Effects of Hydroxychloroquine on Immune Activation and Disease Progression Among HIV-Infected Patients Not Receiving Antiretroviral Therapy

A Randomized Controlled Trial

Nicholas I. Paton, MD, FRCP
Ruth L. Goodall, PhD
David T. Dunn, PhD
Samuel Franzen, BSc
Yolanda Collaco-Moraes, PhD
Brian G. Gazzard, MD, FRCP
Ian G. Williams, MA, FRCP
Martin J. Fisher, FRCP
Alan Winston, MD, MRCP
Julie Fox, MD, MRCP
Chloe Orkin, MRCP
Elbushra A. Herieka, MRCOG
Jonathan G. Ainsworth, FRCP
Frank A. Post, PhD, FCP(SA)
Mark Wansbrough-Jones, FRCP
Peter Kelleher, PhD, MRCPath
for the Hydroxychloroquine Trial Team

Context Therapies to decrease immune activation might be of benefit in slowing HIV disease progression.

Objective To determine whether hydroxychloroquine decreases immune activation and slows CD4 cell decline.

Design, Setting, and Patients Randomized, double-blind, placebo-controlled trial performed at 10 HIV outpatient clinics in the United Kingdom between June 2008 and February 2011. The 83 patients enrolled had asymptomatic HIV infection, were not taking antiretroviral therapy, and had CD4 cell counts greater than 400 cells/μL.

Intervention Hydroxychloroquine, 400 mg, or matching placebo once daily for 48 weeks.

Main Outcome Measures The primary outcome measure was change in the proportion of activated CD8 cells (measured by the expression of CD38 and HLA-DR surface markers), with CD4 cell count and HIV viral load as secondary outcomes. Analysis was by intention to treat using mixed linear models.

Results There was no significant difference in CD8 cell activation between the 2 groups (–4.8% and –4.2% in the hydroxychloroquine and placebo groups, respectively, at week 48; difference, –0.6%; 95% CI, –4.8% to 3.6%; $P=.80$). Decline in CD4 cell count was greater in the hydroxychloroquine than placebo group (–85 cells/μL vs –23 cells/μL at week 48; difference, –62 cells/μL; 95% CI, –115 to –8; $P=.03$). Viral load increased in the hydroxychloroquine group compared with placebo (0.61 $\log_{10}$ copies/mL vs 0.23 $\log_{10}$ copies/mL at week 48; difference, 0.38 $\log_{10}$ copies/mL; 95% CI, 0.13 to 0.63; $P=.003$). Antiretroviral therapy was started in 9 patients in the hydroxychloroquine group and 1 in the placebo group. Trial medication was well tolerated, but more patients reported influenza-like illness in the hydroxychloroquine group compared with the placebo group (29% vs 10%; $P=.03$).

Conclusion Among HIV-infected patients not taking antiretroviral therapy, the use of hydroxychloroquine compared with placebo did not reduce CD8 cell activation but did result in a greater decline in CD4 cell count and increased viral replication.

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INTERNATIONAL HIV TREATMENT guidelines recommend that antiretroviral therapy should be started when the CD4 cell count reaches 350 cells/μL,[1] but resource limitations prevent implementation of this recommendation in many countries. An inexpensive, safe, and well-tolerated intervention that slowed the rate of decline of CD4 cells (and thereby delayed the time of starting combination antiretroviral therapy) would therefore be attractive. One potential therapeutic target for

See also pp 379 and 405.

Author Affiliations: MRC Clinical Trials Unit, London, United Kingdom (Drs Paton, Goodall, Dunn, and Collaco-Moraes); Immunology Section, Imperial College, Chelsea and Westminster Hospital, London (Mr Franzen and Dr Kelleher); Chelsea and Westminster NHS Foundation, NHS Trust, London (Drs Gazzard and Kelleher); Mortimer Market Centre, London (Dr Williams); Brighton and Sussex University Hospitals, NHS Trust, Brighton, United Kingdom (Dr Fisher); St Mary's Hospital, London (Dr Winston); St Thomas' Hospital, London (Dr Fox); Barts and the London NHS Trust, London (Dr Orkin); Royal Bournemouth Hospital, Bournemouth, United Kingdom (Dr Herieka); North Middlesex University Hospital, London (Dr Ainsworth); King's College Hospital and King's College, London (Dr Post); and St George's Hospital, London (Dr Wansbrough-Jones).
The Hydroxychloroquine Trial Team members are listed at the end of this article.
Corresponding Author: Nicholas I. Paton, MD, FRCP, MRC Clinical Trials Unit, Aviation House, 125 Kingsway, London WC2B 6NH, United Kingdom (nick.paton@ctu.mrc.ac.uk).

such an intervention is immune activation, which is considered to play a central causative role in HIV-driven CD4 cell loss by rendering uninfected, resting CD4 and CD8 cells susceptible to spontaneous apoptosis.[2,3] In observational studies, the level of immune activation is independently associated with the subsequent rate of CD4 cell loss.[4-6] A second potential target might be HIV-associated activation of inflammatory and coagulation pathways (as shown by elevated levels of IL-6 and D-dimer, respectively), high levels of which have been shown to predict increased risk of death in both the short- and long-term.[7] An intervention that decreased immune activation or inflammation or both in early HIV disease might therefore be of benefit.

Hydroxychloroquine and its analogue chloroquine have immunomodulatory and anti-inflammatory properties and have been used for decades in the treatment of diverse T-cell–mediated immune diseases such as systemic lupus erythematosus and steroid-resistant graft-vs-host disease.[8] These drugs are thought to interfere with a number of steps in the T-cell activation pathway such as major histocompatibility complex (MHC) class II antigen presentation[9] and T-cell receptor–mediated intracellular calcium signaling.[10] In addition, hydroxychloroquine and chloroquine have been reported to have anti-HIV properties in vitro and in vivo, resulting from their ability to increase endosomal pH, which inhibits posttranslational modification of gp120.[11-14] Hydroxychloroquine is an oral once-daily treatment that has a good long-term safety profile and is of low cost. We hypothesized that this might therefore be an ideal candidate to decrease immune activation and inflammation and thereby slow the progression of early HIV disease.

