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

Clifford G. Banda\textsuperscript{a,b}\#, Fraction Dzinjalamala\textsuperscript{a,b}, Mavuto Mukaka\textsuperscript{a,c,f}, Jane Mallewa\textsuperscript{a,b}, Victor Maiden\textsuperscript{b}, Dianne J Terlouw\textsuperscript{b,d}, David G. Lalloo\textsuperscript{d}, Saye H. Khoo\textsuperscript{e}, Victor Mwapasa\textsuperscript{a,b}\#

\textsuperscript{a}Malawi College of Medicine, Blantyre, Malawi
\textsuperscript{b}Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi
\textsuperscript{c}Oxford Centre for Tropical Medicine and Global Health, Oxford, United Kingdom
\textsuperscript{d}Liverpool School of Tropical Medicine, Liverpool, United Kingdom
\textsuperscript{e}University of Liverpool, Liverpool, United Kingdom.
\textsuperscript{f}Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Running Head: Amodiaquine and antiretroviral therapy

Abstract word count: 216

Text word count: 3771

# Address correspondence to Clifford G Banda, cgbanda@mlw.mw or to Victor Mwapasa, vmwapasa@medcol.mw
There are limited data on the pharmacokinetic and safety profiles of artesunate-amodiaquine in human immunodeficiency virus infected (HIV+) individuals receiving antiretroviral therapy. In a two-step intensive sampling pharmacokinetic trial, we compared area under the concentration-time curve from 0 to 28 days (AUC_{0-28 days}) of an active metabolite of amodiaquine, desethylamodiaquine, and treatment-emergent adverse events between antiretroviral therapy-naive HIV+ adults and those taking nevirapine and ritonavir-boosted lopinavir-based antiretroviral therapy. In step 1, malaria uninfected adults (n=6/arm) received half the standard adult treatment regimen of artesunate-amodiaquine. In step 2, another cohort (n=25/arm) received the full regimen. In step 1, there were no safety signals and significant differences in desethylamodiaquine AUC_{0-28 days} among participants in the ritonavir-boosted lopinavir, nevirapine and antiretroviral therapy-naïve arms. In step 2, compared with the antiretroviral therapy-naïve arm, participants in the ritonavir-boosted lopinavir arm had 51% lower desethylamodiaquine AUC_{0-28 days}, (geometric mean [95% CI]; 23,822 [17,458-32506] vs 48,617 [40,787-57,950] ng.hr/mL, \( p < 0.001 \)). No significant differences in AUC_{0-28 days} were observed between nevirapine and antiretroviral therapy-naïve arms. Treatment-emergent transaminitis was higher in the nevirapine (20% [5/25]) than the antiretroviral therapy naïve (0.0% [0/25]) arm (risk difference 20% [95% CI:4.3-35.7] \( p=0.018 \)). Ritonavir-boosted lopinavir antiretroviral regimen was associated with reduced desethylamodiaquine exposure which may compromise artesunate-amodiaquine’s efficacy. Co-administration of nevirapine and artesunate-amodiaquine may be associated with hepatotoxicity.

**Key words:** Amodiaquine; Antiretroviral therapy; Malaria
INTRODUCTION

Human immunodeficiency virus (HIV) and *Plasmodium falciparum* (*Pf*) malaria infections are endemic in most regions in sub-Saharan Africa (SSA) and co-infections occur frequently. HIV infection increases susceptibility (1–3) and severity of *Pf* malaria (4–6), and reduces the efficacy of antimalarial drugs (7). The World Health Organisation (WHO) recommends initiation of triple antiretroviral therapy (ART) in HIV-positive (HIV+) individuals regardless of CD4 cell count (8).

The recommended ART in SSA contain non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as efavirenz (EFV) and nevirapine (NVP), or protease inhibitors (PIs) such as ritonavir-boosted lopinavir (LPV/r). The WHO also recommends artesunate-amodiaquine (AS-AQ), as one of the first-line treatment for uncomplicated malaria (9).

HIV-malaria co-infected individuals require concurrent treatment with ACTs and ART, potentially resulting in pharmacokinetic interactions (10). Drug information sheets for ACTs caution against concurrent use of ACTs and ART because NNRTIs or PIs and ACTs are metabolized by cytochrome-P (CYP) 450 liver enzymes (particularly, CYP3A4). NNRTIs such as nevirapine and efavirenz usually induce various CYP450 enzymes but are also substrates for CYP450 isoforms (CYP3A4) (11, 12). AQ is rapidly metabolised, mainly by CYP2C8 but also CYP3A4, to its metabolite, desethylamodiaquine (DESAQ), which is responsible for almost all the antimalarial effect (13, 14). This metabolite has a longer half-life and is eliminated slowly compared to AQ (13–19). Thus, co-administration of NNRTI-based ART with AS-AQ could reduce AQ and DESAQ blood concentrations resulting in lower efficacy of AQ. Conversely, HIV protease inhibitors, particularly ritonavir, are potent inhibitors of the CYP3A4 isoform. Co-administration of protease inhibitor-based ART with AS-AQ could lead to elevated AQ and lower DESAQ concentrations, potentially resulting in toxicities or reduced AS-AQ efficacy (20).
To characterize the interactions between AS-AQ and ART, we compared the pharmacokinetic parameters ($AUC_{0-28\ days}$, $C_{\text{max}}$, $t_{\text{max}}$, and $t_{1/2}$) of the longer acting partner drug of AS-AQ, amodiaquine and of its metabolite-DESAQ, and incidence of adverse events in HIV+ adults taking AS-AQ plus NVP-ART or LPV/r-ART and those taking AS-AQ only in a parallel design (two-step) study.

