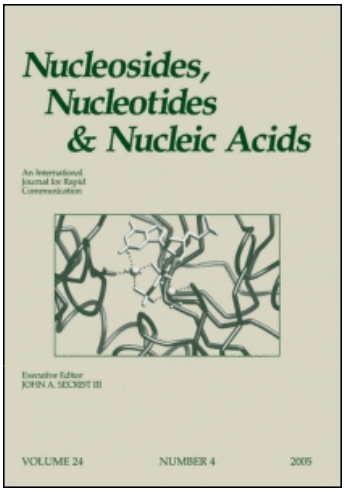

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Efficacy of Topical Acyclovir Monophosphate, Acyclovir, or Penciclovir in Orofacial HSV-1 Infections of Mice and Genital HSV-2 Infections of Guinea Pigs

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EFFICACY OF TOPICAL ACYCLOVIR MONOPHOSPHATE, ACYCLOVIR, OR PENCICLOVIR IN OROFACIAL HSV-1 INFECTIONS OF MICE AND GENITAL HSV-2 INFECTIONS OF GUINEA PIGS

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Dedicated to the memory of Dr. Gertrude B. Elion

ABSTRACT

The purpose of these studies was to compare the efficacy of acyclovir monophosphate (ACVMP), acyclovir (ACV), or penciclovir (PCV) against HSV-1 in an orofacial infection of mice and against ACV sensitive and resistant genital HSV-2 infections of guinea pigs. Treatment was initiated 24, 48, or 72 hours post inoculation with 5% ACVMP, 5% ACV (Zovirax) or 1% PCV (Denavir). In all experiments, similar efficacy was obtained for ACVMP and ACV, whereas PCV was considerably less effective.

INTRODUCTION

Mucocutaneous infections caused by herpes simplex virus type 1 (HSV-1) or 2 (HSV-2) continue to be a problem in a significant portion of the population. In the U.S. it has been estimated that about 20% of the population has clinical manifestations of orolabial infections[1], and genital herpes continues to be one of the most common sexually transmitted diseases. A recent survey indicated that about 22% of people over 12 years of age in the U.S. are seropositive for HSV-2 and, importantly, the seroprevalence of HSV-2 has increased by 30% in the last 20 years[2].

Although parenteral therapy with acyclovir (ACV) has been reasonably effective in reducing severity of primary genital disease[3,4] and prevention of recurrent genital disease[5,6,7,8], topical therapy with ACV (Zovirax) has been largely ineffective for treatment of herpes labialis[9,10]. Some benefit, however, has been reported in the treatment of genital herpes[11,12].

Although ACV administered topically has not been particularly effective against genital and orolabial infections, its lack of efficacy has been attributed in part to the non-penetrating vehicle (polyethylene glycol, PEG) it was formulated in[13]. In other clinical studies utilizing ACV in a vehicle containing propylene glycol (PG) which is thought to enhance penetration results of therapy have been inconsistent[14,15,16,17]. We postulated that ACV monophosphate (ACVMP) in the appropriate vehicle might have superior activity to ACV in that it may penetrate tissue better and be taken up more readily by cells than ACV which must first be phosphorylated by HSV thymidine kinase (TK). It was further postulated that ACVMP might be active against HSV isolates that have lost their TK activity and are resistant to the activity of ACV. A disadvantage of ACVMP is that it would not exhibit the specificity of ACV and would enter both uninfected and infected cells equally, thus incurring the possibility of increased toxicity. However human DNA polymerase α is 30-50 fold more selective for dGTP than for ACV-triphosphate[18]. Penciclovir (PCV) is another acyclic nucleoside analogue whose mechanism of action is similar to that of ACV[19]. It's activity in tissue culture and in experimental animal infections is also similar to that observed with ACV[20,21]. The topical preparation of PCV (Denavir) also contains PG and has been evaluated in one large patient-initiated clinical trial of herpes labialis and demonstrated efficacy against both clinical and laboratory measures[22].

The purpose of the experiments presented in this paper was to compare the efficacy of 5% ACVMP, 5% ACV, and 1% PCV in cutaneous HSV-1 infections of mice and genital HSV-2 infections of guinea pigs. We utilized hairless mice inoculated on the snout with HSV-1 as a model for HSV-1 herpes labialis and guinea pigs inoculated intravaginally with HSV-2 as a model for genital herpes.

