Suppressive Acyclovir Therapy Reduces HIV Cervicovaginal Shedding in HIV- and HSV-2–Infected Women, Chiang Rai, Thailand

Eileen F. Dunne, MD, MPH, Sara Whitehead, MD, MPH,*† Maya Sternberg, PhD,* Sukhon Thepamnuay, RN,‡ Wanna Leelawiwat, MS,† Janet M. McNicholl, MD,*† Surin Sumanapun, MD,‡ Jordan W. Tappero, MD, MPH,*† Taweesap Siriprapasiri, MD, MPH,†§ and Lauri Markowitz, MD*†*

Background: Herpes simplex virus type 2 infection is important in the HIV epidemic and may contribute to increased HIV transmission. We evaluated the effect of suppressive acyclovir therapy on cervicovaginal HIV-1 shedding.

Methods: HIV-1– and herpes simplex virus type 2–coinfected women aged 18–49 years with CD4 counts >200 cells/μL were enrolled in a randomized crossover trial of suppressive acyclovir therapy (NCT00362596, http://www.clinicaltrials.gov). For each woman, monthly plasma and weekly cervicovaginal lavage specimens were collected; the mean of the monthly median cervicovaginal lavage HIV-1 viral load and plasma HIV-1 viral load was compared.

Results: Sixty-seven women were enrolled; at baseline, median CD4 count was 366 cells/μL, and median HIV-1 plasma viral load was 4.6 $\log_{10}$ copies/mL. The mean cervicovaginal lavage HIV-1 viral load was 1.9 (SD 0.8) $\log_{10}$ copies/mL during the acyclovir month and 2.2 (SD 0.7) $\log_{10}$ copies/mL during the placebo month ($P < 0.0001$); the mean decrease in HIV was 0.3 $\log_{10}$ copies/mL. The mean plasma HIV viral load during the acyclovir month (3.78 $\log_{10}$ copies/mL) was reduced compared with the placebo month (4.26 $\log_{10}$ copies/mL, $P < 0.001$).

Conclusions: Acyclovir reduced HIV genital shedding and plasma viral load among HIV-1– and herpes simplex virus type 2–coinfected women. Further data from clinical trials will examine the effect of suppressive therapy on HIV transmission.

Key Words: HIV-1, HSV-2, suppressive acyclovir, HIV transmission

(*J Acquir Immune Defic Syndr* 2008;49:77–83)

Received for publication February 15, 2008; accepted June 11, 2008.

From the *Division of STD Prevention, National Center for HIV, Viral Hepatitis and STD Prevention, Centers for Disease Control and Prevention, Atlanta, GA; †Thailand Ministry of Public Health—U.S. CDC Collaboration, Thailand; ‡Chiang Rai Provincial Public Health Office, Thailand; and §Department of Disease Control, Ministry of Public Health, Thailand.

Supported by Centers for Disease Control and Prevention.

Presented, in part, at the Conference on Retroviruses and Opportunistic Infections, February 25–28, 2007, Los Angeles, CA.

None of the authors of the manuscript have conflicts to report.

Correspondence to: Eileen F. Dunne, MD, MPH, Division of STD Prevention, 1600 Clifton Road, MS E-02, Atlanta, GA 30333 (e-mail: dde9@cdc.gov).

BACKGROUND

Herpes simplex virus type 2 (HSV-2) plays an important role in the HIV-1 epidemic; HSV-2 could facilitate HIV acquisition and/or increase the likelihood of HIV transmission among those who are HIV seropositive and HSV-2 seropositive. Although limited in scope, some studies suggest that HIV transmission is facilitated by HSV-2; studies find that mucosal HIV shedding is higher and more frequent during HSV-2 replication associated with genital ulcer disease,[1] in vivo studies show that HIV and HSV-2 shedding correlate,[2,3] HSV-2 has been associated with significantly higher HIV viremia in some studies,[4,5] and one study reported reductions of HIV viremia among individuals with HSV-2 reactivations treated with HSV-suppressive acyclovir therapy.[6] Recent published randomized trials among women and men coinfected with HIV and HSV-2 have demonstrated reduced HIV genital shedding[7–9] and HIV plasma viral load[8,9] with herpes-suppressive therapy. Randomized trials to evaluate the effect of herpes-suppressive therapy on transmission are greatly needed and currently ongoing or recently completed.[10,11] We conducted a randomized trial to evaluate the effect of suppressive acyclovir on cervicovaginal HIV shedding among HIV-1– and HSV-2–coinfected women in Chiang Rai, Thailand.

