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Disposition of amodiaquine and desethylamodiaquine in HIV-infected Nigerian subjects on nevirapine-containing antiretroviral therapy

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Objectives: Artesunate plus amodiaquine is used for malaria treatment in regions with overlapping HIV endemicity. Co-administration of artesunate/amodiaquine with antiretroviral therapy (ART) may result in drug–drug interactions, but minimal data exist. This study evaluated the impact of nevirapine-based ART, containing a backbone of zidovudine and lamivudine, on the disposition of amodiaquine and its active metabolite, desethylamodiaquine (DEAQ).

Methods: This was an open-label, parallel-group pharmacokinetic comparison between HIV-infected, adult subjects receiving steady-state nevirapine-based ART ($n=10$) and ART-naive subjects (control group, $n=11$). All subjects received a loose formulation of artesunate/amodiaquine (200/600 mg) daily for 3 days, with serial pharmacokinetic sampling over 96 h following the final dose of artesunate/amodiaquine. Amodiaquine and DEAQ were quantified using a validated HPLC method with UV detection. Pharmacokinetic parameters were determined using standard non-compartmental methods.

Results: Exposures to both amodiaquine and DEAQ were significantly lower in the nevirapine-based ART group compared with the control group (amodiaquine AUC_{0-24} 145 versus 204 ng·h/mL, $P=0.02$; DEAQ AUC_{0-96} 14571 versus 21648 ng·h/mL, $P<0.01$). The $AUC_{DEAQ}/AUC_{amodiaquine}$ ratio was not different between groups (ART group 116 versus control group 102, $P=0.67$).

Conclusions: Subjects on nevirapine-based ART had lower exposure to both amodiaquine and DEAQ (28.9% and 32.7%, respectively). Consequently, this may negatively impact the effectiveness of artesunate/amodiaquine in HIV-infected individuals on this ART combination.

Keywords: drug-drug interactions, pharmacokinetics, antimalarial

Introduction

The geographical overlap of HIV and malaria epidemics results in significant malaria and HIV comorbidity. Sub-Saharan Africa is the epicentre of this interaction. Therefore, co-prescription of antimalarials and antiretroviral drugs is inevitable, and characterization of drug–drug interactions between combination antiretroviral therapy (ART) and artemisinin-based combination therapy (ACT) is critical.

Artesunate/amodiaquine is recommended as a first-line ACT by the WHO for treatment of uncomplicated *Plasmodium falciparum* malaria.[1] Amodiaquine is primarily metabolized in the liver by cytochrome P450 2C8 (CYP2C8) to its active metabolite, desethylamodiaquine (DEAQ), which is subsequently eliminated

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via extra-hepatic CYP1A1 and CYP1B1.[2,3] While amodiaquine is more potent than DEAQ, the majority of pharmacological activity is attributed to DEAQ, largely due its long terminal elimination half-life compared with amodiaquine (12 days versus 16 h).[4,5]

Concerns about artesunate/amodiaquine use in HIV-infected patients have emerged.[6–8] A report identified an increased risk of neutropenia with artesunate/amodiaquine treatment among HIV-infected children, particularly those on concomitant ART.[6] Additionally, the use of artesunate/amodiaquine in combination with efavirenz resulted in delayed asymptomatic and transient transaminitis in healthy volunteers.[7] Given concerns for neutropenia and hepatotoxicity, these studies prompted the WHO to caution against the use of artesunate/amodiaquine in individuals receiving ART containing zidovudine or efavirenz, respectively.[9]

The most widely used first-line ART in sub-Saharan Africa is two nucleos(t)ide reverse transcriptase inhibitors, zidovudine plus lamivudine, plus a non-nucleoside reverse transcriptase inhibitor, nevirapine.[10] Of these antiretrovirals, nevirapine is often implicated in drug interactions, as it is both a substrate and an inducer of CYP3A4 and CYP2B6 isoenzymes.[11–13] Although this nevirapine-based ART is not known to specifically induce the enzymes by which amodiaquine or DEAQ are primarily metabolized, nevirapine-based ART plus other antimalarials, including artesunate plus lumefantrine, have resulted in unanticipated pharmacokinetic results;[14,15] therefore, the potential for interaction may exist via an alternative metabolic or drug transport pathway.

