Acyclovir Susceptibilities of Herpes Simplex Virus Strains Isolated from Solid Organ Transplant Recipients after Acyclovir or Ganciclovir Prophylaxis

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We determined the acyclovir (ACV) susceptibilities of herpes simplex virus (HSV) isolates ($n = 18$) recovered from solid organ transplant patients after antiviral prophylaxis with ACV or ganciclovir. All isolates tested were susceptible to ACV (50% inhibitory concentration, $<1 \mu\text{M}$). A clinical review of patients with HSV disease showed that all improved with specific anti-HSV therapy, and no recurrences were reported.

Recurrent infections with herpes simplex virus (HSV) are a major cause of morbidity in patients with bone marrow transplants (BMTs), solid organ transplants (SOTs), and AIDS (7, 8, 15, 20). Acyclovir (ACV), an acyclic guanosine derivative with excellent in vitro and in vivo activities against HSV, has been shown to reduce the risk of HSV infections and HSV-related diseases in BMT and renal transplant recipients (13, 16, 18, 20). In these studies, the administration of ACV (intravenously [i.v.] or orally) for 3 to 12 weeks posttransplantation significantly reduced the incidence of HSV infections from 50 to 80% to less than 10%. Despite its poor in vitro activity against cytomegalovirus (CMV), ACV has been successfully used to prevent CMV infection in kidney transplant and BMT recipients (2, 12). A potential risk associated with the prolonged use of ACV is the emergence of resistant HSV strains; resistant isolates are increasingly being found in persons with AIDS and less commonly in other immunocompromised patients (3, 10, 14, 15). Such infections could lead to progressive disease and death (7, 10, 15). The effect that prolonged exposure to ACV could have in selecting resistant HSV isolates in patients with a lesser degree of immunosuppression (such as transplant recipients) is not well defined. Although a few studies have examined HSV susceptibilities in BMT patients receiving ACV prophylaxis (5, 17), systematic monitoring of ACV susceptibilities has not been done in SOT patients who excrete HSV during or after antiviral prophylaxis. In a large prophylactic trial of ACV for CMV infection conducted at our institution, 2 (3.8%) of 53 kidney transplant recipients randomized to receive ACV developed mucocutaneous HSV infections (2), but the ACV susceptibilities of those HSV isolates were not determined. In the present study, we analyzed the susceptibilities to ACV of HSV isolates from SOT patients with active HSV infections after prophylaxis with ACV or ganciclovir (GCV).

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Frozen HSV isolates recovered from SOT patients who had received CMV prophylaxis with ACV or GCV at our institution were used in the ACV susceptibility studies.

Initial viral cultures were performed only for isolates from patients with lesions suggestive of HSV infection or at the physician's request. ACV susceptibilities were determined by using a commercially available DNA-DNA hybridization assay (HSV Antiviral Susceptibility Test Kit; Diagnostic Hybrids Inc., Athens, Ohio) (19). Different concentrations of ACV (0, 1, 2, and 4 μM) tested in triplicate were used for rapid screening of ACV-resistant HSV. Results were analyzed by using a nonlinear regression analysis program (Systat; Systat Inc., Evanston, Ill.) to determine the 50% inhibitory concentration (IC_{50}) for the isolate. This system has previously been shown to readily detect ACV-resistant HSV isolates ($\text{IC}_{50} \geq 9 \mu\text{M}$) in our laboratory (6). An ACV-susceptible HSV strain (KOS) was used as a control in each assay.

