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Preclinical assessment of topical treatments of herpes simplex virus infection: 5% (*E*)-5-(2-bromovinyl)-2′-deoxyuridine cream*

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Summary

The potential efficacy of topical therapy with (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) for cutaneous herpesvirus infection was evaluated in vitro and in guinea pigs. Drug sensitivity testing against herpes simplex virus type 1 strain E115 revealed an ID_{50} of 0.008 μg/ml for BVDU and 0.19 μg/ml for acyclovir (ACV). In vitro drug diffusion studies showed poor penetration of guinea pig skin by BVDU from the cream compared to BVDU from dimethylsulfoxide (DMSO) (0.04 vs. 1.5 μg/cm^2 per h). 5% BVDU cream, 5% BVDU/DMSO, and 5% ACV in polyethylene glycol (PEG) were then compared in the treatment of experimental dorsal cutaneous HSV-1 infection in guinea pigs. Lesion number, total lesion area and virus titer were reduced by all three formulations compared to control sites treated with the corresponding drug vehicles ($P \leqslant 0.01$). BVDU cream effected a greater reduction in lesion number (20% vs. 13%) and total lesion area (40% vs. 28%) than did ACV/PEG and a significantly greater decrease in virus titer (90% vs. 55%, $P < 0.001$). BVDU/DMSO was clinically twice as effective as BVDU cream ($P \leqslant 0.01$) and reduced lesion virus titers to a similar degree. The results of these studies show that BVDU is a more potent virus-inhibitory

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agent than ACV in vitro and is superior to topical ACV in vivo when formulated in a simple aqueous cream. The marked efficacy of BVDU/DMSO in the animal model demonstrates the potential of this antiviral if drug delivery is improved.

herpes simplex virus; topical antiviral therapy; (*E*)-5-(2-bromovinyl)-2′-deoxyuridine; percutaneous drug absorption; guinea pigs

Introduction

In recent years many new antiviral agents with activity against the herpesviruses have been developed. One of the most promising of these compounds is (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) [9]. When anti-herpesvirus compounds were compared for activity against 18 different isolates of herpes simplex virus (HSV) by De Clercq et al. [3], BVDU was found to have the lowest mean 50% virus inhibitory dose (ID_{50}) for HSV type 1 (HSV-1) strains tested and was 5-fold more active than acyclovir (ACV). Like ACV, BVDU is a nucleoside analogue which is selectively phosphorylated to the monophosphate derivative in virus-infected cells. Its in vitro antiviral index (therapeutic efficacy/toxicity) for HSV-1 is >7000 compared with 1250 for ACV, 27 for idoxuridine (IDU), and 1.7 for trifluorothymidine (TFT) [2]. BVDU is also a potent inhibitor of varicella-zoster virus [11]. In contrast, HSV type 2 is relatively insensitive to BVDU [3]. These differences in activity may be due to the different rates at which the virus-associated kinases catalyze the second step of BVDU phosphorylation from the mono- to the diphosphate [6].

The following experiments were designed to evaluate the efficacy of topical BVDU for the treatment of cutaneous HSV-1 infection. In vitro studies determined the sensitivity of our stock strain of HSV-1, E115, to BVDU and ACV, and drug diffusion studies measured the penetration of BVDU through excised guinea pig skin from test formulations. Topical BVDU and ACV were then compared in the treatment of experimental dorsal cutaneous HSV-1 infection in the Hubler guinea pig model [7]. We have previously shown that inoculation of the skin in this model does not alter the barrier properties of the skin, an important characteristic of the model in order for the results to relate to the early treatment of recurrent human HSV-1 infections [12].

Materials and Methods

Experimental animals and virus

Hartley strain, outbred, female albino guinea pigs, 200–250 g each, were obtained from Charles River Breeding Labs, Wilmington, MA. The virus used in these studies is the laboratory strain HSV-1 E115, originally obtained from Dr. André Nahmias. Virus stock contained $5–10 \times 10^7$ pfu/ml.

