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Herpes Simplex Virus Skin Infection in Hairless Mice: Treatment with Antiviral Compounds

RICHARD J. KLEIN, ALVIN E. FRIEDMAN-KIEN, AND EILEEN BRADY

Departments of Microbiology and Dermatology, New York University School of Medicine, New York, New York 10016

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A hairless mouse-herpes simplex virus skin infection experimental model was used to evaluate the efficacy of the antiviral compounds 9-β-D-arabinofuranosyladenine (ara-A), 5-iodo-2'-deoxyuridine (IUdR), and 6-azauridine (aza-U). Ara-A and IUdR, when administered intraperitoneally by several different dosage schedules, reduced the severity of cutaneous herpetic lesions and the incidence of paralysis and increased significantly the number of survivors. A more rapid healing of the lesions and an increase in the mean survival time also was observed. A delay of 24 to 48 h in the initiation of treatment after the infection was more effective than treatments started at the time of inoculation. Treatment with ara-A was somewhat superior to that with IUdR, but aza-U was totally ineffective. Enhancement of the evolution of the infection was noted after treatment with aza-U.

Recurrent herpes simplex virus (HSV) infection of the skin or mucous membranes of humans has been a considerable source of discomfort and disability. The treatments clinically available at this time for this infection, both topically and/or systemically, have been less than satisfactory.

Recent reports have described an excellent, reproducible experimental laboratory animal infection model involving inoculation of the skin of hairless mice with HSV (1, 5, 6, 11). After HSV inoculation of scarified, lumbosacral hairless-mouse skin, the animals developed a punctate eruption within 3 to 4 days, which rapidly coalesced to form a band-like lesion in 5 to 6 days with neurodermatomic distribution resembling the skin lesions of herpes zoster infection rather than human cutaneous HSV infection. There is evidence that in this skin infection model the virus spreads eventually to the central nervous system (CNS) and causes encephalitis with paralysis and ultimately death (1).

This model provides an experimental system in which a variety of topically or systemically administered antiviral agents may be evaluated in vivo. Underwood (11) and Lieberman et al. (5) used this experimental infection model to evaluate the antiviral effect of several substances including kethoxal, 9-β-D-arabinofuranosyl adenine (adenine arabinoside, ara-A), 1-β-D arabinofuranosylcytosine (cytosine arabinoside, ara-C), interferon, and interferon inducers.

In the present study, the experimental model of HSV infection of hairless mice was used to evaluate the antiviral activity of several compounds to determine their potential usefulness in herpetic infections. 6-Azauridine (aza-U), 5-iodo-2'-deoxyuridine (IUdR), and ara-A have already been shown to have an inhibitory effect upon HSV multiplication in vitro (2, 3, 8), and therapeutic activity in experimental animals and humans has also been demonstrated (4, 7, 9).

MATERIALS AND METHODS

Virus. The S type-1 HSV strain, originally isolated from a human facial skin lesion and passaged in HEp-2 cell cultures, was obtained from Paul E. Came, Schering Corp., Bloomfield, N.J. The strain was propagated in our laboratory in secondary rabbit kidney cell cultures and had a titer of 4×10^6 plaque-forming units per ml when assayed on Vero cell monolayers. Samples of the same batch of virus were stored at −70 C until used throughout the experiments. For the infection of mice, a virus dilution containing 40 skin mean infective dose per ml was used.

Mice. Hairless mice, randomly bred at the New York University School of Medicine animal laboratory facility, were originally derived from the HRS/Y strain of Jackson Laboratories, Bar Harbor, Me. Mice weighing 20 ± 2 g were segregated in groups of 10, according to sex and birth date.

Infection of mice. The procedures used were as described (5), except that mice were not anesthetized prior to infection.

Scoring of the lesions. Mice were examined daily for 14 days. The lesions were graded from 0 to 4 (5). The mean lesion score was determined daily, and the average lesion score was calculated at the end of the observation period from the maximum score attained by the individual mice in each group, irrespective of the day in which it was recorded.

Antiviral compounds. The antiviral agents used in these experiments were obtained through the courtesy of George Galasso, Head of the Antiviral Substances Program, National Institute of Allergy and Infectious Diseases, Bethesda, Md.

