DIMETHYL SULFOXIDE AS A VEHICLE FOR TOPICAL ANTIVIRAL CHEMOTHERAPY*

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INTRODUCTION

Numerous studies of topical antiviral chemotherapy for recurrent herpes simplex labialis and genitalis have failed to show that treatment alters the clinical course of the disease.[1] Investigators have used large numbers of study subjects, meticulous patient follow-up to document the course of lesions, potent antivirals,[2] and early-treatment study design[3]—still without success. We are concerned that delivery of antivirals through the stratum corneum to infected epidermal cells has been inadequate in most of the published trials.

To evaluate the importance of drug delivery experimentally, we have measured the penetration of acyclovir (ACV) through guinea pig skin *in vitro* from different drug vehicles and compared these findings with the efficacy of two topical formulations of the drug in the treatment of an experimental cutaneous herpes simplex virus infection.[4] ACV is a potent new compound with striking *in vitro* activity against herpes simplex virus, a wide toxic/therapeutic ratio, and established clinical activity against cutaneous human HSV infection other than recurrent disease.[5-9]

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MATERIAL AND METHODS

Experimental Animals and Virus

Hartley, outbred, female albino guinea pigs, 200–250 grams each, were obtained from Charles River Breeding Labs (Wilmington, Mass.). The virus used in these studies was the laboratory strain HSV-1 E115. Virus stock used for inoculation of guinea pigs contained 10^7 PFU/ml.

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Penetration of ACV Through Guinea Pig Skin

Guinea pigs were sacrificed and then close-shaved with electric clippers. Clipped, full-thickness skin was removed from the back and sides by dissection and stored in air-tight containers at −20°C until use.

Single-chambered glass diffusion cells were used to measure the flux of ACV from DMSO solution and polyethylene glycol (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 withdrawal of samples and stirring was achieved with a magnetic stir bar. The exposed skin surface was enclosed by a short cylinder having a glass stopper for access and a side-arm containing 1 ml of 44% H_2SO_4 in water (w/w) to maintain a constant 50% relative humidity. Samples were withdrawn from the chamber and analyzed by high performance liquid chromatography. The volume withdrawn was replaced with an equal volume of 0.15 M NaCl. Drug flux ($\mu g/cm^2 \cdot h$) was calculated from the steady-state slope of plots of drug concentration ($\mu g/ml$) versus time (h).

Animal Inoculation

Guinea pigs were anesthetized with 30 mg/kg intraperitoneal sodium pentabarbital. Hair on the dorsum was completely removed with electric clippers and chemical depilatory (Nair®). Undiluted virus stock (0.02 ml) was applied to six different areas and the skin was inoculated at each site by ten activations of a six-pronged spring-loaded vaccination instrument (Sterneedle, Pan Ray Division, Ormont Drug, Englewood, N.J.).

Treatment Regimens

Five percent of ACV in PEG and ACV powder was obtained from Burroughs-Wellcome Co. DMSO was obtained from Sigma Chemical Co. The day of inoculation was designated as Day 0. Treatment was begun 24 hours after inoculation and continued for a total of three days (Days 1-3). Regimens designated as 2×/day were given at 9 AM and 9 PM and 4×/day at 9 AM, 1 PM, 5 PM, and 9 PM. A drug and it's corresponding vehicle were always tested opposite each other at the same rostal/caudal level.

Measures of Drug Efficacy and Statistical Procedures

Regrown hair was removed with depilatory and the number and size of lesions in each treatment site on Day 4 tallied. Lesions enumerated ranged from pinpoint erythema to 2.5 mm diameter vesicles. Results of paired data (drug/drug vehicle) were analyzed by the Wilcoxon signed rank test (10) or a paired *t*-test and other unpaired data by a *t*-test. All probability determinations were two-tailed and $p \leq 0.05$ was considered significant.

TABLE 1

PENETRATION OF ACV THROUGH GUINEA PIG SKIN *IN VITRO*

Exp. No.	Drug/Vehicle	Lag Time (h)*	Flux ($\mu g/cm^2 \cdot h$)†
1	5% ACV/PEG	65	.182
2	5% ACV/PEG	77	.165
3	5% ACV/PEG	37	.069
4	0.5% ACV/DMSO	14	.676
5	0.5% ACV/DMSO	–	.438
6	0.5% ACV/DMSO	–	.284
7	0.5% ACV/DMSO	12	.432

*Time between beginning of the experiment and the intercept of the slope on the x-axis in a plot of drug concentration in the receiver chamber (y) vs. time (x).

†Flux was calculated from the steady-state slope of plots of drug concentration vs. time, the area of the skin surface exposed to drug and the volume of the receiver chamber.

