

BRIEF REPORT

Clinical and Virologic Efficacy of Herpes Simplex Virus Type 2 Suppression by Acyclovir in a Multicontinent Clinical Trial

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Acyclovir suppressive therapy (400 mg twice daily) reduces herpes simplex virus (HSV) type 2–associated genital ulcer disease and lesional HSV shedding. In an international trial of acyclovir for suppression of HSV type 2 to prevent human immunodeficiency virus (HIV) acquisition (HIV Prevention Trials Network 039), acyclovir had a smaller effect on the frequency of genital ulcer disease as well as a smaller effect on the frequency and quantity of lesional HSV DNA in African women and Peruvian men, compared with its effects in men in the United States. The observed regional variation in the clinical and virologic efficacy of acyclovir for HSV suppression warrants further evaluation of determinants of responses to acyclovir. (ClinicalTrials.gov identifier: NCT00076232.)

Herpes simplex virus type 2 (HSV-2) is the most common etiology of genital ulcer disease (GUD) worldwide and has been associated with a 2–4-fold increase in acquisition of human immunodeficiency virus (HIV) [1]. It is hypothesized that in-

creased HIV acquisition risk is conferred by breaches in genital epithelium, as well as genital inflammation during reactivation of herpes simplex virus (HSV). Herpetic ulcerations compromise the integrity of genital epithelium and mucosa and recruit activated CD4⁺ and CD8⁺ T lymphocytes and dendritic cells that may facilitate HIV attachment and infection during sexual intercourse [2].

We recently completed a randomized placebo-controlled trial (HIV Prevention Trials Network [HPTN] 039) to evaluate whether 400 mg of acyclovir twice daily could reduce HIV acquisition among 3172 HSV-2–seropositive men who have sex with men (MSM) in the United States and Peru and women in sub-Saharan Africa by suppressing HSV [3]. This trial failed to demonstrate a protective effect of this regimen on the incidence of HIV acquisition, confirming the results of another trial that tested the same intervention in Tanzanian women [4]. As a secondary objective, HPTN 039 evaluated the impact of acyclovir on symptomatic genital ulcers, as well as on virologic end points, defined by the frequency and amount of HSV detected in genital ulcers that are observed on examination. The efficacy of acyclovir has been extensively characterized in developed countries; however, there is a paucity of data on acyclovir efficacy in resource-poor countries. Although acyclovir has now been added to the World Health Organization list of essential drugs, many sexually transmitted disease treatment clinics in Africa still do not have access to the drug, and the cost of the medication limits its use. In addition, evaluation of acyclovir in resource-poor settings has focused on treatment of GUD and not on prevention of HSV reactivation. Thus, our trial provides novel information about the clinical and virologic efficacy of suppressive acyclovir in resource-poor settings.

Methods. A total of 3127 evaluable HIV-negative, HSV-2–antibody–positive participants were enrolled into the HPTN 039 trial. MSM were enrolled at sites in the United States (Se-

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attle, WA; San Francisco, CA; and New York, NY) and Peru (Lima, Iquitos, and Pucallpa), and women were enrolled at sites in Harare, Zimbabwe; Lusaka, Zambia; and Johannesburg, South Africa. Informed consent was obtained from study participants, and institutional review boards approved the protocol at each participating trial site. Eligibility criteria and screening procedures are described in further detail elsewhere [3].

Participants were randomized to receive 400 mg of acyclovir or a matching placebo twice daily and were seen monthly for 12–18 months. During monthly visits, participants were asked about symptoms of genital herpes in the past 7 days. Clinicians performed a genital examination at all quarterly study visits and at any monthly or interim study visit if symptoms of genital ulcers were reported. The study staff obtained swab specimens from lesions clinically consistent with a herpes recurrence; swab specimens were placed into polymerase chain reaction (PCR) medium, frozen, and shipped to the virology laboratory at the University of Washington. An HSV DNA PCR assay was performed according to validated, previously published procedures [5, 6]. Samples were analyzed using a real-time fluorescent probe-based PCR assay (TaqMan; Applied Biosystems) to quantitate HSV, and results were considered positive for HSV-2 if >3 copies/reaction, or 150 copies/mL of fluid, were detected [7]. Means, medians, and standard deviations of HSV-2 DNA copy counts from genital ulcer specimens were calculated by study arm, and the distribution for \log_{10} HSV PCR titer was plotted by study arm and region. Monthly and quarterly rates of adherence to the study drug regimen were computed on the basis of monthly pill counts from returned study drug bottles and self-reports. Because each participant provided swab samples at up to 6 different study visits, we used generalized estimated equations to analyze numbers of cases of GUD, HSV-2 positivity from lesional swab specimens, and mean \log_{10} HSV copy counts. Models for mean reduction in HSV were adjusted for age and report of genital ulcers in the 3 months prior to enrollment. To explore whether reductions in HSV shedding were affected by adherence to the study drug, analyses were stratified by adherence level as measured by monthly pill counts averaged for the preceding quarter ($\leq 90\%$ and $>90\%$). For counts (ie, numbers of cases of GUD) and binary results (HSV-2 positivity), a log link and negative binomial distribution was used. For continuous outcomes (ie, \log_{10} HSV DNA copy counts), an identity link and normal distribution was used. An independence working correlation and a robust covariance estimate were used in all analyses.

