Hydroxychloroquine Treatment of Patients with Human Immunodeficiency Virus Type 1

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ABSTRACT

Hydroxychloroquine (HCQ), an antimalarial agent used to treat patients with autoimmune diseases, has been shown to suppress human immunodeficiency virus type 1 (HIV-1) replication in vitro in T cells and monocytes by inhibiting post-transcriptional modification of the virus. These in vitro observations have been expanded into an in vivo study of HCQ as a potential anti–HIV-1 agent in HIV-1–infected patients. A randomized, double-blind, placebo-controlled clinical trial was conducted in 40 asymptomatic HIV-1–infected patients who had CD4+ counts between 200 and 500 cells/mm^3. Patients were randomly assigned to receive either HCQ 800 mg/d or placebo for 8 weeks. Virologic and immunologic parameters, including HIV-1 ribonucleic acid (RNA) via use of polymerase chain reaction, viral culture, antigen and mitogen responses, and proinflammatory cytokine levels were measured at the beginning and end of the study. The amount of recoverable HIV-1 RNA in plasma declined significantly in the HCQ group over the 8-week period (P = 0.022), while it increased in the placebo group. The percentage of CD4+ T cells remained stable in the HCQ-treated group (18.1 ± 9.2% before treatment vs 18.6 ± 10.5% after treatment) and fell significantly in the placebo group (21 ± 7% before treatment vs 19.3 ± 6.3% after treatment; P = 0.032). However, this was not reflected as a change in absolute CD4+ counts for either group (HCQ, 262.8 ± 166 cells/mm^3 vs 251 ± 163 cells/mm^3; placebo, 312 ± 121 cells/mm^3 vs 321 ± 124 cells/mm^3). Mitogen- and antigen-specific responses remained constant in the HCQ group while T cell proliferative responses to *Candida* decreased in the placebo group (4.8 ± 3.6 × 10^3 SI [stimulation index] vs 3.0 ± 3.0 × 10^3 SI; P = 0.032). Lastly, serum interleukin 6 levels declined in the HCQ group (14.3 ± 13.5 U/mL vs 12.0 ± 16.7 U/mL; P = 0.023) but not in the placebo group (11.3 ± 8.8 U/mL vs 7.0 ± 11.7 U/mL); this was coincident with a decrease in serum im-

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munoglobulin (Ig)G (2563 ± 1352 mg/mL vs 2307 ± 1372 mg/dL; $P = 0.032$), compared with the placebo group (2733 ± 1473 mg/dL vs 2709 ± 1501 mg/dL). No other parameters, including serum p24 and beta-2 microglobulin levels, were altered by HCQ therapy. HCQ thus may be useful in the treatment of patients with HIV-1 infection.

INTRODUCTION

Numerous agents, either alone or in combination, are available for treating patients with human immunodeficiency virus type 1 (HIV-1).[1] These drugs include both competitive[2–15] and noncompetitive inhibitors of HIV-1 reverse transcriptase[16–19] and HIV-1 protease.[20–23] All these compounds have severe limitations as therapeutic agents because of their significant toxicities[24–27] and the viral resistance associated with their long-term use.[28–34] Consequently, it would be extremely useful to have other, less toxic agents available for treating patients with HIV-1 infection, whether as primary modalities or for use in combination with other drugs.

We have previously demonstrated that hydroxychloroquine (HCQ), an antimalarial agent used to treat patients with autoimmune diseases,[35] suppresses HIV-1 replication in vitro in both T cells and monocytes.[36] HCQ appears to have a different site of action from that of the reverse transcriptase inhibitors zidovudine, 2′,3′-dideoxyinosine, and 2′,3′-dideoxycytidine and the HIV-1 protease and tat inhibitors by blocking posttranscriptional modification of gp120. Treatment of T cells or monocytes before in vitro infection does not prevent viral entry but does produce a noninfectious virus. Other potential sites of action have been identified as well. We have demonstrated that HCQ suppresses the production of interleukin (IL)-6,[37] a cytokine that can upregulate HIV-1 replication in chronically infected cells.[38] Given these in vitro findings, coupled with the proven safety of HCQ in the treatment of autoimmune diseases, we conducted a clinical trial to evaluate HCQ activity in suppressing HIV-1 replication and improving immune function in infected patients.

