

Valacyclovir and Acyclovir for Suppression of Shedding of Herpes Simplex Virus in the Genital Tract

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Background. Valacyclovir exhibits better oral absorption and higher, more prolonged serum concentrations than oral acyclovir. The efficacy of valacyclovir and acyclovir on genital herpes simplex virus (HSV) shedding was assessed in a double-blind, 3-period crossover trial.

Methods. Sixty-nine immunocompetent participants with genital HSV-2 received oral valacyclovir, acyclovir, and matching placebo in random order for 7-week periods. Participants provided daily genital mucosal swabs for HSV detection by viral culture and polymerase chain reaction (PCR).

Results. HSV was detected at least once in 62 (90%) participants by culture and in 68 (98%) by PCR. During placebo, the total HSV shedding rate was 15.4% of days by culture (PCR, 40.2%); the subclinical shedding rate was 6.6% by culture (PCR, 27.1%). Both antivirals were associated with lower HSV shedding by culture (relative risk [RR], 0.03 [95% confidence interval {CI}, 0.01–0.07] for valacyclovir and RR, 0.05 [95% CI, 0.03–0.10] for acyclovir) and PCR (RR, 0.18 [95% CI, 0.12–0.26] for valacyclovir and RR, 0.20 [95% CI, 0.15–0.28] for acyclovir), compared with placebo. No significant differences in frequency and quantity of HSV were detected by PCR between the valacyclovir and acyclovir arms.

Conclusions. Although the suppression of viral replication is not complete, valacyclovir and acyclovir are highly effective in suppressing the frequency and quantity of genital HSV shedding.

Most sexual transmission of herpes simplex virus (HSV) type 2 occurs on days without genital lesions in the source partner [1–3]. Acyclovir reduces both clinical and subclinical shedding of HSV-2 in the genital tract; however, the virus can still be detected by DNA poly-

merase chain reaction (PCR) on 8% of the days during suppressive therapy [4]. Valacyclovir is a prodrug of acyclovir that has better oral absorption and, at high doses, can provide serum levels of acyclovir that are comparable to those of intravenous administration [5, 6]. The projected area under the curve of a 500-mg twice-a-day dose of valacyclovir is ~3 times greater than that of a 400-mg twice-a-day dose of oral acyclovir [7]. Studies in patients with symptomatic recurrent genital herpes have shown that valacyclovir has comparable efficacy to acyclovir [8, 9]. To investigate the efficacy of valacyclovir on the shedding of HSV from genital mucosa, we compared the effect of valacyclovir and acyclovir on viral shedding in persons with recently acquired or frequently recurring genital HSV-2 infection.

PATIENTS, MATERIALS, AND METHODS

Subjects and setting. Healthy men and women who were seropositive for HSV-2 were considered for enrollment. Patients were eligible if they had a diagnosis

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of a first episode of genital HSV-2 infection ≤ 6 months before enrollment. Alternatively, patients were eligible if they had long-standing genital herpes infection and a recurrence rate of ≥ 6 episodes during the preceding year. Patients who had been receiving recent suppressive therapy were eligible if they reported ≥ 6 recurrences/year before initiating suppressive therapy. Suppressive therapy was stopped for at least 1 month before enrollment, and at least 1 recurrence was required during that time. Subjects were excluded if they were immunocompromised, HIV positive, had abnormal renal or hepatic function, or had a history of malabsorption or of ocular HSV. Women of childbearing potential had to use an effective method of contraception throughout the study. Study participants were recruited by newspaper advertisements, flyers, and local health clinics at 2 centers: the University of Washington (UW) Virology Research Clinic in Seattle and the Westover Heights Clinic in Portland, Oregon. Enrollment took place between August 1995 and July 1996. The protocol was approved by human subjects review committees, and all subjects gave written, informed consent.

Study design. The study was a randomized, double-blind, placebo-controlled, 3-period, crossover trial. Sixty-nine subjects received oral acyclovir 400 mg twice a day, valacyclovir 500 mg twice a day, and placebo twice a day, in random order. After 7 weeks of receiving the initial treatment, each participant crossed over to the second treatment for 7 weeks and then to the third treatment for the final 7 weeks. A 1-week washout period of placebo followed the first 2 treatment arms. Participants were stratified by the duration of their HSV-2 infection and sex and were then randomized by blocks at entry. One stratum was composed of patients with a diagnosis of a first episode of genital herpes infection ≤ 6 months before randomization. The other stratum was composed of patients diagnosed with genital herpes > 6 months before randomization and who had a history of ≥ 6 recurrences/year in the absence of suppressive therapy. At the time of enrollment, participants were taught to recognize clinical episodes of HSV, and they visited the clinic every 2 weeks thereafter. During HSV recurrences, participants continued their assigned study treatment and reported to the clinic within 24 h of an outbreak for evaluation.