RESULTS

Characteristics of study participants

In step 1, 18 participants were successfully enrolled and followed up for 28 days, including 1 subject who replaced a participant who was withdrawn following a protocol violation. In step 2, 75 were enrolled and successfully followed up to 28 days, including 2 who replaced those who were lost to follow-up.

Supplementary Table 1 shows baseline characteristics of participants who completed follow-up in steps 1 and 2. In both step 1 and step 2, the majority of participants in all study arms, except the step 1 ART naïve arm, were females. Participants in the LPV/r arm had a tendency towards higher alkaline phosphatase levels at baseline than those in the ART-naïve arm. In step 2, participants in the LPV/r arm had a higher median age than those in the other study arms. The median duration on ART was longer in the LPV/r than the NVP arm. All the participants in step 1 and the majority (80%) in step 2 were on cotrimoxazole prophylaxis.

Pharmacokinetics of AQ and DESAQ and interactions with ART in step 1

PK data were available for 17 of the 18 participants who completed follow-up in step 1. The excluded participant had unquantifiable drug or metabolite concentrations at nearly all follow up time points. AQ concentrations were well below the HPLC assay limit of quantification.
Amodiaquine and antiretroviral therapy

(LLQ=25ng/mL). Therefore, no formal comparisons of AQ PK parameters were performed across the study arms.

As shown in Table 1a, the geometric mean [95% CI] of DESAQ C_max was 60% lower in the LPV/r-ART arm (42 [34-51] ng/mL) compared to the ART-naïve arm (106 [63-179] ng/mL, p=0.006), while no significant difference in DESAQ AUC_0-28 days was observed between the LPV/r-ART (4,128 [1,946-8,758] ng.hr/mL) and the ART-naïve (7,920 [5034-12459] ng.hr/mL, p=0.10) arms. The C_max for DESAQ were similar between participants in the NVP and ART-naïve arms. Similarly, no differences in mean AUC_0-28 days were observed between the NVP-ART and ART-naïve arms. As shown in the concentration-time plot in Figure 1, DESAQ concentration-time profile was notably lower in the LPV/r-ART arm compared to the ART-naïve and NVP-ART arms. There were no significant differences in half-life and T_max of DESAQ between the NVP and ART naïve arms as well as between the LPV/r and ART naïve arms.

Safety assessment in step 1

After AS-AQ administration, one participant in the NVP arm developed headache and chills, which resolved without any treatment and were judged as not related to the study drug. As shown in Table 2a, treatment-emergent grade 3 or 4 neutropenia was observed in the NVP-ART arm (50% [3/6]), LPV/r-ART arm (33% [2/6]) and ART-naïve arm (17% [1/6]). One participant in the AS-AQ plus NVP arm had a car accident which was not thought to be related to the study drug.

Pharmacokinetics of DESAQ and interactions with ART in step 2

In step 2, PK data were available for 74 of the 75 participants who completed follow-up. The excluded participant had unquantifiable drug or metabolite concentrations at nearly all follow up
Amodiaquine and antiretroviral therapy

Similar to our observation in step 1, AQ concentrations in step 2 were well below the HPLC assay limit of quantification (LLQ=25ng/mL).

Table 1b shows that the geometric mean [95% CI] of DESAQ C\textsubscript{max} was 45% lower in the LPV/r-ART arm (248 [199, 310] ng/mL) compared to the ART-naïve arm (448 [374, 534] ng/mL, p<0.001), while DESAQ AUC\textsubscript{0-28 days} was 51% lower in the LPV/r-ART arm (23,822 [17,458-32,506] ng.hr/mL) compared to the ART-naïve arm (48,617 [40,787-57,950] ng.hr/mL, p<0.001).

In contrast, there were no significant differences in AUC\textsubscript{0-28 days} and C\textsubscript{max} between the NVP-treated and the ART naive arms. Also, there were no significant differences in DESAQ T\textsubscript{max} among the ART-naïve, LPV/r-ART and NVP-ART study arms. DESAQ half-life and clearance were significantly shorter and faster, respectively, in the LPV/r-ART arm compared with the ART-naïve arm.

Figure 1 shows the concentration-time plot for DESAQ in the study arms. Similar to the findings in steps 1, DESAQ concentration-time profile in step 2 was notably lower in the LPV/r-ART arm when compared with the ARV-naïve arm.

