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Synergistic Therapy by Acyclovir and A1110U for Mice Orofacially Infected with Herpes Simplex Viruses

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Clinical effects of the administration of a combination of acyclovir (ACV) and compound A1110U (a 2-acetylpyridine thiocarbonothiohydrazone inactivator of herpes simplex virus [HSV] ribonucleotide reductase) on the development of herpetic skin lesions were studied in athymic and hairless mice infected intracutaneously with different HSV type 1 (HSV-1) strains. ACV was administered topically (5%) or orally (5 mg/ml), while A1110U was applied topically (3%). In all but one experiment, the effect of combination therapy was greater than that calculated for the sum of the individual drug effects in limiting the development of herpetic skin lesions in mice. In several experiments, combination therapy totally eliminated all signs of infection. This synergistic chemotherapeutic efficacy was evident in infections caused by ACV-susceptible as well as several ACV-resistant HSV-1 strains. These results indicate that this combination therapy may provide a significant improvement in clinical responses over single-agent topical therapy.

Acyclovir (ACV) is a potent and selective antiherpetic agent that is clinically effective against systemic and local infections caused by herpes simplex viruses (HSV) (12). Widespread clinical use of the drug has revealed several areas in which its effectiveness may be improved.

The first is that better therapy might be designed to treat or prevent the infrequently isolated but clinically resistant strains that may occur during ACV therapy (7, 10, 29). Second, topical ACV is probably not as effective as intravenous ACV against HSV-induced cutaneous disease in humans (26, 35) and does not prevent latent neuronal infections (5, 23). Finally, high-dose intravenous ACV can occasionally be associated with some adverse effects in humans (4, 25).

A recent report (33) describes the potentiation of the antiherpetic effect of ACV by combination with A723U, a 2-acetylpyridine thiosemicarbazone inhibitor of ribonucleotide reductase. The thiosemicarbazones are a group of antiviral agents with activities against DNA and RNA viruses (2, 3, 18, 27). Reports indicate that the antiherpetic activity of the thiosemicarbazones is the result of inhibition of the virus-coded ribonucleotide reductase (37). A1110U, a 2 - acetylpyridine 5 - [(dimethylamino)thiocarbonoyl]thiohydrazone that closely resembles A723U, was recently shown to be a more potent inactivator of HSV type 1 (HSV-1), HSV-2, and varicella-zoster virus ribonucleotide reductases and to potentiate the activity of ACV against these viruses in vitro (34). This dramatic synergy in vitro prompted us to evaluate the combination of A1110U and ACV in vivo.

The athymic mouse model of cutaneous HSV-1 infection was selected because it parallels the circumstances that occur during HSV infection of the human immunocompromised host (14). Furthermore, several types of ACV-resistant mutants produce significant illness in athymic mice, whereas their reduced virulence results in only moderate disease in immunocompetent mice (29).

We also evaluated the clinical effectiveness of combined ACV and A1110U treatment of HSV-1-induced cutaneous disease in normal mice.

(This work was presented in part previously [M. N. Ellis, D. C. Lobe, and T. Spector, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 625, 1986].)

MATERIALS AND METHODS

Viruses. The following HSV-1 strains were used: VL 9014, thymidine kinase positive (wild type [wt]) and ACV susceptible; VL 9013, thymidine kinase (TK) deficient (TK^D) and ACV resistant; SC16-S1 (designated here as VL 8971), TK altered (TK^A) and ACV resistant; VL 10105, ACV resistant by virtue of an altered DNA polymerase. These viruses were described previously (11, 17, 29).

Mice. BALB/c athymic nude (*nu/nu*) female mice 3 weeks old were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.), and were infected at 4 weeks of age. Athymic mice were housed five to a cage and were fed sterile water and food. Hairless (HRS/J) mice were purchased from Jackson Laboratory (Bar Harbor, Maine) and housed five to a cage.

