Antiviral Research 15 (1991) 87–100

ADONIS 0166354291000231

AVR 00462

Synergistic topical therapy by acyclovir and A1110U for herpes simplex virus induced zosteriform rash in mice

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(Received 16 July 1990; accepted 15 October 1990)

Summary

Combination therapy with A1110U, an inactivator of the herpes simplex virus (HSV) and the varicella zoster virus ribonucleotide reductase, and acyclovir (ACV) was evaluated for treatment of cutaneous herpetic disease in athymic mice infected on the dorsum. In this model, infection with HSV produces a 'zosteriform-like' rash that is first visible on day 3 or 4 post-infection (p.i.) and eventually extends from the anterior mid-line to the dorsal mid-line of the affected flank. In untreated mice, the infection is fatal at about day 7 p.i. presumably due to central nervous system involvement. Topical treatment of infections induced by either wild-type (wt) HSV-1 or wt HSV-2 with 3% A1110U in combination with 5% ACV resulted in synergistic ($P<0.01$) reductions in lesion scores. Therapy was also synergistic in mice infected with an ACV-resistant thymidine kinase-deficient mutant and an ACV-resistant TK-altered mutant HSV-1 isolate. Combination therapy was very effective in reducing lesion scores of mice infected with an ACV-resistant HSV-1 DNA polymerase mutant, but did not result in statistically significant synergy ($P = 0.07$) because of the enhanced efficacy of A1110U alone against this virus. These results provide encouragement that the combination of A1110U and ACV may offer an effective therapy for topical treatment of cutaneous HSV infections in humans.

Herpes simplex virus; Murine zosteriform rash model; A1110U; Acyclovir; Synergistic topical therapy; Ribonucleotide reductase

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0166-3542/91/$03.50

Introduction

Acyclovir (ACV) is a potent and highly specific antiviral agent that is currently the treatment of choice for cutaneous herpes simplex virus (HSV) infections. Although many of the patients treated with topical ACV experience reductions in their clinical symptoms (Fiddian et al., 1983a,b; Kinghorn et al., 1983; Van Vloten et al., 1983; Yeo and Fiddian, 1983; Spruance et al., 1984), there are several aspects of this therapy that could be improved (Spruance et al., 1982). A more efficacious treatment may result in a greater reduction in time to vesiculation, duration of vesicles, time to crusting, and time to complete healing after the onset of a recurrent herpes labialis episode. Chemotherapies that involve the combination of two agents with differing modes of action have been shown to be of significant value in the treatment of several diseases caused by infectious agents including HSV (Smith et al., 1982; Fraser-Smith et al., 1984; Hartshorn et al., 1986; Hilfenhaus et al., 1987; Veckenstedt, 1987; Crane and Sunstrum, 1988). The use of combinations of synergistic antiviral agents can result in increased clinical efficacy at lower drug concentrations and may reduce the potential for the emergence of drug-resistant virus strains. Various animal models have been used by investigators to examine the potential of antiviral combination therapies in providing improved therapeutic value over currently available treatments. The combination of ACV with other antiviral agents has been studied in various animal model systems. A combination of vidarabine (Ara-A) and ACV significantly increased the survival time and reduced mortality when compared with the single agents alone in HSV-2 intracerebrally-infected mice (Schinazi et al., 1982) and HSV-2 intranasally-infected mice (Karim et al., 1985; Crane and Sunstrum, 1988). Park et al. (1984) showed that topical treatment with this same combination was more effective in preventing the development of ulcers and death in hairless mice infected facially with HSV-1 than the individual agents. A vaginal model of HSV-2 infection in mice was used by Crane et al. (1984) to examine the efficacy of intraperitoneal treatment with double and triple combination antiviral therapies. They found the Ara-A/ACV combination therapy and a low dose Ara-A/ACV/interferon combination to be synergistic. A combination of ACV and antibody was found to be more effective than ACV alone in the treatment of HSV-1 infections using the zosteriform model (Yamamoto et al., 1985) and an intradermal model (Hilfenhaus et al., 1987) in mice.

