

Title

Effect of antiretroviral therapy on plasma concentrations of chloroquine and desethyl-chloroquine

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Keywords

Chloroquine; antiretroviral therapy; drug-drug interaction

Running title

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Summary

Results of a drug-drug interaction study examining the effects of antiretroviral therapies on concentrations of chloroquine and its metabolite desethyl-chloroquine are presented. The authors found no difference in week 10 or week 12 chloroquine or desethyl-chloroquine plasma concentrations across groups of participants on various antiretroviral regimens. However, efavirenz appeared to inhibit the desethylation of chloroquine.

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The effect of antiretroviral therapy (ART) on chloroquine and desethyl-chloroquine plasma concentrations was evaluated in clinical trial participants. Concentrations did not differ among participants on protease inhibitor-based ART (n=9), efavirenz-based ART (n=15), other ART (n=8), and those not on ART (n=31). Efavirenz appeared to inhibit chloroquine desethylation.

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70 Despite the rise of multidrug resistant malaria parasites over the last several decades,
71 chloroquine retains its clinical relevance. In endemic areas with chloroquine-susceptible,
72 non-falciparum *Plasmodium* spp., it remains a recommended agent for the treatment and
73 prevention of malaria [1]. Regions of sub-Saharan Africa where widespread use of
74 chloroquine was abandoned due to resistance have witnessed re-emergence of susceptible
75 parasites [2], although reports of resistance among *P. vivax* are increasing [3]; and
76 chloroquine continues to be used in some sub-Saharan African communities even where it
77 is not a recommended therapy. It is also used in the treatment of autoimmune conditions.

78 Regions of malaria transmission overlap with those of high human
79 immunodeficiency virus (HIV) prevalence, necessitating co-administration of antimalarial
80 drugs and HIV antiretroviral therapy (ART). However, the potential pharmacokinetic drug
81 interactions of chloroquine and ART in humans have not previously been reported [4].
82 Chloroquine plasma concentrations are mainly driven by its large apparent volume of
83 distribution (100-1,000 L kg⁻¹), and slow clearance that occurs via multiple mechanisms of
84 elimination imparting a long terminal half-life of 20-60 d [5-7]. HIV protease inhibitors are
85 potent cytochrome P450 (CYP) 3A4 inhibitors and may interact with chloroquine—a
86 substrate of various CYP isozymes—to increase chloroquine concentrations and predispose
87 to toxicity [8]. Efavirenz, a non-nucleoside reverse transcriptase inhibitor, is a mixed
88 inhibitor and inducer of CYP isozymes and might thereby increase or reduce concentrations
89 [8-10]. CYP-mediated desethylation of chloroquine produces an active major metabolite,
90 desethyl-chloroquine, buffering against any potential attenuation of drug effect by inducers
91 [11].

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93 **METHODS**

We assessed the pharmacokinetic drug interactions of protease inhibitor- and efavirenz-containing ART regimens on chloroquine to inform the potential need, if any, for dose adjustment of chloroquine in patients on these ART regimens. The study was done in the context of a phase II clinical trial of chloroquine for reducing immune activation in HIV infection [12]. The trial enrolled 70 adults with HIV infection between 2009 and 2012 of whom 65 contributed specimens for pharmacokinetic assays (Figure 1). Chloroquine phosphate was administered orally as a daily dose of 250 mg salt (150 mg base) for 12 weeks, and trough plasma samples were acquired 10 and 12 weeks after the first dose when concentrations approach steady-state. The trial included a group of participants not on ART (n=31). Among those on ART (n=34), 9 were on a protease inhibitor-based regimen, 16 were on an efavirenz-based regimen, 1 was on a regimen containing both a protease inhibitor and efavirenz, and 8 were on a regimen with neither a protease inhibitor nor efavirenz. Protease inhibitors were ritonavir-boosted atazanavir (n=7) and darunavir (n=3). Other ART regimens were combinations of nucleoside reverse transcriptase inhibitors (abacavir, emtricitabine, lamivudine, tenofovir, didanosine) with non-nucleoside reverse transcriptase inhibitors other than efavirenz (etravirine, nevirapine, rilpivirine) and/or the integrase inhibitor raltegravir. The study was approved by the Johns Hopkins University institutional review board.