RESULTS AND DISCUSSION

In Vitro Evaluation

The *in vitro* activity of ACVMP, ACV, and PCV against a variety of HSV type 1 and 2 strains yielded results that were comparable. Both ACV-sensitive and ACV-resistant

strains were evaluated, with ACVMP being effective against ACV-sensitive strains, but ineffective against ACV-resistant strains. PCV had similar levels of activity against ACV-susceptible viruses and had slight activity against ACV-resistant strains (Table 1). These data are similar to those published previously[20]. It is puzzling why ACVMP was not active against HSV strains that lack TK, since the phosphonate nucleotides such as Cidofovir have activity that is comparable to wild type virus. It is possible that ACVMP is either not taken up by cells or is rapidly degraded to ACV upon entry into cells. Studies on uptake or anabolism of ACVMP was beyond the scope of these studies.

In Vivo Evaluation

Prior to the initiation of efficacy studies, the local toxicity of each drug administered topically was determined. There were no toxic effects observed in any of the mice or guinea pigs treated with ACVMP, ACV, or PCV regardless of treatment regimen utilized. Matched vehicle was available for ACVMP, however, since ACV and PCV were obtained commercially, the vehicle for these preparations was not readily available. We have reported previously that the PEG vehicle used for ACV or the PG vehicle for PCV did not alter genital HSV-2 infections or cutaneous HSV-1 infections of mice or guinea pigs when administered under the same conditions as these experiments[21,22]

Orofacial HSV-1 infections of mice.

The purpose of the first experiment was to determine the effects of early treatment with ACVMP or ACV in an HSV-1 orofacial infection. Treatment initiated 24 hours after inoculation prevented both viral replication and the development of lesions in most animals (Table 2). Virus titer-day area under the curve (AUC) values and mean peak virus titers are shown in Table 2A. Virus was recovered from 80% of the vehicle treated mice, but only from 0% or 20% of mice given ACVMP or ACV, respectively. Treatment with ACVMP or ACV significantly reduced lesion virus titer-day AUC and mean peak virus titers. In contrast, PCV at 1% had no effect on infection rates, virus titer-day AUC or peak virus titers. In HSV-1 inoculated animals, 70% treated with vehicle developed lesions on the snout and facial area (Table 2B). Treatment with 5% ACVMP or 5% ACV significantly reduced both the lesion score-day AUC and the mean peak lesion score. Although treatment with 1% PCV reduced both lesion score measures the results were not statistically significant, probably due to the low number of animals that became infected and developed lesions. All three compounds effectively reduced final mortality rates (data not presented). To determine the effect of therapy initiated later in the course

TABLE 1

Comparison of ACVMP and ACV Against HSV Strains

Strain	TK Phenotype	Drug EC_{50} (μg/ml ACVMP	ACV	PCV
HSV-1				
E-377	Wild type	1.5	0.72	0.40
HL-3	Wild type	1.5	1.1	0.24
DM2.1	Deficient	>100	>100	29.1
11359	Deficient	>100	>100	26.4
HSV-2				
MS	Wild type	0.46	0.45	0.60
G	Wild type	0.16	0.13	3.0
AG-3	Deficient	>100	89.3	36.1
12247	Altered	>100	81.2	>83.5

of disease, treatment with all three drugs was initiated at 48 or 72 hours after viral inoculation (Table 3). It should be noted that in this model of infection, HSV-1 replication is first detected on the skin by 48 hours and peak virus titers occur on days 4-6. Lesions first appear on day 4 and peak lesion scores occur during days 7-10 with healing by about day 18 (unpublished results). When treatment was begun 48 hours after viral inoculation, all three compounds reduced the lesion virus titer-day AUC, but only ACVMP and ACV reduced the mean peak virus titer (Table 3A). Both ACVMP and ACV were highly effective in reducing the number of animals that developed lesions and significantly reduced both the lesion score-day AUC and the mean peak lesion score. Although treatment with PCV was less effective, it did reduce both measures of lesion severity (Table 3B). When therapy was delayed until 72 hours both ACVMP and ACV both effectively reduced lesion virus titers, and lesion scores whereas PCV was ineffective (Table 3A,B). In the clinical study evaluating 1% PCV for herpes labialis, it was pointed out that greater efficacy was obtained when treatment was initiated during the early stages of infection[23].

