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Effect of Acyclovir on HIV-1 Set Point among HSV-2 Seropositive Persons during Early HIV-1 Infection

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Abstract

We evaluated whether acyclovir suppression during human immunodeficiency virus type 1 (HIV-1) acquisition reduces HIV-1 set point, increases CD4 cell counts, and selects reverse-transcriptase mutations among 76 HIV-1 seroconverters identified in a placebo-controlled trial of twice-daily acyclovir (400 mg) for the prevention of HIV acquisition in herpes simplex virus type 2 (HSV-2)–seropositive persons (HIV Prevention Trials Network study 039). We found no significant difference in plasma HIV-1 RNA levels ($P<.30$)or CD4 cell counts ($P<.85$) between the acyclovir and placebo recipients. V75I and other mutations in HIV-1 reverse transcriptase reported from in vitro acyclovir studies were not observed. In conclusion, acyclovir suppression during HIV-1 seroconversion and the subsequent 6 months does not affect HIV-1 set point.

Keywords

Acyclovir; HIV-1 seroconversion; HIV-1 viral set point; HSV-2

Introduction

Herpes simplex virus type 2 (HSV-2) is common among persons infected with human immunodeficiency virus type 1 (HIV-1), with dual infection rates ranging from 50-90% [1].

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Daily suppressive therapy with standard doses of acyclovir and valacyclovir reduces both clinical and subclinical HSV-2 shedding, and reduces plasma HIV-1 RNA levels by 0.25-0.5 $\log_{10}$ copies/mL in persons with established HIV-1 infection who are dually infected with HSV-2 [2].

Plasma HIV-1 RNA levels typically reach a steady state by 4-5 months after infection [3]; this viral "set point" correlates with HIV disease progression [4,5]. HSV-2 seropositivity and genital ulcer disease (GUD) have been observed to increase plasma HIV-1 viral load in early HIV-1 infection in some but not all studies [6-8].

We evaluated whether acyclovir reduced HIV-1 viral set point among prospectively identified HIV-1 seroconverters who were HSV-2 seropositive and enrolled in a randomized trial of daily acyclovir versus placebo on HIV-1 acquisition. We also explored whether acyclovir therapy selects for antiretroviral drug resistance mutations in HIV-1, as has been demonstrated *in vitro* [9-11].

Methods

We enrolled HIV-1 seroconverters identified in HPTN 039, a placebo-controlled trial of genital herpes suppression with acyclovir (400 mg twice daily) to reduce the risk of HIV-1 acquisition among 3172 HIV-1 seronegative, HSV-2 seropositive men who have sex with men from the United States and Peru and HIV-1 seronegative, HSV-2 seropositive women from South Africa, Zimbabwe, and Zambia. Participants were followed quarterly for HIV-1 testing and evaluation of GUD, as described elsewhere [12]. There was no difference in HIV-1 acquisition rates in the acyclovir and placebo arms, although genital ulcers due to HSV-2 were reduced significantly [12].

Participants who experienced HIV-1 seroconversion during HPTN 039 were eligible to participate in an ancillary study of HSV-2 suppressive therapy on HIV-1 viral load during the 6 months after their first HIV-1-seropositive test result. Participants were instructed to continue their study medication. Participants and staff remained masked to randomization arm (acyclovir versus placebo) during the ancillary study. Participants who were pregnant, had started antiretroviral therapy, or were unable to enroll before the 60-day window from the first positive HIV test result were excluded.

The study was approved by the Division of AIDS Prevention Science Review Committee, Family Health International Regulatory Affairs, and institutional review boards of all collaborating institutions. All participants provided written informed consent.

At enrollment, HIV-1 seroconverters were asked about symptoms compatible with acute retroviral syndrome since their last HIV-1 seronegative visit. Monthly follow-up visits were conducted to dispense study drug, collect bottles with any unused medication for a pill count and provide condoms as well as adherence and risk-reduction counseling. Blood was drawn for determination of CD4 cell counts and plasma HIV-1 RNA levels at enrollment and months 1, 5, and 6. Clinical evaluations conducted at enrollment and months 1, 3, and 6 included anogenital history and examination.