Results. A total of 459 MSM were enrolled at US sites, a total of 1355 MSM were enrolled at Peruvian sites, and a total of 1358 women were enrolled at African sites. At baseline, 131 (29%) of the US MSM and 227 (17%) of the Peruvian MSM reported having had anogenital herpes symptoms over the prior 3 months; 3% of men from each region received a clinical

diagnosis of GUD at enrollment. In contrast, 443 (33%) of the women reported symptoms over this time period and 225 (17%) received a diagnosis of GUD on examination. During the 18-month study follow-up period, 915 (29%) of the 3172 total participants received a diagnosis of GUD on examination, for a total of 1664 episodes detected. The GUD incidence varied by study arm and population. The overall rate of GUD diagnosis on examination was 55 cases per 100 person-years in the placebo group compared with 30 cases per 100 person-years in the acyclovir group ($P < .001$). For the US MSM, the rate of GUD was 53 cases per 100 person-years in the placebo group, compared with 15 cases per 100 person-years in the acyclovir group. For the Peruvian MSM, the rate of GUD was 34 cases per 100 person-years in the placebo group compared with 16 cases per 100 person-years in the acyclovir group. For the African women, the rate of GUD was 75 cases per 100 person-years in the placebo group, compared with 46 cases per 100 person-years in the acyclovir group. Overall, acyclovir was associated with a 47% reduction in the incidence of GUD (relative risk [RR], 0.53 [95% confidence interval {CI}, 0.46–0.62]); the associated reduction was 71% in US MSM (RR, 0.29 [95% CI, 0.18–0.47]), but it was only 53% in Peruvian MSM (RR, 0.47 [95% CI, 0.36–0.62]) and 39% in African women (RR, 0.61 [95% CI, 0.51–0.74]; $P < .001$ for the difference in treatment effect by region). Thus, we observed statistically significant regional variation in the proportional reduction in the incidence of GUD with acyclovir suppression.

To explore these differences, we examined virologic data obtained from 1468 swab specimens collected from participants with GUD. Overall, 861 (59%) of the 1468 swab specimens tested positive for HSV-2 (630 of 962 in the placebo arm compared with 231 of 506 in the acyclovir arm; $P < .001$). This represented a 63% reduction in the incidence of ulcers with detectable HSV-2, although regional differences persisted. We found an 88% reduction in the incidence of HSV-2–positive breakthrough genital ulcers among enrolled US MSM (97 of 137 in the placebo arm compared with 12 of 43 in the acyclovir arm; RR, 0.12 [95% CI, 0.05–0.29]), but only a 61% reduction in Peruvian MSM (185 of 261 in the placebo arm compared with 71 of 121 in the acyclovir arm; RR, 0.39 [95% CI, 0.28–0.56]), and a 57% reduction in African women (348 of 564 in the placebo arm compared with 148 of 342 in the acyclovir arm; RR, 0.43 [95% CI, 0.34–0.56]; $P < .001$ for the difference in RR between regions). Of the samples with detectable HSV, the mean HSV-2 DNA copy number detected in lesions was reduced by 0.43 \log_{10} . However, as shown in Figure 1, the reduction was highest among US MSM: there was an observed 1.07 \log_{10} reduction with acyclovir (95% CI, 0.33–1.80 \log_{10} copies; $P = .004$) among US MSM, a 0.68 log reduction (95% CI, 0.04–1.32 \log_{10} copies; $P = .04$) among Peruvian MSM, and only a 0.32 \log_{10} reduction (95% CI, 0.01–0.63 \log_{10} copies;

