

## Effect of 9-(2-Hydroxyethoxymethyl)guanine on Herpesvirus-Induced Keratitis and Iritis in Rabbits

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Drugs used for the inhibition of DNA viruses, such as iododeoxyuridine, adenine arabinoside, or trifluorothymidine, are not biochemically selective in their action and also interfere with normal cellular functions. The recently reported 9-(2-hydroxyethoxymethyl)guanine (acycloguanosine) is selectively phosphorylated by viral thymidine kinase but not by normal cellular thymidine kinase. Our present studies show that the acycloguanosine is as effective in treating herpetic keratitis in the rabbit as iododeoxyuridine and trifluorothymidine when given topically as an ointment. It is also effective when given intravenously for the treatment of herpetic iritis and is effective in preventing death from encephalitis in rabbits.

It has been possible to treat a DNA virus infection topically since the discovery of the effect of iododeoxyuridine (IDU) (4), but some toxicity is associated with all drugs. When used topically in the eye, IDU has some toxicity, causing occasional corneal epithelial damage and retarding stromal wound healing. The topical use of adenine arabinoside (Ara-A) in humans has approximately the same toxicity and potency as IDU (6). A more recently reported drug, trifluorothymidine (TFT) (5), is the drug of choice for the treatment of corneal epithelial herpes because of its greater potency and comparable toxicity; however, it, like the others, is not completely selective in inhibiting virus DNA synthesis without inhibiting cellular events.

The search for a systemically active antiviral drug has yielded Ara-A. This drug is promising because it does not inhibit the bone marrow and does not inhibit the host immune system to any great degree. Ara-A was first shown to be active systemically in treating deep virus disease of the eye in a controlled double-blind study (1) and then in treating herpes encephalitis (9). Even with this drug, however, interference with host DNA occurs, and the drug is toxic and not totally selective. A truly selective drug is still needed.

There have been many approaches to the search for biochemical selectivity in the treatment of virus disease with DNA synthesis inhibitors. Greer and co-workers (3) as well as our group used the fact that normal cells cannot phosphorylate a bromodeoxycytidine compound without deamination, whereas herpes simplex virus can, to give some degree of selectivity. The

potency of this system, however, did not seem sufficient for a really promising drug.

Several drugs have been developed that are selectively phosphorylated by viral thymidine kinase but not by normal cellular thymidine kinase. In the past, most of these drugs did not appear to us sufficiently potent to be clinically promising. More recently, acycloguanosine [9-(2-hydroxyethoxymethyl)guanine] was described (2, 8) and appeared more promising. This report describes more detailed studies on this compound to evaluate its topical and systemic activity.

### MATERIALS AND METHODS

**Keratitis studies.** (i) **Topical treatment.** New Zealand white rabbits weighing 1 to 2 kg were infected in both eyes by lightly traumatizing the epithelium, instilling 2 to 3 drops of McKrae herpesvirus suspension into the cul-de-sac, and lightly rubbing the lids over the cornea. Three days later, the eyes were checked for the presence of ulcers by using the slit lamp. Ulcers were graded after fluorescein staining on a basis of 0 to 4, where zero is a normal cornea, 1 is ulcers involving 1/4 of the cornea, and 2 is ulcers involving 1/2 of the cornea, up to 4, which is total corneal ulceration.

Treatment was assigned on a random coded basis, and all evaluations were done on a blind basis. Treatment was given five times a day for 5 days; both eyes of an animal were treated with the same medication.

(ii) **Systemic treatment.** Sixty-three New Zealand white rabbits weighing 1 to 3 kg were infected in the same manner as those receiving topical treatment. Three days after infection, eyes were checked for ulcers, and the severity of keratitis was graded.

Treatment was assigned on a random basis, and all

evaluations were done on a blind basis. Half of the animals served as untreated controls; the other half were treated intravenously twice daily for 6 days with 50 mg of the sodium salt of acycloguanosine per kg. The severity of keratitis was graded on treatment days 1 through 6, and all animals were checked daily for 1 month after the start of treatment for the effect of acycloguanosine on the animals' survival of the viremia which results after ocular infection.

**Iritis study.** New Zealand white rabbits weighing 1 to 2 kg were injected in the anterior chamber of both eyes with 0.1 ml of McKrae herpesvirus. The day after virus injection, treatment was started, and the rabbits were randomly allocated to one of three groups. One group was the untreated control group; one group was treated intravenously with 50 mg of the sodium salt of acycloguanosine per kg twice a day for 8 days; the third group was treated in one eye with 10 mg of the sodium salt of acycloguanosine twice a day by subconjunctival injection for 4 days.