HIV-1 infection was defined as a reactive HIV-1 enzyme immunoassay (EIA) with a positive Western blot result, with repeat confirmatory testing on a sample obtained 2 weeks later performed by the HPTN Central Laboratory at Johns Hopkins University. Plasma HIV-1 RNA assays were batched to minimize intrarun variability and performed at the University of Washington Retrovirology Laboratory, using the Roche Amplicor Monitor Test Kit (version 1.5; dynamic range of 400-750,000 copies/mL). HSV-2 DNA in swabs from anogenital ulcers was tested at the University of Washington Virology Laboratory [20]. CD4 cell counts were

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measured at local laboratories by flow cytometry (FACsCount or FACsCaliber; Becton Dickinson).

Plasma samples collected 6 months after HIV-1 seroconversion were available for HIV-1 resistance testing from 52 of the 76 HIV-1 seroconverters enrolled in the ancillary study, including 33 women (1 from Zimbabwe, 19 from Zambia, and 13 from South Africa) and 19 men (1 from U.S, 18 from Peru). HIV-1 genotyping was performed at the Johns Hopkins University HIV Genotyping Laboratory, using the ViroSeq HIV-1 genotyping system (version 2.8; Celera) and the Prism 3130xl genetic analyzer (Applied Biosystems).

The primary outcome was HIV-1 set point, defined as plasma HIV-1 RNA level 5 and 6 months after enrollment; this time point was chosen to optimize detection of changes in viral set point given greater heterogeneity in viral levels closer to seroconversion. Secondary outcomes were CD4 cell count at 5 and 6 months, incidence of GUD, and symptoms of acute retroviral syndrome.

We estimated 80% power to detect a difference of 0.5 $\log_{10}$ copies/mL in HIV-1 set point with 81 seroconverters, assuming 27 and 54 individuals in the acyclovir and placebo groups, respectively, if acyclovir reduced HIV-1 acquisition by 50%. Analyses were performed using SAS software (version 9.1.3; SAS Institute). A linear mixed-effects model with a random intercept for each participant was fit to the data. The analysis of plasma HIV-1 RNA level or CD4 cell count by treatment arm was adjusted for age, sex, and days from first positive HIV-1 EIA result. The effect of treatment on incidence of symptomatic recurrences of genital ulcers due to HSV-2 after HIV-1 seroconversion was estimated using negative binomial regression, adjusting for the above covariates.

Results

Between October 2003 and November 2007, 148 participants were identified as HIV-1 seroconverters in HPTN 039, of whom 76 enrolled in the ancillary study. Of the 72 participants who did not enroll, 20 became HIV-1 infected before the ancillary study opened and 52 could not be located, declined, or were outside of the 60-day enrollment window. HIV-1 seroconverters enrolled in the ancillary study were similar to those who did not except they were more likely to be female (59% vs. 39%, P= .014).

Forty of the 76 participants were on placebo and 36 on acyclovir. Mean age was 29 years; 59% of participants were women from Africa, 34% were men from Peru, and 7% were men from the United States (Table 1). Women comprised 72% of seroconverters from the acyclovir group and 48% of the placebo group. Adherence to study medication was high: >90% for 80% and 80.6% of placebo and acyclovir recipients, respectively. Only 3 participants were lost to follow-up, 2 from placebo and 1 from acyclovir arms.

Twenty-seven (36%) of 76 HIV-1 seroconverters reported 1 or more symptoms compatible with acute retroviral syndrome since the last visit where they tested negative for HIV-1 infection (Table 2). Placebo recipients were more likely than acyclovir recipients to report symptoms compatible with acute retroviral syndrome (P= .023); this difference persisted after adjustment for sex. All 10 participants who reported 5 or more symptoms of acute retroviral syndrome were in the placebo arm. Mean plasma HIV-1 RNA levels did not differ between acute retroviral symptom categories of 0, 1, 2-4, and ≥5 symptoms (P=.44).

At a median of 6.75 months from estimated time of seroconversion (median for placebo vs acyclovir, 6.69 vs 6.87 months; P= .15), mean plasma HIV-1 RNA levels ± standard deviation were not different by treatment arm (4.11 ± 0.76 $\log_{10}$ copies/mL vs 4.19 ± 0.72 $\log_{10}$ copies/mL for HIV-1 seroconverters in the placebo arm vs acyclovir arm) (Table 2). The difference

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in plasma HIV-1 load at months 5 and 6 (acyclovir minus placebo) in the linear mixed-effects model was 0.16 $\log_{10}$ copies/mL (95% confidence interval [CI]: -0.14 to 0.47 $\log_{10}$ copies/mL, P= .30), adjusting for age, sex, and time from first positive HIV-1 EIA result. Plasma HIV-1 RNA levels were 0.65 $\log_{10}$ copies/mL lower in women compared with men in this model (P<.001). CD4 cell count also did not significantly differ between treatment arms; the difference was -8.3 cells/μL (95% CI, -94 to 77 cells/μL; P= .85) at months 5 and 6.

