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Comparison of Efficacies of Famciclovir and Valaciclovir against Herpes Simplex Virus Type 1 in a Murine Immunosuppression Model

HUGH J. FIELD,[1]* DEEPANKER TEWARI,[1] DAVID SUTTON,[2] AND ALANA M. THACKRAY[1]

Centre for Veterinary Science, Cambridge University Veterinary School, Cambridge CB3 OES,[1] *and SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey RH3 7AJ,*[2] *United Kingdom*

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A mouse model of herpes simplex virus type 1 infection in an immunocompromised host was established by using cyclosporin-A to impair T-cell function. Following inoculation of herpes simplex virus type 1 into the skin of the ear pinna, cyclosporin-A prolonged virus replication in the skin and neural tissues compared with that in immunocompetent mice. This model was used to investigate the activity of famciclovir (FCV) and valaciclovir (VACV), which are oral prodrugs of the antiherpesvirus agents penciclovir and acyclovir, respectively. Both prodrugs gave similar blood profiles of the antiherpesvirus agents in normal and cyclosporin-treated mice. The compounds were administered by the oral route at 50 mg/kg per dose twice daily for 5 days. Both compounds were very effective at clearing infectious virus from the tissues despite the immunosuppression; FCV-treated animals cleared virus from the ear pinna more rapidly than VACV-treated animals. The areas under the concentration-time curve (AUC) for virus replication with time were reduced to 50 and 30% of control values for ear pinna and brain stem, respectively, with VACV therapy and to <5% in both tissues by FCV. When treatment was continued to day 10, the reductions in AUC for ear and brain stem, respectively, were to 33 and 26% of control values with VACV and to <3 and <5% with FCV. However, on cessation of the antiviral treatment, there was a reproducible recurrence of infectious virus in the tissues obtained from VACV-treated mice. The recurrence of infectious virus was also evident after 10 days of treatment with VACV. In mice which had received FCV for 10 or 5 days, there was no resumption of virus replication in the ear pinna or brain stem. When dosing was reduced to once per day, both compounds were less effective at controlling the infection. Nevertheless, no recurrence of infectious virus was observed on cessation of FCV therapy.

For many years, acyclovir (ACV) has been the treatment of choice for various herpes simplex virus (HSV) syndromes (25). However, ACV has poor oral bioavailability, and there is much interest in valaciclovir (VACV), an oral prodrug of ACV (2, 30). Famciclovir (FCV) (26, 29), the oral prodrug of the antiherpes agent penciclovir (PCV) (1, 4, 22), is licensed for the treatment of herpes zoster in immunocompetent patients. Following oral administration to humans, FCV is converted to PCV with an absolute oral bioavailability of 77% (20). The corresponding value for ACV from VACV is 54% (30). The high bioavailability of PCV or ACV from their oral forms offers new opportunities for tackling the more serious manifestations of herpes simplex virus, including infections in the immunocompromised patient (5).

The immunocompromised patient is particularly prone to serious complications resulting from infection by herpesvirus, including cytomegalovirus, varicella-zoster virus, and HSV (15, 17, 18, 24). One problem encountered in treating these infections is the development of drug resistance (8). Several animal models have been employed in order to study the development of resistance during chemotherapy (9), and it has been suggested that better suppression of infection could lead to less resistance (12).

In the present study we have induced immunosuppression in a mouse infection model by means of regular injections of cyclosporin-A (CyA). CyA has been shown to suppress reversibly both cell-mediated and antibody-mediated responses (6). However, its mechanism of action appears to be directed mainly to T cells, in which the important effect appears to be inhibition of the synthesis of interleukin-2. It may also inhibit the expression of interleukin-2 receptors on the T cells that respond to interleukin-2 (16). We report here that when HSV type 1 (HSV-1) was inoculated into mice receiving CyA, the acute phase of virus replication was prolonged. This model was used to evaluate the oral efficacy of FCV and VACV against clinical disease and virus replication in tissue local to the inoculation site and in the central nervous system.

MATERIALS AND METHODS

Virus strain and tissue culture. The virus used in this study was HSV-1 strain SC16. This strain of virus has been extensively characterized in mice (14) and has been widely used previously for studying antiviral compounds (10).

Mice and virus inoculation. BALB/c female mice (Bantin & Kingman, Kingston, Hull, United Kingdom) were purchased at 3 to 4 weeks old and inoculated 1 week later. Virus suspension (20 μl) containing 10^5 PFU was inoculated into the skin of the left ear pinna. Skin thickness was measured daily in individual mice by means of an engineers' micrometer screw gauge. These methods have been described in detail previously (19).

Titration of virus in tissue samples. Tissue samples (ear pinna or brain stem) were obtained not less than 6 h after the last dose of FCV or VACV. Three mice were sampled on each occasion, and their tissues were tested independently by previously published methods (10) with modifications as follows. Tissues were minced with scissors and ground in 1 ml of Eagle's minimal essential medium (EMEM) in glass homogenizers. The samples were subjected to 1 min of ultrasonic vibration in an ice bath. Debris was removed by low-speed centrifugation, and the supernatant was diluted 10- and 100-fold in EMEM. Then, 0.2-ml samples of the supernatant were inoculated onto BHK-21 cell monolayers and virus plaques were counted after 48 h of incubation. All samples were tested and read blind. Area under the concentration-time curve values (AUC) which are quoted in the text were calculated from virus titer-time curves for both ear pinna and brain stem virus titration data.

