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Viral Keratitis-Inhibitory Effect of 9-β-D-Arabinofuranosylhypoxanthine 5'-Monophosphate

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Topical application of 9-β-D-arabinofuranosylhypoxanthine 5'-monophosphate (ara-HxMP) significantly inhibited the development of keratitis induced by types 1 and 2 herpes simplex virus and vaccinia virus in the eyes of rabbits. Parameters for evaluation of efficacy were infectivity (corneal opacity, lesion size, and type), Draize (erythema, conjunctival swelling, and discharge), and reduction in titer of recoverable virus from the eye. When the relative efficacy of the related compounds 9-β-D-arabinofuranosyladenine (ara-A), ara-A 5'-monophosphate (ara-AMP), and ara-Hx was determined against type 1 herpes simplex virus in a parallel experiment, the more water-soluble compounds (ara-HxMP, ara-AMP) were the most effective. The relative efficacy of ara-A was also determined against type 2 herpes and vaccinia virus-induced keratitis. Mortality in rabbits due to central nervous system involvement caused by types 1 and 2 herpes simplex virus was inhibited. Ara-HxMP was not discernibly toxic to the eye at concentrations of at least 20%; efficacy was still discernible with a 0.1% solution.

Treatment of superficial herpes simplex and vaccinial infections is currently accomplished with a relative degree of success by using 5-iodo-2'-deoxyuridine (7, 10, 19), although this drug is somewhat limited in its usefulness due to viral resistance that may develop to it, and on occasion because of lack of human tolerance for the drug (5, 6, 11, 19). There is also some doubt regarding the drug's capability to effectively treat herpes uveitis or deep stromal herpes (6). Among the alternative antiviral drugs for treating these viral eye infections, 9-β-D-arabinofuranosyladenine (ara-A) has attracted considerable attention, and clinical studies on the efficacy of this purine nucleoside have been reported (3, 4, 11). As discussed in previous reports (9, 14, 15, 18), we have synthesized a number of compounds related to ara-A in an effort to develop an improved antiviral drug with properties of antiviral efficacy combined with increased solubility, increased metabolic stability, and reduced toxicity. Of those we have studied to date, 9-β-D-arabinofuranosylhypoxanthine 5'-monophosphate (ara-HxMP; ICN 3952) has been among the most attractive studied in vitro (J. H. Huffman, L. B. Allen, R. L. Tolman, G. L. Revankar, L. N. Simon, R. L. Robins, and R. W. Sidwell, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 14th, San Francisco, Calif., Abstr. 233, 1974), and consequently this drug has been subjected to in vivo experimental studies to further ascertain its antiviral usefulness (1, 2). The efficacy of this drug in keratitis infections induced by types 1 and 2 herpesviruses (HSV/1, HSV/2) and by vaccinia virus (VV) is described in the present report.

MATERIALS AND METHODS

Drug preparation. The ara-HxMP used in these studies was synthesized as the free acid form at this Institute as previously reported (14). The compound was dissolved in 1.4% polyvinyl alcohol, using $NaHCO_3$ to bring the solution to a pH of ~6.5 or used in Lacri-Lube ophthalmic ointment (Allergan Pharmaceuticals, Irvine, Calif.) for the present experiments. Ara-A was obtained from ICN Life Sciences, Cleveland, Ohio. The 5'-monophosphate of ara-A (ara-AMP), and 9-β-D-arabinofuranosylhypoxanthine (ara-Hx), also used in these studies, were synthesized at this Institute.

Viruses. The McKrae and 123 strains of HSV/1, MS strain of HSV/2, and WR strain of VV were used. These viruses have been previously described (16). Approximately 10 50% eye infective doses of each virus were used in the study. This was ~10^7 cell culture 50% infective doses ($CCID_{50}$) for HSV/1, 10^5 $CCID_{50}$ for HSV/2, and 10^6 CCID for VV.

Animals. New Zealand albino female 1- to 2-kg rabbits (Curd's Caviary, Los Angeles, Calif.) were used in all experiments.

