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Review Article

Evaluation of antiviral treatments for recurrent herpes simplex labialis in the dorsal cutaneous guinea pig model

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Summary

Recurrent herpes simplex labialis has proved to be a difficult disease to treat. Despite 25 years of clinical research with established antiviral substances, only small benefits from experimental therapies have been demonstrated. Progress has been slow, in part, because of the time-consuming nature of large, patient-initiated clinical trials. The dorsal cutaneous guinea pig model is a rapid and efficient means to identify topical antiviral formulations with clinical promise. The cumulative results of our studies with 19 different test treatments show that 8 were equal in efficacy to 5% acyclovir ointment, one was worse and ten were better. Two of the treatments found to be better than 5% acyclovir ointment have been studied clinically, with limited but encouraging results. Differences between the guinea pig model and the human illness mandate caution in predicting the degree of clinical efficacy from experimental outcomes. An effective and conservative use of the model is to optimize the topical formulation of a single antiviral substance.

Herpes simplex labialis; Test treatment; Acyclovir; Guinea pig model

Introduction

A variety of animal models has been used to evaluate the efficacy of antiviral chemotherapy for the treatment of mucocutaneous herpes simplex virus (HSV) in-

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fection. These models include infection of the dorsal cutaneous surface of the guinea pig (Hubler et al., 1974), the female guinea pig genitalia (Hsiung et al., 1984; Stanberry et al., 1982), the lumbosacral skin of the hairless mouse (Klein et al. 1979; Lieberman et al., 1973), the lumbosacral skin of the athymic nude mouse (Descamps et al., 1979), the orofacial skin of CD-1 and hairless mice (Klein et al., 1979; Park et al., 1979), and the ears of Swiss white mice (Hill et al., 1975). These models have been used to evaluate a wide variety of antiviral agents administered by the topical, oral, intramuscular or intraperitoneal routes. The virus type employed has most commonly been HSV-1, with the exception of HSV-2 in the female guinea pig genitalia model.

Primary mucocutaneous human HSV infections and HSV infections in immunocompromised hosts are characterized by the formation of multiple and/or large lesions in the ulcer/soft crust stage and a prolonged course during which the disease may progress in severity for several days or weeks before healing begins. In contrast, recurrent human HSV disease in immunocompetent hosts is a brief illness of 7–10 days duration in which maximum lesion severity develops within hours after onset (Spruance et al., 1977; Spruance and Wenerstrom, 1984). These characteristics necessitate that recurrent HSV infections be treated very early, well before the epithelium ruptures. Therefore, penetration of the intact epithelium should be critical to the efficacy of topically administered antiviral substances. Based on these considerations, the predictive power of an animal model testing topical treatments for recurrent human HSV infections depends in part on the application of drug to intact skin.

With regard to the skin as a barrier to topically administered drugs, there are several aspects of existing animal model systems that deserve comment. The process of virus inoculation into the skin of the experimental animal may alter the integrity of the stratum corneum and create an abnormal percutaneous route of drug absorption, such as by needle scratches (Klein et al., 1979; Lieberman et al., 1973), thermal injury with a surgical cautery (Park et al., 1979), or injury produced by tape stripping of mouse ears (Hill et al., 1975). Likewise, preparation of the dorsal skin of guinea pigs has included shaving and chemical depilation (Alenius and Oberg, 1978; Hubler et al., 1974; Schaefer et al., 1977). Examination of the effect of these various procedures on the barrier function of the skin is essential to determine if the model tests the ability of antivirals to diffuse into undisrupted skin. In the female guinea pig genitalia model, topically applied drugs could be absorbed across the introital, vaginal or peritoneal mucosa. Topical therapy in this model might lead to significant systemic drug levels and demonstration of efficacy does not necessarily prove direct drug penetration of the stratum corneum of external cutaneous genital lesions.

Development of the cutaneous HSV infection model in the guinea pig

Teague and Goodpasture (1923, 1924) first showed that the skin of the guinea pig dorsum could be infected with HSV. Inoculation of the scarified skin with pus

from experimental HSV keratitis (rabbits) resulted in lesions, but they were small and infrequent. To increase the susceptibility of guinea pig skin to HSV infection, the skin was pre-treated with coal-tar. Infection could be then regularly produced by scarification, evolved into a zosteriform pattern, and commonly led to infection of the spinal ganglia, neurologic sequellae, and death. Tomlinson and MacCallum (1968) inoculated plucked dorsal guinea pig skin by 20 'pressures' with a triangular needle (vaccination technique) through a suspension containing 10^6 TCID/ml of HSV. Half the animals developed hind leg paralysis. Modification of the procedure to include pre-treatment with HSV immune serum prevented paralysis and did not interfere with the development of viral skin lesions.