Throughout the 161 study days, participants collected daily swabs of genital secretions, as described elsewhere [10–13]. Women swabbed the cervicovaginal, vulvar, and perianal areas, and men swabbed the penile skin and perianal area. Participants also collected a daily mixed swab of the entire genital area and were asked to collect additional swabs of genital lesions during a recurrence, in addition to the regular daily swabs. Each area was swabbed with 2 Dacron swabs held together; 1 was placed in viral culture media and the second in PCR buffer. Samples were collected by courier 3 times/week for delivery to the virology laboratory. Participants maintained a daily diary of med-

ication use, adverse experiences, genital symptoms, and the presence of lesions.

The primary end points of the study were to evaluate the efficacy of valacyclovir and acyclovir for the suppression of subclinical HSV shedding in immunocompetent subjects as measured by culture and PCR, to evaluate the effect of valacyclovir and acyclovir on the amount and distribution of the number of HSV DNA copies detected by quantitative PCR and to evaluate the safety and tolerance of valacyclovir and acyclovir. Secondary end points were to evaluate the efficacy of valacyclovir and acyclovir for the suppression of total (subclinical and lesional) shedding by culture and PCR and to evaluate the efficacy of valacyclovir and acyclovir for the suppression of recurrences.

Laboratory methods. Western-blot analysis was used to detect antibodies to HSV-1 and HSV-2 [14]. Standard serologic testing for HIV was done. Viral isolation procedures were performed by standard tissue-culture methods [15]. A competitive quantitative HSV-DNA PCR assay was performed as described elsewhere [16]. Swabs were set up for culture within 3 days from the time they were collected by the participants. If received in the laboratory > 3 days from the time of collection, the culture swabs were discarded and only PCR was performed.

Statistical analysis. Sample-size calculations were based on a 2-tailed test, to provide 80% power to detect a 50% reduction in subclinical shedding by PCR between acyclovir and valacyclovir. This estimate was based on a 1.67% level of significance (rather than 5%), to adjust for multiple comparisons—that is, each drug versus placebo—as well as the comparison between the 2 drugs. The sample size was further increased to account for dropouts and to facilitate randomization to the 6 different treatment sequences.

The first day of study in each treatment period was excluded from the analysis, because shedding on the first day of a drug cannot be attributed to treatment effect. The 1-week washout periods between treatment arms (i.e., twice-daily placebo administration) were also excluded from the analysis. Total, subclinical, and lesional shedding rates were calculated separately for viral culture and PCR during each of the 3 treatment periods. The total shedding rate was defined as the number of days with positive culture (or PCR) results, divided by the total number of days with culture (or PCR) results. The subclinical shedding rate was defined as the number of days without a genital lesion but with positive viral culture (or PCR) results, divided by the total number of days without lesions. Similarly, the lesional shedding rate was defined as the number of days with observed or reported genital lesions and a positive viral culture (or PCR) result, divided by the total number of days with genital lesions. Recurrences were defined as a period of ≥ 1 successive days on which genital lesions were present.

On days with detectable HSV by PCR, the highest amount of HSV DNA from any genital site or lesion for that day was used

for analysis. This quantity of HSV DNA was summarized by the geometric mean number of HSV DNA copies per milliliter of specimen for each treatment. For total, subclinical, and lesional shedding, generalized estimating equations (GEEs) with Gaussian distribution and robust SEs were used to compare log copy numbers by treatment, while accounting for the multiple observations per person. McNemar's test was used to compare proportion of subjects who were shedding virus, and Wilcoxon's signed rank test was used to compare the number of days with lesions and the duration of recurrences by treatment arm.

Total and subclinical shedding rates were each modeled separately for HSV culture and PCR data. Risk factors for total and subclinical HSV shedding were evaluated by use of GEE models with Poisson distribution, log link, and robust SEs to account for within-person correlation. Period and sequence effects were examined by including the appropriate covariates and interaction terms in the models [17]. Previous studies of oral acyclovir and famciclovir have shown no period or sequence effects with a 2-week washout period between treatments in such crossover studies [4, 18, 19]. Compliance with the regimen was defined as the percentage of pills taken and was assessed by patient diaries and recording the number of unused tablets at clinic visits. All analyses were performed at UW.