Ara-A (Parke, Davis & Co., Ann Arbor, Mich.), obtained as a micronized powder (particle size of 5 μm or less), was prepared as an aqueous suspension which contained 6 mg/0.25 ml (300 mg/kg) and was injected intraperitoneally (i.p.). IUdR (HM Bulk Chemicals, San Diego, Calif.) was administered i.p. as an aqueous suspension containing 2 mg/0.25 ml (100 mg/kg). The ara-A and IUdR suspensions were prepared prior to injection as individual doses in order to ensure a uniform administration of these insoluble compounds. Aza-U (Calbio-Pharmaceuticals, La Jolla, Calif.) was injected i.p. as an aqueous solution containing 5 mg/0.25 ml (250 mg/kg).

Statistical evaluation of the results. The significance of the differences in survival rate, number of animals that developed severe lesions, and incidence of paralysis was evaluated by Fisher's Exact Test. The mean survival time and the average lesion score differences were evaluated by the Student's *t* test.

RESULTS

Evolution of the infection. After cutaneous inoculation with HSV, the evolution of the skin lesions was essentially similar to the pattern described by Lieberman et al. (5). A few animals which did develop cutaneous infection, but which had no CNS involvement, showed complete healing of the skin lesions within 3 to 4 weeks. In very rare instances, mice which developed CNS disease did survive, but with some residual paralysis.

Effect of ara-A. When ara-A was given i.p. in daily doses of 300 mg/kg for 12 days starting at the time of infection, 90 to 100% of the mice survived, and the average lesion score was reduced by 25% (Table 1). Correspondingly, a more rapid healing of the lesions was also apparent (Table 2). The same dose, starting at the time of inoculation, given every 2nd day over a 12-day period seemed to be ineffective, as evidenced by the high average lesion score and no difference in the rate of survival and the

TABLE 1. *Effect of ara-A treatment on HSV-skin infection of hairless mice*[a]

Expt no.	Group	Avg lesion score (cumulative maximum)	P[b]	No. with paralysis	P[c]	No. of deaths	P[c]	Mean survival time (days)	P[b]
1[d]	Control	4.0		7/10		6/10		10.4	
	Ara-A, -ay 0-11	3.0	<0.01	0/10	<0.002	1/10	<0.03	13.5	<0.025
	Ara-A, days 0, 2, 4, 6, 8, 10	4.0		4/10	>0.10	3/10	>0.10	12.3	>0.20
2[d]	Control	4.0		7/10		9/10		8.8	
	Ara-A, day 0-11	3.1	<0.01	0/10	<0.002	0/10	<0.001	14.0	<0.001
	Ara-A, day 2-10	2.8	<0.01	0/10	<0.002	1/10	<0.001	13.6	<0.001
3[d]	Control	4.0		8/10		7/10		9.5	
	Ara-A, day 0-11	3.1	<0.01	2/10	<0.04	2/10	<0.04	12.2	>0.10
	Ara-A, day 1-8	1.8	<0.001	0/10	<0.001	0/10	<0.002	14.0	<0.001
	Ara-A, day 2-9	2.3	<0.001	1/10	<0.005	2/10	<0.04	13.0	<0.025
4[e]	Control	4.0		10/10		8/10		8.7	
	Ara-A, day 1-7	1.4	<0.001	0/10	<0.001	0/10	<0.001	14.0	<0.001

[a] Ara-A was injected i.p. as an aqueous suspension containing 6 mg in 0.25 ml of phosphate-buffered saline.

[b] Probability (Student's *t* test) that the lower average lesion score and the decreased mean survival time was due to chance.

[c] Probability (Fisher's Exact Test) that the decreased rate of paralysis and death was due to chance.

[d] Ara-A in daily doses of 300 mg/kg.

[e] Ara-A in daily doses of 600 mg/kg.