The finding that HSV induces a unique ribonucleotide reductase (RR) that was insensitive to the conventional allosteric inhibition by deoxythymidine triphosphate (reviewed by Spector, 1989) illustrated the potential importance of the HSV RR as a chemotherapeutic target. The unrestricted synthesis of deoxynucleoside triphosphates by the HSV RR was demonstrated when HSV-infected cells treated with ACV showed a large increase in the pool size of deoxyguanosine triphosphate (dGTP), a competitor of ACV triphosphate (ACV-TP) for binding to the HSV DNA polymerase (Spector, 1985). Spector et al. (1989) found that A1110U (2-acetylpyridine 5-[(dimethylamino)thiocarbonyl]thiocarbonohydrazone) greatly increased the antiviral potency of ACV in vitro. Inhibitors of the HSV RR, such as A1110U, prevent the increase in dGTP pools and, by an unknown mechanism,

increase the ACV-TP pool size and thereby reduce the competition by dGTP for the binding of ACV-TP to the HSV DNA polymerase, the target enzyme.

Topical antiviral chemotherapies that make use of this combination are being examined for their potential as more efficacious treatments for cutaneous HSV infections. Initial studies with snout-infected athymic mice demonstrated that topical treatment with the combination of ACV and A1110U caused a synergistic reduction in lesion scores in animals infected with wild-type (wt) or mutant HSV-1 isolates (Ellis et al., 1989).

The murine 'zosteriform rash' back model (Teague and Goodpasture, 1923; Sydiskis and Schultz, 1965; Constantine et al., 1971) has been used to study various components of HSV infection including the pathogenesis of cutaneous HSV infection and the immunological responses generated in the prevention of recurrent disease (Simmons and Nash, 1984). The pathogenesis of HSV infection in mice inoculated on the flank has been shown to be similar to the disease cycle of cutaneous HSV infection in humans (Sydiskis and Schultz, 1965). The disease involves a primary infection at the point of inoculation as well as a secondary component with replication of the virus in skin at a peripheral site. This pattern of infection is similar to that of virus reactivated from the nervous system in recrudescent HSV disease (Simmons and Nash, 1984). In mice infected on the dorsum with HSV, the infection first appears at the site of inoculation on the rear flank. The virus then spreads from the initial site of inoculation into the sensory neurons and returns to the skin via the axons producing a cutaneous dermatomal rash that will extend from the dorsal mid-line to the anterior mid-line of the affected dermatome. This is probably similar to the route by which reactivated virus infects the epidermal cells of the skin in cutaneous HSV infections (Klein, 1985). Although differences in the proposed mechanism for viral spreading in HSV cutaneous disease and varicella zoster virus (VZV)-induced shingles in humans are reported (Croen et al., 1988; Straus, 1989), both viruses are probably transported back to the skin via axons using similar mechanisms. This model is therefore used to examine antiviral therapy on initial virus replication at the site of inoculation as well as effects on virus replication in secondarily infected skin via the involved nerve endings.

The zosteriform model has been used by investigators to evaluate the efficacy of intraperitoneally (Klein et al., 1974; Klein and Friedman-Kien, 1975; De Clercq et al., 1979), topically (Underwood, 1968; De Clercq, 1984) and intradermally (Lieberman, 1973) administered antiviral agents. We have utilized this model to study the efficacy of topical therapies for HSV infections and to confirm the earlier observed synergy between A1110U and ACV (Ellis et al., 1989). The effects of topical therapies on both wt HSV-1 and HSV-2 viruses as well as mutant HSV-1 isolates were studied and are presented herein.