RESULTS

Penetration of ACV Through Guinea Pig Skin

Comparison of the permeation of topical ACV through guinea pig skin as ACV/PEG or ACV/DMSO was accomplished with a single-chamber diffusion cell. Single doses of 250 mg of 5% ACV in 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/DMSO was one-tenth that used in the *in vivo* studies. The results are shown in TABLE 1. The mean flux of 5% ACV from PEG in three experiments was 0.14 ± 0.06 and for four experiments with 0.5% ACV in DMSO, 0.46 ± 0.16 ($\mu g/cm^2 \cdot h$, mean ± SD, $p = 0.02$). A higher concentration of ACV in DMSO would have a proportionately higher flux. In addition, the onset of detectable ACV in the receiver chamber (lag time) occurred more quickly when DMSO was the vehicle (TABLE 1).

TABLE 2

EFFICACY OF ANTIVIRAL NUCLEOSIDES AGAINST EXPERIMENTAL CUTANEOUS HSV INFECTION ON THE DORSUM OF THE GUINEA PIG

Treatment*	Median Number of Lesions on Day 4 at One of the Treatment Sites (range)
Untreated*	29 (17–34)
PEG^{mb}	28 (16–44)
DMSO^{r}	25 (12–36)
5% ACV/PEG^{mb}	23 (6–37)
5% ACV/DMSO^{r}	5 (1–23)

*Sixteen animals were infected at multiple discrete sites on the dorsum and different sites were untreated or received vehicle or antiviral/vehicle treatment as described in the table. Treatments were given for three days beginning 24 hours after inoculation. Formulations containing PEG were applied 4×/day and those containing DMSO 2×/day. A drug and its corresponding vehicle were always tested opposite each other at the same rostral/caudal level. S – shoulder, mb – midback, r – rump.

Comparison of Antiviral/Vehicle Combinations for the Prevention of Experimental HSV-1 Lesions

Sixteen guinea pigs were infected on the dorsum on Day 0 and 24 hours later on Day 1 different sites on the back of each animal were treated with PEG 4×/day for three days, DMSO 2×/day for three days, 5% ACV/PEG 4×/day for three days, or 5% ACV/DMSO 2×/day for three days as explained in Table 2. The number of lesions present in each treatment site were then tallied on Day 4. Analysis of the number of lesions (TABLE 2) showed that ACV/DMSO reduced the lesion count by 80% compared to the contralateral DMSO control areas (median of 5 vs. 25 lesions, $p = 0.001$). Treatment with ACV/PEG was also beneficial but to a far lesser degree, effecting an 18% reduction in the number of lesions compared to PEG alone (median of 23 vs. 28 lesions, $p = 0.002$).

Lesions treated with DMSO were significantly smaller than untreated lesions (TABLE 3, $p < 0.05$). Lesions treated with ACV/PEG were significantly smaller than those treated with PEG ($p < 0.005$) but not meaningfully different from the untreated control ($p > 0.25$).

TABLE 3
THE INFLUENCE OF TREATMENT REGIMENS ON LESION SIZE

Treatment Regimen*	Mean Lesion Diameter ± SEM† (inches)
Untreated	.042 ± .0013
PEG	.046 ± .0016
DMSO	.036 ± .0018
5% ACV/PEG	.040 ± .0012
5% ACV/DMSO	.035 ± .0021

*Animals were treated at different locations on the dorsum with each of the four regimens shown. See TABLE 2 for details.
†Standard error of the mean.

DISCUSSION

The penetration of acyclovir through guinea pig skin *in vitro* was markedly greater with DMSO than when PEG was the vehicle. When 5% ACV in DMSO was compared with 5% ACV in PEG in the treatment of experimental herpes infection in the guinea pig, ACV/DMSO was more effective. The effectiveness of antivirals in DMSO in the guinea pig is likely related to drug penetration, and development of a means to enhance delivery of antivirals to the target cells would appear to be a potentially fruitful next step to further the effectiveness of topical anti-herpesvirus therapy in humans.

Should DMSO be used as a vehicle for topical antiviral therapy in humans? The idea is not new and has been explored by MacCallum and Juel-Jensen,[11] Parker,[12] and Silvestri *et al.*[13] and advocated by Herrmann and Herrmann.[14] Patient acceptability of skin irritation and the odor of DMSO are unsettled issues at present but may depend on the volume of the application and the location of lesions. DMSO-treated lesions were significantly smaller than untreated lesions in this study. Since DMSO itself does not have antiviral activity *in vivo*[12,15] but can affect white blood cell function,[16,17] it is likely that our results occurred from an

anti-inflammatory effect. DMSO has produced changes in the lens of the eye in a wide variety of species of experimental animals, leading some to conclude that eye changes would likely be a consequence of some dose of DMSO in humans.[18]

The potential toxicity of DMSO and the protocol requirements for clinical studies have dampened the enthusiasm and interest of pharmaceutical firms in the U.S.A. for commercial development of this agent. However, DMSO has major potential advantages for enhancing the penetration of topically administered antivirals, which could possibly lead to a clinically beneficial treatment for recurrent human HSV infections. Further experimental and clinical studies are indicated and the feasibility of developing DMSO for topical vehicle use in this country should be reconsidered.

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