TABLE 2

Effect of Early Treatment with ACVMP, ACV, or PCV on Virus Replication and Lesion Severity in an Orofacial HSV-1 Infection of SKH-1 Mice

A Treatment[a]	#Virus Positive/ # Inoculated	Lesion Virus Titer[b]	P-Value	Mean Peak Virus Titer	P-Value
Vehicle –PG	8/10	13.1	---	3.4	---
5% ACVMP-PG	0/10	0	<0.001	0.0	0.001
5% ACV-PEG	2/10	0	<0.001	0.2	0.002
1% PCV-PG	6/10	6.2	0.1	2.4	NS[c]

B Treatment[a]	# With Lesions/ # Inoculated	Lesion Score[b]	P-Value	Mean Peak Lesion Score	P-Value
Vehicle –PG	7/10	12.0	---	3.1±2.0	---
5% ACVMP-PG	3/10	0.3	0.001	0.3±0.4	0.01
5% ACV-PEG	1/10	0.4	0.002	0.2±0.7	<0.01
1% PCV-PG	5/10	3.2	0.1	1.2±1.4	0.06

a. Treatment was initiated 24 hours post inoculation and continued 3 times per day for 7 days.
b. Area under the curve.
c. Not statistically significant when compared to vehicle treated group.

In the orofacial HSV-1 infection of mice, both 5% ACVMP and 5% ACV gave essentially identical results, and altered both virus replication and lesion development when therapy was initiated as late as 72 hours after viral inoculation. Under the same conditions, 1% PCV was effective only when treatment was begun at 24 hours. The difference in efficacy might be explained by the difference in concentration of the compounds, that is 5% ACVMP and ACV versus 1% PCV. However, PCV triphosphate has been reported to persist in HSV infected cells for about 20 hours longer than ACV triphosphate, which could offer PCV a pharmacological advantage[24] and thus negate the concentration difference. Although one can argue that it is not valid to compare ACV

TABLE 3

Effect of Delayed Treatment with ACVMP, ACV, or PCV on Virus Replication and Lesion Severity in an Orofacial HSV-1 Infection of SKH-1 Mice

A

Treatment	#Virus Positive/ # Inoculated	Lesion Virus Titer[a]	P-Value	Mean Peak Virus Titer	P-Value
Vehicle –PG[b]	7/8	14.7	---	4.6±1.4	---
5% ACVMP-PG[b]	6/8	4.6	<0.05	2.7±2.3	0.08
5% ACV-PEG[b]	8/9	5.5	<0.05	2.9±2.2	<0.05
1% PCV-PG[b]	7/8	8.9	0.06	4.0±2.0	NS
5% ACVMP-PG[c]	7/8	6.7	0.08	4.3±1.1	NS[d]
5% ACV-PEG[c]	7/8	5.5	<0.05	3.1±1.6	0.07
1% PCV-PG[c]	8/8	15.0	NS	5.0±0.4	NS

B

Treatment	# With Lesions/ # Inoculated	Lesion Score[a]	P-Value	Mean Peak Lesion Score	P-Value
Vehicle -PG[b]	7/8	13.9	---	2.4±1.2	---
5% ACVMP-PG[b]	2/8	1.2	<0.01	0.3±0.5	<0.01
5% ACV-PEG[b]	2/9	0.5	<0.001	0.3±0.6	<0.01
1% PCV-PG[b]	5/8	5.2	<0.05	1.4±1.3	0.08
5% ACVMP-PG[c]	7/8	4.9	<0.05	1.4±1.0	0.07
5% ACV-PEG[c]	7/8	5.4	<0.05	1.7±1.1	NS
1% PCV-PG[c]	8/8	10.4	NS	2.7±0.6	NS

a. Area under the curve.
b. Treatment was initiated 48 hours post inoculation and continued 3 times per day for 7 days.
c. Treatment was initiated 72 hours post inoculation and continued 3 times per day for 7 days.
d. NS = Not Statistically Significant when compared to vehicle-treated group.

and PCV at different concentrations, we elected to evaluate the products that are currently licensed for use and have been utilized in clinical studies.

Genital HSV-2 infection of guinea pigs.

We have utilized guinea pigs inoculated intravaginally with HSV as a model for genital herpes for more than two decades. The model closely mimics the disease seen in humans and has been predictive for the effectiveness of antiviral agents directed against this disease[22,25,26]. In the first study we compared 5% ACVMP and 5% ACV for their ability to alter viral replication in the vaginal tract, viral replication in external genital lesions and the development and severity of external genital lesions. The results are presented in Table 4. When treatment was initiated 24 hours after viral inoculation, both ACVMP and ACV significantly reduced the vaginal virus titer-day AUC (Table 4A), lesion virus titer-day AUC (Table 4B), and the lesion score-day AUC (Table 4C). In general ACVMP appeared to be slightly more active, however, the vehicle that ACVMP was formulated in, which contained PG, also had a significant effect. In a second experiment the two drugs were evaluated as to their ability to alter a genital HSV-2 infection caused by a virus strain, AG-3, that was resistant to ACV in vitro (Table 1). This experiment was performed to determine if ACVMP might be active against ACV-resistant HSV since it is not dependent on HSV-TK for phosphorylation to its active state. The results summarized in Table 5 clearly indicate that treatment with either ACVMP or ACV had no effect on vaginal virus replication (Table 5A), lesion virus replication (Table 5B), or lesion severity (Table 5C). In a third experiment, we determined the ability of topical ACVMP to prevent infection and replication of HSV-2 when drug was administered 2 hours prior to HSV-2 inoculation. Under these conditions pretreatment with 5% ACVMP reduced infection rates to 70% of control animals and only 40% of animals developed clinical lesions. In addition, vaginal virus titers, lesion virus titers, and lesion scores were reduced significantly (data not presented).