PATIENTS AND METHODS

We conducted a randomized, double-blind, placebo-controlled study in which HCQ 800 mg/d or placebo was given orally to 40 HIV-1–infected patients at the Mount Sinai Medical Center, after providing written informed consent. The treatment assignments were blinded. This trial was conducted to determine the biologic effect of HCQ in HIV-1–infected patients by assessing various parameters of viral load and immunologic function. Because this was a placebo-controlled trial enrolling patients with CD4+ T cell counts between 200 and 500 cells/mm^3 (including some who stopped other antiretroviral therapy for 4 weeks before the study), a higher dose of HCQ (800 mg/d) was given for a shorter period of time (2 months) rather than the 400-mg/d, 6-month dose used routinely in the treatment of patients with rheumatic diseases.[35]

For assessment of viral load, duplicate plasma samples of HIV-1 ribonucleic acid (RNA) were measured by use of polymerase chain reaction (PCR), peripheral blood mononuclear cell (PBMC)-derived virus was measured by use of viral cocultures, and serum p24 antigen levels were determined. For immunologic assessment, we measured the percentage and

total CD4+ count, antigen and mitogen responses, beta-2 microglobulin levels, IL-1, IL-6, and tumor necrosis factor (TNF)-α plasma levels at the beginning and end of the trial period. Successful treatment was defined as a reduction in levels of serum p24, plasma HIV-1 RNA, or cultured virus from the PBMC. Patients were monitored at 2-week intervals for clinical parameters, routine laboratory assessment, including complete blood count and liver chemistry, and compliance with the medication regimen. Compliance was determined by counting pills at each clinical visit. Because retinopathy is the most serious side effect of HCQ,[35] the patients were examined for color discrimination, Amsler grid analysis, and visual acuity by an ophthalmologist at the beginning and end of the 8-week study period.

Both men and women were eligible for this study. Patients who participated had CD4+ counts between 200 and 500 cells/mm^3, were asymptomatic, and were not receiving any antiretroviral agents. Participants who had previously received zidovudine, 2′,3′-dideoxyinosine, or 2′,3′-dideoxycytidine therapy stopped these medications 4 weeks before the start of the clinical trial. All women of childbearing potential had to have negative pregnancy test results 2 weeks before the start of the study and use birth control methods (either oral contraceptives or barrier contraceptives). All of the participants had to have hemoglobulin levels of 8.5 g/dL or greater; neutrophil counts of 1000 cells/mm^3 or greater; platelet counts of 75,000 cells/mm^3 or above; aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase levels less than three times the upper limit of normal; and serum amylase levels less than 1.3 times the upper limit of normal. Patients younger than age 18, women who were breast-feeding, and patients with known G6PD deficiency, were excluded from the study. Patients also were excluded if they had acquired immunodeficiency syndrome (AIDS)-defining infections (eg, *Pneumocystis carinii* pneumonia), malignancies (Kaposi's sarcoma), or were actively abusing alcohol or drugs. Patients with stage 2 AIDS dementia complex were similarly excluded. Patients were withdrawn from the study if they demonstrated noncompliance in taking the study medication.

Viral Load Assessment

Plasma HIV-1 RNA levels were measured in the patient samples[39] before treatment and at the last study visit at week 8. One milliliter of each plasma sample was treated with 0.5 mL of RNAzol B® (Cinna/Biotec, Dallas, Texas) and 0.1 mL of chloroform (Sigma Diagnostics, St. Louis, Missouri). First-strand cDNA synthesis from the RNA was accomplished using reverse transcriptase (MuLV, Bochringer-Mannheim Corporation, Indianapolis, Indiana). The reactions were incubated for 1 hour each at 37 °C. cDNA was amplified using the PCR for 40 cycles in a reaction mixture containing the HIV primers SK145 and SK150B (Digene Diagnostics, Inc., Silver Spring, Maryland). An HIV-1–specific DNA probe complementary to a highly conserved sequence within the SK145 and SK150B region was generated using an oligonucleotide sequencer (Pharmacia Biosystems, Piscataway, New Jersey). The sequence of the 41-base probe was as follows: 5′-TCTATCCCATTCTACAGCTTCCTCATTGATGGTCTCTTTTTA-3′. After heat denaturation of the PCR products, hybridization of the labeled probe and PCR product was carried out at

