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Topical Treatment of Cutaneous Herpes Simplex Virus Infection in Hairless Mice with (*E*)-5-(2-Bromovinyl)-2′-Deoxyuridine and Related Compounds

ERIK DE CLERCQ

Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

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(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (bromovinyldeoxyuridine) was found to suppress the development of herpetic skin lesions and the paralysis and mortality associated therewith in hairless mice inoculated intracutaneously with herpes simplex virus type 1. This protective effect was achieved with bromovinyldeoxyuridine applied topically at 1, 3, or 10% in either dimethylsulfoxide (DMSO), Beeler base, Tween-glycerol-water, 5% Azone (1-dodecylazacycloheptan-2-one) in water, or 5% Azone in DMSO. The optimal vehicle was 5% Azone in DMSO, in which bromovinyldeoxyuridine was effective even at a concentration as low as 0.3%. In its protective activity against cutaneous herpes simplex virus type 1 infection in hairless mice, bromovinyldeoxyuridine was clearly superior to other established antiherpes compounds such as 5-iodo-2′-deoxyuridine, 5-ethyl-2′-deoxyuridine, arabinosyl thymine, and arabinosyl (*E*)-5-(2-bromovinyl) uracil when formulated at 10% in DMSO or Azone-DMSO. However, no activity was noted with any of these drug formulations against cutaneous herpes simplex virus type 2 infection. In contrast, acycloguanosine (acyclovir) proved quite effective in the topical treatment of cutaneous herpes simplex virus type 2 infection when used at 10% in DMSO or at 5% in propylene glycol.

Intracutaneous inoculation of hairless (hr/hr) mice with herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) represents an adequate experimental model for determining the efficacy of topically applied antiviral drugs against primary HSV infections. This model allows one to monitor the effects of the drugs on both local (skin lesions) and systemic (paralysis, death) manifestations of the disease and has been used to establish the effectiveness of such antiviral agents as phosphonoacetic acid (PAA) (16, 17), arabinosyl adenine (AraA) (14, 17), AraA monophosphate (14, 17), acycloguanosine (acyclovir [ACV]) (15), phosphonoformate (PFA) (13), and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (bromovinyldeoxyuridine [BVdU]) (9).

Few studies have focused on the relative efficacy of antiviral compounds in the topical treatment of HSV infections. Alenius and Öberg (3) noted that of five antiviral agents, AraA, arabinosyl cytosine, iododeoxyuridine, ribavirin, and PAA, only PAA showed good therapeutic activity against cutaneous HSV-1 infection in guinea pigs. A similar therapeutic effect was noted for PFA (2), and when compared with ACV, PFA proved clearly superior for topical treatment of HSV-1 skin lesions in guinea pigs (1). In the latter study ACV and PFA were formulated in different (and not necessarily optimal) vehicles, which makes a direct comparison of their efficacy rather difficult. When ACV, PFA, and several other antiviral compounds were compared under standardized conditions, i.e., applied topically at 1% in Beeler base (BB) in athymic nude mice infected intracutaneously with HSV-1, their order of activity was PAA > BVdU ~ ACV > PFA > AraA (10).

The efficacy of antiherpes agents in the topical treatment of cutaneous HSV infection may vary considerably, depending on a number of factors such as the intrinsic antiviral potency of the compound (8), the virus type and strain used, the time at which treatment is started, the concentration at which the drug is applied, the number of applications, and the delivery form (vehicle) used for the drug. The present study was designed to establish the importance of some of these factors, in particular the delivery form and drug concentration, for BVdU and various other antiherpes compounds, viz., ACV, PAA, 5-iodo-2′-deoxyuridine (IdU), 5-ethyl-2′-deoxyuridine (EdU), arabinosyl thymine (AraT), and arabinosyl (*E*)-5-(2-bromovinyl)uracil (BVaraU). BVdU and its congeners were formulated at different concentrations, varying from 0.3 to 10%, in different vehicles (including dimethylsulfoxide [DMSO]), and their efficacy was assessed against cutaneous HSV-1 and HSV-2 infections in hairless mice. These studies bear on the usefulness of BVdU and related compounds in the topical treatment of cutaneous HSV infections in humans. DMSO was included among the vehicles since IdU at 10% in DMSO is currently licensed in some countries (e.g., Belgium) for the topical treatment of herpetic (HSV-1 and HSV-2) skin infections.