To address this, we explored the pharmacokinetic interactions between artesunate/amodiaquine in the context of nevirapine-based ART in HIV-infected adults who were not symptomatically infected with malaria. The pharmacokinetic parameters of the partner drug, artesunate, and its metabolite, dihydroartemisinin (DHA), were previously reported.[14] Herein, the disposition kinetics of amodiaquine and its metabolite DEAQ are described.

Methods

The University of Ibadan/University College Hospital Ethics Committee approved this study (NHREC/05/01/2008a) and informed consent was obtained from all participants. All patient-related activities took place at University College Hospital, Ibadan, Nigeria, between November 2009 and June 2010. HIV-infected Nigerians were enrolled in the study if they met the following inclusion criteria: age ≥18 years, receiving ART containing zidovudine (300 mg), lamivudine (150 mg) and nevirapine (200 mg) twice daily for at least 8 weeks (ART group) or not yet eligible for ART according to the Nigerian national ART guidelines (control group), serum creatinine <1.5 mg/dL and aminotransferases <1.5-fold the upper limit of population normal values. Exclusion criteria were current pregnancy, a history of intolerance of study drugs, use of artesunate- or amodiaquine-containing medications in the preceding 4 weeks, use of known CYP substrates, inducers or inhibitors other than study-related medications, symptoms of malarial infection, total leucocyte count <3000/mm^3 or haemoglobin <10 g/dL.

Study procedures

After enrolment, participants began the standard adult treatment dose of a loose combination of artesunate (200 mg) and amodiaquine (600 mg) once daily for 3 days (Dart®, Swiss Pharma Nigeria Limited, Lagos, Nigeria). Venous blood samples for the quantification of both amodiaquine and DEAQ were collected around the time of the last dose according to the following schedule: pre-dose on study day 2, followed by 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72 and 96 h post-dose (Figure 1). All samples were centrifuged and separated and plasma was stored at −80°C within 30 min of collection. After study completion, plasma samples were shipped on dry ice to the Department of Clinical Pharmacology at the Mahidol-Oxford Tropical Medicine Research Unit in Thailand for drug quantification. Amodiaquine and DEAQ plasma concentrations were quantified by HPLC with UV detection.[16] The lower limit of quantification and limit of detection were 5.0 and 2.5 ng/mL, respectively, for both amodiaquine and DEAQ. The total assay precision was below 8% relative standard deviation at three tested quality control levels (15, 972 and 2916 ng/mL).

Safety measurements

To assess for adverse events related to the study therapy, subjects were interviewed and physically examined at each study visit (days 0, 2, 3, 4, 5 and 6). In addition, all subjects who withdrew from the study prior to completing all study visits were contacted to evaluate them for the presence of any toxicity.

Pharmacokinetic and statistical analyses

The maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}) for both amodiaquine and DEAQ were estimated by visual inspection of the raw data. Using standard non-compartmental methods, plasma pharmacokinetic parameters were obtained for each subject. These parameters included the area under the concentration–time curve (AUC), elimination half-life ($t_{1/2}$), apparent volume of distribution (V/F) and apparent oral clearance (CL/F), where F is the oral bioavailability. AUC_{0-24} was used to describe amodiaquine exposure, while AUC_{0-96} described DEAQ exposure. The AUC ratio of DEAQ to amodiaquine was calculated as a crude measure to explore the potential induction of CYP2C8 by nevirapine-based ART and the consequent increase in amodiaquine metabolism to DEAQ via this pathway.[8]

The Mann–Whitney U-test was used to evaluate significant differences in pharmacokinetic parameters between the independent groups. Univariate analyses of demographic characteristics between groups were performed using Student's t-test for continuous data and either the χ^2 or Fisher's exact test, as appropriate, for categorical data.

Results

Demographic and clinical characteristics

A total of 11 subjects were enrolled in the ART group (10 of whom completed the study procedures), while 24 subjects were enrolled in the control group (11 of whom completed the study). The subjects who failed to complete the study procedures were not evaluated as part of the pharmacokinetic analysis, but are described in the safety analysis. The majority of the subjects (71.4%) were female, the percentage being balanced between the two groups (Table 1). The ART group was slightly older, with a trend towards a lower mean CD4+ cell count (304 versus 492 cells/mm^3, $P=0.3$). Although one subject in each group was asymptomatically infected with *P. falciparum* at enrolment, both subjects were included in the analysis. Those in the ART group had been receiving a stable nevirapine-based ART regimen for a mean duration of 1.7 years (range 6–38 months). Three participants in the control group and five participants in the ART group were taking prophylactic trimethoprim/sulfamethoxazole.

