Herpes Simplex Virus (HSV)–Suppressive Therapy Decreases Plasma and Genital HIV-1 Levels in HSV-2/HIV-1 Coinfected Women: A Randomized, Placebo-Controlled, Cross-Over Trial

Jared M. Baeten,1,2 Lara B. Strick,2 Aldo Lucchetti,6 William L. H. Whittington,2 Jorge Sanchez,6 Robert W. Coombs,2,3 Amalia Magaret,1 Anna Wald,2,4,5 Lawrence Corey,2,3,5 and Connie Celum1,2,4

Departments of 1Global Health, 2Medicine, 3Laboratory Medicine, and 4Epidemiology, University of Washington, and the 5Program in Infectious Diseases, Fred Hutchinson Cancer Research Center, Seattle; 6Asociación Civil Impacta Salud y Educación, Lima, Peru

A randomized cross-over trial of herpes simplex virus type 2 (HSV-2)–suppressive therapy (valacyclovir, 500 mg twice daily, or placebo for 8 weeks, a 2-week washout period, then the alternative therapy for 8 weeks) was conducted among 20 Peruvian women coinfected with HSV-2 and human immunodeficiency virus type 1 (HIV-1) who were not on antiretroviral therapy. Plasma samples (obtained weekly) and endocervical swab specimens (obtained thrice weekly) were collected for HIV-1 RNA polymerase chain reaction. Plasma HIV-1 level was significantly lower during the valacyclovir arm, compared with the placebo arm (−0.26 log10 copies/mL, a 45% decrease [P < .001]), as was cervical HIV-1 level (−0.35 log10 copies/swab, a 55% decrease [P < .001]). Suppressive HSV-2 therapy has the potential to reduce HIV-1 infectiousness and slow HIV-1 disease progression.

Trial registration. ClinicalTrials.gov identifier: NCT00465205.

Herpes simplex virus type 2 (HSV-2) is common among persons infected with HIV-1 (seroprevalence, 50%–90%) [1]. HSV-2 reactivation, including asymptomatic shedding without clinically apparent lesions, has been associated with increased levels of HIV-1 in plasma and genital secretions [2], suggesting HSV-2 reactivation could heighten HIV-1 infectiousness and accelerate HIV-1 disease progression.

Acyclovir and related compounds valacyclovir and famciclovir are routinely used as episodic treatment for symptomatic genital ulcer disease due to HSV-2 and as daily suppressive therapy to decrease the frequency of symptomatic HSV-2 reactivation. Among HSV-2/HIV-1 coinfected individuals, HSV-suppressive therapy decreases symptomatic HSV-2 reactivation and asymptomatic genital HSV shedding [1, 3]. One open-label study found that daily acyclovir therapy was associated with lower plasma HIV-1 levels (by −1/3 log10 copies/mL) [4], and a pooled analysis from the 1990s found high-dose acyclovir therapy, in combination with nucleoside antiretroviral therapy, was associated with a survival benefit [5]. A recent randomized trial among HSV-2/HIV-1 coinfected women found daily valacyclovir reduced plasma and cervicovaginal HIV-1 levels [6].

Thus, HSV-2–suppressive treatment may reduce HIV-1 replication. We conducted a randomized, double-blind, placebo-controlled, cross-over trial of HSV-2–suppressive therapy, with frequent sampling for genital HSV reactivation and plasma and genital HIV-1 levels, among HSV-2/HIV-1 coinfect Peruvian women not on antiretroviral therapy. We hypothesized that suppression of HSV-2 would reduce plasma and genital HIV-1 levels.

METHODS

Eligible women were ≥18 years of age, HIV-1 and HSV-2 seropositive, and had CD4 cell counts >200 cells/μL (the Peruvian cut point for antiretroviral therapy at the time of the study). Exclusion criteria included pregnancy, use of antiretroviral or anti-HSV medications, history of seizure or adverse reaction to any anti-HSV medication, creatinine level >2.0 mg/dL, or hematocrit <30%. The institutional review boards of the University of Washington and Asociación Civil Impacta Salud y Educación approved the protocol. Participants provided written informed consent.