RESULTS

Overall, 69 subjects were enrolled at the 2 sites—47 in Seattle and 22 in Portland. Of these persons, 42 (61%) were women (table 1). The median age of the participants was 32 years, and 66 (96%) were white. Twenty-seven subjects (9 men and 18 women) had a diagnosis of genital HSV infection ≤ 6 months before randomization, and 42 participants (18 men and 24 women) had been diagnosed with genital herpes > 6 months before randomization and had a history of ≥ 6 recurrences/year. Subjects collected evaluable samples on a median of 157 days (range, 59–181 days) for culture and 162 days (range, 59–183 days) for PCR. All participants tested positive for HSV-2 antibody; 9 (33%) of 27 participants with HSV diagnosed ≤ 6 months before the study and 21 (50%) of 42 who had a history of ≥ 6 recurrences/year were also seropositive for HSV-1.

Sixty-seven (97%) of 69 participants completed all 3 arms of the study. Two patients did not complete the study: 1 woman completed only the placebo and valacyclovir arms, and 1 man completed only the placebo arm. One woman with HSV diagnosed ≤ 6 months before the study was assigned to the sequence placebo-acyclovir-valacyclovir but instead received the treatment in the sequence acyclovir-placebo-valacyclovir. No subject withdrew from the study because of adverse events, and no serious adverse events were noted during the study.

Total HSV shedding. Specimens collected on 9287 days for HSV cultures and 9715 days for PCR-based assays were evaluated

in the laboratory. HSV was isolated at least once from mucosal sites in 62 participants (90%) by viral culture. Genital HSV shedding was detected by culture in 59 participants (86%) while they were receiving placebo, compared with 8 participants (12%) receiving valacyclovir ($P < .001$) and 16 participants (24%) receiving acyclovir ($P < 0.001$). HSV was isolated in culture, from at least 1 anatomical site, on 15.4% of the days on which culture was obtained while receiving placebo, compared with 0.5% of days receiving valacyclovir and 0.8% of days receiving acyclovir (table 2), a reduction of 97% and 95%, respectively.

HSV DNA was detected at least once in 68 participants (98%) by PCR. HSV DNA was detected on at least 1 day in mucosal swabs of 64 participants (93%) while receiving placebo, compared with 44 participants (65%) receiving valacyclovir ($P < .001$) and 51 participants (76%) receiving acyclovir ($P = .01$). HSV DNA was detected by PCR, from at least 1 anatomical site, on 40.2% of the days receiving placebo, compared with 7.2% of days receiving valacyclovir and 8.0% of days receiving acyclovir, a reduction of 82% and 80%, respectively.

The temporal effect of antiviral therapy on the frequency of HSV shedding is shown in figure 1A and 1B. With the initiation of valacyclovir or acyclovir, 5 days of therapy were needed to achieve suppression of genital HSV shedding. After the discontinuation of therapy, viral shedding consistently reappeared in 5 days.

Overall, fewer copies of HSV were detected by quantitative PCR while subjects were receiving valacyclovir and acyclovir, compared with that while subjects were receiving placebo. The magnitude of the geometric mean number of PCR copies per milliliter of specimen decreased from $10^{5.2}$ while receiving placebo to $10^{3.9}$ while receiving valacyclovir and $10^{3.6}$ while receiving acyclovir for days with detectable HSV by PCR ($P < .001$ for both treatments vs. placebo, on a logarithmic scale). The decrease in the log geometric mean number with acyclovir was similar to the suppression seen with valacyclovir ($P = .52$).

In the model of predictors of viral shedding (table 3), valacyclovir was associated with a significant decrease in the frequency of total HSV shedding by both viral culture (relative risk [RR], 0.03 [95% confidence interval {CI}, 0.01–0.07]; $P < .001$) and PCR (RR, 0.18 [95% CI, 0.12–0.26]; $P < .001$), compared with placebo, after controlling for gender and HSV history. A similar decrease in the frequency of total HSV shedding was observed with acyclovir, compared with placebo (RR, 0.05 [95% CI, 0.03–0.10] for culture and RR, 0.20 [95% CI, 0.15–0.28] for PCR; $P < .001$ for both). In this model, there were no appreciable differences in total HSV shedding between the valacyclovir and acyclovir arms, both by culture and PCR ($P = .12$ and $P = .49$, respectively). Period and sequence effect parameters were not significantly associated with shedding rates and thus were excluded from the final model.

