Effect of acyclovir on acute and latent herpes simplex virus infections in the rabbit

Melvin D. Trousdale, Edmund C. Dunkel, and Anthony B. Nesburn

Acyclovir, a new potent antiviral drug, was used to treat herpes simplex virus (HSV) infection in the rabbit ocular model. Acyclovir (3% ointment) used topically one to five times a day on acute ocular HSV infection gave beneficial results as measured by a reduction in corneal involvement, conjunctivitis, iritis, and corneal clouding. Topical treatment did not prevent the establishment of latent HSV infection. Intravenous acyclovir used two times a day (50 mg/kg) on rabbits with latent HSV infection appeared to suppress HSV in the nervous system but did not eradicate established latent HSV infection.

Key words: experimental herpes simplex, herpes simplex keratitis, acute herpes infection, latent herpes infection, acycloguanosine, acyclovir, rabbit

The efficacy of epithelial debridement and several antiviral drugs such as idoxuridine, vidarabine, and trifluorothymidine in treating epithelial herpes simplex virus (HSV) keratitis has been documented in double-masked studies.1-5 Thus primary research interest in herpes keratitis has shifted away from treating the acute phase of the disease and has focused on prevention or eradication of deep keratitis and recurrences, which increase the chances of corneal scarring and visual loss. Following the initial episode of HSV keratitis in man, there is approximately a 50% chance of recurrence within 2 years.6 Although antiviral drugs generally reduce the severity and duration of epithelial HSV keratitis, currently available antiviral drugs have not decreased the incidence of recurrence.7,8

Acyclovir (ACV), previously called acycloguanosine (ACG), is a new, specific, and potent antiviral drug (9-(2-hydroxyethoxy-methyl) guanine), which is taken up preferentially by HSV-infected cells.9 The antiviral activity of this drug results from a series of phosphorylation reactions, the first of which is catalyzed by an HSV-specified thymidine kinase. The triphosphorylated form of ACV is both a substrate for and an inhibitor of HSV DNA polymerase. ACV is well tolerated by experimental animals and appears to be more potent at inhibiting HSV replication9-10 and keratitis11,12 than other currently available antiviral drugs. Prior reports of the efficacy of ACV against experimental HSV keratitis have employed topical therapy every 3 to 4 hr five times a day.

The purpose of this preliminary investigation was to determine the effect of ACV on both experimental acute herpes keratitis and latent HSV infection in the rabbit ocular model. We were particularly interested in determining whether the ACV would (1) be effective against acute HSV keratitis in less than the previously used dosage of five times per day, (2) prevent acute ocular HSV from establishing latent infection of the trigeminal

From the Estelle Doheny Eye Foundation and Department of Ophthalmology, USC School of Medicine, Los Angeles, Calif.
Supported in part by NIH grants EY00558 and EY002857 and Discovery Fund.
Submitted for publication Oct. 9, 1979.
Reprint requests: Anthony B. Nesburn, M.D., Virology Department, Estelle Doheny Eye Foundation, 1355 San Pablo St., Los Angeles, Calif. 90033.

1336 0146-0404/80/111336+06$00.60/0 © 1980 Assoc. for Res. in Vis. and Ophthal., Inc.
ganglion, and (3) eradicate HSV from the central nervous system of latently infected animals.

Materials and methods

Virus. McKrae strain HSV-1 was prepared in rabbit kidney (RK) cell cultures and stored at -60°C until needed. The McKrae strain of HSV-1, which has been studied extensively, was selected because it induces a predictable keratitis, a high incidence of latent central nervous system infection, and a high ocular recurrence rate.

Rabbit ocular inoculation. Male albino New Zealand rabbits, weighing 3 to 4 kg, were used. Both eyes were stained with 5% methylene blue and examined by slit-lamp biomicroscopy to detect preinoculation corneal defects. None of the animals used had any significant defects. Both eyes were infected without sacrifice by placing 0.2 ml of HSV-1 (2 × 10⁶ pfu) into the conjunctival cul-de-sac, closing the eye, and rubbing gently for 30 sec.

Ocular examination. Each day the eyes were examined as described above to delineate the epithelial ulceration. At each examination the extent of corneal epithelial involvement, conjunctivitis, corneal clouding, and iritis was recorded as described. These examinations were carried out in a masked fashion. Statistical analysis of data was done by one-way analysis of variance. Duncan's multiple range test was employed to determine the significant differences (p < 0.05) between the treatment groups.

