Antiviral Research, 3 (1983) 253–267
Elsevier

Acyclovir treatment of experimental genital herpes simplex virus infections. I. Topical therapy of type 2 and type 1 infections of mice*

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(Received 30 May 1983; accepted 22 August 1983)

Intravaginal inoculation of mice with herpes simplex virus (HSV) provides a model infection of genital herpes to determine the effectiveness of potential antiviral agents. Topical (intravaginal) treatment with 1% or 5% acyclovir (ACV) in an ointment or gel vehicle initiated 3, 6 or 24 h after inoculation with HSV type 2, significantly inhibited viral replication in the genital tract and usually reduced final mortality. Treatment with 5% ACV initiated 48 or 72 h after infection also reduced vaginal virus titers but did not alter final mortality. When mice were inoculated with HSV type 1 treatment with 5% ACV significantly reduced viral replication in the genital tract when begun as late as 72 h. In HSV-2 infected mice, treatment initiated 3 h but not 24 h after infection prevented the establishment of latent infection in sacral ganglie. These results suggest that topical ACV may be an effective antiviral agent for primary genital herpes in humans.

herpes simplex virus; experimental infections; topical therapy; acyclovir

Introduction

Infections of the human genital tract due to herpes simplex virus type 2 or 1 (HSV-2, HSV-1) are important sexually transmitted diseases in both males and females. It is estimated that in the United States alone, there are at least 20 000–300 000 new cases of

*This work was supported by Public Health Service Contract. No. NOI-AI-42524 from the Antiviral Substances Program of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, U.S.A. This is publication No. 00 from the Cooperative Antiviral Testing Group, Development and Applications Branch, NIAID, NIH.

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genital HSV infections per year [26]. In addition, due to the capacity of HSV to remain in a latent state and cause periodic reactivated disease, there may be 200000–9000000 cases of recurrent infection each year [26]. This frequency of genital HSV disease, the risk of infection for neonates during birth [22], and the association between HSV-2 and cervical carcinoma [23] clearly indicates a need for effective therapy for these infections. Prior to the development of acyclovir treatment of genital or oral mucocutaneous HSV infections with a variety of antiviral agents and other miscellaneous preparations have generally been unsuccessful [1,11,12,21,34,36].

To determine the effectiveness of antiviral agents that have potential for treatment of genital HSV infections of humans we have utilized a model genital HSV infection of mice. After viral inoculation by the intravaginal route, there is local replication in the vaginal tract followed by neural spread of the virus to the central nervous system and death from encephalitis [25]. Antiviral efficacy in this model can be evaluated by determining the effect on local viral replication by monitoring viral titers in vaginal secretions, and against the fatal encephalitis. Since the virus becomes latent in the sacral dorsal root ganglia, the effect of treatment on the establishment and maintenance of latent HSV can also be determined. A variety of antiviral agents including interferon, interferon inducers, adenine arabinoside (ara-A), adenine arabinoside 5'-monophosphate (ara-AMP), 2-deoxy-D-glucose, phosphonoacetic acid (PAA) and phosphonoformic acid (PFA) have been tested in this model infection [15–18, 24]. Of these agents PAA and PFA have been the most effective.

A relatively new drug, 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACV), is a potent inhibitor of HSV replication [33], has a high degree of selectivity for HSV [9] and has been tested in various animal model systems. Topical ACV has been reported to be effective in the treatment of HSV infections of rabbit eyes [2, 13, 30, 33, 38], and the skin of mice [8, 10, 19, 28] and guinea pigs [29]. The purpose of our experiments was to compare the relative susceptibilities of a number of HSV-2 and HSV-1 strains to ACV in tissue culture and to determine the efficacy of topical treatment with ACV on genital HSV-2 and HSV-1 infections in mice.

Materials and methods

Experimental infection

Groups of fifteen 6- to 8-wk-old Swiss Webster mice (Simonsen Laboratories, Gilroy, U.S.A.) were inoculated intravaginally with 0.05 ml of HSV-2 or HSV-1 using a small plastic catheter attached to a syringe. Two hours prior to viral inoculation mice were swabbed with a dacron tipped applicator moistened in medium to remove vaginal secretions. Each animal received approximately 1×10^5 plaque forming units (pfu) of virus.