Table 1. Demographic characteristics of study participants.

Characteristic	HSV diagnosed within past 6 months (n = 27)	Established HSV with frequent recurrences (n = 42)
Age, median (range), years	31 (21–47)	34 (21–67)
No. of men/no. of women (% men)	9/18 (33)	18/24 (43)
Race, no. (%)		
White	25 (93)	41 (98)
Other	2 (7)	1 (2%)
Time since acquisition of genital herpes, median (range)	4.3 months (2.0–6.5)	5.7 years (0.6–21.2)
HSV serologic results, no. (%)		
HSV-2 only	18 (67)	21 (50)
HSV-1 and 2	9 (33)	21 (50)
No. of recurrences during 6 months before study, median (range)	2 (0–6)	4 (0–18)
No. of days in study, median (range)	167 (116–183)	166 (59–192)
No. of days receiving regimen, median (range)		
Acyclovir	49 (0–60)	50 (0–60)
Placebo	50 (48–57)	50 (42–66)
Valacyclovir	51 (48–70)	51 (0–69)
No. of days of cultures, median (range)	154 (68–172)	158 (59–181)
No. of days of PCR samples, median (range)	162 (92–174)	163 (59–183)

NOTE. HSV, herpes simplex virus; PCR, polymerase chain reaction.

Subclinical HSV shedding. Subclinical shedding from the genital tract was detected by culture in 42 (61%) of 69 participants on at least 1 day while they were receiving placebo, compared with 7 (10%) of 68 while they were receiving valacyclovir and 13 (19%) of 67 while they were receiving acyclovir. HSV was cultured in the absence of lesions, from at least 1 anatomical site, on 6.6% of days while receiving placebo, compared with 0.3% of days receiving valacyclovir and 0.6% of days receiving acyclovir (table 2), a reduction of 95% and 91%, respectively.

HSV shedding was detected by PCR in 61 participants (88%) on at least 1 day receiving placebo, compared with 38 partic-

ipants (56%) receiving valacyclovir and 48 participants (72%) receiving acyclovir. HSV DNA was detected by PCR, from at least 1 anatomical site, on 27.1% of the days while receiving placebo, compared with 6.1% of days receiving valacyclovir and 6.4% days receiving acyclovir, a reduction of 77% and 76%, respectively.

For subclinical shedding, the geometric mean number of HSV DNA copies per milliliter of specimen decreased from $10^{4.7}$ while receiving placebo to $10^{3.9}$ while receiving valacyclovir and $10^{3.4}$ while receiving acyclovir ($P = .009$ for valacyclovir and $P < .001$ for acyclovir vs. placebo). Although most reactivation occurred at low copy numbers (figure 2A), shedding

Table 2. Rate of herpes simplex virus (HSV) isolation by viral culture and HSV DNA detection by polymerase chain reaction (PCR) during the study.

Viral shedding	Valacyclovir	Acyclovir	Placebo
Total ^a			
Culture	14/3132 (0.5)	25/3052 (0.8)	477/3103 (15.4)
PCR	233/3254 (7.2)	256/3189 (8.0)	1315/3272 (40.2)
Geometric mean no. of PCR copies/mL ^b	$10^{3.9}$	$10^{3.6}$	$10^{5.2}$
Subclinical			
Culture	10/3038 (0.3)	17/2953 (0.6)	161/2434 (6.6)
PCR	192/3160 (6.1)	196/3084 (6.4)	691/2554 (27.1)
Geometric mean no. of PCR copies/mL ^b	$10^{3.9}$	$10^{3.4}$	$10^{4.7}$
Lesional			
Culture	4/94 (4.3)	7/92 (7.6)	316/669 (47.2)
PCR	41/94 (43.6)	54/98 (55.1)	624/718 (86.9)
Geometric mean no. of PCR copies/mL ^b	$10^{4.1}$	$10^{4.5}$	$10^{5.7}$
Days with genital lesions (%)	98 (2.8)	102 (3.1)	758 (22.1)

NOTE. Data are no. of days positive/total days (%), unless otherwise indicated. Excludes the first day on each treatment and 991 washout days.

^a For PCR-positive samples only.

^b Total shedding in the acyclovir arm includes 1 positive culture and 6 positive PCR results from 1 subject with 7 days of missing accompanying symptom information.

