Treatement Intensification Has no Effect on the HIV-1 Central Nervous System Infection in Patients on Suppressive Antiretroviral Therapy

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Background: Antiretroviral treatment (ART) significantly reduces cerebrospinal fluid (CSF) HIV-1 RNA levels and residual viremia is less frequently found in CSF than in blood. However, persistent intrathecal immunoactivation is common, even after several years of ART. To investigate whether low-level CSF viremia and residual immunoactivation within the central nervous system (CNS) derive from ongoing local viral replication, we conducted a study of treatment intensification in patients on effective ART.

Methods: Ten patients on ART with plasma HIV RNA <50 copies per milliliter for >18 months were included. Intensification was given for in total 8 weeks: 4 weeks with maraviroc or lopinavir/ritonavir (good CNS penetration), and 4 weeks with enfuvirtide (poor CNS penetration). Lumbar punctures were performed 4 weeks before, at intensification commencement, at switchover after 4 weeks, at the conclusion of, and 4 weeks after the intensification period.

Results: No significant changes in HIV RNA, neopterin, β2-microglobulin, immunoglobulin G index, albumin ratio, and CD4+ T-cell count were observed, either in CSF or blood, neither before, during, nor after the intensification periods.

Conclusions: ART intensification did not reduce residual CSF HIV RNA levels or intrathecal immunoactivation in patients on ART. These findings do not support an ongoing viral replication in CNS.

Key Words: antiretroviral drug intensification, cerebrospinal fluid, HIV RNA, viral reservoir

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INTRODUCTION

Combination antiretroviral treatment (ART) has significantly reduced morbidity and mortality for HIV-infected individuals. The large majority of patients initiating ART achieve plasma HIV RNA levels below the limit of detection of clinical assays (20–50 copies/mL). When combination ART became available in 1996, there was hope that it would be possible to eradicate HIV from an infected individual. The hypothesis was that if one could achieve totally suppressed viral replication by ART, short-lived infected CD4+ T-lymphocytes would subsequently die and vanish although no new CD4+ T cells would become infected. However, this hope was destroyed by the discovery of a long-lived latent reservoir of HIV in resting CD4+ T-cells that the immune system or ART cannot reach.1,2 Even though combination ART effectively suppresses plasma viral load to “undetectable” levels, polymerase chain reaction (PCR) assays with higher sensitivities have demonstrated that most HIV-infected patients are actually viremic at low levels.3–5 It has recently been shown that adding another potent antiretroviral drug to the combination regimens of patients with low-level viremia neither reduces the level of residual plasma viremia nor the size of the latent reservoir.6,7 The results of these studies argue against ongoing viral replication as the main source of residual plasma viremia. Instead, this viremia is probably the result of virus released from the stable reservoir in resting CD4+ T cells as those cells become activated, and in smaller part also from other cellular or anatomical reservoirs.8 Chronically infected tissue macrophages in the central nervous system (CNS) have been suggested as an additional viral reservoir.9

ART effectively reduces cerebrospinal fluid (CSF) HIV RNA levels.10,11 Detectable residual viremia (2–20 copies/mL) can also be found in CSF, although less frequently than in...
In addition, many HIV-infected individuals also display signs of increased intrathecal immune activation, even after several years of suppressive ART. This inflammatory response includes activation of macrophages and microglia, measured as increased CSF neopterin levels, and intrathecal immunoglobulin production resulting in an elevated immunoglobulin G (IgG) index. It is unclear whether this persistent immune activation reflects a low-level full-cycle viral replication within cells in the brain or merely release of virus from stable reservoirs.

To determine whether residual CSF viremia and intrathecal immune activation derive from ongoing low-level viral replication in the CNS, we conducted a study of treatment intensification in patients receiving effective ART. Our hypothesis was that if the residual CSF HIV RNA and the persistent inflammatory response in the CNS are driven by ongoing cycles of viral replication, the former would decrease by adding another effective antiretroviral drug that penetrates into the CSF but not by adding a potent drug without the ability to cross the blood–brain barrier (BBB).