Materials and Methods

Mouse model

The zosteriform rash model of HSV infection was used to evaluate topical antiviral therapies. Three-week-old female athymic nude mice (BALB/c Nu/Nu) were obtained from Charles River and used at 4–8 weeks of age. Approximately ten mice per treatment group were infected on the lower right dorsum by either of two routes depending on the virulence of the virus to be studied. For more virulent virus isolates, anesthetized mice were infected by activation of a 'Sterneedle' vaccination gun (Panray Division, Ormont Drug & Chemical Co., Inc.) through a 25 μl droplet of undilute virus inoculum (approximately 10^6 plaque forming units/mouse) placed on the lower right dorsum. For less virulent isolates, mice were infected by abrading the skin (60 times, one way) with a hypodermic needle (25 gauge 7/8 inch) through a 20 μl droplet of inoculum placed on the lower right dorsum. Lesion scores were recorded according to the criteria described in Table 1.

TABLE 1

Criteria used for scoring herpetic cutaneous lesions

Score	Clinical manifestation
0	No visible infection
1	Infection visible only at inoculation site, with some swelling
2	Infection at inoculation site only, with swelling and erythema
3	Infection at inoculation site with discrete lesions forming away from inoculation site
4	Rash visible around half of body but not yet confluent
5	Rash confluent but not yet necrotic, and/or hind limb paralysis
6	Complete rash with necrosis or death

TABLE 2

Description of the viruses studied and their susceptibilities to ACV and A1110U

Virus	HSV type	Phenotype	IC_{50} (μM)[a]	
			ACV	A1110U
BW-sensitive (BW-S)	1	wt[b]	0.5[f]	1.5[f]
BW-resistant (BW-R)	1	TK^{Dc}	5.3[f]	1.1[f]
MS II	2	wt	0.9	1.0
SC16	1	wt	0.5	0.5
SC16/S1	1	TK^{Ad}	7.0[f]	1.7[f]
Polymerase mutant	1	DNA Pol[e]	23	1.3

[a]50% inhibitory concentration as determined by plaque reduction assay in Vero cells.
[b]wt HSV.
[c]Thymidine kinase deficient (TK^D) HSV-1.
[d]Thymidine kinase with decreased utilization of ACV as determined by Darby et al. (1981).
[e]HSV-1 DNA polymerase mutant showing decreased sensitivity to inhibition by ACV-TP.
[f]Data of Ellis et al. (1989).

Viruses, cells and media

Table 2 describes the virus isolates used in these studies. The wt HSV-1 strain, BW-S, and the HSV-1 thymidine kinase-deficient (TK^D) ACV-resistant mutant, BW-R, were described by Sibrack et al. (1982). The origin of the MS II strain of HSV-2 has been described by Kern et al. (1978). The wt HSV-1 strain, SC16 (Hill et al., 1975), and the ACV-resistant thymidine kinase-altered (TK^A) mutant derived from it, SC16/S1 (Darby et al., 1981), were also studied. The HSV-1 DNA polymerase mutant has been described by Parker et al. (1987). Virus stocks were grown in Vero (African green monkey kidney) cells as described by McLaren et al. (1983). Virus infectivity titrations were conducted in Vero cells and stained with 1% crystal violet in 50% methanol at about 4 days p.i. for quantification of virus. ACV and A1110U sensitivities of the virus strains used in these studies were determined by the plaque reduction assay, which is described elsewhere (Sibrack et al., 1982).

Drugs and drug therapy

In all experiments drugs were formulated in modified aqueous cream (MAC) with 0.01% (ethylenedinitrilo)tetraacetic acid (EDTA) added. Usually, 3% A1110U was used in combination with 5% ACV in MAC; however, dose response experiments also examined the efficacy of 5, 2, and 1% A1110U in combination with 5% ACV. In most experiments topical drug was applied to the infected area 3 times/day between 8 a.m. and 5 p.m. Treatments were started at approximately 16 h p.i. and continued through days 4, 5, or 6, except in the experiment with the TK^A mutant, SC16/S1, where treatment was delayed for 48 h and continued through day 6. Sterile cotton-tipped swabs were used for drug applications.