In 1974, Hubler and coworkers addressed the problem of an adequate animal model for cutaneous HSV infections. The authors pointed out that cutaneous infection with HSV in the several existing guinea pig, rat and murine models poorly represented human HSV disease because of death from systemic viremia or central nervous system infection in a high percentage of the animals. The depilated dorsal skin of the guinea pig was demarcated into 6–8 squares with a marking pen. A high titered HSV-1 stock (10^7 pfu/ml) was then applied to each square 0.02 ml and introduced into the skin with a 6-pronged (0.75 mm depth) spring-loaded vaccination instrument (Sterneedle). The procedure resulted in multiple vesicular lesions which crusted and progressed to complete healing in 10–12 days, clinically similar to human cutaneous HSV disease. All of the animals survived. Virus stock containing less than 10^7 pfu/ml failed to consistently produce lesions and inoculation of previously infected animals resulted in abortive, rapidly healing lesions. This basic procedure for producing a uniform, non-fatal infection of guinea pig skin has

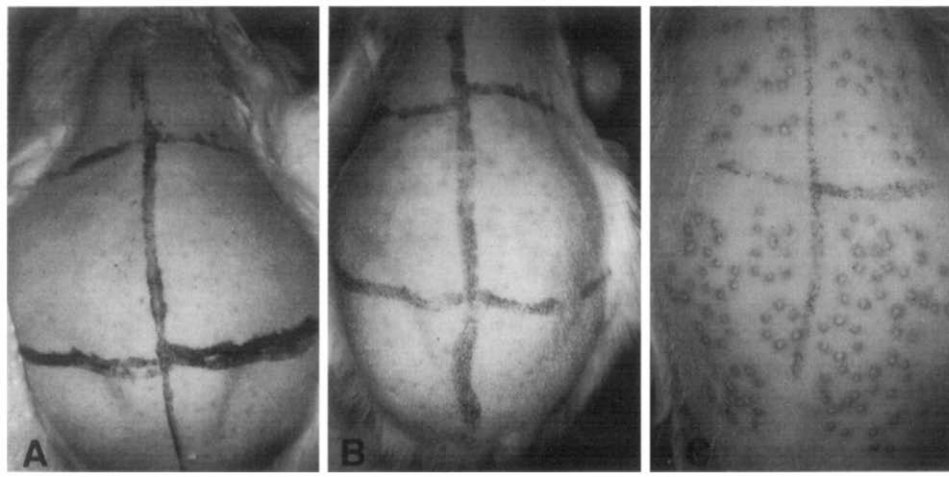
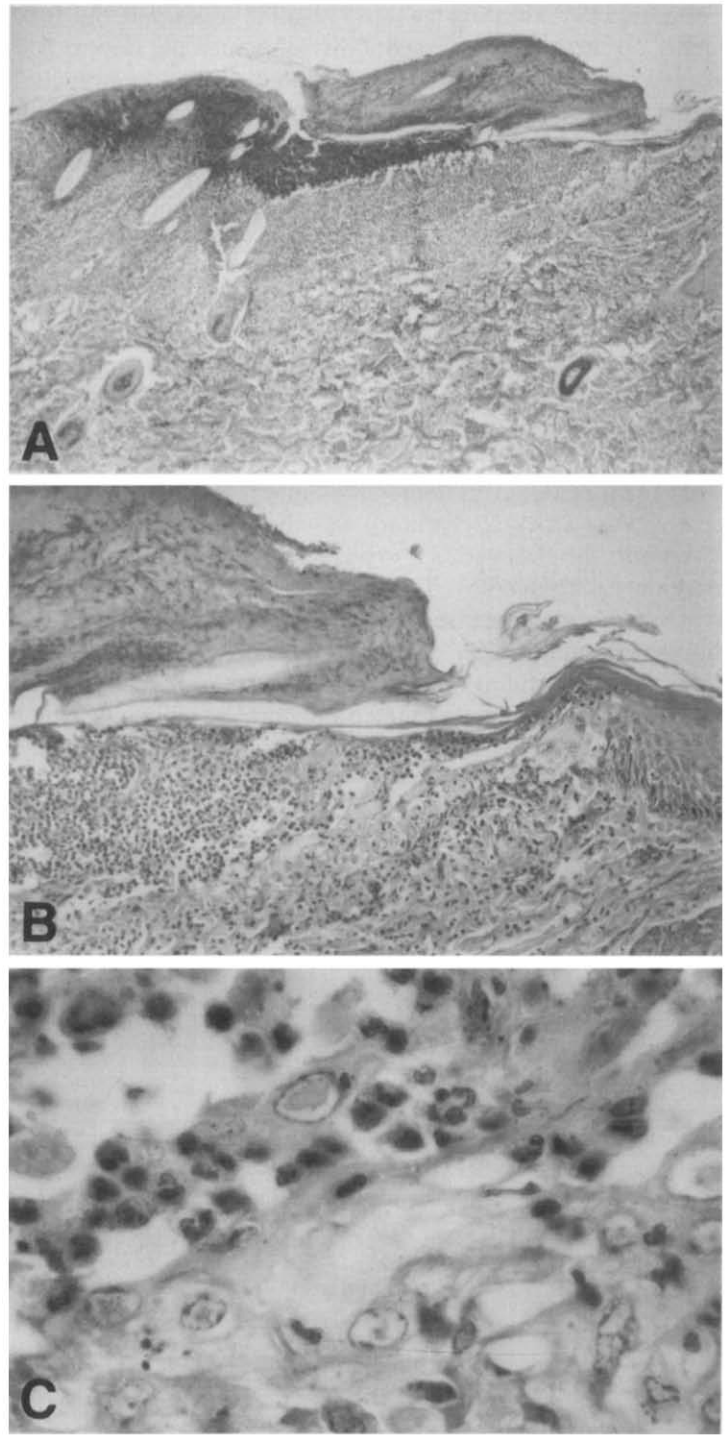


Fig. 1. Appearance of untreated lesions at different times after inoculation of guinea pigs with HSV-1 by multiple punctures with a Sterneedle. A. Day 0, immediately after inoculation. B. Day 1, time of initiation of treatments. C. Day 4, time of assessment of effect of therapy.