METHODS

This randomized, double-blind, placebo-controlled clinical trial was conducted at 10 outpatient sites in the United Kingdom. Participants were recruited between June 2008 and January 2010, and follow-up was completed in February 2011.

Eligible participants were required to be aged 18 to 65 years and to have documented HIV infection; to be naive to antiretroviral therapy or not to have taken therapy in the previous 12 months; and to have a CD4 cell count greater than 400 cells/μL (selected to provide a clear margin above the threshold of 350 cells/μL, the level at which UK national guidelines recommended antiretroviral therapy should be started) and a viral load greater than 1000 copies/mL (selected to exclude patients with minimal HIV stimulation of immune activation) at the time of screening. The principal exclusion criteria were a history of psoriasis, epilepsy, cardiac arrhythmias, depression, or diabetes; chronic liver disease or positive status for hepatitis B surface antigen or hepatitis C virus polymerase chain reaction; suspected primary HIV infection within the previous 12 months or any other major infection or vaccination within the previous 2 months; malignancy; pregnancy; or retinal disease detected on eye screening.

The trial protocol was approved by the Oxfordshire Research Ethics Committee. All participants provided written informed consent.

Randomization and Masking

A computer-generated randomization list with a 1:1 ratio of hydroxychloroquine and placebo was prepared in advance by the trial statistician. Randomization was stratified by study site. Packs of study drug were labeled by the manufacturer with a 3-digit study identifier according to the randomization list. Labels and all packaging were identical apart from the 3-digit study identifier. When an eligible patient was identified, the site completed a randomization form and faxed this to the coordinating trials unit. A study identifier was allocated by computer according to the randomization list. The study identifier was transmitted to the site pharmacist, who then labeled the appropriate bottles of study medication with the patient's trial number. All trial investigators, clinical and laboratory staff, and staff at the central trials unit with the exception of the trial statistician were masked to the treatment allocation. A procedure for emergency unblinding of individual participants was put in place, but unblinding did not become necessary during the trial.

Trial Procedures

Demographic characteristics and clinical history were recorded at the screening visit, and an ophthalmological examination and electrocardiogram were performed. Blood was drawn for routine blood tests, including measurement of glucose-6-phosphate dehydrogenase level, viral load, and CD4 cell count. Viral load and CD4 cell counts were repeated at randomization.

The study medication was provided as identical capsules containing either hydroxychloroquine sulfate, 200 mg (Sanofi-Synthelabo), or lactose placebo. The active and placebo capsules were manufactured by Bilcare Global Clinical Supplies (Europe) using an encapsulation process that served to mask the bitter taste of hydroxychloroquine. Patients were instructed to take 2 capsules once a day with food but advised that they could split the timing of the dose if they developed gastrointestinal intolerance. Treatment was given for a period of 48 weeks.

Patients were seen for follow-up visits at weeks 4, 12, 24, 36, and 48. At each visit, a clinical review was performed that included specific questions about visual symptoms and testing of visual acuity. Patients who reported visual symptoms or those with a deterioration of visual acuity were referred for evaluation by an ophthalmologist. Medication adherence was assessed by patient self-report at each visit, comprising a series of standard questions posed to the patient about changes to the study medication schedule, missed capsules in the previous 2 weeks, missed capsules since the previous visit, and any periods of treatment interruption. Routine safety blood tests (including a full blood cell count and urea, electrolytes, and liver func-

tion tests) were performed in the local laboratory at all visits. CD4 cell count and viral load testing were performed in the local laboratory at all visits except week 4 (ie, every 12 weeks to conform to standard clinical practice at the time). The results of all local laboratory tests were available to the treating physician. A formal ophthalmological examination was repeated at week 48. Patients were invited to attend a final visit at week 60 (12 weeks after stopping study medication) for a final safety assessment, and local laboratory CD4 cell counts and viral load tests were repeated.

At each study visit, additional blood samples were taken for immunological tests performed at the central laboratory. Blood samples were sent by courier to arrive in the central immunology laboratory within a maximum of 4 hours from the time of collection. The results of these tests were not made available to the treating physician. Decisions on when to start antiretroviral therapy were at the discretion of the patients and treating physicians at the sites based on national treatment guidelines. The protocol mandated that a patient should stop trial medication if he or she started antiretroviral therapy during the trial.

A data monitoring committee was appointed to monitor the safety of participants and met periodically to review the data on clinical events and local laboratory results by treatment group. A trial steering committee provided oversight of the trial and included an independent chair and independent physicians and a community representative.

Immunological Analyses

Immunology assays were performed at the central laboratory at the Immunology Section, Imperial College, Chelsea and Westminster Hospital, London, using standardized assays and markers that were already established in this laboratory.