* Corresponding author.

Induction of immunosuppression. CyA was obtained at 50 mg/ml in polyethoxylated castor oil (Sandimmun; Sandoz Pharmaceuticals, Camberley, Surrey, United Kingdom). The drug was administered at a dose of 50 mg/kg by subcutaneous (s.c.) injection on alternate days.

Antiviral compounds and treatment regimen. FCV and VACV were synthesized at the laboratory of SmithKline Beecham following published methods (2, 13). The activities of ACV and PCV, which are the active metabolites of VACV and FCV, respectively, were measured by means of a plaque reduction assay in BHK-21 cells and BALB/c 3T3 cells (11). FCV and VACV were dissolved in double-distilled deionized water and administered orally by gavage to mice in 0.1 ml of a 10-mg/ml solution to give a dose of 50 mg/kg. Therapy was initiated 22 h postinfection (p.i.) and thereafter at 6 p.m. and 8 a.m. each day until the evening of the 5th or 10th day p.i.

Experimental design. A group of 50 control mice (group a) and groups of 30 mice each (groups b to g) were infected with HSV-1 as previously described and treated from 22 h p.i. as follows: group a, no chemotherapy; group b, VACV, 50 mg/kg twice daily to day 5.5 p.i.; group c, VACV, 50 mg/kg twice daily to day 10.5 p.i.; group d, FCV, 50 mg/kg twice daily to day 5.5 p.i.; group e, FCV, 50 mg/kg twice daily to day 10.5 p.i.; group f, VACV, 50 mg/kg once daily to day 5.5 p.i.; and group g, FCV, 50 mg/kg once daily to day 5.5 p.i.

CyA was administered s.c. to all mice from 2 days before virus inoculation and again on days 0, 2, 4, 6, 8, and 10 p.i. All mice were examined daily for clinical signs, and the ear pinnae were measured daily for swelling. Groups of three mice were killed on days 1, 3, 5, 6, 7, 9, 12, and 15. The ear pinnae and brain stems were titrated individually for infectious virus.

Lymphocyte stimulation assay. Lymphocyte suspensions prepared from pooled spleens of groups of three mice were stimulated with mitogens as described previously (23), except that the concentrations of mitogens employed were 1 μg of conconavalin A per ml, 10 μg of *Escherichia coli* lipopolysaccharide per ml, and 1 μg of *Staphylococcus aureus* enterotoxin B per ml. All three mitogens were obtained from Sigma Co., St. Louis, Mo. Stimulated cells were labelled after 48 h of incubation and harvested 18 h later. The stimulation indices were calculated by dividing counts per minute (cpm) from the stimulated cell populations by the cpm from unstimulated cell populations, and the final result was expressed as the percent control value obtained from normal mice.

Immunofluorescence staining and lymphocyte population analysis. The spleen cells obtained as described above were analyzed for subpopulations by means of fluorescence-activated cell scanning with fluorescein isothiocyanate- or RD1-conjugated monoclonal antibodies by direct staining. Cells (10^6) were stained with fluorescein isothiocyanate-anti-Thy 1.2, anti-B220, anti-CD8, and RD1-anti-CD4 (Coulter Immunology), in single-color labelling assays. After labelling, cells were washed thrice with phosphate-buffered saline containing 1% fetal calf serum and 0.1% sodium azide. Cells were fixed in 1% paraformaldehyde and counted. Data were analyzed with "Consort 30" software on FACScan (Becton Dickinson). The results were expressed as percent total positive cells.

Bioavailability in mice. Compounds were administered as a single oral dose at 50 mg/kg. After drug administration, three mice were bled by cardiac puncture under terminal anaesthesia at 15, 60, and 180 min and approximately 8 h. Blood samples (0.2 ml) from each mouse were pooled and treated with an equal volume of 16% (wt/vol) trichloroacetic acid. Following centrifugation to remove blood proteins, the supernatants were treated with 1/5 volume of saturated sodium hydrogen carbonate solution followed by an equal volume of 400 mM ammonium acetate (pH 6.0). Samples were then assayed by high-performance liquid chromatography by a method similar to that previously described (29). The lower limit of detection for both PCV and ACV was 0.15 μg/ml. The AUCs from time zero to 8 h were calculated from blood concentration-time profiles by using the trapezoidal rule.