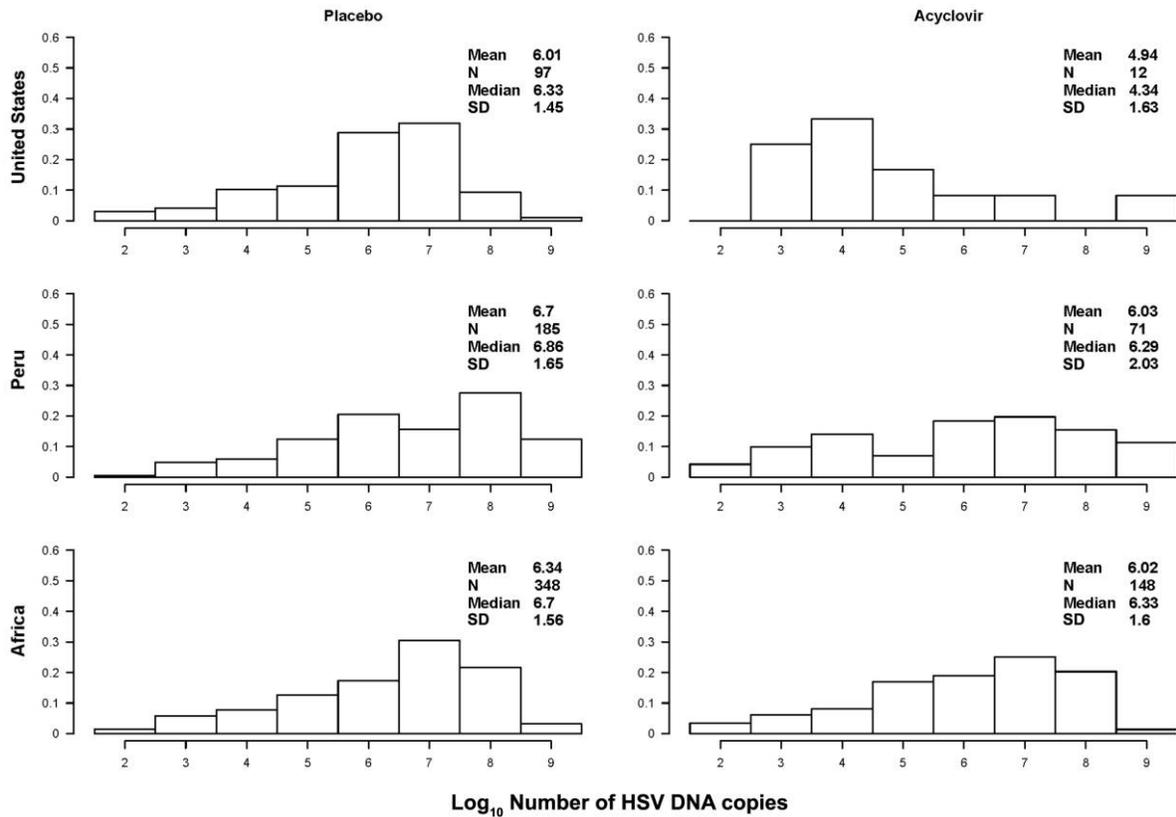


Figure 1. Comparison of herpes simplex virus (HSV) DNA copy counts in 861 swab specimens with detectable HSV from genital ulcers, by study arm and by region. The number of swab specimens (N) and the mean, median, and standard deviation (SD) of the HSV DNA log₁₀ copy count is provided for participants randomized to receive placebo (*left*) or acyclovir (*right*).

$P = .05$) among the African women ($P = .13$ for the difference between regions). As shown in Table 1, these estimates were minimally affected when adjusted for age and reported history of GUD in the 3 months prior to enrollment. In an unadjusted model restricting the analysis to swab specimens with detected HSV-2 collected from participants reporting >90% adherence to the treatment regimen, as measured by average adherence in the preceding quarter ($n = 627$ swab specimens), the mean reduction in the log₁₀ HSV DNA copy count increased mar-

ginally in all groups, but regional differences persisted (1.31 log₁₀ HSV DNA copy reduction in the US MSM, 0.89 in the Peruvian MSM, and 0.47 in the African women; $P = .30$ for the difference between regions).

