ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2002, p. 3042–3044 Vol. 46, No. 9
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.9.3042–3044.2002

Surveillance for Antiviral-Agent-Resistant Herpes Simplex Virus in the General Population with Recurrent Herpes Labialis

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Received 26 December 2001/Returned for modification 29 March 2002/Accepted 28 May 2002

In a general population survey in the United States, the prevalence of antiviral-agent-resistant herpes simplex virus was very low among more than 1,000 isolates from individuals with an episode of recurrent herpes labialis not treated with topical antiviral agents. Two isolates had borderline resistance to acyclovir (0.2%), and all were susceptible to penciclovir.

Between October 1998 and February 1999, a study was undertaken in 10 states across the United States to determine the background prevalence of herpes simplex virus (HSV) resistant to acyclovir or penciclovir among isolates from subjects with an episode of recurrent herpes labialis (RHL) which had not been treated with a topical antiviral agent. Results from other studies with immunocompetent patients with HSV infection have shown that the prevalence of resistant virus is low (1, 2, 3; M. Reyes, J. Graber, N. Weatherall, C. Hodges-Savola, W. C. Reeves, and the Task Force on Herpesvirus Resistance, 11th International Conference on Antiviral Research, April 1998, Antivir. Res. **37:**A44 [abstract], 1998) and stable, despite increasing rates of use of antiviral medication.

Topical penciclovir cream was approved by the Food and Drug Administration in 1996 as a prescription product for the treatment of RHL. Topical acyclovir cream for the treatment of RHL is available in many markets, often without prescription, but has not been approved for use in the United States. The survey formed part of a program to support an application to switch penciclovir from a prescription product to a nonprescription product (one that could be sold over the counter [OTC]). It was anticipated that approval for OTC use will increase the levels of use of penciclovir, and therefore, any future change in the prevalence of resistant virus could be tracked against a background prevalence established prior to the switch to OTC status. At the time of implementation, the survey was sponsored jointly by two pharmaceutical companies and was planned in collaboration with the Task Force on Herpes Simplex Virus Resistance.

(These data were presented at the Thirteenth International Conference on Antiviral Research, Baltimore, Md., April 2000.)

The study was conducted in accordance with good clinical practice and received Essex Institutional Review Board approval. Pharmacists in 47 community-based pharmacies were responsible for the clinical phase of the study (9). The subjects volunteered for the study in response to advertisements placed in the media (9). Eligible subjects were aged ≥12 years and had perioral herpes labialis at the vesicle or soft ulcer stage. No topical treatment with an antiviral agent was permitted prior to completion of the study, although subjects who received any oral antiviral agent for HSV infection were not excluded. Efforts were made to identify individuals who may have been immunocompromised by asking subjects to complete one page of the case report form, which was then sealed in an envelope by the subject to maintain confidentiality and subsequently transferred to the data management team. Subjects responded to questions on medical history (history of cancer, human immunodeficiency virus infection [HIV] or AIDS, or organ transplantation) and completed a checklist of medications that they were taking, use of which may have implied that the subject was immunocompromised. The checklist specified drugs with immunosuppressant activity or drugs used to treat HIV infection or AIDS or other diseases associated with defects of the immune system.

A total of 1,803 subjects were recruited, and each subject was swabbed once. The study population (n = 1,795 subjects with swabs processed for virus isolation) was principally female (67.6%), with a mean age of 37.5 years (standard deviation [SD], 15.34 years). Caucasians formed the largest racial group (89.9%). The preponderance of female Caucasians in the study may reflect that sector of the population that is concerned about RHL and/or that is motivated to seek treatment (4, 7). The mean ± SD number of episodes experienced during the previous 12 months was 5.2 ± 5.26, and the mean age at the first episode was 13.1 years. The mean ± SD duration of an episode of RHL was reported to be 8.1 ± 4.8 days. Lesions were swabbed by the pharmacist. The swabs were stored in transport medium at 4°C and shipped to a central laboratory within 3 to 4 days.

Virus was isolated in primary rabbit kidney cells (8). A cell suspension prepared from each infected culture was used to type the virus by indirect immunofluorescence. Vero cells and MRC-5 cells were used for the plaque reduction assays with acyclovir and penciclovir, respectively. Monolayers in six-well plates were infected with 50 to 100 PFU per well. After adsorption, the inoculum was removed and replaced with culture medium containing methylcellulose (2%) and the test antiviral agent (final concentrations, 0 to 30 μg/ml, tested in duplicate). After incubation for 3 days at 36 ± 1°C in 5 to 7% CO_2, the monolayers were fixed and stained, the plaques were counted,

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TABLE 1. Summary of HSV-1 susceptibility to acyclovir and penciclovir by antiviral use and medical history[a]

Clinical	ACV (Vero cells)			PCV (MRC-5 cells)		
	No. of subjects	Median IC_{50} (μg/ml)[b]	P[c]	No. of subjects	Median IC_{50} (μg/ml)[b]	P[c]
Overall	1,002	0.42		1,004	0.26	
Previous use of an antiviral agent (ACV, FCV, PCV, VCV)						
Used an antiviral agent	331	0.41	0.211	333	0.26	0.649
Never used an antiviral agent	662	0.44		662	0.26	
Current antiviral agent usage (oral ACV, FCV, VCV)						
Using an antiviral agent	49	0.31	0.025	49	0.24	0.488
Not using an antiviral agent	943	0.43		945	0.26	
Immune status						
Potential immunocompromise[d]	37	0.39	0.274	38	0.25	0.377
No immunocompromise	959	0.42		960	0.26	
No. of RHL episodes in past 12 mo						
<4	443	0.43	0.387	443	0.26	0.284
≥4	557	0.42		559	0.26	
Duration of history of RHL						
<16 yr	332	0.39	0.115	333	0.26	0.804
≥16 yr	651	0.44		652	0.26	
Avg duration of an RHL episode						
<6 days	265	0.45	0.223	266	0.27	0.644
≥6 days	735	0.41		736	0.26	