**MATERIALS AND METHODS**

**Participants**

Ten HIV-1–infected patients on combination ART whose plasma HIV RNA was less than 50 copies per milliliter for at least 18 months were recruited for this study. Informed consent was obtained from participants. The antiretroviral drugs chosen for intensification were enfuvirtide 90 mg twice a day, maraviroc 150–600 mg twice a day (dosage dependent upon concomitant drugs), and lopinavir/ritonavir 400/100 mg twice a day. Enfuvirtide is the only fusion inhibitor currently available, whereas maraviroc is a CCR5 coreceptor antagonist that solely has an effect on R5-tropic virus. Both of these drugs provide a potent antiviral effect in patients who are naive to fusion and CCR5 inhibitors. However, enfuvirtide is a large molecule that penetrates poorly across the BBB. Maraviroc, on the other hand, has achieved demonstrable CSF concentrations exceeding inhibitory values. Lopinavir, a potent protease inhibitor with known good penetration into CSF when boosted with ritonavir, was used instead of lopinavir, which made it possible also to include subjects with a protease inhibitor–based regimen.

The study was a randomized controlled trial with a crossover design. All participants were given treatment intensification for a total of 8 weeks as follows: 4 weeks with maraviroc (n = 7) or lopinavir/ritonavir (n = 3) and 4 weeks with enfuvirtide (n = 10). Subjects were randomized as to which of the intensification drugs (CNS-penetrating or nonpenetrating) they were started on; they were switched to the other after 4 weeks.

**Tropism Analysis**

Since all participants were virologically suppressed, we were not able to determine coreceptor use by Trofile analysis (Monogram Biosciences, South San Francisco, CA) before intensification for the 7 patients receiving maraviroc. However, a Trofile test was performed on stored plasma samples from 6 of these patients, and also a genotypic coreceptor tropism determination after sequencing the V3 region from peripheral blood mononuclear cells (PBMC) DNA. We used plasma from the last available date where the participants had HIV RNA >1000 copies per milliliter for the Trofile test. The median time from coreceptor determination to initiation of maraviroc was 6.6 years (range: 4.3–11.0). Patients 3, 6, and 7 had R5 virus, according to the Trofile assay. Dual/mixed tropic virus (D/M) was detected in patient 5. The analysis was unsuccessful in patients 2 and 4. No stored samples were available for patient 1. In addition, the genotypic tropism determination on DNA from PBMC showed that patients 3, 4, 6, and 7 had R5 virus. The genotypic tropism determination failed for patients 2 and 5. The results from the tropism determinations are summarized in Table 1.

This study was approved by the Research Ethics Committee of the University of Gothenburg, Sweden, and the Swedish Medical Products Agency.

**Methods**

Lumbar punctures were performed 4 weeks before (~4 weeks), at the start of intensification (0 weeks), at switch-over (4 weeks), at the conclusion (8 weeks), and 4 weeks after the intensification period (12 weeks). Blood was sampled every second week during these 16 weeks and also 24 weeks after start of intensification.

Plasma and CSF samples were used for determination of HIV RNA levels in a modified COBAS AmpliPrep/COBAS TaqMan HIV-1 Version 2.0 (AC-CT2). This version is a fully quantitative long terminal repeat + GAG reverse transcriptase—polymerase chain reaction multiplex assay with a linear range of $2.0 \times 10^5$–1.0 × 10^2 copies per milliliter and with an analytical sensitivity of 20 copies per milliliter. The assay is subtype independent with high and comparable sensitivities for all subtypes (A–H) of HIV-1 group M. By adding an ultracentrifugation step (50,000g at 4°C for 60 minutes) of 10 mL of specimens and resuspension in 1 mL before the routine COBAS TaqMan Version 2.0 assay, we reached an analytical sensitivity of 2 copies per milliliter (to be published elsewhere).

Enfuvirtide, maraviroc, and lopinavir plasma and CSF concentrations were determined by high-performance liquid chromatography tandem mass spectrometry, methods described elsewhere. To estimate the total CNS penetration of the participants’ regimens, we calculated the CNS penetration effectiveness (CPE) rank for each individual.

