Efficacies of Topical Formulations of Foscarnet and Acyclovir and of 5-Percent Acyclovir Ointment (Zovirax) in a Murine Model of Cutaneous Herpes Simplex Virus Type 1 Infection

JOCELYNE PIRET,1 ANDRÉ DESORMEAUX,1 PIERRETTE GOURDE,1 JULIANNA JUHÁSZ,2 AND MICHEL G. BERGERON1*

Centre de Recherche en Infectiologie1 and Faculté de Pharmacie,2 Université Laval, Québec, Québec, Canada

Received 11 May 1999/Returned for modification 20 July 1999/Accepted 11 October 1999

The topical efficacies of foscarnet and acyclovir incorporated into a polyoxypropylene-polyoxyethylene polymer were evaluated and compared to that of 5% acyclovir ointment (Zovirax) by use of a murine model of cutaneous herpes simplex virus type 1 infection. All three treatments given three times daily for 4 days and initiated 24 h after infection prevented the development of the zosteriform rash in mice. The acyclovir formulation and the acyclovir ointment reduced the virus titers below detectable levels in skin samples from the majority of mice, whereas the foscarnet formulation has less of an antiviral effect. Reducing the number of treatments to a single application given 24 h postinfection resulted in a significantly higher efficacy of the formulation of acyclovir than of the acyclovir ointment. Acyclovir incorporated within the polymer was also significantly more effective than the acyclovir ointment when treatment was initiated on day 5 postinfection. The higher efficacy of the acyclovir formulation than of the acyclovir ointment is attributed to the semisviscous character of the polymer, which allows better penetration of the drug into the skin.

Herpes simplex virus (HSV) type 1 (HSV-1) and HSV type 2 (HSV-2) have the ability to become latent in sensory ganglia and to induce recurrent infections following reactivation (23). The frequencies of recurrent herpetic infections in the U.S. population are estimated to be 50 to 70% for HSV-1 and 23% for HSV-2 (36). Mucosal or skin surfaces are the usual sites of primary infection. Recurrent herpes labialis and herpes genitalis represent the most common clinical manifestations associated with HSV-1 and HSV-2 infections, respectively. Most recurrences are asymptomatic infections, and the shedding of herpesvirus under these conditions represents the most common form of transmission of this disease. Recurrences are associated with physical or emotional stress, fever, exposure to UV light, tissue damage, and immune suppression. The frequency of recurrences has also been correlated with the severity and duration of the initial infection (36). Although herpes is usually a mild disease in immunocompetent individuals, mucocutaneous herpetic infections are troublesome, especially for patients with frequent episodes. Moreover, immunocompromised patients have an increased risk of developing severe and more frequent herpetic infections.

During the past several decades, acyclovir has been the drug of choice for the treatment of herpetic infections. However, the emergence of acyclovir-resistant HSV isolates has been reported for immunocompromised patients (9) as well as for organ and bone marrow recipients (16, 33). Recurrent acyclovir-resistant genital herpes has also been described for an immunocompetent host (13). Foscarnet (trisodium phosphonofluoridate) has a broad antiviral spectrum and in vitro activity against all human viruses of the herpesvirus family, including cytomegalovirus, HSV, and varicella-zoster virus (5, 20). This drug is also effective against acyclovir-resistant HSV and varicella-zoster virus (4, 10, 25–27). Moreover, acyclovir-resistant HSV strains that become resistant to foscarnet may once again be susceptible to acyclovir (28). Because the intravenous administration of foscarnet is limited by the occurrence of nephrotoxic reactions, the development of topical formulations represents an attractive approach for the treatment of mucocutaneous herpetic infections, especially for those caused by acyclovir-resistant strains.

Topical formulations currently available for the treatment of mucocutaneous herpetic infections include 5% acyclovir ointment (Zovirax) and penciclovir cream formulation (Vectavir cold sore cream or Denavir cream in the United States). The currently available treatment, either topical or systemic, has only limited efficacy, particularly against symptomatic recurrent herpes. Treatment of recurrent herpes with topical acyclovir demonstrated no or only limited clinical benefit (6, 18, 22, 30). Wallin et al. demonstrated a limited but significant effect of topical foscarnet cream on time to healing for recurrent genital herpes (34). Conversely, no significant improvements in time to healing or loss of symptoms were observed for recurrent genital herpes in two other clinical trials (2, 24). Patients who received treatment in the prevesicular stage had a slightly reduced number of days with lesions (14). Treatment of herpes labialis in immunocompetent patients with penciclovir cream was reported beneficial for treatment started in the prodrome and erythema stages as well as in the papule and vesicle lesion stages (32).