A
B
C

been utilized by multiple investigators and has been referred to as the 'Hubler' model.

Whereas Hubler et al. (1974) preferred to concentrate the inoculation stabs in one area, we have found it advantageous to carefully space the stab wounds, in order to be able to enumerate the number of lesions developing and to minimize the trauma to the epithelium (Fig. 1). To this end, a higher titered virus inoculum was necessary (5–10 $\times$ 10^7 pfu/ml), which may be conveniently prepared by virus concentration with polyethylene glycol (Lancz, 1973). Selection of an effective virus strain is also an important factor (Alenius and Oberg, 1978). The Sterneedle is no longer commercially available, but another vaccination instrument can be used (Mark 5 Multiple Puncture Apparatus, Bignell Surgical Instruments, Ltd., Littlehampton, West Sussex, BN175BN England).

Fig. 2 illustrates the histology of an experimental cutaneous HSV-1 lesion, 72 h after inoculation of the skin. The infection primarily involved the epidermal layer with focal destruction of the epidermis, leukocytic infiltration of the epidermis and adjacent dermis, and cytologic changes in infected epidermal cells typical for HSV. While the 'stab' inoculation procedure with 0.75 mm vaccination prongs potentially may introduce virus deep into the skin, the primary impact of inoculation was to induce epidermal cell infection, histologically similar to a recurrent lesion of herpes labialis (Huff et al., 1981).

Schaefer et al. (1977) extended our understanding of the model by studies of the kinetics of HSV-1 replication and lesion development. Virus in infected skin was quantified by excision of the infected area, homogenization and centrifugation of the excised tissue and inoculation of susceptible cell monolayers with serial dilutions of the supernatant. The kinetics of clinical lesion development and titers of virus in the skin are shown in Fig. 3. Virus replication rose rapidly to near maximal levels within 24 h after inoculation while the peak clinical lesion score occurred on day 5. These findings were confirmed (Alenius, 1980). Excised skin squares may be minced with scissors, suspended in media, and homogenized by shearing ('Tissuemizer', Tekmar Co., Cincinnati, OH) (Spruance et al., 1984a). Alternatively, the whole skin can be placed in sterile plastic bags and pummeled for 2 min in a Stomacher Lab Blender '80' (Tekmar Co, Cincinnati, OH) (N. Ellis, personal communication).

Multiple infection sites on each animal in the guinea pig model simplifies large, concurrent studies of several topical antiviral agents. Alenius and Oberg (1978) compared 12 different antiviral formulations and described additional features of the model important for therapeutic trials. The variation in lesion severity between different sites was small; the standard deviation of the mean lesion severity score for 402 infection sites was only 11% of the mean. The investigators also

Fig. 2. Histology of dorsal cutaneous HSV-1 infection in the guinea pig. Infection was induced by standard procedures with the Sterneedle and the animal sacrificed 72 h after inoculation. The area of infected skin was excised, fixed in formalin and stained with hematoxylin and eosin. A. 21.5 $\times$, complete lesion. B. 86 $\times$, dermal-epidermal junction of lesion adjacent to normal epidermis. C. 430 $\times$, epidermal cells with characteristic cytopathic effects of HSV infection.

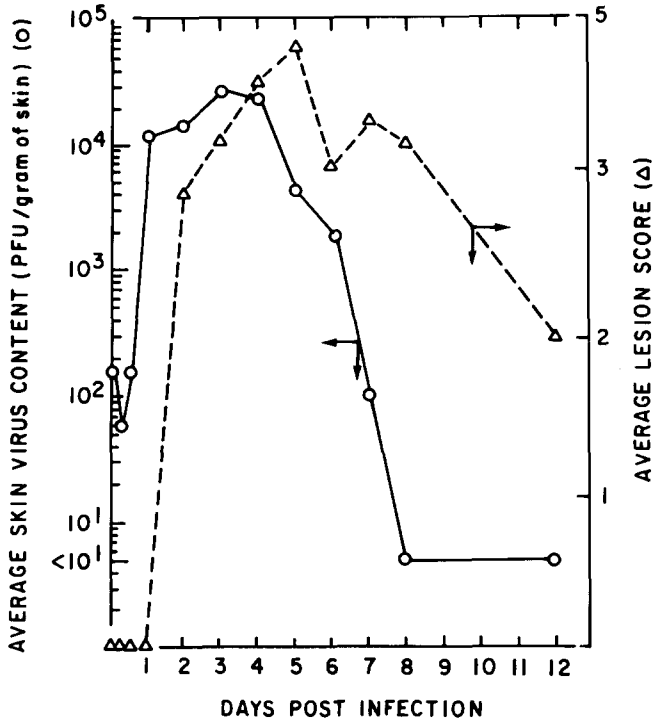


Fig. 3. Time course of HSV-1 infection in the dorsal cutaneous guinea pig model. Reprinted with permission from Schaefer et al. (1977)

showed that variation in lesion severity among multiple infection sites in one animal was less than the variation in severity between infection sites in different animals (outbred guinea pigs). Therefore, test agents were compared with vehicle control formulations at contralateral sites within the same animal. Control areas adjacent to areas treated with active substances were not influenced by the drug nor were there any differences between sites on one animal.