Pharmacokinetic analysis

The amodiaquine pharmacokinetics are summarized in Table 2 and illustrated in Figure 2(a). Amodiaquine clearance increased

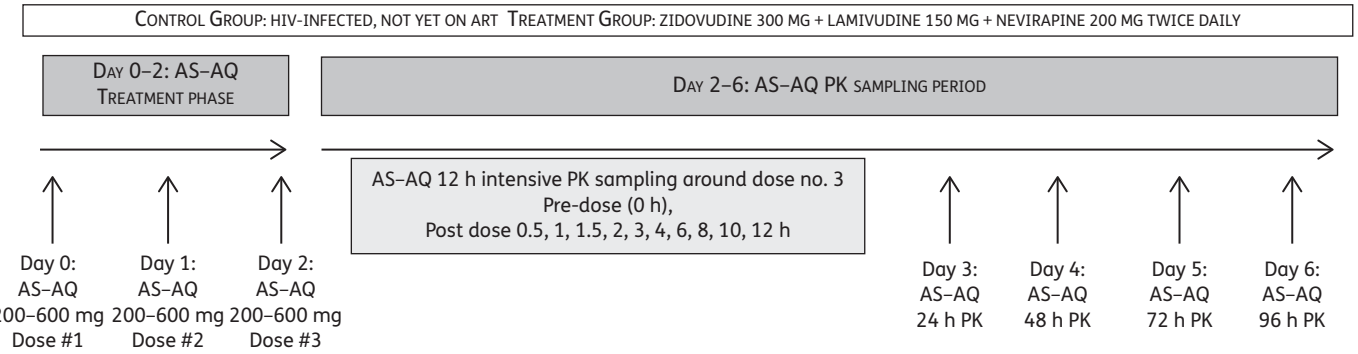

Figure 1. Study schema. ART, antiretroviral therapy; AS–AQ, artesunate/amodiaquine; PK, pharmacokinetic(s).

Table 1. Summary of subject characteristics

	ART group ($n=10$)	Control group ($n=11$)	*P* value
Age (years), mean (SD)	39.7 (13.5)	35.8 (6.4)	0.008
Sex (female), *n* (%)	7 (70)	8 (73)	1.0
Body mass index (kg/m^2), mean (SD)	23.2 (2.9)	22.8 (4.6)	0.6
CD4+ count (cells/mm^3), mean (SD)	304 (229)	492 (157)	0.30
HIV RNA (copies/mL), median (range)[a]	<400 (400–56906)	16394 (2006–48398)[b]	0.10
Withdrawal from study related to adverse events, *n* (%)	1 of 11 (9.1)	12 of 24 (50.0)	0.15
Weakness, *n* (%)	10 (100)	11 (100)	1.0
moderate to severe, *n* (%)	0 (0)	3 (27.2)	
mild, *n* (%)	10 (100)	8 (72.7)	
Gastrointestinal symptoms, *n* (%)	6 (60)	6 (54.5)	1.0
nausea, *n* (%)	4 (40)	6 (54.5)	
vomiting, *n* (%)	1 (10)	2 (18.2)	
diarrhoea, *n* (%)	1 (10)	2 (18.2)	
anorexia, *n* (%)	5 (50)	2 (18.2)	
Dizziness, *n* (%)	3 (30)	0 (0)	0.09

[a]HIV RNA <400 copies/mL reflects the lower limit of quantification of the assay.
[b]$n=10$ patients.

by 50% in the ART group compared with the control group (4165 versus 2775 L/h, $P=0.01$) with a concomitant 2.5-fold increase in the volume of distribution (63761 versus 25837 L, $P=0.04$). This resulted in 28.9% lower amodiaquine median exposure (AUC_{0-24}) in the ART group compared with the control group (145 versus 204 ng·h/mL, $P=0.02$). However, there was no statistical difference in the observed terminal elimination half-life of amodiaquine in the two groups (9.0 versus 6.2 h, $P=0.21$). Although the C_{max} did not differ significantly between groups, T_{max} was significantly shorter in the ART group compared with controls (1.0 versus 3.0 h, $P<0.01$).

The DEAQ pharmacokinetics are summarized in Table 2 and illustrated in Figure 2(b). The median DEAQ exposure (AUC_{0-96}) was 32.7% lower in the ART group compared with the control group (14571 versus 21648 ng·h/mL, $P=0.005$), the difference being similar to that observed with the parent drug. The DEAQ C_{max} and T_{max} were similar between groups. Crude assessment of the conversion of amodiaquine to DEAQ as measured by the exposure ratio ($AUC_{DEAQ}/AUC_{amodiaquine}$) did not show a significant difference between the two groups (ART group 116 versus control group 102, $P=0.67$).