In summary, in the in vitro assays, in an orofacial HSV-1 infection of mice and in a genital HSV-2 infection of guinea pigs, 5% ACVMP had similar activity as 5% ACV. Using the monophosphate of ACV, or using a vehicle containing PG rather than PEG, did not appear to provide a significant difference. In the orofacial model, 1% PCV was clearly less effective than 5% ACVMP or 5% ACV.

TABLE 4

Effect of ACVMP or ACV Treatment on Vaginal Virus Replication, Lesion Virus Replication, and Lesion Severity in a Primary Genital HSV-2, G Infection of Guinea Pigs

A

Treatment[a]	#Virus Positive/ # Inoculated	Vaginal Virus Titer[b]	P-Value	Mean Peak Virus Titer	P-Value
Placebo-PBS	10/10	25.7	---	4.8±0.5	---
Vehicle-PG	7/10	12.3	0.01	2.9±2.4	NS[c]
5% ACVMP-PG	6/10	3.5	<0.01	1.6±1.9	NS
5% ACV-PEG	9/10	11.6	<0.01	3.8±1.6	0.05

B

Treatment[a]	# Virus Positive/ # Inoculated	Lesion Virus Titer[b]	P-Value	Mean Peak Lesion Score	P-Value
Placebo-PBS	10/10	13.5	---	3.9±0.6	---
Vehicle-PG	5/10	5.9	<0.05	1.8±2.0	0.01
5% ACVMP-PG	3/10	1.5	0.01	0.7±1.3	NS[c]
5% ACV-PEG	3/10	1.6	<0.001	1.1±1.8	0.001

C

Treatment[a]	# With Lesions/ # Inoculated	Lesion Score[b]	P-Value	Mean Peak Lesion Score	P-Value
Placebo-PBS	9/10	32.1	---	2.8±1.5	---
Vehicle-PG	6/10	19.0	0.001	1.7±1.8	NS
5% ACVMP-PG	3/10	6.8	0.0001	0.7±1.3	NS
5% ACV-PEG	8/10	11.9	<0.001	1.5±1.2	NS

a. Treatment was initiated 24 hours post inoculation and continued 3 times per day for 7 days.
b. Area under the curve.
c. NS = Not Statistically Significant when compared to appropriate placebo or vehicle-treated group.

TABLE 5

Effect of ACVMP or ACV Treatment on Vaginal Virus Replication, Lesion Virus Replication, and Lesion Severity in a Primary Genital HSV-2, AG-3 Infection of Guinea Pigs

A Treatment[a]	#Virus Positive/ # Inoculated	Vaginal Virus Titer[b]	P-Value	Mean Peak Virus Titer	P-Value
Placebo-PBS	10/10	33.3	---	5.7±0.6	---
Vehicle-PG	10/10	31.6	NS[c]	5.6±0.8	NS
5% ACVMP-PG	10/10	24.0	NS	5.5±1.1	NS
5% ACV-PEG	8/10	26.3	NS	4.7±2.6	NS

B Treatment[a]	# Virus Positive/ # Inoculated	Lesion Virus Titer[b]	P-Value	Mean Peak Virus Titer	P-Value
Placebo-PBS	10/10	21.2	---	4.4±0.6	---
Vehicle-PG	10/10	21.3	NS	4.4±0.6	NS
5% ACVMP-PG	10/10	16.4	NS	4.6±0.6	NS
5% ACV-PEG	9/10	17.9	NS	4.1±2.0	NS

C Treatment[a]	# With Lesions/ # Inoculated	Lesion Score[b]	P-Value	Mean Peak Lesion Score	P-Value
Placebo-PBS	10/10	37.5	---	3.1±0.7	---
Vehicle-PG	10/10	50.8	<0.001↑	4.0±0.6	0.01↑
5% ACVMP-PG	10/10	50.9	NS	3.8±1.0	NS
5% ACV-PEG	9/10	35.8	NS	2.8±1.4	NS

a. Treatment was initiated 24 hours post inoculation and continued three times per day for seven days.
b. Area under the curve.
c. NS = Not Statistically Significant when compared to appropriate placebo or vehicle-treated group.