**Day 7 plasma DESAQ levels by ART arm in step 2**

Compared with the geometric mean concentration [95% CI] of DESAQ at day 7 in the ART-naïve arm (94 [73, 120] ng/ml), the concentration was 52% lower in the LPV/r arm (45 [29, 73] ng/ml, p=0.011) and was 28% lower in the NVP arm (68 [57, 80] ng/ml, p=0.092). However, there were no significant differences in the proportion of participants with Day 7 DESAQ levels below 75ng/ml (a threshold associated with 100% parasitological cure rate (19)) between the LPV/r arm (67%, [14/21]) and the ART-naïve arm (43%, [9/21], p=0.215), and between the ART naïve arm and the NVP arm (56%, [14/25], p=0.554).
Safety assessment in step 2

Overall, gastrointestinal symptoms (such as vomiting or diarrhoea) or neurological symptoms (such as headache) were not reported following intake of AS-AQ in the different study arms. However, as shown in Table 2b, there was a statistically non-significant trend towards higher incidence of grade 3 or 4 treatment emergent neutropenia in the NVP arm (28.0% [7/25] compared to ART-naïve arm (16.0% [4/25], p=0.496). The incidence of grade 3 or 4 post-dosing neutropenia was lower in the LPV/r arm (0.0% [0/25], p=0.110). The incidence of treatment-emergent grade 3 or 4 transaminitis (concurrent ALT and AST elevation) was higher in the NVP arm (20% [5/25]) than the ART naïve arm (0.0% [0/25], risk difference 20% [95% CI: 4.3, 35.7] p=0.018). Similar to the ART-naïve arm, there were no cases of treatment-emergent grade 3 or 4 transaminitis in the LPV/r arm. Two cases of QTc prolongation (change in QTc >60ms from baseline to Cmax) were detected in both the LPV/r-ART arm (8.0%, n=25) and the NVP-ART arm (8.0%, n=25) arms but none were detected in the ART-naïve arm (0.0%, n=25).

No significant differences were found between any of the ART arms and the ART-naïve arm (p=0.490). These cases resolved spontaneously within two weeks of occurrence.

DISCUSSION

In this study, we found that median DESAQ AUC and Cmax were significantly lower in the LPV/r- arm when compared to the ART-naïve arm but no differences were observed in these PK parameters between the NVP and ART-naïve arms. While AS-AQ appeared to be generally tolerated in all study arms, treatment-emergent transaminitis was more common in the NVP-arm than in the ART-naïve arm.

Our findings of insignificant differences in PK parameters of DESAQ between the ART naive and NVP group are in contrast with those from a previous Nigerian open-label parallel-arm PK
Amodiaquine and antiretroviral therapy

study which found a lower DESAC AUC in HIV-infected adults on NVP-based ART than in ART naïve participants (21). These differences could be due to several reasons including genetic differences in CYP450 iso-enzymes of the study populations. Additional studies would be needed to explain the reasons for this discrepancy.

Although highly expressed in the liver, CYP family enzymes, especially CYP3A4 and CYP2C8, are expressed in the small intestinal epithelium and play an active role in the metabolism of drugs (22–24). Findings of significantly reduced DESAC \( C_{\text{max}} \) in the LPV/r arm at full standard dose in step 2 may partly be due to reduced CYP2C8-mediated gut or liver metabolism of AQ to DESAQ. This is plausible as CYP2C8 is the main hepatic P450 isoform that clears AQ and catalyses the formation of DESAQ (13)(25). Consequently, inhibition of CYP2C8 by its known potent inhibitors, LPV and ritonavir (10), are likely to account for the observed reduction in \( C_{\text{max}} \).

Alternatively, the reduced DESAQ AUC in the LPV/r could be as a result of rapid clearance of DESAQ in the LPV/r arm compared to the ART naïve arm. However, this increased clearance is inconsistent with the known inhibitory effects of LPV/r on CYP2C8 (25). DESAQ is eliminated through extrahepatic CYP1A1 and CYP1B1 (25, 26), any potential impact that LPV/r may have on clearance of DESAQ by CYP1A1 and CYP1B1 needs to be further evaluated.

Since DESAQ is responsible for nearly all the antimalarial effect of AQ (13, 14), it is likely that lower DESAQ exposure (reduced \( C_{\text{max}} \) and AUC at full standard dose) in those taking LPV/r may result in lower treatment efficacy or prophylactic effect. Indeed, previous studies which administered amodiaquine base at a dosage of 10 mg/kg/day found that lower day 7 DESAQ concentrations were associated with an increased risk of treatment failure (14)(19). In the study by Strepniewska (19), patients with Day-7 DESAQ concentrations above 75 ng/mL achieved 100% parasitological cure rate while 60% (n=5) of the participants who had Day-7 DESAQ concentrations of below 75 ng/mL had PCR confirmed recrudescent parasitaemia. The daily
Amodiaquine and antiretroviral therapy

and total amodiaquine dose received by participants in step 2 (9.5 mg/kg/day and 28.5 mg/kg, respectively) falls within the middle of WHO’s therapeutic dose range of 7.5 to 15 mg/kg/day for amodiaquine (27)(28)(14). The higher frequency of participants below the 75 ng/mL level in the LPV/r arm suggests that, in this population, the current dosage of AS-AQ may likely result in treatment failure or recurrent malaria infections.

