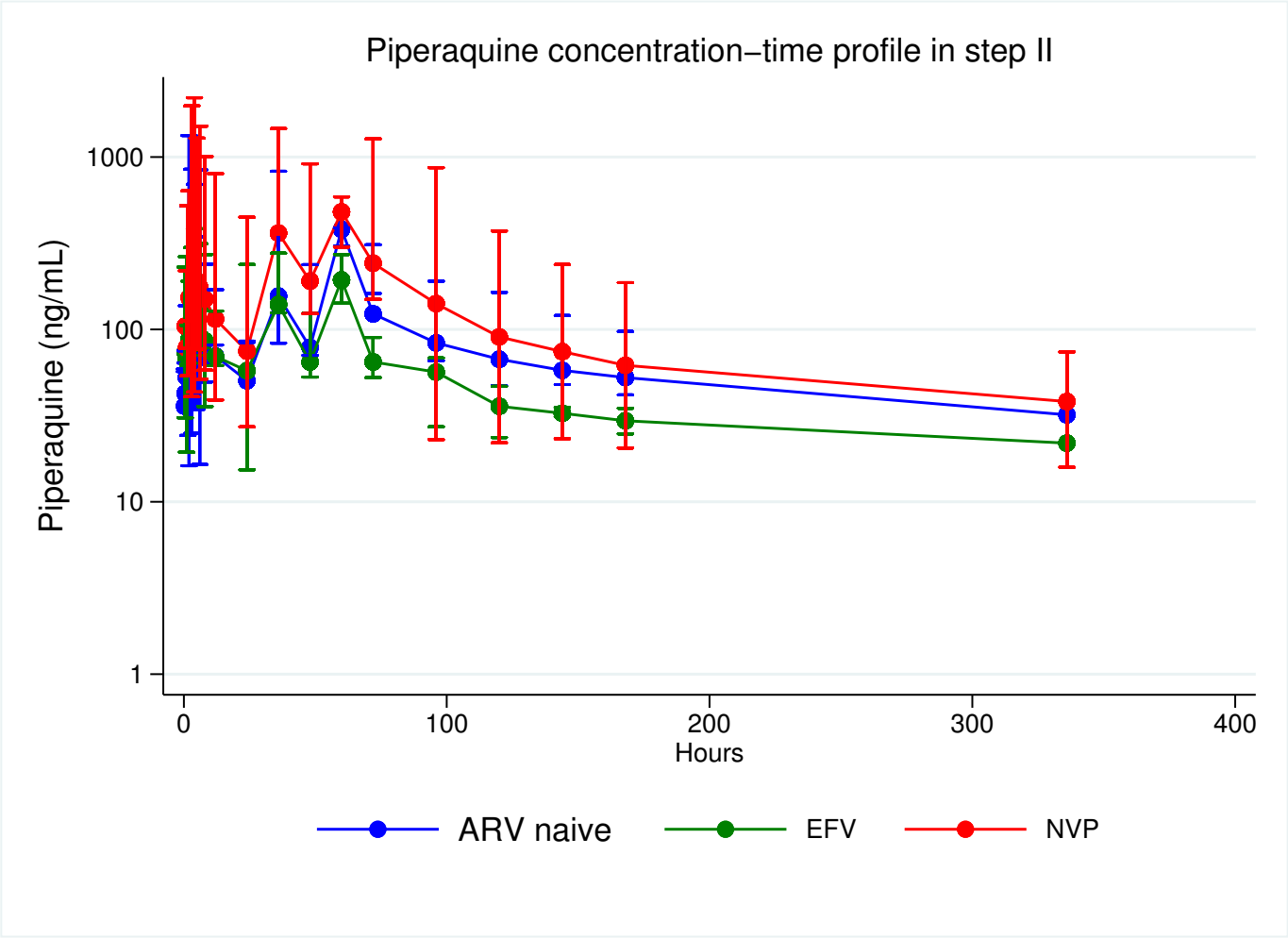

Table 1: Baseline Characteristics for study participants in Step 1 and Step 2

	Step 1					Step 2			
Characteristic	DHA-PPQ+NVP Containing ART N=5	DHA-PPQ +EFV Containing ART N=6	DHA-PPQ +LPV/r Containing ART N=6	DHA-PPQ without ART N=6	P-value	DHA-PPQ+NVP Containing ART N=15	DHA-PPQ +EFV Containing ART N=15	DHA-PPQ without ART N=10	P-value
Gender (n, % female)	3 (50.0)	2 (33.3)	2 (33.3)	4 (66.7)	0.811	13 (86.7)	13 (86.7)	5 (50.0)	0.071
Median age (range, years)	39 (34–62)	43 (36–56)	41 (20–63)	29 (23–46)	0.360	36 (28–44)	36 (24–60)	40 (33–62)	0.060
Mean haemoglobin (SD, g/dL)	13.9 (1.3)	12.7 (1.6)	13.1 (1.6)	12.9 (1.0)	0.633	13.3 (2.1)	13.4 (2.2)	13.9 (2.9)	0.830
Median Body Mass Index (range in kg/m^2)	24.3 (22.0–25.5)	20.4 (18.7–23.1)	19.8 (17.5–25.7)	23.9 (19.9–26.4)	0.071	23.1 (18.0–28.8)	20.9 (16.0–19.0)	21.3 (18.4–27.4)	0.602
Median (range) duration of ART intake at the time of screening (in months)	26.3 (7.0–55.7)	24.5 (15.2–49.9)	65.7 (52.2–86.9)	NA	0.020	47.7 (10.2–80.4)	39.8 (7.1–120.1)	NA	0.371
On Cotrimoxazole prophylaxis, n (%)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	1.000	13 (86.7)	13 (86.7)	7 (70.0)	0.511
