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Phosphonoacetic Acid in the Treatment of Experimental Herpes Simplex Keratitis

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In a rabbit model of herpes simplex corneal ulceration, 5% phosphonoacetic acid solution or ophthalmic ointment suppressed clinical disease and virus replication. The effect of 5% phosphonoacetic acid ointment was equivalent to that of 0.5% idoxuridine ointment in the treatment of this established herpetic eye infection.

In 1973 Shipkowitz et al. reported the suppression of herpes simplex virus (HSV) by phosphonoacetic acid (PAA) in tissue cultures, in herpetic dermatitis in mice, and in herpetic keratitis in rabbits (8). In this first report of the antiviral activity associated with PAA, treatment of corneal HSV infections was begun 2 h after virus inoculation (8). This experimental model is useful for screening purposes, but it bears little relationship to the problems faced in treating established herpes simplex keratitis in humans. In the study presented here, we tested the effect of PAA in the treatment of established HSV corneal infections in rabbits, and compared it with idoxuridine (IDU), the only drug now commercially available to treat herpetic keratitis.

MATERIALS AND METHODS

Female New Zealand white rabbits weighing between 2 and 3 kg were used in the study. Prior to inoculation they were found to be free of corneal disease. HSV RE strain (type 1) with a titer of 10^6 mean tissue culture infective doses per ml was prepared in primary cultures of rabbit kidney cells as previously described (6, 7). To infect the rabbits' eyes, 0.15 ml of the undiluted virus pool (about 10^5 mean tissue culture infective doses) was applied to the cornea of both eyes of each animal without prior scarification. Only animals which developed typical branching (dendritic) ulcers of the corneal epithelium in both eyes were employed in the study.

A single examiner graded corneal epithelial ulceration and opacity by the scale presented in Table 1 (7). The examiner did not know which eyes had received a particular medication. Examinations were done before virus inoculation, and from day 3 to day 28 postinoculation. In each experiment, examinations were performed before viral cultures were taken and prior to treatment during the treatment period.

Because of the variability of corneal disease among different animals, and the similarity of the disease severity between the two eyes of each animal, the effect of topically applied antiviral agent in one eye was compared to no treatment or placebo in the other eye of each animal. The treated eye was scored as "better" if the epithelial lesion was 2 or more steps lower than the control eye, "worse" if it was 2 or more steps higher, and the "same" if the grades were within one step. For corneal opacity a one step difference was regarded as significant. In each experiment, the number of animals with the treated eye better are compared to those with the treated eye worse (ties or the same are discarded for statistical purposes). The level of significance of these differences can then be determined by the sign test from tables calculated on the binomial distribution (2).

Specimens for virus reisolation were obtained by lightly rolling a sterile dry cotton swab over the cornea which was immediately placed in the tissue culture medium and then stored at −70 C. The culture medium and isolation procedure were described previously (6, 7).

The disodium salt of PAA with approximately neutral pH was used throughout this study as a 2 or 5% aqueous solution in distilled water, or as a 5% ophthalmic ointment. (Abbott Laboratories, North Chicago, Ill.) Commercially available preparations of 0.5% IDU ophthalmic ointment and sterile ointment base without medication (as a placebo) were used in some experiments. All treatment was started on day 3 post-virus inoculation and continued daily for 5 days.

RESULTS

To evaluate toxicity, two uninfected rabbits were treated for 3 days with 1 drop of the 5% PAA solution in each eye, eight times a day. Fine punctate lesions of the superficial corneal epithelium which stained with fluorescein occurred during treatment, but disappeared within 48 h after cessation of the medication. The corneal lesions were no more severe than those encountered with topical IDU now used to treat human herpetic keratitis (9).

TABLE 1. *Grading of signs in experimental herpetic keratitis*

Experimental	Scale	Condition
Corneal staining (amount)	0.00	Absence of staining defect
	0.25	1 to 10 pinpoint, pinhead, punctate or small dendritic fluoroscein staining defect(s)
	0.50	11 to 20 pinpoint, pinhead punctate, small geographic, or small dendritic staining defects
	0.75	Staining defects >0.5 lesion score but <1.0; staining defects >21 but less than 25% of the cornea
	1.00-4.00	Staining defects involving approximately 25 to 100% of the cornea
Corneal opacity	0	Clear cornea
	1	Some haze but iris details clearly visible
	2	Iris details obscured but can visualize pupil margin
	3	Completely opaque—cannot visualize iris or pupil

In infected but untreated or placebo-treated eyes, there was a progression from linear (dendritic) corneal ulcers to wider geographic ulceration and opacity with vascularization of the cornea in some animals and resolution by day 21. Treatment was administered from days 3 to 7 postinoculation when corneal ulceration was increasing and virus proliferation was at its height (5, 6).

