Comparison of Topically Applied 5-Ethyl-2'-Deoxyuridine and Acyclovir in the Treatment of Cutaneous Herpes Simplex Virus Infection in Guinea Pigs

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Three percent 5-ethyl-2'-deoxyuridine (EdU) in an aqueous cream base was compared with 5% acyclovir (ACV) in polyethylene glycol ointment and 3% EdU in 95% dimethyl sulfoxide (DMSO) for efficacy in the topical treatment of an experimental dorsal cutaneous herpes simplex virus type 1 infection in guinea pigs. Topical ACV treatment reduced the mean lesion number by 15%, the lesion area by 32%, and the lesion virus titer by 60% when compared with measurements at contralateral sites treated with the vehicle alone. Application of 3% EdU cream was more beneficial, effecting reductions of 29, 44, and 68% in the same measurements. EdU in DMSO was even more effective, reducing the lesion measurements by 39, 60, and 90%, respectively. The penetration of EdU and ACV through guinea pig skin was compared in single-chamber diffusion cells. In the aqueous cream, EdU readily penetrated excised skin and exhibited rates of flux 10-fold greater than those shown by ACV in ointment formulation (0.56 versus 0.05 μg/cm² per h; P = 0.05). The flux of EdU in DMSO was 3.39 μg/cm² per h, six times higher than the flux in the cream vehicle. EdU was more effective than ACV in this experimental animal model, most likely due to better percutaneous drug delivery of EdU from the cream and DMSO vehicles.

The activity of 5-ethyl-2'-deoxyuridine (EdU) against infection with herpes simplex virus (HSV) was first recognized in 1967 (13). In vitro testing by reduction in cytopathic effect has identified the mean 50% inhibitory dose against HSV type 1 (HSV-1) and HSV type 2 (HSV-2) to be 0.3 to 0.5 μg/ml in primary rabbit kidney cells (8) and BHK-21 cells (22). In vivo, EdU has shown efficacy in the rabbit model of HSV keratitis (17) and has been used in Europe for human herpes keratitis (11). EdU was similar in efficacy to vidarabine parenterally in the treatment of HSV encephalitis in mice (7) and topically in the cutaneous HSV infection model in athymic nude mice (10). In both athymic mice and guinea pigs, however, topical acyclovir (ACV) was more effective than EdU (5, 10).

In the studies reported here, we examined the therapeutic efficacy of 3% EdU cream applied topically to the dorsal skin of guinea pigs infected with HSV-1 and the penetration of EdU through guinea pig skin in vitro. The results attained with EdU cream were compared with those for 5% ACV ointment and 3% EdU in 95% dimethyl sulfoxide (DMSO) tested concurrently.

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MATERIALS AND METHODS

Antiviral agents. EdU (3%) in a proprietary gynecological aqueous-cream base and EdU powder were provided by Ortho Pharmaceutical (Canada) Ltd., Ontario, Canada. DMSO was obtained from Sigma Chemical Co., St. Louis, Mo., and a 3% (wt/vol) solution of EdU in 95% DMSO–5% water (vol/vol) was prepared in our laboratory. ACV (5%) in polyethylene glycol (PEG; Zovirax ointment) and ACV powder were obtained from Burroughs-Wellcome Corp., Research Triangle Park, N.C. Drug vehicles without antiviral agents were provided for use as control treatments. [3H]ACV (600 mCi/mmol) was obtained from Moravek Biochemicals, Brea, Calif. [3H]ACV (200 μl) was dissolved with an excess of unlabeled ACV (3 g) in an ethanol–water (70:30) solution at 60°C and recrystallized overnight at room temperature. The recrystallized [3H]ACV had a specific activity of 80 cpm/μg. The purity of [3H]ACV was established by thin-layer chromatography on a cellulose plate with an n-propanol–ammonium hydroxide–water (60:30:10) liquid phase (9).

[3H]ACV (5%) in PEG was formulated by melting PEG at 40°C; recrystallized [3H]ACV was added to 5% by weight, and the mixture was then blended thoroughly at room temperature until the PEG base had resumed semisolid characteristics.

Experimental virus strain. The virus used was a stock of HSV-1 E115 that had a titer of 5 to 10 PFU/ml on Vero cells (20). The concentrations of EDU and ACV that inhibited HSV-1 E115 plaque formation by 50% in Vero cells were 0.49 and 0.14 μg/ml, respectively.

Experimental animals. Hartley strain outbred female albino guinea pigs weighing 200 to 250 g each were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. During treatment, animals were housed in individual cages.