ALT (IU/L)	26 (12–39)	35 (20–44)	20 (15–23)	18 (11–19)	0.024	23 (15–39)	22 (11–38)	21 (17–28)	0.750
% with AST >ULN n (%)	2 (33.3)	4 (66.7)	0 (0.0)	0 (0.0)	0.092	5 (33.3)	3 (20.0)	3 (30.0)	0.743

AST (IU/L)	27 (19–58)	39 (24–46)	29.5 (21–35)	23 (19–27)	0.081	27 (17–52)	29 (21–53)	28 (20–34)	0.524
% with ALT >ULN n (%)	2 (33.3)	3 (50.0)	0 (0.0)	0 (0.0)	0.284	3 (20.0)	3 (20.0)	2 (20.0)	1.000
Creatinine (umol/L)	67 (42–139)	57 (38–67)	73 (44–90)	58 (51–69)	0.332	60 (41–83)	55 (32–69)	59 (47–68)	0.871
% with Creatinine >ULN n (%)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0.221	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Any anemia, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000	2 (22.0)	0 (0.0)	0 (0.0)	0.330
Any leucopenia, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1.000	5 (33.3)	1 (6.7)	3 (30.0)	0.232
Any neutropenia, n (%)	2 (33.3)	2 (33.3)	4 (66.7)	1 (16.7)	0.460	3 (20.0)	5 (33.3)	3 (30.0)	0.741
Any thrombocytopenia, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1.000	4 (26.7)	1 (6.7)	1 (10.0)	0.410
Median CD4 cell count (range, cells/UL)	441 (254–832)	386 (273–757)	422 (375–691)	411 (324–734)	0.670	476 (298–685)	389 (274–1222)	429 (393–888)	0.311

