ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1977, p. 577-581

Vol. 12, No. 5
Printed in U.S.A.

Latent Herpes Simplex Virus Infections in Sensory Ganglia of Mice After Topical Treatment with Adenine Arabinoside and Adenine Arabinoside Monophosphate†

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Received for publication 22 June 1977

Adenine arabinoside (Ara-A) and Ara-A monosphosphate (Ara-AMP) ointments were able to prevent the fatal outcome of herpes simplex virus (HSV)-induced skin infection of the lumbosacral area in hairless mice. Ara-A and Ara-AMP had no irritating effect on the skin, but in a number of animals a protracted healing time of the skin lesions after the treatment was observed. The compounds conferred only a partial protection against the establishment of latent HSV infection in the spinal root ganglia of the treated animals. The immune response, as judged from levels of HSV-specific neutralizing serum antibody titers, was not impaired by the antiviral treatment.

Since the original demonstration of the in vitro inhibitory effect of adenine arabinoside (Vidarabine; Ara-A) against herpes simplex virus (HSV) has been described (10), a large number of studies have confirmed the in vivo effectiveness of the compound, both in experimental animal models and in certain clinical forms of HSV infections in humans (see 9). Ara-A seems to possess an advantage over other antiviral drugs, such as 5-iodo-2'-deoxyuridine and phosphonoacetic acid (PAA), in that no drug-resistant HSV mutants seem to emerge (4). In addition, Ara-A is effective in the treatments of 5-iodo-2'-deoxyuridine-resistant (8) and PAA-resistant (5) HSV infections in rabbits and mice, and Ara-A monosphosphate (Ara-AMP) has been shown to be effective in the treatment of 5-iodo-2'-deoxyuridine-resistant HSV-induced keratitis in rabbits (2).

The effectiveness of any chemotherapeutic agent active against HSV cannot be judged solely by the increase in survival rate or by a decrease in the intensity of lesions observed in experimental infections. The ability of a compound to prevent the establishment of latent HSV infections in the sensory ganglia of animals surviving the primary infection has to be taken into consideration as well. It has been shown (7, 13) that PAA ointments can greatly reduce, or suppress completely, the initiation of latent ganglionic infection in mice after cutaneous inoculation of HSV.

† Publication no. 30 from the Cooperative Antiviral Testing Group of the Antiviral Substances Program, Infectious Disease Branch, National Institute of Allergy and Infectious Diseases.

In the present paper, we have evaluated the ability of topically applied Ara-A and Ara-AMP ointments to prevent the development of skin lesions, spread to the central nervous system, and death, as well as the establishment of latent ganglionic infections in the surviving animals after percutaneous inoculation of HSV type 1 in the lumbosacral area of hairless mice. The immune response of the animals after antiviral treatment was likewise investigated.

MATERIALS AND METHODS

The type 1 HSV strain, the human foreskin cells, and the hairless mice utilized, as well as procedures for the infection of mice and scorings of lesions, were identical to those described in an earlier publication (6). The techniques for neutralizing antibody assay, detection of latent HSV infection in spinal root ganglia, and identification of virus isolates have been outlined in a previous report (7). Details of methods and techniques are given in footnotes to tables.

Chemicals. Ara-A (Vidarabine) as a 10% aqueous gel and Ara-AMP powder were kindly provided by Parke, Davis & Co., Ann Arbor, Mich. The 5 and 10% Ara-AMP ointment and placebo ointments in a petrolatum base were prepared in the facilities of the New York University Medical Center.