Inoculation of mice. Groups of 8 to 10 mice (under light ether anesthesia) were inoculated on the snout by scarification with a 25-gauge needle followed by rubbing for 15 s with a cotton-tipped applicator soaked in an appropriately diluted virus suspension (approximately 10^6 PFU per mouse). The mice were inspected daily (through day 10 or 14 postinfection [p.i.]) for cutaneous lesions, which were scored by a method previously described (29).

Antiviral compounds. For oral administration, ACV was dissolved in sterile distilled water. A1110U at 3% or ACV at 5% or a combination of the two was prepared as a suspension with 4% cetyl alcohol as a thickener and 0.01% EDTA in either a modified aqueous cream (MAC) or 1,3-butanediol. The poor solubility of these compounds necessitates the use of 1,3-butanediol, and it was described previously (28).

Treatment of mice. Topical drug was applied to the area of inoculation at various intervals p.i. as described below. Treatment was administered between 8 a.m. and 5 p.m. at the frequencies described below. In several experiments, ACV was administered in the drinking water. In others, it was made up in a single-agent topical preparation or combined with A1110U (see above). During the studies, we

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TABLE 1. HSV strains

Strain	Phenotype	ED_{50} (μM)[a]	
		ACV[b]	A1110U
VL 9014	TK positive (wt)	0.5	1.5
VL 9013	TK deficient (TK^D)	5.3	1.1
VL 8971	TK altered (TK^A)	7.0	1.7
VL 10105	DNA polymerase altered	8.8	1.3

[a] ED_{50}, 50% effective dose.
[b] As determined by the plaque reduction method in Vero cells.

encountered an occasional transient skin irritation in animals treated with A1110U alone or in combination with ACV.

Antiviral susceptibility assay. Virus strains used in these studies were tested for susceptibility to drug inhibition in a standard plaque reduction assay (29).

Statistical analysis. The area under the curve (AUC) of mean lesion scores versus days p.i. was analyzed by the Kruskal-Wallis test (30). 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 expected value for the AUC was calculated by the following formula (19, 38):

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

The expected AUC was compared for statistical significance with the observed AUC by Student's t test (1). If the observed inhibition is significantly greater than that expected from the sum of independent interactions, the combination is synergistic (38).

RESULTS

Drug susceptibilities. The ACV and A1110U 50% effective doses determined for the HSV-1 strains used in these experiments are shown in Table 1. VL 9014 (wt) and VL 9013, its TK^D mutant, were approximately 10-fold different in susceptibility to ACV. However, they were of equivalent susceptibility to A1110U. Two additional ACV-resistant mutants used in these studies, designated here as VL 8971 and VL 10105, were equally susceptible to A1110U while clearly resistant to ACV. The in vitro data indicated that the two drugs had different modes of action with no cross resistance.

Effects of combined topical therapy on wt HSV-1 infection of mice. The efficacy of combined topical treatments was first evaluated in athymic mice (Table 2, experiment 1). Infected areas were treated topically with 3% A1110U or 5% ACV or with a combination of 3% A1110U and 5% ACV beginning 24 h p.i. Topical treatments were administered four times per day for 8 days, with only three treatments on day 1. The development of facial herpetic lesions in untreated mice was similar to previously reported observations (17). While virus shedding was not evaluated in this experiment, previous studies have shown that statistically significant reductions in lesion score are associated with reduced quantities of virus in the skin (14). A significant reduction in lesion scores was recorded in all treatment groups compared with controls. However, the greatest reduction in lesion scores resulted from treatment with 3% A1110U plus 5% ACV, which showed significant synergism ($P < 0.001$).

Because of the efficacy of 5% ACV in the early treatment of infections initiated by wt virus, the effect of delayed treatment (48 h p.i.) was studied with these same topical therapies (Table 2, experiment 2). Inoculated areas were treated four times per day for 5 days. Lesion scores in untreated animals and mice receiving 3% A1110U were not statistically different. Animals treated with 5% ACV in MAC had significant reductions in lesion scores compared with untreated or 3% A1110U-treated mice. However, animals receiving 3% A1110U plus 5% ACV had the greatest reduction in lesion scores ($P < 0.001$). A synergistic interaction between the two drugs was observed.