In our experience with the model, we have observed a rostral-caudal gradient in lesion severity, more severe lesions developing on the rump than at higher locations on the back (Spruance et al., 1984a). However, drug efficacy (difference between active substance and contralateral vehicle control) appears to be independent of position on the rostral-caudal axis.

Evaluation of clinical lesion severity in the Hubler guinea pig model has been most commonly done by a lesion scoring system, assigning an incremental numeric value as lesions evolved in size and pathologic maturity (Alenius and Oberg, 1978; Schaefer et al., 1977). The total lesion experience was expressed by a plot of average daily scores and time or by cumulative scores. This system takes all phases of the experimental disease into account, but has the disadvantage of subjective lesion observations and arbitrary quantitative relationships between disease stages.

An alternative method has been to measure lesions once at the point of maximum severity, 4 days after infection, by counting the number of lesions; measuring lesion diameter with the aid of Polaroid photographs and a calibrated magnifying lens; and calculating the total lesion area. The same lesions were then excised for virus quantitation (Spruance et al., 1984a). This procedure is quantitative and objective; permits a direct correlation of the clinical and virologic outcomes; and evaluates the ability of a test agent to affect maximum lesion severity. The disadvantage is a lack of information about the impact of treatment on healing.

Barrier function and biochemistry of guinea pig skin

In 1982, we reported the results of a large, multicenter trial of 5% acyclovir (ACV) ointment for recurrent herpes labialis in which there was no clinical benefit from treatment (Spruance et al., 1982). When we investigated the several factors that might have contributed to this failure, we found there was little information in the literature on the penetration of antiviral compounds through skin. Herpes labialis occurs on the cutaneous side of the lips or on the hairless vermillion border. The surface of the vermillion border is epidermis characterized by a prominent granular layer, an identifiable stratum lucidum, and a slightly thickened stratum corneum. At the point of labial apposition, the epithelium becomes abruptly non-keratinizing oral mucosa (Dimond and Montagna, 1976). Studies in our laboratory showed that the in vitro penetration of ACV from ointment formulation through excised guinea pig skin was slight (Spruance et al., 1984a). To examine the in vivo implications of this observation, the Hubler guinea pig model was chosen because the inoculation process, by multiple needle puncture, appeared to be minimally traumatic to the skin and would likely not create an artificial route of entry through the skin for a topically applied substance. A protocol of brief, early treatment was designed, primarily during the erythema and papular stages of the infection, comparable to treatment of the early stages of herpes labialis when the skin is unbroken. When administered by this protocol, ACV ointment was only of minor benefit against experimental HSV-1 infection. When ACV was delivered through the skin by formulation in dimethyl sulfoxide (DMSO), markedly improved results were obtained (Spruance et al., 1984a). The results suggested that poor skin penetration was a factor in the failure of ACV ointment for the treatment of herpes labialis, and that improvements in the formulation of this antiviral agent might be associated with greater clinical success.

Because of our desire to have an experimental animal model which tested the ability of an antiviral agent to penetrate into the skin, we examined the effect of our guinea pig skin preparation procedures and our method of virus inoculation on the in vitro skin penetration of ACV (Spruance et al., 1984a). Chemical depilation is known to increase skin permeability (Wahlberg, 1972). However, treatment in our model system was begun 24 h after depilation and inoculation, allowing time for the skin to recover. As shown below in Table 1, the permeability coefficient (K_p) for ACV 24 h after depilation (Expt. 2) was the same as control

Table 1

Effect of skin trauma on the permeability coefficient (K_p) for ACV from 0.5% solution in DMSO for guinea pig skin in double-chambered glass diffusion cells, adapted from Spruance et al. (1984a)

Experiment No.	Skin condition	Time interval between procedure and collection of skin	Determinations (N)	K_p (cm/h × 10^3)
1	Control skin[a]	0	4	1.47 ± 0.95[b]
2	Depilatory	24	2	1.15 ± 0.67
3	Depilatory and punctured	24	2	1.40 ± 0.48

[a]Close-clipped with electric shears.
[b]Mean ± standard deviation.

skin. Similarly, 24 h after both depilation and mock infection (Expt. 3), K_p was unchanged.

The ability of drugs to diffuse through the skin would be less critical if the barrier properties of the stratum corneum were altered by the process of infection. To examine this question, Freeman and Spruance (1986) excised infected guinea pig skin when the infections were 24 and 72 h old and compared the penetration of ACV through these infected skin specimens with the penetration of ACV through uninfected control skin. The results are shown in Table 2. The data indicated that no major increase in skin permeability could be attributed to early HSV infection. Ericson et al. (1985) found a 43–59% increase in permeability with mock infection and an 85–134% increase with HSV-1 infected guinea pig skin using the nucleosides (*R*)-9-[3,4-dihydroxybutyl]guanine and 9-[4-hydroxybutyl]guanine as penetrants. The reason for these differing results is unclear.