MATERIALS AND METHODS

Mice. Hairless (hr/hr) mice that were 25 to 30 days old and weighed 15 to 20 g were used throughout all experiments. The mice were bred by backcross and intercross of the homozygous parents. The hr/hr mouse strain was initially obtained from D. Gil (Central Animal Laboratory, Utrecht, The Netherlands). The mice were housed under conventional conditions in groups of five and given food and drinking water ad libitum. Female and male mice were used at ramdom in all experiments.

Viruses. The origins of HSV-1 strain KOS and HSV-2 strain 196 have been described previously (8). Virus stocks were prepared in primary rabbit kidney cell cultures. The titers of the HSV-1 KOS and HSV-2 196 virus stocks were $10^{6.7}$ and $10^{5.7}$ PFU/ml, respectively.

Test compounds. BVdU was synthesized by R. Busson and H. Vanderhaeghe (Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium) by a

procedure similar to that described by Jones et al. (12). IdU was obtained from Ludeco (Brussels, Belgium), and EdU was kindly provided by E. Mauz (Robugen GmbH, Esslingen, Federal Republic of Germany). AraT and BVaraU were gifts from H. Machida (Yamasa Shoyu Co., Choshi, Japan). ACV and PAA were from Wellcome Research Laboratories (Research Triangle Park, N.C.) and Abbott Laboratories (North Chicago, Ill.), respectively. The 5% ACV cream (Zovirax) was supplied by A. P. Fiddian (Wellcome Research Laboratories, Beckenham, England); this formulation is based upon propylene glycol as the solvent (4, 11).

Vehicles. The following vehicles were used for drug formulation: DMSO; BB (15 g of cetyl alcohol, 1 g of cera alba, 10 g of propylene glycol, 2 g of sodium lauryl sulfate, and enough water to make 100 g); polyethylene glycol (PEG); TGW (0.1% Tween 80, 10% glycerol, and water [pH 6.0]; AZW (5% Azone in water); and AZDMSO (5% Azone in DMSO). Azone (1-dodecylazocycloheptan-2-one) (20) was provided by V. J. Rajadhyaksha (Nelson Research, Irvine, Calif.).

Virus inoculation. The mice were infected intracutaneously in the lumbosacral area with either HSV-1 or HSV-2. The skin was scratched with a scarificator and inoculated with HSV-1 KOS at $10^{4.7}$ PFU/0.05 ml per mouse or with HSV-2 196 at $10^{3.7}$ PFU/0.05 ml per mouse.

Treatment. Unless stated otherwise, the animals were treated topically with the indicated formulation four times a day for 5 days, starting immediately after virus infection.

Scoring. Three parameters of infection were followed: (i) skin lesions presenting as an epidermal ulceration, which were considered positive if at least 0.5 cm long; (ii) paralysis of the hind legs; and (iii) mortality. Those mice that developed paralysis invariably had concomitant skin lesions. The data are presented as the number of survivors, including the number with skin lesions only and the number with skin lesions plus paralysis, on days 4, 6, 8, 10, 15, and 20 after virus infection. Also indicated is the percent survivors at day 20.

RESULTS

The 50% effective doses of BVdU, IdU, EdU, AraT, ACV, and PAA against HSV-1 KOS and HSV-2 196 in vitro (primary rabbit kidney cell cultures) have been reported previously (8). The 50% effective dose of BVaraU for HSV-1 KOS has also been reported (5); its 50% effective dose for HSV-2 196 in primary rabbit kidney cells was 70 μg/ml (E. De Clercq, unpublished data).

When used at 10% in DMSO in hairless mice infected intracutaneously with HSV-1 KOS, IdU suppressed the development of skin lesions and paralysis of the hind legs and reduced the mortality rate from 100 to 30% (Table 1). A similar protective effect was exhibited by AraT. EdU, however, did not significantly alter the course of the infection, nor did it decrease the final mortality rate by more than 10%. In contrast, BVdU conferred complete protection against HSV-1 infection and reduced the mortality rate from 100 to 0%. Under similar conditions, BVaraU proved significantly less protective than BVdU, as it reduced the mortality rate to only 50%.