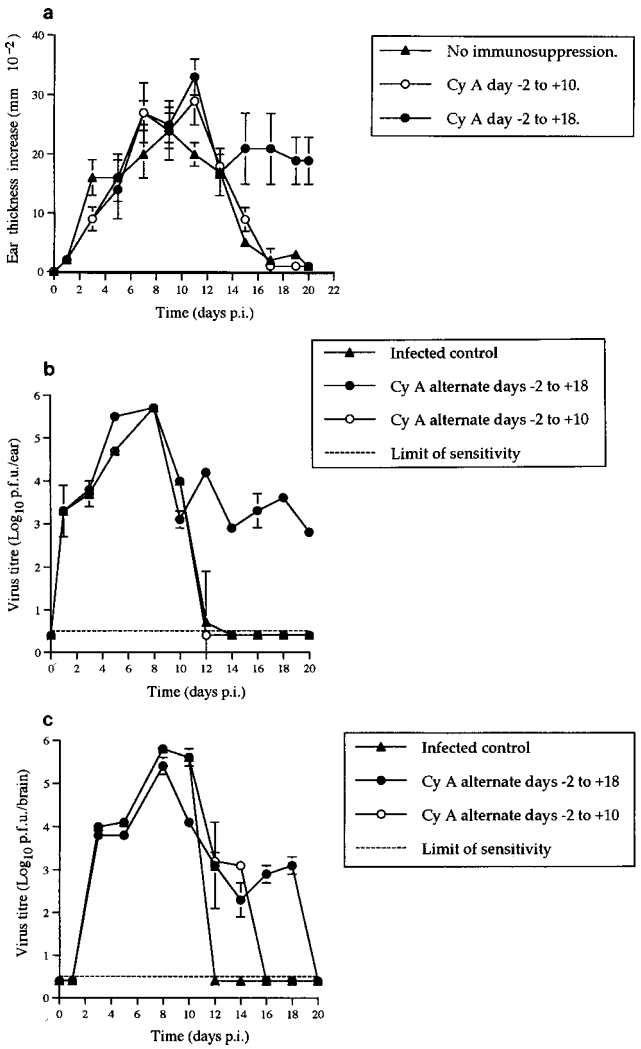

FIG. 1. Effect of CyA on HSV infection in the mouse ear. Mice were treated on alternate days with CyA (50 mg/kg/day) s.c. from 2 days before virus inoculation until 10 or 18 days p.i. All mice were inoculated into the skin of the ear pinna with 10^5 PFU HSV-1. Vertical bars represent standard deviations; error bars have been omitted from those points where the error is very small. (a) Virus-induced inflammation in the ear pinnae measured by means of ear thickness; eight mice were measured at each time point. (b) Titers of infectious virus in the ear; three mice were tested at each time point. (c) Titers of infectious virus in the brain stem; three mice were tested at each time point.

RESULTS

Characterization of the model. Eight days after commencing CyA dosing on alternate days, splenic lymphocytes were collected and analyzed by fluorescence-activated cell scanning. The results indicated that in mice without immunosuppression, B cells and T cells formed 27 and 30% of the total lymphocyte population, respectively, with 16% CD4 cells and 6% CD8 cells. No change occurred in the relative proportions of B and T cells, nor were there changes in the proportions of CD4- or CD8-bearing subpopulations of T cells following administration of CyA for 8 days. The figures were 25, 28, 15, and 5% for B, T, CD4, and CD8 cells, respectively.

Spleen cells were also tested by means of a lymphocyte stimulation assay. There was a significant reduction in the responses to the two T-cell mitogens, conconavalin A and *S. aureus* enterotoxin B, to approximately 60% control (data not shown). However, there was no reduction in the response to lipopolysaccharide, a B-cell mitogen. Infection by inoculation of HSV into the ear produced a small rise in stimulation (104%).

To confirm that antiviral chemotherapy did not interfere with T-cell immunosuppression, lymphocytes from mice that received either virus alone, CyA alone, or CyA plus antiviral therapy were tested for responsiveness to mitogens. However, the administration of either VACV or FCV twice per day for 5 days had no effect on the extent of the reduction in the T-cell response to mitogen.

The induction of immunosuppression had no measurable effect on the extent of clinical signs (e.g., ear thickness; Fig. 1a) or virus replication either in the skin of the ear (Fig. 1b) or in the brain (Fig. 1c) during the first 10 days of the infection. However, while infectious virus was cleared from ear and brain stem by days 12 and 16 p.i., respectively, in mice receiving CyA up to day 10, with continued injections of CyA on alternate

TABLE 1. Oral bioavailability of FCV and VACV in mice with or without CyA-induced immunosuppression

Therapy[a]	PCV or ACV AUC (0–8 h) following FCV or VACV administration, respectively	
	First dose	Fifth dose
FCV and CyA	13	10
VACV and CyA	12	8
FCV alone	12	7
VACV alone	13	8

[a] Compounds were administered orally at 50 mg/kg twice daily from day 1 to day 5. CyA was administered subcutaneously at 50 mg/kg on alternate days from 2 days before virus infection.

days until day 18, virus replication persisted in both sites. It was notable that ear swelling declined rapidly when CyA treatment was discontinued at day 10, and this was consistent with the rapid clearance of infectious virus from the ear pinna of these mice.