In experiment 1, the animals were divided into two groups: one group of four animals was treated in the right eye with 2% PAA solution, and five other animals received 5% PAA solution. The solutions were applied topically eight times a day during a 9-h period. In addition, 5% PAA ophthalmic ointment was applied into the treated right eyes of both groups each night. No treatment was given to the left eyes in this experiment. Eyes treated with 5% PAA solution had less ulceration and corneal opacity than the matched control eyes 7 and 11 days after inoculation (Table 2; Fig. 1). By day 14, however, the untreated corneas had improved enough so that both treated and control eyes exhibited similar degrees of epithelial disease. While two of four PAA-treated animals had suppression of epithelial disease on day 7 (Table 3), 5% PAA was clearly more effective. Thus the 5% concentration was used in subsequent experiments.

In experiment 2, 12 animals received 5% PAA ophthalmic ointment in one eye and a placebo ointment in the other eye four times a day during the treatment period. Corneal ulceration and opacity were less severe in a significant number of animals, but the effect was less marked after day 14 postinoculation (Table 4). In this experiment, virus cultures taken on day

TABLE 2. *Five percent PAA solution compared to placebo (experiment 1)*

Experimental	Day	Condition[a]		
		Better	Same	Worse
Epithelial keratitis	7	5[b]	0	0
	11	5[b]	0	0
	14	2	3	0
Opacity	7	5[b]	0	0
	11	4	1	0
	14	3	2	0

[a] Condition of the PAA-treated eye.
[b] $P < 0.05$, sign test (2).

TABLE 3. *Two percent PAA solution compared to placebo (experiment 1)*

Experimental	Day	Condition[a]		
		Better	Same	Worse
Epithelial keratitis	7	2	1	1
	11	0	3	1
	14	0	2	0
Opacity	7	0	4	0
	11	1	2	1
	14	0	0	2

[a] Condition of the PAA-treated eye.

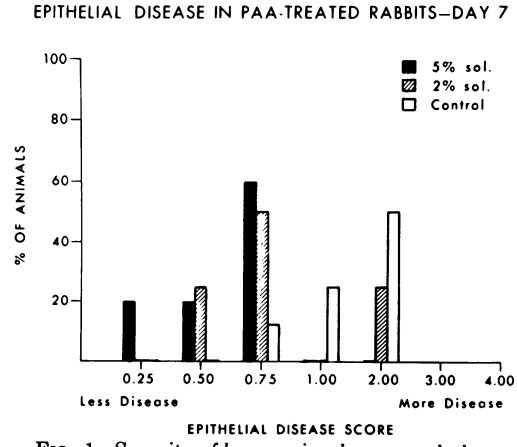

FIG. 1. *Severity of herpes simplex corneal ulcers of the cornea in rabbits 7 days after inoculation and after 4 days of treatment. Each bar represents the percent of all animals in each group with the particular score of epithelial disease.*

TABLE 4. *Five percent PAA ointment compared to placebo*

Experimental	Day	Condition[a]		
		Better	Same	Worse
Epithelial keratitis	7	11[b]	1	0
	10	10[b]	2	0
	14	4	6	1
Opacity	7	7[b]	5	0
	10	6[b]	6	0
	14	5	6	0

[a] Condition of PAA-treated eye.
[b] $P < 0.05$, sign test.

TABLE 5. *Virus recovery in treated hepetic keratitis*

Experimental	Eyes yielding isolates			
	3[a]	7	10	21
Experiment 2				
PAA ointment (5%)	12/12[b]	6/12	4/12	0/4
Placebo	12/12	12/12	1/12	0/4
Experiment 3				
PAA ointment (5%)	ND[c]	ND	5/10	ND
IDU ointment (0.5%)	ND	ND	6/10	ND

[a] Day postinoculation.
[b] Number of eyes yielding virus/number of eyes tested.
[c] ND, not done.

