ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1984, p. 10–15
0066-4804/84/010010-06$02.00/0

Vol. 25, No. 1

Penetration of Guinea Pig Skin by Acyclovir in Different Vehicles and Correlation with the Efficacy of Topical Therapy of Experimental Cutaneous Herpes Simplex Virus Infection

S. L. SPRUANCE,[1]* M. B. McKEOUGH,[1] AND J. R. CARDINAL[2]

Department of Medicine and the Center for Infectious Diseases, Diagnostic Microbiology and Immunology, School of Medicine,[1] and Department of Pharmaceutics, College of Pharmacy,[2] University of Utah, Salt Lake City, Utah 84132

Received 18 July 1983/Accepted 10 October 1983

Inadequate penetration of antiviral agents through the stratum corneum of the skin may be one of the limiting factors in the topical therapy of recurrent cutaneous herpes simplex virus infections in humans. In vitro studies of the penetration of the nucleoside analog acyclovir (ACV) through guinea pig skin demonstrated a marked increase in drug flux when ACV was formulated in dimethyl sulfoxide (DMSO), compared with water or polyethylene glycol (PEG) as the vehicle. To examine whether the increased transcutaneous flux of ACV effected by DMSO was meaningful in vivo, topical 5% ACV in DMSO was evaluated for the treatment of cutaneous herpes simplex virus infection in guinea pigs and compared with topical 5% ACV in PEG. When compared with infection sites treated with the vehicle alone, ACV in DMSO produced a greater percent reduction than did ACV in PEG in median lesion number (8 versus 58%; $P < 0.001$), median lesion area (35 versus 73%; $P = 0.001$), and median lesion virus titer (21 versus 84%; $P = 0.08$). We conclude that DMSO is a highly effective vehicle for topical administration of ACV and is superior to PEG in our model. Careful choice of vehicle and consideration of transcutaneous penetration may be important for realization of the full potential of topical antiviral therapy in humans.

Development of an effective topical therapy for recurrent mucocutaneous herpes simplex virus (HSV) infections in immunologically normal humans has been difficult (16). Acyclovir (ACV) is a new anti-herpesvirus compound which is 10- to 150-fold more potent in vitro than older drugs such as vidarabine or iodoxuridine (IDU). In addition, ACV is phosphorylated selectively to an active form in virus-infected cells and is nontoxic to uninfected mammalian cells over a wide range of concentrations (22). The value of ACV applied intravenously, orally, and topically in the treatment of severe HSV infections in immunosuppressed hosts and in primary genital HSV infections has been documented (5, 7, 15, 25, 27). However, the results of treatment of recurrent mucocutaneous HSV infections in normal subjects with topical 5% ACV in polyethylene glycol (PEG; Zovirax) were disappointing. Topical treatment of recurrent herpes simplex labialis and recurrent herpes genitalis effected a reduction in the excretion of virus from the lesions, but there was no change in the clinical course of infection (7, 24).

Assessment of the reasons for the clinical failure of topical ACV therapy in the treatment of recurrent herpes labialis and genitalis has led to the conclusion that either ACV was applied too late to affect the natural course of recurrent HSV disease or that it did not penetrate the stratum corneum and reach infected cells in the lower layers of the epidermis in adequate concentrations (24). To evaluate the latter possibility, we studied the flux of ACV through guinea pig skin in vitro from different drug vehicles and have compared these findings with the in vivo efficacy of two drug vehicle combinations in the topical treatment of an experimental HSV infection (10).

* Corresponding author.

MATERIALS AND METHODS

Experimental animals and virus. Hartley strain, outbred, female albino guinea pigs weighing 200 to 250 g each were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. The virus used in these studies was the laboratory strain HSV-1 E115, originally obtained from André Nahmias, Emory University, Atlanta, Ga. Virus stock for inoculation of guinea pigs was prepared in Vero cells (Flow Laboratories, Inc., Rockville, Md.) and contained 1×10^7 to 4×10^7 PFU/ml.

Penetration of ACV through guinea pig skin. Guinea pigs were shaved closely with electric clippers and sacrificed with ether. Care was taken to ensure that the stratum corneum was not injured during the process of clipping. Full-thickness skin was removed from the back and sides by dissection and stored in airtight containers at −20°C until use.