Analysis of T-cell activation and apoptosis subsets was determined using flow cytometry. Monoclonal antibodies used for staining included CD3 allophycocyanin (APC)-Cy7, CD4 peridinin-chlorophyll-protein complex, CD8 APC, CD27 phycoerythrin (PE), CD38 PE, HLA-DR fluorescein isothiocyanate (FITC), caspase-3 FITC, Ki67 PE (all from BD BioSciences), CD28 FITC, and CD45RA phycoerythrin cyanin (PC)-7 (Beckmann Coulter). The activation status of CD8 and CD4 cells was evaluated by measuring the percentage of cells expressing both CD38 and HLA-DR. Coexpression of CD38 and HLA-DR on CD8 cells is a widely used marker of immune activation and is associated with rate of CD4 cell loss in chronic untreated HIV-1 infection.[15]

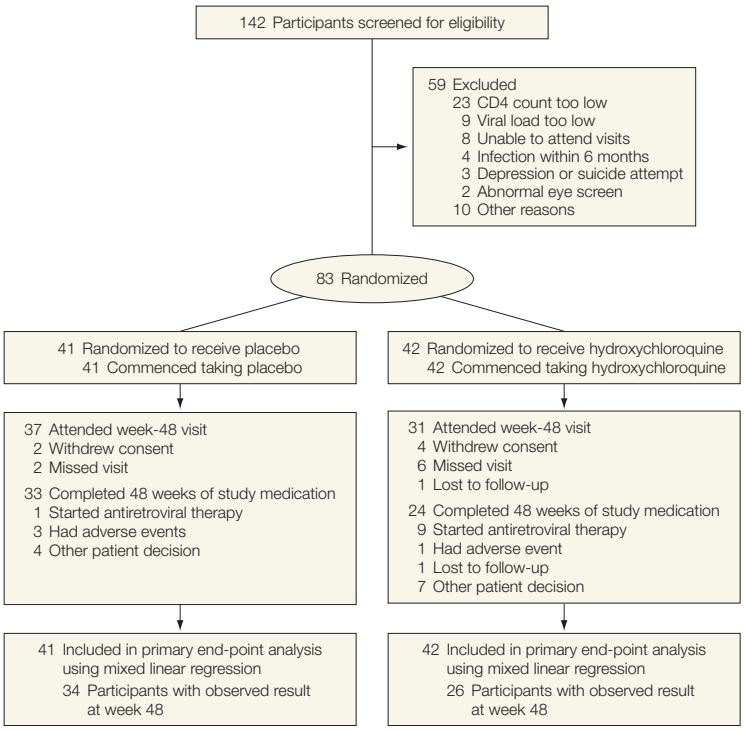
Instrument settings for the flow cytometer were checked on a daily basis using single and rainbow beads to ensure that voltage thresholds and coefficient of variation met target values. Single color staining of T cells from a study patient was used to control for spectral overlap. A standard operating procedure was used to minimize variation in the performance of the tests and data analysis. Isotypes controls for HLA-DR and CD38 to assess nonspecific monoclonal antibody binding were run on each patient sample. To maximize reproducibility, a fixed threshold of 0.5% was used for determining the proportion of CD8 cells considered to be positive for both CD38 and HLA-DR markers in the specimen sample. The threshold was based on previously unpublished studies of immune activation conducted in the central laboratory used for this trial. The percentages of CD4 and CD8 cells that have the naive, effector, and central memory phenotypes were assessed using monoclonal antibodies to CD45RA and CD27 and CD28.

In brief, 100-µL EDTA-anticoagulated whole blood was incubated with conjugated monoclonal antibodies to CD3, CD4, CD8, and markers of cellular activation and memory phenotypes for 30 minutes at room temperature. Red cell lysis was performed with a Beckmann Coulter TQ Prep. Cell acquisition was performed on an LSRII flow cytometer (BD Biosciences) with at least 30 000 CD8 and CD4 cell events. FACS Diva software (version 6.1.2, BD Biosciences) was used for data analysis. One of us (P.K.) cross-checked all flow cytometric dots to ensure that acquisition and analysis of flow cytometric data met specified standard operating procedure criteria for T-cell activation and naive/memory phenotype. The average intra-assay coefficient variation for CD8 cell activation was 10%.

T-cell apoptosis and proliferation were measured in peripheral blood mononuclear cells (PBMCs) at baseline and weeks 12 and 24. PBMCs were isolated from lithium-heparin anticoagulated blood within 4 hours of collection and were then stored at –140°C in freezing medium (10% DMSO in fetal bovine serum [both Sigma-Aldrich]) at a density of 107 cells/mL. Analyses were performed on stored samples in 1 batch at the completion of the trial. After thawing, surface staining for CD4 and CD8 markers was performed by incubating suspended cell samples with anti-CD4 and anti-CD8 monoclonal antibodies for 20 minutes. Caspase 3 (as an indicator of apoptosis) and Ki67 (as an indicator of proliferation) staining were performed using the BD Cytofix/Cytoperm kit reagents as per manufacturer's instructions (BD Biosciences). The proportion of cells expressing these surface markers was measured by flow cytometry.

Absolute CD4 and CD8 cell counts and percentages were measured by incubating 100 µL of EDTA-anticoagulated whole blood with the following monoclonal antibodies for 20 minutes at room temperature: FITC-conjugated anti-CD45, phycoerythrin-cyanin red 5.1 (PC5) conjugated anti-CD3, phycoerythrin Texas red (ECD) conjugated anti-CD8, and rhodamine 1 (RD1) conjugated anti-CD4 (all antibodies from Beckmann Coulter). Erythrocytes were then lysed with a Beckmann Coulter TQ Prep. A 100-µL sample of flow-count fluorospheres was then added to each sample. T-cell counts and percentages were then evaluated by using a Cytomics FC500 flow cytometer (Beckman Coulter).

Figure 1. Trial Profile



Proinflammatory cytokines were measured on serum that had been separated within 4 hours of blood draw and stored at −70°C until time of analysis. Analyses were performed on stored samples in 1 batch at the completion of the trial. IL-1β, IL-6, IL-8, tumor necrosis factor (TNF), granulocyte-macrophage colony-stimulating factor, TNF-soluble receptors p55 and p75, and the TNF-related apoptosis-inducing ligand receptor DR5 were measured using fluorescence bead technology (Biomedical Diagnostics) as previously described.[16] An ultrasensitive enzyme-linked immunosorbent assay was used to determine IL-6 levels (R&D Systems). D-dimer was measured in plasma using a commercial assay (TriniLIA Auto Dimer kit; Tcoag Ireland).