Effects of combination treatment on TK^D mutant infection of mice. Snout infection of athymic or hairless (HRS/J) mice with TK^D HSV-1 resulted in an attenuated pattern of cutaneous disease with herpetic skin lesions progressing slowly. Peak lesion scores appeared 6 days after inoculation of mice

TABLE 2. Treatment of TK-positive (wt) HSV-1 infections of athymic mice

Treatment regimen	Mean lesion score (±SEM) on day after virus inoculation:					AUC (±SEM)		
	3	5	7	10	14	Observed[a]	Expected[b]	Synergy[c]
Expt 1 ($n = 6$)[d]								
None	0.4 (±0.2)	2.0 (±0.4)	3.6 (±0.2)	4.0 (±0.0)	4.0 (±0.0)	36.2 (±1.4)[1]		
5% ACV[e]	0.2 (±0.2)	0.0 (±0.0)	0.0 (±0.0)	0.3 (±0.3)	2.2 (±0.6)	6.8 (±2.6)[2]		
3% A1110U[e]	0.0 (±0.0)	0.0 (±0.0)	0.8 (±0.5)	2.2 (±0.5)	3.7 (±0.3)	17.7 (±3.4)[3]		
5% ACV + 3% A1110U[e]	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)[4]	3.4 (±1.4)	Yes ($P < 0.001$)
Expt 2 ($n = 10$)[f]								
None	1.3 (±0.2)	3.0 (±0.0)	3.8 (±0.1)	4.0 (±0.0)		28.3 (±0.5)[1]		
5% ACV[g]	1.5 (±0.2)	1.4 (±0.2)	1.4 (±0.2)	3.0 (±0.0)		17.1 (±0.7)[2]		
3% A1110U[g]	1.5 (±0.2)	2.4 (±0.2)	3.2 (±0.1)	4.0 (±0.0)		27.1 (±0.6)[1]		
5% ACV + 3% A1110U[g]	0.7 (±0.2)	0.4 (±0.2)	0.1 (±0.1)	1.4 (±0.2)		6.6 (±0.6)[3]	16.4 (±0.9)	Yes ($P < 0.001$)

[a] The AUCs of lesion scores were compared by the Kruskal-Wallis test. Means with the same numerical superscript are not significantly ($P < 0.05$) different.
[b] The expected value for the AUC was calculated as described in the text.
[c] Difference between observed and expected AUC for the combination was significantly greater than expected from independence by two-sample t test.
[d] Athymic mice were treated topically 24 h p.i., with three treatments on day 1 and four treatments on days 2 to 8.
[e] Topical preparation in 1,3-butanediol.
[f] Athymic mice were treated topically 48 h p.i., with four treatments on days 2 to 5.
[g] Topical preparation in MAC.

TABLE 3. Treatment of TK^D HSV-1 infections of mice

Treatment regimen	Mean lesion score (±SEM) on day after virus inoculation:				AUC (±SEM)		
	3	5	7	10	Observed[a]	Expected[b]	Synergy[c]
Expt 1 (n = 8)[d]							
None	1.4 (±0.3)	1.6 (±0.3)	1.3 (±0.3)	0.5 (±0.4)	9.5 (±2.2)[1]		
5% ACV[e]	0.4 (±0.2)	1.0 (±0.0)	0.3 (±0.2)	0.1 (±0.1)	3.6 (±0.7)[2]		
3% A1110U[e]	0.5 (±0.3)	0.6 (±0.2)	0.6 (±0.2)	0.0 (±0.0)	3.5 (±0.8)[2]		
5% ACV + 3% A1110U[e]	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)[3]	1.3 (±0.6)	Yes ($P < 0.05$)
Expt 2 (n = 5)[f]							
None	1.0 (±0.3)	2.2 (±0.2)	3.0 (±0.0)	3.4 (±0.2)	19.9 (±0.9)[1]		
ACV (5 mg/ml) orally[g]	0.2 (±0.2)	0.4 (±0.2)	0.4 (±0.2)	0.6 (±0.2)	3.5 (±1.3)[2]		
3% A1110U[e]	1.3 (±0.2)	0.5 (±0.3)	2.0 (±0.0)	2.0 (±0.4)	11.0 (±0.8)[3]		
ACV (5 mg/ml) orally[g] + 3% A1110U[e]	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)[4]	1.9 (±0.8)	Yes ($P < 0.05$)