METHODS

Design

This was a triple-blind, placebo-controlled, randomized crossover trial of suppressive acyclovir (800 mg administered twice daily for 1 month). Participants were randomized to a blinded study sequence of either acyclovir or placebo in the first study month, followed by a washout (no product), followed by the other product in the third study month. The sequence of intervention was determined based on random allocation in blocks of 10; trained staff not involved in the study used the generated sequence to label study product bottles with sequential study numbers from 1 to 67.

This study was designed with a 1-month washout period, assumed to be adequate to ensure no carryover effect as the half-life of acyclovir in the setting of normal creatinine clearance is 2.5 hours.[12] As it is unclear how menstrual cycle

affects HIV shedding,[13] all women began the study at the beginning of their menstrual cycle.

A crossover design was used to minimize the number of patients needed to assess treatment effects because the within-person variability of HIV shedding is smaller than the between-person variability.

Participants

Women aged 18–49 years with regular or no menses and serum antibodies to HIV-1 and HSV-2, and not eligible for antiretroviral therapy by Thai national guidelines (ie, CD4 count >200 cells/μL and without opportunistic infection),[14] were eligible for the study. Exclusion criteria included the following: serum creatinine >1.4 mg/dL; high-grade squamous intraepithelial lesion on Pap test; receiving antiretroviral therapy; and pregnancy, breast-feeding, or intent to become pregnant during the study period.

Objectives

The objective of the study was to determine if there was a reduction in HIV-1 $\log_{10}$ copies of RNA from cervicovaginal lavage (CVL) specimens among participants during suppressive acyclovir therapy compared with placebo.

Preenrollment Procedure

At the first screening visit, written informed consent was obtained in Thai and blood samples were collected for HIV-1 and HSV-2 testing, and rapid plasma regain for syphilis and CD4 cell count. At a second screening visit, all HIV-1–infected women were referred to Chiang Rai Regional Hospital for an HIV care assessment to determine eligibility for antiretroviral therapy. At a third screening visit, cervical and vaginal swabs were collected to test for reproductive tract infections including chlamydia, gonorrhea, trichomoniasis, candidiasis, and bacterial vaginosis. In addition, CVL was collected for HIV-1 and HSV-2, and blood was collected for baseline HIV-1 plasma viral load. Women with reproductive tract infections at screening were eligible for enrollment 2 weeks after appropriate treatment.

Study Procedure

Eligible women were scheduled for enrollment at the start of expected menses (±5 days). Each study month started with a woman's menstrual cycle (day 1 being the start of menses) and ended with her next menses. At enrollment, women were given detailed information about study procedures and completed a standardized assessment of comprehension before providing written informed consent. At 3 clinic visits each month (day 7, day 14, and day 21 of the menstrual cycle), participants were asked about compliance with study assessments and symptoms and had a clinical examination, which included a pelvic examination and collection of CVL. Once each month, women were tested for reproductive tract infections and pregnancy and had a plasma specimen collected for HIV-1 viral load. Women who had genital pain and a physical finding consistent with genital ulcer disease were asked to contact study staff, suspend study drug (but continue other study assessments), and take episodic therapy (acyclovir 400 mg three times a day) for 7 days.

Blood was collected for plasma HIV-1 RNA at a randomly assigned weekly visit during months 1 and 3.

Compliance

Compliance with medication was determined using 3 methods: a weekly pill count, a daily diary card, and Medication Event Monitoring System caps (Aprex Corp, Fremont, CA).

Human Subjects Issues

The study protocol was reviewed and approved by the Ethics Review Committee of the Thailand Ministry of Public Health and the Institutional Review Board of the Centers for Disease Control and Prevention. The trial was registered in the National Institutes of Health clinical trials database (http://www.clinicaltrials.gov, NCT00362596).

Laboratory Methods

Serologic Assessment for HIV-1, HSV-2, and Creatinine

Whole-blood specimens were clotted, centrifuged at 1100*g*, and aliquotted into 5 × 1 mL aliquots. One aliquot was used to determine creatinine levels using the Jaffe method (Olympus Diagnostica GmbH, Hamburg, Germany). Separate aliquots were used to determine HSV-2 infection using the HerpeSelect2 IgG enzyme-linked immunosorbent assay (Focus Technologies, Cypress, CA); 1.1 was used to define seropositivity. HIV-1 infection was determined using Determine HIV-1/2 (Abbott Laboratories, Tokyo, Japan) rapid test kit and the Genetic Systems HIV-1/HIV-2 Plus O EIA (Bio-Rad, Redmond, WA) test kit. If discordant HIV results were obtained, then the specimen was retested with DoubleCheckII HIV 1 &2 (Orgenics, Yavne, Israel).