Statistical Analysis

The primary end point was the change from baseline to week 48 in activation of CD8 cells, as shown by percentage of cells expressing CD38+ and HLA-DR+. The main secondary end points were change in CD4 cell activation, IL-6 level, absolute CD4 cell count, and HIV viral load from baseline to week 48.

The sample size was estimated for the primary end point as follows. We proposed that a reduction in CD8 cell activation from 46% to 35% (ie, a 25% reduction) would be a realistic goal of hydroxychloroquine therapy given the magnitude of the changes usually seen with antiretroviral therapy.[17,18] Models indicate that this level of reduction would be expected to decrease the relative hazard for disease progression by at least 50%.[19] The standard deviation for the reduction was estimated as 15%.[17] With a 2-sided α of .05 and a power of 90%, a total of 80 patients would be required. This estimate did not take account of the availability of repeated measures and was therefore considered to be conservative.

For the efficacy analysis, the change in CD8 cell activation from baseline (taken as the average of values from screening and randomization visits) was assessed using a linear mixed model. This approach accounts for individual variation in intercept and slope and therefore implicitly adjusts for any baseline differences between the groups. The analysis was performed on all eligible patients who were randomized into the study, but values obtained after initiation of antiretroviral therapy were ignored. *P* values were derived from the parameter representing the difference between the 2 group-specific slopes. A sensitivity analysis that included site as a fixed effect (after combining the 4 smallest sites) showed no evidence of a difference ($P=.66$, χ^2_6 test). Secondary outcome parameters were compared between groups using a similar approach, apart from change in levels of IL-6 and D-dimer (where random intercept-only models were used because of limited data) and change in viral load (where a piecewise linear model with inflection at week 18 was used as the change was clearly nonlinear). The adherence level was calculated from individual patient self-reports as the percentage of days during the study follow-up period (from randomization to either the date of the last visit, study withdrawal, or initiation of antiretroviral therapy—whichever occurred first) when the patient did not miss any doses of study drug. The proportion of patients reporting good adherence (above 90%) was compared between groups using a χ^2 test. For the safety analysis, all adverse events occurring up to week 60 were included except for events that occurred after initiation of antiretroviral therapy (n=21). The frequency of adverse events was compared using a 1-sided Fisher exact test. All other *P* values are 2-sided. All analyses were performed using Stata version 12.1 (StataCorp).

RESULTS

FIGURE 1 shows the trial profile. A total of 83 eligible participants were enrolled and randomly assigned to study treatment (41 placebo, 42 hydroxychloroquine). The demographic and baseline clinical characteristics of the participants were similar between the 2 groups (TABLE 1). One participant had previously taken antiretroviral

therapy and stopped 22 months prior to randomization, and all others were therapy naive.

Of the enrolled participants, 75 (90%) attended the week-24 visit and 68 (82%) attended the week-48 visit. The reasons for attrition are shown in Figure 1. Twenty-five participants discontinued study medication prior to the week-48 visit, 17 in the hydroxychloroquine group (9 started antiretroviral therapy, 1 had an adverse event, 6 were patient decision, 1 lost to follow-up) and 8 in the placebo group (1 started antiretroviral therapy, 3 had an adverse event, 4 were patient decision). All patients who initiated antiretroviral therapy did so as a result of their CD4 cell count falling below 350 cells/μL, the standard threshold for initiating therapy recommended in clinical practice guidelines at that time. Self-reported adherence to study medication was good and equal in both treatment groups (more than 90% of prescribed doses were taken by 81% of patients in the hydroxychloroquine group and 83% in the placebo group; $P=.82$).

As shown in TABLE 2 and eFigure 1 (available at http://www.jama.com), there was no difference between treatment groups in the change of CD8 cell activation as measured by the percentage of cells expressing CD38 and HLA-DR (–4.8% and –4.2% in the hydroxychloroquine and placebo groups, respectively; difference, –0.6%; 95% CI, –4.8% to 3.6%; $P=.80$). There were no significant differences between groups in change in CD4 cell activation or levels of D-dimer and IL-6 (Table 2), nor in change in expression of Ki67 on CD4 or CD8 cells or in other inflammatory cytokines (eTable), although increased expression of caspase 3 on CD8 cells was observed in the hydroxychloroquine group ($P=.03$).

Patients taking hydroxychloroquine had a significant reduction in total CD4 cell count (–85 cells/μL vs –23 cells/μL at week 48; difference, –62 cells/μL; 95% CI, –115 to –8; $P=.03$) compared with placebo (Table 2 and FIGURE 2). In a sensitivity analysis, the difference remained after adjusting for baseline viral load by including it as a covariate in the model. The time to first CD4 cell count below 350 cells/μL was significantly shorter in the hydroxychloroquine group (eFigure 2). Total CD8 cell count and total lymphocyte count were also significantly reduced in the hydroxychloroquine group by week 48, but the percentage of CD4 and CD8 cells was unchanged (Table 2). There was a significant reduction in the proportion of central memory (RA-CD27+) CD8 cells (–5.9% vs –0.9%; difference, –5.0%; 95% CI, –7.6% to –2.4%; $P<.001$) and an increase in proportion of terminally differentiated (RA+CD27–) CD8 cells (5.1% vs 1.0%; difference, 4.1%; 95% CI, 2.1% to 6.1%; $P<.001$) in the hydroxychloroquine group compared with placebo (Table 2), but no change in naive or effector CD8 cells. Similar patterns were observed for CD4 cell subsets (eTable).