In this study, we used a polymer composed of polyoxypropylene and polyoxyethylene as a new vehicle for acyclovir and foscarnet to evaluate if the semiviscous character of this galenic form could allow efficient drug penetration into the skin, thereby increasing the efficacies of these drugs against HSV-1 cutaneous lesions in mice. The topical efficacies of acyclovir and foscarnet incorporated into the polymer matrix were also compared with that of the commercially available 5% acyclovir ointment.

* Corresponding author. Present address: Centre de Recherche en Infectiologie, Centre Hospitalier Universitaire de Québec, Pavillon CHUL, 2705 Blvd. Laurier, Sainte-Foy, Québec, Canada G1V 4G2. Phone: (418) 654-2705. Fax: (418) 654-2715. E-mail: Michel.G.Bergeron@crchul.ulaval.ca.
VOL. 44, 2000  TOPICAL FORMULATIONS AGAINST HSV-1 INFECTION 31

MATERIALS AND METHODS

Drugs. Acyclovir (9-[(2-hydroxyethoxy)methyl]guanine) and foscarnet (trisodium phosphonoformate) were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). (1,3,5,6-Tetrasodium-2-cyclohexane-1,2-dicarboxylate and [15N]foscarnet were obtained from Morinaga Co., Calif.). The commercially available 5% acyclovir ointment was obtained from our local hospital pharmacy.

Preparation of the topical formulations. For the formulations of acyclovir and foscarnet, we used a polymer (gel) composed of polyoxypropylene and polyoxyethylene suspended in phosphate buffer (200 mM, pH 6.0) at a concentration of 18% (w/w). We selected a pH of 6.0 to correspond with the pH of the skin. For the formulation of foscarnet, the drug was first dissolved in phosphate buffer (200 mM, pH 6.0) at a concentration of 6 or 1% (w/w). The pH of the solution was then readjusted to 6.0 by adding a small amount of 1 N HCl. The solution was then mixed under agitation at 4°C with an equal volume of the polymer solution prepared in the same buffer to obtain a final foscarnet concentration of 3 or 0.5% (w/w). For the formulation of acyclovir, the antiviral agent was first dissolved in dimethyl sulfoxide (DMSO) and then mixed at 4°C with the polymer powder and phosphate buffer (200 mM, pH 6.0) to obtain a final drug concentration of 5, 3, or 1% (w/w). The pH of the formulation of acyclovir was then adjusted to 6.0. The final amount of DMSO present in the formulation was 12.5%.

Virus strain. HSV-1 strain F (American Type Culture Collection, Manassas, Va.) was propagated in Vero (African green monkey kidney) cells (American Type Culture Collection) in Eagle’s minimum essential medium (Canadian Life Technologies, Burlington, Ontario, Canada) supplemented with 0.22% sodium bicarbonate, 100 U of penicillin-streptomycin per ml, 2 mL-glutamine, and 2% fetal bovine serum (EMEM + 2% FBS) to obtain a viral inoculum of 1.5 x 10^6 PFU/ml.

Plaque reduction assay. Vero cells seeded in 24-well plates (Costar, Montréal, Québec, Canada) were infected with approximately 100 PFU of HSV-1 strain F in 0.5 ml of EMEM + 2% FBS for 2 h at 37°C in a 5% CO2 atmosphere. Cell sheets were washed twice with fresh culture medium, overlaid with 0.5 ml of 0.5% SeaPlaque agarose (Marine Colloids, Rockland, Maine) in EMEM + 2% FBS containing increasing amounts of the drug under study, and incubated for 2 days at 37°C. Cells were then fixed with 10% formaldehyde in phosphate-buffered saline for 20 min, washed with deionized water, and stained with 0.05% methylene blue.