55 °C for 15 minutes. The probe-target duplex was then separated from the unhybridized probe by use of gel electrophoresis. Gels were dried under vacuum at 70 °C for 90 minutes and then autoradiographed. Regions of the gel producing bands corresponding to the probe-target duplex were excised and radioactivity measured in a beta-scintillation counter. This assay has been used previously to quantitate RNA.[40,41] Determinations were made at the beginning and end of the 8-week study period. The PCR had a lower limit of detection of 10^2 copies which corresponded to approximately 100 counts per minute (cpm). Correlative analyses were performed at Roche Laboratories (Burlington, North Carolina) in six paired samples using a quantitative RNA PCR. Although the current PCR measured cpm and the Roche assay measured viral copies/mL, there was a correlation between their increases and decreases.

p24 antigen levels were determined in the culture supernatants of serially diluted (2×10^6 to 1×10^2) phytohemagglutinin (PHA)-stimulated (1 μg/mL) patient PBMC co-cultured with PHA-stimulated (1 μg/mL) normal control PBMC (2×10^6) in Rosewell Park Memorial Institute 1640 (GIBCO, Grand Island, New York) supplemented with 10% IL-2 (Boehringer-Mannheim Corporation), 1% penicillin/streptomycin (GIBCO), and 2 mM L-glutamine (GIBCO); this is henceforth called complete medium (CM), according to previously established methods[42] where a commercially available kit (Du Pont Company, Wilmington, Delaware) was used. Supernatants were then sampled at weekly intervals and supplemented with CM and uninfected PBMC (2×10^6) for a total of 4 weeks. A culture was considered to be positive if p24 levels in the culture supernatant exceeded 1000 pg on a single determination, or more than 100 pg/mL on two occasions. The dilution of PBMC required to produce a positive culture was the reciprocal of the viral titer, and titers of infectious HIV-1 were expressed as 0.5, 5, 50, 500, 5000, or 10,000 tissue culture infective doses (TCID) per 10^6 PBMC. Determinations were made at the beginning and end of the 8-week study period. Supernatants from the patients' PBMC were maintained in culture for 4 weeks, but samples for p24 were done biweekly. These determinations were done at the beginning and end of the study.

Similar to the p24 antigen levels determined from the culture supernatants of the PBMC, serum levels of p24 were measured under acid dissociation conditions before treatment and at the end of the 8-week study using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Du Pont).

Immunologic Assessment

At the beginning and end of the study, CD4+ count, percentage of CD4+ cells, absolute lymphocyte count, and white blood cell (WBC) count were measured by using previously established methods.[43] Beta-2 microglobulin was measured by competitive ELISA using a commercially available kit (Immunotech, Marseilles, France).

Mitogen and antigen stimulations were performed by using previously described methods.[43] Briefly, PBMC (1×10^5 cells/well) isolated at the beginning and end of the study were cultured in CM in the presence or absence of either PHA (1 μg/mL) (GIBCO), Con A (10 μg/mL) (GIBCO) and pokeweed mitogen (1 μg/mL) (GIBCO), for 72 hours, or in the presence

or absence of tetanus toxoid (40 μg/mL) (Wyeth-Ayerst Laboratories, Philadelphia, Pennsylvania) or *Candida* (10 μg/mL) (Hollister-Stier, West Haven, Connecticut) for 96 hours. During the last 18 hours of culture, 1 μCi ^{3}H thymidine was added to each well, harvested, and counted as previously described.[40] Each assay was performed in parallel with normal control PBMC to account for interassay variability. The data were expressed as counts per minute for triplicate wells. These results were compared with the proliferative responses of unstimulated T cells. The stimulation index (SI) was computed using the following formula: SI = T cells + antigen or mitogen (cpm)/unstimulated T cells (cpm).

Plasma levels of IL-1-α and IL-1-β were measured using commercially available ELISA kits (ARI Systems, Paint Branch, Maryland). Plasma levels of TNF-α also were measured by using ELISA (Endogen, Inc., Boston, Massachusetts).

The IL-6–dependent murine B9 plasmacytoma (a gift of L. May, PhD, Rockefeller University, New York) was used to measure IL-6 in the patients' plasma before and after the study.[37,44] In these studies, serial dilutions of test plasma were added to 5×10^3 B9 cells and cultured in CM for 3 days. One μCi ^{3}H thymidine was added during the last 16 hours of culture and the cells were then cultured and processed for scintillation counting. Standard IL-6 curves were obtained using known concentrations of recombinant IL-6 (provided by E. Siden, PhD, Mount Sinai School of Medicine). The specificity of the bioassay was demonstrated by total inhibition of proliferation of B9 cells by a polyclonal anti–IL-6 antibody.