When IdU, EdU, and BVdU were applied topically at 10% in DMSO to hairless mice infected intracutaneously with HSV-2 196, none of the drugs had a substantial effect on the symptoms of the infection and the ensuing mortality rate (Table 1). Yet under the same conditions, ACV proved highly efficacious. When applied at 10% in DMSO, ACV offered complete protection against HSV-2 infection, with an increase in the survival rate from 0 to 100%. When used at 1% in DMSO, ACV offered only transient protection and an increase in survival rate from 0 to 20%.

The effects of topical BVdU treatment on HSV-1 infection were then examined at different concentrations of the drug in different vehicles (Table 2). Irrespective of the vehicle (DMSO, BB, PEG, TGW, AZW, or AZDMSO), BVdU completely suppressed all manifestations of the disease when used at 10%, but even at 3 or 1% it effected complete protection in the majority (70 to 100%) of the mice. Whereas

TABLE 1. Effects of BVdU, EdU, IdU, BVaraU, AraT, and ACV at 10% in DMSO on the development of herpetic skin lesions, paralysis of the hind legs, and mortality of hairless mice inoculated intracutaneously with HSV-1 KOS or HSV-2 196[a]

HSV strain and compound tested	No. of survivors (no. with skin lesions only/no. with skin lesions plus paralysis) on the following day after virus inoculation:						% Survivors
	4	6	8	10	15	20	
HSV-1 KOS							
BVdU	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
EdU	10 (0/0)	9 (4/3)	3 (1/1)	1 (0/0)	1 (0/0)	1 (0/0)	10
IdU	10 (0/0)	10 (0/0)	10 (1/0)	10 (1/1)	7 (0/0)	7 (0/0)	70
BVaraU	10 (0/0)	10 (2/0)	10 (1/2)	8 (0/1)	5 (0/0)	5 (0/0)	50
AraT	10 (0/0)	10 (0/0)	9 (0/0)	8 (0/0)	7 (0/0)	7 (0/0)	70
DMSO	10 (0/0)	8 (3/5)	1 (0/1)	0	0	0	0
No treatment	10 (0/0)	6 (0/6)	0	0	0	0	0
HSV-2 196							
BVdU	10 (3/0)	10 (3/7)	0	0	0	0	0
EdU	10 (2/0)	10 (5/2)	6 (1/3)	3 (1/1)	1 (0/1)	0	0
IdU	10 (1/0)	9 (4/2)	5 (2/2)	3 (1/1)	1 (0/0)	1 (0/0)	10
ACV	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
ACV[b]	10 (0/0)	10 (4/0)	8 (3/2)	6 (1/4)	2 (0/0)	2 (0/0)	20
DMSO	10 (4/0)	8 (5/3)	0	0	0	0	0
No treatment	10 (3/0)	8 (5/2)	5 (0/5)	0	0	0	0

[a] Ten mice were used in each experiment.
[b] At 1% in DMSO.

TABLE 2. Effects of BVdU at 0.3, 1, 3, or 10% in different vehicles on the development of herpetic skin lesions, paralysis of the hind legs, and mortality of hairless mice inoculated intracutaneously with HSV-1 KOS