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Reprints or correspondence: Jared M. Baeten, University of Washington, 901 Boren Ave., Ste. 1300, Seattle, WA 98104 (jbaeten@u.washington.edu).
Women were randomly assigned to receive either valacyclovir, 500 mg orally twice daily, or matching placebo. Randomization was 1:1, in blocks of 10. After 8 weeks, participants crossed over to the alternative treatment for an additional 8 weeks. Treatment periods were separated by a 2-week washout. Every 2 weeks, medication was dispensed and adherence was assessed by pill counts. The study drug was supplied by GlaxoSmithKline. Open-label valacyclovir, 1 g twice daily for 3 days, was dispensed, in addition to the study drug, for symptomatic herpes recurrences. Investigators were blinded to treatment assignments.

Each day at home, participants self-collected swab specimens from the genital and perianal skin into a single cryovial of HSV polymerase chain reaction (PCR) medium. Participants kept a daily diary of genital symptoms. Three times weekly, women returned to the clinic for collection of an endocervical swab specimen for quantification of HIV-1; swabs were placed into 500 µL of guanidinium solution [7]. Once weekly, blood was collected for plasma HIV-1 RNA determination. Samples were frozen at −70°C within 8 h of collection, with the exception of home-collected swabs for HSV, which were delivered to the research clinic by participants every 2–3 days and then frozen at −70°C.

HIV-1 serostatus was confirmed by ELISA and Western blot analysis. HSV-2 serostatus was determined by ELISA (HerpeSelect-2; Focus Technologies) and confirmed by Western blot analysis at the University of Washington.

Screening for genital tract infections included culture of cervical specimens for Neisseria gonorrhoeae on modified Thayer-Martin media, rapid plasma reagin and treponema-specific testing (treponema-specific microhemagglutination assay) for syphilis, and vaginal wet mount for bacterial vaginosis, Trichomonas vaginalis, and candidiasis. An additional specimen was batch-tested for N. gonorrhoeae and Chlamydia trachomatis (Aptima; Gen-Probe) at the University of Washington upon study completion.

HSV DNA in anogenital swabs was detected by use of PCR methods described elsewhere [8]. The lower limit of HSV detection was 500 copies/swab (2.70 log_{10} copies/swab). HIV-1 RNA was quantified in plasma and cervical specimens using an independently validated TaqMan real-time PCR assay, as described elsewhere [7]. The lower limit of HIV-1 quantification was 60 HIV-1 RNA copies/mL (1.78 log_{10} HIV-1 RNA copies/mL) for plasma and 188 HIV-1 RNA copies/swab (2.27 log_{10} HIV-1 RNA copies/swab) for cervical swab specimens.

A sample size of 20 was estimated to provide >80% power to detect a change of 0.3 log_{10} copies/swab in cervical HIV-1 RNA, the primary end point, on the basis of variance measures seen in longitudinal studies [9]. The cross-over design was chosen for its efficiency in sample size, as within-person HIV-1 RNA variability is generally less than between-person variability.

Analyses were intent-to-treat and were performed using SPSS (version 15.0; SPSS) and S-Plus (version 7.0; Insightful). HIV and HIV-1 concentrations were log_{10} transformed. Generalized estimating equations were used to compare the frequency of HSV and HIV-1 detection, and linear mixed-effects models were used to assess the quantities of HSV and HIV-1 observed while a participant was receiving valacyclovir versus those observed when the participant was receiving placebo.

RESULTS

Twenty HSV-2/HIV-1 coinfected women were enrolled. An additional 19 were screened and excluded: 10 had CD4 cell counts <200 cells/µL and 9 were HSV-2 seronegative. The median age was 28 years (range, 21–47 years), and the median CD4 cell count was 372 cells/µL (range, 229–850 cells/µL). Nine participants had previously taken zidovudine monotherapy for prophylaxis against mother-to-child HIV-1 transmission. One woman had serologic evidence of syphilis and was treated prior to enrollment; no other genital tract infections were detected. Four participants used hormonal contraception. Four participants reported a history of symptomatic herpes, of whom 3 had used acyclovir for treatment of primary genital herpes. No participant had used acyclovir for treatment or suppression of recurrent genital herpes.