Animal inoculation and treatment. Guinea pigs were inoculated with HSV-1 E115 in six different areas on the depilated dorsum by multiple shallow punctures as originally described by Hubler (15). This procedure regularly produces 30 to 50 pustules at each infection site when care is taken not to overlap the punctures (20). Treatments were begun 24 h after inoculation at 0900 and were repeated at 0900, 1300, 1700, and 2100 h each day for a total of 3 days. Approximately 200
TABLE 1. Effect of topical therapy with EdU and ACV on the severity of an experimental cutaneous HSV-1 infection of guinea pigs

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Measure of lesion severity (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of lesions</td>
</tr>
<tr>
<td>5% ACV in PEG</td>
<td>29 ± 9</td>
</tr>
<tr>
<td>PEG vehicle</td>
<td>34 ± 11</td>
</tr>
<tr>
<td>3% EdU cream</td>
<td>20 ± 11</td>
</tr>
<tr>
<td>Cream vehicle</td>
<td>28 ± 13</td>
</tr>
<tr>
<td>3% EdU in 95% DMSO</td>
<td>19 ± 8</td>
</tr>
<tr>
<td>95% DMSO vehicle</td>
<td>31 ± 12</td>
</tr>
</tbody>
</table>

* Each antiviral formulation was tested 15 times.

to 250 mg of cream or ointment or 100 μl of solution of antiviral formulation was applied at each of these times. A drug formulation and its corresponding drug vehicle were always tested opposite each other at the same rostral-caudal level on the guinea pig dorsum (1). The three different drug formulations and their vehicles were tested once on each animal. On the day after completion of the treatment regimen, the dorsum of each animal was again depilated, and each infection site was evaluated with respect to numbers of lesions, diameter of lesions, total lesion area, and titer of virus in the excised site by methods previously described (20).

Penetration of EdU through guinea pig skin. Guinea pigs were sacrificed with ether and shaved closely with electric clippers, and full-thickness skin was clamped across the opening of a water-jacketed single-chambered glass diffusion cell (Crown Glass Co., Somerville, N.J.). The temperature of the receiver solution was kept at 37°C by circulating water through the outer jacket or left at room temperature (25°C). The glass cylinder clamped above the skin specimen was covered with Parafilm in the experiments at 25°C or left open to ambient conditions in experiments conducted at 37°C. Drug was applied to the exposed skin surface, and samples were withdrawn from the chamber at various time intervals and assayed for EdU by Derek Ilse, Ortho Pharmaceutical (Canada) Ltd., using the high-pressure liquid chromatography method of B. Hempel (14) or assayed for [³H]ACV in our laboratories by using a liquid scintillation counter. Drug flux was calculated from the steady-state slope of plots of drug concentration versus time by linear regression. This procedure has been detailed elsewhere (20).

Statistical procedures. Paired data (drug and drug vehicle) were evaluated by the Wilcoxon signed-rank test. The percent differences in various measures of lesion severity effected by each formulation in comparison to its vehicle control were compared with the efficacy of other formulations by a Mann-Whitney rank-sum procedure. Other data were analyzed by Student's t-test. All probability determinations were two-tailed, and a P value of ±0.05 was considered to be significant.

RESULTS

Efficacy of topical EdU in the dorsal cutaneous guinea pig model. Fifteen animals were inoculated with HSV-1 E115 as described above at six locations as on the dorsum, and the infection sites were treated topically for 3 days with 3% EdU cream, the cream vehicle, 3% EdU in 95% DMSO, 95% DMSO, 5% ACV in PEG, or PEG. Lesion severity at the different treatment sites was assessed on day 4 by lesion number, area, and virus titer. The results for each antiviral formulation and its corresponding vehicle control are shown in Table 1. Treatment with EdU cream compared to the cream alone reduced the mean lesion number, total lesion area, and lesion virus titer by 29, 44, and 68%, respectively. Application of 3% EdU in DMSO was more effective in reducing lesion severity (39, 60, and 90%, respectively), whereas ACV produced lesser benefit (15, 32, and 60%, respectively). For each of the formulations, the differences between treatments with drug and vehicle were statistically significant (P = 0.001 to 0.03). Mild to moderate erythema was seen in areas treated with EdU cream and cream base. There was no evidence of systemic drug toxicity with any of the treatments.

The percent reductions in lesion severity effected by each formulation were compared. The differences between topical ACV and EdU cream were not statistically significant. The differences between EdU in DMSO and the other two formulations were significant for lesion area and lesion virus titer (P = 0.002 to 0.04).