Safety analysis

Thirteen out of 24 (54.2%) enrolled subjects in the control group withdrew from the study prior to completing all pharmacokinetic sampling. Moderate to severe weakness ($n=10$) and mild weakness and dizziness ($n=2$) after starting artesunate/amodiaquine were the reasons for withdrawal cited by subjects; one subject withdrew from the study without any documentation of adverse events. Two of the subjects with moderate to severe weakness also complained of anorexia and abdominal pain. Among the

Table 2. Pharmacokinetic parameter estimates of amodiaquine and DEAQ

	ART group ($n=10$), median (IQR)	Control group ($n=11$), median (IQR)	*P* value
Amodiaquine			
C_{max} (ng/mL)	16.7 (14.5–21.5)	24.6 (18.7–26.2)	0.09
T_{max} (h)	1.0 (0.5–1.5)	3.0 (1.0–4.0)	<0.01
CL/F (L/h)	4165 (3264–5695)	2775 (2116–3807)	0.01
V/F (L)	63761 (37388–81968)	25837 (16551–41628)	0.04
$t_{1/2}$ (h)	9.0 (6.1–18.6)	6.2 (5.0–13.6)	0.21
AUC_{0-24} (ng·h/mL)	145 (105–185)	204 (158–284)	0.02
DEAQ			
C_{max} (ng/mL)	495 (457–578)	650 (342–797)	0.67
T_{max} (h)	2.0 (1.5–8.0)	4.0 (2.0–10.0)	0.40
AUC_{0-96} (ng·h/mL)	14571 (12662–18732)	21648 (19022–23564)	<0.01
$AUC_{DEAQ}/AUC_{amodiaquine}$ ratio	116 (88–138)	102 (73–142)	0.67