7 postinoculation, the final day of treatment, yielded virus in 6 of 12 PAA-treated eyes compared to 12 of 12 nontreated control eyes ($P < 0.01$, Fisher's exact test) (Table 5).

In experiment 3, 10 animals received 5% PAA ointment applied into one eye and 0.5% IDU ophthalmic ointment in the other eye four times a day during the treatment period. There was a minimal difference in the severity of disease between the two antiviral regimens, with slightly more suppressive effect in the 5% PAA ointment treated eyes 10 days postinoculation (Table 6; Fig. 2). Virus cultures done on day 10 postinoculation (3 days after treatment was stopped) showed no significant differences in the virus-inhibitory effect of the two antiviral agents (Table 5).

DISCUSSION

PAA, given as disodium phosphonoacetate, appears to produce a statistically significant suppression of experimental herpes keratitis in rabbits when used as a 5% preparation of either ointment or solution. The magnitude of the antiviral effect appears to be at least equivalent to that of IDU. These experiments support the initial study by Shipkowitz et al. (8) in which PAA concentrations of 0.5% or more administered starting 2 h after virus inoculation was effective in preventing corneal disease. Thus, while PAA had a prophylactic effect at 0.5% concentrations, a concentration of 5% was necessary to achieve a therapeutic effect. While a lower concentration of the agent might be therapeutically effective, our findings suggest that the 2% solution is suboptimal.

The mode of action of PAA is yet unknown. A similar compound, benzylphosphonic acid, has been shown to have some antiviral activity against the encephalomyocarditis virus, a picornavirus unrelated to herpesvirus (3, 4). It has been suggested that the drug may interfere with the production or utilization of small molecular precursors of nucleic acid and/or protein, or partially inhibit the formation or action of the virus-induced enzymes. (4) Similarly, little is known of the pharmacologic properties of PAA.

TABLE 6. *Five percent PAA ointment compared to 0.5% IDU ointment*

Experimental	Day	Condition[a]		
		Better	Same	Worse
Epithelial keratitis	7	4	3	3
	10	5[b]	5	0
	14	3	4	3
Opacity	7	0	9	1
	10	1	8	1
	14	1	7	1

[a] Condition of PAA-treated eye.
[b] $P < 0.05$; sign test.

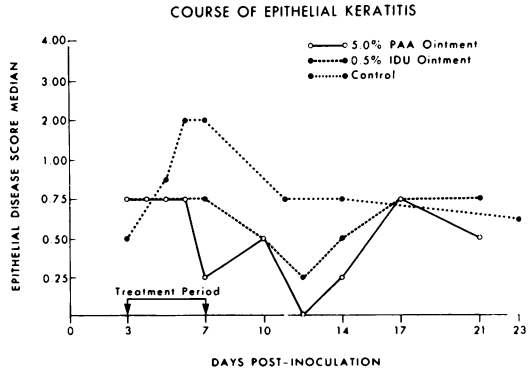

FIG. 2. *Course of HSV epithelial ulceration in rabbits treated with PAA, IDU, or placebo. The plotted values represent the median scores of disease intensity for all rabbits in each group on the designated days.*

Since herpes virus appears to proliferate in the deep cornea and anterior uvea (1), data on the penetration of topically applied PAA into the deeper structures of the eye would be of interest for predicting the usefulness of this compound in clinical situations.

Comparison of the drug-treated to placebo-treated eye in the same animal is a widely used strategy in experimental ocular therapeutics. The severity of experimental ocular herpes infection varies considerably from animal to animal but is usually the same in the two eyes of a single animal. The amount of a topically applied antiviral drug that might be transferred to the nontreated eye by the grooming activities of the animal or in the blood stream are trivial in view of the concentrations needed to produce a measurable effect. This justification could not be applied, however, to drugs administered by subconjunctival or intraocular injection.

One interesting finding in these studies was the persistence of virus in 15 of 32 PAA- and IDU-treated eyes compared to 1 of 12 control eyes (Table 5). We have encountered this phenomenon in studies of herpetic keratitis treated with IDU (5) and proflavine-mediated photodynamic inactivation (7). This virus "rebound" in treated eyes suggests that these antiviral agents, in suppressing the replication of the virus, may also interfere in some way with the local host response to the virus infections. Although the development of resistance to the antiviral compounds may also account for this persistence of virus, this would be unlikely with such a short period of exposure to drug.

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