Virus strains, media, cell cultures, and virus assays

Laboratory passaged strains of known type designated MS, X-79 and E-377 were

obtained from A. Nahmias, Emory University, Atlanta, GA, U.S.A. Strain E-196 was obtained from H. Haines, University of Miami, Miami, U.S.A. All other strains utilized were isolated from oral (HL-3, HL-34) or genital (Heeter, Wilson) lesions from patients seen in our Herpes Study Clinic. The type 1 strains were E-377, HL-3, HL-34 and Wilson and the type 2 strains MS, X-79, E-196 and Heeter. The type 2 strain, MS, and the type 1 strain E-377 were used for the animal studies. The media utilized, preparation of cell cultures and assays for HSV have been described previously [14].

Antiviral drug

The ACV was provided through the Antiviral Substances Program (NIAID, NIH) and supplied as a powder and a 1% or 5% ointment suspended either in polyethylene glycol (PEG) or a vaginal gel (GEL) by Burroughs Wellcome Company, Research Triangle Park, U.S.A. Placebo preparations for each vehicle were also provided. In all animal experiments, ACV was administered intravaginally in a volume of 0.1 ml.

Susceptibility of HSV strains in vitro

The sensitivity of type 2 and type 1 strains to ACV was determined by a 50% plaque reduction assay in mouse embryo fibroblast (MEF) or human foreskin fibroblast (HFF) cells. Confluent cell monolayer cultures were inoculated with 20–50 pfu of each strain and incubated at 37°C for 1 h. Then serial 2-fold dilutions of ACV in twice concentrated minimal essential medium were mixed with an equal volume of 1.0% agarose and the mixture added to the culture plates. After incubation for 48 h the cells were stained with neutral red and plaques counted [14].

Assay for HSV in vaginal secretions

Vaginal swabs for quantitation of virus were obtained from both placebo and drug-treated animals on days 1, 3, 5, 7 and 10 after viral inoculation. The swabs were placed in 1.0 ml of tissue culture media, vortexed, and frozen at −70°C until assayed on sencondary rabbit kidney (RK) cells for the presence of HSV. Viral titers are expressed as $\log_{10}$ pfu/ml of media in which the swab was placed. For each experimental group of 15 animals, the mean HSV titer of the vaginal secretion specimens collected on days 1–10 were used to calculate a mean virus titer–day area under the curve [16].

Receovery of latent HSV

Surviving mice were killed 45–60 days after intravaginal inoculation. The lumbosacral spinal column was removed aseptically and the dorsal root ganglia were dissected out and removed. Eight to ten lumbosacral ganglia were recoverable from each mouse. The intact ganglia from each mouse were placed into a 35-mm Petri dish with a confluent monolayer of RK cells. The monolayers were observed for 21 days for the appearance of viral cytopathic effect (CPE). All virus positive cultures were confirmed

by plating 0.2 ml of supernatant onto a fresh RK monolayer and observing for typical HSV CPE [32].

Statistical analysis

Differences in infection rates and final moratlity rates were evaluated using the Fisher Exact test. The in vitro sensitivity results and the areas under the virus titer–day curves in the genital infection were compared using the Mann–Whitney *U* test. A *P* value of <0.05 was considered significant.

Results

Susceptibility of HSV strains to ACV in mEF and HFF cells

The susceptibility of 4 strains each of HSV-2 and HSV-1 was determined in both MEF and HFF cells. The mean values from two separate experiments are listed in Table 1. In MEF cells both type 2 and type 1 strains were inhibited by approximately 0.02 μg/ml, whereas in HFF cells 0.3 to 0.7 μg/ml was required for 50% inhibition when compared to control cultures. The MS and E-377 strains of HSV used in the animal experiments were as susceptible to ACV as the other strains tested.

TABLE 1

Susceptibility of type 2 and type 1 strains of herpes simplex virus (HSV) to acyclovir in mouse embryo fibroblast (MEF) and human foreskin fibroblast (HFF) cells

Virus strain	50% inhibitory levels (μg/ml ± SD)[a]	
	MEF cells	HFF cells
HSV type 2		
MS	0.04 ± 0.005	0.68 ± 0.10
X-79	0.02 ± 0.0007	0.31 ± 0.06
E-196	0.01 ± 0.002	NT[b]
Heeter	0.01 ± 0.0007	0.33 ± 0.05
Mean ± SEM	0.02 ± 0.01	0.44 ± 0.21
HSV type 1		
E-377	0.01 ± 0.004	0.31 ± 0.14
HL-3	0.01 ± 0.0007	NT
HL-34	0.01 ± 0.002	0.31 ± 0.04
Wilson	0.03 ± 0.02	0.55 ± 0.10
Mean ± SEM	0.015 ± 0.01	0.39 ± 0.14

[a] Mean values from two experiments ± 1 SD.