Statistical analysis

The area under the curve (AUC) of mean lesion scores versus days p.i. for each treatment group was computed using the trapezoidal rule as described by Pollard (1977) and analyzed by the Kruskal-Wallis test (Siegal, 1956) for statistically significant differences. The probability of a synergistic interaction between the drugs was analyzed by comparing the observed AUC for the combination therapy with the expected AUC for theoretically combining the two agents. The following formula was used to calculate the expected value for the sum of the AUCs for two independent agents (Harvey, 1978; Spruance et al., 1984):

$$\left[\frac{(AUC_{ACV}) \quad (AUC_{A1110U})}{(AUC_{control}) \quad (AUC_{control})}\right](AUC_{control}) = \text{expected AUC}$$

Student's *t*-test (Afifi and Azen, 1972) was used to compare the expected AUC with the observed AUC for statistical significance. The combination therapy is considered synergistic if the observed inhibition is significantly greater than that expected from the sum of the independent interactions.

Results

Acyclovir and A1110U sensitivities of viruses studied

Table 2 shows the ACV and A1110U 50% inhibitory concentrations (IC_{50}) for the HSV strains studied in these experiments. The wt HSV-1 virus, BW-S, was approximately tenfold more sensitive to ACV than its thymidine kinase deficient mutant BW-R. A second independent wt HSV-1 isolate, SC16, was also sensitive to ACV while the TK^ mutant derived from it, SC16/S1, was considerably less sensitive. The HSV-2 used in these studies (MS II) was slightly less sensitive to ACV than the HSV-1 strains. The HSV-1 DNA polymerase mutant was the most resistant to ACV of the isolates studied. The A1110U sensitivities of all the virus isolates studied were not significantly different.

Combination therapy for wild-type HSV-1 and HSV-2 infected mice

Fig. 1 (A and B) and Table 3 (experiments 1 and 2) present the results of topical treatment begun on day 1 (approximately 16 h p.i.) and applied 3 times per day through day 5 for the two wt HSV-1 strains studied, SC16 and BW-S. A topical combination therapy of 3% A1110U and 5% ACV prevented lesions from appearing in almost all of the mice infected with either of these viruses. In both experiments the AUC for combination therapy was significantly less ($P<0.01$) than the AUC for treatment with ACV alone. In ACV-treated mice infected with either of the HSV-1 isolates, a flare-up of lesions was evident by approximately day 7 (2 days after the cessation of treatment) and lesion scores

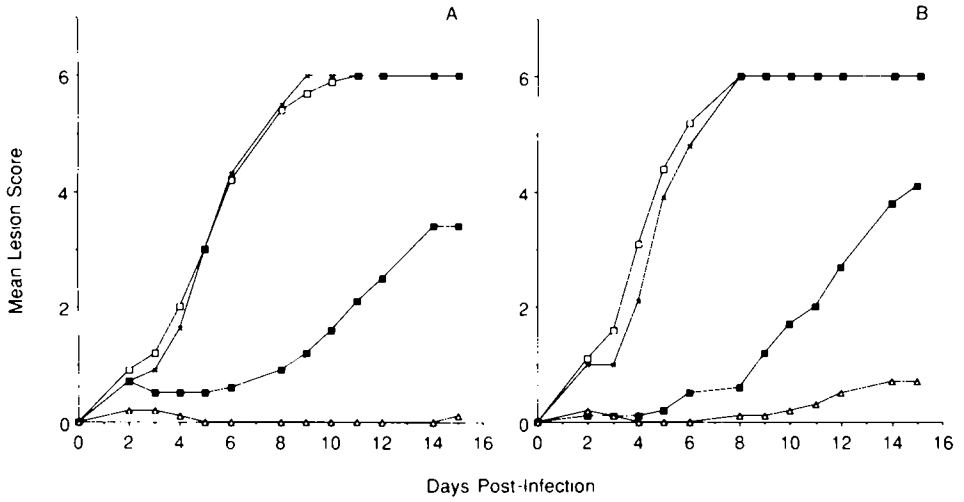

Fig. 1. Effect of topical treatment on wt HSV-1 back-infected athymic mice. Eleven mice per treatment group were scratch-infected with either the SC16 strain (A) or the BW-sensitive (B) strain of HSV-1. Three treatments per day were begun 16 h p.i. and continued through day 5. (□ □) No treatment; (■ ■) 5% ACV; (X- X) 3% A1110U; (△- △) 5% ACV + 3% A1110U.