Experimental design. The corneal epithelium of

each eye was anesthetized with 0.5% proparacaine HCl (Allergan) and uniformly scratched three times horizontally and vertically, using an inoculating needle. Two drops of a suspension containing virus in minimum essential medium with Earle balanced salt solution, 0.1% $NaHCO_3$, and 1% sorbitol was then added to each eye. The eyelids were held closed and massaged lightly for 1 min. Each compound in polyvinyl alcohol was added to the eyes hourly from 8 a.m. to 7 p.m. daily for 7 to 8 days beginning 24 h after virus inoculation. Drug in ophthalmic ointment was applied at 8 p.m. each day of treatment. Virus control eyes received polyvinyl alcohol only hourly and ophthalmic ointment without drugs at the end of the day. Because of the heavy initial inoculum on scarified corneas, discrete punctate lesions were visible by initiation of treatment. The scratches particularly exhibited infection as seen under fluorescein staining, and considerable erythema was manifested in the conjunctiva. Four rabbits (eight eyes) were used for each drug level and for virus controls. The eyes were examined and scored daily or every other day both grossly and, after fluorescein staining, by slit lamp biomicroscope for infectivity and for Draize response as previously described (15, 16). In this method, scores of 0 (uninfected) to 4 (maximal severity) were assigned to each reading of lesion size and corneal opacity, and the total of the two scores was multiplied by 10. Similar scores were assigned for erythema, chemosis, and discharge, and the total of these three Draize scores was multiplied by 2. The combined, weighted infectivity and Draize scores for each treatment group were plotted against time. Rabbits whose eyes were anesthetized and scratched but exposed to sterile minimum essential medium only were similarly treated with ara-HxMP in parallel in each experiment as toxicity controls.

As an added parameter for measuring antiviral efficacy, the concentration of recoverable virus from the eyes during therapy was determined in selected experiments. To accomplish this, sterile cotton swabs were rolled over each eye on the days designated before initiation of the daily therapy. Each swab was placed in 2 ml of minimal essential medium containing 0.25% $NaHCO_3$, 5% fetal bovine serum, and 50 μg of gentamicin per ml and frozen at −70 C. When all samples were collected, each was thawed and the supernatants were titered in BHK-21 cells. Presence of virus was determined by cytopathic effect formation as seen by microscopic examination of these cells incubated at 37 C for 72 h, and a $CCID_{50}$/0.2 ml of each sample was calculated.

RESULTS

Studies with HSV/1. Three experiments were run to determine the efficacy of ara-HxMP treatment on HSV/1-induced keratitis. In the first study, ara-HxMP and ara-A were compared with the McKrae strain of virus, using 0.5% concentrations of each. Marked inhibition was seen with each drug, with ara-HxMP exhibiting the more pronounced effect (Fig. 1). No toxicity to the eye was discernible. A second experiment (Fig. 2) compared the efficacy of 0.1% ara-HxMP with 0.1% ara-Hx against the same virus. A moderate inhibition was seen at this relatively low drug level. Ara-HxMP exerted the greater inhibitory effect. In the third experiment strain 123 of HSV/1 was used, which tends to be more neurotropic (17) than the McKrae strain in our hands. In this experiment, ara-HxMP, ara-Hx, ara-A, and ara-AMP were tested in parallel, using 0.2 and 0.02 M concentrations of each. For ara-HxMP, this concentration corresponded to 7 and 0.7%, respectively. The infected animals began exhibiting signs of neurological involvement by day 6 after infection, and deaths occurred by days 9 through 12 although in the 0.2 M ara-HxMP- and ara-AMP-treated rabbits no deaths occurred. Ara-HxMP and ara-AMP in both concentrations had an approximately equal effect on inhibiting the viral keratitis. Ara-A and ara-Hx had lesser activity. The results of this study are summarized in Fig. 3 and 4. Treatment with each compound at 0.2 and 0.02 M