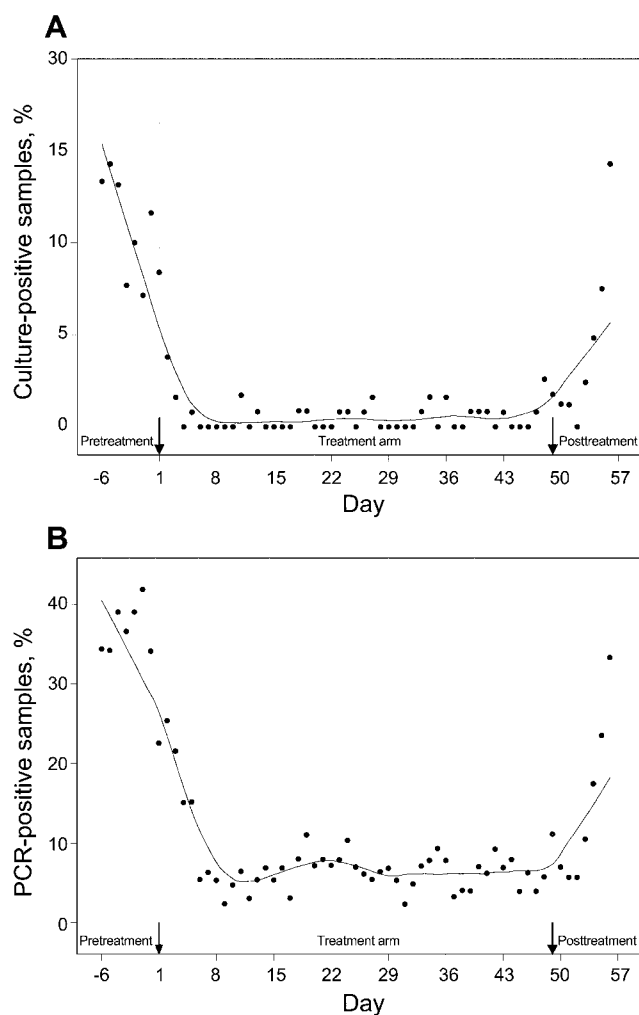


Figure 1. Shedding frequency before the initiation and after the completion of antiviral therapy. Data from both the acyclovir and valacyclovir arms are pooled in this figure. Arrows with extended dotted lines mark the first and last day of treatment with acyclovir or valacyclovir. The curve represents a locally weighted smoothed curve of daily shedding data. *A*, Culture; *B*, polymerase chain reaction (PCR).

with a high number of HSV DNA copies could also be detected. While subjects were receiving placebo, 27% of samples from subclinical shedding episodes had $\geq 10^{6.3}$ HSV DNA copies/mL versus 17% during the valacyclovir arm and 4% during the acyclovir arm.

In the model of predictors of subclinical viral shedding, treatment with valacyclovir significantly decreased the frequency of subclinical shedding compared with placebo (table 3), by both viral culture (RR, 0.05 [95% CI, 0.02–0.12]; $P < .001$) and PCR (RR, 0.22 [95% CI, 0.14–0.35]; $P < .001$), after controlling for sex and HSV history. A similar decrease in the frequency of subclinical HSV shedding was observed with acyclovir, compared with placebo (RR, 0.09 [95% CI, 0.04–0.17] for culture and RR, 0.24 [95% CI, 0.18–0.32] for PCR). There was no

statistically significant difference between the valacyclovir and acyclovir treatments, both by culture and PCR ($P = .07$ and $P = .70$, respectively).

Days with genital lesions and duration of recurrences.

Genital lesions were reported by 63 participants (91%) during the study. Of the 221 genital recurrences, 181 (82%) were observed in the clinic on at least 1 day of the episode. Thirty recurrences (14%) occurred during valacyclovir therapy, 31 (14%) during acyclovir therapy, 123 (56%) during placebo, 35 (16%) during the washout periods, and 2 (1%) on day 1 of a study arm. Genital lesions were reported on 758 days (22.1%) while subjects were receiving placebo (table 2), compared with 98 days (2.8%) while subjects were receiving valacyclovir ($P < .001$) and 102 days (3.1%) while subjects were receiving acyclovir ($P < .001$), a reduction of 87% and 86%, respectively.