Studies with ACV and idoxuridine (IDU) in different vehicles have shown the potential range of transcutaneous drug delivery for antiviral nucleosides and the

Table 2

Effect of HSV-1 infection on the penetration of ACV through guinea pig skin in single-chambered glass diffusion cells from 5% ointment and cream formulations, adapted from Freeman and Spruance (1986)

Drug vehicle	No. of experiments	Control skin	ACV penetration (μg/cm²/h)	
			Skin infected	
			24 h	72 h
Polyethylene glycol	3	0.07 ±0.01[a]	0.07 ± 0.02	
Polyethylene glycol	3		0.06 ± 0.01	0.06 ± 0.01
Aqueous cream	6	0.38 ± 0.05	0.45 ± 0.04	
Aqueous cream	6		0.63 ± 0.06	0.71 ± 0.13

[a] Mean ± standard error of the mean.

Table 3

Penetration of ACV and IDU from different vehicles through human and guinea pig skin in vitro, adapted from Freeman et al. (1986)

Drug	Drug vehicle	Drug penetration ($\mu g/cm^2/h$)	
		Human skin	Guinea pig skin
5% ACV	Polyethylene glycol	0.06 ± 0.04*	0.05 ± 0.01
	Aqueous cream	0.42 ± 0.05	0.36 ± 0.12
	95% DMSO	3.31 ± 0.79	4.10 ± 0.26
5% IDU	Polyethylene glycol	0.01 ± 0.001	0.23 ± 0.01
	Aqueous cream	0.36 ± 0.01	0.17 ± 0.05
	95% DMSO	10.39 ± 1.08	3.69 ± 0.33

* Mean ± standard error of the mean, n = 3–5.

dependence of drug flux on the vehicle (Table 3). The vehicle influence affected ACV and IDU to the same degree and could be demonstrated with both human and guinea pig skin (Freeman et al., 1986). Drug efficacy in the Hubler guinea pig model has been shown to correlate with the degree of skin penetration, whether determined in vitro in glass diffusion chambers (Freeman and Spruance, 1986) or in vivo by measurement of IDU concentrations in tape strippings of the stratum corneum (Sheth et al., 1987).

High concentrations of thymidine have been found in guinea pig skin (20 μM) and much lower amounts in human skin (0.5 μM) (Harmenberg et al., 1985). This natural enzyme substrate can antagonize the antiviral activity of nucleoside analogues in cell cultures (Harmenberg, 1983; Larsson et al., 1983). HBG was inactive in the dorsal cutaneous guinea pig model despite good activity against HSV-1 in cell culture and evidence of adequate skin penetration, leading to the conclusion that the lack of in vivo activity was due to competition from endogenous thymidine (Ericson et al., 1985). Biochemical and pharmacokinetic factors important to understanding the efficacy of anti-herpesvirus agents in animal models were recently well-reviewed in this journal (Datema et al., 1987).

Effect of virus type and strain, drug dosage and route of administration on the efficacy of test agents

The dorsal cutaneous guinea pig model has been used to study treatment of infections by more than one HSV type or strain. HSV-1 strain CS and HSV-1 strain E115, the former ten times more sensitive to inhibition by recombinant interferon-alpha A in human foreskin fibroblast monolayers, were used to infect guinea pigs and the in vivo efficacy of topical recombinant interferon-alpha A against the two strains was compared. There was no significant difference in drug efficacy (Freeman et al., 1987). We concluded that either the in vitro inhibitory activity of interferon was not an accurate measure of the activity of interferon in the guinea pig; or the activity of interferon administered topically in the guinea pig was so

Table 4

Effects of topical therapy with 5% BVDU in DMSO on experimental cutaneous HSV-1 and HSV-2 infections of guinea pigs

HSV type	Therapy[a]	No. of lesions	Percent reduction by BVDU compared to vehicle control	Lesion area (mm^2)	Percent reduction by BVDU compared to vehicle control	Lesion virus titer (log_{10}/pfu/ml)	Percent reduction by BVDU compared to vehicle control
1	DMSO	40 ± 6[b]		189 ± 51		3.0 ± 0.8	
	BVDU	17 ± 6	58[c]	68 ± 32	64	2.1 ± 1.1	87
2	DMSO	50 ± 6		280 ± 51		4.3 ± 0.2	
	BVDU	41 ± 8	18	195 ± 47	30	3.8 ± 0.4	68

[a] Each active formulation was compared with its vehicle control 10 times.
[b] Mean ± standard deviation.
[c] All drug vs. drug vehicle differences were statistically significant. In addition, the degree of drug efficacy with HSV-1 was significantly greater than with HSV-2 ($P<0.01$ for all measures).

negligible that differences between viral strains in sensitivity to interferon could not be manifested. Alenius et al. (1982) infected guinea pigs with two HSV-1 strains differing 2-fold in their in vitro sensitivity to ACV, and treated both infections topically with 5% ACV ointment. A reduction in lesion severity was accomplished only for infection by the more sensitive strain. We inoculated guinea pigs intracutaneously with either HSV-1 strain E115 or HSV-2 strain MS, the former 650-fold more sensitive to (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) by plaque reduction assay (ID_{50} 0.008 vs. 5.2 μg/ml) (Freeman et al., 1985; E. Kern, personal communication). The infections were treated 4 times a day for three days beginning 24 h after inoculation with topical 5% BVDU in DMSO. As shown in Table 4, both infections improved with treatment, but a significantly greater therapeutic effect was seen with HSV-1 disease. Neither topical 10% BVDU in DMSO or systemic (ip) BVDU could be shown to reduce the mortality from cutaneous HSV-2 infection in hairless mice (De Clercq, 1984; De Clercq and Zhang, 1981).