Plasma concentrations of chloroquine and desethyl-chloroquine were determined by a newly developed and validated method using solid-phase extraction and liquid chromatography with tandem mass spectrometry at the Department of Clinical Pharmacology, Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand. The range of quantitation was 1.13-702 ng ml⁻¹, and the observed total assay coefficient of variation was < 5% in all quality control samples in accordance with US Food and Drug Administration requirements [13]. Forty-three participants had two replicates at identical

time points due to duplicate aliquots. For these participants, the average concentration at each time point was used. Differences in median plasma drug concentrations were assessed by Kruskal-Wallis one-way analysis of variance, Wilcoxon rank-sum test for unadjusted pairwise comparisons, and linear regressions of pooled and unpooled week 10 and 12 data for adjusted analyses. To best approximate a Gaussian distribution, square-root transformed concentrations were used for linear regression models based on results of skewness and kurtosis tests of normality. The sample size afforded 80% power to detect a one-fold difference (-50 to 100% change) in mean plasma drug concentrations among all subgroups with a two-tailed α of 0.05. All statistical analyses were done using Stata 14.0 (StataCorps, College Station, Texas, USA).

RESULTS

Study Population

Participants were predominantly male (91%) and those on ART tended to be older than those off ART ($P < 0.001$) (Table 1). A few were on medications with potential for pharmacokinetic interactions including proton pump inhibitors (PPIs) and systemic triazoles. There were no reported toxicities related to chloroquine, including among the 5 trial participants for whom no specimen was available. Three participants had only a week 10 sample, of which two were not on ART and one was on a non-protease inhibitor, non-efavirenz-based ART regimen; and one participant on a protease inhibitor-based regimen contributed only a week 12 sample. One participant on an efavirenz-based regimen had undetectable drug concentrations and was excluded from the primary analysis for presumed non-adherence. A sensitivity analysis including this participant in all models did not significantly influence results.

Effect on Chloroquine and Desethyl-Chloroquine Plasma Concentrations

Between-group comparisons showed no differences in drug concentrations across any of the groups, including after adjustment for age, gender, weight, and concomitant use of a PPI or triazole in both pooled and unpooled analyses. The median week 10 plasma concentration of chloroquine was 227 ng ml⁻¹ (interquartile range [IQR]: 147-352 ng ml⁻¹) and of desethyl-chloroquine 184 ng ml⁻¹ (IQR: 89-333 ng ml⁻¹). The respective median week 12 plasma concentrations were 235 ng ml⁻¹ (IQR: 156-317 ng ml⁻¹) and 197 (IQR: 71-342 ng ml⁻¹) (Figure 2). There were The single participant on combined efavirenz and protease inhibitor therapy had week 10 and 12 chloroquine concentrations of 252 and 317 ng ml⁻¹, and desethyl-chloroquine concentrations of 87 and 148 ng ml⁻¹. Among participants with high outlying plasma concentrations, three were on a protease-inhibitor-based regimen, one was on an efavirenz-based regimen, two were on other ART regimens, three were not on ART, and none were taking a PPI or systemic triazole.