Double- and single-chamber glass cells for measuring drug diffusion were constructed to our specifications by the Department of Chemistry, University of Utah. The double-chamber cells were similar to those described by Michaels et al. (14) and were composed of two separate 25-ml compartments designed to be clamped together across a 3.0-cm-diameter channel in the side of each compartment. Each compartment had a port for the withdrawal of samples and a second opening for stirring. Skin specimens were held between the cells with a pressure clamp, with the stratum corneum facing the donor solution. The donor compartments contained either a 5-mg/ml (0.5%) solution of ACV in dimethyl sulfoxide (DMSO) or a saturated solution (1.49 mg/ml) of ACV in 0.15 M NaCl. The receiver solutions were 0.15 M NaCl. Samples (200 μl) were withdrawn from the receiver solutions over time and replaced with 200 μl of 0.15 M NaCl. All experiments were conducted at room tempera-

ture (≈22°C). Samples from the receiver compartment were analyzed on a Waters high-pressure liquid chromatography system with a reverse-phase C-18 column (Merck & Co., Inc., Rahway, N.J.). The mobile phase was a 9.5:1 water-methanol mixture. Permeability coefficients (K_p) were calculated from the slopes of plots made according to the following equation (29):

$$\ln\left[1 - \left(\frac{v_1 + v_2}{v_1}\right)\frac{C_2}{C_{1,0}}\right] = -\left(\frac{1}{v_1} + \frac{1}{v_2}\right)K_pAt \quad (1)$$

where V_1 is the volume of the donor solution, V_2 is the volume of the receiver solution, C_2 is the drug concentration in the receiver solution at time t, $C_{1,0}$ is the initial concentration in the donor solution, and A is the membrane area (7.07 cm^2) in the channel between the donor and receiver compartments. The steady-state flux of drug (J), in micrograms per centimeter squared per hour, was calculated from Fick's law:

$$J = K_p\Delta C \quad (2)$$

where $\Delta C = C_{1,0} - C_2$ (29).

Single-chambered cells were used to measure the flux of ACV from PEG ointment. The receiver chamber had a volume of 5.7 ml and was filled with 0.15 M NaCl. Skin was clamped across a 1.6-cm-diameter opening at the top of the cell, with the stratum corneum facing upwards. There was a single port for the withdrawal of samples, and stirring was done with a magnetic stir bar. The exposed skin surface was enclosed by a short cylinder with a glass stopper for access and a side arm containing 1 ml of 44% H_2SO_4 in water (wt/wt) to maintain a constant 50% relative humidity. Samples were withdrawn from the chamber and analyzed by high-pressure liquid chromatography as described above. Drug flux (J; micrograms per centimeter squared per hour) was calculated from the steady-state slope of plots of drug concentration (micrograms per milliliter) versus time (hours).

Animal inoculation. Guinea pigs were anesthetized with 30 mg of intraperitoneal sodium pentobarbital per kg. Hair on the dorsum from the shoulders to the rump was removed with electric clippers as described above, followed by two 5- to 10-min applications of a chemical depilatory (Nair). A grid of six areas was demarcated with a pen, outlining infection sites on either side of the midline at dorsal levels corresponding to the shoulders, midback, and rump by the method of Hubler et al. (10). Undiluted virus stock (0.02 ml) was applied to each different area and introduced through the skin at well-spaced sites by 10 activations of a six-pronged, spring-loaded vaccination instrument (Sterneedle; Ormont Drug, Englewood, N.J.). The length of the vaccination prongs was 0.75 mm.

Treatment regimens. ACV-PEG ointment (5%) and ACV powder were obtained from Burroughs Wellcome Co., Research Triangle Park, N.C., and DMSO was obtained from Sigma Chemical Co., St. Louis, Mo. The concentration of ACV in ACV-PEG was confirmed by high-pressure liquid chromatography. ACV-PEG and PEG were applied with gloved fingertips in an amount sufficient to cover the infected area. ACV (5% [wt/vol]) in DMSO and DMSO were applied with a cotton-tipped applicator in an amount sufficient to leave the treatment site with a moistened appearance. The amount of ACV applied to each treatment site in one dose was ca. 10 mg for the ACV-PEG formulation and 5 mg for ACV-DMSO. The day of inoculation was designated as day zero. Treatment was begun 24 h after inoculation and continued for a total of 3 days (days 1 to 3). Regimens designated as two times per day were given at 9 a.m. and 9 p.m., and those designated as four times per day were given at 9 a.m., 1 p.m., 5 p.m., and 9 p.m. The drug and the corresponding drug vehicle were always tested opposite each other at the same rostral-caudal level. A treatment or vehicle was tested only once on each animal. Other details are described below.