Antiviral sensitivity determination

The sensitivity of HSV-1 E115 to BVDU and ACV was assayed on Vero cells in 6-well tissue culture plates. Confluent monolayers were inoculated with 200 µl of virus stock in a dilution which gave 50–70 plaques/well in control wells ($10^{-5.3}$). After 1 h incubation the infected monolayers were overlaid with 2 ml of modified Eagle's minimal essential medium containing 0.5% agarose, 5% heat-inactivated fetal bovine serum, antibiotics and serial 2-fold dilutions of antiviral drug in the test wells. Each drug dilution was tested in triplicate. The plates were then incubated for 4–5 days in a 5% CO_2 atmosphere at 37°C, and the number of plaques was determined by staining with neutral red. The drug concentrations ($\log_{10}$ µg/ml) were plotted against the percent reduction in plaques compared to controls, and the concentration of drug producing a 50% reduction in plaque count (ID_{50}) was determined by linear regression analysis of the data.

Penetration of BVDU through guinea pig skin

Guinea pig skin was shaved, excised, and clamped in single-chambered glass diffusion cells as previously described [12]. The epidermal surface of the skin was treated once with 250 mg of 5% BVDU in Beeler base (BVDU/BB, BVDU cream) or 100 µl of 5% BVDU in 95% dimethylsulfoxide (DMSO). Samples (200 µl) were intermittently withdrawn from the receiver chamber of the cell over a 140 h period. All experiments were conducted at room temperature. Samples were analyzed for BVDU concentration by high performance liquid chromatography [14]. The results were expressed as µg/ml vs. time, and the steady state slopes of these plots used to calculate flux (J, µg/cm^2 per h).

Animal inoculation and treatment regimens

Guinea pigs were inoculated with undiluted virus stock (0.02 ml) on each of 6 areas on the back using a spring-loaded vaccination instrument [12]. Five percent BVDU/BB, Beeler base, and BVDU powder were provided by one of the authors (E.D.C.). Five percent ACV in polyethylene glycol (PEG) and PEG were obtained from Burroughs-Wellcome Co., and DMSO from Sigma Chemical Co. was used to prepare 95% DMSO in water (v/v). Beeler base (BB) consists of 15 g cetyl alcohol, 1 g cera alba, 10 g propylene glycol, 2 g sodium dodecyl sulfate and enough water to make 100 g. BVDU/BB, BB, ACV/PEG and PEG were applied with gloved fingertips in an amount sufficient to cover the infected area (250 mg). Five percent (w/v) BVDU in 95% DMSO (BVDU/DMSO) and DMSO were applied with a cotton-tipped applicator in an amount sufficient to leave the treatment site with a moistened appearance (100 µl). The day of inoculation was designated as day 0. Treatment was begun 24 h after inoculation and continued for a total of 3 days (days 1–3). Treatments were given 4 times a day at 9 a.m., 1 p.m., 5 p.m. and 9 p.m. Drug and the corresponding 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 in Results.

Skin homogenates and assays of virus infectivity

Animals were sacrificed with ether on day 4 after infection and the full-thickness

skin of the back from each treatment area was removed, minced, and homogenized in tissue culture medium. Debris was pelleted by centrifugation, and the supernatants were removed and stored frozen at -70°C until assay for infectivity was performed in duplicate on Vero cells using methods previously described [12].

Measures of drug efficacy and statistical procedures

On day 4, regrown hair was removed with depilatory and the number of lesions in each treatment site tallied. Lesion diameter was measured from photographs of the depilated backs using a calibrated hand lens. Differences between drug-treated and vehicle-treated sites were examined by the Wilcoxon signed-rank procedure. Lesion severity at drug-treated sites was expressed as percent of the result at the contralateral vehicle-treated site. To compare the efficacy of different formulations, the percent reductions effected by each drug formulation compared to its vehicle were examined by the Mann–Whitney rank-sum test. The slope of plotted data was determined by linear regression. Comparisons of drug flux were performed with Student's *t*-test. All probability determinations were two-tailed and $P \leqslant 0.05$ was considered significant.

Results

Sensitivity of HSV-1 to BVDU and ACV

The antiviral sensitivity of HSV-1 E115 was determined by the plaque reduction procedure. The concentrations of BVDU and ACV producing a 50% reduction in plaque count were 0.008 and 0.19 μg/ml, respectively.