Effect on Metabolic Conversion to Desethyl-Chloroquine

Within-group comparisons of chloroquine and desethyl-chloroquine concentrations were made to explore the possible influence of ART regimens on metabolic conversion of chloroquine. For both week 10 and pooled week 10 and 12 data, participants on efavirenz-containing regimens had chloroquine concentrations approximately twice those of desethyl-chloroquine, compared to no difference in all other groups (efavirenz-based: 333 vs. 146 ng ml⁻¹, $P=0.049$; no ART: 214 vs. 206 ng ml⁻¹, $P=0.67$; protease inhibitor-based: 190 vs. 136 ng ml⁻¹, $P=0.79$; other ART: 219 vs. 208 ng ml⁻¹, $P=0.92$) (above P values are for unpooled week 10 data; for efavirenz-based regimens at week 12: $P=0.093$, and pooled week 10 and 12: $P=0.007$). The difference remained significant after conditioning on age,

sex, weight, and use of PPI or triazole, suggesting that efavirenz inhibits desethylation of chloroquine.

DISCUSSION

We found no significant differences in plasma concentrations of chloroquine or its metabolite, desethyl-chloroquine, among individuals on protease inhibitor-based ART, efavirenz-based ART, non-protease inhibitor/non-efavirenz based ART, and those not on ART. Efavirenz appeared to reduce metabolic conversion of chloroquine to desethyl-chloroquine, although not to an extent that significantly influenced exposure to one or the other.

Desethylation of chloroquine is catalyzed in part by CYP 3A4 and 2C8, the former inhibited by both protease inhibitors and efavirenz and the latter by efavirenz, while relying also on non-enzymatic processes that govern drug disposition [8, 14, 15]. The observed difference in efavirenz- but not protease inhibitor-containing regimens therefore suggests a role of other, non-CYP mediated mechanisms such as drug transporter effects.

There were limitations to the study design. The results presented here apply only to the dose of chloroquine used in the trial, 150 mg base daily, which is between the World Health Organization recommended antimalarial prophylaxis (300 mg base weekly) and treatment (25 mg kg⁻¹ base over three days) adult doses, and comparable to those used to treat rheumatologic diseases (e.g. 150 mg base once to twice daily). The low dose of chloroquine relative to the malaria treatment dose may have reduced the sensitivity of the study to detect changes in concentrations among groups, although the direction of change is unlikely to be affected by dose. We did not assess the pharmacokinetic impact of chloroquine on ART. Chloroquine was administered to adults with HIV infection who did

not have malaria infection, limiting generalizability to other populations. A strength of the study design was inclusion of a concurrent control group not taking ART.

To the best of our knowledge, this is the first reported formal study of pharmacokinetic interactions between chloroquine and ART. Coadministration of chloroquine 150 mg base daily with ART was well tolerated and chloroquine disposition was not significantly impaired. Future drug-drug interaction studies employing malaria treatment and prevention doses of chloroquine in HIV-infected individuals on ART would be further informative.

Notes

Acknowledgements. We thank the study volunteers, as well as Linda Boone and Ellyn Sherman of the AIDS Clinical Trial Group Leadership and Operations Center, Boston, Massachusetts, USA, and Kyle Whitson and Alex Benns of the Biomedical Research Institute, Rockville, Maryland, USA for their logistical support. We also thank Dr. Noreen Hynes, Director of the Johns Hopkins University School of Medicine Travel and Tropical Medicine Clinic, for her review of and comments to the manuscript.

Financial support. This research was supported by the AIDS Clinical Trial Group (UMI AI068636). M.M.I. receives support from the National Institute of General Medical Sciences (T32-GM-066691), the Pharmaceutical Research and Manufacturers of America Foundation, the Burroughs Wellcome Fund, and from the Sherrilyn and Ken Fisher Center for Environmental Infectious Diseases, Division of Infectious Diseases of the Johns Hopkins University School of Medicine. The Mahidol-Oxford Tropical Medicine Research Unit is supported by the Wellcome Trust of Great Britain.