Plaque assay for virus infectivity. Test fluids were diluted 1:10, and 0.2-ml samples of each dilution were inoculated in duplicate onto confluent layers of Vero cells in six-well plastic tissue culture plates (Linbro). After 1 h, the cell monolayers were overlaid with 2 ml of modified Eagle minimal essential medium (Flow Laboratories) containing 0.5% agarose, 5% heat-inactivated fetal bovine serum, and antibiotics. The monolayers were then incubated for 6 to 7 days in a 5% CO_2 atmosphere at 37°C, and the number of plaques was determined by staining with neutral red.

Skin homogenates. Animals were sacrificed with ether on day 4 after infection, and the full-thickness skin of the back was removed by dissection. The square of skin from each of the six treatment areas was minced with a scissors and then homogenized in 4 ml of tissue culture medium (Tissumizer; Tekmar Co., Cincinnati, Ohio). Debris was pelleted by centrifugation, and the supernatants were frozen at −70°C until assay for infectivity was performed.

Measures of drug efficacy and statistical procedures. Regrown hair was removed with a depilatory, and the number of lesions in each treatment site on day 4 was tallied. Lesions enumerated ranged from erythema to 2.5-mm-diameter vesicles. The diameter of individual lesions was determined by a hand lens from photographs, and the total lesion area was computed for each infection site. Paired data (drug-drug vehicle) were evaluated by the Wilcoxon signed-rank test, with the value for the drug expressed as a percentage of the result for the vehicle. The percent differences between the drug treatment and the vehicle control for ACV-PEG and ACV-DMSO were compared by the Mann-Whitney rank sum procedure (6). Other data were analyzed by Student's t test. All probability determinations were two tailed, and $P \leq 0.05$ was considered to be significant.

RESULTS

In vitro penetration of ACV through guinea pig skin. In vitro skin permeation studies were performed to examine the influence of the drug vehicle on the rate of ACV penetration through guinea pig skin. We first examined the flux of ACV through guinea pig skin in double-chamber diffusion cells, using aqueous 0.15 M NaCl solutions as both the donor and receiver solvents. The mean value of the permeability coefficient for ACV through clipped skin was 0.34×10^{-3} cm/h (Table 1, experiment 1). From this value and the value of the aqueous solubility (1.49 mg/ml), the maximum steady-state flux of ACV through guinea pig skin with 0.15 M NaCl as the donor solution was calculated to be $(0.34 \times 10^{-3}$ cm/h) × (1.49 mg/ml − 0) = 0.51 μg/cm^2 per h (equation 2).

The rate of skin penetration of drugs can be enhanced by certain vehicles which affect the barrier properties of the skin. Examples include urea, alcohol-water mixtures, and DMSO (4, 20). The rate of penetration of ACV through guinea pig skin was not greatly enhanced through the use of ethanol-water solvent mixtures at a variety of compositions or by urea at a concentration of 10% (wt/vol) (J. R. Cardinal and S. L. Spruance, unpublished data). In contrast, DMSO

TABLE 1. Permeability coefficients (K_p) for ACV through guinea pig skin for different skin conditions and drug solvents

Expt no.	Skin procedure	Donor solvent/ receiver solvent	No. of determinations	K_p[a]
1	Clipped[b]	Water/water[c]	5	0.34 ± 0.22
2	Clipped[b]	DMSO/water	4	1.47 ± 0.95
3	Clipped, depilatory[d]	DMSO/water	2	1.15 ± 0.67
4	Clipped, depilatory, and punctured[d]	DMSO/water	2	1.40 ± 0.48

[a] Mean ± standard deviation (centimeters per hour × 10^3).
[b] No time interval between procedure and collection of skin.
[c] 0.15 M NaCl.
[d] Time interval between procedure and collection of skin, 24 h.

was found to greatly accelerate the rate of penetration of ACV through clipped guinea pig skin. The average value of the permeability coefficient of ACV with DMSO as the donor solvent is 1.47×10^{-3} cm/h, an approximate fourfold increase in the permeability coefficient relative to 0.15 M NaCl ($P < 0.05$) (Table 1, experiment 2). In addition, because the solubility of ACV in DMSO is much greater than in water, $C_{1,0}$ is larger, and the net flux of ACV through skin can be greatly enhanced (equation 2). For a 5% solution of ACV in DMSO, the steady-state flux of ACV would be 73.5 μg/cm² per h, a net increase in flux of 150-fold over the value obtained when 0.15 M NaCl is the donor solvent.