Serum immunoglobulin (Ig) levels of IgG, IgM, and IgA were determined by a commercial laboratory (MetPath, Teterboro, New Jersey).

HCQ Levels

In the HCQ group, plasma concentrations of HCQ and its metabolites (desethylhydroxychloroquine, desethylchloroquine, and bidesethylchloroquine) were measured at the end of the study by use of a high-performance liquid chromatographic method previously described.[45]

Statistical Analysis

All data are given as mean ± SD. Study parameters were compared at the end of the 8-week study period by use of previously established methods[46] utilizing paired Student's *t* tests.

RESULTS

Forty patients were recruited for the trial, 20 in the HCQ group and 20 in the placebo group. Thirty-eight patients completed the clinical trial, 19 in the HCQ group, and 19 in the placebo group. Two patients (1 in the placebo group and 1 in the HCQ group) were withdrawn from the study because of noncompliance. Seven of the patients in the placebo group and six patients in the HCQ group had received antiretroviral therapy before entering the study. The HCQ and placebo groups were matched for age and sex. There were 15 men and 4 women in the HCQ group ranging in age from 29 to 52 years (mean age, 39.1 ± 6.6 years). There were 18 men and 1 woman in the placebo group ranging in age from 25 to 62 years (mean age, 40.6 ± 12.5 years). There were no changes in the results of routine laboratory tests.

Patients had no adverse reactions to the study drug, and the ophthalmologic examinations (Amsler grid, color discrimination, and visual acuity) before and after the study were unchanged.

Viral Load Assessment

The antiviral effect of HCQ was evaluated by measuring the plasma levels of HIV-1 RNA with cultured virus before and after treatment. HIV-1 RNA was reverse transcribed, amplified, and then quantified by ligation with an HIV-1–specific labeled probe. All analyses were performed at least twice, with a correlation between the studies. Total plasma levels of HIV-1 RNA decreased significantly in the HCQ group (range, 98 to 2517 cpm; mean, 168 ± 144 cpm vs 311 ± 331 cpm; $P = 0.022$) during the 8-week study period (table). As shown in the figure, which represents the ratio of pretreatment to posttreatment plasma HIV-1 RNA measured as a bound, radiolabeled probe (cpm), plasma HIV-1 RNA levels fell significantly ($P = 0.022$) after HCQ therapy in 12 of the 17 patients (values greater than 1) in whom the PCR was performed. HIV-1 RNA levels remained the same in 4 patients (values of approximately 1) and rose in 2 patients (values less than 1). By contrast in the placebo group, total plasma levels of HIV-1 RNA increased over the 8-week period (range, 201 to 2153 cpm; mean = 222 ± 215 cpm vs 302 ± 224 cpm; NS). Plasma HIV-1 RNA levels increased in 11 patients (values less than 1), remained the same in 2 patients (values of approximately 1), and fell in 5 patients (values greater than 1). There were no clearly defined characteristics in the HCQ-treated patients whose HIV-1 RNA levels increased (n = 3) or in those patients in the placebo group whose HIV-1 RNA levels decreased (n = 5).

The assay used here has been previously utilized as an appropriate assessment of viral load.[47] However, other groups have reported variability in such an assay system.[48] Therefore, we compared the results obtained with the study PCR with the quantitative PCR from Roche Laboratories. In 6 paired samples (3 in the HCQ group and 3 in the placebo group), there was a correlation between the study PCR and the Roche PCR, thus validating the current system. An attempt was made to correlate the decrease in HIV-1 RNA levels with a change in serum p24 levels and cultured virus. Because of the chosen study population (asymptomatic HIV-1–infected patients with CD4+ counts between 200 and 500 cells/mm^3), 20 (11 in the HCQ group and 9 in the placebo group) of the 38 patients had no detectable serum p24 and 19 had negative viral cultures. There was a decrease in serum p24 levels in the HCQ group (16.0 ± 18.7 pg/mL vs 12.6 ± 19.1 pg/mL) compared with the placebo group (1.3 ± 3.1 pg/mL vs 1.3 ± 3.4 pg/mL) (table). Additionally, there was considerable variability in culturing HIV-1 from PBMC, as had been previously described with this assay system.[42] However, in those patients (n = 20, 11 in the HCQ group and 9 in the placebo group) in whom virus could be identified by use of p24 antigen capture after a 4-week culture period, there was a decrease in the amount of recoverable virus in the HCQ-treated group (58.1 ± 129 TCID vs 5.3 ± 22.9 TCID) compared with the placebo group (0.7 ± 2.4 TCID vs 0.6 ± 2.4 TCID) (table). Thus these in vivo effects of HCQ support the previously described in vitro findings.

