

Acyclovir-Resistant Genital Herpes Among Persons Attending Sexually Transmitted Disease and Human Immunodeficiency Virus Clinics

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Background: Genital herpes is epidemic in the United States; long-term acyclovir therapy is common; and long-term use of antimicrobials in suppressive doses favors development of resistance.

Objective: To determine the prevalence of and risk factors for acyclovir-resistant genital herpes.

Methods: We identified and attempted to enroll all patients 18 years or older with suspected genital herpes who attended 22 sexually transmitted disease and human immunodeficiency virus (HIV) clinics in the United States between October 1996 and April 1998. We conducted standardized interviews of all consenting patients. Lesions were cultured, and isolates were typed as herpes simplex virus (HSV) 1 or HSV-2 and tested for acyclovir sensitivity (using a 50% inhibitory concentration of 2 µg/mL) by plaque reduction, which was independently confirmed.

Results: Herpes simplex virus was isolated from 2088 of 3602 patients, and 90.2% of isolates were HSV-2. Fif-

teen isolates, all HSV-2, were acyclovir resistant. Three (0.18%) of 1644 HIV-negative patients had acyclovir-resistant isolates (95% confidence interval [CI], 0.04%-0.5%); resistance was associated with oral ($P<.006$) and topical ($P<.001$) acyclovir use. Twelve (5.3%) of 226 HIV-positive patients yielded resistant HSV isolates (95% CI, 2.8%-9.1%); resistance was associated with oral acyclovir use ($P<.001$), duration of the current episode ($P<.001$), history of recurrent genital herpes ($P<.01$), and low CD4 cell count ($P<.05$).

Conclusions: In the 15 years following licensure of acyclovir, resistance to the drug remains low among immunocompetent patients. However, 5% of HIV-positive patients had resistant HSV-2 isolates. Continued surveillance is essential to monitor changes in acyclovir resistance and to characterize the clinical and public health importance of acyclovir-resistant HSV.

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GENITAL HERPES is epidemic in the United States.¹ From 1970 to 1995, physician visits for genital herpes increased 10-fold.^{2,3} Between 1978 and 1990, the seroprevalence of herpes simplex virus (HSV) 2, the primary causative agent of genital herpes, increased 32% in the United States, infecting 22% of persons 12 years or older.⁴ Genital herpes disproportionately affects women, adolescents, and disadvantaged populations and is a risk factor for acquisition and transmission of human immunodeficiency virus (HIV).

At least 45 million US residents are seropositive for HSV-2, but fewer than 10% of them report a history of genital herpes.⁴ Furthermore, HSV-2-infected persons experience frequent subclinical recurrences.⁵ Thus, behavior modification alone is not an appropriate prevention strategy for HSV-2 infections, and primary prevention through vaccination has not been successful.⁶ However, daily suppressive therapy with acyclovir significantly reduces the fre-

quency of recurrences and asymptomatic shedding.⁷ The safety and efficacy of acyclovir have been documented among patients receiving daily therapy for as long as 6 years.^{8,9} Therefore, long-term antiviral therapy might be a method for controlling genital herpes.

However, long-term use of antimicrobials in suppressive doses may favor development of resistance.¹⁰ Numerous studies of acyclovir-resistant HSV infections among immunocompromised patients have been published,¹¹⁻¹⁹ but most are case reports, and there are few substantive studies of acyclovir resistance in immunocompetent patients. Prevalence estimates of acyclovir-resistant HSV among immunocompromised persons and immunocompetent persons have varied widely because of the lack of a standard definition for acyclovir resistance. Thus, our objectives were to estimate the prevalence of acyclovir-resistant genital herpes among patients attending sexually transmitted disease and HIV clinics, to establish baseline prevalence estimates for monitoring

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temporal trends of resistance, and to describe risk factors for acyclovir resistance.

METHODS

SURVEILLANCE SYSTEM

Between October 1996 and April 1998, we enrolled patients with genital herpes from 22 public health, university hospital, and private sexually transmitted disease and HIV clinics in Atlanta, Ga; Birmingham, Ala; Chicago, Ill; Columbus, Ohio; Denver, Colo; Houston, Tex; Indianapolis, Ind; Nassau Bay, Tex; New Orleans, La; New York, NY; San Francisco, Calif; and Seattle, Wash. Study site coordinators received training in enrollment and sample- and data-collection procedures.