A comparison of the permeation through guinea pig skin of ACV in PEG and ACV in DMSO was accomplished with a single-chamber diffusion cell. Since ACV in PEG is largely a suspension and the concentration of ACV solubilized in PEG is unknown, K_p could not be determined for ACV-PEG. Therefore, the results of these experiments are expressed as the flux of ACV (J; micrograms per centimeter squared per hour) for each preparation. Single doses of 250 mg of 5% ACV-PEG and 100 μl of 0.5% ACV in DMSO were applied to the exposed skin surface of the diffusion apparatus. The volume of the dose corresponded to the amount used for treatment of experimental HSV infection, but the concentration of ACV in DMSO was 1/10th that used in the in vivo studies (Fig. 1). The mean flux of 5% ACV from PEG in three experiments was 0.14 ± 0.06 μg/cm² per h and, for 0.5% ACV in DMSO, 0.46 ± 0.16 μg/cm² per h; $P = 0.02$. A higher concentration of ACV in DMSO would have a proportionately higher flux (Fick's Law; equation 2).

Preparation and manipulation of the skin in the animal model could affect the rate of ACV penetration and generate misleading therapeutic results. The application of a depilatory is known to decrease the resistance of the skin to drug penetration (28), and inoculation of the skin by puncture of the stratum corneum could leave pathways of low resistance. To examine how the depilation and the inoculation process might affect the transfer of ACV through guinea pig skin and influence the treatment results, experiments were designed to obtain skin for the in vitro studies which would mimic the condition of the skin at the onset of drug administration. In the animal model, the hair is clipped, a depilatory is applied twice, the virus is applied with a vaccination gun, and 24 h later, drug treatment regimens are initiated. Shown in Table 1 are K_p values determined for ACV in DMSO for skin collected (i) immediately after clipping (experiment 2),