Viral load increased significantly in the hydroxychloroquine group compared with placebo (0.61 vs 0.23 $\log_{10}$ copies/mL at week 48; difference, 0.38 $\log_{10}$ copies/mL; 95% CI, 0.13 to 0.63; $P=.003$). The increase in viral load was apparent by week 12 (FIGURE 3), and

Table 1. Baseline Characteristics

	No. (%)	
	Hydroxychloroquine (n = 42)	Placebo (n = 41)
Age, mean (SD)	37.1 (7.7)	38.3 (10.8)
Age categories, y		
18 to <35	17 (40.5)	18 (44)
35-45	17 (40.5)	13 (32)
>45 to 65	8 (19)	10 (24)
Sex		
Male	40 (95)	37 (90)
Female	2 (5)	4 (10)
Ethnicity		
White	36 (86)	37 (90)
Black	4 (10)	3 (7)
Other[a]	2 (5)	1 (2)
Mode of infection		
Sex between men	38 (90)	36 (88)
Sex between men and women	4 (10)	4 (10)
Blood contact, nontransfusion	0	1 (2)
Time since HIV diagnosis, median (IQR), y	3.0 (1.7-5.6)	2.5 (1.7-3.5)
CD4 count, cells/μL		
<400	12 (29)	10 (24)
400-499	11 (26)	11 (27)
500-599	10 (24)	11 (27)
≥600	9 (21)	9 (22)
Median (IQR), cells/μL	492 (392-557)	492 (404-586)
HIV RNA, copies/mL		
<5000	4 (10)	5 (12)
5000-19 999	16 (38)	25 (61)
20 000-99 999	15 (36)	8 (20)
≥100 000	7 (17)	3 (7)
Mean (SD), $\log_{10}$ copies/mL	4.3 (0.5)	4.1 (0.5)
CD8+ CD38+ HLA-DR+, cells/μL		
<100	4 (10)	13 (32)
100-199	11 (26)	11 (27)
200-299	12 (29)	8 (20)
≥300	15 (36)	9 (22)
Median (IQR), cells/μL	282 (146-386)	159 (83-252)

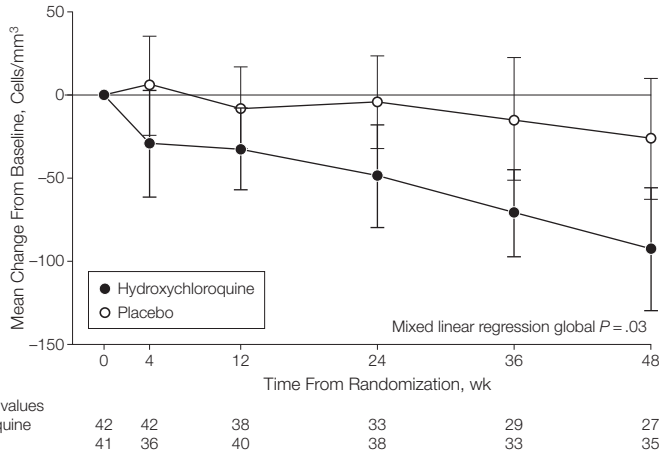
Abbreviation: IQR, interquartile range.
[a]Hispanic, Asian, or mixed race.

Table 2. Changes in Immunological and Inflammatory Parameters and HIV Viral Load From Baseline to Week 48[a]

	Baseline, Mean (SD)		Change at Week 48, Mean (95% CI)			
Marker[b]	Hydroxychloroquine	Placebo	Hydroxychloroquine	Placebo	Hydroxychloroquine – Placebo	*P* Value[c]
CD8 CD38+ DR+, %	28.7 (13.7)	21.5 (13.6)	–4.8 (–7.9 to –1.6)	–4.2 (–7.1 to –1.3)	–0.6 (–4.8 to 3.6)	.80
CD4 CD38+ DR+, %	6.2 (3.5)	4.8 (2.8)	1.2 (0.2 to 2.2)	0.0 (–0.9 to 0.9)	1.2 (–0.1 to 2.6)	.08
IL-6, $\log_{10}$ pg/mL	0.64 (0.60)	0.73 (0.60)	–0.05 (–0.27 to 0.16)	–0.04 (–0.24 to 0.15)	–0.01 (–0.27 to 0.24)	.94
D-dimer, $\log_{10}$ ug/L	2.16 (0.31)	2.14 (0.22)	–0.04 (–0.15 to 0.06)	0.02 (–0.07 to 0.12)	–0.07 (–0.19 to 0.06)	.29
CD4 cell count/μL	492 (114)	509 (121)	–85 (–125 to –45)	–23 (–60 to 14)	–62 (–115 to –8)	.03
CD4 cells, %	27.0 (6.6)	29.4 (7.3)	–2.4 (–4.0 to –0.8)	–0.7 (–2.2 to 0.08)	–1.7 (–3.9 to 0.5)	.12
CD8 cell count/μL	1020 (356)	961 (393)	–104 (–188 to –21)	22 (–55 to 99)	–126 (–238 to –14)	.03
CD8 cells, %	53.4 (9.4)	51.9 (9.8)	1.2 (–0.7 to 3.1)	0.9 (–0.9 to 2.7)	0.3 (–2.3 to 2.9)	.83
Total lymphocyte count/μL	2056 (467)	2006 (620)	–294 (–408 to –181)	–95 (–204 to 14)	–199 (–346 to –53)	.01
CD8RA– CD27+, % central memory	40.1 (9.7)	37.6 (14.3)	–5.9 (–7.8 to –4.0)	–0.9 (–2.6 to 0.9)	–5.0 (–7.6 to –2.4)	<.001
CD8RA+ CD27+, % naive	28.3 (9.9)	27.7 (11.5)	–0.2 (–1.6 to 2.1)	–0.7 (–2.4 to 1.1)	0.9 (–1.6 to 3.4)	.47
CD8RA– CD27–, % effector	9.1 (5.8)	10.2 (6.8)	0.6 (–0.3 to 1.5)	0.5 (–0.3 to 1.3)	0.1 (–1.1 to 1.3)	.90
CD8RA+ CD27–, % terminally differentiated	22.6 (7.9)	24.5 (12.3)	5.1 (3.6 to 6.6)	1.0 (–0.4 to 2.4)	4.1 (2.1 to 6.1)	<.001
HIV RNA, $\log_{10}$ copies/mL[b]	4.33 (0.48)	4.11 (0.53)	0.61 (0.37 to 0.85)	0.23 (0.08 to 0.38)	0.38 (0.13 to 0.63)	.003