In vitro penetration of EdU through guinea pig skin. The ability of EdU and ACV to penetrate guinea pig skin from the different formulations was studied in vitro in a single-chambered glass diffusion cell at 25 and 37°C. Fresh, closely-clipped guinea pig skin was clamped across the top of the chamber, and 250 mg of 3% EdU cream, 250 mg of 5% [³H]ACV ointment, or 100 μl of 3% EdU in DMSO, amounts corresponding to the quantities used for treatment in the animal experiments, was applied at time zero. The results of experiments at room temperature (25°C) with the top of the cell covered or at 37°C with the top of the cell exposed to ambient conditions are shown in Fig. 1. The calculated mean flux values for each experiment are presented in Table 2. EdU readily penetrated guinea pig skin from the aqueous cream formulation. In contrast, the flux of ACV from PEG was minimal, and concentrations of ACV in the receiver chamber never exceeded 1 μg/ml even after 5 days. The flux of EdU from aqueous cream was enhanced threefold when the temperature was elevated from 25 to 37°C and the treated skin surface was left open to ambient conditions, whereas these changes had little effect on ACV penetration. At 37°C, the flux of EdU from cream was 10-fold that of ACV from PEG (P = 0.05). The highest rates of drug penetration occurred with EdU when DMSO was the vehicle.

DISCUSSION

In these studies, we have compared 3% EdU cream with 5% ACV ointment and 3% EdU in 95% DMSO in the topical treatment of cutaneous HSV disease. In guinea pigs with experimentally induced cutaneous HSV-1 infection, EdU cream reduced total lesion area by 44% compared with placebo-treated control lesions. In the same model, 5% ACV ointment reduced lesion area by 32%. EdU in aqueous cream penetrated guinea pig skin well in vitro, as much as 10-fold faster than ACV. The ability of EdU to penetrate skin from the cream formulation may explain its greater activity in the animal model, since EdU has less virus-inhibitory activity in vitro than ACV. When the penetration of EdU was further enhanced with DMSO, the therapeutic benefit in the animal model was twofold greater than that of ACV ointment (60% versus 32%; P = 0.003).

In the present diffusion experiments, experimental conditions different from prior studies (19, 20) were used to examine whether circumstances more closely approximating in vivo conditions would affect the rate of drug penetration. The rate of penetration of EdU from aqueous cream was
threefold greater at 37°C under ambient conditions than at 25°C with the skin chamber sealed. One reason for the increase is that drug flux is proportional to molecular diffusivity, which is determined in part by temperature (degrees Kelvin) (18). Further, because of the low ambient humidity in Utah, evaporation of water from aqueous cream likely occurs with the chamber uncovered, increasing the effective concentration of EdU and creating a steeper gradient across the skin (20). Interestingly, the rate of EdU penetration at 37°C appears to increase with time (Fig. 1, open triangles), consistent with the latter explanation. We conclude that formulations with volatile components such as EdU cream are most accurately evaluated in vitro at body temperature and ambient humidity.

We found that ACV formulated in PEG penetrated guinea pig skin poorly. These results, determined with [3H]ACV, confirm the findings of a prior study (20) in which ACV was measured by high-pressure liquid chromatography. There is no evidence as yet that ACV is less soluble in the stratum corneum than are other nucleoside antiviral agents. However, in addition to our ACV data, there is considerable evidence that PEG is a poor topical vehicle for a variety of molecules when skin penetration is the criterion (2-4). We have also observed that trifluorothymidine in PEG penetrates guinea pig skin at one-fourth the rate of trifluorothymidine in water (N. Sheth, D. Freeman, and S. Spruance, manuscript in preparation). PEG may be a poor vehicle in part because it is hygroscopic and dehydrates the stratum corneum (3). Clinical trials in Europe with topical ACV in a different vehicle (modified aqueous cream) have shown favorable results (12).

The antiviral action of EdU is similar to that of ACV. EdU binds selectively to HSV thymidine kinase, is ineffective against thymidine kinase-negative mutants of HSV, and has a high therapeutic index when concentrations of drug necessary to affect cellular functions are compared with the amounts required for virus inhibition (6, 8). EdU triphosphate is an effective substrate for HSV-induced DNA polymerase, and the mechanism of the virus-inhibitory action of the drug may be competitive inhibition of viral DNA polymerase or incorporation of the nucleotide analog into viral DNA or both (16).

Although topical treatment of recurrent cutaneous HSV disease has been discouraging, failure to combine early treatment with a penetration-enhancing formulation may well account for the limited benefits seen to date (20, 21). The present studies have shown that EdU cream and EdU in DMSO penetrate skin well and that they have efficacy comparable or superior to that of topical ACV ointment in the treatment of an experimental HSV-1 infection. Further studies with topical EdU are warranted to examine its possible role in the treatment of human mucocutaneous herpes simplex virus disease.

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LITERATURE CITED

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