Doseage schemes have a marked effect on the outcome of treatment in the dorsal cutaneous guinea pig model. When topical treatment of HSV-1 infections was begun 48 h after inoculation, twice daily 10% IDU in DMSO was ineffective (Alenius and Oberg, 1978) while treatment 4 times a day with 3% ACV in DMSO was active (Alenius et al., 1982). When begun 24 h after inoculation, twice daily 3% IDU in DMSO was markedly effective (Sheth et al., 1987). Beginning oral ACV treatment (drinking water) immediately after inoculation did not improve the efficacy compared to therapy initiated 24 h post-infection (Table 5). In contrast, the activity of systemic (im) interferon in the model, measured by percent reduction in total lesion area, was 85, 58 and 11%, respectively, for treatment begun −24, + 0.5, and +24 hours in relation to inoculation (Freeman et al., 1987). The relationship of efficacy to frequency of administration has been examined for three formulations of IDU in DMSO administered 1, 2, 3, or 4 times a day. For each of

Table 5

Effects of oral acyclovir therapy on experimental cutaneous HSV-1 infection of guinea pigs

Therapy[a]	No. of lesions	Percent reduction by ACV compared to control	Lesion area (mm^2)	Percent reduction by ACV compared to control	Lesion virus titer (log_{10}pfu/ml)	Percent reduction by ACV compared to control
Plain drinking water	49 ± 5[b]		254 ± 42		4.9 ± 0.3	
ACV in drinking water	50 ± 5	−2	176 ± 28	31[c]	4.1 ± 0.5	84[c]

[a]Ten animals received 5 mgs/ml ACV in the drinking water, beginning 24 h after infection with HSV-1, and 10 control animals took plain water. The results were the same when treatment was begun immediately after inoculation (data not shown).

[b] Mean ± standard deviation.

[c] Significantly different from control value (rank sum test, $P < 0.01$).

the three formulations, efficacy reached a plateau at a dosing frequency of 3 times a day (Sheth et al., 1987).

Comparison of the activity of an antiviral agent in the guinea pig model when given by different routes of administration has enhanced our ability to interpret the results of topical therapy. Combined studies of systemic (im) and topical interferon in the model demonstrated that this antiviral substance was effective systemic therapy but too big a molecule for topical administration (Freeman et al., 1987). Because of the widespread use of ACV capsules for the treatment of HSV infections, including two recent clinical trials in recurrent herpes labialis (Raborn et al., 1987; Spruance et al., 1986b), it was of interest for us to study oral ACV therapy in the guinea pig model and compare efficacy with the topical ACV treatments. Attempts to administer ACV to guinea pigs by intermittent gavage led to variable and unpredictable blood levels (S.L. Spruance, unpublished data). Therefore, ACV was administered in the animals' water bottles as a 5 mg/ml solution, a procedure which results in blood levels of ACV in the guinea pig comparable to peak serum levels in human subjects following ingestion of a 200 mg capsule (Ellis and Barry, 1985). Treatment was begun 24 h after infection. The results of the experiment are shown in Table 5. Animals receiving ACV in drinking water had significantly lower total lesion area (31% reduction) and lesion virus titers (84% reduction) compared to control animals. The efficacy of ACV by the oral route of administration was slightly more than that which we have seen by topical therapy with 5% ACV ointment in the model (greater reduction in lesion virus titers); comparable to the efficacy of 5% ACV cream; and considerably less than the efficacy of topical 5% ACV in DMSO (Table 6). The similarity between the efficacy of oral ACV and ACV cream in the model is of interest since these preparations have both had small positive effects on the severity of recurrent herpes labialis (Spruance, 1988). Since enhanced delivery of ACV by formulation in DMSO is associated with better therapeutic efficacy in the model, increased delivery of ACV

Table 6

Efficacy of topical antiviral formulations in the dorsal cutaneous guinea pig model of HSV-1 infection

Expt. No.	Test treatment[b]			Percent reduction in lesion severity by test treatment compared to vehicle control[a]			Percent reduction in lesion severity by 5% ACV ointment compared to ointment base control			Source
				Lesion						
	Antiviral	Concentration	Vehicle	No.	Area	Virus titer	No.	Area	Virus titer	
1	ACV	5%	DMSO	58*	73*	84	8	35	21	Spruance et al. (1984a)
2	BVDU	5%	Aqueous cream	20	40	90*	13	28	55	Freeman et al. (1985)
	BVDU	5%	DMSO	55*	69*	94*				
3	EDU	3%	Aqueous cream	29	44	68	15	32	60	Spruance et al. (1985)
	EDU	5%	DMSO	39	60*	90*				
4	ACV	5%	Aqueous cream	19*	31	75	−8	19	60	Spruance et al. (1986a)
	PFA	0.3%	Aqueous cream	36*	52*	80				
	PFA	3%	Aqueous cream	54*	73*	90*				
5	ARA-A	5%	DMSO	44*	53*	60	−13	8	37	
	IDU	5%	DMSO	75*	81*	99*				
6	TFT	1%	Azone and propylene glycol	32	70*	97	18	46	90	Spruance et al. (1984b)