There were no significant changes in the absolute CD4+ count before and after treatment in either the HCQ- or placebo-treated group (262 ± 166 cells/mm^3 vs 251 ± 163 cells/mm^3 and 312 ± 121 cells/mm^3 vs 321 ± 124 cells/mm^3, respectively) (table). However, there was a decrease in the percentage of CD4+ T cells in the placebo group 21 ± 7.0% vs 19.3 ± 6.3%; $P = 0.032$) compared with the HCQ group (18.1 ± 9.2% vs 18.6 ± 10.5%). Beta-2 microglobulin levels, absolute lymphocyte counts (ALC), and WBC counts were unchanged in the two groups (table).

Immunologic Assessment

To further describe the effect of HCQ on immune function, we evaluated mitogen and antigen responses in both the HCQ and placebo groups at the beginning and end of the study. HCQ has been used to treat patients with a variety of autoimmune diseases and has also been reported to blunt mitogen- and antigen-specific responses.[49,50] Proliferative responses to PHA, Con A, PWM, *Candida*, and tetanus toxoids were measured. There were significant decreases in the proliferative responses to *Candida* (4.8 ± 3.6 × 10^3 SI vs 3.0 ± 3.0 × 10^3 SI; $P = 0.032$) over the 8-week period in the placebo group, but not in the HCQ group (data not shown). PHA responses for the placebo group decreased in 14 patients and increased in 5. By contrast, in the HCQ group, the PHA response was reduced in only 5 patients but increased or remained the same in 14. Con A responses in the placebo group decreased in 12 patients, and increased or remained the same in 7; in the HCQ group, the response decreased in 7 but remained the same or increased in 12. No change in response to PWM or tetanus was evident in either group. There appeared to be no correlation between increases in the percentage of CD4+ T cells or increases in antigen or mitogen responses and decreased HIV-1 RNA levels.

Infection with HIV-1 has been correlated with increased circulating levels of IL-1-α, IL-1-β, TNF-α, and IL-6, all of which have been associated with cachexia and hypergammaglobulinemia. IL-6 has been shown to play an important role in HIV-1 infection by upregulating HIV-1 in chronically infected HIV cells.[38] We previously demonstrated that HCQ inhibits the production of IL-1-α and IL-6 in vitro by inhibiting posttranscriptional modification.[37] When the effect of HCQ on plasma levels of IL-1-α, IL-1-β, TNF-α, and IL-6 was assessed, there were no differences in IL-1-α, IL-1-β, and TNF-α before or after treatment in either the HCQ or placebo groups. There was, however, a statistically significant decrease in the plasma levels of IL-6 after treatment in the HCQ group (14.3 ± 13.5 U/mL vs 12.0 ± 16.7 U/mL; $P = 0.023$) but not in the placebo group (11.3 ± 8.8 U/mL vs 7.0 ± 11.7 U/mL). As a potential consequence of the decrease in IL-6 levels, there was a significant decrease in serum IgG levels in the HCQ group (2563 ± 1352 mg/dL vs 2307 ± 1372 mg/dL; $P = 0.032$) but not in the placebo group (2733 ± 1473 mg/dL vs 2709 ± 1501 mg/dL). This decrease was selective for IgG as there were no statistically significant decreases in IgA or IgM levels. These findings are consistent with previous reports[51–53] that HCQ therapy decreases immunoglobulin levels selectively in patients with rheumatoid arthritis and Sjögren's syndrome.