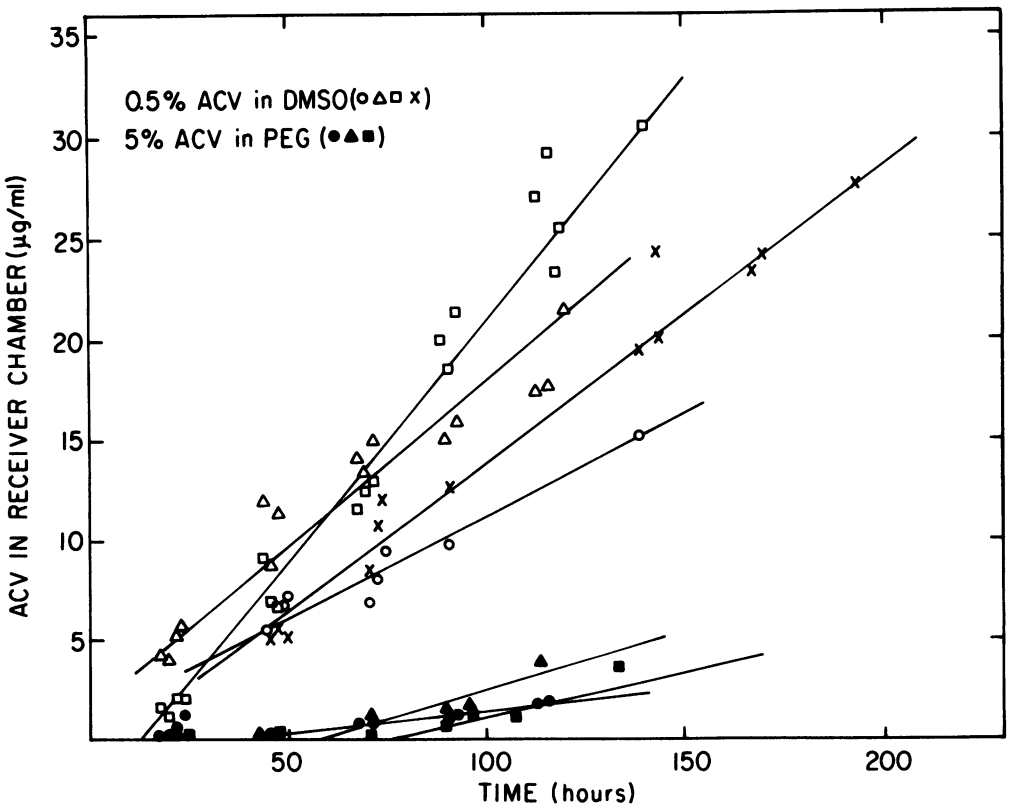

FIG. 1. Penetration of ACV through guinea pig skin in vitro. The apparatus was a single-chamber diffusion cell. A total of 250 mg of 5% ACV in PEG or 100 μl of 0.5% ACV in DMSO was applied to the stratum corneum at time zero. Each of the different symbols and corresponding lines represent data from one experiment.

(ii) 24 h after clipping and depilation (experiment 3), and (iii) 24 h after clipping, depilation, and 60 sterile skin punctures (experiment 4). The permeability coefficients obtained from the manipulated skins at a time corresponding to the start of treatment do not differ significantly from the values obtained from control skins which were prepared only with electric clippers. Although K_p may be increased immediately after the application of Nair and multiple punctures, our results imply that 24 h is sufficient time for the normal barrier properties of guinea pig skin to be restored.

Efficacy of topical ACV-PEG and ACV-DMSO against experimental cutaneous HSV-1 infection. Sixteen guinea pigs were infected in six different areas on the dorsum. Drug and drug vehicles were tested in pairs on opposite sides of the midline. PEG and 5% ACV-PEG were applied four times per day for 3 days on the two midback sites, and DMSO and 5% ACV-DMSO were given two times per day for 3 days on opposing sides at the level of the rump. The two infection sites on the shoulders were untreated. Treatments were begun 24 h after inoculation at a time when lesions were just beginning to form. Treatment duration was 3 days, so the results might reflect penetration of intact skin and lesion prevention rather than therapy of established lesions. Analysis of the number of lesions at each site on day 4 showed that ACV-DMSO reduced the median lesion count by 80% compared with the contralateral DMSO control areas (5 versus 25 lesions; $P = 0.001$). Treatment with ACV-PEG effected an 18% reduction in the median number of lesions compared with PEG alone (22 versus 30 lesions; $P = 0.002$).

There is growing evidence that the guinea pig back has a biological gradient along the rostral-caudal axis (3). We have found that fewer numbers of lesions develop rostrally (S. L. Spruance, unpublished data). Because this gradient could affect the degree of drug efficacy, we were unable to directly compare ACV-PEG and ACV-DMSO with the initial experimental design. A second experiment was performed in which the two combinations of drug and drug vehicle were tested an equal number of times on the midback and on the rump. The effect of ACV on lesion area and virus titer were included as additional measures of efficacy.

For the second experiment, 16 animals were infected at four sites on the midback and rump. Treatment with ACV-PEG, PEG, ACV-DMSO, and DMSO was administered as drug-drug vehicle pairs on opposite sides of the midline as described above, except that the different formulations were alternately applied to the midback or rump areas, and DMSO was given four times per day instead of twice. The efficacy of drug treatment at the midback and rump was similar, and the data were combined. For statistical analysis, the percent difference for each drug formulation compared with the corresponding vehicle was derived and applied in a signed-rank procedure (drug versus drug vehicle) or rank sum test (ACV-PEG versus ACV-DMSO). The results for each preparation on three measures of lesion severity are shown in Table 2. ACV-PEG and ACV-DMSO treatments significantly reduced all measures of lesion severity compared with the vehicle controls ($P = <0.001$ to 0.004), but the effect of ACV-DMSO was much greater. ACV-DMSO produced a greater change than ACV-PEG in median lesion number (8% versus 58%; $P < 0.001$), median lesion area (35% versus 73%; $P = 0.001$), and median lesion virus titer (21% versus 84%; $P = 0.08$).