Animal model. Female hairless mice (SKH1; 5 to 7 weeks old; Charles River Breeding Laboratories Inc., St. Constant, Québec, Canada) were used throughout this study. Mice were anesthetized by intraperitoneal injection of a mixture containing 70 mg of ketamine hydrochloride (Rogar/STB Inc., Montréal, Québec, Canada) and 11.5 mg of xylazine (Miles Canada Inc., Etobicoke, Ontario, Canada) per kg of body weight. The virus was inoculated on the lateral side of the body in the left lumbar skin area. The skin was scratched six times in a crossed-hatch pattern with a 27-gauge needle held vertically. A viral suspension (5 x 10^4 PFU/50 µl) was rubbed for 10 to 15 s on the scarified skin area with a cotton-tipped applicator saturated with EMEM + 2% FBS. The scarified area was protected with adhesive tape (Tegaderm; 3M Canada, London, Ontario, Canada) to prevent accidental systemic administration that could result from licking and grooming. For treatments initiated 5 days after infection (i.e., at the onset of the zosteriform rash), the corn cushion was removed and the scarified area was closed with surgical tape. The porous inner wall of the aperture of the corn cushion was made impermeable with tissue adhesive ( Vet-bond, St. Paul, Minn.) prior to use to prevent drug absorption by the back of the animal which could act as a reservoir for the accumulation of the formulations. The aperture of the corn cushion was also closed with surgical tape. Mice were then returned to their cages and observed twice daily.

Treatments. Different treatment regimens were evaluated in this study. For treatments initiated 5 days after infection (i.e., at the onset of the zosteriform rash), the surgical tape closing the aperture of the corn cushion was removed and the scarified area was closed with a cotton-tipped applicator saturated with cold water to remove gel or ointment remaining from the last application. Fifteen microliters of the polymer alone, of the polymer containing foscarnet or acyclovir or a similar amount of acyclovir ointment was applied to the scarified area. The aperture of the corn cushion was closed with surgical tape to avoid systemic administration that could result from licking and grooming. For treatments initiated 5 days after infection (i.e., at the onset of the zosteriform rash), the corn cushion was removed. The entire zosteriform lesion was treated with 50 µl of the foscarnet or acyclovir formulation or a similar amount of acyclovir ointment. The treated area was protected with adhesive tape (Tegaderm; 3M Canada, London, Ontario, Canada) to prevent accidental systemic treatment that could occur due to licking of drug on the treated lesion. The treated area was cleaned with a cotton-tipped applicator saturated with cold water to remove gel or ointment remaining from the last application. Three daily treatments were given at 8:00 a.m., 2:00 p.m., and 9:00 p.m., as these times represent convenient times for self-application by patients. Seven to 13 animals per group were used for all experiments. The efficacies of the different treatments were evaluated by use of lesion scores, survival rates, and viral titers in skin samples. We used a Chi-square test between treatment groups were undertaken in this study.

Determination of viral titers in skin samples. The extent of inhibition of HSV-1 replication in skin samples of mice was determined 5 days after virus inoculation. Peritoneal cavity experiments showed that viral titers in skin samples were maximum on days 4 and 5 postinfection. In brief, mice were sacrificed, and the site of virus inoculation and the lower flank (a skin area located between the virus inoculation site and the ventral midline but not touching the inoculation site) were excised. Skin samples were maintained in Hank’s balanced salt solution (Canadian Life Technologies) at 4°C, blotted, weighed, and diluted with 1 ml of EMEM + 2% FBS. Viruses were released from skin samples by three cycles of sonication for 10 s each with a 5-s interval. The suspension obtained was centrifuged (1,000 x g for 15 min at 4°C). The supernatant was collected and stored at −80°C until use. Titration of viruses in skin samples was done by determining PFU on Vero cells in cultures. We used a method essentially similar to that described for the plaque reduction assay, except that infection of cells with viruses extracted from skin samples was done by centrifuging the plates (700 x g for 45 min at 20°C).