[b] Not tested.

Treatment of genital HSV-2 infections of mice with ACV suspended in PEG (ACV-PEG)

In the initial experiment, groups of 15 mice were inoculated intravaginally (ivg) with HSV-2 and treated ivg twice daily (every 12 h) for 5 days with placebo-PEG, 1% ACV-PEG or 5% ACV-PEG beginning 6 h after viral inoculation. The placebo-treated group was compared with the untreated control group, and the groups that received ACV were compared with the placebo. When placebo-PEG was initiated 6 h after infection (Fig. 1, top), the geometric mean peak virus titer was reduced from 10^5 pfu/ml (area = 34.1) in the untreated control group to 10^3 pfu/ml (area 23.4, $P<0.05$). In the group given 1% ACV-PEG the mean virus titer was reduced to 10^2 pfu/ml (area = 17.9, $P<0.05$), and in the group given 5% ACV-PEG to about $10^{1.5}$ pfu/ml (area = 8.3, $P<0.001$). The mortality and mean day of death (MDD) of mice treated with either 1% or 5% ACV-PEG were also reduced significantly (Table 2, Expt. 1).

In a second experiment, groups of mice were inoculated ivg with HSV-2 and treated ivg 4 times daily (every 6 h) for 5 days with either placebo-PEG, 1% ACV-PEG or 5% ACV-PEG beginning 24 h after viral inoculation. There were no differences in vaginal virus titers or areas under the curve between untreated control mice and those treated

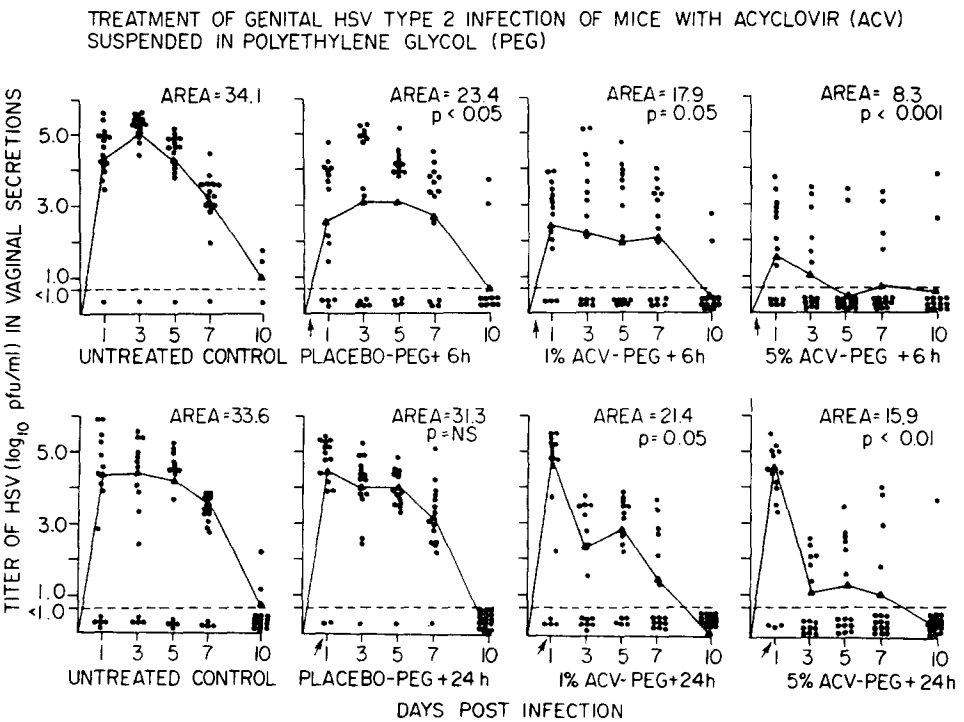

Fig. 1. Treatment of a genital HSV type 2 infection of mice with acyclovir (ACV) suspended in polyethylene glycol (PEG), twice daily (top) or 4 times daily (bottom) for 5 days. Treatment by the intravaginal route was initiated 6 or 24 h after infection. The mean HSV titer in vaginal secretions on days 1, 3, 5, 7 and 10 is indicated by triangles.