CD4 T-Lymphocyte Determinations

Lymphocyte immunophenotyping was performed on whole blood using a 2-color panel (IMK-lymphocyte; Becton Dickinson, San Jose, CA) and BD FACScan flow cytometer.[15]

HIV-1 and HSV-2 Assessments From CVL Specimens

Ten milliliters of sterile phosphate-buffered saline was injected into the cervical/vaginal area using a 20-mL syringe: 25% each toward the right and left vaginal walls and 50% toward the cervical os. CVL samples were vortexed, and 1 or 2 mL of CVL supernatant was used to quantitate HIV-1 RNA using a modified Amplicor Monitor HIV-1 version 1.5 assay (Roche Diagnostic Systems, Branchburg, NJ) where the extraction method was modified by using NucliSens lysis buffer and a silica-based extraction technique (Biomerieux, Boxtel, The Netherlands) instead of Amplicor lysis buffer and alcohol precipitation. The internal control from the Amplicor kit was added in the extraction step to quantitate HIV-1 RNA in the samples. Amplification and detection were done according to the package insert. The lower limit of detection was 40 copies/mL, and the lower limit of quantitation was 80 copies/mL.

One milliliter of whole CVL was used to quantitate HSV-2 using a TaqMan-based real-time polymerase chain reaction assay (McNicholl J, MD, unpublished data, 2008).

Briefly, 1 mL of whole CVL was extracted as above. Ten microliters of the extracted sample was used to amplify and detect HSV-2 in 25 μL polymerase chain reaction. Primers and probes for the HSV-2 glycoprotein G and the human RNaseP (as an internal control) genes were used, and detection was performed on a Rotor-Gene 3000. HSV-2 DNA (ABI Advance Biotechnology, Columbia, MD) was used to develop a standard curve for HSV-2 quantitation. The analytical sensitivity of the real-time polymerase chain reaction assay was approximately 1–10 genomic copies/reaction mixture.

HIV-1 Plasma Viral Load

HIV-1 RNA load in plasma was quantified using the Amplicor HIV-1 Monitor Test, version 1.5. The lower limit of quantitation for the standard assay was 400 copies/mL.

Reproductive Tract Infections, Pregnancy Evaluation, and Semen Analysis

Serologic testing for syphilis was done using the rapid plasma regain (Becton Dickinson, Sparks, MD) method and, if positive, confirmed with the Serodia *Treponema pallidum* passive particle agglutination (Fujirebio, Tokyo, Japan). Cervical swab specimens were tested for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using the COBAS Amplicor CT/NG test (Roche Molecular Systems, Branchburg, NJ). Vaginal swabs were collected to test for *Trichomonas vaginalis* infection using the InPouch TV assay (Biomed Diagnostics, White City, OR), candida infection by Gram stain, and bacterial vaginosis using Nugent criteria.[16] The OneStep ABAcard p30 Semen Detection test (Abacus Diagnostics, Inc, West Hills, CA) was used on a vaginal swab specimen to test for semen contamination. Pregnancy was determined using the tester point-HCG, OneStep urine pregnancy test (Ulti Med Products, Ahrensburg, Germany).

Sample Size

The sample size was determined to detect a minimum of 0.5 $\log_{10}$ reduction in CVL HIV-1 shedding among women taking acyclovir compared with women taking placebo. Assuming no period or carryover effects, the sample size was calculated based on a paired *t* test, a 2-sided significance level of 0.05, assuming an SD on the log scale of 1, and a correlation of 0.6 between paired CVL samples from the same woman.[17] With loss to follow-up of 10%, and up to 40% of subjects with nondetectable CVL HIV-1 shedding, a sample size of 65 had 80% power to detect a significant reduction in HIV shedding.