TABLE 3

Effects of topical treatment on wt HSV-1 and HSV-2 infected athymic mice

Topical treatment	Number of mice	Area under the curve (± SEM)		
		Observed[a]	Expected if additive[b]	Synergy[c]
Expt. 1: HSV-1 wt (SC16)[d]				
None	11	59.5 (± 1.2)[1]		
5% ACV	11	19.5 (± 2.3)[2]		
3% A1110U	11	59.5 (± 0.7)[1]		
3% A1110U + 5% ACV	11	0.4 (± 0.3)[3]	19.5 (± 2.3)	Yes (P<0.01)
Expt. 2: HSV-1 wt (BW-S)[e]				
None	10	65.4 (± 0.8)[1]		
5% ACV	11	19.0 (± 2.8)[3]		
3% A1110U	11	62.7 (± 0.5)[2]		
3% A1110U + 5% ACV	11	3.0 (± 1.8)[4]	18.2 (± 2.8)	Yes (P<0.01)
Expt. 3: HSV-2 wt (MS II)[f]				
None	11	64.9 (± 1.4)[1]		
5% ACV	9	33.9 (± 5.7)[2,3]		
3% A1110U	10	46.7 (± 7.9)[2]		
1% A1110U + 5% ACV	11	22.9 (± 4.4)[3]	24.4 (± 6.0)[g]	No (P=0.5)
3% A1110U + 5% ACV	10	5.3 (± 3.6)[4]	24.4 (± 6.0)	Yes (P<0.01)
5% A1110U + 5% ACV	10	8.3 (± 4.5)[4]	24.4 (± 6.0)[g]	Yes (P<0.01)

[a]The AUCs of mean lesion scores were compared by the Kruskal-Wallis test. Means with the same numerical superscript are not significantly (P<0.05) different.

[b]AUC expected values were calculated as described in Materials and Methods.

[c]Combination therapy was synergistic if the difference between observed and expected AUCs was significantly greater than that expected from sum of the independent interactions by two-sample t-test.

[d]Mice were treated topically 16 h p.i. with three treatments on days 1–5.

[e]Mice were treated topically 16 h p.i. with three treatments on days 1–5.

[f]Mice were treated topically 16 h p.i. with three treatments on days 1–6.

[g]The data obtained for 3% A1110U as a single agent was used in the calculations for determining synergy in combination experiments with 1% or 5% A1110U and 5% ACV. Since 3% A1110U had a weak effect, we assumed that the effects at 1% and 5% would not be significantly different.

continued to increase through day 14. The AUC for treatment with 3% A1110U alone was very similar to the AUC for the no treatment group. Significant synergism was observed with the combination therapy in experiments with both isolates (P<0.01).

An experiment to determine the effectiveness of the combination therapy in the treatment of HSV-2 infections was conducted with different doses of A1110U in combination with 5% ACV. The results of this experiment are shown in Fig. 2 and Table 3 (experiment 3). The AUC for the 5% or 3% A1110U combinations with ACV were significantly reduced when compared to the AUC for ACV alone. Treatment with 3% A1110U alone was slightly better than no treatment. There was also significant synergy (P<0.01) observed for both the 5% and 3% A1110U combinations with ACV. However, the synergy was lost when the dose of A1110U was reduced to 1%. There was a flare-up of lesions evident by day 3 (3 days prior to the cessation of treatment) in mice treated with ACV alone. Mice treated with