EXPERIMENTAL

In Vitro Efficacy

The activity of ACVMP, ACV, and PCV against HSV strains *in vitro* was determined using a plaque reduction assay in human foreskin fibroblast (HFF) cells, as previously reported[27].

In Vivo Evaluation

5% ACVMP in a propylene glycol (PG) base was provided through the Antiviral Substance Program, NIAID, NIH, by Triangle Pharmaceuticals, Inc., Durham, N.C. 5% ACV in PEG (Zovirax) and PCV in a PG formulation (Denavir) was obtained through UAB Hospital Pharmacy. SKH-1 hairless immunocompetent mice (Charles River Laboratories) were anesthetized with ketamine/xylazine mixture i.p. and tattooed (Harvard Apparatus, Holliston, Mass.), for individual identification. The snout area, composed of a triangular shaped area over the nasal bones from the nose-bridge to the eyes, was lightly abraded with a Dremmel tool with a #113 tungsten-carbide engraving bit[28]. Care was taken to avoid bleeding. Groups of 8-10 SKH-1 mice were inoculated orofacially (snout) with 5 x 10^5 pfu/ml of HSV-1, E-377 using a dacron swab soaked in a virus suspension that titered 2 x 10^6 pfu/ml. Swabs of the snout area were taken on days 1-7 and 10 for quantitation of viral replication. Lesions were scored and mortality recorded daily for 21 days.

Female Hartley guinea pigs weighing 300-350 grams (Charles River Laboratories), were used in these studies. In order to increase the efficiency of HSV infection, guinea pigs were swabbed with a dry dacron applicator one hour prior to vaginal inoculation to remove vaginal secretions. Vaginal inoculation with HSV-2 was accomplished using a dacron tipped swab soaked in an appropriate virus concentration that would result in a 90-100% infection rate (1 x 10^5 pfu/ml), which was then inserted into the vaginal tract and rotated approximately 10 times. To determine the effect of treatment on virus replication, swabs of vaginal secretions were obtained on days 1, 3, 5, 7, and 10 after viral inoculation. Swabs were placed in 2.0 ml of tissue culture media (10% fetal bovine serum, 2 mM L-glutamine, 200 units/ml penicillin, 50 μg/ml gentamicin, 3.3 μg/ml Amphotericin B) and frozen at -70°C until titrated for HSV on rabbit kidney fibroblast cells using a microtiter CPE assay. Genital lesion virus replication was evaluated by obtaining lesion swabs on days 3, 4, 5, 6, 7, 8, and 10 post inoculation. Lesion severity

was scored on a 0 to +5 scale in 0.5 increments, from days 3-21[22,25,26]. Vehicle treated groups were compared to PBS-placebo treated groups. Animals that received therapy were compared to the appropriate vehicle treated group. Virus titer-day AUC, mean peak virus titers, lesion score-day AUC, and mean peak lesion scores were compared using the Mann-Whitney u rank sum test. A p-value of 0.05 or less was considered significant.

Treatment with ACVMP, ACV, or PCV in the HSV-1 orofacial infection of mice.

In the first experiment, groups of mice were inoculated orofacially with HSV-1 and treatment was initiated 24 hours after inoculation and continued three times a day for seven days. Animals were treated with 5% ACVMP, 5% ACV, or 1% PCV. Control animals were treated with placebo (ACVMP vehicle) three times per day for seven days. In the second study, we evaluated the effect of delaying treatment until either 48 or 72 hours post inoculation. Mice were inoculated and treated as above.

Treatment with ACVMP or ACV in a genital HSV-2 infection of guinea pigs.

In the first experiment, groups of 10 guinea pigs were inoculated intravaginally (ivg), with 1 x 10^5 pfu of HSV-2, G and treatment was initiated 24 hours after inoculation and continued three times a day for seven days. Animals were treated with placebo, PG vehicle, 5% ACVMP-PG, or 5% ACV-PEG. Toxicity was also evaluated in this experiment using uninfected animals treated as stated above. In the second study, efficacy against an ACV resistant virus (AG-3) was tested. Guinea pigs were inoculated with 4 x 10^5 pfu of HSV-2, AG-3 and treated as above. The next study evaluated the efficacy of pretreatment with ACVMP. Animals were inoculated with HSV-2, G two hours after ivg treatment with ACVMP-PG.

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