Statistical Analysis

All enrolled women were included in the analysis regardless of compliance, early withdrawal from the study, detection of semen in a specimen, or HSV-2 episodic acyclovir therapy, after an intent-to-treat strategy. There were 5 persons lost to follow-up, all of whom were initially randomized to the placebo/acyclovir sequence and dropped out before the second period. To obtain descriptive statistics during a given month, all the available data are used; however, in presenting descriptive statistics and inferences for within-period differences, only data for subjects who contributed data to both periods are used. Continuous variables are presented as medians with the ranges or means with SDs. After reviewing probability plots, a logarithmic base 10 transformation was applied to positively skew continuous data such as plasma and CVL HIV-1 viral load.

The primary outcome of HIV-1 shedding was left censored meaning that for some observations the actual value was not observed but rather known to be less than some value defined by the measurement limit(s). Although the approach of substituting half the reporting limit for values which fall below the defined threshold (eg, assigning a value of 40 copies when the quantification limit is 80 copies) is used extensively in the applied literature, it is well known to exhibit bias and high variability as compared with other methods now available.[18–21] We used regression on order statistics, censoring all nondetectable and nonquantifiable values at 80 copies/mL.[18] The primary outcome of CVL shedding of HIV-1 was measured on a weekly basis during each of the 3 monthly periods. For each month, the median of the 3 weekly values was used to represent the summary value for that woman during that period. We applied regression on order statistics (ROS) methods to the monthly medians to obtain an unbiased estimate of the mean. SEs, 95% confidence intervals (CIs), and *P* values for ROS estimated means were obtained using bias-corrected accelerated bootstrap method based on 1500 bootstrap samples.[22]

Additional summary measures of the monthly CVL HIV-1 and HSV-2 data included the following: shedding at least once and shedding at all 3 weekly visits. Shedding was defined as a binary outcome, such that a nondetectable was considered no shedding and a nonquantifiable or quantifiable reading was considered shedding.

For analyses comparing the 2 independent groups, like randomized treatment sequences, the Peto–Prentice test was used for CVL HIV-1 shedding and Wilcoxon Rank Sum test was used for all other continuous variables. For within-group comparisons, the paired Prentice–Wilcoxon test was used for CVL HIV-1 shedding and the Wilcoxon sign test was used for all other continuous variables.[23,24] For categorical variables, χ^2 tests for between-group comparisons and McNemar test for within-group comparisons were used.

For multivariate analyses, standard longitudinal methods were applied. We categorized CVL HIV and HSV shedding as binary outcomes and applied generalized estimating equations with a Poisson distribution, an exchangeable working correlation matrix, and a log link to provide adjusted relative risks and *P* values.[25] The empirical variance–covariance matrix was used for all inferences and CIs. For the plasma viral load, we applied linear mixed models.[26] All statistical analyses in this paper were performed in both SAS version 9.1.3 and R version 2.3.1 (R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org) with the NADA package (Lopaka Lee, US NADA http://water.usgs.gov/software/nada_r.html).[21]

RESULTS

One hundred sixty-six women were assessed for eligibility, and 69 (42%) were determined to be eligible. The most common reasons for ineligibility were as follows: CD4 count <200 cells/μL (n = 34), HSV-2 seronegativity (n = 22), HSV-2 negative and CD4 count < 200 cells/μL (n = 4), and a variety of other reasons (n = 27) (Fig. 1).