HSV isolation from tear film. Daily conjunctival swab cultures (6 days/week) were taken to document the rate of spontaneous HSV shedding into the tear film. Samples for virus isolation were taken with a sterile cotton swab, which was rotated in the upper fornix, gently swept across the cornea, and rotated in the lower fornix. The swab was immediately inoculated into our standard medium over the monolayer culture of secondary RK cells.

HSV isolation from tissue. Surgical stimulation of the trigeminal ganglion induces the recurrence of HSV and makes HSV more easily recoverable from nervous tissue. Ocular and nervous system tissues (corneal epithelial, trigeminal ganglion, midbrain, and cerebellum) were removed aseptically, and virus isolation was attempted by the more sensitive enzymatic cell dispersion (ECD) technique. Briefly, tissue was minced and suspended in phosphate-buffered saline and treated with 2% collagenase (10 µl/0.1 gm of tissue) at 37°C for 15 min. This tissue was trypan
tenized with 0.25% EDTA-trypsin at 37°C for 30 min. The enzymatic reaction was terminated by adding fetal calf serum to a final concentration of 10%. The dispersed cells were immediately placed on secondary RK indicator cell monolayers. All cultures were incubated at 37°C in 5% CO₂ atmosphere and read every other day.

Identification of HSV isolates. All positive HSV cultures were confirmed by repassing in RK cell cultures. All negative cultures were blind-passed in RK cell cultures and observed for at least 2 weeks before being recorded as negative. Identity of all isolates was confirmed by standard microneutralization tests with known anti-HSV serum.

Infectivity assay. Samples to be assayed for infectious HSV were freeze-thawed three times. An aliquot was serially diluted and inoculated onto confluent secondary RK cells in 60 mm plastic Petri dishes. These cultures were then incubated for 60 min at 37°C, and 5 ml of a 1% methylcellulose overlay were added. After incubation at 37°C for 3 days, 1 ml of a 1:5000 dilution of neutral red (GIBCO) was added to the overlay, and plaques were counted 24 hr later.

Experiment 1. Topical ACV treatment of acute HSV keratitis. Experiment 1 compared the effect of ACV 3% ointment (Burroughs Wellcome Co., Research Triangle Park, N. C.) applied one, three, or five times a day against placebo applied five times a day for 10 days. The placebo was the same vehicle used in ACV ointment.

On the basis of corneal epithelial involvement 3 days after inoculation, rabbits were assigned to provide three equally matched groups, and treatment was initiated. At this time all eyes showed...
early signs of dendritic keratitis. Bilateral topical application of a small (3 to 4 mm) ribbon of appropriate ointment was placed into the lower cul-de-sac for 10 consecutive days.

In 2 to 4 weeks after ACV therapy, all rabbits were sacrificed. Portions of both trigeminal ganglia, left and right midbrain, and cerebellum were tested for infectious and latent HSV by the ECD method.

Experiment 2. Intravenous ACV treatment of latent HSV infection. Eleven rabbits were used in the intravenous treatment experiment. ACV (50 mg/kg) was injected intravenously twice a day. Virus shedding was determined, and portions of the central nervous system were tested as described in experiment 1.

Rabbits 1 to 6 were stimulated by surgery 1 day following completion of chemotherapy in an attempt to reactivate latent HSV, and rabbits 7 to 11 were similarly stimulated 30 days after the ACV regimen.

Results

Topical treatment of acute herpetic keratitis. In experiment 1, we established the effect of different dosing schedules of topical ACV 3% ointment on rabbits with acute HSV-1 eye infections. The degree of corneal epithelial involvement, conjunctivitis, corneal clouding, and iritis related to the dose (one, three, or five applications per day) is presented in Figs. 1 to 4. Compared to placebo-treated controls (five times a day), ACV treatment resulted in a beneficial response which was significantly different (p < 0.05) regardless of the number of daily drug applications. When the animals were treated with ACV either three or five times a day, progression of corneal lesions was stopped within 24 hr, and this was significantly better than treatment once a day (Fig. 1). The severity and duration of conjunctivitis, corneal clouding, and iritis was also reduced significantly (p < 0.05) by ACV treatment (Figs. 2 to 4).