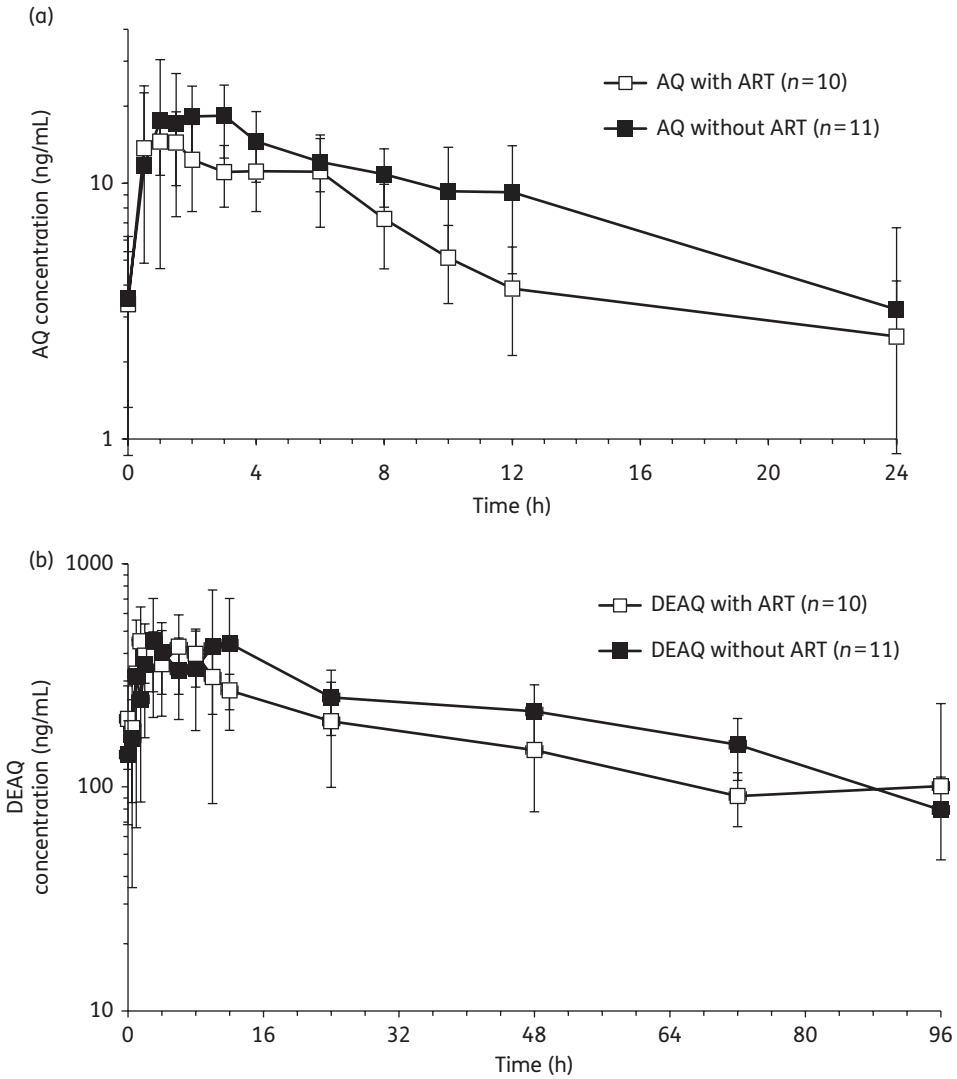

Figure 2. (a) Mean (±SD) plasma concentration versus time profile of amodiaquine (AQ) 0–24 h after the third and last dose of 200 mg of artesunate plus 600 mg of amodiaquine. (b) Mean (±SD) plasma concentration versus time profile of DEAQ 0–96 h after the third and last dose of 200 mg of artesunate plus 600 mg of amodiaquine.

11 subjects in the control group who completed the study, 3 (27.3%) complained of moderate to severe weakness, 8 (72.7%) complained of mild weakness, 6 (54.5%) reported mild gastrointestinal symptoms and 1 (9.0%) reported dizziness (Table 1).

In contrast, only 1 out of 11 (9.0%) enrolled subjects in the ART group withdrew from the study due to mild weakness and diarrhoea after starting artesunate/amodiaquine and no subject complained of moderate to severe weakness. All 10 subjects (100%) in the ART group who completed the study complained of mild weakness after starting artesunate/amodiaquine. Additionally, 6 of 10 subjects (60.0%) in the ART group reported mild gastrointestinal symptoms and 3 subjects (30.0%) reported mild dizziness during the treatment course of artesunate/amodiaquine (Table 1).

Discussion

We found significantly lower exposures to both amodiaquine and DEAQ in the ART group compared with HIV-infected, ART-naive subjects. The mechanism for the observed impact of nevirapine-based ART containing a backbone of zidovudine and lamivudine on amodiaquine exposure in this study is unclear. Neither zidovudine nor lamivudine inhibit or induce CYP enzymes and are therefore not expected to impact the metabolism of amodiaquine or DEAQ. However, nevirapine is a known inducer of the CYP3A4 and CYP2B6 isoforms, yet its impact upon the CYP2C8 isoform, the primary route of amodiaquine metabolism to DEAQ, or the extrahepatic routes (CYP1A1 and CYP1B1) of subsequent DEAQ elimination has not been clearly elucidated *in vivo*.[2,3,8,11–13]

While this study was not designed to evaluate the exact mechanism of the interaction, we assessed the ratio of metabolite (DEAQ) and parent (amodiaquine) drug AUCs (DEAQ:amodiaquine), with the assumption that a significant induction of CYP2C8 by nevirapine-based ART would lead to a higher ratio of DEAQ/amodiaquine in the ART group compared with controls. If this assumption were correct, the similar DEAQ/amodiaquine ratio between the nevirapine and control groups would not support the induction (or inhibition) of CYP2C8 by ART unless the ART simultaneously impacts the CYP1A1 and CYP1B1 pathways for DEAQ, resulting in similar proportions of metabolite to drug at any given time. Alternatively, the observed reduction of both substrate and metabolite could be due to a difference in the drug fraction of an oral dose that reaches the systemic circulation. Further mechanistic evaluations of these observed pharmacokinetic changes, including the potential role of drug transporters, are required to elucidate the extent and cause of this interaction.

The pharmacokinetics of amodiaquine and DEAQ are highly variable between subjects, as demonstrated by the wide range of pharmacokinetic parameter estimates observed in prior evaluations, making comparison of our results with prior publications challenging.[17–21] This high inter-subject variability is likely the cumulative result of numerous factors, including various drug dosage forms,[21] drug–drug interactions,[17] the presence of acute malaria,[20] age,[22] body weight[22] and pharmacogenetics.[8,23] In fact, artesunate co-administration has been reported to decrease exposure to amodiaquine.[17] Given that amodiaquine is not recommended in the absence of artesunate for the treatment of malaria, the inclusion of artesunate in both study groups was essential to determine any excess impact of ART on amodiaquine and DEAQ disposition during typical clinical administration of this ACT.