TABLE 2

Effect of early treatment with acyclovir (ACV) in polythylene glycol (PEG) on the mortality of mice inoculated intravaginally with HSV-2

Treatment	No. infected/ No. inoculated (%)		No dead/ No. infected (%)		MDD
Expt. no. 1[a]					
None	14/15	93	14/14	100	9.3
PEG + 6 h	11/15	73	10/11	91	11.6[b]
1% ACV-PEG + 6 h	12/15	80	7/12	68[c]	13.1[d]
5% ACV-PEG + 6 h	9/15	60	3/9	33[b]	15.7[d]
Expt. no. 2[e]					
None	10/15	67	10/10	100	11.4
PEG + 24 h	13/15	87	13/13	100	10.2
1% ACV- 24 h	11/15	73	8/11	73	12.6[c]
5% ACV-PEG + 24 h	12/15	80	5/12	42[b]	15.0[b]

[a] Treatment with 0.1 ml of ACV-PEG administered intravaginally twice daily for 5 days.
[b] $P<0.01$.
[c] $P<0.05$.
[d] $P<0.001$.
[e] Treatment with 0.1 ml of ACV-PEG administered intravaginally 4 times daily for 5 days.

with placebo-PEG (Fig. 1, bottom). In mice treated with 1% ACV-PEG, the mean virus titers were reduced significantly (area = 21.4, $P = 0.05$). In animals treated with 5% ACV-PEG, there was even a greater reduction in mean HSV titers (area = 15.9, $P<0.01$). The effect of ACV treatment on infection rates, final mortality rates and the MDD of these same animals is summarized in Table 2, Expt. 2. There were no differences in final mortality or MDD between untreated and placebo-treated control mice. Treatment with 1% ACV did not alter mortality but did increase the MDD. In mice treated with 5% ACV there was a significant reduction in final mortality and an increase in the MDD.

Effect of treatment with ACV-GEL on a genital HSV-2 infection of mice

To determine the effectiveness of ACV in another vehicle, a vaginal GEL, the placebo-GEL, 1% of 5% ACV-GEL was administered ivg to mice 4 times daily (every 6 h) beginning 3 or 24 h after viral inoculation. The results of this experiment are shown in Fig. 2. When pacebo-GEL was initiated 3 h after infection, the mean virus titer was reduced from 10^5 pfu/ml (area = 37.3) in the untreated control to about 10^3 pfu/ml (area = 23.2, $P<0.01$). In the group of mice given 1% ACV-GEL the mean virus titer was reduced to 10^1 pfu/ml (area = 8.0, $P<0.05$). Treatment with 5% ACV-GEL completely inhibited viral repication in the genital tract of most of the mice (area = 1.5, $P<0.01$). When therapy was initiated 24 h after infection, a time when most animals had viral titers of 10^3–10^6 pfu/ml in vaginal secretions, there was a significant drop in mean viral titers to about 10^1 pfu/ml in the groups treated with