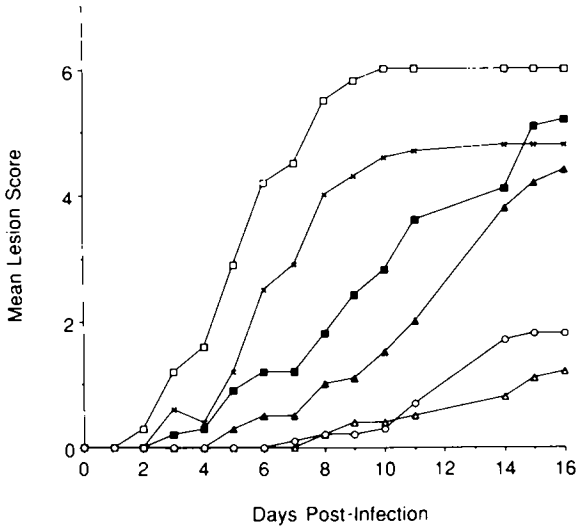

Fig. 2. Effect of topical treatment with 5% ACV in combination with varying doses of A1110U on wt HSV-2 (MS II) back-infected athymic mice. Ten mice per treatment group were vaccination gun-infected on the right dorsum. Topical drug was applied 3 × per day on days 1–6. (□——□) No treatment; (■- -■) 5% ACV; (X X) 3% A1110U; (▲ ▲); 1% A1110U + 5% ACV; (△——△) 3% A1110U + 5% ACV; (○ ○) 5% A1110U + 5% ACV.

the 5% and 3% combination therapies displayed no lesions until day 8 (2 days after treatment ended).

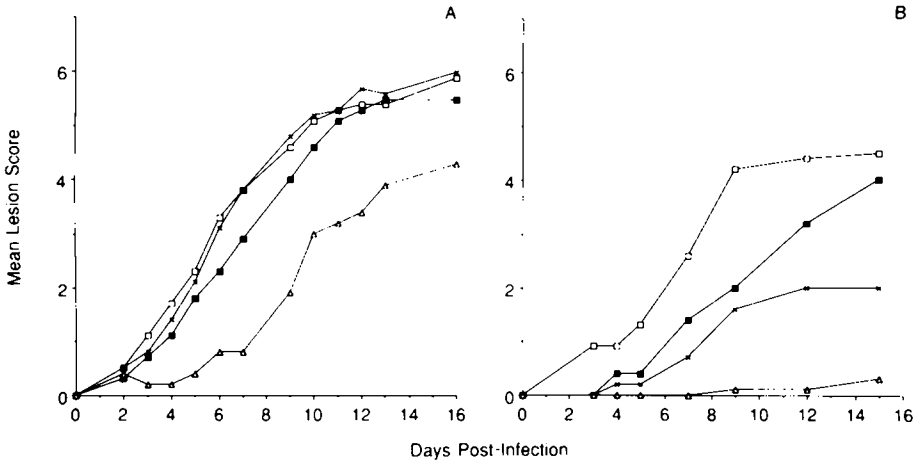

Fig. 3. Effect of topical treatment on (A) TK^{A} or (B) DNA polymerase mutant HSV-1 back-infected nude mice. In both experiments mice were vaccination gun-infected on the right dorsum. In (A) mice were topically treated 3 × per day on days 2–6 while in (B) mice were treated 3 × per day on days 1–5. (□——□) No treatment; (■- -■) 5% ACV; (X——X) 3% A1110U; (△——△) 3% A1110U + 5% ACV.

Combination therapy for ACV-resistant HSV-1 mutants

The results of infections by a TK^A and a DNA polymerase mutant of HSV-1 are shown in Fig. 3 and Table 4 (experiments 1 and 2). As indicated in the graphs, combination therapy of 3% A1110U and 5% ACV was significantly more effective than either single agent alone in reducing lesion scores in mice infected with these mutants. In mice infected with the TK^A virus the combination therapy was synergistic ($P<0.05$), whereas the lesion scores of ACV-treated mice were not different from scores of either the untreated mice or mice treated with A1110U alone. The combination was almost statistically synergistic ($P<0.07$) in infections initiated with the DNA polymerase mutant. Treatment with 3% A1110U appeared more effective against DNA polymerase mutant-infected mice than treatment with 5% ACV, although the difference was not statistically significant.