Our finding of a higher incidence of neutropenia in the NVP-ART arm than the ART naïve arm is consistent with results from a previous Ugandan study which found an increased risk of neutropenia in children receiving AQ-AS and ART (20). Although blood levels of AQ and AS were not measured in the Ugandan study, the observed cases of neutropenia could have been due to high AQ or DESAQ levels. NVP has been associated with granulocytopenia as a marker of hypersensitivity (29). Any potential synergistic role of AQ and NVP in causing neutropenia or other haematological abnormalities requires further understanding. Additionally, administration of AS-AQ in our study was associated with transient liver function abnormalities, especially in people taking NVP-based ART. This finding is similar to significant increases in liver transaminase levels observed in a previous study when AS-AQ was co-administered with an NNRTI (efavirenz) (30). NVP is independently associated with hepatotoxicity (31, 32), so is AQ (33, 34). Thus, combining these drugs may have an additive hepatotoxic effect. The observed cases of transaminitis in the NVP arm could have been due to an increase in NVP concentrations following co-administration with AQ or a result of a synergistic effect of NVP and AQ as previously experienced among individuals taking an NNRTI (efavirenz) and AQ (30). Since we did not measure NVP concentrations, we were unable to ascertain the pharmacokinetic changes in steady state concentrations of NVP after administration of AQ and the impact this may have on incidence of transaminitis. Despite the fact that haematological and hepatic abnormalities found in our study were not clinically significant and did not persist beyond two weeks, our findings suggest that caution should be exercised when co-administering AS-AQ...
and NVP or the need for careful monitoring of liver function and haematological changes in malaria-infected HIV+ patients taking AS-AQ, particularly those taking AS-AQ plus NVP.

The present study was not adequately powered to detect adverse events such as cardiac toxicity. In our study, AQ levels were below the HPLC assay limit of quantification possibly due to lack of sensitivity of the assay in detecting very low plasma drug concentrations. Although this study was not aimed at examining dose proportionality between the two steps, the inability to observe this and to detect significant differences in PK parameters across arms and between steps may have been due to a very small sample size in step 1 relative to step 2 and the use of the parallel-arm design, which is more prone to effects of inter-individual anthropometric and genetic variations than a cross-over design. Genetic polymorphisms in CYP 450 iso-enzymes may have contributed to wide interquartile ranges of DESAQ PK parameters observed within each study arm. However, our study sample size is unlikely to have missed large (>2-fold) clinically important differences in AUC across the study arms. Future studies should explore dose linearity when AS-AQ is administered with antiretroviral drugs, assess the effect of genetic polymorphisms on the pharmacokinetics of DESAQ, quantify any changes in plasma ART levels when co-administered with antimalarial drugs and explore any potential impact of artesunate on the metabolism of amodiaquine when co-administered with antiretroviral drugs.

In conclusion, this study found significant PK interactions between LPV/r and AS-AQ, and signals of transaminitis and neutropenic effects among those taking NVP and AS-AQ. The clinical therapeutic implications of these findings in malaria-infected individuals on ART need further evaluation.
Amodiaquine and antiretroviral therapy

MATERIALS AND METHODS

Study Design

We conducted an open-label, parallel arm, pharmacokinetic (PK) trial at Queen Elizabeth Central Hospital, Malawi, from August 2010 to March 2013. The study was implemented in two steps;

1. In step 1 (N=18) [PACTR2010030001871293], we administered half adult oral doses of AS-AQ (1 tablet of Coarsucam™, Sanofi-Aventis containing AS/AQ 100mg/270mg) at 0, 24 and 48 hours, to HIV+ malaria-negative individuals in the following arms: (i) those on NVP-d4T-3TC, (ii) those on AZT-3TC-TDF-LPV/r and (ii) antiretroviral naive individuals which served as a control arm. step 1 served as a safety evaluation step, checking for unexpected clinical toxicities or interactions.

2. In step 2 (N=75) of the study [PACTR2010030001971409], after review of step 1 safety data by an independent Data Safety Monitoring Board (DSMB), full treatment doses of AS-AQ (2 tablets of Coarsucam™, Sanofi-Aventis, each containing AS/AQ 100mg/270mg) were administered to additional HIV+ individuals in the same arms and at the same time intervals as in step 1.

All doses of AS-AQ were administered with water only as recommended by Sanofi-Aventis.

Study Population

The target population for both steps were HIV+ male and non-pregnant female adults aged ≥18 years residing in Blantyre or neighbouring districts of Thyolo and Chiradzulu. Individuals were eligible if they had been on NVP-ART or LPV/r-ART for ≥ 6 months and had CD4 cell count ≥
Amodiaquine and antiretroviral therapy

250 cells/mm\(^3\). At the beginning of the study, HIV+ antiretroviral naive individuals were eligible for recruitment into the study if they had a CD4 cell count ≥ 250/mm\(^3\) but this cut-off point was increased to ≥350/mm\(^3\) when the new WHO criteria for ART initiation was implemented in Malawi in July 2011 (35). Other inclusion criteria were body weight ≥40kgs, willingness to be admitted in the hospital for 3 days, to remain within the study sites and be contacted by phone or at home during the course of the study.

We excluded subjects who met any of the following criteria:

i. Body Mass Index ≤18.5kg/m\(^2\)
ii. Haemoglobin concentration <8.5 g/dL
iii. Reported use of any antimalarial drugs within the preceding 4 weeks
iv. Reported hypersensitivity to any of the ACTs
v. Receipt of other drugs which are known inhibitors or inducers of P450 enzymes or P-glycoprotein (except cotrimoxazole prophylaxis)
vi. History of regular intake of alcohol (>twice/week), tobacco (>3 times/week) or use of illicit drugs
vii. History or evidence of pre-existing liver, kidney or heart disease, including conductive abnormalities on electrocardiographs (\(QTc\) interval >450ms in men, >470ms in females)
viii. Clinical and/or laboratory evidence of \(Pf\) malaria, hepatitis B, pneumonia, tuberculosis, bacteraemia or laboratory evidence of potentially life threatening disorders
ix. Karnofsky score of <80%

Sample size:
The sample size in step 1 was 6 for each of the three arms. This approach 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
Amodiaquine and antiretroviral therapy

drug levels. The sample size for step 2 was 25 per arm which gave at least 90% power to detect a two-fold increase in the DESAQ AUC in any of the AS-AQ plus ART arms, assuming a mean DESAQ AUC of 154 ng/ml/hr (standard deviation of 150 ng/ml/hr (2)) in the AS-AQ control arm, at the 5% significance level.