Neopterin levels were analyzed by a commercially available radioimmunoassay (Henningtest Neopterin; BRAHMS, Berlin, Germany), with a normal reference value of <8.8 nmol/L in serum and <5.8 nmol/L in CSF. β2-microglobulin was measured by nephelometry in an accredited laboratory. The in-house reference values were age-dependent, as follows: <1.8 mg/L in serum for individuals between 20 and 49 years of age; <2.1 mg/L for individuals >49 years of age. In CSF, the reference values are <1.2 mg/L for individuals ≤49 years old and <1.8 mg/L for individuals >49 years of age.
Intrathecal IgG synthesis was determined on the basis of the IgG index, defined as [CSF IgG (mg/L)/serum IgG (g/L)]/[CSF albumin (mg/L)/serum albumin (g/L)]. The reference value for the IgG index was <0.63. The CSF/plasma albumin ratios (calculated as CSF albumin (ng/mL)/blood albumin (g/L)) were used as an index of BBB/blood–CSF barrier disruption.

Determination of the peripheral blood CD4+ T-cell count was measured by direct immunofluorescence in a flow cytometer.

Genotypic coreceptor tropism determination was performed after sequencing the V3 region from PBMC DNA. A fragment of the HIV-1 env gene was amplified with the GeneAmp high fidelity PCR system (Applied Biosystems, Foster City, CA) and a nested PCR protocol, with outer primers 6540 (HXB2 nucleotide position 6540–6560) and 7701 (position 7701–7721) and inner primers 6561 (position 6561–6580) and 7645 (position 7645–7667). Sequencing of the V3 region was done with 3 degenerate internal primers: 5′-AGYRCACTAAATYGACATCG-3′ (forward primer 1); 5′-TCAACHAATRTCTGTAATGTT-3′ (forward primer 2) and 5′-ATTTCTGGRTCTYCCKCTTG-3′ (reverse primer) and the BigDyeTerminator Cycle Sequencing kit v. 3.1 (Applied Biosystems). For prediction of the coreceptor tropism, the clonal geno2pheno prediction algorithm (http://coreceptor.bioinf.mpi-inf.mpg.de/index.php) was used with a false-positive rate set at 10%.

**Statistical Analysis**

Descriptive group statistics are presented as median (range) values. Log10 transformation was applied to all HIV RNA levels in the Figures. We used paired t tests to evaluate changes associated with the intensification. Significance was assessed at the P < 0.05 level.

**RESULTS**

**Study Participants**

Eight of the 10 participants were male. Their median age was 52 years (range 38–62). Patient characteristics are presented in Table 1. Subtypes had previously been determined for 7 of the patients; 4 had subtype B and 1 each had subtypes A, C, and CRF 01_AE. All were on a regimen consisting of 3 or 4 antiretroviral drugs. The patients had been virally suppressed (HIV RNA < 50 copies/mL) for a median of 6.5 years (range: 1.7–10.7). At baseline, the median plasma viral load was 4.8 copies per milliliter (range <2–21.3), and in CSF <2 copies per milliliter (range: <2–11.2). The median peripheral CD4+ T-cell count was 465 cells per microliter. Six had CSF neopterin levels above the normal reference value, with a median concentration of 7.3 nmol/L. CSF β2-microglobulin levels were elevated in seven participants (median 1.7 mg/L). The IgG index was above the normal reference value in 6 of the patients at baseline (median 0.65).

**Effect of Treatment Intensification**

Six participants initially received an antiretroviral drug with good CNS penetration, and the remaining four received enfuvirtide first. Seven of 10 participants had detectable CSF viremia on at least 1 occasion. Individual results for CSF and plasma viral loads, and, neopterin are presented for the first and second groups of patients in Figure 1. There were no significant changes in CSF and plasma HIV RNA levels, CSF and plasma neopterin, CSF and serum β2-microglobulin, IgG index, albumin ratio, or CD4+ T-cell count during any of the intensification periods (Figs. 2 and 3).