Potential conflicts of interest. C.F. reports serving as a paid consultant for Merck, GlaxoSmithKline, ViiV Healthcare, Cipla, and Mylan Pharmaceuticals. M.M.L. has received grant support from Gilead. Other authors declare no conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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262 **Table 1. Characteristics of Study Participants**

Characteristic	No ART, n=31	PI-based, n=9	EFV-based, n=15	Other ART, n=8
Age in years, median (IQR)	39 (30-44)	50 (46-52)	50 (42-55)	50 (49-56)
Female gender, no. (%)	3 (10)	1 (11)	1 (7)	1 (13)
Weight in kg, median (IQR)	80 (70-92)	81 (69-82)	73 (62-88)	75 (71-89)
HIV viremia, no. (%) [*]	31 (100)	1 (11)	0 (0)	0 (0)
Concomitant medications, no. (%)				
Proton pump inhibitor	4 (13)	2 (22)	2 (13)	1 (13)
Triazole	2 (7)	1 (11)	2 (13)	1 (13)
Week 10 drug concentration, ng ml ⁻¹ , median (IQR)				
Chloroquine	214 (139-279)	190 (138-320)	333 (149-360)	219 (171-383)
Desethyl-chloroquine	206 (94-332)	136 (65-504)	146 (73-234)	208 (135-406)
Ratio of chloroquine:desethyl-chloroquine	1.04	1.40	2.28 ^{**}	1.05
Week 12 drug concentration, ng ml ⁻¹ , median (IQR)				
Chloroquine	210 (159-290)	222 (136-311)	294 (160-366)	176 (79-337)
Desethyl-chloroquine	225 (72-342)	282 (71-462)	146 (53-320)	170 (64-329)
Ratio of chloroquine:desethyl-chloroquine	0.93	0.79	2.01	1.04

Abbreviations: ART, antiretroviral therapy; PI, protease inhibitor; EFV, efavirenz; IQR, interquartile range; HIV, human immunodeficiency virus. Triazoles include fluconazole and itraconazole. "Other ART" regimens included combinations of nucleoside reverse transcriptase inhibitors (abacavir, emtricitabine, lamivudine, tenofovir, didanosine) with non-nucleoside reverse transcriptase inhibitors other than efavirenz (etravirine, nevirapine, rilpivirine) and/or the integrase inhibitor raltegravir. (*) Defined as quantifiable HIV RNA at any time during the study period. (**) Participants on EFV-containing regimens had chloroquine concentrations more than twice those of desethyl-chloroquine ($P=0.049$), compared to no statistically significant differences within other study arms.

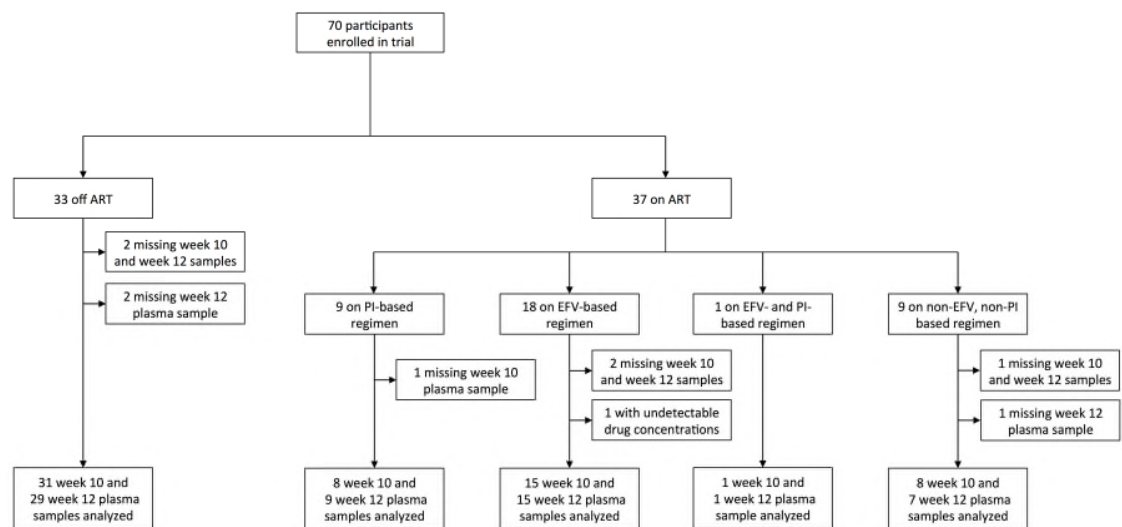


Figure 1. Flow chart of study participants included in the pharmacokinetic interaction analysis.