The results of the topical treatment of athymic mice infected with an HSV-1 TK deficient mutant virus are shown in Fig. 4 and Table 4 (experiment 3). In this experiment the AUC for the combination therapy was significantly less than the AUC for ACV alone. 3% A1110U was as effective as ACV in this experiment. Even during the treatment period, mice treated with either of the single agents

TABLE 4

Effects of topical treatment on mutant HSV infections in athymic mice

Topical treatment	Number of mice	Area under the curve (± SEM)		
		Observed[a]	Expected if additive[b]	Synergy[c]
Expt. 1: HSV-1 TK altered[d]				
None	12	56.6 (± 7)[1]		
5% ACV	12	50.5 (± 5.4)[1]		
3% A1110U	12	56.8 (± 3.9)[1]		
3% A1110U + 5% ACV	11	31.0 (± 6.9)[2]	50.5 (± 7.2)	Yes ($P<0.05$)
Expt. 2: HSV-1 DNA Pol mutant[e]				
None	8	38.9 (± 8.8)[1]		
5% ACV	10	24.3 (± 5.5)[1,2]		
3% A1110U	9	14.8 (± 7.5)[2,3]		
3% A1110U + 5% ACV	10	1.0 (± 0.5)[3]	9.2 (± 5.9)	No ($P=0.07$)
Expt. 3: HSV-1 TK deficient[f]				
None	11	47.8 (± 2.5)[1]		
5% ACV	12	32.3 (± 1.9)[2]		
3% A1110U	12	31.1 (± 1.3)[2]		
3% A1110U + 5% ACV	10	11.5 (± 2.7)[3]	21.0 (± 1.9)	Yes ($P<0.01$)

[a]The AUCs of mean lesion scores were compared by the Kruskal-Wallis test. Means with the same numerical superscript are not significantly ($P<0.05$) different.

[b]AUC expected values were calculated as described in Materials and Methods.

[c]Combination therapy was synergistic if the difference between observed and expected AUCs was significantly greater than that expected from sum of the independent interactions by two-sample *t*-test.

[d]Mice were treated topically 48 h p.i. with three treatments on days 2–6.

[e]Mice were treated topically 16 h p.i. with three treatments on days 1–5.

[f]Mice were treated topically 16 h p.i. with three treatments on days 1–5 and 1 treatment on day 6.

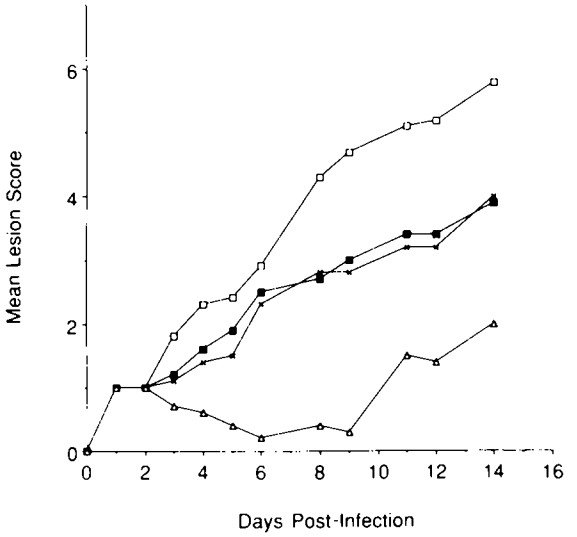

Fig. 4. Effect of topical treatment on TK^D HSV-1 back-infected nude mice. Twelve mice per treatment group were scratch-infected on the lower right dorsum. Mice were treated 3 × per day on days 1–5 and once on day 6. (□ -—□) No treatment; (■—- ■) 5% ACV; (X—-X) 3% A1110U; (△——△) 3% A1110U + 5% ACV.

showed only slightly reduced lesion scores when compared to untreated animals. Mice receiving combination therapy showed greatly reduced lesion scores during treatment and new lesions did not begin to develop until 6 days post-treatment. The combination therapy was significantly synergistic ($P<0.01$).