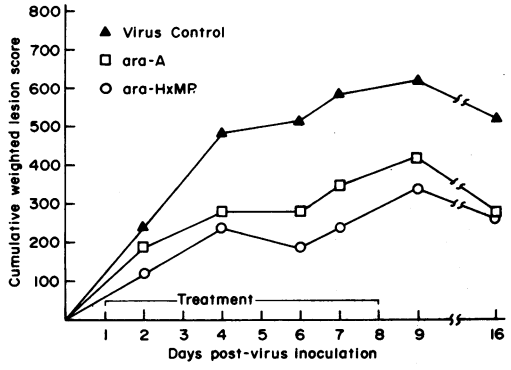

FIG. 1. *Effect of 0.5% ara-HxMP and ara-A on type 1 herpes keratitis in rabbits.*

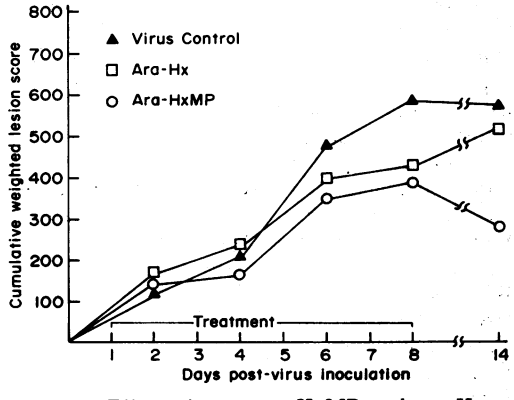

FIG. 2. *Effect of 0.1% ara-HxMP and ara-Hx on type 1 herpes keratitis in rabbits.*

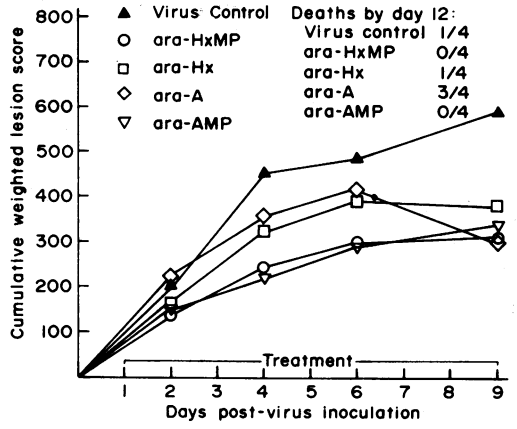

FIG. 3. *Effect of 0.2 M ara-HxMP, ara-Hx, ara-A, and ara-AMP on type 1 herpes keratitis in rabbits.*

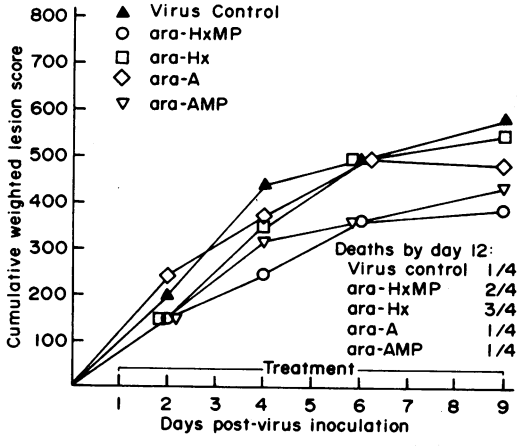

FIG. 4. *Effect of 0.02 M ara-HxMP, ara-Hx, ara-A, and ara-AMP on type 1 herpes keratitis in rabbits.*

concentrations reduced the titers of virus recovered from the eye (Table 1). Ara-HxMP and ara-AMP had the greatest relative efficacy using this parameter of evaluation.

Studies with HSV/2. Ara-HxMP at 0.2 and 0.02 M concentrations inhibited the development of keratitis in rabbit eyes induced by this neurotropic strain of HSV/2 (Fig. 5). Ara-A, utilized in the same experiment, had a slightly greater efficacy. In this study, the eye infection was manifested by corneal opacity, punctate, dendritic, and geographic lesions, conjunctival redness, and swelling; this progressed to marked signs of encephalitis in the virus control animals by day 12 of the infection. Both drugs at 0.2 M concentration prevented the occurrence of signs of central nervous system involvement. Treatment by concentrations of each drug reduced the titers of virus recoverable from the treated