All evaluations were done on a blind basis, and the iritis was graded on a 0 to 4 scale as follows: 1, mild iris hyperemia, clear cornea; 2, moderate iris hyperemia, clear cornea; 3, severe iris hyperemia, stromal haze; 4, severe iris hyperemia, moderate stromal haze, hypopyon, or hyphema.

**Statistical analyses.** Statistical analyses of the clinical grades of ulcers or iritis were done using the nonparametric Kruskal-Wallis analysis. This was done by Y. C. Patel of the Department of Biometry of LSU Medical Center.

**Drugs.** For topical treatments, commercially available IDU (Stoxil; Smith, Kline & French Laboratories, Philadelphia, Pa.) and Ara-A (Vira-A; Warner Lambert/Parke-Davis Pharmaceutical Research Laboratories, Detroit, Mich.) ointments were used. A 10% ointment of 5-iodo-5'-amino-2',5'-dideoxyuridine (AIU) was prepared in a white petrolatum base, the same as used in the other ointments. The acycloguanosine, TFT, and placebo ointments and the sodium salt of the acycloguanosine were supplied by Burroughs Wellcome Co., Research Triangle Park, N.C.

**Virus employed.** The McKrae herpesvirus was isolated on primary rabbit kidney cells from a patient with recurrent herpetic keratitis. It has been passaged a total of 14 times in either primary human embryonic kidney or HEp-2 cells. For keratitis studies, five times the minimal infective dose in the rabbit eye was used. For the iritis studies, the virus was diluted to produce an iritis which became clinically apparent the day after injection and which lasted approximately 14 days. The titer of the McKrae virus used was  $1.6 \times 10^7$  plaque-forming units per ml; for the Hicks virus it was  $3.8 \times 10^7$  per ml. The Hicks virus is a type II virus obtained from A. Nahmias.

**In vitro assay.** A dose-response curve was determined using monolayers of Vero cells in 4-ounce (ca. 0.12-liter) plastic bottles. The virus, in a 0.2-ml volume, was placed over the monolayer and allowed to adsorb at 37°C for 45 min. One hundred twenty-five plaque-forming units of the McKrae virus or 149 plaque-forming units of the Hicks virus was added to each bottle. After the adsorption was completed, both drug and complete media (containing basal medium with Earle salts, 2% fetal calf serum, glutamine, sodium

bicarbonate, and antibiotics) were added. Methyl cellulose or agar overlays were not used because of the possibility that they might interfere with the diffusion of the drug to the monolayer. At 48 h, when the control exhibited discrete plaques, all the cultures were fixed and stained with crystal violet, the plaques were counted in an illuminator, and the percent inhibition was determined. The 50% tissue culture effective dose represents the concentration of drug that inhibited 50% of the plaques.

Two different viruses were used, the McKrae strain (a clinical isolate) and the Hicks strain (type II from A. Nahmias). The AIU did not go into solution at 37°C for several hours, but the supernatant from the AIU suspension was plaqued at 20, 15, 10, 5, and 2 µg/ml.

## RESULTS

In a comparison of 3% acycloguanosine, 3% Ara-A, 0.5% IDU, and placebo ointments, it was found that the acycloguanosine- and IDU-treated animal eyes had statistically significantly less severe keratitis on treatment days 4 and 5 than did the placebo- or Ara-A-treated groups. There was no difference between the IDU- and the acycloguanosine-treated eyes (Table 1).

When the acycloguanosine, IDU, TFT, AIU, and placebo ointments were compared (the grading was done early in the course of treatment to determine whether any difference would occur early), it was found that the acycloguanosine, IDU, and TFT were effective and similar. All promoted healing of the keratitis and were statistically significantly better than the placebo and the AIU treatments (Table 1).

The intravenous treatment of herpetic keratitis with the sodium salt of acycloguanosine allowed significant healing of corneal ulcers on all days that the eyes were examined (Table 2). Treatment with the acycloguanosine also had a marked effect on the death rate. At day 9 after infection, untreated control animals began to die of encephalitis (Fig. 1). At 30 days after the start of treatment, 23 of the 32 untreated controls had died, whereas only 8 of the 31 treated animals had died.

The intravenous and subconjunctival treatment of herpetic iritis with the sodium salt of acycloguanosine produced a significant lessening of the iritis on treatment days 3 and 4 (Table 3). By a nonparametric "sign test" there was no difference between the eyes of control rabbits and the untreated eyes of rabbits whose other eye received subconjunctival acycloguanosine. The subconjunctival treatment could only be given for 4 days because of the irritation, but the intravenous treatment did not appear toxic, and treated animals gained weight at the same rate as the untreated controls.