TABLE 2. *Effect of ara-A treatment on the evolution of HSV skin lesions in hairless mice*

Expt no.	Group	No. of animals with severe lesions (>2+) on day after infection												Maximum positive (%)	P^a
		3	4	5	6	7	8	9	10	11	12	13	14		
1[c]	Control	0/10[b]	4/10	7/10	10/10	10/10	8/8	6/6	2/4	2/4	2/4	0/4	0/4	100	
	Ara-A, day 0–11	0/10	0/10	4/10	7/10	5/10	4/10	3/10	2/9	2/9	1/9	0/9	0/9	70	>0.1
	Ara-A, day 0–11 every 2nd day	0/10	3/10	7/10	10/10	10/10	9/9	8/8	7/8	5/7	5/7	3/7	3/7	100	
2[c]	Control	0/10	1/10	6/10	10/10	10/10	6/6	5/5	3/3	1/1	1/1	1/1	1/1	100	
	Ara-A, day 0–11	0/10	0/10	0/10	4/10	7/10	6/10	6/10	5/10	4/10	2/10	1/10	1/10	70	>0.1
	Ara-A, day 2–10	0/10	0/10	1/10	6/10	2/10	1/10	1/10	1/10	0/9	0/9	0/9	0/9	60	<0.05
3[c]	Control	0/10	0/10	1/10	6/9	9/9	5/7	2/5	2/3	1/3	0/3	0/3	0/3	100	
	Ara-A, day 0–11	0/10	0/10	1/10	7/10	8/10	7/10	4/8	3/8	1/8	0/8	0/8	0/8	80	>0.2
	Ara-A, day 1–9	0/10	0/10	0/10	2/10	2/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	20	<0.001
	Ara-A, day 2–10	0/10	0/10	0/10	4/10	4/10	2/10	2/10	1/8	1/8	0/8	0/8	0/8	40	<0.01
4[d]	Control	0/10	0/10	4/10	9/10	10/10	4/4	3/3	3/3	2/2	1/2	1/2	0/2	100	
	Ara-A, day 1–6	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10	<0.001

[a] Probability (Fisher's Exact Test) that the decreased number of mice with severe lesions was due to chance.
[b] Number of mice with severe lesions per number of survivors.
[c] Daily dose of 300 mg of ara-A per kg.
[d] Daily dose of 600 mg of ara-A per kg.

mean survival time (Table 1). Starting the treatment 1 or 2 days after the infection had approximately the same protective effect on the survival of the mice as had treatment started at the time of infection (Table 1); however, a significant decrease in the severity of the lesions and a marked reduction of healing time was seen in the former group of animals (Tables 1 and 2). The best results were obtained when a daily dose of 600 mg/kg was given over a period of 6 days and by starting the treatment 24 h after infection (Table 1).

Effect of IUdR. IUdR in doses of 100 mg/kg and given daily from the time of inoculation for 12 days significantly reduced the severity of the HSV-induced lesions and increased mouse survival (Table 3). When treatment was started 1 day after infection, an even lower average lesion score was observed and lesions healed faster (Tables 3 and 4). In those animals in which treatment was begun on the 3rd day after infection, at the time when the earliest lesions usually became evident, the course of infection was not altered. Infections progressed exactly as in the untreated control group (Tables 3 and 4).

Effect of aza-U. No protection against the HSV infection in mice was afforded by aza-U treatment in doses of 250 mg/kg when given daily for up to 10 days after inoculation. Although no toxic effect of the drug was demonstrable, there was significant ($P < 0.05$) reduction of the mean survival time of treated, infected animals as compared with that of the untreated, infected control group.

DISCUSSION

In this study, the protective effect of several drugs reported to have an inhibitory action on HSV was evaluated in vivo. The use of cutaneous HSV infection in hairless mice as an experimental model offers the advantage of excellent reproducibility and high susceptibility to infection. Furthermore, this model provides two parameters for measuring any antiviral effects: (i) ease of observation of the lesions on hairless skin and (ii) the development of paralysis and mouse deaths. The relatively high dose of virus was used to ensure a maximal lesion score and a mortality rate greater than 50%.

When infected animals were given ara-A i.p. from day 0 to day 11 after inoculation in doses of 300 mg/kg (total dose 3,600 mg), a marked reduction in skin lesion severity was seen, and protection from fatal spread to the CNS was

TABLE 3. *Effect of IUdR treatment[a] on HSV skin infection in hairless mice[b]*

Group	Avg lesion score (cumulative maximum)	P[c]	No. paralysis	P[d]	No. of deaths	P[d]	Mean survival time (days)	P[c]
Virus control	4.0		10/10		10/10		7.4	
IUdR, day 0-12	2.6	<0.001	2/10	<0.001	4/10	<0.01	12.4	<0.001
IUdR, day 1-12	1.9	<0.001	2/10	<0.001	4/10	<0.01	12.3	<0.001
IUdR, day 3-12	4.0		10/10		9/10	>0.5	7.9	>0.5

[a] IUdR (100 mg/kg) was injected i.p. daily as an aqueous suspension containing 8 mg of IUdR per ml of phosphate-buffered saline.