Bioavailability in mice. Following oral administration of a single 50-mg/kg dose of FCV or VACV to normal mice, the blood concentration-time curves for PCV and ACV in either normal or CyA-treated mice were almost identical, leading to very similar AUCs (Table 1). Comparison of these data with those obtained following 5 days of dosing showed that there was no significant alteration in PCV or ACV blood levels in either normal or CyA-treated mice (Table 1). No residual PCV or ACV (<0.2 μg/ml) was detected in blood from 8 h onwards after the last dose (data not shown). On the basis of this observation together with the 50% effective doses of PCV and ACV in BHK-21 cells and the initial sample dilution, any residual drug within tissues would not interfere with virus quantitation.

Antiviral activity in cell culture. PCV and ACV had similar activities against HSV-1 strain SC16 in BALB/c 3T3 cells; the 50% effective doses (± standard deviation) were 0.02 ± 0.01 and 0.01 ± 0.01 μg/ml, respectively. In BHK-21 cells, the cell line used to quantify virus in tissue samples, PCV was less active than ACV (0.2 ± 0.1 and 0.03 ± 0.01 μg/ml, respectively).

Effect of antiviral therapy on HSV-1 infection in CyA-treated mice. The murine immunosuppression model was employed to compare the effects of the two antiviral compounds, FCV and VACV, on the course of HSV infection. Oral therapy commenced 22 h following virus inoculation with the last dose given on either day 5.5 or day 10.5. Both compounds markedly

TABLE 2. Clinical signs in immunosuppressed mice with or without oral antiviral therapy[a]

Clinical signs (n = 21)	% showing sign with antiviral therapy[b]		
	None	FCV	VACV
Erythema	100	24	38
Ear paralysis	62	0	0
Ear lesions[c]	67	0	0
Other neurological signs	33	0	10
Death	52	0	0

[a] Mice were infected in the left ear pinna with HSV-1 (10^5 PFU per animal).
[b] Compounds were administered orally at 50 mg/kg twice daily from day 1 to day 5. CyA was administered subcutaneously at 50 mg/kg on alternate days from 2 days before virus infection.
[c] Ear lesions are defined as one or more vesicles (visible to the naked eye), blisters, crusts, or scabs. This does not include erythema or swelling.

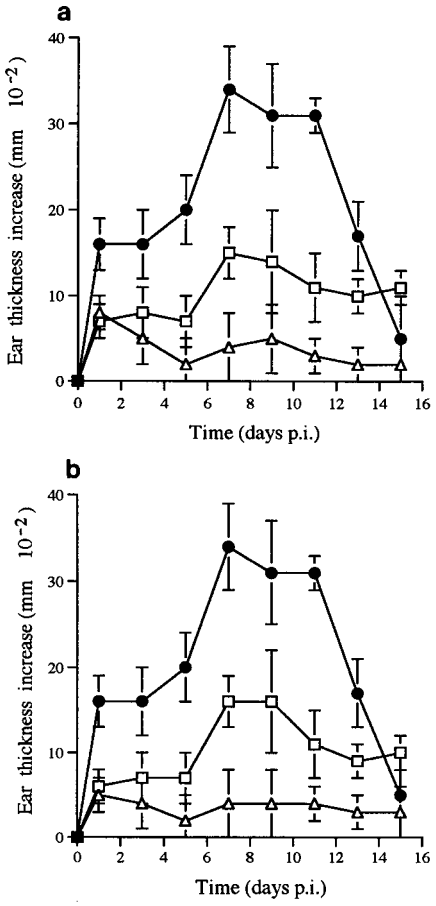

FIG. 2. Effect of antiviral therapy on HSV infection in mice immunosuppressed with CyA. Mice were inoculated into the skin of the ear pinna with 10^5 PFU of HSV-1. CyA was given at 50 mg/kg s.c. on alternate days starting 2 days before infection and continuing for 10 days p.i. FCV or VACV was given by the oral route at 50 mg/kg twice per day starting at 22 h p.i. for the number of days indicated below. Ear thickness was measured in groups of eight mice. Vertical bars indicate standard deviations. (a) Ear thickness; antiviral therapy continued until 5.5 days p.i. (b) Ear thickness; antiviral therapy continued until 10.5 days p.i. Symbols: ●, infected control; △, FCV; □, VACV.

reduced clinical signs (Table 2) and also the ear swelling response to virus infection (Fig. 2a and 2b).

Both treatments reduced virus replication in the ear (Fig. 3a) and brain stem (Fig. 3b) during the 5-day treatment period. FCV was more effective than VACV in limiting HSV infection in the ear (Fig. 3a). The AUC for FCV (<5% control) was less than that observed for VACV (50 and 30% of control for ear and brainstem, respectively) and titers were significantly lower on several individual days. Highly significant differences between either treatment and the untreated controls were also observed at multiple time points. By day 6, no infectious virus was detected in any of the groups receiving VACV or FCV. However, infectious virus reappeared in the brains and ears of all mice which had been treated with VACV. This rebound was not observed in mice treated with FCV.