7	Triacontanol	5%	Branch chain ester base	9	17	41	7	22	64	
8	Ribavirin	10%	Site release base	17	36	50	16	32	56	
	Ribavirin	10%	Ointment	10	25	53				
	Ribavirin	10%	Aqueous cream	15	33	37				
	Ribavirin	5%	Propylene glycol lotion	10	34	38				
9	Interferon***		Gel	10	2**	21**	11	30	68	Freeman et al. (1987)
10	Heparin/zinc		Gel	1	8	2	2	16	26	
			Lipstick	2	6	15				

[a] Percent differences between mean lesion severity at drug-treated infection sites compared to the vehicle-treated sites are shown. Data are derived from comparison of 8–15 drug and vehicle-treated sites 4 days after inoculation. A positive value indicates a reduction in lesion severity by the test compound. For statistical analysis, paired data were evaluated by the Wilcoxon signed rank test, utilizing the percent differences between $\log_{10}$ derivatives of the drug and drug vehicle results. These percent differences were also used as a means to compare the efficacy of different antivirals by the Mann-Whitney rank sum procedure. All probability determinations were two-tailed, and $P \leq 0.05$ was considered to be significant.

[b] Treatments were given 4 times a day for three days beginning 24 h after inoculation, except for formulations which were irritating to the skin and administered 1–2 times a day (PFA, TFT). ACV, BVDU, PFA (Foscarnet), EDU (5-ethyl-2′-deoxyuridine), TFT and recombinant interferon-alpha A were obtained through pharmaceutical industry sponsors as previously documented (see refs. mentioned under Source). Triacontanol, a mixture of long-chain hydrocarbons, was provided by Royal Pharmaceutical Products, Inc., Bountiful, UT; ribavirin (Virazole) in different vehicles by Viratek, Costa Mesa, CA; and the combination of heparin and zinc (Bonactin) by Ciba Consumer Pharmaceuticals, Edison, NJ. ARA-A was obtained from Sigma Chemical Co., St. Louis, MO; and IDU and DMSO were provided by Research Industries, Salt Lake City, UT. Five percent ACV ointment (Zovirax Ointment) was generously supplied throughout these studies by Burroughs-Wellcome Co., Research Triangle Park, NC.

* Significantly better ($P < 0.05$) therapeutic effect compared to ACV ointment.

** Significantly less ($P < 0.05$) therapeutic effect compared to ACV ointment.

*** Recombinant interferon-alpha A.

by the oral or topical route is a logical means to pursue a better treatment for herpes labialis.

Evaluation of antiviral substances for use against recurrent herpes labialis by comparison with ACV ointment in the guinea pig model

Numerous investigators have used the cutaneous guinea pig model to screen test compounds for in vivo antiviral efficacy (Alenius, 1980; Alenius and Oberg, 1978; Alenius et al., 1982; Amtmann et al., 1985; Burkhardt and Wigand, 1983; Collins and Oliver, 1982; Ericson et al., 1985; Freeman et al., 1985, 1987; Oill et al., 1978; Park et al., 1980; Schaefer et al., 1977; Shipman et al., 1986; Spruance et al., 1984a,b, 1985, 1986a; Tomlinson and MacCallum, 1968). Table 6 summarizes our treatment studies of multiple topically administered antivirals in different drug vehicles. The results of ten experiments with 12 different antivirals and 19 different topical formulations are reported. For trifluorothymidine (TFT) and interferon, only the formulation that produced maximum efficacy in the model is shown from among 3 and 5 formulations tested, respectively (Freeman et al., 1987; Spruance et al., 1985). The results are expressed as the percent reduction in lesion severity measures by the test treatment compared to vehicle-treated infection sites. All studies included 5% ACV ointment (Zovirax Ointment, Burroughs-Wellcome Co.) as a concurrent control therapy since the lack of efficacy of this product against herpes labialis is known ((Spruance, 1988). An asterisk marks the instances when the efficacy of the test treatment was significantly greater than ACV ointment.

A wide variety of outcomes was observed. In experiments nos. 1–5, the nucleoside antivirals, when formulated in DMSO to enhance drug delivery, reduced lesion area 53–81%, superior to the effect on area by nucleosides in aqueous creams (31–44%). Aqueous cream formulations of BVDU and ACV, in turn, were more effective than 5% ACV ointment, although these differences were less pronounced.

For each experiment, three measures of lesion severity were used to assess drug effect: lesion number, lesion area and lesion virus titer. The measures were affected by drug therapy in the following order: virus titer > lesion area > number of lesions. The greater effect of treatment on lesion area compared to number of lesions reflects a regular reduction in the size of lesions by active compounds. The finding that lesion virus titers were affected to the largest degree supports the contention that the antiviral activity of the test compounds was responsible for the clinical benefits. Using 48 data points from experiment no. 4, we found a significant correlation between reduction in total area and reduction in lesion virus titer (R=0.61, and P<.001).