Table 2a: Piperaquine pharmacokinetic parameters for participants in step 1

	Study groups				Geometric Mean Ratio [90% CI] (p-value)		
	ART naïve n=6	NVP n=5[§]	LPV/r n=6	EFV n=6	NVP/ART naïve	LPV/r/ART naïve	EFV/ART naïve
$AUC_{0\text{-}28\ days}$ hr.ng/mL	33385 [26131-42652]	43632 [31383-60662]	38300 [27256-53802]	18914 [14144-25291]	1.31 [0.86-1.99] (0.290)	1.15 [0.75-1.76] (0.589)	0.57 [0.38-0.83] (0.029)
C_{max} (ng/mL)	350 [252-485]	637 [453-897]	327 [263-406]	253 [156-412]	1.82 [1.13-2.94] (0.061)	0.94 [0.63-1.39] (0.775)	0.72 [0.40-1.32] (0.371)
t_{max} (hr)	3 [2-60]	4 [3-5]	60 [60-60]	3 [2-60]	0.573[a]	0.049[a]	1.000[a]
$t_{1/2}$ (hr)*	332 [174-631]	319 [262-388]	455 [186-1114]	227 [120-432]	0.36 [0.49-1.89] (0.915)	1.37 [0.44-4.31] (0.636)	0.68 [0.36-1.30] (0.658)

PK parameters are presented as geometric mean [90% confidence interval] except t_{max} which is presented as median [interquartile range].

P-value is calculated using analysis of variance (ANOVA) in Stata 15.0, α= 0.1

ART=antiretroviral therapy; NVP=Nevirapine-based ART; EFV=Efavirenz-based ART; LPV/r=Ritonavir boosted lopinavir based ART

C_{max}=maximal concentration, t_{max}=time to reach maximal concentration, $t_{1/2}$=drug elimination half-life

$AUC_{0\text{-}28\ days}$ =area under the concentration-time curve from 0 to 28 days

§: One participant did not complete follow up and was excluded from analysis

a: p-value only, calculated using Wilcoxon rank sum test, α= 0.05

* Half-life estimation excluded below lower limit of quantification values

Table 2b: Piperaquine pharmacokinetic parameters for participants in step 2

	Study groups			Geometric Mean Ratio [90% CI] (p-value)	
	ART naïve n=10	NVP n=15	EFV n=15	NVP/ART naïve	EFV/ART naïve
$AUC_{0\text{-}28\ days}$, hr.ng/mL	27573 [23208-32759]	36747 [28419-47516]	15792 [13094-19048]	1.33 [0.98-1.82] (0.179)	0.57 [0.44-0.74] (0.002)
C_{max} (ng/mL)	430 [315-587]	557 [424-731]	245 [175-343]	1.30 [0.85-1.96] (0.314)	0.57 [0.36-0.90] (0.065)
t_{max} (hr)	60 [60-60]	60 [36-60]	60 [24-60]	0.841[a]	0.441[a]
$t_{1/2}$ (hr)*	136 [72-255]	76 [36-160]	49 [27-90]	0.56 [0.21-1.51] (0.356)	0.36 [0.15-0.87] (0.072)
C_{d7} (ng/mL)[#]	53 [39-71]	62 [46-84]	39 [32-48]	1.17 [0.76-1.83] (0.519)	0.74 [0.51-1.07] (0.469)

PK parameters are presented as geometric mean (90% confidence interval) with exception of t_{max} which is given as median [interquartile range].

P-value is calculated using analysis of variance (ANOVA) in Stata 15.0, α= 0.1

ART=antiretroviral therapy; NVP=Nevirapine-based ART; EFV=Efavirenz-based ART.

C_{max}=maximal concentration, t_{max}=time to reach maximal concentration, $t_{1/2}$=drug elimination half-life.

$AUC_{0\text{-}28\ days}$=area under the concentration-time curve from day 0 to 28, C_{d7}= day 7 piperaquine concentration

* Half-life estimation excluded below lower limit of quantification values for each participant

a: p-value only, calculated using Wilcoxon rank sum test, α= 0.05

#: Day 7 piperaquine n=22, below lower limit of quantification values excluded resulting in number of observations as follows ART naïve=2, NVP=10, EFV=10