**Concentrations of Intensifying Drugs**

To evaluate whether each patient received adequate doses of the drug administered, we analysed the plasma concentrations of the particular drug at the end of each intensification period.

All patients had therapeutic plasma concentrations of maraviroc, lopinavir, and enfuvirtide. Median maraviroc plasma concentration was 94.9 ng/mL (range: 21.4–478.0). Enfuvirtide concentrations ranged from 658 to 7485 ng/mL (median 2417 ng/mL). For the three patients receiving intensification with lopinavir/ritonavir, the plasma lopinavir concentrations were 5644, 7304, and 8831 ng/mL.
As previously reported, maraviroc was detectable in all 7 CSF samples with a median concentration of 3.63 ng/mL (range: 1.83–12.2). All CSF samples were at least 3-fold above the median EC\textsubscript{90} for maraviroc (http://www.emea.europa.eu/humandocs/PDFs/EPAR/celsentri/emea-combined-h811en.pdf).

CNS Penetration

The median CPE rank\textsuperscript{24} of ART regimens for all participants was 2.5 (range: 1.0–3.5) before intensification. Individual ranks are presented in Table 1.

DISCUSSION

Successful antiretroviral therapy most often leads to sustained viral suppression in both plasma and CSF, with HIV RNA levels below the limits of detection of commercial assays.\textsuperscript{29} The development of novel quantitative assays has made it possible to determine HIV RNA levels down to \(\leq 1\) copy per millilitre.\textsuperscript{3} These new techniques have demonstrated residual low-level plasma viremia in the range of 1–20 copies/mL in a majority of patients on effective ART.\textsuperscript{3,5,30} When highly sensitive PCR assays (limits of detection 2–2.5 copies/mL) were used on CSF samples from successfully treated patients, the number of patients with detectable HIV in CSF has ranged from 0% to 41%, depending on the quantitation method used and the cohort studied.\textsuperscript{12,23,31}

Possible sources of residual plasma viremia are ongoing cycles of viral replication not fully suppressed by ART (for example, in anatomical compartments such as the CNS, where drug concentrations may not be sufficient to inhibit HIV replication) or the intermittent release of virus from cellular reservoirs. Augmenting the power of an already effective regimen by adding another potent drug—preferably one with a different mode of action—should reduce the level of viremia insofar as it originates from ongoing rounds of viral replication. Treatment intensification with lopinavir/ritonavir, atazanavir/ritonavir, or efavirenz did not reduce residual plasma HIV RNA levels.\textsuperscript{6} Enfuvirtide intensification has recently been reported as having no effect on the size of the reservoir of latently infected resting CD4\textsuperscript{+} T cells.\textsuperscript{7} In our study, we show that treatment intensification has no effect either on residual CSF HIV RNA levels or intrathecal immune activation over the course of 4 weeks with an antiretroviral drug that penetrates into the CNS. In agreement with previous studies, we could not detect any significant changes in the level of residual plasma viremia during the total intensification period of 8 weeks. In addition, no effect was observed on peripheral immune activation, as measured by serum neopterin and \(\beta_2\)-microglobulin levels.
After initiation of combination ART, there is a sudden pronounced decrease of plasma viremia attributed to the rapid elimination of cell-free virus and clearance of productively infected CD4+ T cells. This is followed by a second slower phase of decay that has been ascribed to the elimination of cells of the monocyte-macrophage lineage with half-lives between 1 and 4 weeks. The productively infected cells in the CNS are perivascular macrophages and microglia, the major phagocytic and antigen-presenting cells in the CNS. There are several factors that enable these cell types to act as viral reservoirs. First, the life span of differentiated tissue macrophages can encompass a few days up to several months, depending on the tissue. Contrary to activated CD4+ T cells, these cells are not affected by the cytopathic effect of HIV to the same extent, which may lead to continuous low-level virus production during the natural life span of these cells. Perivascular macrophages are replaced by cells from the bone marrow. Microglia, a type of macrophage found in the CNS, can persist for much longer than macrophages, and their role as an HIV reservoir may be significant. Not only are microglia able to proliferate in situ, they are also replenished by monocytes from the periphery (although probably only to a minor degree), suggesting that this cell compartment is capable of surviving for the normal human life span.