HCQ Levels

The finding that levels of HIV-1 RNA did not decrease in some HCQ-treated patients suggests that the effect of HCQ may be concentration related. Therefore, we measured blood levels of HCQ and its major metabolites in the HCQ-treated group. Unmetabolized HCQ is the active drug and its major metabolites, desethylhydroxychloroquine and bidesethylhydroxychloroquine, correlate with HCQ levels. The mean HCQ level in the treated patients was 316.3 ng/mL, but there was a wide range of values (27 to 1000.4 ng/mL). Study parameters in patients whose HCQ levels were above the mean were compared with those of patients whose levels were below the mean. There was no correlation between higher HCQ levels and either a decrease in HIV-1 RNA levels or an increase in CD4+ T cells, beta-2 microglobulin levels, mitogen and antigen responses, or IL-6 levels. However, the patient with the highest HCQ level had the best response to HCQ. Absolute CD4+ T cells in this patient increased from 200 to 400 cells/mm^3, the percentage of CD4+ T cells increased from 11% to 34%, and plasma levels of HIV-1 RNA decreased from 225 to 135 cpm. There was also improvement in mitogen responses (PHA, 19.7 vs 244.5 SI; Con A, 4.6 vs 84.6 SI; PWM, 13.0 vs 31.0 SI). IL-6 levels also were reduced (4.0 U/mL vs 0 U/mL) along with serum IgG levels (5668.0 mg/dL vs 5342.8 mg/dL).

DISCUSSION AND CONCLUSION

Any agent used to treat patients with HIV-1 infection should be able to inhibit HIV-1 replication. HCQ significantly reduced the levels of detectable RNA in plasma (figure) in 12 of the 19 patients given the drug. There was also a reduction in the amount of cultured virus from PBMC from the HCQ group compared with the placebo group but owing to the variability in HIV-1 isolation and the asymptomatic status of the study participants, these differences did not achieve statistical significance.

The study was limited because all of the patients were asymptomatic with a low viral load. It is also possible that the patients in the HCQ group had a greater viral load than those in the placebo group, making it that much more likely to document an anti–HIV-1 effect. The study evaluating patients with more advanced stages of HIV-1 infection might be more telling in this regard. Also, patients were only studied for an 8-week period. While anti–HIV-1 effects have been demonstrated for zidovudine, 2′,3′-dideoxyinosine, 2′,3′-dideoxycytidine, nevirapine, and HIV-1 protease inhibitor in short periods of time, clinical responses to HCQ take longer.[52] Hence, there were no significant changes in absolute CD4+ levels, WBC, ALC, or percentage of CD4+ cells in the HCQ-treated patients (table). Interestingly, there was a statistically significant decrease ($P = 0.032$) in the percentage of CD4+ cells in the placebo group (table). Although the decrease in the percentage of CD4+ cells in the placebo group was unusual, it may represent stabilization of immune function in the HCQ group. Absolute CD4+ levels may be an incomplete surrogate marker, especially in asymptomatic patients, owing to the variability of the WBC count and percentage of lymphocytes.[54] There was no statistically significant decrease in serum beta-2 microglobulin levels, although this

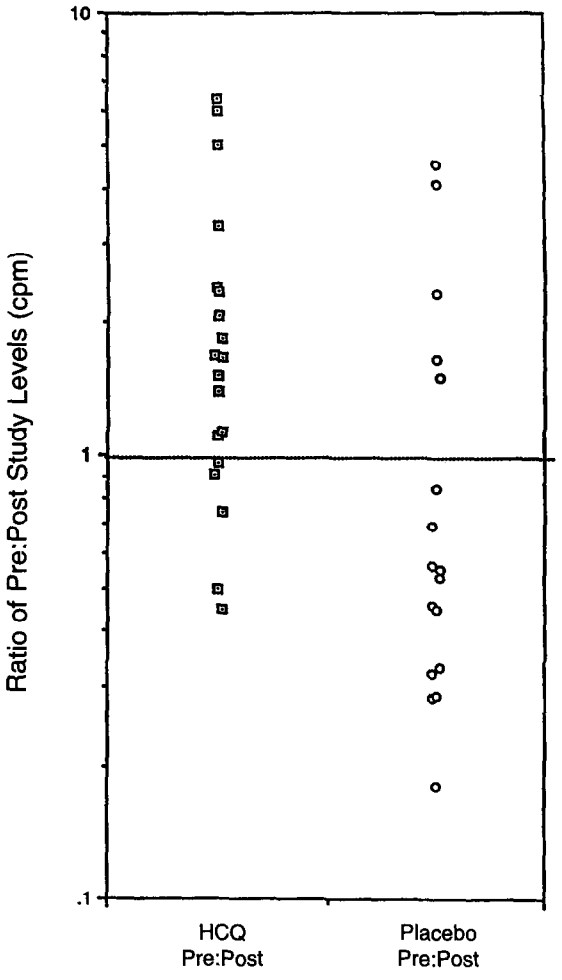

Figure. Plasma human immunodeficiency virus type 1 ribonucleic acid levels in the hydroxychloroquine (HCQ) and placebo groups before and after the 8-week study period as assessed by the use of a semiquantitative polymerase chain reaction (PCR). Data are expressed as a ratio of pretreatment:posttreatment (Pre:Post) study levels measured as incorporated radiolabel counts per minute after PCR.