TABLE 2. Effect of topical treatment on experimental cutaneous HSV-1 infection by three measures of lesion severity

Preparation evaluated	Median values (range)		
	No. of lesions	Total lesion area (mm^2)	Virus titer (log_{10} PFU/ml)
PEG	53 (38–64)	119 (76–204)	4.5 (3.2–5.2)
5% ACV-PEG	49 (26–58)	77 (25–99)	4.4 (2.8–4.9)
DMSO	38 (24–58)	51 (28–103)	4.0 (3.2–4.9)
5% ACV-DMSO	16 (5–36)	14 (5–41)	3.2 (2.7–4.3)

DISCUSSION

Treatment with topical 5% ACV-PEG does not alter the clinical course of recurrent herpes simplex labialis or genitalis (7, 24). Since the drug is a potent antiviral agent effective against herpes simplex virus disease under other clinical circumstances, we have attributed the failure of topical therapy in recurrent infections to either late initiation of treatment or inadequate penetration of the drug through the stratum corneum (24). A recently completed trial of topical ACV-PEG against prodromal and erythema-stage herpes labialis has yielded negative results, which makes it unlikely that late treatment alone is responsible for the lack of efficacy (S. L. Spruance, C. S. Crumpacker, L. E. Schnipper, E. R. Kern, S. Marlow, J. Modlin, K. A. Arndt, and J. C. Overall, Jr., Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 187, 1982). The present experimental studies have shown: (i) ACV penetrated very poorly through guinea pig skin in vitro as a 5% preparation in PEG; (ii) when tested in the Hubler guinea pig model, topical ACV-PEG was of minor benefit, similar to the human trials against recurrent herpes infection; (iii) ACV had greatly enhanced penetration through guinea pig skin when DMSO was the vehicle; and (iv) topical treatment with ACV-DMSO produced excellent results in the animal model. Collectively, these observations indicate that transcutaneous drug penetration should be given major consideration in the design of future topical antiviral chemotherapy for recurrent human HSV disease.

Before reports by us (Clin. Res. 30:60A, 1982) and Alenius et al. (1), topical ACV-PEG was highly effective in a variety of animal models of cutaneous HSV disease, including the guinea pig dorsum (18, 22), female guinea pig genitalia (11), mouse lip (17), lumbosacral and orofacial skin of hairless mice (12), and lumbar skin of athymic nude mice (9). Topical ACV-PEG may have succeeded in these studies because the inoculation process altered the integrity of the stratum corneum or alternate routes of absorption were available, so that the ability of the formulation to penetrate stratum corneum was not a critical determinant of the outcome. For example, inoculation in several mouse models was achieved by needle scratches (9, 12) or by thermal injury with a surgical cautery (17), procedures which are likely to have abrogated the barrier properties of the skin. In the guinea pig genital model, ACV may have achieved significant systemic levels by absorption across the introital or vaginal mucosa (11). The study on the guinea pig dorsum by Park et al. (18) was similar to ours. However, if inoculation stabs were close together or superimposed, more damage to the skin may have occurred than with the widely spaced injections we employed, or increased drug penetration may have resulted from their repeated use of a depilatory (28).

Although for many years guinea pigs have been known to be susceptible to experimental HSV infection, Hubler et al. showed in 1974 that inoculation of the dorsal skin with HSV-1, using a specific vaccination instrument (Sterneedle), produced multiple, small pustular herpes lesions that ran a benign, self-limited course of 10 to 12 days, a duration

similar to that of recurrent human herpetic disease (10). Subsequently, Schaefer et al. described a procedure to quantitate the virus content of infected skin, and Schaefer et al. (21) and Alenius and Oberg (2) used the model to evaluate antiviral drugs. The present report has determined that Sterneedle punctures do not increase the diffusion of ACV through guinea pig skin and has correlated transcutaneous flux of ACV with in vivo efficacy. We have also observed a rostral-caudal gradient in the number of lesions which can be induced, with fewer lesions developing at rostral sites on the dorsum. Drug efficacy appears to be independent of location. Alenius and Oberg (2) did not find differences in lesion severity score attributable to site, whereas Paquette and Hughes (Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, S38, p. 241) have reported that HSV-1 titers in guinea pig skin are markedly higher at caudal dorsal than at rostral dorsal sites of inoculation. Auerbach and Auerbach (3) described a host of rostral-caudal biological differences in animals, possibly related to a primary embryonic gradient that occurs with the establishment of the nervous system. Lesion site should be examined as a potential variable when the Hubler guinea pig model is used.