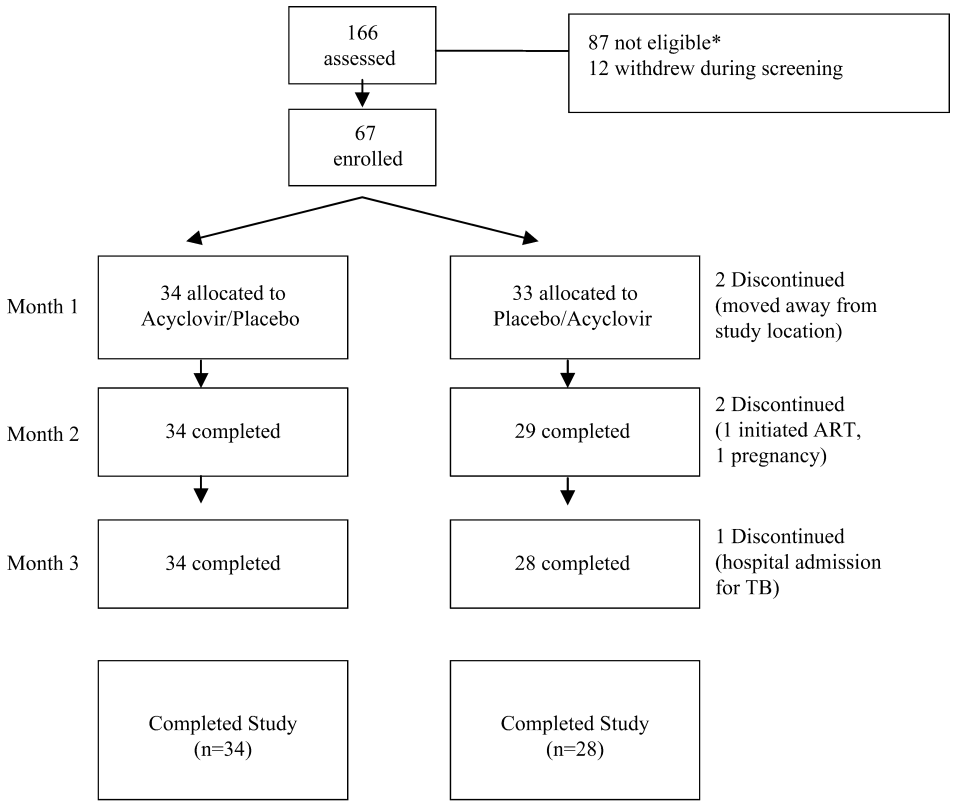

FIGURE 1. Flow of participants in the randomized placebo-controlled trial of suppressive acyclovir therapy, Chiang Rai, Thailand.

ART=Antiretroviral therapy TB=tuberculosis
*HSV-2 negative (N= 22), CD4 <200 (N=34), HSV-2 negative and CD4 <200 (N=4), Other (N=27)

Sixty-seven women were enrolled in the trial, 34 were randomized to acyclovir followed by placebo, and 33 to placebo followed by acyclovir. During the study, 5 participants in the treatment sequence of placebo followed by acyclovir dropped out, 1 due to pregnancy and 2 each due to moving and HIV-related hospitalizations. Eighty-four percent of the 67 women returned to all 3 weekly visits in each of the 3 months (total of 9 weekly visits). Of the 11 women who did not have all 9 weekly visits, 5 women were lost from the placebo/acyclovir group before the acyclovir phase and 6 women missed a weekly visit and therefore do not have all their weekly visits recorded; however, some of the weekly visits are available for each of the 3 months. Episodic acyclovir-suppressive therapy was given to 3 participants during the study.

All 34 women on the acyclovir–placebo sequence and 28 on the placebo–acyclovir sequence completed the study. Mean adherence to acyclovir dosing as assessed by pill count, diary card, and Medication Event Monitoring System caps was 97.2%, 97.4%, and 97.2% of doses, respectively, and for placebo dosing 97.1%, 97.3%, and 96.3%, respectively. Semen was detected in 10 (1.7%) of 568 CVL specimens collected during the study. Of the 10 CVLs with semen detected, 4 had HIV-1 detected, of these, 2 were detected during the washout month.

Women randomized to the 2 different treatment sequences did not differ significantly by demographic, behavioral, clinical, and laboratory characteristics (Table 1), except for median household income and occupation (data not shown). The median age was 33 years (range 22–46 years), the median number of sex partners in the last year was 1 (range 0–2), and 19.4% had a history of a sexually transmitted infection. Forty-two percent of women reported a prior history of symptoms consistent with genital herpes.

Overall, 75% of women had detectable CVL HIV-1 at baseline. Median CD4 count was 366 (range 209–930) cells/μL, median HIV-1 plasma viral load was 4.6 (range 2.9–5.7) $\log_{10}$ copies/mL, and median CVL HIV-1 viral load was 1.9 (range 0–4.1) $\log_{10}$ copies/mL (Table 1). There was no significant difference in baseline laboratory values among women on different sequences.

Prevalence of symptoms commonly attributed to acyclovir, such as headache, nausea, and diarrhea, did not significantly differ among women taking acyclovir or placebo during the study; nausea (14.3% of women on acyclovir vs 13.6% on placebo), headache (33.3% on acyclovir vs 27.3% on placebo), and diarrhea (12.7% on acyclovir vs 7.6% on placebo).

Evaluation of weekly CVLs during the study demonstrated that a higher percentage of women had HIV-1 detected at least once during the placebo month compared with the acyclovir month (80.3% vs 64.5%, $P < 0.01$) (Table 2). Differences in HSV-2 shedding in CVLs were also observed; overall, 42.4% of women had HSV-2 detected at least once during the placebo month compared with 1.6% during the acyclovir month ($P < 0.01$).