Ethics

The study conformed to the principles of the International Conference on Harmonization on Good Clinical Practice and was approved by the College of Medicine Research Ethics Committee (COMREC) in Malawi. Written informed consent, to participate in the study, was sought from potential participants.

Screening and enrolment

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. Based on the results from screening procedures which were available within 7 days, potential study participants were informed about their eligibility to participate in the study. 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 follow-up procedures

While participants were hospitalized, blood samples for PK assays were collected in heparin vacutainer tubes, pre-treatment and at the following post-treatment times: 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 60 and 72 hours. After discharge from hospital, blood samples were taken at 4, 5, 6, 7, 14, 21 and 28 days. Immediately after collection, samples were spun in a refrigerated centrifuge and the separated plasma was temporarily frozen in liquid nitrogen before being transferred to a -80°C freezer until PK analyses.

Participants were monitored for 28 days after administration of the first study dose to detect clinical adverse events. Blood samples to detect haematological, renal and liver function abnormalities were collected at 12, 48 and 72 hours and days 7, 14, 21 and 28. Participants were monitored for treatment emergent adverse events (AEs), defined as any clinical or subclinical abnormality which was absent before dosing with AS-AQ but emerged post dosing, or a clinical or subclinical abnormality which was present before dosing with AS-AQ but worsened post-dosing. Severity of AEs was graded using the DAIDS criteria (36). In addition, 12-lead ECGs were performed pre-dosing, 2 hours after the first dose and 2 hours after the last dose in step 2 to assess Fridericia-corrected (37) QT interval.

Pharmacokinetic assays

Plasma samples were analysed for AQ and DESAQ levels using a validated HPLC-UV assay adopted and transferred to Malawi-Liverpool Wellcome Trust Clinical Research Programme in Blantyre, Malawi from Liverpool School of Tropical Medicine. The PK laboratory in Blantyre participated in WWARN’s External Quality Assurance programme (38). Briefly, AQ/DESAQ and the internal standard (Quinidine) were recovered from plasma using liquid extraction (diethyl/tert-butyl ether). The supernatant was evaporated to dryness in a vacuum concentrator at 25°C. The
Amodiaquine and antiretroviral therapy

residue was re-dissolved in 200 µl of the reconstitution mobile phase: Water–Acetonitrile–
Triethylamine (85:15:1, v/v/v; pH 3) and 75 µL was injected into the chromatograph (Agilent
1100). The optimum detection wavelength for each drug was 345 nm. The lower limit of
quantification (LLOQ) of the HPLC-UV assay was 25 ng/mL for the drugs AQ/DESAQ. Extracted
plasma PK samples were run in batches. 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, 1500 and 3000 ng/mL for AQ/DESAQ). For
batch assay to pass the measured concentrations, 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 LLOQ. In
addition, 75% of each calibration curve’s concentrations had to lie within +/-20% and +/-15% of
the nominal concentration at the LLOQ or all other concentrations, respectively. The mean
interassay precision for low, medium and high QCs was 15%, 9% and 6% respectively.

Data analyses

Plasma concentrations of AQ/DESAQ were analysed using non-compartmental
pharmacokinetic analysis (NCA), employing the trapezoidal rule with cubic splines. Observed
AQ/DESAQ concentrations below 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$_{0-28}$ days, maximum concentration [$C_{\text{max}}$], time to maximum
concentration [$t_{\text{max}}$] and terminal elimination half-life [$t_{1/2}$]). We used STATA 15.0 for the NCA
and to compare PK parameters. The Two-sample Wilcoxon rank-sum (Mann-Whitney-U test)
was used to test any significant differences in PK parameters between each ACT/ART arm and
the control arm ($\alpha=0.05$). Geometric means and their 95% confidence intervals have been
reported. 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.
Amodiaquine and antiretroviral therapy

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.
ACKNOWLEDGEMENTS

We thank all trial participants, and Prof Joel Tarning for his valuable comments and advice on PK assays and the draft manuscript. We also thank Dr Paolo Denti for his assistance with technical aspects of non-compartmental analysis, and Prof Steve Ward for supporting with laboratory training of study personnel in PK assay methods. This work was supported by European & Developing Countries Clinical Trials Partnership (EDCTP) [IP.07.31060.003]. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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).
Amodiaquine and antiretroviral therapy

REFERENCES

1. Laufer MK, van Oosterhout JJG, Thesing PC, Thumba F, Zijlstra EE, Graham SM, Taylor TE, Plowe CV. 2006. Impact of HIV-Associated Immunosuppression on Malaria Infection and Disease in Malawi. J Infect Dis 193:872–878.

2. Rosenthal PJ. 2006. Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. Lancet 356:1051–1056.

3. Hewitt K, Steketee R, Mwapasa V, Whitworth J, French N. 2006. Interactions between HIV and malaria in non-pregnant adults: evidence and implications.