With regard to safety, we observed a high rate of mild weakness in subjects who received at least one dose of artesunate/amodiaquine during the study period and higher rates of moderate to severe weakness among those in the control arm than those in the ART group. This raises concerns about the tolerability of artesunate/amodiaquine in HIV-infected subjects. However, high rates of adverse events with artesunate/amodiaquine are not uncommon. In a recent study, 1144 (30.1%) of 3708 adult patients receiving artesunate/amodiaquine reported an adverse event, 70% of which were mild to moderate.[24]

The relationship between antimalarial pharmacokinetics and therapeutic effectiveness is pivotal to assessing the implications of these findings. Therapeutic response has been associated with assessments of exposure at single timepoints (day 7 concentrations), as these measurements have been associated with overall exposure (AUC) for ACT partner drugs.[25–30] However, the relationship between drug exposure and therapeutic efficacy for amodiaquine has not been clearly established; therefore, the optimal single-timepoint drug concentration or overall drug exposure is still unknown and may vary by transmission setting and other demographic characteristics. One pharmacodynamic model based on treatment of *Plasmodium vivax* in a pregnant population suggested a DEAQ threshold concentration of 7 ng/mL was needed to reduce the risk of *P. vivax* recurrence by 50%, or 131 ng/mL for a 95% risk reduction,[5] supporting the presence of a concentration–response relationship for amodiaquine. It remains to be seen whether the decrease in exposure observed in our ART group increases the potential risk for treatment failure in malaria-infected patients.

A report by German *et al*.[7] describes the only other pharmacokinetic evaluation of ART in combination with artesunate/amodiaquine published to date. In this study with healthy volunteers, enrolment was stopped after two subjects developed transaminitis at 34 and 42 days after receiving the combination of efavirenz plus artesunate/amodiaquine (alanine aminotransferase peak of 206 and 868 U/L, respectively). Elevated amodiaquine levels likely contributed to the observed hepatotoxicity, as cases of amodiaquine-induced hepatotoxicity have been reported previously.[31,32] A potential mechanism for this interaction may be the described moderate *in vitro* inhibition of CYP2C8 by efavirenz.[8,33] While we are encouraged by the lack of long-term transaminitis measured 1 year after study enrolment (data not shown) in our participants, a more rigorous assessment of the impact of nevirapine-based ART with this ACT is needed.

Given that treatment of both HIV and malaria requires combination therapy, to evaluate the clinical implications of antiretroviral and antimalarial interactions it is essential to include a full combination regimen, rather than an individual drug. However, this makes attribution of any observed effect to an individual drug challenging. While a randomized or crossover study design may be ideal, these study designs may not be ethically feasible in evaluating drug–drug interactions in this setting for a number of reasons. First, nevirapine is contraindicated in immunocompetent subjects due to the significant risk of hepatotoxicity, preventing nevirapine-based ART drug–drug interaction studies in healthy volunteers. Further, it is not advisable to delay potentially life-saving ART pending completion of the pharmacokinetic evaluation. An additional limitation is that we are unable to comment

on the extended pharmacokinetic profile of DEAQ, which would have been informative. Importantly, we are also unable to comment on pharmacokinetic and pharmacodynamic relationships in the context of malaria, which may alter the drug disposition.[26]

In summary, lower exposures to both amodiaquine and its active metabolite, DEAQ, were observed in subjects receiving nevirapine-based ART containing a backbone of zidovudine and lamivudine. In contrast, we previously reported higher exposure to the co-administered artesunate when given with nevirapine-based ART in the same group of subjects.[14] While the clinical significance of the pharmacokinetic changes in artesunate and amodiaquine and their active metabolites is unknown, these results raise concern about the effectiveness of artesunate/amodiaquine in subjects receiving this ART regimen, particularly after the first several days of therapy when only the long-acting partner drug is present in the circulation. Given the widespread availability of artesunate/amodiaquine in diverse settings and the expanding use of ART in HIV-infected subjects throughout the world, these results highlight the importance of conducting pharmacokinetic studies in endemic settings, as well as continued pharmacovigilance, to evaluate the safety and efficacy of this ACT in combination with ART.

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Transparency declarations

None to declare.

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