Some drug vehicles alone can influence the course of experimental cutaneous HSV-1 infection in the guinea pig. DMSO has anti-inflammatory activity, in part as a scavenger of hydroxyl radicals and inhibitor of polymorphonuclear leukocyte function (Fox and Fox, 1983; Repine et al., 1983), and is licensed for the treatment of interstitial cystitis (Sant, 1987). We infected 12 guinea pigs at two sites on

the dorsum with HSV-1, treated one site with 95% DMSO 4 times a day for three days beginning 24 h after inoculation, while the contralateral site on each animal was untreated. In comparison to the untreated site, DMSO reduced the median number of lesions 37% (P=0.002), the median lesion area 62% (P=0.002), and the median lesion virus titer 46% (P= 0.12). Alenius et al. (1982) found no significant difference either between titers of HSV-1 in untreated and DMSO-treated guinea pig skin. Azone has been reported to be virucidal (Leonard et al., 1987) but does not appear to have antiviral activity in vivo (Spruance et al., 1984b). Polyethylene glycol (PEG) does not effect clinical lesion severity or lesion virus titers in the guinea pig (Spruance and Wenerstrom, 1984).

Discussion

The dorsal cutaneous guinea pig model has several important advantages as a screening procedure for antiviral treatments of recurrent herpes labialis or other cutaneous HSV infections. First, the infection is easy to initiate and infection sites of comparable severity on the same or different animals are readily produced. As a result of the small variance encountered, relatively few animals are necessary to perform a treatment study. As many as six infection sites can be utilized on one animal. All animals are available for evaluation since the infection is mild and does not lead to complications or mortality. Discreet lesions develop which can be readily quantitated, both clinically and virologically. The evolution of lesion stages parallels herpes labialis, going through erythema, papule, vesicle and hard crust, to lesion healing. Importantly, during the erythema/papule stages, we have demonstrated in our laboratory that the barrier function of infected guinea pig skin is comparable to control skin. Thus, our experimental infection model, exogenously and traumatically derived, in contrast to recurrent herpes labialis, nevertheless maintains a normal physiologic impediment to antiviral penetration, a necessary test for candidate therapies of recurrent HSV infections developing endogenously within keratinized epidermal surfaces. While no animal skin perfectly models human skin, we have found reasonable comparability among three nucleoside antivirals (ACV, IDU, TFT) for which concurrent drug diffusion studies in human and guinea pig skin have been performed (Freeman et al., 1986; Spruance et al., 1984b).

There are also important differences between the model and recurrent herpes labialis. Guinea pig skin has a high concentration of thymidine, and this factor should be considered in evaluating experimental results in the model with compounds whose anti-herpesvirus activity in vitro is antagonized by this natural substrate (Harmenberg, 1983; Larsson et al., 1983). The experimental animal disease is a primary infection. The guinea pig has a natural, although as yet undefined, resistance to primary infection in the dorsal skin (when inoculated by shallow stab wounds) which usually limits the experimental infection to a brief illness. Seeding of the dorsal neural ganglia has not been reported, nor spontaneous recurrences, nor could lesions be reactivated on the dorsum of previously infected animals by exposure to ultraviolet light (S.L. Spruance, unpublished data). However, the

gradual progression of lesions in the guinea pig to maximum severity 4–6 days after inoculation is still much slower than the evolution of herpes labialis, where multifocal inoculation of the skin and a rapid secondary immune response result in maximum lesion severity within 24 h after onset (Spruance et al., 1977; Spruance and Wenerstrom, 1984). Thus, treatment of the progressive lesion stages, the logical target of antiviral chemotherapy, is easily accomplished in the animal model but only with difficulty in the human disease. In addition, lesion pathology is the result of two processes: viral-directed cytolysis and the host inflammatory response to viral antigens. Data from a human model of experimental ultraviolet light-induced herpes labialis has suggested that the inflammatory response can be the predominant factor in some lesions, which creates further difficulties for antiviral substances in the treatment of this illness (McKeough et al., 1988). In summary, antiviral agents have more time to act in the guinea pig and will be far more effective in the model than one should anticipate in recurrent human HSV disease. This 'sensitivity' of the model to therapy can be advantageous when the efficacy of different antivirals or formulations are compared.

To estimate the potential of test compounds for the treatment of herpes labialis, we have evaluated each agent relative to ACV ointment, a preparation which has received extensive clinical evaluation against the human disease, with essentially negative results (Spruance, 1988). By the criteria of a statistically significant difference for at least one lesion measure and a trend toward superior effect in the other measures, 10 of 17 new treatments tested were better than ACV ointment, eight were similar and one was inferior. Two of the 10 superior formulations (ACV cream and PFA cream) have received evaluation in human trials against herpes labialis, and evidence of a small clinical benefit has been noted for each (Spruance, 1988).

The degree of clinical benefit achievable in recurrent herpes labialis is a moot question at this point. The limited results with oral acyclovir against this disease in normal, non-immunosuppressed subjects should caution us not to anticipate large gains from antiviral therapy (Raborn et al., 1987; Spruance et al., 1986b). However, in light of the moderate results with oral acyclovir in the present animal model and the existence of other treatment modalities more successful in the guinea pig than oral acyclovir, we are inclined to withhold final judgement on the treatment of herpes labialis until more potent treatments have received clinical evaluation.

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