RESULTS

Effect of Ara-A and Ara-AMP on the evolution of HSV skin infection in hairless mice. The 10% Ara-A ointment provided good protection against the fatal outcome of the HSV skin infections (Table 1). The overall mortality rate was only 8.6%, as compared to 84% observed in placebo- or nontreated mice. However, the compound did not suppress the development of viral

TABLE 1. *Effect of Ara-A and Ara-AMP on HSV-induced skin infection in hairless mice*

Expt	Treatment[a]	Maximum lesion score[b]	Mortality rate[c]	Mean survival time (days)[d]
1	Placebo ointment	3.80	9/10	7.40
	Ara-A, 10%	2.90	0/10[e]	14.00[e]
	Ara-AMP, 10%	3.55	6/10	10.50
2	None	2.95	6/10	11.90
	Ara-A, 10%	1.80	0/10[e]	14.00[e]
	Ara-AMP, 10%	2.70	0/10[e]	14.00[e]
3	None	3.48	27/30	8.80
	Ara-A, 10%	2.80	3/15[e]	13.70[e]
	Ara-A, 10%[f]	2.90	5/16[e]	13.60[e]
	Ara-AMP, 5%	3.57	13/15	10.30
	Ara-AMP, 5%[f]	3.37	11/15	9.50

[a] Treatment was initiated 3 h after virus inoculation (except when otherwise indicated) and was applied four times daily over a period of 5 days. The same inoculum used throughout the experiments consisted of a virus preparation containing 5×10^5 plaque-forming units/ml.

[b] Graded on a scale from 0 to 4. Figures represent means of the highest score of individual mice in each group.

[c] Numerator = number of dead mice, denominator = number of inoculated mice.

[d] Over an observation period of 14 days.

[e] Statistically significant; Fisher's exact test for mortality rates and Student's *t* test for mean survival time.

[f] Treatment initiated 24 h after virus inoculation.

skin lesions. Their intensity was lower than in untreated mice, but in about 25% of the animals the healing of the lesions was somewhat protracted (Fig. 1). When the initiation of the treatment was delayed until 24 h after the infection, the survival rate, although lower, was not significantly different from the group in which treatment was initiated 3 h after infection (experiment 3, Table 1).

Ara-AMP, as a 10% ointment, displayed somewhat irregular results (experiments 1 and 2, Table 1). The overall mortality rate was 30%, compared to 75% in placebo- or nontreated groups of mice (experiments 1 and 2, Table 1). The difference is statistically significant ($P < 0.01$). A delay in the healing process of the lesions after Ara-AMP treatment was likewise observed (Fig. 1). The 5% Ara-AMP treatment initiated at 3 or 24 h was without any effect (Experiment 3, Table 1).

Latent HSV infections in spinal root ganglia after antiviral treatment. The frequency of latent infections in placebo- or nontreated mice was 62% (Table 2). Treatments with either Ara-A or Ara-AMP ointments initiated 3 h after infection four times daily for 5 days were able to reduce the number of detectable latent infections (43 and 33%, respectively); however, the decreases induced by Ara-A or Ara-AMP were statistically not significant.

The time elapsed from the initiation of ganglia cultures and the appearance of virus-induced cytopathic effect is shown in Table 3. In placebo-treated or untreated mice, only 17% of the positive cultures were detected between days 11 and 30 of cocultivation, whereas in Ara-A-treated animals the frequency of late cytopathic effect was 60%. This difference is statistically significant ($P < 0.01$). Where the Ara-A treatment was delayed until 24 h after infection, the frequency of positive ganglia cultures detected between days 11 and 30 of cocultiva-

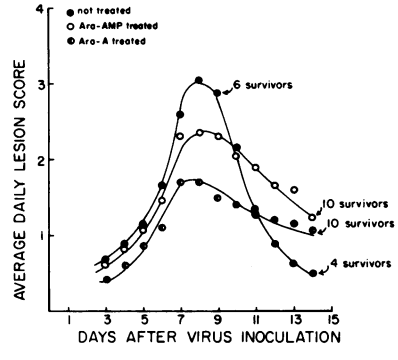

FIG. 1. *Evolution of skin lesions in HSV-infected mice treated with 10% Ara-A (◐), 10% Ara-AMP (○), or placebo (●) ointments. The treatment initiated 3 h after infection was applied on the site of virus inoculation four times daily, over a period of 5 days. Each point represents the average daily lesion score of the surviving mice in each group graded on a scale from 0 to 4.*

tion was not significantly different from that observed in nontreatment animals (67 versus 83%).