BVdU concn (%)	Vehicle	No. of mice	No. of survivors (no. with skin lesions only/no. with skin lesions plus paralysis) on the following day after virus inoculation:						% Survivors
			4	6	8	10	15	20	
10	DMSO	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
3	DMSO	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
1	DMSO	10	10 (0/0)	10 (2/0)	10 (0/3)	8 (0/1)	8 (0/1)	7 (0/0)	70
0.3	DMSO	10	10 (0/0)	6 (2/3)	2 (1/1)	1 (0/1)	1 (0/1)	1 (0/1)	10
0	DMSO	20	20 (2/0)	9 (1/8)	0	0	0	0	0
10	BB	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
3	BB	10	10 (0/0)	10 (1/0)	10 (0/2)	9 (0/1)	8 (0/0)	7 (0/0)	70
1	BB	10	10 (0/0)	10 (0/0)	10 (0/3)	9 (0/2)	7 (0/0)	7 (0/0)	70
0.3	BB	10	10 (0/0)	10 (6/4)	4 (0/4)	1 (0/1)	0	0	0
0	BB	20	20 (4/0)	6 (1/5)	0	0	0	0	0
10	PEG	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
3	PEG	10	10 (0/0)	10 (1/0)	10 (0/1)	9 (0/0)	9 (0/0)	9 (0/0)	90
1	PEG	10	10 (0/0)	10 (1/0)	10 (0/1)	9 (0/0)	9 (0/0)	9 (0/0)	90
0.3	PEG	10	10 (0/0)	9 (5/4)	3 (1/2)	1 (0/1)	1 (0/1)	1 (0/1)	10
0	PEG	20	20 (2/0)	15 (2/13)	4 (0/4)	0	0	0	0
10	TGW	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
3	TGW	10	10 (0/0)	10 (1/0)	10 (0/1)	9 (0/0)	9 (0/0)	9 (0/0)	90
1	TGW	10	10 (0/0)	10 (2/1)	8 (0/1)	7 (0/0)	7 (0/0)	7 (0/0)	70
0.3	TGW	10	10 (0/0)	9 (4/5)	0	0	0	0	0
0	TGW	20	20 (4/0)	16 (0/16)	2 (0/2)	0	0	0	0
10	AZW	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
3	AZW	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
1	AZW	10	10 (0/0)	10 (2/0)	10 (2/0)	8 (0/0)	8 (0/0)	8 (0/0)	80
0.3	AZW	10	10 (0/0)	10 (4/3)	3 (0/1)	2 (0/0)	2 (0/0)	2 (0/0)	20
0	AZW	20	20 (4/0)	12 (2/10)	1 (0/1)	0	0	0	0
1	AZDMSO	10	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
0.3	AZDMSO	10	10 (0/0)	10 (1/0)	9 (0/0)	9 (0/0)	9 (0/0)	9 (0/0)	90
0	AZDMSO	10	10 (0/0)	10 (8/2)	5 (0/5)	0	0	0	0

all control mice eventually succumbed to the infection, treatment with BVdU at 1% resulted in an average survival rate of 80%, whether the drug was formulated in DMSO, BB, PEG, TGW, AZW, or AZDMSO.

When applied at 0.3% in DMSO, BB, PEG, TGW, or AZW onto the skin of HSV-1-infected mice, BVdU temporarily delayed the appearance of symptoms but did not markedly affect the final mortality rate (Table 2). However, when BVdU was applied at 0.3% in AZDMSO, it suppressed the development of HSV-1 infection and increased the survival rate from 0 to 90%. Thus, AZDMSO turned out to be the most appropriate of all vehicles examined for topical use of BVdU in hairless mice.

When BVdU, EdU, and IdU were formulated at 10% in AZDMSO and reexamined for their inhibitory effects on cutaneous HSV-1 and HSV-2 infection in hairless mice (Table 3), EdU and IdU proved clearly more effective in suppressing the disease than when applied at 10% in DMSO (Table 1). For BVdU no increase in activity could be noted, as it already gave optimal protection when used at 10% in DMSO. Although it increased the potency of the drugs against HSV-1 infection, AZDMSO did not substantially potentiate the activity of either BVdU, EdU, or IdU against HSV-2 infection (Tables 1 and 3).

An ACV formulation based on propylene glycol and termed "cream" (11) has previously been found to be superior to ACV formulated in ointment (based on polyethylene glycol) in the topical treatment of cutaneous HSV infections in guinea pigs (4). This 5% ACV cream was successful in 100% of the mice infected with HSV-1 (Table 3). It also proved successful in the topical treatment of HSV-2 infection, but only in 50% of the mice.