4. Cohen C, Karstaedt A, Frean J, Thomas J, Govender N, Prentice E, Dini L, Galpin J, Crewe-Brown H. 2005. Increased Prevalence of Severe Malaria in HIV-Infected Adults in South Africa. Clin Infect Dis 41:1631–1637.

5. Grimwade K, French N, Mbatha DD, Zungu DD, Dedicato M, Gilks CF. 2004. HIV infection as a cofactor for severe falciparum malaria in adults living in a region of unstable malaria transmission in South Africa. AIDS 18:547–54.

6. Chalwe V, Van geertruyden J-P, Mukwamataba D, Menten J, Kamalamba J, Mulenga M, D’Alessandro U. 2009. Increased risk for severe malaria in HIV-1-infected adults, Zambia. Emerg Infect Dis 15:749; quiz 858.

7. Van Geertruyden J-P, Mulenga M, Mwananyanda L, Chalwe V, Moerman F, Chilengi R, Kasongo W, Van Overmeir C, Dujardin J, Colebunders R, Kestens L, D’Alessandro U. 2006. HIV-1 immune suppression and antimalarial treatment outcome in Zambian adults with uncomplicated malaria. J Infect Dis 194:917–925.

8. WHO. 2017. WHO | Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. WHO.

9. WHO. 2017. WHO | Guidelines for the treatment of malaria. Third edition. WHO.
Amodiaquine and antiretroviral therapy

10. Khoo S, Back D, Winstanley P. 2005. The potential for interactions between antimalarial and antiretroviral drugs. AIDS 19:995–1005.

11. Malaty LI, Kuper JJ. 1999. Drug interactions of HIV protease inhibitors. Drug Saf 20:147–69.

12. Walubo A. 2007. The role of cytochrome P450 in antiretroviral drug interactions. Expert Opin Drug Metab Toxicol 3:583–598.

13. Adjei GO, Kristensen K, Goka BQ, Hoegberg LCG, Alfrangs M, Rodrigues OP, Kurtzhals JAL. 2008. Effect of concomitant artesunate administration and cytochrome P4502C8 polymorphisms on the pharmacokinetics of amodiaquine in Ghanaian children with uncomplicated malaria. Antimicrob Agents Chemother 52:4400–6.

14. Hietala SF, Bhattarai A, Msellem M, Röshammar D, Ali AS, Strömberg J, Hombhanje FW, Kaneko A, Björkman A, Ashton M. 2007. Population pharmacokinetics of amodiaquine and desethylamodiaquine in pediatric patients with uncomplicated falciparum malaria. J Pharmacokinet Pharmacodyn 34:669–686.

15. Mwesigwa J, Parikh S, McGee B, German P, Drysdale T, Kalyango JN, Clark TD, Dorsey G, Lindegardh N, Annerberg A, Rosenthal PJ, Kamya MR, Aweke F. 2010. Pharmacokinetics of artesunate-lumefantrine and artesunate-amodiaquine in children in Kampala, Uganda. Antimicrob Agents Chemother 54:52–9.

16. Navaratnam V, Ramanathan S, Wahab MSA, Siew Hua G, Mansor SM, Kiechel J-R, Vaillant M, Taylor WRJ, Olliaro P. 2009. Tolerability and pharmacokinetics of non-fixed and fixed combinations of artesunate and amodiaquine in Malaysian healthy normal volunteers. Eur J Clin Pharmacol 65:809–21.

17. Rijken MJ, McGready R, Jullien V, Tarning J, Lindegardh N, Phyo AP, Win AK, Hsi P, Cammas M, Singhavikanon P, White NJ, Nosten F. 2011. Pharmacokinetics of amodiaquine and desethylamodiaquine in pregnant and postpartum women with Plasmodium vivax malaria. Antimicrob Agents Chemother 55:4338–42.
Amodiaquine and antiretroviral therapy

18. Winstanley PA, Simooya O, Kofi-Ekue JM, Walker O, Salako LA, Edwards G, Orme ML, Brekenridge AM. 1990. The disposition of amodiaquine in Zambians and Nigerians with malaria. Br J Clin Pharmacol 29:695–701.

19. Stepniewska K, Taylor W, Sirima SB, Ouedraogo EB, Ouedraogo A, Gansané A, Simpson JA, Morgan CC, White NJ, Kiechel J-R. 2009. Population pharmacokinetics of artesunate and amodiaquine in African children. Malar J 8:200.

20. Gasasira AF, Kamya MR, Achan J, Mebrahtu T, Kalyango JN, Ruel T, Charlebois E, Staedke SG, Kekitiinwa A, Rosenthal PJ, Havlir D, Dorsey G. 2008. High Risk of Neutropenia in HIV-Infected Children following Treatment with Artesunate plus Amodiaquine for Uncomplicated Malaria in Uganda. Clin Infect Dis 46:985–991.

21. Scarsi KK, Fehintola FA, Ma Q, Aweeka FT, Darin KM, Morse GD, Akinola IT, Adedeji WA, Lindegardh N, Tarning J, Adedeji OO, Parikh S. 2014. Disposition of amodiaquine and desethylamodiaquine in HIV-infected Nigerian subjects on nevirapine-containing antiretroviral therapy. J Antimicrob Chemother 69:1370–6.

22. Obach RS, Zhang QY, Dunbar D, Kaminsky LS. 2001. Metabolic characterization of the major human small intestinal cytochrome p450s. Drug Metab Dispos 29:347–52.