[a] Changes at week 48 are changes from baseline predicted using mixed linear regression models.
[b] All parameters were measured at the central laboratory other than HIV RNA, which was measured at site local laboratories.
[c] *P* value from parameter representing the difference in slopes between the 2 groups.

Figure 2. Change in CD4 Cell Count During the Trial by Treatment Group



Changes from baseline are based on empirical data, supplemented by individual predicted values for missing data obtained using a linear mixed model. Bars indicate 95% CIs around mean values.

nearly all patients in the hydroxychloroquine group had some degree of increase in viral load above baseline (eFigure 3A), but this resolved on discontinuation of treatment (eFigure 3B).

TABLE 3 shows all clinical adverse events reported by more than 5% of patients in either treatment group. Adverse events were similar in the 2 groups apart from a higher rate of influenza-like illness in the hydroxychloroquine group (29% vs 10%, *P* = .03) with a non-significant increase in upper respiratory tract infections observed in the hydroxychloroquine group. Most of the clinical adverse events were mild, with only 5 classified as grade 3 (1 in the hydroxychloroquine group, 4 in the placebo group) and none classified as grade 4 using the 1992 National Institute of Allergy and Infectious Diseases Division of AIDS toxicity grading scale. There were 6 serious adverse events: 4 in the hydroxychloroquine group (Hodgkin disease at week 11, pneumonia at week 21, severe otitis externa at week 26, and gastroenteritis at week 46, all considered unrelated to study medication) and 2 in the placebo group (tuberculosis at week 28 and acute exacerbation of chronic obstructive pulmonary disease at week 44). Thirty-four participants in the hydroxychloroquine group had a formal eye examination performed at the end of the study or at the time of study drug discontinuation, and none had evidence of hydroxychloroquine-associated retinopathy or other new abnormalities detected. There were 2 grade 3 laboratory adverse events (thrombocytopenia and raised triglycerides, both in the placebo group) and no grade 4 laboratory adverse events reported.

COMMENT

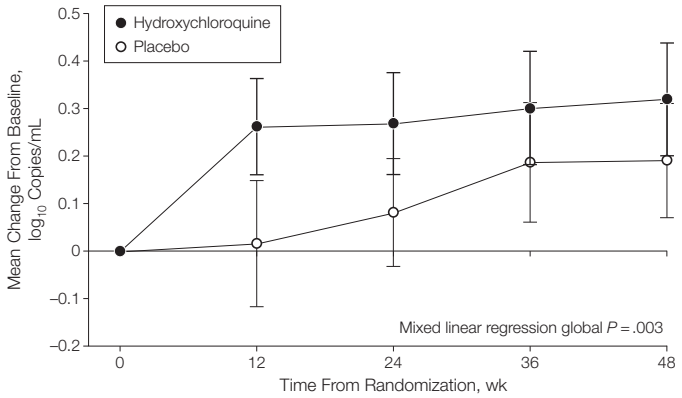
This randomized, double-blind, placebo-controlled clinical trial has demonstrated that hydroxychloroquine is not of benefit in modifying HIV disease course in patients with high CD4 cell counts who have not yet started antiretroviral therapy. Contrary to our original hypothesis, we found that hydroxychloroquine accelerated the decline in CD4 cell count and shortened the time to starting antiretroviral therapy. This unexpected finding contrasts with the re-

sults of previous small short-term comparative trials of hydroxychloroquine in HIV infection that showed no change in CD4 cell count when given as monotherapy in a dose of 800 mg per day for up to 16 weeks[20,21] and either no change or a modest increase in CD4 cells when combined with other antiretroviral drugs.[22-24] The CD8 cell count and total lymphocyte count declined in parallel with the CD4 cell count, indicating that the CD4 cell change was part of a generalized lymphopenia. It is unlikely that the cellular changes were driven by the observed increase in viral load because the effects remained after adjustment for viral load. These changes may be a direct effect of hydroxychloroquine, although they have not been reported in trials of this drug in rheumatoid arthritis or other conditions.[25]

Our finding that hydroxychloroquine increased HIV viral load was also unexpected, given that a number of in vitro studies have shown that chloroquine and hydroxychloroquine decrease HIV replication,[11-14] although one study has shown an increase in HIV infectivity in cells treated with chloroquine.[26] The 2 previous clinical trials of hydroxychloroquine in antiretroviral-naive patients observed reductions in viral load of 0.3 $\log_{10}$ copies/mL and 0.4 $\log_{10}$ copies/mL, which were not statistically different from placebo and zidovudine comparators, respectively.[20,21] The increase in viral load of approximately 0.4 $\log_{10}$ copies/mL that we observed in the hydroxychloroquine group may have resulted from down-regulation of the innate immune response to HIV, an effect previously reported with chloroquine in vitro.[27] The increase in HIV viral load was observed in the majority of patients who received hydroxychloroquine, and this is consistent with an indirect, immune-mediated mechanism. The reason for the divergent findings from the earlier studies is unclear. The earlier studies were of short-term treatment (8 to 16 weeks) using a dose of hydroxychloroquine of 800 mg per day, twice the dose we used. The effects of hydroxychloroquine on endosomal pH and HIV replication in vitro are known to be dose-dependent,[11] and it is possible that greater direct effects on viral replication at a higher dose may outweigh a tendency to increased viral replication resulting from reduction of immunological control of virus replication. However, the dose of hydroxychloroquine that we used is the maximum recommended dose for long-term use, and it would be inappropriate to pursue investigation of a higher dose for this potential long-term treatment indication.