Other groups of mice were treated continuously for 10 days. Ear pinna and brain stem HSV titers were consistently and substantially lower in mice receiving FCV compared with those receiving VACV (Fig. 3c and 3d), with the AUC reduced to <5% of control compared with 33 and 26% for ear and brain stem tissues, respectively, from mice treated with VACV. By day 6, no virus was detected in tissues from mice treated with

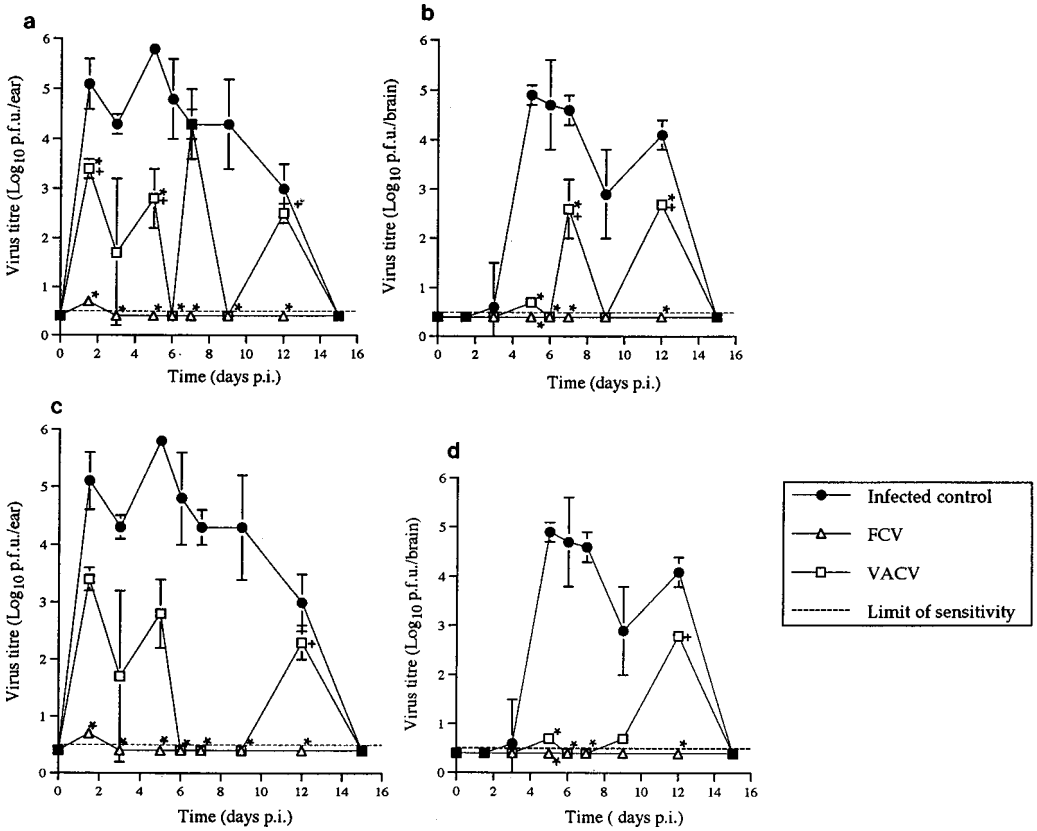

FIG. 3. Effect of antiviral therapy (twice daily) on HSV infection in mice immunosuppressed with CyA. Mice were inoculated into the skin of the ear pinna with 10^5 PFU of HSV-1. CyA was given at 50 mg/kg s.c. on alternate days starting 2 days before infection and continuing for 10 days p.i. FCV and VACV were given by the oral route at 50 mg/kg twice per day starting at 22 h p.i. and continued for the number of days indicated below; three mice were tested at each time point. Vertical bars indicate standard deviations; error bars have been omitted from those points where the error is very small. Differences in virus titers at individual time points were tested by the Student's *t* test (for unpaired data, two-tailed); points that differ significantly from the control ($P < 0.01$) are marked by a star (*). Where the titer in VACV-treated mice is significantly different from FCV-treated mice ($P < 0.01$) the points are marked with a cross (+). (a) Titers of infectious virus in the ear; antiviral therapy continued until 5.5 days p.i. (b) Titers of infectious virus in the brain stem; antiviral therapy continued until 5.5 days p.i. (c) Titers of infectious virus in the ear; antiviral therapy continued until 10.5 days p.i. (d) Titers of infectious virus in the brain stem; antiviral therapy continued until 10.5 days p.i.

either compound. When treatment with VACV was discontinued on day 10 p.i., however, 2.5 and 3.0 $\log_{10}$ PFU were detected in ear tissues (Fig. 3c) and brain tissues (Fig. 3d), respectively, on day 12 p.i. Again, following FCV treatment, no virus was detected.