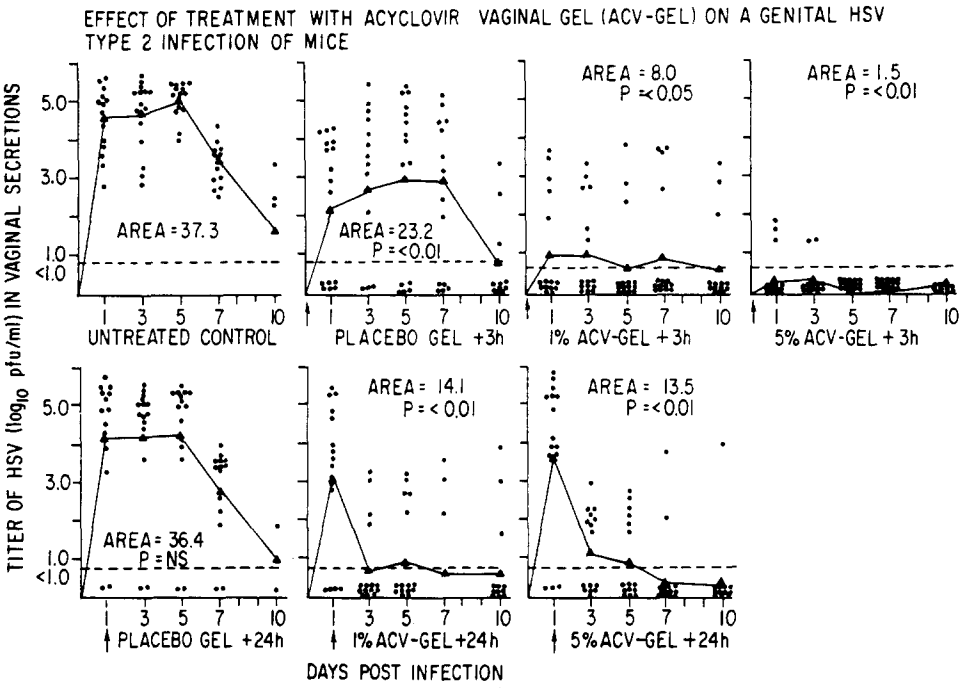

Fig. 2. Treatment of a genital HSV type 2 infection of mice with Acyclovir (ACV) suspended in a vaginal gel (GEL), 4 times daily for 5 days. Treatment by the intravaginal route was initiated 3 or 24 h after infection. The mean HSV titer in vaginal secretions on days 1, 3, 5, 7 and 10 is indicated by triangles.

either 1% (area = 14.1, $P<0.01$) or 5% (area = 13.5, $P<0.01$) ACV-GEL. The effect of treatment on infection rate, mortality and MDD of these animals is summarized in Table 3. Both concentrations of ACV initiated 3 h after infection significantly decreased the infection rate, and the 5% ACV-GEL prevented death in infected animals. When therapy was initiated 24 h after viral inoculation, both 1% and 4% ACV-GEL significantly reduced mortality and increased the MDD.

Since both ACV preparations were effective in inhibiting HSV-2 replication in the genital tract of mice when administered early in the course of infection, an additional experiment was performed to compare directly the two preparations using the same treatment regimen. Groups of mice were inoculated ivg with HSV-2 and treatment with either placebo (PEG or GEL), 1% ACV (in PEG or GEL), or 5% ACV (in PEG or GEL) 4 times daily for 5 days was initiated 24 h after viral inoculation. In mice treated with either preparation of 1% or 5% ACV, there were no significant differences in vaginal virus titers between the PEG or GEL formulations. All 4 groups were significantly different from their respective placebo-treated controls (data not presented).

Since both preparations appeared to be equally effective in inhibiting HSV replication in the genital tract and the PEG preparation had greater shelf stability the

TABLE 3

Effect of early treatment with acyclovir (ACV) suspended in vaginal gel (GEL) on the mortality of mice inoculated intravaginally with HSV-2

Treatment[a]	No. infected/ No. inoculated (%)		No dead/ No. infected (%)		MDD
None	15/15	100	14/15	93	9.6
GEL + 3 h	12/15	80	10/12	83	11.3
1% ACV-GEL + 3 h	6/15	40[b]	6/6	100	13.2[c]
5% ACV-GEL + 3 h	3/15	20[d]	0/3	0	-
GEL + 24 h	13/15	87	13/13	100	9.1
1% ACV-GEL + 24 h	12/15	80	7/12	58[c]	13.3[b]
5% ACV-GEL + 24 h	12/15	80	4/12	33[d]	15.0[b]

[a] Treatment with 0.1 ml of ACV-GEL administered intravaginally 4 times daily for 5 days.
[b] $P<0.01$.
[c] $P<0.05$.
[d] $P<0.001$.

ACV-PEG was selected as the formulation to be used in subsequent animal studies and clinical trials. The placebo effect seen with both preparations in the previous experiments is thought to be due to mechanical interference with infection, rather than an antiviral effect since it was observed only when treatment was initiated at 3 or 6 h post infection.

Effect of late therapy with ACV-PEG on a genital HSV type 2 infection of mice

Since treatment initiated 3, 6 or 24 h after infection was highly effective in reducing virus titers in the vaginal tract of mice, the next experiment was designed to determine the effect of treatment initiated later during the course of infection. Groups of animals were inoculated with HSV-2 and treatment with 5% ACV-PEG was initiated 24, 48 or 72 h after infection. The effect of therapy on vaginal virus titers is shown in Fig. 3. If the values for the entire area under the curve are used in the analysis 6p), only treatment begun at 24 h had a significant effect, however, when only the portion of the curve after the onset of therapy is utilized (p'), then treatment initiated at both 48 h and 72 h was also effective. The effect of treatment on final mortality and MDD is listed in Table 4. Due to the low infection rates, significant protection could be demonstrated only in the group that received treatment beginning 24 h after infection. It is possible that with larger numbers, significant protection with later could be obtained.