Finally, a selected number of established (8) antiherpes (HSV-2) compounds were compared for their protective effects against cutaneous HSV-2 infection in hairless mice (Table 4). When applied twice daily at 5% in BB for 10 days, PAA and ACV reduced the mortality rate from 100 to 20 and 40%, respectively. When evaluated under the same conditions, AraT and EdU did not cause any change in the final mortality rate.

DISCUSSION

BVdU has proven efficacious in the systemic and topical treatment of various experimental HSV-1 infections, including cutaneous herpes infection of athymic nude mice and guinea pigs, orofacial herpes infection of mice, herpetic encephalitis in mice, and herpetic keratitis and uveitis (iritis) in rabbits (reviewed in references 6 and 7). When applied topically at 5% in BB to hairless mice infected with HSV-1, BVdU suppressed the development of skin lesions and reduced the death rate from 82 to 12% (9). However, the same treatment had no effect on cutaneous HSV-2 infection in hairless mice (9).

The present study was aimed at further evaluating the effectiveness of BVdU in the topical treatment of cutaneous

TABLE 3. Effects of BVdU, EdU, and IdU at 10% in AZDMSO and of ACV as a 5% cream (Zovirax) on the development of herpetic skin lesions, paralysis of the hind legs, and mortality of hairless mice inoculated intracutaneously with either HSV-1 KOS or HSV-2 196[a]

HSV strain and compound tested	No. of survivors (no. with skin lesions only/no. with skin lesions plus paralysis) on the following day after virus inoculation:						% Survivors
	4	6	8	10	15	20	
HSV-1 KOS							
BVdU	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
EdU	10 (0/0)	10 (0/0)	10 (0/0)	9 (0/0)	6 (0/0)	6 (0/0)	60
IdU	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
AZDMSO	10 (0/0)	10 (9/1)	3 (0/3)	0	0	0	0
Zovirax	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	10 (0/0)	100
No treatment	10 (7/0)	3 (0/3)	0	0	0	0	0
HSV-2 196							
BVdU	10 (0/0)	8 (6/2)	1 (0/1)	0	0	0	0
EdU	10 (0/0)	10 (6/0)	5 (0/3)	3 (0/1)	2 (0/0)	2 (0/0)	20
IdU	10 (0/0)	10 (5/0)	3 (1/2)	0	0	0	0
AZDMSO	10 (0/0)	10 (7/1)	3 (1/1)	1 (0/0)	1 (0/0)	1 (0/0)	10
Zovirax	10 (0/0)	10 (0/0)	10 (0/0)	8 (0/1)	5 (0/0)	5 (0/0)	50
No treatment	10 (0/0)	10 (5/3)	4 (1/2)	1 (0/0)	1 (0/1)	1 (0/1)	10

[a] Ten mice were used in each experiment.

HSV infection in hairless mice. Experiments were specifically designed to determine the optimal vehicle and minimum drug concentration required to achieve protective activity against herpetic skin lesions, paralysis, and mortality in hairless mice inoculated intracutaneously with HSV-1 and HSV-2.

BVdU was highly effective in the topical treatment of cutaneous HSV-1 infection in hairless mice, irrespective of the vehicle in which it was delivered (DMSO, BB, PEG, TGW, AZW, or AZDMSO). The optimal vehicle appeared to be AZDMSO. Azone has been found to enhance the percutaneous penetration of various chemical agents such as antibiotics, fluorouracil, and glucocorticoids (20). Recently, W. M. Shannon et al. (W. M. Shannon, L. Westbrook, W. I. Higuchi, R. Vaid Yanathan, and D. C. Baker, Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 149, 1983) reported that AraA, AraA-2',3'-diacetate, and ACV showed enhanced penetration and efficacy in the topical treatment of HSV-1 skin infections in hairless mice when the antiviral drugs were formulated in Azone.

Azone can be applied neat to human skin without any irritation (20). Likewise, 5% Azone in either water or DMSO did not prove irritating to the hairless mouse skin. When formulated in AZDMSO, BVdU was effective at a concentration as low as 0.3% in suppressing HSV-1 skin infection and mortality associated therewith in hairless mice (Table 2). This is a much lower concentration than that generally prescribed for other topical antiherpes drugs, i.e., PFA (3% [1]), ACV (5% [11, 19]), arildone (8%). In fact, arildone (Win 38030 [18]) did not show any effect on cutaneous HSV-1 infection in hairless mice when applied at either 8% in the recommended cream form (Winthrop Laboratories, Div. Sterling Drug Inc., New York, N.Y.) or 10% in DMSO (data not shown).