[a] The AUCs of lesion scores were compared by the Kruskal-Wallis test. Means with the same numerical superscript are not significantly ($P < 0.05$) different.
[b] The expected value for the AUC was calculated as described in the text.
[c] Difference between observed and expected AUC for the combination was significantly greater than expected from independence by two-sample t test.
[d] Hairless (HRS/J) mice were treated topically 24 h p.i., with four treatments on days 1 to 4.
[e] Topical preparation in 1,3-butanediol with 4% cetyl alcohol.
[f] Athymic mice were treated topically beginning 3 h p.i., with three treatments on day 0 and four treatments on days 1 to 4. ACV was administered in drinking water beginning 3 h p.i. and continuing through day 4.
[g] ACV made up to required concentration in sterile distilled water.

with TK^D virus. In hairless mice, these lesions eventually heal, while in athymic mice, these indolent ulcers remain for many weeks with slight progression (29).

In our initial experiment, hairless mice were infected with TK^D HSV-1 (summarized in Table 3, experiment 1). Mice received topical 5% ACV and/or 3% A1110U beginning 24 h p.i. and continuing four times per day through day 4. Mice receiving either topical drug had significant reductions in lesion scores. However, the greatest reduction in lesion scores was observed in animals receiving combined topical therapy. These mice had no lesions during the entire observation period.

The efficacy of topical A1110U and oral ACV therapy was studied in athymic mice infected with TK^D HSV-1 (Table 3, experiment 2). Beginning 3 h after infection, mice received ACV in the drinking water at a dose of 5 mg/ml. A second group of mice received 3% topical A1110U, with three treatments on day 0 and four treatments per day for the next 4 days. A third group received oral ACV and topical A1110U on the same dosing regimen as described for animals receiving the single agents. While animals receiving ACV or A1110U alone had significant reductions in lesion scores compared with untreated controls, mice receiving the combination had the most improved clinical responses, with no detectable lesions. Furthermore, virus could not be recovered from skin samples taken from infected snouts of these animals.

Effects of combination treatment on DNA polymerase mutant infection of mice. Infection of athymic mice with a DNA polymerase mutant virus resulted in a course of infection similar to that described for TK^D virus (Table 4). Therapy was initiated 3 h p.i., as in the previous study. Treatment of mice with oral ACV in the drinking water at a dose of 5 mg/ml produced significant reductions in lesion scores ($P < 0.05$). Treatment with topical 3% A1110U or a combination of topical A1110U plus oral ACV (5 mg/ml) produced substantially better reductions in lesion scores than did oral ACV alone ($P < 0.05$). While reductions in lesion scores were the lowest with combination therapy, these scores were not significantly different from those of mice receiving only 3% A1110U, and synergy was not observed ($P > 0.5$).

The enhanced efficacy of combination therapy in mice infected with attenuated ACV-resistant mutants suggested that even better clinical responses might be possible in mice infected with fully pathogenic ACV-resistant virus.