In vivo skin penetration studies. Mice were infected cutaneously with HSV-1 in order to obtain a fully developed zosteriform rash. On day 5 postinfection, a corn cushion was placed on the inoculation site of infected mice. A maximal mean lesion score was observed during the first 4 days following infection, and only infected mice, no pathological signs of cutaneous infection were visible during the first 4 days following infection, and only the scarified area remained apparent. On day 5, herpetic skin lesions began to appear on some mice in the form of small vesicles distant from the inoculation site. On day 6, almost all untreated infected mice developed herpetic skin lesions in the form of a 4- to 5-mm-wide band extending from the spine to the ventral midline of the infected dermatome, similar to zoster-like infections. A maximal mean lesion score was observed on day 8. The mean lesion score decreased thereafter from day 12 to day 15 because of spontaneous regression of cutaneous
lesions in some mice. For mice treated with the polymer alone, we observed a pattern largely similar to that for untreated infected mice, suggesting that the polymer alone had no therapeutic effect on the development of lesions. However, infected mice treated with all three drug formulations showed a significant reduction of the mean lesion score compared to untreated infected mice and mice treated with the polymer alone (Table 2). The decrease in the mean lesion score was less pronounced in mice receiving the polymer containing 0.5% foscarnet (data not shown). Acyclovir incorporated into the polymer at concentrations of 1, 3, and 5% demonstrated a dose-dependent effect in reducing the mean lesion score of infected mice, but the differences between doses were not significant (data not shown).

Figure 1B shows the corresponding survival rates of the animal groups mentioned above. Fifty percent of untreated infected mice died from encephalitis between day 7 and day 10. In mice receiving the polymer alone, the lethality of infection was 60%, suggesting once again that the polymer alone had no therapeutic effect against the infection. On the other hand, all mice treated with the polymer containing 3% foscarnet or 5% acyclovir or with the acyclovir ointment survived the infection. In mice treated with a formulation containing 0.5% foscarnet, the survival rate was 90% ($P < 0.001$), whereas the survival rates of mice treated with topical formulations containing 1 and 3% acyclovir were 90% ($P < 0.05$) and 100% ($P < 0.001$), respectively (data not shown).

Figure 2 shows viral titers measured in skin samples corresponding to the inoculation site (Fig. 2A) and to the lower flank (Fig. 2B) on day 5 postinfection. The polymer alone could not significantly reduce the virus content either at the site of inoculation or in the lower flank. Treatment with the polymer containing 3% foscarnet resulted in a significant decrease in the virus content in skin samples. This decrease was more pronounced at the inoculation site than in the lower flank. Of prime interest, acyclovir incorporated into the polymer and acyclovir ointment caused a marked and significant reduction (often below the limit of detection of the assay) of the viral titers both at the inoculation site and in the lower flank.

**Effect of reducing the number of treatments.** Figure 3A shows the time evolution of the mean lesion scores for untreated infected mice and for infected mice treated with a single application of polymer containing 3% foscarnet or 5% acyclovir or with the acyclovir ointment given at 24 h postinfection. Preliminary experiments demonstrated that the polymer alone did not exert any effect on the development of herpetic cutaneous lesions in this treatment regimen (data not shown). For mice treated with the formulation containing 3% foscarnet, the evolution of the mean lesion score was similar to that observed for untreated infected mice. Of prime interest, the topical formulation containing 5% acyclovir reduced significantly the development of cutaneous lesions compared to the results for untreated infected mice and mice treated with the acyclovir ointment (Table 2), which exerted only a modest effect. The formulation containing 3% foscarnet given only once delayed mortality but could not increase the survival rate compared to that of untreated infected mice (Fig. 3B). However, 5% acyclovir incorporated into the polymer significantly reduced the lethality of the infection ($P < 0.001$), but the acyclovir ointment did not. On the other hand, viral titers determined at the inoculation site and in the lower flank of mice treated with a single application of the polymer alone, polymer containing 3% foscarnet or 5% acyclovir or with 5% acyclovir ointment on day 5 postinfection were not markedly decreased compared to those in untreated infected mice (data not shown).

The topical efficacies of the different treatments were also evaluated after daily treatment for 4 days starting at 24 h postinfection. No major differences in efficacy could be seen between the polymer formulations containing 5% acyclovir or 3% foscarnet and the acyclovir ointment (data not shown). In addition, all topical treatments used in this regimen significantly increased survival rates ($P < 0.05$).