Treatment of genital HSV-1 infection of mice with ACV-PEG

Since primary genital HSV infections in humans can also be due to type 1 strains and recurrent herpes labialis is almost always caused by HSV-1, the next experiment was designed to determine the effectiveness of ACV in the treatment of a genital

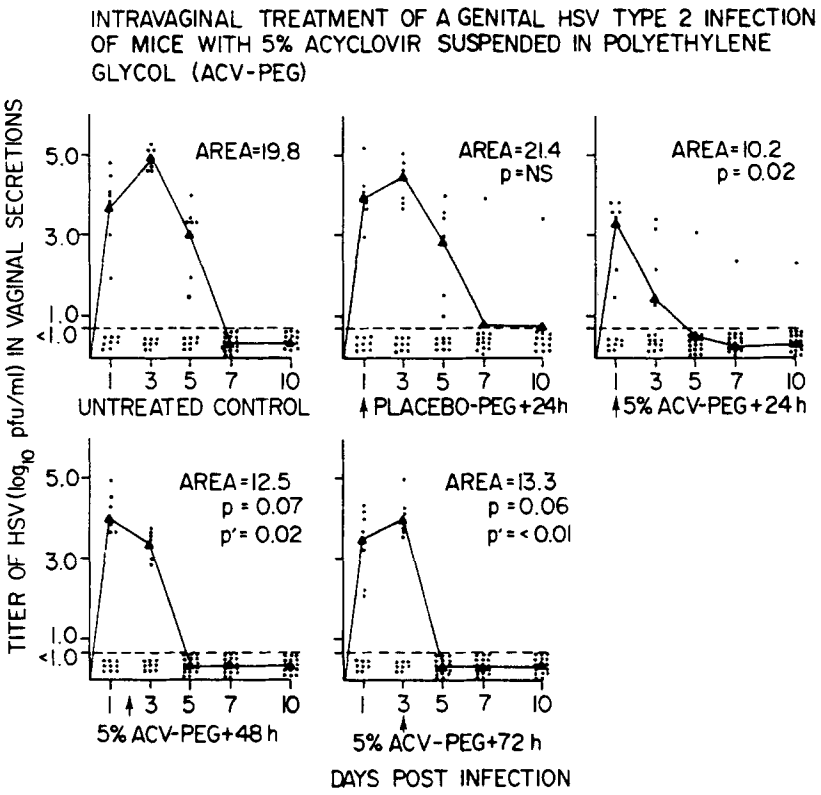

Fig. 3. Treatment of a genital HSV type 2 infection of mice with Acyclovir (ACV) suspended in polyethylene glycol (PEG), 4 times daily for 5 days. Treatment by the intravaginal route was initiated 24, 28 or 72 h after infection. The mean HSV titer in vaginal secretions on days 1, 3, 5, 7 and 10 is indicated by triangles.

TABLE 4

Effect of late treatment with 5% Acyclovir (ACV) in polyethylene glycol (PEG) on the mortality of mice inoculated intravaginally with HSV-2

Treatment[a]	No. infected/ No. inoculated (%)		No dead/ No. infected (%)		MDD
None	7/15	47	6/7	86	10.3
PEG + 24 h	6/15	40	5/6	83	11.0
ACV-PEG + 24 h	7/15	47	1/7	14[b]	11.0
ACV-PEG + 48 h	6/15	40	2/6		
ACV-PEG + 72 h	7/15	47	2/7	29	10.0

[a] Treatment with 0.1 ml of 5% ACV-PEG administered intravaginally 4 times daily for 5 days.

[b] $P<0.05$.

