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Preliminary Communication

2′-Nor-2′-deoxyguanosine is an effective therapeutic agent for treatment of experimental herpes keratitis

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Summary

2′-Nor-2′-deoxyguanosine (2′NDG), previously reported by us to effectively treat acute herpes simplex infections in mice, was used therapeutically to significantly enhance healing of established herpetic corneal lesions and prevent stromal disease in rabbits. Treatment using 0.06% 2′NDG drops (5 times daily) starting 3 days after infection resulted in more rapid healing of corneal epithelial lesions, rapid resolution of conjunctival inflammation, and prevention of stromal clouding compared to placebo-treated animals. In comparative dose–response titrations, the relative potency of 2′NDG to acyclovir was 6.4, which was significant. In addition, soluble ophthalmic inserts were developed for delivery of 2′NDG. Once a day treatment using ophthalmic inserts which released 100 μg 2′NDG significantly enhanced corneal and conjunctival healing and prevented stromal disease; 2′NDG eye drops (100 μg) delivered once a day were also effective in inhibiting the progression of corneal lesions. These results indicate that 2′NDG may be therapeutically effective in treatment of herpes keratitis, and further suggest that for use as eye drops or in an ophthalmic insert, 2′NDG may be effective even if applied once per day.

Experimental herpes keratitis; 2′-Nor-2′-deoxyguanosine; Ocular inserts

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Introduction

9-{[2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine (2′NDG) was first demonstrated by Ashton et al. (1982) and Smith et al. (1982) to be a potent inhibitor of herpes simplex virus replication. In subsequent publications, this compound, also referred to as BIOLF-62 (Smith et al., 1982), DHPG (Smee et al., 1983) and BW759 (Rollinson and White, 1983) has been shown to be a more potent inhibitor than acyclovir (ACV) of herpes simplex virus types I (HSV-1) and II (HSV-2) infections both in cell cultures and in mice and of human cytomegalovirus and Epstein-Barr virus infections in cell culture (Cheng et al., 1983; Davies et al., 1984; Field et al., 1983; Lin et al., 1984; Mar et al., 1983; Tocci et al., 1984).

In recent studies from our laboratories (Davies et al., 1984), 2′NDG was shown to be highly effective when applied topically for therapeutic treatment of orofacial HSV-1 infection in mice. These findings established that effective localized treatment could be initiated days after infection. We have now extended these therapeutic topical studies to demonstrate the efficacy of 2′NDG for treatment of herpes keratitis in rabbits. In addition, the present data demonstrate the potential utility of an ophthalmic insert as an alternate method of delivering effective 2′NDG therapy.

Materials and Methods

Ocular HSV-1 infection

The unscarified eyes of New Zealand white rabbits (3–4 kg) were bilaterally inoculated with 10^5 PFU McKrae strain HSV-1 per eye by dropping the virus suspension into the lower cul-de-sac and gently rubbing the lid over the cornea for 30 sec. After 3 days the eyes were evaluated for corneal ulcers using either a hand-held ophthalmoscope or a slit-lamp biomicroscope. Animals were then placed into balanced treatment groups of two with matched corneal involvement in all four eyes.

Study design

Observations using an ophthalmoscope or slit lamp were made daily on day 3 through day 10 post-inoculation (PI). Corneal, conjunctival and stromal evaluations were made daily prior to treatment based on the scoring system of Wander et al. (1980). Briefly, after fluorescein staining, the corneal epithelium was graded from 0 (normal) to 4 (total corneal ulceration). Drug treatments were begun on day 3 and continued through day 7. Treatment consisted of instilling a 20 μl drop of indicated drug, a 3-mm strip of ointment or the indicated ocular insert into the lower cul-de-sac. Animals receiving 5 daily treatments were dosed at 3-h intervals, while twice daily treatments were administered at an eight hour interval.