Eighteen HSV isolates recovered from 14 SOT recipients (6 kidney, 4 kidney-pancreas, 2 pancreas, and 2 liver recipients) were studied. HSV strains were isolated from bronchoalveolar lavage fluids ($n = 4$), throat swab or sputum samples ($n = 8$), esophageal or duodenal biopsy specimens ($n = 2$), genital or anal swabs ($n = 2$), urine ($n = 1$), and an eye swab ($n = 1$). Six HSV isolates were recovered from 3 patients after ACV prophylaxis (mean length of prophylaxis, 74.7 days; range, 42 to 140 days), and 12 isolates were recovered from 11 patients after GCV prophylaxis (mean length of prophylaxis, 8.6 days; range, 7 to 13 days). HSV strains were isolated a mean of 215 days (range, 82 to 310 days) after transplantation from patients who received ACV prophylaxis and a mean of 86 days (range, 27 to 194 days) after transplantation for those who received GCV prophylaxis (Table 1). Eight patients had received additional antiviral therapy (other than primary prophylaxis) before HSV was isolated, as indicated in Table 2. The ACV susceptibilities of 14 isolates were determined. All isolates were susceptible to ACV (IC_{50} s, less than 1 μM). Eleven isolates were identified as HSV type 1, and three isolates were identified as HSV type 2. The remaining four isolates were not tested because they were obtained from the same patient at closely spaced intervals (three isolates) or failed to regrow in culture (one isolate).

Clinical data for these 14 patients are summarized in Table 2. Six patients were asymptomatic, whereas eight patients had possible or definitive clinical evidence of HSV disease (two with mucocutaneous infections, one with keratitis, one

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TABLE 1. SOT patients excreting HSV by form of CMV prophylaxis^a

Prophylaxis	No. of patients	No. of HSV isolates	Mean time (days [range]) for isolation after Tx	Mean duration (days [range]) of Px
ACV	3	6	215 (82–310)	74.7 (42–140)
GCV	11	12	86 (27–194)	8.6 (7–13)

^a Tx, transplantation; Px, prophylaxis.

with esophagitis, and four with possible pneumonitis [culture-positive bronchoalveolar lavage fluid]). Three of the last four patients also had evidence of pulmonary CMV infection and were treated i.v. with GCV. None of the patients developed active HSV infections during prophylaxis. All patients with HSV-related disease responded clinically to antiviral therapy, and no recurrences were reported, but sequential HSV cultures were not always performed.

Infections with HSV isolates that are resistant to ACV have been described in persons with AIDS (7, 9). These infections are characterized by chronic, large ulcerative mucocutaneous lesions that do not improve during i.v. ACV therapy and tend to recur (7). Infections with ACV-resistant HSV have also been reported among other groups of immunocompromised patients receiving multiple courses of ACV therapy (4–6, 10, 14). In a retrospective study performed at our institution, 7 (4.7%) of 148 immunocompromised patients treated with ACV were infected with ACV-resistant HSV (6). In all seven patients, these infections were associated with clinical disease. The role of antiviral prophylaxis in selecting resistant viruses is controversial. Gold and Corey (8) reported that continuous low levels of ACV during chronic oral therapy could select resistant strains of HSV. In contrast, chronic antiviral prophylaxis could result in low or absent viral replication, thus decreasing the likelihood of mutations and the opportunity for development of resistance. A limited number of studies have monitored the

antiviral susceptibilities of HSV strains isolated from BMT patients receiving antiviral prophylaxis. Shepp et al. (17) used a quantitative plaque reduction assay to determine the in vitro susceptibilities of 20 HSV isolates from 19 BMT patients who shed HSV during a 4-week trial of ACV given i.v. One ACV-resistant HSV isolate (IC₅₀, 4.1 mg/liter, or 18.2 μM) was shed during the course of prophylaxis, and two other resistant isolates (IC₅₀s, 5.6 mg/liter [24.9 μM] and 14 mg/liter [62.2 μM]) were recovered from two placebo recipients during subsequent ACV therapy (17). In another trial comparing 5-week ACV prophylaxis with placebo in 49 BMT recipients (20), ACV-resistant HSV isolates were not found among 33 patients who developed active HSV infections. In contrast, a recent report by Ljungman et al. (10) described three BMT patients with fatal pneumonia caused by ACV-resistant, thymidine kinase-deficient HSV type 1. All three patients had previously received ACV prophylaxis (until day 30 after the transplant) and multiple courses of ACV therapy. Determination of the prevalence of HSV resistance during and after antiviral prophylaxis in SOT patients is important, because CMV prophylaxis with ACV and GCV is being provided more frequently (1, 2, 11). In the present study, we did not identify any ACV-resistant HSV strains among patients with active HSV infections following ACV or GCV prophylaxis. On the basis of our sample size, the 95% confidence interval for the rate of shedding of ACV-resistant HSV was 0 to 20%. Furthermore, all patients with possible or definitive HSV-related diseases improved following antiviral therapy, and no recurrences were reported. Although we did not perform surveillance cultures for HSV, the fact that we did not find ACV-resistant HSV is reassuring. The high-dose ACV regimen used after transplantation for CMV prophylaxis (2) and the relative lower degree of immunosuppression of these patients (compared with BMT or human immunodeficiency virus-infected patients) could explain why ACV-resistant HSV isolates were not recovered. In conclusion, HSV infections that occur in SOT recipients after antiviral prophylaxis are caused by ACV-susceptible viruses; consequently, these infections should be