TABLE 1. *Effect of topical treatment with ara-HxMP, ara-Hx, ara-A, and ara-AMP on concentration of recoverable virus from type 1 herpes virus-infected rabbit eyes*

Compound	Concn (M)	Mean virus titer on day:[a]		
		3	6	9
Virus control	—.	2.8	1.4	0.4
Ara-HxMP	0.2	2.3	0.8[b]	0.1
Ara-HxMP	0.02	1.9	1.5	0.0
Ara-Hx	0.2	2.4	0.8[b]	0.8
Ara-Hx	0.02	2.1	1.3	0.3
Ara-A	0.2	2.4	1.4	0.8
Ara-A	0.02	2.6	1.1	0.4
Ara-AMP	0.2	2.1	0.9	0.1
Ara-AMP	0.02	2.3	1.4	0.2

[a] Mean of eight eyes per group; titer expressed as $\log_{10}$ $CCID_{50}$/0.2 ml.

[b] $P < 0.05$ (Student's t test).

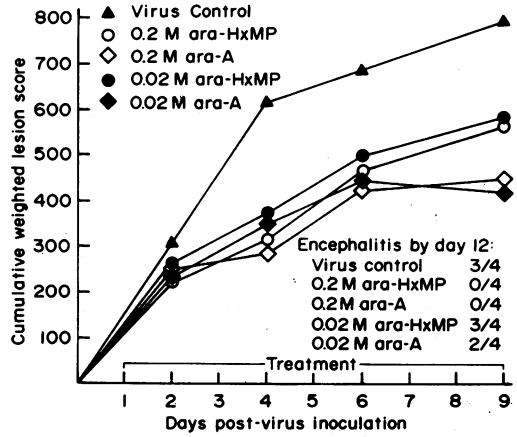

FIG. 5. *Effect of 0.2 and 0.02 M ara-HxMP and ara-A on type 2 herpes keratitis in rabbits.*

eyes on days 3, 6, and 9 (Table 2), although no statistical difference was seen.

Studies with VV. Keratitis induced in rabbits by VV was markedly inhibited by topical treatment with both ara-HxMP and ara-A (Fig. 6). Concentrations of 0.2 and 0.02 M were used. This form of keratitis was characterized by marked dendritic infections that rapidly progressed to geographic lesions covering the majority of the cornea. Draize signs of infection were also very apparent, typified by severe conjunctival inflammation, discharge, and swelling. In the drug-treated eyes, this infection was limited to barely discernible punctate lesions, a mild conjunctival erythema, and virtually no discharge or swelling of the eyelid and nictitating membrane. A pronounced iritis occurring in the virus control eyes was not appar-

TABLE 2. *Effect of topical treatment with ara-HxMP and ara-A on the concentration of recoverable virus from type 2 herpes virus-infected rabbit eyes*

Compound	Concn (M)	Mean virus titer on day:[a]		
		3	6	9
Virus control	—	1.0	0.9	0.9
Ara-HxMP	0.2	0.3	0.3	0.4
Ara-HxMP	0.02	0.5	0.1	0.1
Ara-A	0.2	0.2	0.0	0.4
Ara-A	0.02	0.8	0.1	0.1

[a] Mean of eight eyes per group; titer expressed as $\log_{10}$ $CCID_{50}$/0.2 ml.

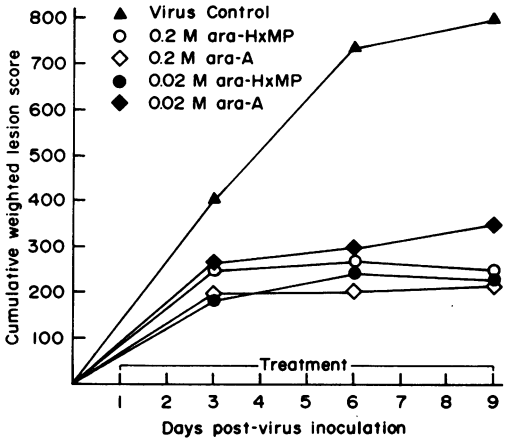

FIG. 6. *Effect of 0.2 and 0.02 M ara-HxMP and ara-A on vaccinial keratitis in rabbits.*

ent in any of the treated eyes. Marked virus titer reductions occurred as a result of therapy by both concentrations of ara-HxMP and by the 0.2 M concentration of ara-A (Table 3). The 0.02 M ara-A dosage was less efficacious on sampling days 6 and 9 in this experiment.