The median duration of recurrence was shortened from 4 days while subjects were receiving placebo to 3 days while subjects were receiving valacyclovir ($P = .01$) and to 2 days while subjects were receiving acyclovir ($P = .02$). HSV was cultured, in the presence of lesions, on 47.2% of days while subjects were receiving placebo, compared with 4.3% of days while subjects were receiving valacyclovir and 7.6% of days while subjects were receiving acyclovir.

HSV shedding by PCR, in the presence of lesions, decreased from 86.9% of days while subjects were receiving placebo, compared with 43.6% of days while subjects were receiving valacyclovir and 55.1% of days while subjects were receiving acyclovir. In addition, during recurrences, significantly fewer copies of HSV were detected by quantitative PCR while subjects were receiving valacyclovir and acyclovir, compared with placebo (figure 2*B*). The magnitude of the geometric mean number of HSV DNA copies per milliliter of specimen decreased from $10^{5.7}$ while subjects were receiving placebo to $10^{4.1}$ on valacyclovir and $10^{4.5}$ on acyclovir ($P < .001$ for both vs. placebo). The decrease in HSV DNA with valacyclovir was similar to the suppression seen with acyclovir ($P = .69$).

Difference in site-specific shedding.

Total shedding from various sites was evaluated by culture (table 4). Among women, HSV shedding was observed most frequently while they were receiving placebo in swabs obtained from the vulva (11.1% of days) and perianal area (11.2% of days). Among men, HSV shedding was observed most frequently while they were receiving placebo from swabs obtained from the penis (7.5% of days). The frequency of HSV shedding was suppressed by 75%–95% at all genital sites with both valacyclovir and acyclovir.

Compliance with valacyclovir and acyclovir regimens.

Overall, the full dose of study drug was taken on 97.7% of the days, as assessed by pill counts at the 2-week clinic visits. Fifty-three (79%) of 67 participants who completed the study had $\geq 95\%$ compliance in both the acyclovir and valacyclovir treatment arms.

Table 3. Multivariate analysis of risk factors for total and subclinical viral shedding by viral culture and polymerase chain reaction (PCR).

Type of test	Total viral shedding		Subclinical shedding	
	RR (95% CI)	P	RR (95% CI)	P
Viral culture				
Valacyclovir vs. placebo	0.03 (0.01–0.07)	<.001	0.05 (0.02–0.12)	<.001
Valacyclovir vs. acyclovir	0.54 (0.25–1.16)	.12	0.55 (0.28–1.05)	.07
Women vs. men	2.40 (1.61–3.59)	<.001	3.71 (1.84–7.48)	<.001
HSV diagnosed ≤6 months vs. >6 months ago	1.48 (0.99–2.22)	.05	2.90 (1.45–5.80)	.003
PCR				
Valacyclovir vs. placebo	0.18 (0.12–0.26)	<.001	0.22 (0.14–0.35)	<.001
Valacyclovir vs. acyclovir	0.87 (0.60–1.28)	.49	0.93 (0.63–1.36)	.70
Women vs. men	1.28 (0.93–1.75)	.13	1.28 (0.83–1.97)	.27
HSV diagnosed ≤6 months vs. >6 months ago	1.57 (1.20–2.05)	.001	2.23 (1.57–3.18)	<.001

NOTE. CI, confidence interval; HSV, herpes simplex virus; RR, relative risk.

We evaluated whether shedding episodes while receiving study medications were related to compliance with both doses of antiviral therapy. Compliance with the study drug during the week before each positive culture or PCR specimen obtained during treatment with acyclovir or valacyclovir was determined. There were 39 positive cultures obtained during therapy with

acyclovir or valacyclovir. Eight of these occurred during the first 5 days of antiviral therapy. Of the remaining 31 positive cultures, only 4 (13%) were preceded by <100% compliance with the antiviral medication during the preceding week. Similarly, 489 specimens that were positive by PCR were obtained during treatment with acyclovir or valacyclovir; 101 of these