Table I presents the frequency of HSV isolation from the nervous system of rabbits 2 to 4 weeks following topical ACV therapy of acute ocular infection. HSV was detected most frequently in trigeminal ganglia. At least one trigeminal ganglion of each rabbit tested (21 out of 21) was positive for HSV. Fourteen of 21 rabbits, or 66%, had HSV isolated from at least one midbrain tissue sample. Four of 21 (19%) cerebellum tissue samples were positive for HSV. Frequency of topical ACV did not influence these isolation rates.

Even when the ACV treatment was administered topically five times a day for 30 days to six rabbits, HSV was readily isolated from the trigeminal ganglia, midbrain, and cerebellum (unpublished data).

Topical ACV therapy of HSV-infected rabbit eyes showed no toxicity changes which could not be explained by infection itself. No
Acyclovir effect on acute and latent infections

Toxic reaction was ever detected in the cornea of four normal control rabbits receiving ACV or placebo ointment five times a day for 30 days.

**Intravenous treatment of latent HSV infection.** The number of independent episodes of virus shedding into the tear film during this study ranged from two to nine (unpublished data). An episode of HSV shedding is defined as when virus is isolated from the same eye for 1 or more consecutive days.

In rabbits 1 to 6, HSV was not detectable in the tear films during the period of chemotherapy nor during 2 hr intervals taken for 24 hr following stimulation (Table II). However, one or both trigeminal ganglia of rabbits 1 to 6 were positive for HSV. HSV was not isolated from the midbrain or cerebellar tissue specimens from these rabbits.

In rabbits 7 to 11, which were stimulated 30 days following completion of the regimen, three rabbits (8, 9, and 11) shed HSV in tear film during the treatment period. Each rabbit had from one to six episodes of HSV shedding during the 30 days following chemotherapy.

HSV was isolated from the tear film of both eyes in four of five rabbits following stimulation. Seven of ten corresponding trigeminal ganglia were positive for HSV. One additional ganglion was positive, although no virus was shed into the tear film. The yield of virus from midbrain samples was lower than that of trigeminal ganglia. Three of 10 midbrain tissue samples had detectable HSV, as did two of five cerebellum specimens tested.

**Discussion**

ACV is a guanine derivative with potent antiviral activity.10 The unique feature of ACV is the selectivity of its antiviral action, which results from activation of the drug by viral thymidine kinase.19 This specific antiviral drug has been reported to be effective both in vitro and in vivo against HSV-1, HSV-2, varicella-zoster virus, and monkey B virus. The potency, selectivity, and nontoxic properties of ACV provide the theoretical possibility for its being used to (1) prevent HSV from establishing latent infection in the

![Fig. 4. Measure of corneal clouding during topical treatment of experimental HSV infection with ACV 3% ointment.](image)

**Table I. Experiment I. Isolation of HSV from rabbits following experimental acute ocular infection treated topically with ACV.**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Tri-geminal</th>
<th>Mid-brain</th>
<th>Cere-bellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. (ACV once a day)</td>
<td>7/7</td>
<td>6/7</td>
<td>1/7</td>
</tr>
<tr>
<td>B. (ACV 3 times a day)</td>
<td>7/7</td>
<td>3/7</td>
<td>1/7</td>
</tr>
<tr>
<td>C. (ACV 5 times a day)</td>
<td>5/5</td>
<td>4/5</td>
<td>2/5</td>
</tr>
<tr>
<td>D. (Placebo 5 times a day)</td>
<td>2/2</td>
<td>1/2</td>
<td>0/2</td>
</tr>
</tbody>
</table>

Percent positive 100 66 19

Values are number positive/total tested.

*ECD procedure with secondary RK cell cultures was used for HSV isolation.

E Two rabbits in Group C died before the study was terminated.

trigeminal ganglia and (2) eradicate HSV from the nervous system of existing latent infections.

In our rabbit ocular model, the course of acute herpetic keratitis induced by McKrae strain of HSV is quite predictable. Corneal involvement appears about day 3 and progresses until most of the cornea is covered with dendritic or geographic lesions by day 7 or 8. Severity of conjunctivitis, corneal clouding, and iritis accompanying this disease parallels the corneal involvement. Without chemotherapy, the acute phase of the disease resolves within 10 to 14 days, although corneal clouding may continue longer. Virtually
all surviving animals are latently HSV-infected; that is, after the acute phase of the disease has completely healed, there are periodic spontaneous episodes of shedding of HSV into the tear film. Several investigators have incriminated ganglionic cells as a reservoir for infectious virus. We confirmed that topical 3% ACV ointment gives favorable results when used on acute ocular HSV infections. This therapeutic effect was present even when the drug was applied once daily. No clinical significant difference was noted between applications given three and five times daily, indicating that treatment three times a day might be clinically effective. This could be important because patient compliance is not always optimum.