**TABLE 1. Criteria used for the evaluation of herpetic cutaneous lesions**

<table>
<thead>
<tr>
<th>Score</th>
<th>Appearance of the lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No visible infection</td>
</tr>
<tr>
<td>1</td>
<td>Infection at inoculation site in scarification area only</td>
</tr>
<tr>
<td>2</td>
<td>Infection at inoculation site only, with swelling, crusts, and erythema</td>
</tr>
<tr>
<td>3</td>
<td>Infection at inoculation site, with discrete lesions forming away from inoculation site</td>
</tr>
<tr>
<td>4</td>
<td>Rash visible around half of body but not yet confluent</td>
</tr>
<tr>
<td>5</td>
<td>Rash confluent but not yet necrotic or ulcerated</td>
</tr>
<tr>
<td>6</td>
<td>Complete rash with necrosis or ulceration, hind limb paralysis, bloating, and death</td>
</tr>
</tbody>
</table>

*Adapted from Lobe et al. (17).*
Effect of delaying the treatments. Figure 4 shows the time evolution of the mean lesion score (Fig. 4A) and survival (Fig. 4B) for untreated infected mice and for infected mice treated three times daily for 4 days starting on day 5 postinfection with the polymer alone, with the polymer containing 3% foscarnet or 5% acyclovir, or with the acyclovir ointment. The results showed that treatment with the polymer alone, with the polymer containing 3% foscarnet, or with the acyclovir ointment exerted a modest but not significant effect compared to the results obtained for untreated infected mice. In contrast, a significant reduction of the mean lesion score was observed for mice treated with the polymer containing 5% acyclovir compared to untreated infected mice and mice treated with the polymer alone or the acyclovir ointment. The results also showed that treatment with the polymer alone, with the polymer containing 3% foscarnet, or with the acyclovir ointment increased the survival rates of infected mice (P < 0.05). Of prime interest, all mice treated with the polymer containing 5% acyclovir survived the infection (P, <0.001).

In vivo skin penetration of antiviral agents. Figure 5 shows the distributions of foscarnet and acyclovir in skin tissues of uninfected (Fig. 5A, C, and E) and infected (Fig. 5B, D, and F) mice at 24 h after topical application either in phosphate buffer or in the polymer matrix to determine whether the polymer could increase the penetration of antivirals into the skin. The distributions of both formulations of foscarnet and of the buffered solution of acyclovir in the stratum corneum tape strips of uninfected and infected mice were similar. In contrast, the incorporation of acyclovir into the polymer matrix markedly increased the amount of drug recovered in the stratum corneum of both uninfected and infected mice (P, <0.05) and infected (P, <0.005) mice, the increased drug penetration being more pronounced in infected mice. No or negligible amounts of foscarnet were found in the underlying epidermis and dermis of uninfected mice irrespective of the carrier used for the drug application. The concentrations of foscarnet in the epidermis and dermis of infected mice were higher when the drug was incorporated into the polymer, but a high variability between mice was observed. The concentrations of acyclovir were higher than those of foscarnet in the epidermis and dermis of both uninfected and infected mice irrespective of the carrier used. The concentrations of acyclovir incorporated into the polymer were 6.1- and 3.3-fold higher than that of the drug in the buffered solution in the epidermis of uninfected and infected mice, respectively. The concentrations of acyclovir administered in the polymer matrix were 3.9- and 4.1-fold higher than that of the drug in the buffered solution in the dermis of uninfected and infected mice, respectively. Infection of mice did not significantly increase the amount of acyclovir in the epidermis or in the dermis.

Figure 6 shows the concentration of acyclovir in the plasma of infected and infected mice at 24 h after topical application either in phosphate buffer or in the polymer matrix. Similar concentrations of acyclovir were found in the plasma of uninfected mice for both formulations. The concentration of acyclovir in the plasma of infected mice was 2.3-fold higher when the drug was incorporated into the polymer matrix than when it was present in the buffered solution. Infection of mice resulted in a fourfold increase in the concentration in plasma of acyclovir administered within the polymer matrix. No or negligible amounts of foscarnet were recovered in the plasma of uninfected or infected mice, respectively (data not shown).