DISCUSSION

These data indicate that the synthetic purine nucleotide ara-HxMP has significant efficacy against experimentally induced ophthalmic infections of rabbit eyes caused by HSV/1, HSV/2, and VV. The keratitis-inhibitory effect was seen by inhibition of both the inflammatory (Draize) reaction to the viruses and the development of the virus-induced lesions, as well as reduction in titer of infectious virus recoverable from the surface of the eye. Toxicity determinations to date indicate that ara-HxMP is nontoxic to rabbit eyes when used at levels at least as high as 20%; since a moderate inhibitory effect was seen at concentrations as low as 0.1%, a therapeutic index (maximum tolerated dose

TABLE 3. *Effect of topical treatment with ara-HxMP, ara-Hx, ara-A, and ara-AMP on concentration of recoverable virus from vaccinia virus-infected rabbit eyes*

Compound	Concn (M)	Mean virus titer on day:[a]		
		3	6	9
Virus control	—	1.7	2.3	0.9
Ara-HxMP	0.2	0.1[b]	0.0[b]	0.0[b]
Ara-HxMP	0.02	0.0[b]	0.1[b]	0.0[b]
Ara-A	0.2	0.0[b]	0.0[b]	0.0[b]
Ara-A	0.02	0.1[b]	0.6	0.6

[a] Mean of eight eyes per group; titer expressed as $\log_{10}$ $CCID_{50}$/0.2 ml.

[b] $P < 0.001$ (Student's t test).

divided by the minimum inhibitory dose) of 200 was therefore obtained for the drug.

Studies run in parallel suggest that ara-HxMP is at least as effective, if not more effective, than ara-A. Increased efficacy may be due predominantly to the greater water solubility of ara-HxMP, since in vitro investigations (Huffman et al., Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif. Abstr. 233, 1974) show ara-A to be moderately more potent in antiviral effect. It is significant that in these studies the other water-soluble compound, ara-AMP, also had an increased efficacy over the less soluble drugs. Such an observation has been previously reported (15).

Studies have been reported showing that ara-A deaminates to ara-Hx in the eye (12), although in a recent report Pavan-Langston et al. showed that ara-Hx was approximately five times less potent than ara-A when used against established herpes simplex keratoconjunctivitis (13). Ara-HxMP appeared to be more potent against keratitis than was ara-Hx in our study, suggesting that it may not be exerting its antiviral action as the dephosphorylated compound.

Some comment should be made if one attempts to compare our data with those derived from the above-cited study of Pavan-Langston et al. (13). Although the McKrae strain of HSV/1 was used in each study, we induced an ocular infection by first scratching the cornea rather severely, whereas those investigators did not scarify the eye. In addition, our viral inoculum was 10-fold higher than that used by the Pavan-Langston group. As a result, the infections produced progressed at very different rates, and the degree of severity probably also differed considerably. Hence, that group could initiate treatment at a later period of time and

get an apparently greater degree of efficacy. We have utilized the more severe infection in order to obtain greater uniformity in all animals infected.

The central nervous system involvement seen in these studies probably occurs as a result of the virus progressing through the cornea, and thence up the trigeminal ganglia to the brain. In the cases where treated rabbits did not develop this encephalitis, it is presumed that the drug inhibited the viral infection from progressing through the cornea. Such a supposition correlates with the visual examination in which the corneal infection was markedly subdued.

The relatively high therapeutic index of ara-HxMP combined with its water solubility and ability to control viral infections of the brain (1) suggests the practicality of using the drug against deoxyribonucleic acid virus-induced eye diseases in man. Still to be determined are the drug's efficacy against established deep-seated virus infections using subconjunctival injections.

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