Mechanical trigeminal ganglia stimulation and ECD procedure normally increase the isolation rate of HSV from all central nervous system tissues in our rabbit model. When surgical stimulation and ECD were carried out 1 day following completion of ACV chemotherapy in latently infected animals, HSV was detectable only in trigeminal ganglia. The failure to recover virus from midbrain and cerebellum immediately after cessation of treatment could result from eradication of drug-susceptible, replicating virus in these tissues. More ACV may reach the midbrain and cerebellum than reaches the trigeminal ganglia. Virus-infected cell populations may also have differential susceptibility to ACV. By contrast, when stimulation and ECD were performed 30 days after completion of systemic ACV therapy, HSV was detected more widely in the nervous system. The pattern and isolation rates at 30 days after treatment resemble those we have encountered in numerous assays of the central nervous system in untreated infected animals. This suggests that systemic ACV in the doses given suppresses HSV in the nervous system. Later, the midbrain and cerebellum may become HSV-positive because of reinoculation of these tissues with virus from the trigeminal ganglia reservoir. Perhaps more HSV exists in the trigeminal ganglia than in the midbrain or cerebellum. Although these results could be attributed theoretically to residual drug in the tissue affecting our indicator cell layer, this seems unlikely because of extensive washing involved in the ECD procedure prior to inoculation of the cell cultures.

Table II. Isolation of HSV from latently infected rabbits stimulated to induce virus reactivation either 1 or 30 days following completion of ACV intravenous chemotherapy

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Trigeminal stimulated</th>
<th>Tear film</th>
<th>Trigeminal ganglion</th>
<th>Midbrain</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OS OD</td>
<td>Right Left</td>
<td>Right Left Cerebellum</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Left</td>
<td>- -</td>
<td>- -</td>
<td>- + - - -</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Left</td>
<td>- -</td>
<td>- -</td>
<td>- + - - -</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Left</td>
<td>- -</td>
<td>- -</td>
<td>- + - - -</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Left</td>
<td>- -</td>
<td>- -</td>
<td>- + - - -</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Left</td>
<td>- -</td>
<td>- -</td>
<td>- + - - -</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Left</td>
<td>- -</td>
<td>- -</td>
<td>- - - - -</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Right</td>
<td>- -</td>
<td>- -</td>
<td>- - - - -</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Left</td>
<td>+ +</td>
<td>- -</td>
<td>+ - - - -</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Left</td>
<td>+ +</td>
<td>+ +</td>
<td>- - - + +</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Right</td>
<td>+ +</td>
<td>+ +</td>
<td>+ - + - +</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Right</td>
<td>+ +</td>
<td>+ +</td>
<td>- + + - +</td>
<td></td>
</tr>
</tbody>
</table>

*Acyclovir was given (50 mg/kg) i.v. in ear or femoral vein twice a day for 14 days.
*Rabbits 1 to 6 were stimulated one day following completion of chemotherapy. Rabbits 7 to 11 were stimulated 30 days after chemotherapy.
*Positive HSV isolation is designated by a plus (+). If the cell cultures failed to induce the presence of HSV after 2 weeks of culturing, the results were reported as negative (-).
Field et al.\textsuperscript{22} reported that ACV reduced or prevented the establishment of latent infection in a mouse ear pinna model. However, he also found that ACV was not effective against an existing latent infection. In a mouse ocular model, Pavan-Langston et al.\textsuperscript{23} also reported that ACV reduced the establishment of latent HSV in trigeminal ganglia. Both of these reports suggest that in mice, replication of HSV in the trigeminal ganglia is a continuous event because ACV is effective only when the herpesvirus is replicating. Our rabbit ocular HSV model which mimics human herpetic ocular disease also suggests that virus replication occurs during latency. HSV was detected in the rabbit tear film cultures both during and after virus chemotherapy. This would seem to indicate that the therapeutic regimen employed had little or no effect on established latent HSV infection.

ACV offers a nontoxic and highly HSV-specific therapy which would seem to have the potential for eradicating HSV in the nervous system when or if the virus is in a replicating state. Further experiments with different schedules and routes of administration must be carried out to determine the optimal use of ACV in the treatment of clinical herpetic disease.

REFERENCES