This trial was intended as a proof-of-concept trial prior to a subsequent large-scale clinical trial to look at benefits such

Figure 3. Change in Viral Load



Changes from baseline are based on empirical data, supplemented by individual predicted values for missing data obtained using a linear mixed model. Bars indicate 95% CIs around mean values.

Table 3. Clinical Adverse Events With a Frequency of 5% or Greater in Either Group

	No. of Participants (%) [95% CI]		
	Hydroxychloroquine (n = 42)	Placebo (n = 41)	*P* Value[a]
Upper respiratory tract infection	18 (43) [28-59]	10 (24) [12-40]	.06
Diarrhea	11 (26) [14-42]	12 (29) [16-40]	.47
Flu-like illness	12 (29) [16-45]	4 (10) [3-23]	.03
Blurred vision	5 (12) [4-26]	7 (17) [7-32]	.36
Lethargy/fatigue	8 (19) [9-34]	3 (7) [2-20]	.11
Rash/erythema	4 (10) [3-23]	4 (10) [3-23]	.63
Night sweats	5 (12) [4-26]	2 (5) [1-17]	.23
Dizziness	5 (12) [4-26]	2 (5) [1-7]	.23
Depression	4 (10) [3-23]	3 (7) [2-20]	.51
Headache	4 (10) [3-23]	2 (5) [1-7]	.35
Lower respiratory tract infection	4 (10) [3-23]	2 (5) [1-7]	.35
Nausea	3 (7) [1-19]	3 (7) [2-20]	.65
Abdominal pain	2 (5) [1-16]	4 (10) [3-23]	.33
Oral or genital HSV infection	4 (10) [3-23]	2 (5) [1-7]	.35
Insomnia	3 (7) [1-19]	2 (5) [1-7]	.51
Back pain	4 (10) [3-23]	0 (0) [0-9]	.06
Dry skin/itching	3 (7) [1-19]	1 (2) [0-13]	.32
Bloating/flatulence	3 (7) [1-19]	1 (2) [0-13]	.32
Adverse events leading to treatment discontinuation	1 (2) [0-13]	3 (7) [2-20]	.30
One or more serious adverse events	4 (10) [3-23]	2 (5) [1-7]	.35

Abbreviation: HSV, herpes simplex virus.
[a]Fisher exact test, 1-sided.

as preserving CD4 cell counts and decreasing clinical end points. We chose CD8 cell activation as the primary end point because previous in vitro studies have indicated that hydroxychloroquine may block various steps on the T-cell activation pathway[9,10] and because we did not expect a small trial to have sufficient power to detect a difference in CD4 cell counts. We did not find evidence of a significant effect of hydroxychloroquine on CD8 cell activation. It is possible that there was an effect of hydroxychloroquine in decreasing immune activation but that this was blunted by the increase in viral load (which would tend to increase immune activation). However, after adjusting for viral load, there was still no effect of hydroxychloroquine apparent on immune activation, and so this seems an unlikely explanation for the absence of an effect.

It is also unlikely that the absence of effect on immune activation can be explained by poor adherence to hydroxychloroquine. Adherence was reported to be good (and equal between the treatment groups), and there was a clear departure from baseline/normal trajectory for a number of parameters in the hydroxychloroquine group (viral load, total lymphocyte count, CD4 and CD8 cell counts), which can only be explained by a biological effect of the drug to which patients were adherent. Even if there was underreporting of missed doses, the short-term effect would be small as hydroxychloroquine has a very long half-life (months). Although there was no effect of hydroxychloroquine per se, we did observe a decline of immune activation in both treatment groups. The reasons for this are unclear but may be attributable to an effect of regression to the mean or perhaps to a placebo effect; inflammatory immune responses have been shown to be susceptible to conditioning.[28]

Several small and uncontrolled studies have examined the effects of chloroquine or hydroxychloroquine on immune activation. One study of 8 patients taking chloroquine but no concomitant antiretroviral therapy for 8 weeks reported a decrease (from 8% to 5.5%) in the percentage of memory CD8 cells expressing HLA-DR and CD38. Immune activation in the total CD8 cell population was not measured, and only 2 placebo participants were evaluable, so no statistical comparison of these changes with a control group was possible.[29] A second uncontrolled study examined the effect of hydroxychloroquine at 400 mg per day for 6 months in 20 HIV-infected patients who were taking antiretroviral therapy but who had suboptimal CD4 cell increases and found no significant decrease in the percentage of memory (CD45RO+) CD8 cells expressing CD38 during hydroxychloroquine therapy, although this population increased (from approximately 0.9% to 1.7%, significant within group) after cessation of hydroxychloroquine.[23] The lack of an effect of hydroxychloroquine on proinflammatory cytokines that we found contrasts with an earlier study that showed the drug decreased levels of IL-1 and IL-6.[30,31]

Hydroxychloroquine was well tolerated with only 1 patient stopping treatment because of adverse effects and no serious adverse events attributed to this drug. In view of the theoretical concerns about retinal toxicity, we included careful clinical monitoring of eye symptoms as well as a formal ophthalmological assessment at baseline and the end of study. This was a more intensive approach than is usually practiced in the monitoring of hydroxychloroquine for treatment of connective tissue diseases, but we felt it to be important to document the absence of ocular toxicity given that we were investigating a potential treatment indication in a population of asymptomatic patients. We found no excess reported blurred vision in the hydroxychloroquine group, and there was no evidence of hydroxychloroquine-related eye disease on ophthalmological follow-up. This confirms the findings of an earlier study that showed no evidence of retinopathy when hydroxychloroquine was used for up to 3 years in HIV-infected patients.[24]