In the same experiment, two groups of mice were treated with VACV or FCV at the same dose but once per day only. In these groups, the reductions in ear swelling (Fig. 4a) and virus titers in skin and the central nervous system were less marked compared with control mice (Fig. 4b and 4c) than in animals treated twice daily. The AUC was reduced to 43 and 24% of control in ear and brain stem, respectively, by VACV therapy and to 29 and 6% of control, respectively, in the same tissues by FCV. However, it was notable that the titers of virus continued to fall in mice that had been treated once per day with FCV when treatment was discontinued after 5 days and there was no delayed recurrence of infectious virus in either site during the period of observation (to day 15 p.i.). By contrast, recurrence of infectious virus was observed in mice treated once per day with VACV (Fig. 4c).

DISCUSSION

The effects of CyA have been reported to be highly specific for T-cell function (16). This was confirmed in the present study. The drug is not thought to be cytotoxic and, as expected, the relative proportions of T and B cells were unaffected. There was a small increase in the lymphocyte response to stimulation with lipopolysaccharide, which is known to be a B-cell mitogen, and a marked reduction in the response to two different mitogens which are T-cell specific. Consistent with the effect on T-cell function, mice given CyA showed persistent virus replication and ear swelling. It was notable that neither virus replication nor ear swelling was markedly increased during the first few days of the infection and virus was readily cleared from the tissues as soon as the immunosuppressive treatment was withheld. This is entirely consistent with previous reports (6, 16), which suggest that the drug is not anti-inflammatory and that its effects are quickly reversed. The coadministration of FCV did not affect the immunosuppressive effect of CyA. In addition, CyA treatment did not alter the absorption or metabolism of either FCV or VACV. The plasma pharmacokinetics of PCV and ACV from FCV and VACV, respectively, were similar to each other and not altered by the dosing period or by immunosuppression by CyA. CyA has been reported to be nephrotoxic under some circumstances (3), but this did not appear to be a factor in the present experiment.

Both compounds were remarkably effective in preventing the development of clinical signs and clearing virus from the tissues, including the central nervous system. Another interest-

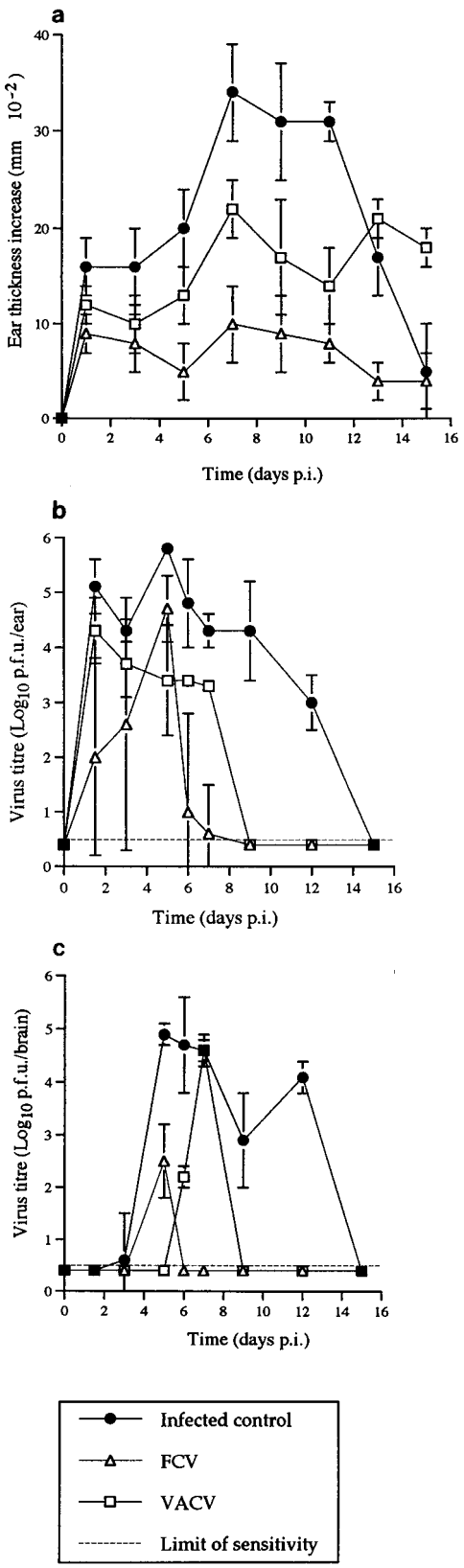

FIG. 4. Effect of antiviral therapy (once daily) on HSV infection in mice immunosuppressed with CyA. Mice were inoculated into the skin of the ear pinna with 10^5 PFU of HSV-1. CyA was given at 50 mg/kg s.c. on alternate days starting 2 days before infection and continuing for 10 days p.i. FCV and VACV were given by the oral route at 50 mg/kg once a day from 22 h p.i. until day 5.5 p.i. Ear thickness increase was measured in groups of eight mice. Vertical bars indicate standard deviations; error bars have been omitted from those points where the error is very small. (a) Virus-induced inflammation in the ear pinnae measured by means of ear thickness. (b) Titers of infectious virus in the ear; three mice were tested at each time point. (c) Titers of infectious virus in the brain stem; three mice were tested at each time point.

ing finding was the recurrence of infection in mice on cessation of VACV therapy. This occurred even in mice which had been treated with VACV for 10 days. It is possible that these two key observations are interrelated in some way. Overall, FCV appeared to be the more effective compound in the model, despite there being little difference between ACV and PCV in terms of inhibitory activity in murine cells, measured by plaque reduction assay. However, it was notable that, although reduction of the dose schedule of FCV to once per day caused a decrease in efficacy, when FCV therapy was discontinued after 5 days, virus replication rapidly declined to undetectable levels and did not recur.