TABLE 2. Clinical and laboratory data from SOT patients who received ACV or GCV for CMV prophylaxis and who shed HSV^a

Patient no.	Form of HSV infection	HSV isolates		Antiviral regimen (day) ^b	
		Source	Day post-Tx	Prophylaxis	Treatment
1 ^c	Lung	BAL	45	GCV (10)	GCV (7)
2	Lung	BAL	42	GCV (12)	ACV (6)
		Sputum ^d	36		
3 ^c	Lung	BAL	169	GCV (9)	GCV (28)
4 ^c	Lung	BAL ^d	67	GCV (7)	No Rx
5	Mucocutaneous	Skin	82	ACV (42)	No Rx
		Throat ^d	83		
6	Mucocutaneous	Genital	57	GCV (7)	No Rx
7	Esophagitis	Esophageal biopsy	252	ACV (42)	GCV (21)
		Duodenal biopsy ^d	252		
8	Keratitis	Eye	107	GCV (7)	GCV (12)
9	Asymptomatic	Throat	27	GCV (13)	No Rx
10	Asymptomatic	Sputum	27	GCV (7)	No Rx
11	Asymptomatic	Sputum	310	ACV (140)	GCV (14)
		Throat	306		
12	Asymptomatic	Throat	194	GCV (7)	GCV (9)
13	Asymptomatic	Urine	142	GCV (7)	GCV (7)
14	Asymptomatic	Saliva	37	GCV (7)	No Rx

^a Tx, transplantation; BAL, bronchoalveolar lavage fluid; Rx, treatment.

^b Regimen and day before HSV isolation.

^c CMV was isolated in culture from the same specimen.

^d Susceptibility to ACV was not tested.

treated conventionally with ACV. Yet, antiviral susceptibility studies of HSV isolates are recommended for infections that do not respond to standard antiviral therapy or that occur during antiviral prophylaxis.