We found an excess of influenza-like illness and upper respiratory tract infection in the hydroxychloroquine group. A pandemic of H1N1 influenza occurred during the follow-up period of this trial, and it is therefore likely that a number of these reports represent genuine cases of influenza. Although chloroquine decreases influenza virus replication in vitro, our findings of an increased risk of influenza are consistent with the results of a clinical trial of chloroquine for the prevention of influenza that suggested a higher rate of influenza in participants taking chloroquine.[32,33] We can postulate that the same cellular immune changes that may have led to enhanced HIV replication in the present trial also led to a permissive environment for influenza virus replication.

Hydroxychloroquine and chloroquine are very similar in structure, and the biological mechanisms of action appear identical. They have equivalent effects on endosomal pH and gp120 production in vitro,[11] and there is no basis to prefer one drug over the other on grounds of likely efficacy for treating HIV. It is unlikely that the effects would have differed had we performed this trial with chloroquine. We chose hydroxychloroquine in preference to chloroquine for this trial because it is generally regarded to have a better safety profile and thus appeared more suitable for long-term use in HIV infection.[34] It is unlikely that short-term courses of chloroquine for treating acute malaria in HIV-coinfected patients would have a significant effect on the course of HIV disease, but in areas of high HIV prevalence, prolonged use of chloroquine or hydroxychloroquine for treating rheumatological or other conditions may best be avoided until a patient has been shown to be HIV antibody negative.

In conclusion, hydroxychloroquine did not decrease immune activation but had a detrimental effect on CD4 cell count and increased HIV viral replication in patients with chronic HIV infection who were not receiving antiretroviral therapy. Alternative interventions are needed to reduce immune activation and disease progression in early HIV infection. The findings do not rule out the possibility that hydroxychloroquine may decrease immune activation

and inflammation in patients taking antiretroviral therapy. Given the potential for complex and unpredictable effects, interventions for immune activation must be evaluated rigorously in adequately powered randomized controlled trials.

Author Contributions: Dr Paton had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Paton, Goodall, Dunn, Gazzard, Kelleher.
Acquisition of data: Paton, Goodall, Dunn, Franzen, Collaco-Moraes, Gazzard, Williams, Fisher, Winston, Fox, Orkin, Herieka, Ainsworth, Post, Wansbrough-Jones, Kelleher.
Analysis and interpretation of data: Paton, Goodall, Dunn, Franzen, Collaco-Moraes, Gazzard, Williams, Fisher, Winston, Fox, Orkin, Herieka, Ainsworth, Post, Wansbrough-Jones, Kelleher.
Drafting of the manuscript: Paton, Goodall, Dunn, Collaco-Moraes, Kelleher.
Critical revision of the manuscript for important intellectual content: Paton, Goodall, Dunn, Franzen, Collaco-Moraes, Gazzard, Williams, Fisher, Winston, Fox, Orkin, Herieka, Ainsworth, Post, Wansbrough-Jones, Kelleher.
Statistical analysis: Paton, Goodall, Dunn.
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The Hydroxychloroquine Trial Team: *Investigators and Site Personnel:* Barts and The London (Chloe Orkin, MRCP; James Hand; Carl De Souza, RN, MSc); Brighton and Sussex University Hospital (Martin Fisher, FRCP; Tara Maher; Lisa Heald); Chelsea and Westminster Hospital (Brian Gazzard, MD, FRCP; Katrina Dennis; Jessica Taylor); St Georges Hospital (Mark Wansbrough-Jones, FRCP; Beverly Edwards, BSc); St Mary's Hospital Paddington (Alan Winston, MD, MRCP; Peita-Lee Ambrose; Scott Mullaney, BSN, MSc); UCL-Mortimer Market Centre (Ian Williams, MA, FRCP; Steve O'Farrell); King's College Hospital (Frank Post, MBBS, PhD; Lucy Campbell, BSc, MSc; Emily Wandolo, MSc); North Middlesex University Hospital (Jonathan Ainsworth, FRCP; Anele Waters, BSc); Royal Bournemouth Hospital (Elbushra Herieka, MRCOG; Henry Wilding); St Thomas' Hospital (Julie Fox, MD, FRCP; Isabelle Jendrulek, BA, MA). *Central Immunology Laboratory:* Peter Kelleher, PhD, MRCPath; Melanie Hart, PhD; Louise Greathead, MChem, MSc; Rebecca Metcalf, PhD; Samuel Franzen, BSc. *MRC Clinical Trials Unit:* Alejandro Arenas-Pinto, MBBS, PhD; Brian Angus, MD, FRCP; Abdel Babiker, PhD; Ruth Goodall, PhD; David Dunn, PhD; Yolanda Collaco-Moraes, PhD; Kay Taylor; Debbie Johnson, BSc; Patrick Kelleher; Suzie Hennings, BSc, MSc; Fleur Hudson, BA; Filippo Pacciarini, PhD; Liz Brodnicki; Nicholas Paton, MD, FRCP. *Trial Steering Committee:* Andrew Freedman (Chair), MD, FRCP; David Chadwick, PhD, FRCP; Toju Cline-Cole, BSc, MA; Brian Gazzard, MD, FRCP; Abdel Babiker, PhD; Nicholas Paton, MD, FRCP. *Data Monitoring Committee:* Tim Peto, BM, DPhil, FRCP; David Lalloo, MD, FRCP.
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