STUDY PARTICIPANTS

We attempted to enroll all patients 18 years or older suspected of having active genital herpes. Patients from whom HSV was isolated at the first visit were not enrolled on subsequent visits, but those whose specimens were initially negative were eligible for enrollment if they had another episode of genital herpes. The study adhered to human experimentation guidelines of the US Department of Health and Human Services. All participants were volunteers who gave informed consent.

Participants were queried in English or Spanish for demographic characteristics, sexual proclivity and activity in the past year, history of genital herpes, acyclovir use, and HIV infection (including the date and results for their most recent tests for CD4 cells and viral load). Their HIV status was not independently confirmed.

LABORATORY CULTURE AND SENSITIVITY TESTING

Suspected anogenital herpetic lesions were recorded and sampled, and specimens were immediately placed into M4-3 Multi-Microbe transport media (Microtest, Snellville, Ga) for overnight transport on ice to ViroMed Laboratories, Minneapolis, Minn. Within 2 hours of arrival at the laboratory, 100 μ L of each specimen was inoculated into duplicate tubes of primary rabbit kidney cells. Cultures were observed daily for 5 days for HSV cytopathic effect. Positive cultures were harvested for typing, acyclovir sensitivity screening, and long-term storage. The HSV type was determined by staining with type-specific, fluorescein-labeled monoclonal antibodies (Syva Microtrak, Wicklow, Ireland).

Harvests from primary cultures were screened by plaque-reduction assay^{20,21} for acyclovir resistance. In brief, virus (50-200 plaque-forming units/well) was screened in two 6-well plates seeded with Vero cells. The titer of the input virus was determined in a third plate. Isolates were tested in duplicate against acyclovir concentrations of 30, 10, 3, 1, 0.3, and 0.1 μ g/mL. Each set of assays included 2 controls: a wild-type acyclovir-sensitive HSV-2 strain (MS-2) and an acyclovir-resistant thymidine kinase (TK)-deficient HSV-2 isolate (strain 15486). To inhibit secondary plaque formation, cultures were overlaid with media containing human immune serum globulin or methylcellulose. Plates were incubated in a humidified atmosphere (37°C, with 5%-7% carbon dioxide) for 3 days. Cells were fixed by using Safefix (Biochemical Sciences, Swedesboro, NJ) and stained with crystal violet. Plaques were counted manually on a light box. A dose-response curve was generated, and the median 50% inhibitory concentration (IC₅₀) of acyclovir was calculated in micrograms per milliliter. Isolates with an IC₅₀ of 2 μ g/mL or higher were sent to 2 independent laboratories for confirmatory testing (Earl R. Kern, PhD, University of Alabama at Birmingham, and Michelle Davis, PhD, Glaxo Wellcome, Research Triangle Park, NC). Iso-

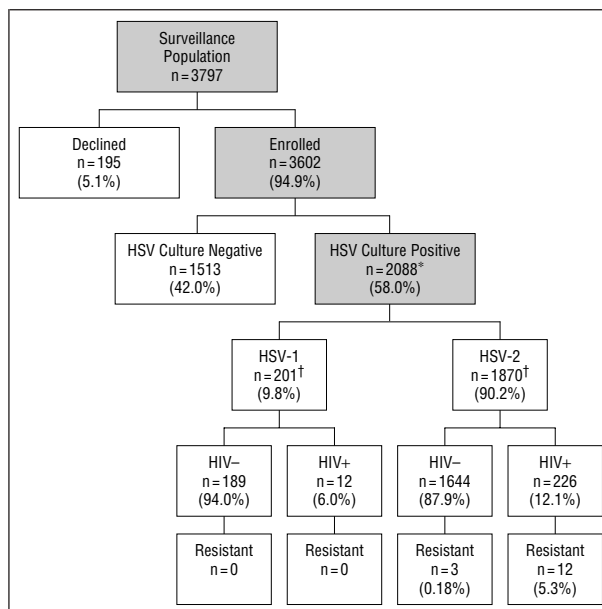


Figure 1. Flowchart of patient enrollment, results of herpes simplex virus (HSV) culture, human immunodeficiency virus (HIV) status, and findings of acyclovir-sensitivity testing. Asterisk indicates that 1 isolate in this group included HSV-1 and HSV-2 and was dropped from the analysis; dagger, the group does not include 3 HSV-1-positive patients and 14 HSV-2-positive patients with unknown HIV status.

lates confirmed as having an IC₅₀ of 2 μ g/mL or higher at both laboratories were considered to be acyclovir resistant.²¹ Four isolates with IC₅₀ values of approximately 2 μ g/mL in the screening assay were not confirmed as resistant and were not included in the analysis.