Effects of combination treatment on TK^A mutant infection of mice. Preliminary studies indicated that the course of infections initiated by TK^A virus in hairless or athymic mice

TABLE 4. Treatment of DNA polymerase mutant HSV-1 infection of athymic mice[a]

Treatment regimen	Mean lesion score (±SEM) on day after virus inoculation:				AUC (±SEM)		
	3	5	7	10	Observed[b]	Expected[c]	Synergy[d]
None	2.2 (±0.2)	3.0 (±0.0)	3.1 (±0.1)	3.4 (±0.2)	22.9 (±0.6)[1]		
ACV (5 mg/ml) orally[e]	0.4 (±0.2)	2.0 (±0.2)	2.1 (±0.2)	2.6 (±0.2)	15.7 (±1.2)[2]		
3% A1110U[f]	0.2 (±0.1)	0.7 (±0.3)	1.1 (±0.3)	2.2 (±0.4)	7.7 (±1.5)[3]		
ACV (5 mg/ml) orally[e] + 3% A1110U[c]	0.2 (±0.1)	0.4 (±0.2)	0.6 (±0.2)	1.3 (±0.2)	4.3 (±0.9)[3]	5.3 (±1.1)	No ($P > 0.5$)

[a] Athymic mice were treated topically beginning 3 h p.i., with three treatments on day 0 and four treatments on days 1 to 4. ACV was administered in the drinking water at 3 h p.i. and continued through day 4. n = 10.
[b] The AUCs of lesion scores were compared by the Kruskal-Wallis test. Means with the same numerical superscript are not statistically ($P < 0.05$) different.
[c] The expected value for the AUC was calculated as described in the text.
[d] Difference between observed and expected AUC for the combination is not significantly greater than expected from independence by two-sample t test.
[e] ACV made up to required concentration in sterile distilled water.
[f] Topical preparation in 1,3-butanediol with 4% cetyl alcohol and 0.01% EDTA.

TABLE 5. Treatment of TK^A HSV-1 infections of mice[a]

Treatment regimen	Mean lesion score (±SEM) on day after virus inoculation:				AUC (±SEM)		
	3	5	7	10	Observed[b]	Expected[c]	Synergy[d]
None	0.6 (±0.2)	2.1 (±0.4)	3.0 (±0.5)	3.5 (±0.5)	18.3 (±2.8)[1]		
ACV (5 mg/ml) orally[e]	0.6 (±0.2)	1.0 (±0.3)	2.0 (±0.4)	3.3 (±0.4)	12.4 (±2.2)[1,2]		
3% A1110U[f]	0.0 (±0.0)	0.7 (±0.3)	1.8 (±0.4)	2.9 (±0.5)	10.2 (±1.8)[2]		
ACV (5 mg/ml) orally[e] + 3% A1110U[f]	0.0 (±0.0)	0.0 (±0.0)	0.1 (±0.1)	0.4 (±0.2)	0.7 (±0.4)[3]	6.9 (±2.3)	Yes ($P < 0.02$)

[a] Athymic mice were treated 3 h p.i., with three treatments on day 0 and four treatments on days 1 to 4. ACV was administered in the drinking water beginning 3 h p.i. and continued through day 4. $n = 8$.
[b] The AUCs of lesion scores were compared by the Kruskal-Wallis test. Means with the same numerical superscript are not significantly ($P < 0.05$) different.
[c] The expected value for the AUC was calculated as described in the text.
[d] Difference between observed and expected AUC for the combination was significantly greater than expected from independence by two-sample t test.
[e] ACV made up to required concentration in sterile distilled water.
[f] Topical preparation in MAC.

was quite similar to and as severe as cutaneous disease caused by wt virus (15, 27). The TK of this virus readily phosphorylates thymidine but not ACV (11). Therefore, the effectiveness of combined therapy against infections initiated by ACV-resistant TK^A HSV-1 was evaluated. Infected athymic mice were treated topically from 3 h p.i. through day 4. Mice received three treatments on day 1 of infection and four treatments per day for the next 4 days. A second group of mice received ACV in the drinking water at a dose of 5 mg/ml beginning 3 h p.i. and continuing through day 4. The final group of mice received both oral ACV and topical A1110U. Mice that received ACV alone did not have significant reductions in lesion scores when compared with controls (Table 5). Mice that received 3% A1110U had statistically significant reductions in lesions compared with control mice ($P < 0.05$). The most significant reductions in lesions were again observed in mice that received combination therapy. This interaction of the two drugs was synergistic ($P < 0.02$). Similar results (data not shown) occurred when topical ACV (5%) was combined with topical A1110U against this same virus strain.