**DISCUSSION**

In the present study, we have evaluated the efficacies of foscarnet and acyclovir incorporated within a polymer matrix in comparison with that of acyclovir ointment in a murine model of cutaneous HSV-1 infection. The zosteriform model, even though it involves only the primary infection, provides a useful analog of recurrent disease (3). In this model, the virus is inoculated in the skin, where the primary infection occurs. From this site, the virus spreads, probably by retrograde axonal flow, to sensory ganglia and the central nervous system. Thereafter, the virus reaches axons that innervate skin within the same dermatome as the inoculation site, spreads via orthodegrade flow to the skin, and produces herpetic lesions within the affected dermatome. In our model, viral titers measured 24 h postinfection at the inoculation site of untreated infected mice were below the detectable level (data not shown), suggesting that the virus was disseminated to sensory ganglia. Thereafter,
the virus returned to the skin and was again detectable at the inoculation site on day 2 postinfection. Ijichi et al. (11) also reported that viral titers measured at the inoculation site gradually decreased and were undetectable 12 h after infection, whereas high viral titers were recovered 24 h postinfection. Viral titers determined both at the inoculation site and in the lower flank reached a maximum value on day 5 postinoculation and then rapidly decreased on day 7. Virus clearance in cutaneous HSV infections is mediated by the host immune system and is essentially a property of CD4\(^+\) T cells (19). In spite of the complete clearance of the virus by day 7, severe ulcerations continued from day 6 to day 12 and might have been partly due to nonspecific cellular immune responses of infected mice (29).

If adequate virus clearance does not occur, the virus spreads into the central nervous system, and mice develop encephalitis and ultimately die. Viral titers were detectable in the brains of some mice as soon as day 6 postinfection (data not shown), and mice began to die at about day 7.

All treatments given three times daily for 4 days and initiated 24 h postinfection demonstrated a marked therapeutic effect on the development of herpetic cutaneous infections, on the basis of mean lesion scores. The formulation of acyclovir within the polymer matrix and the acyclovir ointment reduced viral titers in skin samples below detectable levels, whereas the formulation of foscarnet within the polymer matrix exerted less of an effect on this parameter. Because in our model, virus
returns to the skin between 24 and 48 h after inoculation, the marked therapeutic effect observed with all topical treatments given 24 h postinfection should correspond to an inhibition of secondary virus dissemination. This type of treatment could thus mimic the situation in which a patient initiates therapy at the onset of the prodrome phase. Reducing the treatment to a single application of topical formulations given 24 h after the infection resulted in a significantly higher efficacy of acyclovir incorporated within the polymer matrix than of the acyclovir ointment.

A delay in the initiation of topical treatments to 5 days postinfection was characterized by an increase in the mortality of infected mice treated with the formulation of foscarnet and with the acyclovir ointment given three times daily for 4 days. Many authors suggest that the observed lack of efficacy of topical treatments in delayed therapy could be due to the fact that the phase of virus replication in the zosteriform model occurs before the appearance of symptoms and the initiation of treatment. Kristofferson et al. reported that topical treatments with foscarnet initiated 12, 24, and 48, or 72 h after infection were markedly effective, moderately effective, and ineffective, respectively (14). Lee et al. (15) also showed that the topical efficacy of acyclovir in 1- and 2-day-delayed treatments was essentially the same as that for treatment started immediately after infection, while the topical efficacy of a 3-day-delayed treatment was much lower. Ijichi et al. (11) also showed reduced effects of late therapy with 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl)uracil cream. Furthermore, as previously mentioned, a lack of therapeutic effect on disease development was reported when treatment was initiated on the appearance of the first clinical signs of infection in clinical trials. Interestingly, the results clearly showed that the application of acyclovir incorporated within the polymer on day 5 postinfection was significantly more efficacious than the acyclovir ointment, as evidenced by the reduced mean lesion scores. At this time, the zosteriform rash has started to develop and large amounts of virus have reached the skin. This type of treatment could thus mimic the situation in which a patient initiates therapy at the appearance of lesions. We could not, however, determine viral titers in skin samples of treated animals because untreated infected mice began to die at about day 7 or 8 postinfection. In addition, in untreated infected mice that survived the infection, virus tended to disappear from the skin after day 7, making the interpretation of results difficult (data not shown).