No evidence was observed for the selection of resistant virus mutants during the course of the experiment up to day 16 p.i. Thus, virus isolates obtained from the samples which yielded virus after rebound were tested and found to be equally sensitive to both drugs in comparison with the inoculum. While the development of resistant HSV is known to be associated with chemotherapy in the immunocompromised host, this does not appear to be a factor in the present experiments.

It is of interest to compare the results of the present experiment with results published previously with the same infection model although without immunosuppression. Field et al. (10) reported a recurrence of virus growth in the ears of mice 5 days after the onset of once-daily i.p. therapy with acycloguanosine, now known as ACV. It was reported that the recurrence of infection was reproducible and, while the generation of resistance was ruled out, no other explanation was forthcoming. In addition, Sutton and Boyd (21) have reported that PCV therapy, but not ACV therapy, led to prolonged suppression of virus replication on cessation of therapy. These data were obtained in an intraperitoneal HSV-1 infection model in DBA-2 mice. Furthermore, the observation of virus recurrence of infectious virus made with VACV in the present study has now been confirmed in several additional experiments both with and without immunosuppression. We have no explanation at present, but in individual mice there have been several occasions on which virus has been detected in the brain whereas ear tissue remained negative. This suggests that the site for virus reactivation or persistence is most likely in neural tissue, and this possibility is now the subject of further investigation. However, the results obtained in the present experiment suggest that, while PCV and ACV share a broadly similar mechanism of action (27) (for example, both are phosphorylated by HSV thymidine kinase and in both cases the nucleoside triphosphate interferes with HSV DNA polymerase), the two compounds clearly behave differently as shown by the manner in which they inhibit virus replication in the present murine model. These results appear to demonstrate a key difference between FCV and VACV in vivo which cannot be explained by the plasma pharmacokinetic properties or in vitro potency of the two compounds. However, the intracellular pharmacokinetics may be of critical importance. For example, it has been reported that PCV-triphosphate is much more stable than ACV-triphosphate within herpesvirus-infected cells (7, 28). The half-life of PCV-triphosphate is reported to be 10 to 20 h in MRC-5 cells, whereas the equivalent value for ACV-triphosphate is approximately 1 h (27). The implications

of this difference in half-life for the effects of the two compounds on HSV replication in vivo have yet to be explored fully, and this may be an important factor in the present study.

In conclusion, these results demonstrate that FCV and VACV affect the virologic course of HSV infection differently in this murine model of immunosuppression. Further studies are needed to understand the reasons for these differences.