All 20 participants completed the study. Women took a median of 100% (range, 99%–100%) of the dispensed study tablets; this did not differ by study arm (P = .5, by McNemar’s test). No serious adverse events were reported. Six women were treated with open-label valacyclovir for herpes recurrences on a total of 43 study days, including 31 (2.8%) of 1120 placebo days.

Each participant returned 100% of 112 daily self-collected swab specimens for detection of genital HSV reactivation (2240 swabs total). HSV was detected at least once for all participants; 54 swab specimens (2.4%) could not be analyzed because of PCR inhibition. HSV was detected significantly less often during valacyclovir administration, compared with placebo administration (40 [3.7%] of 1092 samples vs. 242 [22.1%] of 1094 samples) (table 1). By participant, HSV was detected in 0%–19.2% and 1.8%–69.6% of swabs during valacyclovir and placebo administration, respectively. Among those swab samples with detectable HSV, the quantity was significantly lower, by 0.67 log_{10} copies/swab, when participants were receiving valacyclovir, compared with placebo. Most HSV shedding was asymptomatic: women reported genital ulcers on 23 (2.1%) of 1120 valacyclovir days and 49 (4.6%) of 1120 placebo days.

All participants provided once-weekly plasma samples for the duration of follow-up (320 samples total; 2 could not be analyzed). Plasma HIV-1 levels were greater than the limit of quantification for 313 samples (98.4%). The mean HIV-1 plasma viral load was significantly lower, by 0.26 log_{10} copies/mL, during the valacyclovir arm, compared with the placebo arm—a 45% decrease. Plasma HIV-1 RNA levels were lower, similar (within 0.1 log_{10} copies/mL), and higher during valacyclovir therapy in 11, 5, and 4 women, respectively (figure 1A).
Cervical HIV-1 RNA levels were lower, similar (within 0.1 log10) compared with the placebo arm—a 55% decrease. HIV-1 levels remained statistically significant (0.24 log10 copies/swab) respectively (figure 1). The effect of valacyclovir on cervical whom 7 had lower plasma HIV-1 levels), 5, and 2 women, registration (71.1%) of 374 swab specimens collected during placebo administration, versus 266 (71.1%) of 347 swab specimens collected during placebo administration (P < .001). The mean cervical HIV-1 RNA level was significantly lower, by 0.35 log10 copies/swab, during the valacyclovir arm, compared with the placebo arm—a 55% decrease. Cervical HIV-1 RNA levels were lower, similar (within 0.1 log10 copies/swab), and higher during valacyclovir therapy in 13 (of whom 7 had lower plasma HIV-1 levels), 5, and 2 women, respectively (figure 1B). The effect of valacyclovir on cervical HIV-1 levels remained statistically significant (0.24 log10 copies/swab; P < .001) in additional sensitivity analyses in which each participant’s mean cervical HIV-1 RNA concentration during the placebo arm was imputed for swab samples that were PCR inhibited (i.e., estimating no effect of valacyclovir on these days).

**DISCUSSION**

In this placebo-controlled, cross-over trial, with multiple observations per participant, HSV-suppressive therapy reduced plasma and genital HIV-1 levels by 50% among HSV-2/HIV-1 coinfected women with intermediate levels of immunosuppression who were not on antiretroviral therapy. Adherence to the study medication and compliance with sample collection were very high. Our findings strongly support the hypothesis that HSV-2 reactivation increases plasma and genital HIV-1 levels.

Our results are consistent with those of a recent trial involving women from Burkina Faso, which found that 12 weeks of HSV-suppressive therapy with valacyclovir, 1 g daily, reduced plasma HIV-1 level by 0.53 log10 copies/mL and cervicovaginal lavage fluid HIV-1 level by 0.29 log10 copies/mL [6]. Among Peruvian men in a cross-over study similar to the present investigation, HSV-suppressive therapy reduced plasma and rectal HIV-1 levels by 0.33 and 0.16 log10 copies/mL, respectively [7]. Together, these studies indicate that short-term HSV-suppressive therapy reduces plasma HIV-1 levels by ~0.25–0.5 log10 copies/mL and anogenital HIV-1 levels by a slightly lesser amount.