Symptomatic HSV-2 recurrences (genital ulcers positive for HSV-2 by polymerase chain reaction) occurred less frequently in HIV-1 seroconverters receiving acyclovir than among those receiving placebo, with an incidence of HSV-2 of 0.32 and 0.55 episodes per person-year, respectively (P= .07). All 3 seroconverters who had multiple episodes of GUD during the post-seroconversion follow-up were in the placebo arm (Table 2). Among those with detectable HSV-2 DNA in anogenital swabs, median quantity of HSV-2 DNA was 6.90 $\log_{10}$ copies/mL (range, 3.78, 8.26) and 6.50 $\log_{10}$ copies/mL (range, 3.45, 8.35) for placebo and acyclovir recipients, respectively (P= .91). The risk of HSV-2 GUD was higher after seroconversion for placebo recipients than before seroconversion: risk ratios were 1.91 (95% CI: 1.02-3.55) for placebo recipients and 1.29 (95% CI, 0.43-3.88) for acyclovir recipients.

HIV-1 genotyping results were obtained for 42 (80.8%) of the 52 samples tested, including 22 participants in the acyclovir arm and 20 participants in the placebo arm (1 from the United States, 1 from Zimbabwe, 18 from Peru, 12 from Zambia, and 10 from South Africa). None of the samples had mutations at the following positions in HIV-1 reverse transcriptase that have been associated with acyclovir selection in vitro: V75I, T69N, W71X, R72X, Q151M, and M184V [9,10].

Discussion

This is the first study, to our knowledge, to examine the effect of HSV-2 suppression on plasma HIV-1 set point in HSV-2 seropositive persons during HIV-1 acquisition and the subsequent 6 months. We found no significant difference in plasma HIV-1 load or CD4 cell count in the acyclovir versus placebo arms a median of 7 months after HIV-1 seroconversion. However, we did note a greater incidence of acute retroviral symptoms among seroconverters who were taking placebo, compared to those on acyclovir. Consistent with an earlier report [13], we observed an increased incidence of symptomatic genital ulcer recurrences due to HSV-2 in placebo recipients in the 6 months after HIV-1 seroconversion compared to that before seroconversion.

The lack of difference in plasma HIV-1 RNA levels between HIV-1 seroconverters receiving acyclovir versus placebo during seroconversion and early HIV-1 infection contrasts with results from the largest study of acyclovir suppressive therapy in HIV-1/HSV-2 dually-infected persons with established HIV-1 infection that demonstrated a reduction in plasma HIV-1 viral levels of 0.25 $\log_{10}$ copies/mL [2]. A significantly higher HIV-1 load was observed among 256 seroconverters who reported symptoms of GUD in the prior 10 months than those who did not (4.71 vs 4.32 $\log_{10}$ copies/mL; P= .01) as well as among HSV-2 seropositive adults than HSV-2 seronegative adults (4.56 vs 4.06 $\log_{10}$ copies/mL; P<.01) an estimated 5 months after HIV acquisition [8].

We found no resistance mutations in HIV-1 reverse transcriptase among the HIV-1 seroconverters on suppressive acyclovir. Our finding contrasts with those of in vitro studies which used very high doses of acyclovir in HIV infectivity models with activated CD4 T cells or tonsillar lymphoid explants [9,10] and found V75I and other mutations in HIV-1 reverse transcriptase [10,11] that may confer resistance to some nucleoside reverse transcriptase inhibitors (NRTIs) used to treat HIV-1 infection [14]. However, the V75I mutation has a

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significant fitness cost, and it is possible that any selective advantage conferred by acyclovir resistance did not outweigh the fitness cost at the lower dose of acyclovir used in this study [14].

Limitations of the study included that not all HIV-1 seroconverters from the main HPTN 039 study were enrolled, which reduced the power to detect a smaller difference in HIV-1 set-point; that plasma samples were not always available from some seroconverters; and that HIV-1 levels were at times insufficient to assess NRTI mutations. Assessment of acute retroviral syndrome symptoms relied on retrospective reporting.