Discussion. In this large, multisite, international trial of suppressive HSV-2 therapy, the standard dose of twice daily acyclovir reduced the incidence of GUD recurrence by half overall, reduced the incidence of ulcers with detectable HSV-2 by 63%, and reduced the HSV DNA copy count in samples

Table 1. Reductions in Herpes Simplex Virus (HSV) DNA Copy Count in Patients Who Received Acyclovir, Compared with Patients Who Received Placebo, by Region

Variable	No. of swab specimens	Mean reduction in log ₁₀ copy count (95% CI)				
		Overall	<i>P</i> for overall value ^a	US MSM	Peruvian MSM	African women
Unadjusted	861	0.43 (0.15–0.71)	.003	1.07 (0.33–1.80)	0.68 (0.04–1.32)	0.32 (0.01–0.63)
Adjusted ^b	861	0.46 (0.18–0.75)	.001	1.12 (0.40–1.84)	0.70 (0.05–1.34)	0.32 (0.01–0.64)
>90% Adherence ^c	627	0.57 (0.25–0.89)	.001	1.31 (0.20–2.42)	0.89 (0.15–1.63)	0.47 (0.11–0.83)

NOTE. Patients were HSV-positive patients with genital ulcer disease (GUD). CI, confidence interval; MSM, men who have sex with men.

^a *P* value for overall reduction associated with acyclovir compared with that associated with placebo.

^b Generalized estimated equation multivariate analysis adjusted for age and baseline report of GUD symptoms in the 3 months prior to enrollment.

^c Unadjusted analysis restricted to swab specimens collected from MSM and women with >90% average quarterly adherence to the study drug regimen, as measured by monthly pill count.

from those ulcers by 0.43 log₁₀ copies. However, we observed significant regional variation in the incidence of GUD diagnosis on examination, as well as the frequency and amount of HSV detected in specimens from ulcers in US and Peruvian MSM and African women. These differences by region in the quantity of HSV-2 detected in swab specimens from genital herpes lesions persisted after adjusting for level of adherence to antiviral therapy in the preceding quarter, and thus they are unlikely to be explained by inadequate drug intake. Other possible explanations for the lower efficacy of acyclovir on GUD-associated HSV-2 shedding in populations outside the United States, particularly in Africa, include strain variation, resulting in inherent acyclovir resistance among HSV strains from Africa, or unappreciated differences in acyclovir absorption or pharmacokinetics. Detailed studies with frequent assessment of genital shedding combined with drug level evaluation may elucidate the mechanism underlying our observations.

The substantial body of evidence establishing the efficacy of acyclovir suppression on clinical and subclinical HSV shedding comes from studies of US and European cohorts [8, 9]. For example, a recent study by Gupta and colleagues [10] that enrolled men and women at research clinics in the Northwest United States observed a 1.2–1.6 log₁₀ reduction in HSV DNA copy number by means of PCR in a comparison of acyclovir and valacyclovir with placebo; this result mirrors our estimates for US participants. Despite increased use of acyclovir worldwide for syndromic management of HSV-2, data are limited on the efficacy of suppressive HSV therapy in resource-poor settings. However, over the past several years, studies in Peru [11] and Africa [12] evaluating HSV-2 suppression with both drugs to reduce HIV shedding and disease progression among HIV-infected men and women have also shown efficacy in reducing HSV-2 viral shedding, on the basis of molecular diagnostic methods similar to those used in the present study. The authors of the Tanzanian trial [4] suggested that suboptimal adherence to a regimen of 400 mg of acyclovir twice daily may account for the lack of efficacy in reducing the incidence of HIV acquisition among the HIV-negative women enrolled in their study, as evidenced by a lower than anticipated reduction in HSV shedding from cervicovaginal lavage specimens. However, in our study, adherence rates, measured primarily by pill count, were >90% on average [3]. Moreover, our GUD analysis showed only marginal improvements in the reduction of HSV shedding across all groups when the analysis was restricted to those with the highest level of adherence.

A limitation of this analysis is that we did not perform HSV cultures at the time of GUD swab sampling, which would have permitted us to phenotype viral isolates for acyclovir sensitivity, nor did we perform genotypic assays to determine whether populations outside the United States had a greater prevalence of acyclovir resistance, although future studies are planned. Given

that acyclovir resistance is infrequent among immunocompetent persons from settings with high levels of background acyclovir use [13], it is unlikely that resistance from this mechanism would be observed in Peru and Africa, where acyclovir availability remains very limited.