In the topical treatment of cutaneous HSV-1 infection in hairless mice, BVdU was far more effective than several other established (8) antiherpes compounds, such as IdU, EdU, BVaraU, and AraT. This is clearly illustrated by the comparative effectiveness of the compounds when delivered at 10% in DMSO (Table 1). That BVdU would be more effective than IdU, EdU, BVaraU, and AraT could be expected from the relative potency of these agents against HSV-1 in cell culture (5, 8).

Although BVdU was effective at 0.3% (in AZDMSO) against cutaneous HSV-1 infection, it did not show any activity against HSV-2 infection, even if formulated at 10% (in either DMSO or AZDMSO). Again, this is in agreement with the different sensitivities of HSV-1 and HSV-2 toward BVdU in cell culture (8), as BVdU does not inhibit HSV-2 replication in cell culture unless it is used at a concentration that is at least 100-fold higher than that required for inhibition of HSV-1 replication.

Like BVdU, IdU and EdU were unsuccessful in protecting hairless mice against cutaneous HSV-2 infection, whether

TABLE 4. Effects of PAA, ACV, AraT, and EdU at 5% in BB on the development of herpetic skin lesions, paralysis of the hind legs, and mortality of hairless mice inoculated intracutaneously with HSV-2 196

Compound[a]	No. of mice	No. of survivors (no. with skin lesions only/no. with skin lesions plus paralysis) on the following day after virus inoculation:						% Survivors
		4	6	8	10	15	20	
PAA	10	10 (0/0)	10 (0/0)	9 (0/0)	9 (0/0)	8 (0/0)	8 (0/0)	80
ACV	10	10 (0/0)	10 (1/0)	10 (0/5)	9 (0/6)	7 (0/4)	6 (0/3)	60
AraT	10	10 (0/0)	10 (9/0)	7 (2/5)	3 (0/3)	0	0	0
EdU	10	10 (0/0)	9 (6/3)	2 (0/2)	0	0	0	0
BB	20	20 (0/0)	16 (12/3)	3 (0/2)	0	0	0	0

[a] Applied twice daily for 10 days, starting on the day of infection.

the drugs were applied at 10% in DMSO (Table 1) or in AZDMSO (Table 3). This is rather surprising, as IdU and EdU were effective against HSV-1 infection in hairless mice when applied at 10% in AZDMSO (Table 3), and, since their potency against HSV-2 in cell culture is comparable to their potency against HSV-1 (8, 21), they may have been expected to suppress HSV-2 infection in vivo.

The cutaneous HSV-2 model infection in hairless mice seemed adequate to demonstrate the in vivo activity of PAA and ACV against HSV-2 (Table 4). When formulated at 5% in BB, both PAA and ACV suppressed the development of HSV-2 infection in hairless mice. This was also the case (Table 3) with ACV formulated at 5% in its recommended cream form (4, 11).

In all experiments treatment was initiated immediately after virus inoculation. This protocol does not allow a direct extrapolation to the situation in humans, where treatment can be started only upon appearance of the clinical symptoms. However, the experiments were designed primarily to assess the comparative efficacy of a number of antiviral compounds, when formulated in different vehicles, in the topical treatment of HSV-1 and HSV-2 skin infections.

Our findings point to the effectiveness of BVdU, delivered at 0.3% in AZDMSO or at 1 to 10% in any other vehicle, in the topical treatment of cutaneous HSV-1 infection, as well as its ineffectiveness, even if delivered at 10%, in the topical treatment of HSV-2 infection.