The *in vitro* testing showed that the 50% effective dose for acycloguanosine was 0.3 µg/ml

TABLE 1. Topical treatment of experimental herpetic keratitis in rabbits

Treatment ointment <sup>a</sup>	No. of eyes	Severity of keratitis <sup>b</sup> on treatment day:				
		1	2	3	4	5
3% acycloguanosine	28				0.20 <sup>c,d</sup>	0.0 <sup>c,d</sup>
	20	0.42	0.38 <sup>d,e</sup>	0.24 <sup>d</sup>	0.17 <sup>d</sup>	0.10 <sup>d,e</sup>
0.5% IDU	26				0.20 <sup>c,d</sup>	0.18 <sup>c,d</sup>
	20	0.42	0.49	0.31 <sup>d</sup>	0.15 <sup>d,e</sup>	0.11 <sup>d</sup>
3% Ara-A	28				1.63	1.53
10% AIU	20	0.47	1.44	1.80	1.92	1.95
3% TFT	20	0.38	0.25 <sup>d,e</sup>	0.14 <sup>d,e</sup>	0.05 <sup>d,e</sup>	0.14 <sup>d</sup>
Placebo	40				2.39	2.29
	32	0.48	1.50	2.26	2.12	2.42

<sup>a</sup> Treatments were given five times a day for 5 days. Both eyes of an animal received the same treatment.

<sup>b</sup> Keratitis was graded on a basis of 0 to 4: 0, normal cornea; 4, completely ulcerated cornea.

<sup>c</sup> Significantly better than Ara-A at the 1% level.

<sup>d</sup> Significantly better than placebo at the 1% level.

<sup>e</sup> Significantly better than AIU at the 1% level.

TABLE 2. Systemic treatment of experimental herpetic keratitis in rabbits

Treatment <sup>a</sup>	No. of rabbits	Severity of keratitis <sup>b</sup> on treatment day:					
		1	2	3	4	5	6
Acycloguanosine	31	1.25	1.14	1.02	0.78	0.86	0.63
Untreated	32	1.35	1.53	1.51	1.51	1.54	1.23

<sup>a</sup> Treatment, started 3 days after infection, was 50 mg of acycloguanosine intravenously per kg, twice daily for days 3 to 9.

<sup>b</sup> Keratitis was graded on a basis of 0 to 4: 0, normal cornea; 4, completely ulcerated cornea.

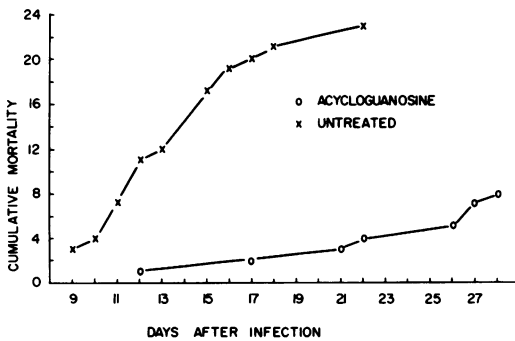


FIG. 1. New Zealand white rabbits infected in both eyes with McKrae herpesvirus were treated intravenously twice daily with 50 mg of acycloguanosine per kg of body weight from days 3 to 8. The untreated control animals began to show signs of encephalitis and to die 9 days after infection. At 30 days after infection 23 of the 32 untreated controls had died, whereas only 8 of the 31 treated animals had died.

for McKrae herpes and 0.8  $\mu\text{g}/\text{ml}$  for the type II Hicks virus. The 50% tissue culture effective dose of IDU was 1.2  $\mu\text{g}/\text{ml}$  for McKrae herpes and 3.6  $\mu\text{g}/\text{ml}$  for the type II virus; that of TFT was 1.2  $\mu\text{g}/\text{ml}$  for the McKrae herpes; AIU did not have any measurable activity.

## DISCUSSION

These studies compare the drug acycloguanosine with other presently used compounds in an antiviral test system that has proven to be highly predictive for humans. Quantitative analysis of the rabbit keratitis model, as done in this laboratory, has been an almost perfect predictor for the drugs IDU, bromodeoxyuridine, cytosine arabinoside, Ara-A, and TFT. In this system, acycloguanosine seems to be approximately as active as the presently used compounds, and although the differences are not significant, its activity is close to that of TFT, presently by far the most potent topical agent. This potency was demonstrated by topical administration without signs of local toxicity, suggesting that the drug may be extremely useful when used topically. It appears that AIU has a slight effect on keratitis, and it may be that this activity would be more apparent with a different strain of virus such as that used by Puliafito et al. (7).