Regional differences in HSV-2 shedding, despite suppressive therapy, were unexpected and may have several implications. Potential biological explanations require further exploration to assess differential effects of acyclovir by population. Thus far, liquid chromatography performed on study drug stored in the field has confirmed expected drug potency [3], and studies are currently underway to assess pharmacokinetics and GUD healing among African women treated with standard episodic acyclovir dosing. In addition, HSV-2 genotypic strain variation has been reported [14], and although an association with lower susceptibility to acyclovir has not been observed, further study of viral polymorphisms as a potential explanation for the regional differences in acyclovir response is warranted. Of note, although our study relied on pill count and self-report to measure adherence to the study regimen, as is the standard for most biomedical prevention trials, these methods may overestimate adherence [15]. Finally, efforts to identify a safe and effective HSV-2 vaccine must continue, to reduce the burden of new HSV-2 infections globally and potentially reduce HIV transmission risk in those regions most affected by the epidemic.

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References

1. Freeman EE, Weiss HA, Glynn JR, et al. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. *AIDS* **2006**; 20:73–83.
2. Zhu J, Hladik F, Woodward A, et al. Persistence of HIV-1 receptor-positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. *Nat Med* **2009**; 15:886–892.
3. Celum C, Wald A, Hughes J, et al. Effect of aciclovir on HIV acquisition in herpes simplex virus 2 seropositive women and men who have sex with men: a randomized, double-blind, placebo controlled trial. *Lancet* **2008**; 371:2109–2119.
4. Watson-Jones D, Weiss HA, Rusizoka M, et al. Effect of herpes simplex suppression on incidence of HIV among women in Tanzania. *N Engl J Med* **2008**; 358:1560–1571.
5. Jerome K, Huang M, Wald A, et al. Quantitative stability of DNA after extended storage of clinical specimens as determined by real-time PCR. *J Clin Microbiol* **2002**; 40:2609–2611.
6. Corey L, Huang ML, Selke S, Wald A. Differentiation of herpes simplex virus 1 and 2 in clinical samples by a real-time Taqman PCR assay. *J Med Virol* **2005**; 76:350–355.
7. Magaret A, Wald A, Huang ML, et al. Optimizing PCR positivity cri-

- terion for detection of herpes simplex virus DNA on skin and mucosa. *J Clin Microbiol* **2007**; 45:1618–1620.
8. Douglas JM, Critchlow C, Benedetti J, et al. A double blind study of oral acyclovir for suppression of recurrences of genital herpes simplex virus infection. *N Engl J Med* **1984**; 310:1551–1556.
 9. Wald A, Zeh J, Barnum G, Davis LG, Corey L. Suppression of sub-clinical shedding of herpes simplex virus type 2 with acyclovir. *Ann Intern Med* **1996**; 124:8–15.
 10. Gupta R, Wald A, Krantz E, et al. Valacyclovir and acyclovir for suppression of shedding of herpes simplex virus in the genital tract. *J Infect Dis* **2004**; 190:1374–1381.
 11. Zuckerman RA, Lucchetti A, Whittington WL, et al. Herpes simplex virus (HSV) suppression with valacyclovir reduces rectal and blood plasma HIV-1 levels in HIV-1/HSV-2-seropositive men: a randomized, double-blind, placebo-controlled crossover trial. *J Infect Dis* **2007**; 196:1500–1508.
 12. Cowan FM, Pascoe SJ, Barlow KL, et al. A randomised placebo-controlled trial to explore the effect of suppressive therapy with acyclovir on genital shedding of HIV-1 and herpes simplex virus type 2 among Zimbabwean sex workers. *Sex Transm Infect* **2008**; 84:548–553.
 13. Bacon TH, Levin MJ, Leary JJ, Sarisky RT, Sutton D. Herpes simplex virus resistance to acyclovir and penciclovir after two decades of antiviral therapy. *Clin Microbiol Rev* **2003**; 16:114–128.
 14. Norberg P, Kasubi MJ, Haarr L, et al. Divergence and recombination of clinical herpes simplex virus type 2 isolates. *J Virol* **2007**; 81:13158–13167.
 15. Berg K, Arnsten J. Practical and conceptual challenges in measuring antiretroviral adherence. *J Acquir Immune Defic Syndr* **2006**; 43:S79–S87.