23. Peters WH, Kock L, Nagengast FM, Kremers PG. 1991. Biotransformation enzymes in human intestine: critical low levels in the colon? Gut 32:408–12.

24. Bergheim I, Bode C, Parlesak A. 2005. Distribution of cytochrome P450 2C, 2E1, 3A4, and 3A5 in human colon mucosa. BMC Clin Pharmacol 5:4.

25. Li X-Q, Björkman A, Andersson TB, Ridderström M, Masimirembwa CM. 2002. Amodiaquine clearance and its metabolism to N-desethylamodiaquine is mediated by CYP2C8: a new high affinity and turnover enzyme-specific probe substrate. J Pharmacol Exp Ther 300:399–407.

26. Aweeka FT, German PI. 2008. Clinical Pharmacology of Artemisinin-Based Combination Therapy. Antimicrob Agents Chemother.
Amodiaquine and antiretroviral therapy

Therapies. Clin Pharmacokinet 47:91–102.

27. WHO | Guidelines for the treatment of malaria. Third edition.

28. WorldWide Antimalarial Resistance Network (WWARN) AS-AQ Study Group TWARN

29. Boehringer Ingelheim Pharmaceuticals I. 2005. Viramune ® (nevirapine) Tablets & Oral Suspension. Boehringer Ingelheim Pharm Inc Ridgefield, CT 06877 USA 1:4–28.

30. German P, Greenhouse B, Coates C, Dorsey G, Rosenthal PJ, Charlebois E, Lindegardh N, Havlir D, Aweeka FT. 2007. Hepatotoxicity Due to a Drug Interaction between Amodiaquine plus Artesunate and Efavirenz. Clin Infect Dis 44:889–891.

31. Luz M-C, Marina N, Juan G-L, Vincent S. 2003. Incidence of Liver Injury After Beginning...
Amodiaquine and antiretroviral therapy

Antiretroviral Therapy with Efavirenz or Nevirapine. HIV Clin Trials 4:115–120.

32. Sanne I, Mommeja-Marin H, Hinkle J, Bartlett JA, Lederman MM, Maartens G, Wakeford C, Shaw A, Quinn J, Gish RG, Rousseau F. 2005. Severe Hepatotoxicity Associated with Nevirapine Use in HIV-Infected Subjects. J Infect Dis 191:825–829.

33. Shimizu S, Atsumi R, Itokawa K, Iwasaki M, Aoki T, Ono C, Izumi T, Sudo K, Okazaki O. 2009. Metabolism-dependent hepatotoxicity of amodiaquine in glutathione-depleted mice. Arch Toxicol 83:701–707.

34. Clarke JB, Neftel K, Kitteringham NR, Park BK. 1991. Detection of antidrug IgG antibodies in patients with adverse drug reactions to amodiaquine. Int Arch Allergy Appl Immunol 95:369–75.

35. Clinical Management of HIV in adults and Children: Malawi Integrated Guidelines for Providing HIV Services.

36. DAIDS. 2004. Division of Aids Table for Grading the Severity of Adult and Pediatric Adverse Events Publish Date: December, 2004 Division of Aids Table for Grading the Severity of Adult and Pediatric Adverse Events Publish Date: December, 2004 1–20.

37. Fridericia LS. 2003. The duration of systole in an electrocardiogram in normal humans and in patients with heart disease. Ann Noninvasive Electrocardiol. Blackwell Science Inc.

38. Lourens C, Watkins WM, Barnes KL, Sibley CH, Guerin PJ, White NJ, Lindegardh N. 2010. Implementation of a reference standard and proficiency testing programme by the World Wide Antimalarial Resistance Network (WWARN). Malar J 9:375.
Amodiaquine and antiretroviral therapy

**LEGEND**

**Figure 1:** Desethylamodiaquine concentration-time profile (semi-log scale) in step 1 (left; n=17) and step 2 (right; n=74) following oral administration of half and full standard artesunate-amodiaquine adult treatment courses, respectively, among HIV infected ART naïve (blue), those on nevirapine- (red) and ritonavir-boosted lopinavir-based (green) antiretroviral therapy. Below limit of quantification concentrations are not included (resulting in observation time up to 144 hours in step 1 and 504 hours in step 2). Data are presented as mean (95% confidence interval).
Desethylamodiaquine concentration–time profile in step 1 and 2
Table 1a: Desethylamodiaquine pharmacokinetic parameters for participants in step 1

| Study groups          | Geometric Mean Ratio (p-value) |
|-----------------------|--------------------------------|
|                       | ART naïve | NVP | LPV/r | NVP/ART naïve | LPV/r/ART naïve |
|                       | n=5*      | n=6 | n=6   |               |                 |
| AUC\(_0\text{-}28\text{days},\text{hr}\cdot\text{ng/mL}\) | 7,920 (5,034-12,459) | 6,091 (3,096-11,983) | 4,128 (1,946-8,758) | 0.77 (0.465) | 0.52 (0.100) |
| C\(_{\text{max}}\) (ng/mL) | 106 (63-179) | 75 (54-105) | 42 (34-51) | 0.71 (0.273) | 0.40 (0.006) |
| T\(_{\text{max}}\) (hr) | 60 (36-60) | 60 (3-60) | 60 (36-60) | (0.562)* | (0.484)* |
| t\(_{1/2}\) (hr) | 59 (9-381) | 88 (23-331) | 75 (16-334) | 1.49 (0.715) | 1.27 (0.715) |

PK parameters are presented as geometric mean (95% confidence interval) except for T\(_{\text{max}}\), which is reported as median (range). P-value for the ratio is calculated using Wilcoxon rank sum test in Stata 15.0.