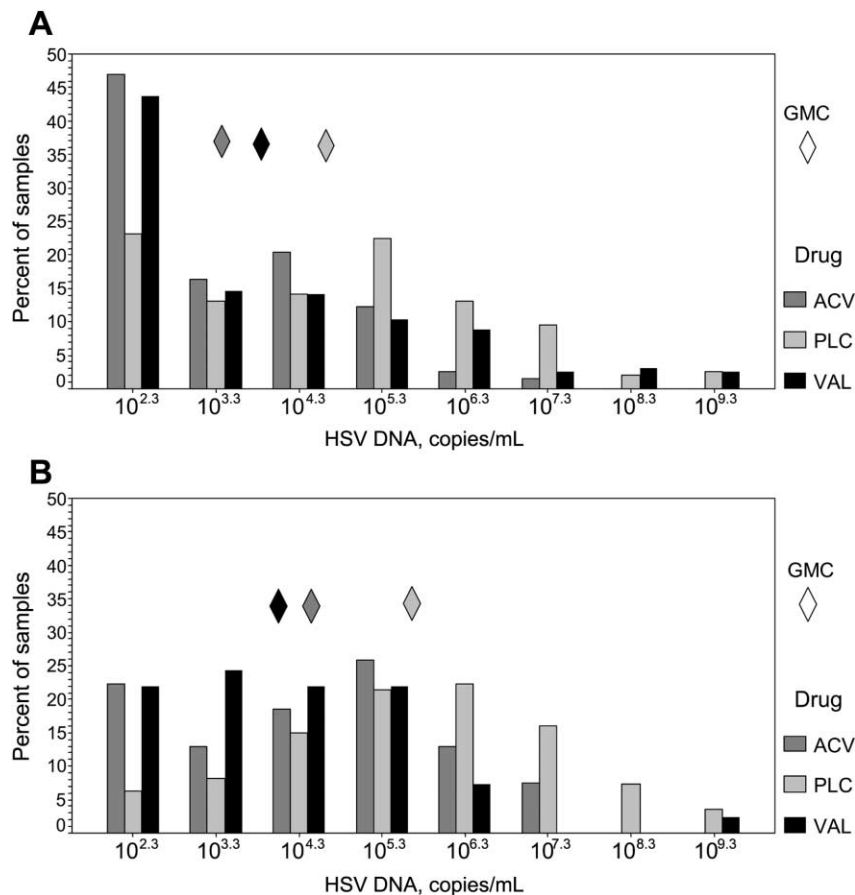


Figure 2. Distribution of the quantity of herpes simplex virus (HSV) DNA obtained from genital swabs during subclinical and lesional HSV reactivation. Valacyclovir (VAL; black bars) and acyclovir (ACV; dark gray bars) are compared with placebo (PLC; light gray bars). Geometric mean no. of HSV copies (GMC) for each treatment (reported in the text) is indicated by diamonds. A, Subclinical; B, lesional.

Table 4. Frequency of herpes simplex virus (HSV) isolation, by anatomic site and treatment arm.

Site	Valacyclovir	Acyclovir	Placebo
Men			
Penile skin	5/1227 (0.4)	7/1213 (0.6)	93/1240 (7.5)
Perianal	3/1226 (0.2)	1/1212 (0.1)	12/1241 (1.0)
Women			
Cervix	3/1901 (0.2)	6/1836 (0.3)	108/1858 (5.8)
Vulva	5/1903 (0.3)	10/1837 (0.5)	206/1862 (11.1)
Perianal	2/1903 (0.1)	7/1838 (0.4)	208/1860 (11.2)

NOTE. Data are no. of HSV-positive samples/total no. of samples (%).

occurred during the first 5 days of antiviral therapy. Of the remaining 388 specimens, only 39 (10%) were preceded by <100% compliance with the study medication during the preceding week.

DISCUSSION

The present results demonstrate several issues about the natural history of HSV reactivation in the genital tract and the effects of antiviral therapy on reactivation. HSV reactivation in both men and women is frequent, especially when measured by quantitative PCR. Treatment with valacyclovir or acyclovir is effective in reducing subclinical and total HSV shedding. The frequency of both total and subclinical shedding decreased with antiviral therapy by 91%–97% when measured by culture and by 76%–82% when measured by PCR. This effect was seen at all anatomic sites and in both men and women. Additionally, the quantity of HSV shed and the number of recurrences were also significantly reduced.

The subclinical reactivation of HSV accounted for a large proportion of total HSV shedding observed during the study. While subjects were receiving placebo, nearly one-third of all days with positive HSV cultures occurred on days without any reported lesions. Using the more-sensitive PCR method, one-half of the total days with HSV DNA detected while subjects were receiving placebo occurred in the absence of lesions. While subjects were receiving therapy with acyclovir or valacyclovir, most of the culture- and PCR-positive days were subclinical in nature.