Immune response after antiviral treatment. Surviving groups of Ara-A- and Ara-AMP-treated mice reinoculated with HSV 1 month after the primary infection did not develop any skin lesions. The specific HSV serum antibody response was found to be similar in nontreated or placebo-treated and in Ara-A- or Ara-AMP-treated groups of mice (Table 4). Antibody titers increased up to 2 months after the primary infection, with a slight decline observed after 3 months in all groups. Reinoculation of mice stimulated a secondary rise in antibody response, with about a fourfold increase in titer 1 month after challenge. There seems to be no relationship between antibody titer and the presence of latent ganglionic infection. Titers around 1:1,000 were detected in both mice with (seven animals) and mice without latent infection (eight animals). Likewise, titers around 1:200 were observed in two mice with latent infection and five mice in which no virus was de-

TABLE 2. *Latent HSV infection in spinal ganglia of hairless mice after topical treatment with Ara-A and Ara-AMP*

Treatment[a]	Latent infection[b]	P[c]
None or placebo	18/29 (62%)[d]	
Vidarabine, 10%, 3 h postinfection	10/23 (43%)	>0.10
Vidarabine, 10%, 24 h postinfection	6/11 (54%)	>0.50
ARA-AMP, 10%, 3 h postinfection	3/10 (33%)	>0.10
Ara-AMP, 5%, 24 h postinfection	2/3 (67%)	>0.50

[a] As described in Table 1.

[b] Detected by cocultivation of spinal root ganglia fragments in a human foreskin fibroblast cell line (FS-7). Numerator = number of positive cultures, denominator = number of mice tested.

[c] Probability that decreased HSV isolation rate from cocultivated ganglia is due to change (chi-square test).

[d] Number includes placebo- and nontreated mice from experiments described in Table 1 and from other experiments previously performed under identical conditions.

TABLE 3. *Latent stage of HSV in cocultivated ganglia from mice treated with antiviral agents*

Treatment of mice	No. of positive cultures detected at:		
	5–10 days[b]	11–20 days[b]	21–30 days[b]
None or placebo	15	3	0
Ara-A, 10%, days 0–5	4	5	1
Ara-A, 10%, days 1–5	4	2	0
Ara-AMP, 10%, days 0–5	2	1	0
Ara-AMP, 5%, days 1–5	2	0	0
Total (%)	27 (69)	11 (28)	1 (3)

[a] Ganglia fragments were seeded on a human foreskin cell line (FS-7) and observed for 30 days. During this interval the medium was changed once. All virus isolates were identified as HSV type 1 by neutralization with type-specific rabbit sera.

[b] Time elapsed from initiation of culture to appearance of cytopathic effect.

TABLE 4. *HSV-specific serum antibody titers in hairless mice after topical treatment with Ara-A and Ara-AMP*[a]

Treatment	Titer (log_{10} units) at:				
	Month after primary infection			Month after challenge infection	
	1	2	3	1	>3
None or placebo	2.15 ± 0.23[b]	2.84 ± 0.14	2.76 ± 0.14	NT[c]	NT
Vidarabine, 10%, days 0–5	2.43 ± 0.38	3.02 ± 0.43	2.54 ± 0.23	3.16 ± 0.54	2.50 ± 0.12
Vidarabine, 10%, days 1–5	NT	2.80 ± 0.28	2.37 ± 0.12	NT	NT
Ara-AMP, 10%, days 0–5	3.03 ± 0.20	2.96 ± 0.50	2.87 ± 0.12	3.58 ± 0.57	NT

[a] Twofold dilutions of individual sera were mixed with equal volumes of 100 to 200 plaque-forming units of HSV type 1 and incubated for 30 min at 37°C in a water bath. Human foreskin fibroblasts were grown in MicroTest II plastic trays (Falcon Plastics). The growth medium was removed, and duplicate wells were inoculated with 0.2 ml of serum-virus mixture. The highest serum dilutions that protected 50% of the wells from the virus-induced cytopathic effect was taken as the serum antibody titer. Numbers represent the averages of titers from five to eight individual mice ± standard deviation.