In vitro skin penetration of BVDU through guinea pig skin

The skin penetration of BVDU, formulated as 5% BVDU/BB and 5% BVDU/DMSO, was studied in single-chambered glass diffusion cells. Single doses of 250 mg cream or 100 μl BVDU/DMSO solution were applied to the exposed stratum corneum side of the skin, doses corresponding to the amounts used for the treatment of the experimental HSV infection. The results are shown in Fig. 1. BVDU penetrated from the cream formulation slowly and, with the exception of one experiment, the concentration of BVDU in the receiver chamber was less than 5 μg/ml after 100 h. In contrast, when in the DMSO base, BVDU penetrated the skin quickly and accumulated in high concentrations in the receiver chamber (mean of 50 μg/ml at 96 h). The mean flux of BVDU from the cream base was 35-fold less than from DMSO (0.04 vs. 1.5 μg/cm^2 per h, $P = 0.008$), and 3-fold less than the flux of ACV from PEG (0.14 μg/cm^2 per h) determined previously by the same methods [12]. Thus, the relative order of drug flux from these formulations was BVDU/DMSO >> ACV/PEG > BVDU/BB.

Efficacy of topical BVDU/BB, BVDU/DMSO and ACV/PEG, against experimental cutaneous HSV-1 infection

Thirteen guinea pigs were infected in 6 different areas on the dorsum. The different infection sites were either left as untreated controls or treated with one of the following

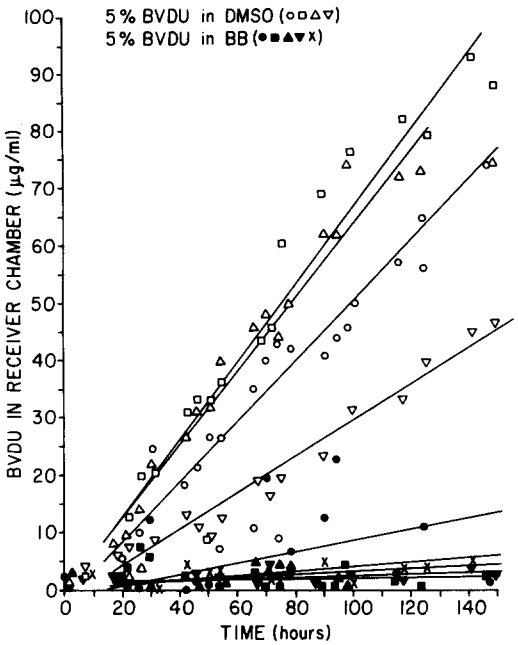

Fig. 1. In vitro penetration of guinea pig skin by BVDU from different vehicles. The concentration of drug in the receiver chamber of a glass diffusion apparatus is shown as a function of time. The different symbols indicate data for one experiment. Drugs were applied to the skin at time zero. Mean flux of BVDU/BB = 0.04 and of BVDU/DMSO = 1.5 (μg/cm² per h, $P = 0.008$).

regimens 4 times a day for 3 days: BB, 5% BVDU/BB, DMSO, 5% BVDU/DMSO, PEG or 5% ACV/PEG. Treatment was begun 24 h after inoculation, at which time lesions were just beginning to appear. Each treatment was paired on all occasions with its vehicle control applied at the contralateral infection site, and the position used on the back (shoulder, midback or rump) was rotated among the test preparations.

On the 4th day, the lesions were counted and measured, and the skin was harvested for virologic studies (Fig. 2). Lesion number, total lesion area and skin virus titer were used to assess efficacy. Position on the guinea pig back was examined and excluded as a variable affecting drug/vehicle differences, and the results from different dorsal locations were then combined.

When the results were examined, all 3 antiviral formulations were significantly more active than the vehicle controls by all measures of lesion severity ($P \leqslant 0.01$, Table 1). The antiviral effect was consistently greater than the clinical effect, and this was most notable with BVDU cream. BVDU/DMSO effected the most dramatic results: lesion count was reduced by 55%, total lesion area by 69% and virus titer by 94% compared with DMSO alone.