Discussion

We examined the effects of a topical combination of A1110U and ACV in treating HSV-infected athymic mice using the zosteriform rash model of infection. This reproducible infection model is a useful tool for the evaluation of topical antivirals since it incorporates a convenient infection procedure with a simple, objective scoring system. Infections, induced by either a vaccination gun or by light abrasion of the skin with a hypodermic needle, were highly reproducible. The resulting infection produces large lesions that are easily scored and treated with topical therapies. Additionally, HSV isolates with different pathogenicities were tested with this model.

Our results show that a topical therapy combining 3% A1110U with 5% ACV applied three times per day for 5 or 6 days produced synergistic antiviral effects and resulted in substantially reduced lesion scores for all the wt HSV-1 and HSV-2 strains tested. Therapy against the TK^D and TK^A mutant virus isolates was also significantly synergistic. The combination therapy was very effective and nearly statistically synergistic (P = 0.07) in the infection with the HSV-1 polymerase

mutant studied. However, because of the increased efficacy of A1110U alone against this mutant and the smaller number of animals used in this study, statistical significance was not obtained. Treatment with A1110U was previously found to be more effective than treatment with ACV in mice infected with another DNA polymerase mutant (Ellis et al., 1989). We do not know the reason for this increased sensitivity in vivo.

The flare-up of lesions observed when therapy is discontinued is a significant feature of HSV infection and therapeutic treatment of athymic mice (Ellis et al., 1989). In the zosteriform model, higher lesion scores are indicative of an infection spreading to secondary sites, which have become infected as a result of virus replication in ganglia that supply nerves to the dermatomes. Since the area treated was confined to the inoculation site, treatments which resulted in less flare-up of lesions were probably more effective in inhibiting virus replication at the site of inoculation. In our studies, discontinuation of topical ACV therapy resulted in the development of new lesions (flare-up) with both the wt HSV-1 isolates tested, whereas combination therapy was successful in preventing the development of infection in the majority of the mice treated (only 2 of 22 mice had high lesion scores). A flare-up of lesions was also seen with infections initiated by the HSV-2 strain. However, new lesions began forming on ACV-treated mice during the treatment period. While a few mice treated with the combination did exhibit a flare-up of lesions, these lesions were delayed and began to form only after treatment was stopped.

The in vitro ACV resistance of the mutant HSV isolates studied (see Table 2) was also observed in the in vivo experiments conducted with these viruses. Lesion scores of mice infected with the TK^D and TK^A mutants and treated with either ACV or A1110U did not differ and progressively increased during the course of treatment. These treatments were no more effective than no treatment in the TK^A initiated infection and only marginally more effective than no treatment in the TK^D initiated infection. In contrast, lesion scores of these mice treated with the combination therapy were very low and did not increase until several days after treatment was stopped. The results of this study suggest that extended treatment with the combination might eliminate any sign of infection. Although the ACV-resistant DNA polymerase mutant produced a less severe infection, ACV treatment was only moderately effective and did not prevent the onset of lesions during treatment. The combination therapy for this infection was almost totally effective.

In summary, our studies indicate that the combination of A1110U with ACV is significantly more effective than ACV alone in reducing the zosteriform rash in mice caused by dorsal infections by both wt and mutant HSV isolates. These results offer additional encouragement that this type of therapy will be an effective topical treatment for cutaneous HSV infections in humans.

Acknowledgements

The authors wish to thank Gerald Hajian for statistical analysis, Susan Kelley for drug preparation, Jack Hill for technical assistance and William Phelps for critical review of the manuscript.

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