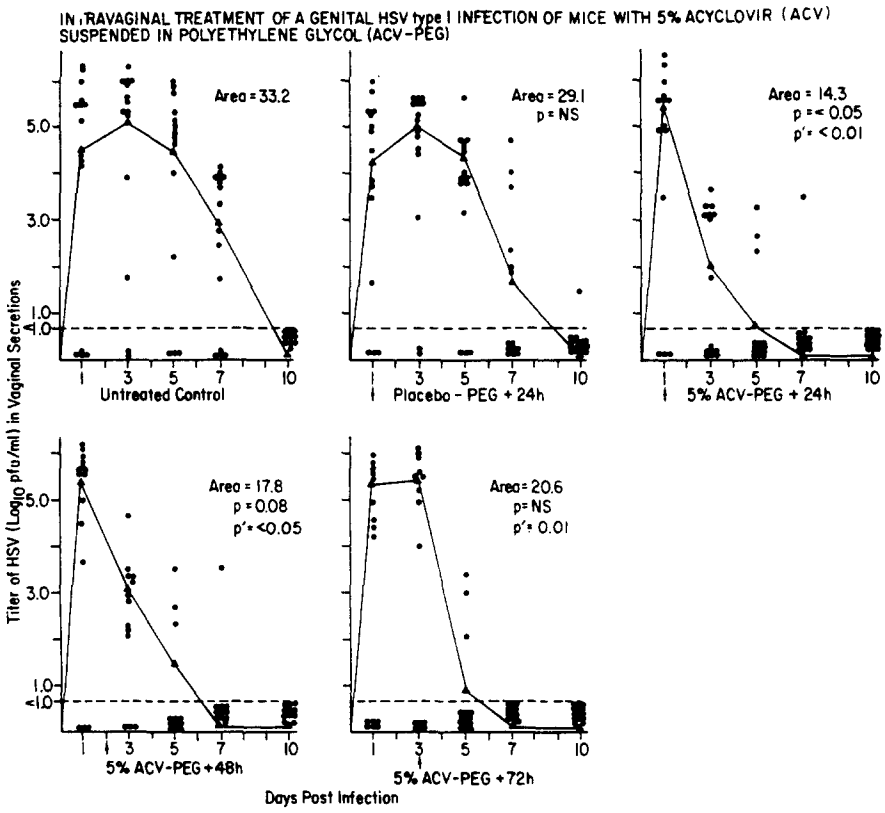

Fig. 4. Treatment of a genital HSV type 1 infection of mice with Acyclovir (ACV) suspended in polyethylene glycol (PEG), 4 times daily for 5 days. Treatment by the intravaginal route was initiated 24, 48 or 72 h after infection. The mean HSV titer on days 1, 3, 5, 7 and 10 is indicated by the triangles.

HSV-1 infection of mice. The effect of treatment with 5% ACV–PEG on HSV-1 replication in the genital tract when begun 24, 48 or 72 h after infection is shown in Fig. 4. Analysis of the curve from the time of initiation of therapy (p') indicated that treatment begun at all 3 times effectively reduced vaginal virus titers. The type 1 strain of HSV used in these studies is relatively non-lethal when inoculated intravaginally so an effect on mortality could not be determined.

Effect of treatment with ACV on the establishment of latent infection

To determine the effect of ACV treatment on the establishment of HSV latency in sacral ganglia, surviving animals from some of the previous experiments were sacrificed, the dorsal root ganglia removed and tested for the presence of latent HSV. The results from these experiments are in Table 5. Due to the high mortality rate in some groups and the low infection rate due to early ACV treatment in others, there were few

TABLE 5

Effect of treatment with ACV on infection, mortality and ganglionic latency rates in mice inoculated intravaginally with HSV-2

Treatment[a]	No. infected/ No. inoculated (%)		No. dead/ No. infected (%)		No. infected Survivors	No positive latent HSV/ No. infected Survivors (%)	
Expt. No. 1							
None	15/15	100	14/15	93	1	NT	
GEL + 3 h	10/15	67	10/10	100	0	--	
1% ACV-gel + 3 h	6/15	40	4/6	67	2	2	100
5% ACV-gel + 3 h	3/15	20	0/3	0	3	0	0
Expt. No. 2							
None	10/15	67	10/10	100	0	--	
Placebo-gel + 24 h	12/15	80	12/12	100	0	--	
1% ACV-gel + 24 h	10/15	67	4/10	40	6	3	50
5% ACV-gel + 24 h	14/15	93	4/14	29	10	6	60
Placebo-PEG + 24 h	13/15	87	13/13	100	0		
1% ACV-PEG + 24 h	11/15	73	8/11	73	3	2	67
5% ACV-PEG + 24 h	12/15	80	5/12	42	7	3	43

[a] Treatment with 0.1 ml of drug 4 times daily for 5 days.

infected animals available for examiniation. Treatment with 5% ACV beginning 3 h after infection appeared to prevent the establishment of latency, however, if therapy with either 1% of 5% ACV in GEL or PEG was delayed until 24 h after infection, a time when virus is already in the peripheral nerves, there was no alteration in the number of mice with latent HSV infection.