![FIGURE 2](image1.png)

**FIGURE 2.** No changes were observed in (A) HIV RNA, (B) CD4+ T-cell count, (C) neopterin, and (D) β2-microglobulin in blood before, during, or after the 8-week intensification period. Blue squares indicate mean values for all 10 participants, and the bars, the 95% confidence interval. Black dotted lines demonstrate the normal reference values, and blue dotted lines represent baseline values of each parameter.

![FIGURE 3](image2.png)

**FIGURE 3.** No changes were observed in (A) CSF HIV RNA, (B) CSF neopterin, (C) CSF β2-microglobulin, (D) IgG index, and (E) albumin ratio before, during, or after the 8-week intensification period. Results for the 6 participants receiving initial intensification with CNS-penetrating drug (maraviroc or lopinavir/r) are presented in the upper panel and for the 4 patients receiving enfuvirtide first in the bottom panel. Values are presented as mean and the 95% confidence interval. Black dotted lines demonstrate the normal reference values, and red dotted lines represent baseline values of each parameter.
Macrophages also have the capacity to spread HIV to CD4+ T cells, further enabling them to act as a reservoir for HIV.\textsuperscript{37}

The CNS is often considered a “sanctuary site” where antiretroviral drug concentrations are generally lower than in blood. Our participants were all on effective ART regimens but with varying degrees of CNS penetration. However, there were no differences in the effect of treatment intensification among participants with high or low CPE ranks. In addition to low drug concentrations in CSF, the activity of some antiretroviral drugs, such as the protease inhibitors, may be inferior in chronically infected macrophages, compared with their effect in lymphocytes.\textsuperscript{38}

Despite having several years of virological suppression in CSF, a substantial proportion of patients continue to have signs of macrophage/microglia activation and intrathecal IgG production.\textsuperscript{15} One possible explanation for persistent immune activation in the CNS could be viral replication within or close to the CSF or within brain tissue. However, we found no changes in CSF neopterin levels, β2-microglobulin levels, or IgG index before, during, or after the intensification period. Our results, therefore, do not support the hypothesis that persistent intrathecal immune activation is driven by residual viral replication in the CNS. Other plausible explanations for this phenomenon could be reseeding of the CNS with virus from the periphery, autoimmune phenomena, or a self-supporting state of cellular activation.\textsuperscript{39,40} The humoral immune response might also be the result of a nonspecific immunological reaction because HIV-specific antibodies constitute only a minor part of intrathecally produced antibodies or a consequence of autoimmune reactions triggered by HIV.\textsuperscript{41}

A limitation of this study is the relatively small number of participants. Another potential limitation could be the relative brevity of the intensification period.

In clinical practice, a tropism test is required before initiation of maraviroc therapy. In our study, this was not possible because the patients concerned had been on effective ART for an extensive period. By using the Trofile assay on stored samples or V3 sequencing from PBMCs at baseline, or both, we were able to confirm that at least 4 of the 7 patients who had received maraviroc had R5-tropic virus in plasma. In general, R5 viruses predominate during initial stages of infection, and most HIV strains that infect macrophages utilize the CCR5 coreceptor.\textsuperscript{42} Even in patients with more advanced disease and X4 virus in plasma, a considerable proportion of HIV strains isolated from the CNS are R5 viruses.\textsuperscript{43–46} This is consistent with the key role played by macrophages in HIV CNS infection.\textsuperscript{33,47}

The results from this study are interesting in the view of HIV persistence and reservoirs but have no direct implications in clinical practice. In conclusion, treatment intensification with a potent CNS-penetrating antiretroviral drug does not reduce residual CSF HIV RNA levels or intrathecal immune activation. These findings argue against the hypothesis that ongoing cycles of viral replication are the main source of residual CSF viremia and intrathecal immune.

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