One interpretation of our results is that GUD is a consequence and marker of immunosuppression in early HIV-1 infection, rather than a determinant of higher HIV-1 viremia. Although we observed a reduction in clinical HSV-2 recurrences in HIV-1 seroconverters randomized to acyclovir compared to placebo, we found little difference in HSV-2 DNA quantity between treatment arms in participants who had HSV-2 detected from genital swabs. This finding is consistent with our observation from the main HPTN 039 trial that suppression of HSV-2 DNA was lower than expected in Peruvian men and African women [15].

In summary, we observed no significant difference in plasma HIV-1 RNA levels in HSV-2 seropositive women and men who have sex with men who experienced HIV-1 seroconversion and continued acyclovir suppressive therapy or placebo during 6 months after seroconversion. Additional studies are needed to assess whether more potent interventions, such as antiretroviral therapy, can reduce plasma HIV-1 RNA levels and modify disease course in early HIV-1 infection.

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Table 1

Characteristics of Herpes Simplex Virus Type 2-Seropositive Human Immunodeficiency Virus Type 1 (HIV-1) Seroconverters Who Received Placebo versus Acyclovir

Characteristic	Placebo (*n*=40)	Acyclovir (*n*=36)
Age, mean (IQR), years	30 (24-35)	28 (21-33)
Sex		
Women	19 (48)	26 (72)
Men	21 (53)	10 (28)
Region		
Africa	19 (48)	26 (72)
United States	3 (7)	2 (6)
Peru	18 (45)	8 (22)
Circumcised (among men), proportion (%)	0/21 (0)	0/10 (0)
Genital ulcers during the 3 months before study enrollment		
Any self-reported	8 (20)	6 (17)
Any genital ulcers noted on exam from main study	5 (13)	4 (11)
Time in days from first HIV-positive visit to ancillary study enrollment, mean (IQR), days	41 (31-52)	45 (35-56)

NOTE. Data are no. (%) of participants, unless otherwise indicated. IQR, interquartile range.

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Table 2

Genital Herpes Ulcer Outbreaks, Acute Retroviral Symptoms, Plasma Human Immunodeficiency Virus Type-1 (HIV-1) RNA Levels, and CD4 Cell Counts during the First 6 Months after HIV-1 Seroconversion

Outcome	Placebo (*n*=40)	Acyclovir (*n*=36)
Genital ulcers positive for HSV by PCR[a]		
0 episodes	31 (78)	29 (81)
1 episode	6 (15)	7 (19)
2 or 3 episodes	3 (7)	0 (0)
Acute retroviral symptoms[b]		
0 per person	24 (60)	25 (70)
1 per person	1 (2.5)	3 (8)
2 per person	2 (5)	4 (11)
3 per person	1 (2.5)	1 (3)
4 per person	2 (5)	3 (8)
≥ 5 per person	10 (25)	0 (0)
Plasma HIV-1 RNA level, mean ± SD, $\log_{10}$ copies/mL		
Months 0, 1, 5, and 6	4.15 ± 0.71	4.22 ± 0.79
Month 0	4.11 ± 0.63	4.28 ± 0.84
Month 1	4.26 ± 0.69	4.22 ±0.92
Month 5 and 6[c]	4.11 ± 0.76	4.19 ± 0.72
CD4 cell counts, mean ± SD, cells/μL		
Months 0, 1, 5, and 6	485 ± 182	500 ± 202
Month 6	438 ± 171	469 ± 186

NOTE. Data are no. (%) of participants, unless otherwise indicated. HSV, herpes simplex virus; PCR, polymerase chain reaction; SD, standard deviation.

[a] Total no. of episodes during the 6-month period.

[b] P= .023, χ^2 test for trend. The 6 most commonly reported symptoms (placebo vs acyclovir recipients) were fever (33% vs 17%), headache (28% vs 11%), fatigue (25% vs 11%), sore throat (25% vs 8%), myalgias (23% vs 8%) and swollen glands (25% vs 6%).

[c] The difference in plasma HIV-1 RNA levels (acyclovir minus placebo) in the linear mixed-effects model was 0.16 $\log_{10}$ copies/mL (95% confidence interval, -0.14 to 0.47 $\log_{10}$ copies/mL, P= .30).