ART=antiretroviral therapy; NVP=Nevirapine-based ART; C\(_{\text{max}}\)=maximal concentration, T\(_{\text{max}}\)=time to reach maximal concentration, t\(_{1/2}\)=drug elimination half-life.

AUC\(_{\text{0-28\text{days}}}\)=area under concentration-time curve from 0 hours to 28 days.

* One participant did not have quantifiable DESAQ concentrations at nearly all follow up time points and was excluded from analysis.

a: p-value only, calculated using Wilcoxon rank sum test.
Table 1b: Desethylamodiaquine pharmacokinetic parameters for participants in step 2

| Study groups        | Geometric Mean Ratio (p-value) |
|---------------------|--------------------------------|
|                     | ART naive                      | NVP                           | LPV/r                       | NVP/ART naive | LPV/r/ART naive |
|                     | n=25                           | n=25                          | n=24*                       |               |                 |
| AUC 0-28 days, hr.ng/mL | 48,617 (40,787-57,950)         | 43,016 (38,300-48,313)        | 23,822 (17,458-32,506)      | 0.88 (0.308)  | 0.49 (0.0005)  |
| Cmax (ng/mL)       | 448 (374-534)                  | 360 (322-403)                 | 248 (199-310)               | 0.80 (0.067)  | 0.55 (0.0003)  |
| Tmax (hr)          | 60 (1.5-96)                    | 60 (3-60)                     | 60 (2-72)                   | 0.887*        | 0.248*         |
| t1/2 (hr)          | 166 (121-227)                  | 234 (201-272)                 | 90 (58-140)                 | 1.41 (0.037)  | 0.54 (0.023)   |

PK parameters are presented as geometric mean (95% confidence interval) except Tmax, which is reported as median (range). P-value for the ratio is calculated using Wilcoxon rank sum test in Stata 15.0; \( \alpha = 0.05 \).

ART = antiretroviral therapy; NVP = Nevirapine-based ART; LPV/r = ritonavir-boosted Lopinavir-based ART; Cmax = maximal concentration; Tmax = time to reach maximal concentration; t1/2 = drug elimination half-life.

AUC0-28 days = area under concentration-time curve from 0 hours to 28 days

* One participant did not have quantifiable DESAQ concentrations at nearly all follow up time points and was excluded from analysis

a: p-value only compared using Wilcoxon rank sum test
### Table 2a: Summary of DAIDS Grade 3 or 4 Treatment-emergent adverse events in Step 1

| DAIDS (Grade 3 or 4) Treatment-emergent abnormalities | AS-AQ (Without ART) N=6 | AS-AQ +NVP N=6 | AS-AQ +LPV/r N=6 |
|-------------------------------------------------------|-------------------------|----------------|------------------|
| n (%)                                                  | n (%)                   | n (%)          |
| Haematological events                                 |                         |                |
| Anaemia                                               | 0 (0)                   | 1 (17)         | 0 (0)            |
| Leucopenia                                            | 0 (0)                   | 0 (0)          | 0 (0)            |
| Lymphopenia                                           | 0 (0)                   | 0 (0)          | 0 (0)            |
| Neutropenia                                           | 1 (17)                  | 3 (50)         | 2 (33)           |
| Thrombocytopenia                                      | 0 (0)                   | 0 (0)          | 1 (17)           |
| Biochemical events                                    |                         |                |
| Elevated ALT and AST                                  | 0 (0)                   | 0 (0)          | 0 (0)            |
| Raised Creatinine                                     | 0 (0)                   | 0 (0)          | 0 (0)            |
| Cardiac events                                        |                         |                |
| QTc prolongation                                      | NA                      | NA             | NA               |

NA: ECG assessment not conducted in step 1
Table 2b: Treatment-emergent DAIDS Grade 3/4 abnormalities in Step 2

| DAIDS (Grade 3 or 4) Treatment-emergent abnormalities | AS-AQ (Without ART) N=25 | AS-AQ +NVP N=25 | AS-AQ +LPV/r N=25 |
|------------------------------------------------------|---------------------------|-----------------|-------------------|
|                                                      | n (%)                     | n (%)           | n (%)             |
| **Haematological events**                             |                           |                 |                   |
| Anaemia                                               | 1 (4)                     | 0 (0)           | 0 (0)             |
| Leucopenia                                            | 0 (0)                     | 0 (0)           | 0 (0)             |
| Lymphopenia                                           | 1 (4)                     | 1 (4)           | 0 (0)             |
| Neutropenia                                           | 4 (16)                    | 7 (28)          | 0 (0)             |
| Thrombocytopenia                                      | 0 (0)                     | 2 (8)           | 0 (0)             |
| **Biochemical events**                                |                           |                 |                   |
| Elevated ALT and AST                                 | 0 (0)                     | 5 (20)          | 0 (0)             |
| Raised Creatinine                                     | 0 (0)                     | 0 (0)           | 0 (0)             |
| **Cardiac events**                                    |                           |                 |                   |
| QTc prolongation                                      | 0 (0)                     | 2 (8)           | 2 (8)             |