In a final experiment, the effects of different concentrations of A1110U in combination with ACV (Table 6) were examined. Hairless mice were infected with TK^A virus. Treatment was begun 24 h p.i. and continued three times per day for 5 days. Infected mice were divided into groups that received one of the following doses in MAC: 5% ACV, 3% A1110U, 5% ACV–0.3% A1110U, or 5% ACV–3% A1110U. Mice that received 3% A1110U alone had a significant reduction of herpetic lesions compared with controls ($P < 0.05$). Mice receiving ACV alone did not have significant reductions in lesion scores compared with control mice. Mice that received the lower concentration of A1110U (0.3%) in combination with 5% ACV had significantly greater reductions in lesions compared with mice that received a single agent ($P < 0.05$). However, mice receiving A1110U at the higher concentration (3%) with 5% ACV had reductions in lesion scores that were significantly greater than those observed in 5% ACV–0.3% A1110U-treated mice ($P < 0.05$). The combination of 5% ACV plus 3% A1110U was synergistic ($P < 0.005$).

DISCUSSION

In recent years, many groups have attempted to discover synergistic combinations of antiviral compounds. The advantages of such combinations are reduced doses of potentially toxic compounds, reduced potential for the emergence of drug-resistant viruses, and increased antiviral potency. Several combinations of antiviral agents have shown synergistic effects in vitro (24, 31). Since the early report of additive effects of interferon and vidarabine (6), several other reports have described enhanced therapeutic activity of various types of interferons in combination with nucleosides (15, 16, 20–22, 36). However, the basis for these antiviral combinations was primarily empirical.

More recently, several investigators have attempted the rational design of synergistic combinations of antiviral agents. Spector et al. (33, 34) have shown that an inhibitor of HSV ribonucleotide reductase (A723U or A1110U) results in a synergistic potentiation of the activity of ACV against HSV-1, HSV-2, and varicella-zoster virus replication in vitro. These novel strategies take advantage of the importance and function of the HSV ribonucleotide reductase in HSV replication (8, 13, 32).

TABLE 6. Dose-response combination therapy of TK^A HSV-1 infection of hairless mice[a]

Treatment regimen	Mean lesion score (±SEM) on day after virus inoculation:				AUC (±SEM)		
	3	5	7	10	Observed[b]	Expected[c]	Synergy[d]
None	2.1 (±0.1)	4.0 (±0.0)	4.0 (±0.0)	3.9 (±0.1)	28.9 (±0.2)[1]		
5% ACV[e]	2.0 (±0.0)	3.6 (±0.2)	4.0 (±0.0)	3.8 (±0.2)	27.7 (±0.4)[1,2]		
3% A1110U[e]	1.4 (±0.2)	3.4 (±0.2)	4.0 (±0.0)	4.0 (±0.0)	26.0 (±0.8)[2]		
5% ACV + 0.3% A1110U[e]	0.5 (±0.2)	2.7 (±0.2)	3.3 (±0.2)	1.6 (±0.3)	19.0 (±0.9)[3]		
5% ACV + 3% A1110U[e]	0.0 (±0.0)	2.0 (±0.3)	2.7 (±0.2)	1.7 (±0.4)	13.9 (±1.3)[4]	24.9 (±2.9)	Yes ($P < 0.005$)

[a] Hairless (HRS/J) mice were treated topically beginning 24 h p.i. and continued three times per day for 5 days. $n = 10$.
[b] The AUCs of lesion scores were compared by the Kruskal-Wallis test. Means with the same numerical superscript are not statistically ($P < 0.05$) different.
[c] The expected value for the AUC was calculated as described in the text.
[d] Difference between observed and expected AUC for the combination was significantly greater than expected from independence by two-sample t test.
[e] Topical preparation made up in MAC.