Antiviral compounds

2′NDG and ACV were prepared at the Merck, Sharp & Dohme Research Lab-

oratories, Rahway, NJ. 2'NDG and ACV solutions and suspensions were prepared in an isotonic buffered vehicle (pH 6.6) containing 35 mmol each of sodium phosphate mono and dibasic USP, 0.012% benzalkonium chloride USP, 0.35% sodium chloride USP and 2% povidone USP. With suspensions, the powdered drug was ground with an agate mortar and mixed with vehicle to achieve the final dispersion. Sonication for 3–5 min assured homogenous and agglomerate-free dispersions. Viroptic® 1% ophthalmic solution and Zovirax® 3% ointment (manufactured by Burroughs-Wellcome Co.) were used as applied commercially. Isotonic buffered vehicle without added antiviral drugs served as placebo control.

Ophthalmic inserts

Ophthalmic inserts were prepared by mixing the required quantity of 2'NDG with vehicle containing polyvinyl alcohol, mannitol USP and glycerol USP. Sufficient water was added to form a slurry so that 10 μl contained 100 μg 2'NDG and excipients to yield a final insert weight of 3 mg. Aliquots of the 10 μl slurry were deposited onto Teflon-coated metal plates, dried at ambient conditions overnight, and further dried in a vacuum desiccator. The 2 mm wafer inserts were then removed and assayed for weight and content uniformity. Upon dissolution of the insert in 5 ml of isotonic phosphate buffer (pH 7.4) at 37°C, 100% of 2'NDG was released within 30 min as measured by high-performance liquid chromatography. For treatment of infected rabbit eyes, a single insert was placed into the lower cul-de-sac. The insert began to dissolve within seconds and was completely solubilized within 1 h.

Analysis of data

Statistical analyses were performed on corneal epithelium conjunctival and stromal scores at each time, and the average of each of those scores over the eight days of observation. Analysis of variance on average scores, at each time, was followed by Duncan's multiple range test (Duncan, 1955) to determine significant differences ($P < 0.05$) between treatment groups. Average score over time was analyzed by the same methods. Dose titration data were used to calculate a relative potency between test preparations, by standard bioassay techniques (Finney, 1978).

Results and Discussion

Comparative therapeutic efficacy of 2'NDG and acyclovir

In separately controlled experiments, 2'NDG or acyclovir were administered as eye drops 5 times daily at 3-h intervals, starting 3 days PI and continued for 4 days. Efficacy was determined by significant reduction of corneal lesion involvement, development of conjunctivitis and evidence of stromal clouding, compared to control groups treated with the isotonic buffered vehicle alone. Dose response data on corneal lesion development are shown in Table 1. Significant reduction of corneal epithelial lesions was repeatedly achieved using 0.06 or 0.25% 2'NDG. Sim-

TABLE 1

Efficacy of 2'NDG and acyclovir in treatment of HSV-1 infection of rabbit corneal epithelium

Treatment regimen[a]			Severity of corneal lesions ave. lesion score[b] (± S.E.M.)		
Drug	conc. (%)	μg del./day	Expt. 1	Expt. 2	Expt. 3
2'NDG	0.25	250	0.50[c](0.06)	0.18[c](0.04)	0.29[c](0.04)
	0.06	63	1.18[c](0.04)	0.73[c](NA)	
	0.02	20	1.58 (0.00)		
	0.005	5	1.74 (0.15)		
Acyclovir	1.0	1000		0.34[c](0.13)	
	0.25	250		1.78 (0.19)	
	0.06	63		1.54 (0.78)	
	0.02	20		2.24 (0.09)	
Zovirax®	3.0			0.36[c](0.04)	0.29[c](0.12)
Viroptic®	1.0	1000			0.58[c](0.002)
Placebo			1.62 (0.04)	2.38 (0.23)	2.41 (0.35)

[a] Administered as drops or ointment (Zovirax®) 5 times daily.

[b] Means of average lesion score and standard errors based on 2-rabbits, except placebo groups in Expts. 1 and 3 (4 rabbits) and 0.06% 2'NDG in Expt. 2 (1 rabbit).

[c] Significantly different ($P<0.05$) from placebo-treated group for that experiment.

NA = Not applicable, mean based on 1 rabbit.