In subconjunctival administration, acycloguanosine is extremely irritating, probably because of the high pH necessary to keep the compound in solution. In this study, subconjunctival treatment with acycloguanosine produced a clear-cut effect on herpetic iritis that was localized to the

TABLE 3. Systemic treatment of experimental herpetic iritis in rabbits

Treatment	No. of eyes	Severity of iritis <sup>a</sup> on treatment day:							
		2	3	4	6	7	8	9	10
Acycloguanosine									
Intravenous <sup>b</sup>	28	2.3	0.8 <sup>c</sup>	0.8 <sup>c</sup>	1.0	0.8	0.7	0.4	0.2
Subconjunctival <sup>d</sup>	15	2.9	0.9 <sup>c</sup>	1.0 <sup>c</sup>	1.5	1.1	0.9	0.6	0.4
Untreated fellow eye <sup>e</sup>	15	2.7	1.4	1.7	1.5	0.9	0.7	0.6	0.3
Untreated control	30	2.7	2.6	2.4	1.7	1.2	0.7	0.6	0.3

<sup>a</sup> Iritis was graded on a 0 to 4 basis: 0, normal eye; 4, severe iritis, stromal haze, hypopyon, or hypHEMA.

<sup>b</sup> Dosage of 50 mg/kg intravenously, twice daily on days 1 to 8.

<sup>c</sup> Statistically better than placebo at the 1% level.

<sup>d</sup> Dosage of 10 mg subconjunctivally, twice daily on days 1 to 4.

<sup>e</sup> Of rabbits receiving subconjunctival injection in one eye only.

treated eye and did not involve the deep infection of the opposite eye. This suggests a selective local absorption, rather than the presence of sufficiently high blood levels to give a generalized systemic effect. No toxicity to the eye itself was noted with subconjunctival administration in spite of rather severe conjunctival redness.

In systemic use, the acycloguanosine was extremely effective in treating deep ocular infection. In addition, without apparent toxicity, it also prevented death. These studies indicate that not only does the drug have the kind of safety capability which one would hope for from a drug such as this, which is poorly phosphorylated by the normal cellular thymidine kinase, but more importantly it has a very high degree of antiviral potency while retaining this great safety factor. Although many more studies are required before human use can be contemplated, present studies indicate that this is an extremely active, potent, and relatively safe antiviral compound.

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#### LITERATURE CITED

1. Abel, R., Jr., H. E. Kaufman, and J. Sugar. 1975. Intravenous adenine arabinoside against herpes simplex keratouveitis in humans. *Am. J. Ophthalmol.* 79:659-664.
2. Elion, G. B., P. A. Furman, J. A. Fyfe, P. deMiranda, L. Beauchamp, and J. Schaeffer. 1977. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. *Proc. Natl. Acad. Sci. U.S.A.* 74:5716-5720.
3. Greer, S., I. Schildkraut, T. J. Zimmerman, and H. E. Kaufman. 1975. 5-Halogenated analogs of deoxycytidine as selective inhibitors of the replication of herpes simplex viruses in cell culture and related studies of intracranial herpes simplex virus infections in mice. *Ann. N.Y. Acad. Sci.* 255:359-365.
4. Kaufman, H. E. 1962. Clinical cure of herpes simplex keratitis by 5-iodo-2'-deoxyuridine. *Proc. Soc. Exp. Biol. Med.* 109:251-252.
5. Kaufman, H. E., and C. Heidelberger. 1964. Therapeutic antiviral action of 5-trifluoromethyl-2'-deoxyuridine. *Science* 145:585-586.
6. Pavan-Langston, D., and C. H. Dohlman. 1972. A double blind clinical study of adenine arabinoside therapy of viral keratoconjunctivitis. *Am. J. Ophthalmol.* 74:81-88.
7. Puliafito, C. A., N. L. Robinson, D. M. Albert, D. Pavan-Langston, T.-S. Lin, D. C. Ward, and W. H. Prusoff. 1977. Therapy of experimental herpes simplex keratitis in rabbits with 5-iodo-5'-amino-2',5'-dideoxyuridine. *Proc. Soc. Exp. Biol. Med.* 156:92-96.
8. Schaeffer, H. J., L. Beauchamp, P. deMiranda, G. B. Elion, D. J. Bauer, and P. Collins. 1978. 9-(2-Hydroxyethoxymethyl)guanine activity against viruses of the herpes group. *Nature (London)* 272:583-585.
9. Whitley, R. J., S. J. Soong, R. Dolin, G. J. Galasso, L. T. Ch'ien, C. A. Alford, and the collaborative study group. 1977. Adenine arabinoside therapy of biopsy-proved herpes simplex encephalitis. *N. Engl. J. Med.* 297:289-294.