TABLE 1. Baseline Demographic, Clinical, and Laboratory Characteristics of Enrolled Women

Characteristic	Overall (N = 67) n (%)	Treatment: Acyclovir–Placebo (n = 34) n (%)	Treatment: Placebo–Acyclovir (n = 33) n (%)	*P*
History of any STI	3 (19.4)	7 (20.6)	10 (30.3)	0.88*
Ever have symptoms consistent with herpes	28 (41.8)	14 (41.2)	14 (42.4)	0.64*
	Median (range)	**Median (range)**	**Median (range)**	
Age, yrs	33 (22–46)	33 (27–46)	33.5 (22–42)	0.24†
Years since HIV diagnosis	4.5 (0.5–15)	5.3 (0.5–13.0)	3.9 (0.6–15.0)	0.68†
No. sex partners last year	1 (0–2)	1 (0–1)	1 (0–2)	0.76†
CD4 count, cells/μL (range)	366 (209–930)	374 (210–930)	316 (209–806)	0.41†
Baseline HIV-1 plasma viral load, $\log_{10}$ copies/mL (range)	4.6 (2.9–5.7)	4.6 (2.9–5.7)	4.7 (3.0–5.7)	0.36†
Baseline HSV-2 CVL, $\log_{10}$ copies/mL (range)	0 (0–5.7)	0 (0–5.7)	0 (0–2.5)	0.28†
Baseline HIV-1 CVL, $\log_{10}$ copies/mL (range)	1.9 (0–4.1)	1.9 (0–4.1)	1.9 (0–3.7)	0.97†

*χ^2 test.
†Wilcoxon Rank Sum test.
STI, sexually trasmitted infection.

There was a statistically significant reduction in HIV-1 from CVLs among women on acyclovir compared with women on placebo; this was found in both unadjusted and adjusted measurements (adjusted for reproductive tract infections, baseline HSV-2 and HIV-1 from CVL, plasma HIV-1, CD4 count, sequence, and period) (Table 2, Fig. 2). The proportion of women with CVL HIV-1 by weekly visit was reduced (Fig. 3). The estimated mean HIV-1 $\log_{10}$ copies/mL in CVL among the 62 participants who completed the study was 1.9 (SD 0.8) during acyclovir therapy and 2.2 (SD 0.7) during placebo therapy ($P < 0.0001$, paired Prentice–Wilcoxon test); the mean decrease in HIV-1 among these participants was 0.3 $\log_{10}$ copies of HIV. Among the 46 women with detectable CVL HIV-1 at baseline and evaluated in both periods, the mean decrease in HIV copies/mL while on acyclovir was 0.35 $\log_{10}$ copies (SD 0.39, $P < 0.01$).

Only 6 women had an increase in CVL HIV $\log_{10}$ copies during acyclovir therapy. Twenty-one (34%) women had no change, and the majority of these women (86%) had nondetectable CVL HIV-1. Thirty-four women had a reduction in CVL HIV-1 while taking acyclovir; the mean reduction of HIV-1 genital shedding in this group was 0.44 $\log_{10}$ HIV-1 RNA copies/mL.

There was also a statistically significant reduction in plasma HIV viral load during the acyclovir treatment month compared with the placebo month (Table 2). The mean plasma HIV-1 viral load was 3.78 (95% CI: 3.50 to 4.05) $\log_{10}$ copies/mL during the treatment month compared with 4.26 (95% CI: 4.07 to 4.44) $\log_{10}$ copies/mL during the placebo month (a mean reduction of 0.47 $\log_{10}$ copies/mL). At baseline and during the washout month, the median plasma HIV viral loads were 4.54 and 4.46 $\log_{10}$ copies/mL, respectively.