The magnitude of effect of therapy on genital HSV suppression with valacyclovir was comparable to that achieved with acyclovir. We chose the dose of valacyclovir 500 mg twice daily, because our study was initiated before studies that established the clinical efficacy of once-daily therapy [8, 20], and our goal was to maximize the potential advantage of valacyclovir over acyclovir. Valacyclovir is rapidly converted to acyclovir after absorption and achieves higher plasma levels of acyclovir than oral preparations of acyclovir [5]. The present study data show that neither valacyclovir nor acyclovir leads to 100% suppression of HSV detection on mucosal surfaces. Nonetheless, the clinical and virological effect of both antivirals is pronounced.

For total, subclinical, and lesional shedding (measured by the number of culture- and PCR-positive days), there was a trend in favor of valacyclovir—patients who received valacyclovir shed virus on a smaller percentage of days than when they received acyclovir. However, the observed difference of 5% in subclinical shedding rates by PCR was small and was far below the hypothesized 50% reduction on which we calculated our sample size. Whether a much larger study would show better suppression with valacyclovir versus acyclovir is unknown. It is unclear why occasional episodes of high copy numbers of HSV DNA shedding were seen while subjects were receiving daily therapy. A detailed analysis of compliance showed no association between missed pill use (which was very infrequent) and breakthrough shedding. Whether the limits of suppression that we have described can be further reduced by higher doses of drug or the addition of compounds with alternative mechanisms of action (e.g., helicase inhibitors) are questions that remain unanswered.

The subclinical shedding rate of this cohort was 6.6% by culture, which is higher than the rates of 1%–4% in immunocompetent hosts that have been described elsewhere [11–13]. This rate likely reflects the high risk factors of this cohort, recent HSV-2 acquisition [21], and frequent recurrences [4, 12]. The study population was specifically chosen to be “high shedders,” to facilitate the detection of potential differences between the antiviral agents. The frequency of HSV reactivation, as defined by lesions and the degree of reduction in genital lesions by valacyclovir and acyclovir, was similar to previous studies of these medications in persons with frequently recurring genital herpes [8].

The effect of antiviral treatment on shedding has a time lag. Viral suppression is not immediate and takes 5 days to achieve. After the cessation of therapy, the suppression effect lingers and returns to pretreatment levels in 5 days. This lag may reflect the inhibition of viral replication that occurs not only at the mucosal but also at the neuronal level. Of interest, reactivation of HSV-2 on buttock sites after ultraviolet light exposure occurs after ~5 days [22], which suggests that this is the time required for the virus to become evident at the mucosa after reactivation.

Our results help elucidate the dynamics of mucosal HSV detection. Both clinical and subclinical reactivation of HSV in an individual may result in the sexual transmission of HSV to their partner. Subclinical reactivation is unrecognized and may occur sporadically and frequently [12, 23]; hence, it is felt to be the major mode of HSV transmission [1]. The significance of suppressing HSV shedding with antiviral medication was recently demonstrated in a large international, randomized, placebo-controlled trial [24]. In that study of >1400 heterosexual, HSV-2 antibody-discordant couples, once-daily valacyclovir (500 mg) reduced the risk of transmitting symptomatic genital herpes by 75% and overall acquisition of HSV-2 by 48%. A shedding substudy ($n = 89$ subjects) that used a similar pro-

tolocol indicated that once-daily valacyclovir also reduced the frequency of viral shedding as measured by PCR by 73%, a rate similar to that observed in the present study. It is unclear whether there are any definitive correlates between frequency of reduction in subclinical shedding and transmission. Developing such a surrogate measurement for transmission requires more study. However, it is reassuring to see that a reduction in transmission is associated with a reduction in viral shedding on mucosal surfaces.

The strengths of the present study include the large number of participants for an investigation of this type; daily sample collection, which provided >20,000 specimens for analysis; a low attrition rate; and a high rate of adherence to study drug and procedures. In addition, the majority of genital lesions were confirmed by a clinician, which minimized the misclassification of lesional days of shedding as subclinical days. Our study population was highly selected for interest in such a labor-intensive study and was mostly white. The effect of race on HSV reactivation has not been evaluated. Although a history of recognized genital herpes is found less frequently among African Americans with HSV-2 antibody in the United States [25], shedding has not been systematically studied in other populations. Preliminary data from Africa, focusing on HIV seropositive women, suggest that HSV reactivation is common in that population as well [26–28].

In summary, we found that valacyclovir and acyclovir are highly effective in suppressing the frequency and quantity of HSV shedding. Current antivirals do not achieve an absolute cessation of shedding, but the effect on HSV shedding is sustained beyond the half-life of the drug.

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