[b] Mice were infected by rubbing into the scarified skin a suspension of HSV (Schering strain) containing 4×10^5 plaque-forming units per ml.

[c] Probability (Student's *t* test) that the lower average lesion score and the increased mean survival time in treated mice was due to chance.

[d] Probability (Fisher's Exact Test) that the lower incidence of paralysis and death was due to chance.

TABLE 4. *Effect of IUdR treatment (100 mg/kg daily) on the evolution of HSV skin lesions in hairless mice*

Maximum group	No. of animals with severe lesions (>2+) on day												Maximum positive (%)	P[a]
	3	4	5	6	7	8	9	10	11	12	13	14		
Control	0/10[b]	3/10	9/10	10/10	9/9	3/3	2/2	0/0					100	
IUdR, day 0-12	0/10	0/10	0/10	5/10	6/10	5/10	3/9	0/8	0/7	0/6	0/6	0/6	60	<0.05
IUdR, day 1-12	0/10	0/10	0/10	1/10	1/10	2/10	0/9	0/7	0/7	0/6	0/6	0/6	20	<0.001
IUdR, day 3-12	0/10	1/10	6/10	8/10	5/7	5/5	2/2	2/2	1/1	1/1	0/0		100	

[a] Probability (Fisher's Exact Test) that the decreased number of mice with severe lesions was due to chance.

[b] Number of mice with severe lesions per number of survivors.

prevented in 90 to 100% of the treated animals. No protection was seen when the administration of the compound was spaced at 48-h intervals. Sloan et al. (10) performed three daily subcutaneous (s.c.) injections of ara-A with a total amount of 3,000 mg/kg or s.c. injections twice daily for four 2-day periods with intervals of 3 days between each period (total amount 4,000/mg/kg). Effects were equally good with both schedules, and that experiment suggests that our once-every-second-day treatment schedule failed not because of spaced administration of the drug, but because of an insufficient total amount of the drug (1,800 mg/kg given). By the 7th day Sloan et al. had given a total dose of 2,000 mg/kg, whereas we had only given a total dose of 1,200 mg/kg. In another study (5), two daily intradermal (i.d.) injections of 125 mg/kg for 14 days resulted in good protection. One has to conclude that the route of administration (i.p., i.d., or s.c.) and the treatment schedules influence treatment results less than the total amount of ara-A administered. It must, however, be noted that treatment is more effective when extended over a period of several days, because it has been shown that when a total amount of 3,000 mg/kg was given within the first 2 days after infection, no therapeutic effect was obtained (10). In our experiments, the best results were obtained with a total dose of 3,600 mg administered over a period of 6 successive days.

The use of the HSV-hairless mouse model for the evaluation of IUdR as an antiviral agent showed that a total dose of 1,000 mg of IUdR reduced the severity of skin lesions and the incidence of paralysis and death, and also increased the mean survival time of infected animals. The same favorable effect seen with ara-A when the initiation treatment was delayed for 2 days after inoculation was also observed with IUdR. Mice treated on the 3rd day after the herpetic lesions had developed did not benefit from IUdR treatment.

Lack of protection and enhancement of the severity of HSV infection by aza-U treatment was manifested by a decrease in the mean survival time in the aza-U-treated mice as compared with that of the HSV-infected untreated controls. The potentiation of HSV infection by aza-U may be related to the observation (5) that HSV-infected mice treated with ara-C similarly exhibited a 100% mortality as compared with the 60% death rate in the infected, untreated control group ($P < 0.05$). We attribute these effects to the toxicity of these compounds, which may influence the course of infection by diminishing the natural resistance of the treated mice. Drug toxicity also might explain the few deaths observed after ara-A and IUdR treatments in HSV-infected mice, deaths not necessarily attributable only to the antiviral activity failure of these compounds.

The better antiviral effect achieved when treatment with either ara-A or IUdR was delayed for 24 or 48 h after infection could have been caused by the toxicity of these compounds, by the stress produced by the physical handling of the animals, or by the injection itself, which could have promoted the spread of the infection.

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