The 3 drug formulations were then evaluated relative to each other by comparing the percent reductions in severity effected by each antiviral over its contralateral vehicle control. When BVDU cream and ACV/PEG were compared, BVDU showed

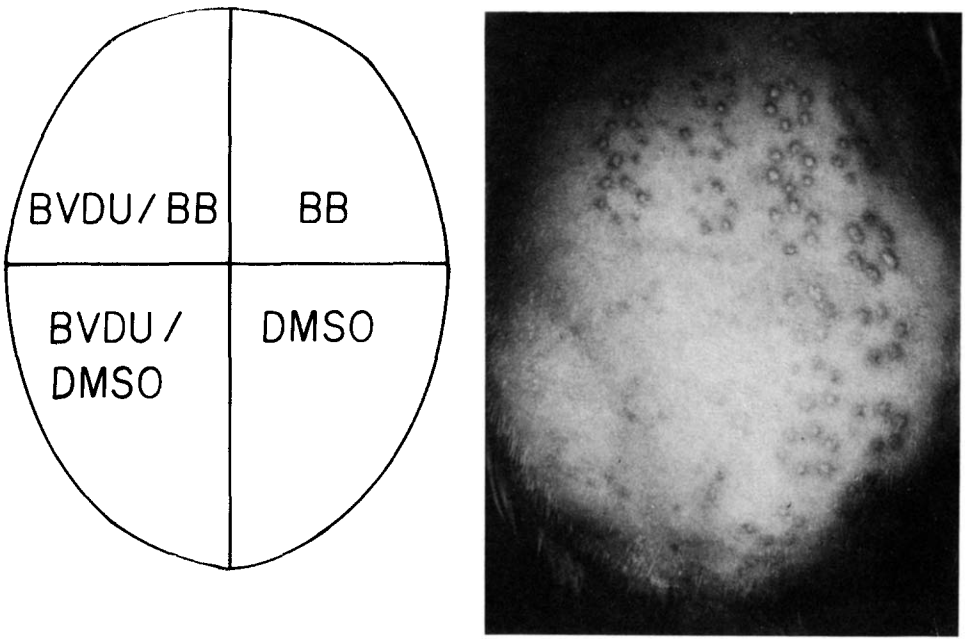

Fig. 2. The dorsal surface of an infected guinea pig on day 4. The drawing on the left shows the treatments given the animal in the photograph.

a greater percent reduction in lesion count (20% vs. 13%) and total lesion area (40% vs. 28%) and was significantly more effective than ACV/PEG in reducing lesion virus titer (90% vs. 55%, $P = 0.0003$). BVDU/DMSO was more potent than BVDU/BB in reducing lesion count (55% vs. 20%, $P = 0.0004$), total lesion area (69% vs. 40%, $P = 0.01$), and lesion virus titer (94% vs. 90%, $P = 0.13$).

Discussion

The present studies have compared topical BVDU with ACV in the dorsal cutaneous guinea pig model of HSV-1 infection. The in vitro inhibitory potency of BVDU and ACV was determined for the test HSV-1 strain, and the role of skin penetration in topical drug efficacy was examined by in vitro skin diffusion studies and formulation of BVDU in DMSO. The test HSV-1 strain (E115) was 20-fold more sensitive to BVDU than to ACV. The skin penetration of BVDU from the cream base was poor (0.04 $\mu g/cm^2$ per h) and one-third that previously reported for ACV from PEG [12]. In the animal model, topical BVDU/BB was moderately more effective than ACV/PEG. These results suggest that the greater viral inhibitory potency of BVDU compared to ACV was manifested by greater activity in vivo, but that poor skin penetration by BVDU from BB limited the extent of the clinical difference. Acceleration of BVDU skin penetration with DMSO resulted in a major therapeutic effect on lesion number

TABLE 1

Effect of 5% BVDU in BB, 5% BVDU in DMSO, and 5% ACV in PEG on experimental cutaneous HSV-1 infection