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LITERATURE CITED

1. **Alenius, S., M. Berg, F. Broberg, K. Eklind, B. Lindborg, and B. Öberg.** 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., Z. Dinter, and B. Öberg.** 1978. Therapeutic effect of trisodium phosphonoformate on cutaneous herpesvirus infection in guinea pigs. Antimicrob. Agents Chemother. **14**:408–413.
3. **Alenius, S., and B. Öberg.** 1978. Comparison of the therapeutic effects of five antiviral agents on cutaneous herpesvirus infection in guinea pigs. Arch. Virol. **58**:277–288.
4. **Collins, P., and N. M. Oliver.** 1982. Acyclovir treatment of cutaneous herpes in guinea pigs and herpes encephalitis in mice. Am. J. Med. **73A**:96–99.
5. **De Clercq, E.** 1982. Comparative efficacy of antiherpes drugs in different cell lines. Antimicrob. Agents Chemother. **21**:661–663.
6. **De Clercq, E.** 1983. The chemotherapy of herpesvirus infections with reference to bromovinyldeoxyuridine and other antiviral compounds, p. 295–315. *In* C. H. Stuart-Harris and J. Oxford (ed.), Problems of antiviral therapy. Academic Press, Inc., London.
7. **De Clercq, E.** 1983. Antiviral activity of 5-substituted pyrimidine nucleoside analogues. Pure Appl. Chem. **55**:623–636.
8. **De Clercq, E., J. Descamps, G. Verhelst, R. T. Walker, A. S. Jones, P. F. Torrence, and D. Shugar.** 1980. Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. J. Infect. Dis. **141**:563–574.
9. **De Clercq, E., and Z.-X. Zhang.** 1982. Differential effects of *E*-5-(2-bromovinyl)-2′-deoxyuridine on infections with herpes simplex virus type 1 and type 2 in hairless mice. J. Infect. Dis. **145**:130.
10. **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.
11. **Fiddian, A. P., J. M. Yeo, R. Stubbings, and D. Dean.** 1983. Successful treatment of herpes labialis with topical acyclovir. Br. Med. J. **286**:1699–1701.
12. **Jones, A. S., G. Verhelst and R. T. Walker.** 1979. The synthesis of the potent anti-herpes virus agent, *E*-5-(2-bromovinyl)-2′-deoxyuridine and related compounds. Tetrahedron Lett. **45**:4415–4418.
13. **Klein, R. J., E. DeStefano, E. Brady, and A. E. Friedman-Kien.** 1979. Latent infections of sensory ganglia as influenced by phosphonoformate treatment of herpes simplex virus-induced skin infections in hairless mice. Antimicrob. Agents Chemother. **16**:266–270.
14. **Klein, R. J., and A. E. Friedman-Kien.** 1977. Latent herpes simplex virus infections in sensory ganglia of mice after topical treatment with adenine arabinoside and adenine arabinoside monophosphate. Antimicrob. Agents Chemother. **12**:577–581.
15. **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.
16. **Klein, R. J., A. E. Friedman-Kien, A. A. Fondak, and E. Buimovici-Klein.** 1977. Immune response and latent infection after topical treatment of herpes simplex virus infection in hairless mice. Infect. Immun. **16**:842–848.
17. **Klein, R. J., A. E. Friedman-Kien, and P. B. Yellin.** 1978. Orofacial herpes simplex virus infection in hairless mice: latent virus in trigeminal ganglia after topical antiviral treatment. Infect. Immun. **20**:130–135.
18. **Pancic, F., B. A. Steinberg, G. D. Diana, P. M. Carabateas, W. G. Gorman, and P. E. Came.** 1981. Antiviral activity of Win 41258-3, a pyrazole compound, against herpes simplex virus in mouse genital infection and in guinea pig skin infection. Antimicrob. Agents Chemother. **19**:470–476.
19. **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 acyclovir in polyethylene glycol. J. Infect. Dis. **146**:85–90.
20. **Stoughton, R. B.** 1982. Enhanced percutaneous penetration with 1-dodecylazacycloheptan-2-one. Arch. Dermatol. **118**:474–477.
21. **Teh, C.-Z., and S. L. Sacks.** 1983. Susceptibility of recent clinical isolates of herpes simplex virus to 5-ethyl-2′-deoxyuridine: preferential inhibition of herpes simplex virus type 2. Antimicrob. Agents Chemother. **23**:637–640.