An important consideration in the treatment of herpetic mucocutaneous infections is the delivery of adequate amounts of drugs at the site(s) of infection (31). It is well known that

FIG. 3. Time evolution of the mean lesion score and survival of hairless mice infected cutaneously with HSV-1 strain F and treated 24 h postinfection with a single application of the polymer containing 3% foscarnet ( ), the polymer containing 5% acyclovir ( ), or the acyclovir ointment ( ). Untreated infected mice ( ) were used as controls. Values represent the means for 7 to 13 animals per group.

FIG. 4. Time evolution of the mean lesion score and survival of hairless mice infected cutaneously with HSV-1 strain F and treated later after infection with the polymer alone ( ), the polymer containing 3% foscarnet ( ), the polymer containing 5% acyclovir ( ), or the acyclovir ointment ( ). Untreated infected mice ( ) were used as controls. Treatment was started on day 5 postinfection and was repeated three times daily for 4 days. Values represent the means for 7 to 10 animals per group.
DMSO, at a concentration of higher than 70%, has the ability to accelerate the skin penetration of a variety of substances mainly because it elutes components of the stratum corneum, delaminates the horny layer, and denatures proteins (35). However, the small amount of DMSO (12.5%) in the formulation of acyclovir could not explain its better efficacy. Skin penetration studies revealed that the concentrations of acyclovir recovered in different parts of the skin (stratum corneum, epidermis, and dermis) were significantly higher when the drug was administered in the polymer matrix than when it was administered in a buffered solution. We thus proposed that the incorporation of acyclovir into the polyoxypropylene-polyoxyethylene polymer could lead to a better targeting of sites of viral replication, most probably because of the semiviscous character of this galenic form, which could allow efficient drug penetration into the smallest irregularities of the skin. In infected mice, the presence of crusts due to the scarification and to the zosteriform rash resulted in a significantly higher concentration of acyclovir in the stratum corneum compared to uninfected mice. In addition, we observed an increase in the systemic concentration of acyclovir, which could treat encephalitis or a disseminated disease and might therefore explain the difference in mortality observed with the polymer formulation.

We previously showed that the incorporation of foscarnet into the polymer matrix markedly increased its efficacy over that of the drug contained in a buffered solution (21). However, the efficacy of the formulation of foscarnet was lower than that of acyclovir, irrespective of the schedules of administration tested. This result can be attributed to its high anionic character, which limits its intracellular penetration and thereby

![Graphs showing the distribution of foscarnet and acyclovir in skin tissues of uninfected and infected mice](https://example.com/graph.png)
limits its efficacy compared to that of acyclovir, which is neutral at a physiological pH (8). In that respect, studies performed in our laboratory with Franz diffusion cells and a polytetrafluoroethylene membrane (pore size, 5 μm), which mimics the hydrophobic property of human skin (12, 37), have indicated that acyclovir incorporated within the polymer diffuses at least 30 times more efficiently than foscarnet through such a membrane (data not shown). This result suggests that differences in interactions between the polymer matrix and these two antiviral agents could markedly influence their penetration into the skin. Skin penetration studies indeed revealed that the concentrations of foscarnet in the different skin layers as well as in the plasma were lower than those of acyclovir irrespective of the carrier used for drug administration. Moreover, the difference in the sensitivities of HSV-1 strain F to both drugs may also contribute to the better efficacy of the formulation of acyclovir. Descamps et al. reported a similar ranking for the topical efficacies of these two drugs given as a 1% water-soluble ointment against cutaneous HSV-1 infections in mice (7). Conversely, Alenius et al. showed that acyclovir, both in DMSO and in propylene glycol, is consistently less active than a foscarnet cream when tested on HSV-1 cutaneous lesions in guinea pigs (1).

In conclusion, our results showed that a polymer composed of polyoxypropylene and polyoxyethylene could represent a suitable vehicle for antiviral agents for topical treatments of herpetic cutaneous lesions. Such an approach could also be convenient for the treatment of genital herpes. Studies should now be undertaken to define the respective roles of topical efficacy, which takes place in basal skin or mucosal layers, and systemic efficacy in treating herpetic lesions either prior to or at the time of the appearance of symptoms.

ACKNOWLEDGMENTS

This study was supported by a grant from Infectio Recherche Inc. We thank Rabeca F. Omar and Gay Boivin for constructive comments and helpful discussions. We also thank Hélène Cormier for technical assistance.

REFERENCES