The use of DMSO to enhance the activity of a topical antiviral agent was described by Tomlinson and MacCallum in 1968 (26) in their studies of IDU for the treatment of HSV infection in guinea pigs. The improved results with IDU in DMSO in the animal model led to a human trial of 5% IDU in DMSO against recurrent herpes labialis in a small number of subjects (13). The results were clinically impressive but not statistically significant (16). Results with topical IDU in DMSO for the treatment of recurrent genital herpes have been mixed (19, 23). Studies in which DMSO is used have been limited in the United States because it is an investigational substance regulated by the Food and Drug Administration, but our findings and the similar results of Alenius et al. (1) with ACV suggest that continued study of DMSO as a potential vehicle is warranted. In the present study, DMSO alone reduced lesion severity (Table 2). This effect may be due to its antiinflammatory activity (8).

Reducing the clinical severity of recurrent HSV infections by topical therapy has been a long and frustrating endeavor for many investigators (16). The rapid natural healing of the lesions, the great variability in severity of untreated lesions, and the barrier effect of the stratum corneum appear to be the major difficulties. The present studies have identified the importance of drug penetration in the guinea pig model and should stimulate drug vehicle development for human applications.

ACKNOWLEDGMENTS

We thank Gordon Flynn for advice and support and James Reading for statistical consultation.

This work was supported in part by contract NO1-AI-52532 from the National Institute of Allergy and Infectious Diseases and the National Institute of Dental Research.