Measures of lesion severity	Treatment group[a]								
	BVDU/BB	% diff.[c] (*P*)	BB	BVDU/DMSO	% diff.[c] (*P*)	DMSO	ACV/PEG	% diff.[c] (*P*)	PEG
No. of lesions	39 ± 8[b]	20% (0.003)	49 ± 10	17 ± 6	55% (0.01)	39 ± 14	43 ± 7	13% (0.002)	52 ± 5
Total lesion area (mm^2)	59 ± 21	40% (0.001)	101 ± 24	16 ± 9	69% (0.01)	68 ± 44	71 ± 19	28% (0.003)	101 ± 18
Lesion virus titer (log_{10} pfu/ml)	3.2 ± 0.5	90% (0.001)	4.5 ± 0.5	2.1 ± 0.6	94% (0.01)	3.7 ± 0.6	3.8 ± 0.4	55% (0.001)	4.2 ± 0.4

[a] BVDU/BB and ACV/PEG were compared with their controls 13 times, BVDU/DMSO with its control 8 times.

[b] All data are mean ± S.D.

[c] Mean of the percent differences between the drug-vehicle pairs.

and area, which was approximately 2-fold greater and significantly different from the effects of BVDU in BB.

The topical use of BVDU has been previously evaluated in comparison with topical ACV in several mouse models of cutaneous HSV-1 infection. Descamps and co-workers [5] compared 13 anti-herpes agents, all formulated in Beeler base, in the treatment of cutaneous infection of athymic nude mice with HSV-1 strain KOS. Virus was inoculated intracutaneously by scarification, and treatment was started immediately. 1% BVDU/BB and 1% ACV/BB were considered equally effective in delaying and/or suppressing the development of skin lesions and in increasing mean survival time. BVDU showed an advantage over ACV when the drug concentrations were lowered to 0.1% in BB. At this dose, BVDU still inhibited the development of skin lesions, while 0.1% ACV showed no improvement over the control group. Park et al. [8] used the hairless mouse and initiated infection by inoculation of the McKrae HSV-1 strain on the forehead. Topical 3% and 5% BVDU in PEG were compared with 5% ACV/PEG and PEG alone. The authors concluded that the therapeutic efficacy of topical BVDU was comparable to that of ACV. However, BVDU was more effective than ACV in preventing death when therapy was initiated late.

The results in mice [5,8] and the present report provide evidence that there can be differences in experimental therapeutic effectiveness among nucleosides which have high degrees of in vitro viral inhibitory potency. To demonstrate these differences, the experimental treatment protocol must not be so favorable for a positive outcome that both agents are highly and equally successful. Dose reduction [5], delay in treatment [8], and restriction of drug penetration by the stratum corneum, as in the present studies, appear to be useful protocol modifications for this purpose. BVDU may be useful for forms of human HSV disease that are difficult to treat such as recurrent herpes labialis in immunologically normal persons [13].

In the present study, DMSO alone reduced clinical lesion severity (Table 1), an effect which may be due to its anti-inflammatory activity [4]. Previous reports have shown conflicting results when the effect of DMSO alone on virus titers has been examined [1,12]. We have examined this question by infecting 12 guinea pigs with HSV-1 and comparing DMSO-treated areas with contralateral untreated areas. The virus titers (mean $\pm$ S.D., $\log_{10}$ pfu/ml) were 4.8 ± 0.4 and 4.4 ± 0.8 for the untreated and DMSO-treated areas respectively ($p = 0.12$, sign rank test; S.L. Spruance, unpublished data).

Comparison of antivirals is not necessarily best accomplished by formulating agents in the same vehicle, since differences in drug solubility can affect the partition coefficient and cause differences in drug flux through the skin [10]. Both the activity of antivirals in cell culture and the skin penetration of antivirals from various vehicles can be studied in vitro and used to interpret experimental therapeutic findings. A possible combined expression of these in vitro characteristics is the ratio of skin penetration as the drug flux, J ($\mu g/cm^2$ per h), to antiviral activity, ID_{50}. As the value of J/ID_{50} increases, so might the clinical efficacy. When J/ID_{50} is determined for the drug formulations used in the current set of experiments, the values correspond to the relative order of efficacy of the formulations in the treatment of infection in the animal model: BVDU/DMSO > BVDU/BB > ACV/PEG. We are currently determin-

ing J/ID_{50} for additional antiviral formulations and correlating the results with the effect of each formulation in the animal model.

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