Discussion

The HSV-2 and HSV-1 strains tested were equally sensitive to the action of ACV in MEF and HFF cells. Similar results have been reported by others [33, 6]. In MEF cells the mean inhibitory level for ACV was 0.02 μg/ml, whereas in human cells 0.4 μg/ml was required. The levels required in human cells are similar to those reported by Schaeffer et al. [33], Crumpacker et al. [5] and De Clercq [7]. In other studies not presented here, the mean inhibitory level of ACV for inhibition of HSV strains in rabbit and guinea pig cells was similar to that observed in human cells (unpubl. obs.). These data and those reported by De Clercq [7] indicate that ACV is about 100-fold more active against HSV in MEF cells than in cells from other species.

In the HSV-2 genital infection of mice, ACV treatment initiated 3 h after infection reduced significantly the number of animals that became infected. When treatment with ACV was initiated 6 h or later post infection there was no effect on infection rates. Mortality of infected animals was significantly reduced when treatment was begun as late as 24 h after infection. In all experiments 5% ACV was more effective in reducing final mortality than was 1% ACV. Therapy with either 1% or 5% ACV was highly effective in reducing viral replication in the genital tract when initiated at 3, 6 or 24 h. Again 5% ACV appeared to be more effective than the 1% preparations. Additionally, treatment with 5% ACV significantly altered vaginal virus titers when begun as late as 72 h after HSV-2 inoculation. In mice infected ivg with HSV-1, 5% ACV initiated as late as 72 h after inoculation also effectively reduced viral replication in the genital tract.

In comparisons with other antivirals tested in this model infection in our laboratory, SCV appears to be the most active. Topical application of ara-A, ara-AMP [15] iododeoxyuridine (unpubl. results) and 2'-deoxy-D-glucose [18] has failed to alter vaginal viral titers in this model. PAA and PFA applied topically are highly effective in reducing viral replication in the vaginal tract if treatment is initiated by 24 h after infection [15–17]. Some inhibition of viral replication and more rapid clearance of virus from the genital tract was observed with prophylactic or early treatment using murine exogenous interferon or interferon inducers [24].

In a number of other experimental HSV infections, investigators have reported results similar to those obtained in our studies. In HSV-1 infections of rabbit eyes, topical therapy with 3% ACV initiated 3 days after viral inoculation was highly effective in reducing the severity of keratitis and irritis [2, 13, 30, 33, 38]. When mice were inoculated with HSV-1 or HSV-2 in the orofacial or lumbosacral area, topical treatment with 5% initiated 24–48 h after infection also effectively inhibited the development of skin lesions [19, 27, 28]. In guinea pig skin infected with HSV-1,

topical therapy with 1–5% ACV was effective in reducing the severity of lesions when treatment was begun 1–3 days after infection [29, 33].

In our experiments, topical treatment with ACV initiated 3 h, but not 24 h, after HSV-2 inoculation prevented the establishment of latent HSV infection in the few dorsal root ganglia tested. Similar results with topical treatment using ACV has also been reported by other investigators in their model systems [10, 19, 20, 27, 28, 31, 38]. This suggests that, between 3 and 24 h after viral challenge, HSV enters peripheral nerve endings, and is transported up the nerves to sensory ganglia, and that this aspect of the viral infection is not amenable to topical antiviral therapy.

The results from our studies and those of others suggest that topical therapy with ACV may be effective in human mucocutaneous HSV infections by modifying the severity of these lesions. In patients with initial genital herpes that were treated topically with ACV, the mean duration of viral shedding from lesions, mean duration of pain or itching and mean time to healing were reduced significantly compared with placebo-recipients [3, 4]. In two clinical trials in patients with recurrent herpes genitalis or labialis, topical therapy with ACV reduced excretion of virus from lesions, but did not result in a substantial beneficial clinical effect [3, 37]. It may be that therapy in these studies was initiated too late in the course of the recurrent infection to alter lesion severity [35]. Further clincal studies are presently underway to determine the effect of earlier topical treatment and the use of sustemic ACV mucocutaneous HSV infections. The results in the murine model of genital herpes indicate that ACV is the most active topical antiviral tested to date in our laboratory. The data demonstrating that topical ACV also may provide some benefit in the treatment of initial genital herpes in humans, further substantiates the predictability of this experimental infection.

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