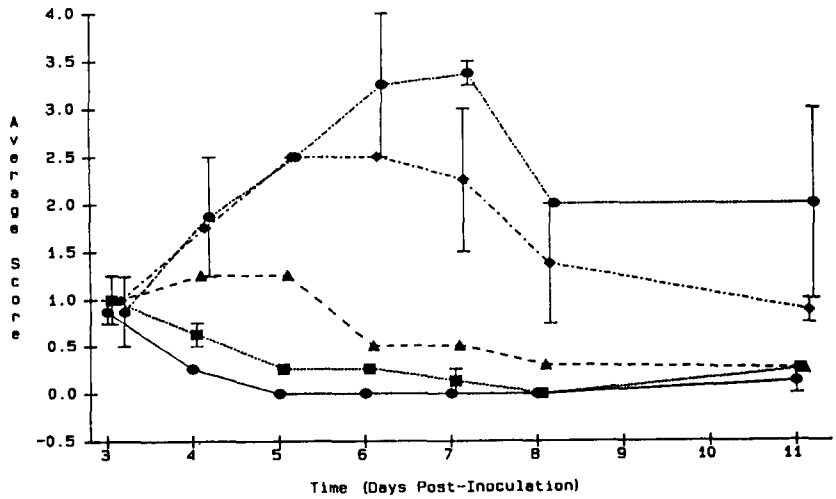

Fig. 1. Corneal epithelium lesion development (mean ± S.E.M.) in placebo and drug treated HSV-1 infected rabbits. Eye drops were administered as described in Materials and Methods to rabbits starting 3 days after eye infection. ●—●, 0.25% 2'NDG; ▲— —▲, 0.06% 2'NDG; ◆— —◆, 0.25% ACV; ■----■, 3% Zovirax; ●—·—●, placebo. S.E.M. are shown only for placebo and 0.06% 2'NDG for clarity of presentation.

ilar reduction in corneal lesions could be attained using 1% acyclovir. Commercial preparations of 3% acyclovir (Zovirax®) or 1% trifluorothymidine (Viroptic®) were comparably effective. The relative potency of 2'NDG to acyclovir was 6.4, which was significant. The kinetics of corneal lesion development for treatment with 0.25 or 0.06% 2'NDG, 0.25% acyclovir, or Zovirax® (3%), determined in experiment 2 are shown (Fig. 1). Significant reduction in average lesion severity compared to placebo treated rabbits was achieved for animals treated with 0.25 or 0.06% 2'NDG or Zovirax® within two days of therapy (day 5 p.i.). Following initiation of therapy, no increase in lesion severity occurred in these groups and lesions progressively resolved. Both 2'NDG and acyclovir were effective at 0.02% or higher concentrations in significantly reducing the severity of conjunctivitis and preventing stromal clouding (Table 2).

2'NDG treatment using ophthalmic inserts or eye drops in once-per-day therapy

Acyclovir (Zovirax®) and trifluorothymidine (Viroptic®) have been accepted for human use in the therapy of herpes keratitis. Both are effective drugs, as demonstrated in experimental and clinical settings (Bauer et al., 1979; Collum et al., 1983; Jensen et al., 1982; McNeill and Kaufman, 1979; Trousdale et al., 1980); however, both require multiple treatments per day for effective therapy. Development of a drug for once a day treatment could be a significant advance in the therapy of herpes keratitis.

We have effectively used 2'NDG for once a day treatment, delivering 100μg of 2'NDG to the HSV infected cornea either by eye drop or in a solid formulation

TABLE 2

Efficacy of 2'NDG and acyclovir in prevention of HSV-1 infection conjunctivitis and stromal clouding

Drug[a]	Conc. (%)	Ave. ophthalmic measure[b] (± S.E.M.)			
		Severity of conjunctivitis		Severity of stromal clouding	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2
2'NDG	0.25	0.26[c](0.15)		0[c](–)	
	0.06	0.61[c](0.00)		0.03[c](0.03)	
	0.02	1.51[c](0.54)		0.71[c](0.60)	
	0.005	2.14 (0.39)		1.14 (0.19)	
Acyclovir	1.0		0.14[c](0.15)		0[c](–)
	0.25		0.63[c](0.27)		0[c](–)
	0.06		0.45[c](0.23)		0[c].07(0.07)
	0.02		1.75 (0.18)		0[c].32(0.25)
Placebo		1.95 (0.05)	1.59 (0.41)	1.44 (0.12)	0.61 (0.18)

[a] Administered as drops 5 times daily.