LITERATURE CITED

1. **Alenius, S., M. Berg, F. Broberg, K. Eklind, B. Lindborg, and B. Oberg.** 1982. Therapeutic effects of foscarnet sodium and acyclovir on cutaneous infections due to herpes simplex virus type 1 in guinea pigs. J. Infect. Dis. **145**:569–573.
2. **Alenius, S., and B. Oberg.** 1978. Comparison of the therapeutic effects of five antiviral agents on cutaneous herpesvirus infection in guinea pigs. Arch. Virol. **58**:277–288.
3. **Auerbach, R., and W. Auerbach.** 1982. Regional differences in the growth of normal and neoplastic cells. Science **215**:127–134.
4. **Chandrasekaran, S. K., P. S. Campbell, and A. S. Michaels.** 1977. Effect of dimethylsulfoxide on drug permeation through human skin. AIChE J. **23**:810–816.
5. **Chou, S., J. G. Gallagher, and T. C. Merigan.** 1981. Controlled clinical trial of intravenous acyclovir in heart-transplant patients with muco-cutaneous herpes simplex infections. Lancet **i**:1392–1394.
6. **Colton, T.** 1974. Statistics in medicine, p. 1–372. Little, Brown, & Co., Boston.
7. **Corey, L., A. J. Nahmias, M. E. Guinan, J. K. Benedetti, C. W. Critchlow, and K. K. Holmes.** 1982. A trial of topical acyclovir in genital herpes simplex virus infections. N. Engl. J. Med. **306**:1313–1319.
8. **de la Torre, J. D. (ed.).** 1983. Biological actions and medical applications of DMSO. Ann. N.Y. Acad. Sci. **411**:1–402.
9. **Descamps, J., E. De Clercq, P. J. Barr, A. S. Jones, R. T. Walker, P. F. Torrence, and D. Shugar.** 1979. Relative potencies of different anti-herpes agents in the topical treatment of cutaneous herpes simplex virus infection of athymic nude mice. Antimicrob. Agents Chemother. **16**:680–682.
10. **Hubler, W. R., Jr., T. D. Felber, D. Troll, and M. Jarratt.** 1974. Guinea pig model for cutaneous herpes simplex virus infection. J. Invest. Dermatol. **62**:92–95.
11. **Kern, E. R.** 1982. Acyclovir treatment of experimental genital herpes simplex virus infections. Proceedings of a symposium on acyclovir. Am. J. Med. **73(1A)**:100–108.
12. **Klein, R. J., A. E. Friedman-Kien, and E. DeStefano.** 1979. Latent herpes simplex virus infections in sensory ganglia of hairless mice prevented by acycloguanosine. Antimicrob. Agents Chemother. **15**:723–729.
13. **MacCallum, F. O., and B. E. Juel-Jensen.** 1966. Herpes simplex virus skin infection in man treated with idoxuridine in dimethyl sulphoxide. Results of double-blind controlled trial. Br. Med. J. **2**:805–807.
14. **Michaels, A. S., S. K. Chandrasekaran, and J. E. Shaw.** 1975. Drug permeation through human skin: theory and *in vitro* experimental measurement. AIChE J. **21**:985–996.
15. **Mitchell, C. D., S. R. Gentry, J. R. Boen, B. Bean, K. E. Groth, and H. H. Balfour, Jr.** 1981. Acyclovir therapy for mucocutaneous herpes simplex infections in immunocompromised patients. Lancet **i**:1389–1392.
16. **Overall, J. C., Jr.** 1979. Dermatologic diseases, p. 305–384. *In* G. J. Galasso, T. C. Merigan, and R. A. Buchanan (ed.), Antiviral agents and viral diseases of man. Raven Press, New York.
17. **Park, N.-H., D. Pavan-Langston, and S. L. McLean.** 1979. Acyclovir in oral and ganglionic herpes simplex virus infections. J. Infect. Dis. **140**:802–806.
18. **Park, N.-H., D. Pavan-Langston, S. L. McLean, and J. H. Lass.** 1980. Acyclovir topical therapy of cutaneous herpes simplex virus infection in guinea pigs. Arch. Dermatol. **116**:672–675.
19. **Parker, J. D.** 1977. A double-blind trial of idoxuridine in recurrent genital herpes. J. Antimicrob. Chemother. **3**(Suppl. A):131–138.
20. **Polano, M. K., and M. Ponec.** 1980. Bioavailability and effects of various vehicles on percutaneous absorption, p. 67–78. *In* P. Mauvais-Jarvis, C. F. H. Vockers, and J. Wepierre (ed.), Percutaneous absorption of steroids. Academic Press, Inc., New York.
21. **Schaefer, T. W., M. Lieberman, J. Everitt, and P. O. Came.** 1977. Cutaneous herpes simplex virus infection of guinea pigs as a model for antiviral chemotherapy. Am. N.Y. Acad. Sci. **284**:624–631.
22. **Schaeffer, H. J., L. Beauchamp, P. deMiranda, and G. B. Elion.** 1978. 9-(2-hydroxyethoxymethyl)guanine activity against viruses of the herpesgroup. Nature (London) **272**:583–585.
23. **Silvestri, D. L., L. Corey, and K. K. Holmes.** 1982. Ineffectiveness of topical idoxuridine in dimethyl sulfoxide for therapy for genital herpes. J. Am. Med. Assoc. **248**:953–959.
24. **Spruance, S. L., L. E. Schnipper, J. C. Overall, Jr., E. R. Kern, B. Wester, J. Modlin, G. Wenerstrom, C. Burton, K. A. Arndt, G. L. Chiu, and C. S. Crumpacker.** 1982. Treatment of herpes simplex labialis with topical 5% acyclovir in polyethylene glycol. J. Infect. Dis. **146**:85–90.
25. **Straus, S. E., H. A. Smith, C. Brickman, P. deMiranda, C.**

McLaren, and R. E. Keeney. 1982. Acyclovir for chronic mucocutaneous herpes simplex virus infection in immunosuppressed patients. Ann Intern. Med. **96**:270–277.

26. **Tomlinson, A. H., and F. O. MacCallum.** 1968. The effect of 5-iodo-2′-deoxyuridine on herpes simplex virus infections in guinea-pig skin. Br. J. Exp. Pathol. **49**:277–282.
27. **Wade, J. C., B. Newton, C. McLaren, N. Flournoy, R. E. Keeney, and J. D. Meyers.** 1982. Intravenous acyclovir to treat mucocutaneous herpes simplex virus infection after marrow transplantation. Ann. Intern. Med. **96**:265–269.
28. **Wahlberg, J. E.** 1972. Impairment of skin barrier function by depilatories. J. Invest. Dermatol. **59**:160–162.
29. **Zentner, G. M., J. R. Cardinal, J. Feijen, and S. Z. Song.** 1979. Progestin permeation through polymer membranes. IV. Mechanism of steroid permeation and functional group contributions to diffusion through hydrogel films. J. Pharm. Sci. **68**:970–975.