marker is a relatively weak indicator of disease progression (table). The decrease in mitogen and antigen responses in the placebo group but not in the HCQ group suggests that HCQ prevents a decline in immune function (table). This stabilization concurred with previous findings that HCQ blunted an immune response to ra-

Table. Clinical parameters in patients receiving hydroxychloroquine (HCQ) or placebo.

	HCQ (n = 19)		Placebo (n = 19)	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
CD4+ cells/mm^3	263 ± 166	251 ± 163	312 ± 121	321 ± 124
% CD4+	18 ± 9	19 ± 10	21 ± 7	19 ± 6*
WBC/mm^3 × 10^3	4 ± 2	4 ± 2	5 ± 3	5 ± 4
ALC/mm^3	1823 ± 750	1357 ± 850	1875 ± 823	2087 ± 937
Beta-microglobulin (mg/mL)	4 ± 3	4 ± 3	4 ± 2	4 ± 3
Serum p24 (pg/mL)	16 ± 19	13 ± 19	1 ± 3	1 ± 3
TCID	58 ± 129	5 ± 23	1 ± 2	1 ± 2
PCR (cpm)	311 ± 331	168 ± 144†	222 ± 215	302 ± 224
PCR (viral copies/mL)	5136 ± 836	1334 ± 899	835 ± 136	988 ± 455

WBC = white blood cell count; ALC = absolute lymphocyte count; TCID = tissue culture infective doses; PCR = polymerase chain reaction; cpm = counts per minute.
*P = 0.032, compared with pretreatment values.
†P = 0.022, compared with pretreatment values.

bies vaccine in Peace Corps workers[49] and mitogen responses in patients receiving chloroquine for rheumatoid arthritis.[51,52]

HCQ may have other sites of action in HIV-1 infection. IL-6 has been described as a co-factor in disease progression. Several agents, including pentoxyphylline,[55] have been reported to decrease IL-6 levels in vitro. Elevated IL-6 levels in HIV-1 infection[56] and increased circulating IL-6 may be important co-factors for the development of non–Hodgkin's lymphoma and Kaposi's sarcoma.[57] In our study, HCQ reduced plasma IL-6 levels. Selective inhibition of IL-6 by HCQ has been demonstrated in vitro as well.[37] Serum IgG levels were also significantly reduced in the HCQ group, which may have been related to the reduction in the IL-6 levels, although there has been no direct correlation between IL-6 and immunoglobulin levels.[58] The reduction in immunoglobulin levels may be important because autoantibodies may contribute to the autoimmune diseases and CD4+-cell depletion observed in HIV-1 infection.[59]

We attempted to find the factors that would account for the variability in response seen with HCQ therapy. Previous studies did not relate blood levels of HCQ to a better and more rapid clinical response[45] in rheumatoid arthritis treatment. Recently, Tett et al[60] demonstrated that increased blood levels of HCQ (>1000 ng/mL) were related to a better clinical response in the treatment of patients with rheumatic diseases. A similar effect may have occurred in this study. While increased plasma or tissue levels of HCQ have been associated with an increased risk of ocular toxicity, recent studies have demonstrated the safety of the 800-mg dose for extended periods of time.[61] Because HCQ interferes with gp120 production at a site distinct from zidovudine, 2′,3′-dideoxyinosine, or 2′,3′-dideoxycy-

tidine, it may be especially effective in combination with established anti–HIV-1 agents. Preliminary laboratory experiments have demonstrated additive in vitro antiviral effects between HCQ and either zidovudine, 2′,3′-dideoxycytidine, or 2′,3′-dideoxyinosine.[62]

HCQ also has the additional benefit of inhibiting IL-6 production, which may be an important co-factor in the development of non–Hodgkin's lymphoma and Kaposi's sarcoma. In conclusion, a larger study is needed using a population with a more advanced stage of HIV to better assess the efficacy of HCQ as an anti–HIV-1 agent.

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