[a] No meaningful comparisons of the potency of acyclovir and penciclovir can be made because the isolates were tested in different cell lines. Abbreviations: ACV, acyclovir; FCV, famciclovir; PCV, penciclovir; VCV, valacyclovir.
[b] The mean ± SD IC_{50} of acyclovir for all isolates was 0.53 ± 0.39 μg/ml; that of penciclovir was 0.28 ± 0.11 μg/ml. For sensitive reference strain SC16, the mean ± SD IC_{50} of acyclovir in all assays was 0.57 ± 0.34 μg/ml; that of penciclovir was 0.20 ± 0.17 μg/ml. For resistant HSV-1 reference strain DM21, the mean ± SD IC_{50}s of acyclovir ranged from 14.35 to >30 μg/ml; the mean ± SD IC_{50}s of penciclovir for HSV-1 DM21 ranged from 2.26 to >30 μg/ml.
[c] Medians within each subgroup were compared by the Wilcoxon rank sum test with the *t*-test approximation.
[d] Based on subjects' reported medication and medical histories.

and the 50% inhibitory concentrations (IC_{50}s) were calculated. Two standard HSV type 1 (HSV-1) reference strains were included in all assays, SC16 (which is sensitive to acyclovir and penciclovir) and DM21 (a thymidine kinase-negative mutant which is resistant to acyclovir and penciclovir). Summary IC_{50} data for these strains are provided in Table 1.

The 1,803 cultures yielded 1,087 isolates, all of which were typed as HSV-1. A total of 1,002 isolates were tested for their susceptibilities to acyclovir and penciclovir, and 2 additional isolates were also tested for their susceptibilities to penciclovir. The breakpoint for defining resistance to acyclovir was an IC_{50} ≥2 μg/ml (5). Two criteria were applied to define penciclovir resistance: (i) an IC_{50} ≥2 μg/ml and (ii) an IC_{50} three or more times greater than the mean IC_{50} of penciclovir for all isolates in the survey (≥0.84 μg/ml).

Two HSV-1 isolates were confirmed to be resistant to acyclovir (IC_{50}s, 3.21 and 2.41 μg/ml, respectively), but resistance was borderline in this assay and in the original assay (IC_{50}s, 2.21 and 4.35 μg/ml, respectively). Both isolates were sensitive to penciclovir (IC_{50}s, 0.38 and 0.34 μg/ml, respectively). The two subjects with acyclovir-resistant HSV-1 isolates were immunocompetent; one subject had no history of antiviral agent use, and the other subject reported prior use of acyclovir (not during the episode evaluated for the present study).

All other isolates were susceptible to acyclovir; therefore, the prevalence of acyclovir-resistant HSV-1 isolates was 0.20% (2 of 1,002 isolates tested; 95% confidence interval, 0.02 to 0.72%). A total of 1,004 HSV-1 isolates were tested for their susceptibilities to penciclovir, and none was found to be resistant (0 of 1,004 isolates tested; prevalence, 0.00%; 95% confidence interval, 0.00 to 0.37%). These results are consistent with data from a similar survey conducted in the United Kingdom at a time when topical acyclovir had been available OTC for 5 years and topical penciclovir had been available as a prescription medicine for 2 years (1). One resistant HSV-1 isolate which was cross resistant to acyclovir and penciclovir was identified (1 of 924 isolates tested for susceptibility to acyclovir; 1 of 915 isolates tested for susceptibility to penciclovir; prevalence, 0.1%) (1). Other surveys with immunocompetent populations, predominantly, patients with genital herpes, have reported a prevalence of HSV resistance of 0.1 to 0.7% (2, 3; Reyes et al., Antivir. Res. **37:**A44 [abstract], 1998). The historical prevalence of acyclovir-resistant HSV isolates among untreated, immunocompetent patients, as measured by the plaque reduction assay, is 0.3% (6). Resistant HSV is more common in immunocompromised patients; for example, in a surveillance study by Reyes et al. (Reyes et al., Antivir. Res. **37:**A44 [abstract], 1998), 4 of 62 (6.5%) isolates from HIV-infected patients were resistant to acyclovir.

IC_{50} data from the present survey were analyzed to investigate differences in the susceptibilities of isolates from the study subjects to acyclovir and penciclovir on the basis of their reported use of antiviral agents, immunocompetence, or history of RHL (Table 1). For isolates from subjects who reported use

of antiviral medication to treat herpesvirus infections, the median IC_{50} was similar to the median IC_{50} for isolates from subjects who had never used antiviral medication both for acyclovir ($P = 0.211$) and for penciclovir ($P = 0.649$). For the small number of isolates ($n = 49$) from subjects who used oral acyclovir, famciclovir, or valacyclovir at the time of the study, the median IC_{50} of acyclovir (0.31 μg/ml) was significantly lower than that for isolates from subjects who were not using antiviral medication (0.43 μg/ml) ($P = 0.025$). This difference is not clinically important and may simply have arisen by chance; the parallel comparison for penciclovir showed no significant difference ($P = 0.488$). No other comparisons reached statistical significance, including that for immune status, although the number of subjects in this survey who may have been immunocompromised was low (Table 1).

In conclusion, the prevalence of resistant HSV-1 strains among isolates from the general population with RHL is low, consistent with earlier surveys with immunocompetent patients with HSV infection, despite the increasing rate of use of herpesvirus-specific antiviral agents over the past two decades.

We thank S. Khan and S. Modin for contributions to the study.

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