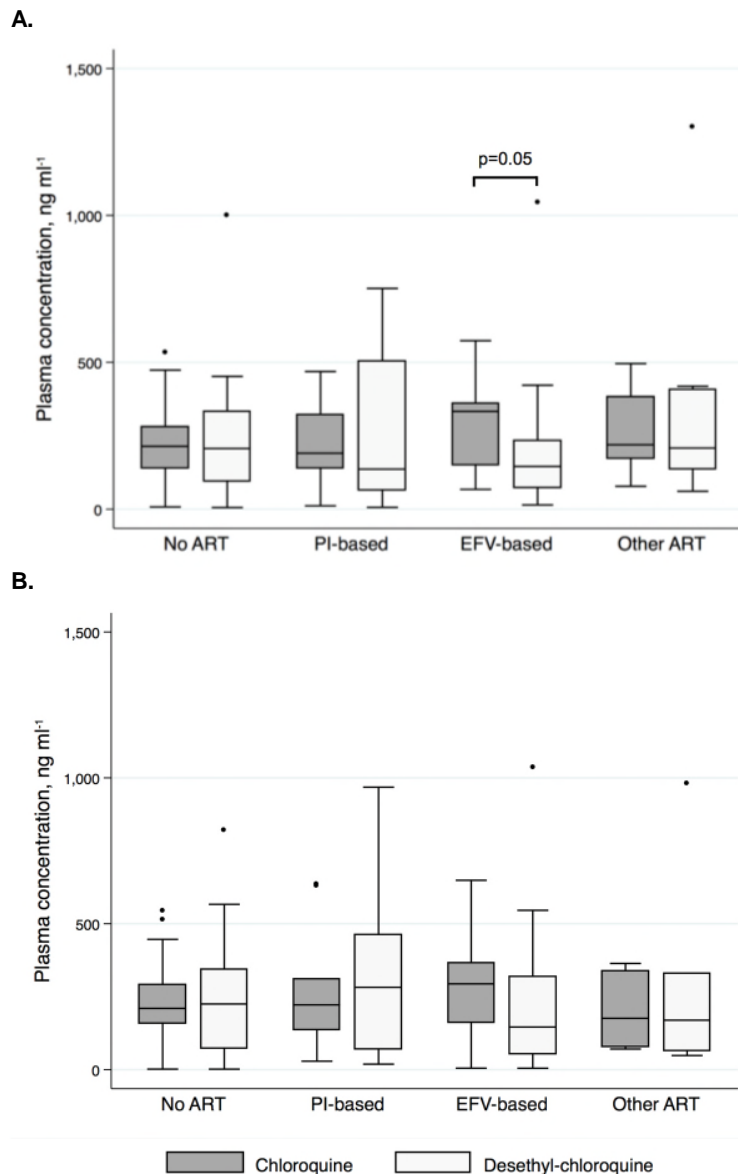


Figure 2. Standard box-and-whisker plot showing median values, upper and lower quartiles, and outliers of plasma concentrations of chloroquine and desethyl-chloroquine in adults with human immunodeficiency virus infection on and off antiretroviral therapy (ART) after 10 (panel A) and 12 (panel B) weeks of chloroquine 150 mg base daily. PI, protease inhibitor. EFV, efavirenz. “Other ART” regimens included combinations of nucleoside reverse transcriptase inhibitors (abacavir, emtricitabine, lamivudine, tenofovir, didanosine) with non-nucleoside reverse transcriptase inhibitors other than efavirenz (etravirine, nevirapine, rilpivirine) and/or the integrase inhibitor raltegravir.