[b] Tested after 2 weeks.

[c] NT, Not tested.

tected in the ganglia (Table 5). The difference is statistically not significant.

DISCUSSION

Our data show that Ara-A and Ara-AMP ointments are able to prevent the fatal outcome of HSV-induced skin infection in hairless mice. However, in contradistinction to PAA (7, 13), the compounds confer only a partial protection against the establishment of latent HSV infections in the spinal root ganglia of the animals and are not able to prevent the development of skin lesions. Unlike PAA (7), Ara-A and Ara-AMP have no irritating effect on the skin, but in a number of animals a protracted healing time of the skin lesions after the treatment was observed.

The increased mortality observed among Ara-AMP-treated mice in one experiment (Table 1, experiment 1) might be related to the fact the petrolatum-base ointments have a somewhat poorer adherence to the mouse skin than aqueous gels. In subsequent experiments, care was taken to apply the ointment to the skin very firmly to insure its adherence.

Since Ara-A's are potent in vitro virus inhibitors, their inability to prevent the establishment of HSV after primary infection might be due to poor adsorption of the compounds. However, virus migration towards the nerve endings has to be somewhat restricted, since the compounds are able to prevent the fatal outcome of the primary infection. As the antibody response of Ara-A and Ara-AMP is similar to that observed in placebo- or nontreated mice, the drugs might also have retarding effect upon virus penetration into the nerve endings. The pathogenesis of HSV skin infection in the lumbosacral area during antiviral treatment should provide certain clarifications of these aspects. Experiments along these lines are in progess in our laboratory.

Experiments with PAA treatment have shown that prevention of the establishment of latent infection is associated with low or undetectable levels of neutralizing antibodies in the mouse serum (7), indicating an early cessation of virus multiplication and thus a poor antigenic stimulation.

It has been shown in vitro assays (11), in rabbits (Z. S. Zam, Y. M. Centifanto, and H. E. Kaufman, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. no. 139, 1974) and humans (12), that Ara-A does not impair the cell-mediated immune response. Our results show that the humoral immune response, as expressed by neutralizing antibody titers, is likewise not affected. Results in humans (12), using a complement fixation technique, are similar, although the method appears less sensitive in revealing differences in antibody titers.

Studies in humans have shown that topical Ara-A treatment did not modify the course of recurrent herpes progenitalis (1). Although the pathogenic mechanisms of primary and recurrent HSV infections are different, it appears that topical Ara-A treatment of HSV lesions of the skin cannot prevent or eradicate the latent infection of the sensory ganglia. This has been confirmed (Klein and Friedman-Kien, manuscript in preparation) in a facial HSV infection model in hairless mice in which the rate of latent infection detected in the trigeminal ganglia of Ara-A- and Ara-AMP-treated mice was between 70 and 90%, very close to the 100% of the nontreated survivors. Similar findings were observed in a genital infection, HSV type 2 infection, in mice (3). A 5% PAA ointment was able to both reduce the rate of the virus replication in the genital tract and prevent the fatal outcome of the infection, whereas 10% Ara-A or Ara-AMP ointments were unable to affect virus multiplication in the genital tract or to protect the HSV-infected mice from death.

TABLE 5. *Serum HSV antibody titers and latent virus in the spinal ganglia of Ara-A- and Ara-AMP-treated mice*

Serum HSV antibody titer	Spinal ganglia cultures	
	Negative	Positive (%)
≤2.30	5	2 (29)[a]
2.35–2.90	13	12 (48)
≥2.95	8	7 (47)

[a] Numbers in parentheses are percentage rates of positive ganglia cultures.

ACKNOWLEDGMENTS

The excellent technical assistance of Eileen Brady is gratefully acknowledged.

This study was supported by Public Health Service contract N01-02131 from the Infectious Disease Branch, National Institute of Allergy and Infectious Diseases.

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