STATISTICAL ANALYSES

We analyzed data using SAS (SAS Institute, Cary, NC) and StatXact-3 (Cytel Software, Cambridge, Mass) software. The significance of association was estimated by using *P* values and 95% confidence intervals (CIs). Univariate significance tests used the Fisher exact test for dichotomous variables and the Wilcoxon signed-rank test for ordered categorical variables; *P* ≤ .05 was considered significant.

RESULTS

ENROLLMENT

Of 3797 eligible patients, 3602 (94.9%) participated in the study (**Figure 1**). More women than men (odds ratio [OR], 1.5; 95% CI, 1.1-2.0) and more blacks than non-blacks (OR, 2.7; 95% CI, 1.9-3.8) declined enrollment. No other demographic differences were noted between those who enrolled and those who declined to enroll. Approximately 50% of enrollees were black, 40% were white, and 10% reported another race; 346 (10%) were Hispanic. More than half the enrollees were aged between 18 and 29 years (mean, 31 years; range, 18-93 years), and 511 (14.3%) reported being HIV positive.

ACYCLOVIR RESISTANCE

Of 3602 specimens, HSV was isolated from 2088 (58.0%): 1884 isolates (90.2%) were HSV-2 and 204 were HSV-1.

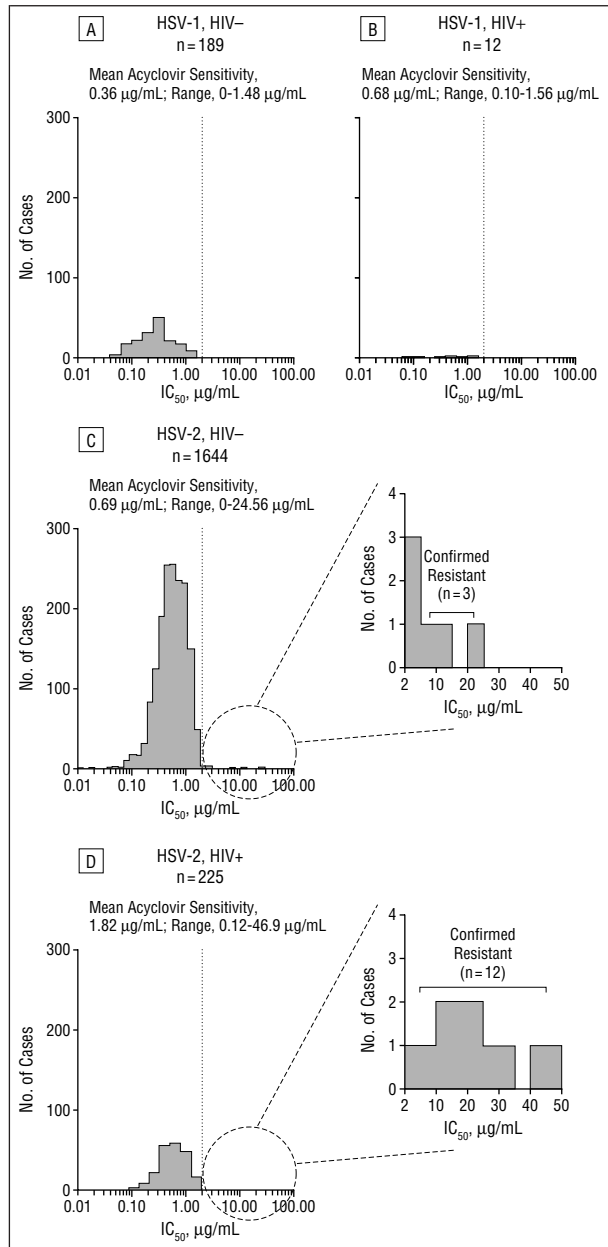


Figure 2. Number of herpes simplex virus (HSV) cases (by subtype and human immunodeficiency virus [HIV] status) plotted against acyclovir sensitivity (measured by median 50% inhibitory concentration [IC_{50}] in micrograms per liter). Isolates with IC_{50} of 2 $\mu\text{g/mL}$ (cutoff represented by dotted vertical line) or higher in the screening assay were subsequently tested in 2 independent laboratories for confirmation of the results.