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REFERENCES

- Baily, T. C., E. P. Trulock, N. A. Ettinger, G. A. Storch, J. D. Cooper, and W. G. Powderly. 1992. Failure of prophylactic ganciclovir to prevent CMV disease in recipients of lung transplants. *J. Infect. Dis.* **165**:548-552.
- Balfour, H. H., Jr., B. A. Chace, J. T. Stapleton, R. L. Simmons, and D. S. Fryd. 1989. A randomized, placebo-controlled trial of oral acyclovir for the prevention of CMV disease in recipients of renal allografts. *N. Engl. J. Med.* **320**:1381-1387.
- Chatis, P. A., and C. S. Crumpacker. 1991. Analysis of the thymidine kinase gene from clinically isolated acyclovir-resistant herpes simplex viruses. *Virology* **180**:793-797.
- Collins, P., B. A. Larder, N. M. Oliver, S. Kemp, I. W. Smith, and G. Darby. 1989. Characterization of a DNA polymerase mutant of herpes simplex virus from a severely immunocompromised patient receiving acyclovir. *J. Gen. Virol.* **70**:375-382.
- Collins, P., and N. M. Oliver. 1986. Sensitivity monitoring of herpes simplex virus isolates from patients receiving acyclovir. *J. Antimicrob. Chemother.* **18**(Suppl. B):103-112.
- Englund, J. A., M. E. Zimmerman, E. M. Swierkosz, J. L. Goodman, D. R. Scholl, and H. H. Balfour, Jr. 1990. Herpes simplex virus resistant to acyclovir. *Ann. Intern. Med.* **112**:416-422.
- Erlich, K. S., J. Mills, P. Chatis, G. J. Mertz, D. F. Busch, S. E. Follansbee, R. M. Grant, and C. S. Crumpacker. 1989. Acyclovir-resistant herpes simplex virus infections in patients with acquired immunodeficiency syndrome. *N. Engl. J. Med.* **320**:293-296.
- Gold, D., and L. Corey. 1987. Acyclovir prophylaxis for herpes simplex virus infection. *Antimicrob. Agents Chemother.* **31**:361-367.
- Hill, E. L., G. A. Hunter, and M. N. Ellis. 1991. In vitro and in vivo characterization of herpes simplex virus clinical isolates recovered from patients infected with human immunodeficiency virus. *Antimicrob. Agents Chemother.* **35**:2322-2328.
- Ljungman, P., M. N. Ellis, R. C. Hackman, D. H. Shepp, and J. D. Meyers. 1990. Acyclovir-resistant herpes simplex virus causing pneumonia after marrow transplantation. *J. Infect. Dis.* **162**:244-248.
- Merigan, T. C., D. G. Renlund, S. Keay, M. R. Bristow, V. Starnes, J. B. O'Connell, S. Rosta, D. Dunn, P. Gamberg, R. M. Ratkovec, W. E. Richenbacher, R. C. Millar, C. DuMond, B. DeAmond, V. Sullivan, T. Cheney, W. Buhles, and E. B. Stinson. 1992. A controlled trial of ganciclovir to prevent cytomegalovirus disease after heart transplantation. *N. Engl. J. Med.* **326**:1182-1186.
- Meyers, J. D., E. C. Reed, D. H. Shepp, M. Thornquist, P. S. Dandliker, C. A. Vicary, N. Flournoy, L. E. Kirk, J. H. Kersey, E. D. Thomas, and H. H. Balfour, Jr. 1988. Acyclovir for prevention of cytomegalovirus infection and disease after allogeneic marrow transplantation. *N. Engl. J. Med.* **318**:70-75.
- Pettersson, E., T. Hovi, J. Ahonen, A. P. Fiddian, K. Salmela, K. Hockerstedt, B. Eklund, E. V. Willebrand, and P. Hayry. 1985. Prophylactic oral acyclovir after renal transplantation. *Transplantation* **39**:279-281.
- Sacks, S. L., R. J. Wanklin, D. E. Reece, K. A. Hicks, K. L. Tyler, and D. M. Coen. 1989. Progressive esophagitis from acyclovir-resistant herpes simplex. *Ann. Intern. Med.* **111**:893-899.
- Safrin, S., T. Assaykeen, S. Follansbee, and J. Mills. 1990. Foscarnet therapy for acyclovir-resistant mucocutaneous herpes simplex virus infection in 26 AIDS patients: preliminary data. *J. Infect. Dis.* **161**:1078-1084.
- Seale, L., C. J. Jones, S. Kathpalia, G. G. Jackson, M. Mazes, M. S. Maddux, and D. Packham. 1985. Prevention of herpes virus infections in renal allograft recipients by low dose oral acyclovir. *JAMA* **254**:3435-3438.
- Shepp, D. H., P. S. Dandliker, N. Flournoy, and J. D. Meyers. 1985. Once daily intravenous acyclovir for prophylaxis of herpes simplex virus reactivation after marrow transplantation. *J. Antimicrob. Chemother.* **16**:389-395.
- Stoffel, M., J. P. Squifflet, Y. Pirson, M. Lamy, and G. P. J. Alexandre. 1987. Effectiveness of oral acyclovir prophylaxis in renal transplant recipients. *Transplant Proc.* **19**:2190-2193.
- Swierkosz, E. M., D. R. Scholl, J. L. Brown, J. D. Jollick, and C. A. Gleaves. 1987. Improved DNA hybridization method for detection of acyclovir-resistant herpes simplex virus. *Antimicrob. Agents Chemother.* **31**:1465-1469.
- Wade, J. C., B. Newton, N. Flournoy, and J. D. Meyers. 1984. Oral acyclovir for prevention of herpes simplex virus reactivation after marrow transplantation. *Ann. Intern. Med.* **100**:823-828.