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REFERENCES

1. **Bacon, T. H., and R. F. Schinazi.** 1993. An overview of the further evaluation of penciclovir against herpes simplex virus and varicella-zoster virus in cell culture highlighting contrasts with acyclovir. Antivir. Chem. Chemother. **4**(Suppl. 1)**:**25–36.
2. **Beauchamp, L. M., G. F. Orr, P. de Miranda, T. Burnette, H. J. Schaeffer, and T. A. Krenitsky.** 1992. Amino acid ester prodrugs of acyclovir. Antivir. Chem. Chemother. **3:**157–164.
3. **Bennett, W. M., and J. P. Pulliman.** 1983. Cyclosporine nephrotoxicity. Ann. Intern. Med. **99:**851–854.
4. **Boyd, M. R., S. Safrin, and E. R. Kern.** 1993. Penciclovir: a review of its spectrum of activity, selectivity, and cross-resistance pattern. Antivir. Chem. Chemother. **4**(Suppl. 1)**:**3–11.
5. **Carrington, D.** 1994. Prospects for improved efficacy with antiviral prodrugs: will valaciclovir and famciclovir meet the clinical challenge? Int. Antivir. News **2:**50–52.
6. **Dale, M. M., and J. C. Foreman (ed.).** 1989. Textbook of immunopharmacology, 2nd ed. Blackwell Scientific, Oxford.
7. **Earnshaw, D. C., T. H. Bacon, S. J. Darlison, K. Edmonds, R. M. Perkins, and R. A. Vere Hodge.** 1992. Mode of antiviral action of penciclovir in MRC-5 cells infected with herpes simplex virus type 1 (HSV-1), HSV-2, and varicella-zoster virus. Antimicrob. Agents Chemother. **36:**2747–2757.
8. **Ellis, M. N., J. L. Martin, D. C. Lobe, J. D. Johnsrude, and D. W. Barry.** 1986. Induction of acyclovir resistant mutants of herpes simplex virus type 1 in athymic nude mice. J. Antimicrob. Chemother. **18**(Suppl. B)**:**95–101.
9. **Field, H. J.** 1988. Animal models in the evaluation of viral chemotherapy, p. 67–84. *In* H. J. Field (ed.), Antiviral agents, the development & assessment of antiviral chemotherapy, vol. 1. CRC Publications, Boca Raton, Fla.
10. **Field, H. J., S. E. Bell, G. B. Elion, A. A. Nash, and P. Wildy.** 1979. Effect of acycloguanosine treatment on acute and latent herpes simplex infections in mice. Antimicrob. Agents Chemother. **15:**554–561.
11. **Field, H. J., and S. E. Goldthorpe.** 1989. Antiviral drug resistance. Trends Pharm. Sci. **10:**333–337.
12. **Goldthorpe, S. E., M. R. Boyd, and H. J. Field.** 1992. Effects of penciclovir and famciclovir in a murine model of encephalitis induced by intranasal inoculation of herpes simplex type 1. Antivir. Chem. Chemother. **3:**37–47.
13. **Harnden, M. R., R. L. Jarvest, M. R. Boyd, D. Sutton, and R. A. Vere Hodge.** 1989. Prodrugs of the selective antiherpesvirus agent 9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]guanine (BRL39123) with improved absorption properties. J. Med. Chem. **32:**1738–1743.
14. **Hill, T. J., H. J. Field, and W. A. Blyth.** 1975. Acute and recurrent infection with herpes simplex virus in the mouse: a model for studying latency and recurrent disease. J. Gen. Virol. **28:**341–353.
15. **Jeffries, D. J.** 1989. The spectrum of cytomegalovirus infection and its management. J. Antimicrob. Chemother. **23**(Suppl. E)**:**1–10.
16. **Kahan, B. D.** 1989. Cyclosporine. N. Engl. J. Med. **321:**1725–1738.
17. **Maclean, H., and B. Dhillon.** 1993. Cytomegalovirus retinitis: diagnosis and treatment. Int. J. STD AIDS **4:**322–325.
18. **Meyers, J. D.** 1988. Management of cytomegalovirus infection. Am. J. Med. **107:**809–816.
19. **Nash, A. A., H. J. Field, and R. Quartey-Papafio.** 1980. Cell-mediated immunity in herpes simplex virus-infected mice: induction, characterisation and antiviral effects of delayed type hypersensitivity. J. Gen. Virol. **48:**351–357.
20. **Pue, M. A., and L. Z. Benet.** 1993. Pharmacokinetics of famciclovir in man. Antivir. Chem. Chemother. **4**(Suppl. 1)**:**47–55.
21. **Sutton, D., and M. R. Boyd.** 1993. Comparative activity of penciclovir and acyclovir in mice infected intraperitoneally with herpes simplex virus type 1 SC16. Antimicrob. Agents Chemother. **37:**642–645.
22. **Sutton, D., and E. R. Kern.** 1993. Activity of famciclovir and penciclovir in HSV-infected animals: a review. Antivir. Chem. Chemother. **4**(Suppl. 1)**:**37–46.
23. **Tewari, D., J. M. Whalley, D. N. Love, and H. J. Field.** 1994. Characterisation of immune responses to baculovirus-expressed equine herpesvirus type 1 glycoproteins D and H in a murine model. J. Gen. Virol. **75:**1735–1741.
24. **Thomson, M. H., and D. J. Jeffries.** 1989. Ganciclovir therapy in iatrogenically immunosuppressed patients with cytomegalovirus disease. J. Microb. Chemother. **23**(Suppl. E)**:**61–70.
25. **Tilson, H. H., C. R. Engle, and E. B. Andrews.** 1993. Safety of acyclovir: a summary of the first ten years experience. J. Med. Virol. **41**(Suppl. 1)**:**67–73.
26. **Vere Hodge, R. A.** 1993. Famciclovir and penciclovir. The mode of action of famciclovir including its conversion to penciclovir. Antivir. Chem. Chemother. **4:**67–84.
27. **Vere Hodge, R. A., and Y. C. Cheng.** 1993. The mode of action of penciclovir. Antivir. Chem. Chemother. **4**(Suppl. 1)**:**13–24.
28. **Vere Hodge, R. A., and R. M. Perkins.** 1989. Mode of action of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL 39123) against herpes simplex virus in MRC-5 cells. Antimicrob. Agents Chemother. **33:**223–229.
29. **Vere Hodge, R. A., D. Sutton, M. R. Boyd, M. R. Harnden, and R. L. Jarvest.** 1989. Selection of an oral prodrug (BRL 42810; famciclovir) for the antiherpesvirus agent BRL 39123 [9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine; penciclovir]. Antimicrob. Agents Chemother. **33:**1765–1773.
30. **Weller, S., M. R. Blum, M. Doucette, T. Burnette, D. M. Cederberg, P. de Miranda, and L. M. Smiley.** 1993. Pharmacokinetics of the acyclovir prodrug valaciclovir after escalating single and multiple-dose administration to normal volunteers. Clin. Pharm. Ther. **54:**595–605.