Fifteen isolates, all HSV-2, were confirmed as acyclovir resistant ($IC_{50} \geq 2 \mu\text{g/mL}$; range, 4.5-46.9 $\mu\text{g/mL}$; mean, 20.7 $\mu\text{g/mL}$) (**Figure 2**). The mean IC_{50} was 14.8 $\mu\text{g/mL}$ (median, 11.5 $\mu\text{g/mL}$; range, 8.2-24.6 $\mu\text{g/mL}$) for the 3 acyclovir-resistant isolates from HIV-negative patients and 22.2 $\mu\text{g/mL}$ (median, 19.5 $\mu\text{g/mL}$; range, 4.5-46.9 $\mu\text{g/mL}$) for the 12 acyclovir-resistant isolates from patients reporting HIV positivity.

PREVALENCE OF ACYCLOVIR RESISTANCE

Of 1644 HSV-2 isolates from HIV-negative patients, 3 (0.18%) were resistant to acyclovir (95% CI, 0.04%-0.5%)

as were 12 (5.3%) of 226 isolates from HIV-positive patients (95% CI, 2.8%-9.1%) (**Table 1**). No statistically significant differences were observed in the prevalence of acyclovir resistance by age, sex, race, or ethnicity.

RISK FACTORS FOR ACYCLOVIR RESISTANCE

Table 2 summarizes risk factors for having acyclovir-resistant HSV-2. Among HIV-negative patients, resistance was significantly associated with having used acyclovir or an analogue in the past and with use of topical acyclovir for the current outbreak. Among HIV-positive patients, resistance was significantly associated with having recurrent genital herpes and ever having used acyclovir or an analogue. In addition, HIV-positive patients with acyclovir-resistant isolates had lesions of significantly longer duration than HIV-positive patients with sensitive isolates (mean, 43 days; median, 30 days vs mean, 19 days; median, 6 days; $P < .001$), and they had lower CD4 cell counts (mean, 59 and median, 24 vs mean, 176 and median 72 cells/mL; $P < .05$). Age, sex, race, ethnicity, and sexual activity in the past 12 months were not associated with acyclovir resistance in either subgroup.

COMMENT

Consistent with previous findings, we identified acyclovir-resistant HSV-2 infection in 0.18% of HIV-negative and 5.3% of self-reported HIV-positive patients.¹⁴ Our results provide baseline prevalence estimates for acyclovir resistance of genital herpes in ethnically diverse and geographically distributed patients attending sexually transmitted disease and HIV clinics in the United States. Given the increasing prevalence of genital herpes infection and disease, coupled with the rising use of acyclovir and its analogues, surveillance should be continued to monitor the potential spread of acyclovir-resistant genital herpes.

All 15 patients with acyclovir-resistant HSV-2 isolates reported previous episodes of recurrent genital herpes and previous oral acyclovir use in the past year. Of 12 HIV-positive patients with resistant isolates, 11 reported CD4 cell counts of less than 125/mL, previous lesion duration of at least 2 months, and mean acyclovir use of 80 days (range, 0.5-10.2 years). Thus, acyclovir resistance was significantly associated with prior acyclovir use, a finding that supports previous reports^{15,17,22} and suggests that most resistance develops because of the selective pressure created by acyclovir use rather than acquisition of resistant virus from infected sex partners. The finding is also consistent with reports that acyclovir-resistant HSV can readily be selected by growth in the presence of the drug.^{23,24} However, prospective studies have found no differences in acyclovir susceptibility of HSV isolates over time in patients who receive suppressive therapy.²⁵⁻²⁷

The low prevalence of acyclovir resistance among immunocompetent patients with genital herpes could reflect reduced pathogenicity of HSV-2 with mutations in the TK gene and HSV-TK mutants resistant to acyclovir exhibit attenuation in the mouse model.²³ However, TK-deficient, acyclovir-resistant HSV can cause progressive disease in humans and maintain pathogenicity for mice.^{15,28} In our study, genital lesions persisted significantly longer

Table 1. Characteristics of Patients With Acyclovir-Resistant HSV-2 Isolates by HIV Status*