Our in vitro results indicated that several types of ACV-resistant mutants (TK^D, TK^A, and DNA polymerase altered) are susceptible to A1110U, with 50% effective doses of 1.1, 1.7, and 1.3 μM, respectively. Since none of the mutants were resistant to both ACV and A1110U, we decided to evaluate ACV and A1110U as a potential combination therapy for HSV-infected mice.

In most experiments, topical or oral ACV plus topical A1110U was significantly more effective than the calculated sum of treatments with either drug alone. This enhanced antiviral activity was demonstrated in infections initiated by ACV-susceptible as well as ACV-resistant viruses. The interaction between ACV and A1110U was significantly synergistic in treating infections in which ACV alone was effective as well as in treatment of infections in which the efficacy of ACV was less consistently effective.

The finding of synergism between ACV and A1110U in the treatment of ACV-resistant strains of HSV-1 was unexpected. Earlier studies showed that dGTP levels increase after treatment of HSV-1-infected cells with ACV (33, 34). Since increased pools of dGTP would competitively impede the binding of ACV triphosphate to HSV DNA polymerase, inhibitors of HSV ribonucleotide reductase, such as A723U and A1110U, were used to prevent the buildup of dGTP (33, 34). However, they also caused the ACV triphosphate pools to increase markedly. Thus, it is likely that the 100-fold increase in the ratio of ACV triphosphate to dGTP facilitated the binding of ACV triphosphate to HSV DNA polymerase and accounted for the synergy. The finding that high concentrations of ACV were effective against TK^D and TK^A mutant viruses in the present study indicates that ACV triphosphate can be formed even though these mutants have severely decreased capacity to phosphorylate ACV. Furthermore, the occurrence of synergy between ACV and A1110U suggests that A1110U augments the activation of ACV and blocks the buildup of dGTP in infections with the mutants as well.

Our results with infection of athymic mice with a DNA polymerase mutant of HSV-1 (Table 4) represent the only instance in which the two drugs did not act synergistically. Although the HSV-1 mutants used in these studies had similar in vitro susceptibilities to A1110U, the DNA polymerase mutant was considerably more responsive to A1110U in vivo than anticipated. As with the situation in which ACV was effective alone, the effectiveness of A1110U left little room for improvement by combination therapy. Perhaps synergy could have been demonstrated with delayed treatment.

A unique feature of infection and treatment of athymic mice is the flare-up of lesions once therapy is stopped (14, 29). Virus that initiates this reemergent disease represents incompletely eradicated virus still present in the skin or virus activated from the ganglia. Complete virions have been found in ganglionic neurons of laboratory animals 24 h after primary infection, and in most instances topical treatment initiated at this time does not prevent establishment of latent infection (5, 9, 23). Interestingly, in two of our experiments (Table 2, experiment 1; Table 3, experiment 2), this post-therapy flare-up was not observed in mice receiving the antiviral combination beginning 3 or 24 h p.i. Yet, animals receiving ACV that had very reduced lesion scores during the clinical scoring period (through day 10) had the usual flare-ups shortly after therapy was stopped. Studies are in progress to evaluate the effects of the combination on viral latency.

In summary, the present results indicate that ACV and A1110U act synergistically to moderate HSV-1 infection of mice. This synergistic interaction is based on the unique modes of action of the two drugs and the interrelatedness of their modes of action. Our experiments suggest that combination therapy with these agents is effective against ACV-resistant HSV-1 strains and offers greater clinical potency than present topical preparations for the treatment of cutaneous herpetic disease.

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