TABLE 2. HIV-1 and HSV-2 Detection During Study: Placebo and Acyclovir Months

	Placebo (n = 66)		Acyclovir (n = 62)		Unadjusted Difference Between Acyclovir and Placebo		Adjusted Difference Between Acyclovir and Placebo	
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean (SD)	95% CI, *P*	Mean	95% CI, *P*
HIV-1 $\log_{10}$ copies/mL, plasma	4.26	4.07 to 4.44	3.78	3.50 to 4.05	−0.46	−0.61 to −0.31, <0.01	−0.43	−0.56 to −0.29,* <0.01
Monthly medians (ROS)								
HIV-1 $\log_{10}$ copies/mL, CVL†	2.2 (0.7)	1.9 to 2.4†	1.9 (0.8)	1.6 to 2.1†	−0.32 (0.3)	−0.19 to −0.48,† <0.01‡	−0.33	—, <0.01§
HIV-1 shedding	%	n/N	%	n/N	RR	95% CI, *P*	RR	95% CI, *P*
Detected at least once	80.3	53/66	64.5	40/62	0.80	0.69 to 0.93, <0.01	0.81	0.69 to 0.94,* <0.01
Detected at all visits	39.4	26/66	33.9	21/62	0.86	0.62 to 1.19, 0.36	0.94	0.67 to 1.30,* 0.69
HSV-2 shedding	%	n/N	%	n/N	RR	95% CI, *P*	RR	95% CI, *P*
Detected at least once	42.4	28/66	1.6	1/62	0.04	0.01 to 0.28, <0.01	0	0.006 to 0.33,* <0.01
Detected at all visits	3.0	2/66	0.0	0/62	0.00	—, <0.01	NA	NA

ROS, regression on order statistics; NA, not available.
*Adjusted for reproductive tract infections, baseline HSV-2, baseline HIV-1 from CVL, plasma HIV-1, CD4, sequence, and period using generalized estimating equations for binary shedding outcomes and linear mixed model for plasma HIV.
†95% CIs based on 1500 bootstrap samples using bias-corrected accelerated bootstrap methods.
‡*P* value based on paired Prentice–Wilcoxon test.
§Peto–Prentice test, adjusted for period effect (*P* value for period effect = 0.36).

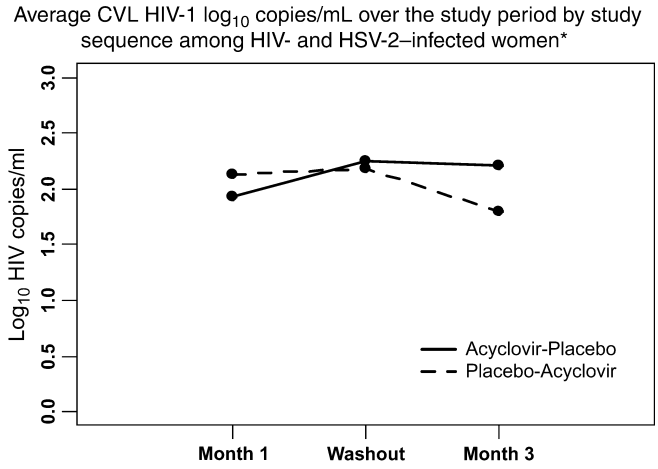

FIGURE 2. *Estimated reduction -0.31log $_{10}$ copies/mL [95% CI; (-0.9, -9.2)], P = value < 0.01, adjusted for period effect.

DISCUSSION

This is the first randomized, controlled crossover trial of suppressive acyclovir therapy in HIV-1– and HSV-2–coinfected women which found a significant reduction of genital HIV-1 shedding. All women in our study were relatively immunocompetent, and most women (58%) did not report having recognized symptoms of anogenital herpes before the study. This study provides laboratory evidence suggesting that HIV-1 transmission may be reduced with suppressive antiviral therapy and lends further biologic plausibility to the possible reduction in HIV transmission that is being evaluated in ongoing prevention trials.

These data are similar to other published studies demonstrating a reduction of genital HIV-1 shedding and plasma HIV associated with suppressive antiviral therapy for herpes among women, although methods varied across studies. In the recently published study by Nagot et al,[8] there was a statistically significant reduction in quantity of HIV shedding (0.29 $\log_{10}$ copies/mL) and frequency of shedding. This finding was found among women similar to ours (immunocompetent HIV-1– and HSV-2–coinfected women not taking or eligible for highly active antiretroviral therapy) in Burkina Faso. However, Nagot et al[8] used a somewhat different clinical trial design (parallel cohort design), a different antiviral (valacyclovir), a different method to evaluate genital HIV-1 and HSV-2 shedding (CVL enriched with a cervical swab), and a different method to account for nondetectables. In another cohort study of HIV-1–infected women on highly active antiretroviral therapy, women with baseline HIV-1 shedding had a significant reduction of genital HIV-1 shedding with suppressive valacyclovir therapy, both in the proportion of visits with detectable HIV-1 shedding (odds ratio 0.27, 95% CI: 0.07 to 0.99) and the quantity of genital HIV-1 RNA during these visits (-0.71 $\log_{10}$ copies/mL, 95% CI: -1.27 to -0.14).[7] Our study, and the study by Nagot, demonstrated a significant reduction in plasma HIV, which may have contributed to the reduction in genital HIV shedding.[10]