Characteristic	HIV Negative		HIV Positive	
	Total No.	Resistant (95% CI)	Total No.	Resistant (95% CI)
Overall	1644	0.18 (0.04-0.53)	226	5.31 (2.77-9.09)
Age, y				
<40	1410	0.14 (0.02-0.51)	157	3.82 (1.42-8.13)
≥40	234	0.43 (0.01-2.36)	69	8.70 (3.26-17.97)
Sex				
Male	989	0.20 (0.02-0.73)	143	6.99 (3.40-12.48)
Female	648	0.15 (0.00-0.86)	82	2.44 (0.30-8.53)
Race				
Black	828	0.00 (0.00-0.44)	127	6.30 (2.76-12.03)
White	650	0.46 (0.10-1.34)	73	4.11 (0.86-11.54)
Other	161	0.00 (0.00-2.27)	26	3.85 (0.10-19.64)
Ethnicity				
Hispanic	140	0.00 (0.00-2.60)	43	4.65 (0.57-15.81)
Non-Hispanic	1476	0.20 (0.04-0.59)	173	5.78 (2.81-10.37)

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HSV, herpes simplex virus.

*Several categories do not sum to total because of missing values. Unless otherwise indicated, data are percentages.

Table 2. Risk Factors Associated With Acyclovir-Resistant HSV-2 by HIV Status*

Risk Factor	HIV Negative (n = 1644)			HIV Positive (n = 226)		
	Pos/Tested (%)	P Value	OR	Pos/Tested (%)	P Value	OR (95% CI)
HSV recurrence		.09	Undefined		.01*	Undefined
Yes	3/746 (0.4)			12/152 (7.9)		
No	0/898			0/74		
Ever use acyclovir or an analogue		.005*	Undefined		.001*	Undefined
Yes	3/289 (1.0)			12/118 (10.2)		
No	0/1355			0/108		
Current acyclovir or analogue use		<.001*	Undefined		.42	1.76 (0.45-6.86)
Yes	3/79 (3.8)			3/37 (8.1)		
No	0/1565			9/189 (4.8)		
Oral acyclovir or analogue† use		>.99	Undefined		.002*	7.11 (2.05-24.63)
Yes	0/65			8/55 (14.6)		
No	3/1579 (0.2)			4/171 (2.3)		
Topical acyclovir use		<.001*	Undefined		.29	2.18 (0.44-10.71)
Yes	3/66 (4.6)			2/20 (10.0)		
No	0/1578			10/196 (4.9)		

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HSV, herpes simplex virus; OR, odds ratio.

*All P values are based on the Fisher exact 2-tailed test of comparison with patients whose HSV-2 isolates were sensitive to acyclovir. Undefined ORs were undefined because all resistant isolates were found in 1 cell.

†Valacyclovir or famciclovir.

among HIV-positive patients with acyclovir-resistant herpes than among HIV-positive patients with sensitive isolates. However, this correlation was not observed among immunocompetent patients, perhaps because they could more effectively suppress infection or because of small sample size. Previous studies have not reported an association between acyclovir sensitivity of isolates and clinical response.²¹ Prospective studies of patients with resistant genital herpes are warranted to further explore the relationship between in vitro and clinical resistance.

The strong association of acyclovir resistance with current use of topical acyclovir among our HIV-negative patients was interesting. We considered whether acyclovir residue collected during sampling influenced virus isolation. However, the proportion of persons who used only topical acyclovir to treat their current episode was the same among those whose lesions were (4.4%)

and those whose lesions were not (4.3%) culture positive for HSV. Thus, acyclovir resistance likely had no effect on outgrowth of virus in the primary culture. Physicians should counsel patients against the use of topical acyclovir for genital herpes infections. Besides affording little or no clinical efficacy for lesion resolution, topical use may enhance development of resistance.

In the treatment of genital herpes, the absence of efficacious vaccines, the increasing use of antiviral agents in immunocompromised patients, and the possible use of acyclovir for primary prevention have created an environment conducive to the emergence of acyclovir resistance. We found that exposure to acyclovir was the most important factor associated with resistance, but we did not address whether this resistance is permanent or transient. We did not document acyclovir-resistant HSV in patients with first-episode genital herpes, and be-

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cause of small numbers, we cannot accurately estimate the magnitude of primary acyclovir resistance in this population.

The occurrence of acyclovir resistance documented in our study was similar to that estimated in smaller studies over the 15 years since licensure of acyclovir, providing some reassurance that resistance is not rapidly increasing. However, mathematical models predict that substantial lags in emergence of resistance occur when acyclovir use in the population is low (as it has been since licensure).^{29,30} Within the next decade, antiviral medications may assume an importance and penetrance similar to that held by antibiotics. For this reason, the use of antiviral drugs must be guided by continued surveillance for emerging resistance.

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