[b] Means of average ophthalmic measure and standard errors based on 2 rabbits, except placebo group in Expt. 1 (4 rabbits), and 0.06% 2'NDG in Expt. 2 (1 rabbit).

[c] Significantly different ($P<0.05$) from placebo treated group for that experiment.

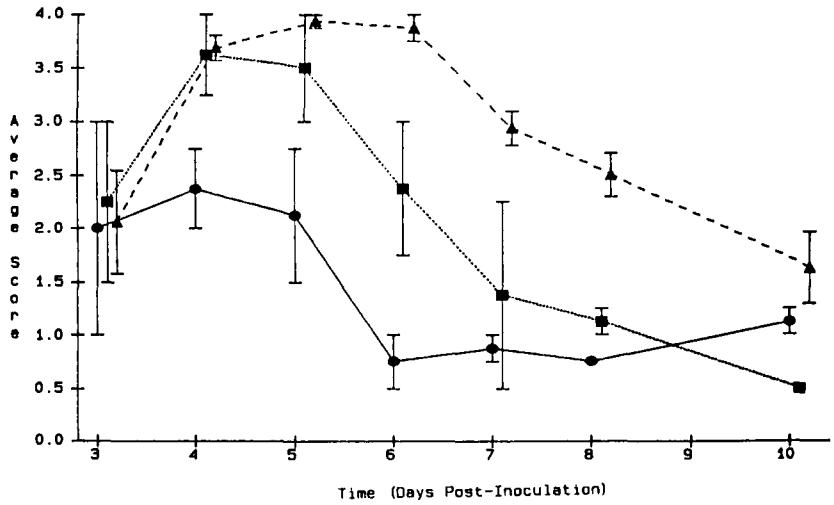

Fig. 2. Corneal epithelium lesion development (mean ± S.E.M.) in HSV-1 infected rabbits treated once daily using 100 μg 2'NDG in eye drops or in ophthalmic inserts. ■----■, 2'NDG eye drops; ●—●, 2'NDG ophthalmic inserts; ▲— — —▲, placebo.

ophthalmic insert. Treatment was initiated 3 days p.i. and continued for 4 days. Both treatment regimens effectively altered the development of corneal lesions compared to placebo treated control rabbits (Fig. 2). However, more rapid cessation of lesion development occurred using insert-deliverd 2'NDG, suggesting that 2'NDG released from inserts was the more effective therapy. After 5 days of therapy (7 days p.i.), lesion healing was comparable for both 2'NDG inserts and eye drop therapy. Placebo eye drop and placebo insert treated eyes showed comparable lesion development and the data are combined in Fig. 2. Both eye drop and ophthalmic insert therapy for delivery of 2'NDG resulted in complete prevention of stromal clouding, which developed in all placebo treated animals. In uninfected rabbit eyes, daily treatment using ophthalmic inserts containing 100 μg 2'NDG did not show any signs of irritation, including a lack of conjunctivitis or alteration of the corneal epithelium.

The use of 2'NDG for treatment of experimental herpetic eye infections has been previously reported by Smith et al. (1984) and Trousdale et al. (1984). These studies demonstrated that ointment preparations of 2'NDG were effective in limiting the progression of corneal lesions and virus shedding in HSV-1 infected rabbits. Smith et al. (1982, 1984) also noted the lack of toxicity in 1% 2'NDG treated rabbit eyes. In the present studies, 2'NDG was prepared as an eye drop solution, which was significantly more effective than similar preparations of ACV; these observations are in agreement with Trousdale et al. (1980, 1984). Unlike the results of Trousdale et al. (1980, 1984), however, our data indicate that 2'NDG may be effective if used therapeutically once daily in treatment of herpes keratitis. Although the data are preliminary, the present results suggest that use of a sustained release vehicle for 2'NDG may make once daily eye treatment a practicle therapeutic regimen.

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