There are important strengths and limitations to this study. A strength is our study design; the crossover trial design allows for evaluation of all women both on and off therapy and period effects. A limitation is that our study was limited to 3 months and participants were only on acyclovir for 1 month; a study for several months would be useful to answer the

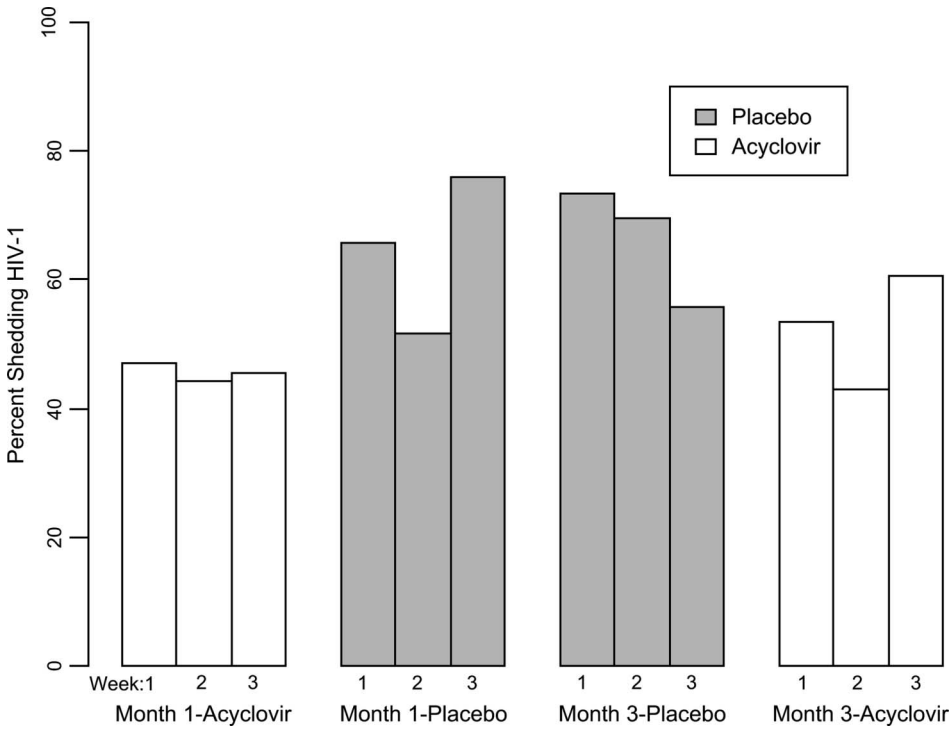

FIGURE 3. Proportion of women with detectable CVL HIV-1 by weekly visit and sequence, HIV- and HSV-2–infected women.

question about duration of therapy and impact on genital HIV-1 shedding. Another limitation is that we evaluated a surrogate end point for transmission and we cannot conclude that the reduction of HIV genital shedding found in our study translates into reduction in HIV transmission. We demonstrated that HIV-1 shedding was reduced but not eliminated; it is unclear as to what level and duration of HIV-1 shedding are necessary for transmission.

Our study demonstrated that suppressive antiviral therapy for herpes reduces HIV genital shedding, the source for most heterosexual HIV transmission worldwide. Our study is the first to find reduced genital and plasma HIV among women using acyclovir, a commonly available and inexpensive antiviral. The reduction in genital HIV shedding was statistically significant and similar to other studies at a reduction of 0.3 $\log_{10}$ copies/mL HIV-1 during 1 month of acyclovir. Although trials of suppressive therapy among HSV-2–infected persons did not find a significant reduction in HIV acquisition, the public health community awaits results on the trials of HSV suppression to reduce HIV transmission to determine if this intervention will be successful.[11]

ACKNOWLEDGMENTS

The authors would like to acknowledge Drs. Clyde Hart, Chen Cheng, Tammy Evans-Strickfaden, and Ron Ballard for input on the laboratory testing and analysis for this study.

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