The mechanism by which genital HSV reactivation increases systemic and genital HIV-1 levels is not well understood. In a cross-sectional study involving 36 HSV-2/HIV-1 coinfected Kenyan women, HSV-2 shedding was associated with higher chemokine levels, increased numbers of activated CD4 cells, and immature dendritic cell depletion in the cervix, suggesting local immunologic factors that may increase HIV-1 replication [10]. Laboratory studies have found that HSV increases HIV-1 transcription through HSV-encoded proteins binding to the HIV long terminal repeat [11].

Higher plasma HIV-1 viral load is a strong predictor of faster HIV-1 disease progression and greater infectiousness [12], and genital HIV-1 level is also likely a marker of infectivity. Early studies found zidovudine monotherapy decreased plasma HIV-1 RNA by 0.25–0.5 log10 copies/mL (similar to the effect in the present study) [13], and this modest reduction in HIV-1 levels was accompanied by decreased mortality [14]. Unlike zidovudine monotherapy, which loses clinical benefits for an individual as zidovudine-resistant HIV-1 variants are selected, the benefits of anti-HSV medications on HIV-1 replication are unlikely to be attenuated with time, since the effect on HIV-1 is mediated through HSV suppression and acyclovir resistance rarely develops, even in immunocompromised individuals treated for extended periods [1].

Long-term HSV-suppressive therapy is safe among persons with HIV-1 [1]. Studies with comparable doses of acyclovir and valacyclovir (a prodrug of acyclovir) have shown comparable effects on the suppression of HSV reactivation [3], al-
though valacyclovir achieves higher peak serum levels than does acyclovir. In this study, HSV was detected on 3.7% of days during the valacyclovir arm, demonstrating that HSV suppression was not complete at a dose of 500 mg administered twice daily. Most HSV reactivation in this study was asymptomatic, emphasizing the need for suppressive HSV therapy rather than episodic treatment only in response to clinical herpes recurrences.

Moreover, while HSV suppression reduced systemic and genital HIV-1 levels overall in this study, the magnitude of the re-

Figure 1. HIV-1 concentrations in plasma (A) and cervical (B) samples, for each study participant, stratified by treatment arm (black lines and circles, placebo; gray lines and squares, valacyclovir). Circles and squares represent mean values, and brackets indicate 1 SD. Undetectable levels were assigned values between 0 and the lower limit of quantification.
duction varied across individual participants, with some demonstrating no reduction in HIV-1 levels with HSV suppression. Future studies will determine whether higher doses of HSV-suppressive therapy might have greater effects on HIV-1 concentrations and will evaluate the characteristics of individuals who demonstrate a greater or lesser “response” to HSV suppression. The Burkina Faso study suggested that anti-HSV therapy may have greater effects on HIV-1 levels among those with higher CD4 cell counts or who had received suppressive therapy for a longer time [6]; our sample size did not permit assessment of these factors.

An additional limitation of our study was the modest frequency of cervical swab sample PCR inhibition. However, valacyclovir reduced endocervical HIV-1 levels even in a sensitivity analysis that conservatively accounted for PCR inhibition.

In conclusion, this trial demonstrated that HSV suppression with daily valacyclovir significantly reduced plasma and genital HIV-1 concentrations in HSV-2/HIV-1 coinfected women who had moderate immunosuppression and were not receiving antiretroviral therapy. The ultimate impact that HSV suppression may have on HIV-1 transmission is unknown. Recently completed clinical trials found that HSV suppression failed to reduce HIV-1 acquisition among HSV-2 seropositive women and men, despite strong epidemiologic evidence associating HSV-2 with HIV-1 susceptibility [15]. In contrast, our results offer surrogate marker evidence, through systemic and genital HIV-1 levels, that HSV suppression may reduce HIV-1 infectivity. Ongoing large clinical trials among HSV-2/HIV-1 coinfected persons will assess whether the short-term effects of HSV-suppressive therapy seen in the present study translate into decreased HIV-1 transmission and slower HIV-1 disease progression.

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References