

HSV-1 Shedding by Iontophoresis of 6-Hydroxydopamine followed by Topical Epinephrine

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The development of an animal model for herpes simplex virus (HSV) reactivation is of great importance in studying HSV latency, reactivation, recurrence, and chemotherapy. Epinephrine iontophoresis to the cornea can induce HSV type 1 (HSV-1) ocular shedding from latently infected rabbits. In the present experiment, adrenergic induction of HSV-1 was enhanced by iontophoresis of 6-hydroxydopamine (6-HD) to the rabbit cornea followed by topical epinephrine to the eye. Eye swabs were utilized to determine HSV-1 shedding in the tear film. The combined treatment was performed on selected days during 66 to 292 days (mean 159) postinoculation, resulting in HSV-1 shedding from 100% of the eyes (17/17) within 6 days after the 6-HD treatment. The onset of initial shedding was as early as day 1. The highest frequency (93%) of shedding occurred on day 2. The mean duration of consecutive HSV-1 sheddings was 3.2 days. The importance of prior spontaneous HSV-1 ocular sheddings relative to this induced HSV-1 reactivation system also was demonstrated. Latently infected rabbits that shed virus spontaneously could be induced to shed virus at a much higher frequency and for a longer duration than rabbits that had not shed virus spontaneously. Iontophoresis of 6-HD produced ocular adrenergic supersensitization and with topical epinephrine it induced HSV-1 ocular shedding in 100% of eyes. This new model of induced HSV-1 ocular shedding will be useful for investigation of adrenergic mechanisms that may be involved in HSV reactivation and recurrence. Invest Ophthalmol Vis. Sci 24:1588-1594, 1983

Previous investigations in our laboratory have demonstrated that epinephrine iontophoresis to the eye induced a high frequency of HSV-1 shedding in the preocular tear film from HSV-1 latently infected rabbits.¹⁻³ Reactivation of HSV by administering epinephrine to rabbits has been reported by others,⁴⁻⁷ but not as consistently nor as high a frequency as after epinephrine iontophoresis. A model for viral reactivation involving manipulation of the adrenergic system could aid in understanding the mechanism(s) of latency and reactivation.

Epinephrine iontophoresis to the cornea induced a higher frequency of shedding than systemic administration,^{1,4,5} which appears to indicate the importance of drug penetration in high concentrations. Furthermore, epinephrine is a neurohormone of the sympathetic nervous system which can activate sympathetically innervated structures. The superior cervical ganglion, as well as the trigeminal ganglion, harbors

HSV-1 during the latent infection.^{1,3,8,9} Thus, the action of epinephrine upon the sympathetic structures at the peripheral site might be a trigger to reactivate HSV-1 harbored in the superior cervical ganglion.

The norepinephrine congener 6-hydroxydopamine (6-HD) causes selective and reversible degeneration of sympathetic terminals in the anterior segment of the eye.¹⁰ After this chemical sympathectomy, the previously innervated structures demonstrate supersensitivity to extremely dilute solutions of epinephrine.^{11,12} Indeed, 6-HD has been administered in treatment of primary open-angle glaucoma to augment the effect of the topical application of epinephrine.^{11,13,14} Furthermore, iontophoresis causes eight times more penetration of 6-HD into the aqueous humor than corneal bathing, and 20 times more than instillation.¹⁵ Thus, after iontophoresis, drugs attain a higher concentration and remain for longer periods in the ocular tissues.^{15,16} In HSV-1 intraocular infections, intraperitoneal injection of 6-HD increased HSV-1 replication in the superior cervical ganglion of mice in the acute phase, while it decreased the subsequent prevalence of the establishment of latency.¹⁷

We designed experiments that would alter the adrenergic system by the production of ocular supersensitivity by 6-HD and assessed the role of supersensitivity in reactivation of HSV-1. Iontophoresis of 6-HD to

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Table 1. Detection of HSV-1 from the tear film

Experiment	Group	Treatment	Number of rabbits	PI days (mean \pm SEM)	Positive eyes (%)		Average duration of shedding (days)
					Total eyes		
1	A	6-HD (iontophoresis) [†] Epinephrine (topical)	10	159 \pm 18	17/17 (100%)		3.2
	B	6-HD (iontophoresis)	7	160 \pm 14	6/12 (50%)*		0.9
	C	Epinephrine (topical)	7	85 \pm 17	6/10 (60%) [†]		1.4
	D	Debridement	8	175 \pm 20	2/16 (12%)* ^{‡§}		0.19
2	E	6-HD (iontophoresis) [†] Epinephrine (topical)	5	132 \pm 12	2/9 (22%)*		0.22
	F	6-HD (iontophoresis)	5	107 \pm 17	0/9 (0%) [†]		0
	G	Epinephrine (topical)	5	134 \pm 0.9	2/10 (20%)*		0.20

Rabbits in Groups A, B, C, and D (Experiment 1) had spontaneously shed HSV-1 at least once in both eyes. Rabbits in Groups E, F, and G (Experiment 2) never had a spontaneous HSV-1 positive eye swab in the latent period (days 21–160 P.I.). All eyes had dendritic lesions in the acute phase, and all eyes in Groups E, F, and G were swabbed at least 25 times prior to any treatments. The conditions for each treatment are given in the text.

* Significant difference ($P < 0.001$) from Group A (χ^2 -test).

[†] Significant difference ($P < 0.005$) from Group A.

[‡] Significant difference ($P < 0.05$) from Group B.

[§] Significant difference ($P < 0.05$) from Group C.

^{||} No significant difference from Group C.

the cornea enhanced the pharmacologic action of topical epinephrine for inducing ocular HSV-1 shedding in the latently infected rabbit.

Materials and Methods

Virus Inoculation

Both unscarified corneas of New Zealand albino rabbits (1.5–2.0 kg) were inoculated with a 50- μ l suspension of HSV-1 McKrae strain (0.2 – 1.0×10^6 PFU/ml) that had been prepared in primary rabbit kidney cell (PRK) monolayers and titrated on green monkey kidney cell (CV-1) cultures. At 72 hours postinoculation, all eyes had typical corneal epithelial dendritic ulcers as determined by slit-lamp examination using fluorescein stain.

Determination of Viral Shedding

HSV-1 ocular shedding was detected from eye swabs taken with sterile, Dacron[®]-tipped applicators that were rotated gently in the upper cul-de-sac, then lightly across the cornea and into the lower cul-de-sac, and rested in the lower fornix for 5 seconds for maximum tear film absorption.¹⁸ Special attention was given to swabbing so that mechanical trauma to the corneal epithelium was minimized. The Dacron swabs were placed in culture tubes containing PRK monolayers and incubated overnight at 37 C in 5% CO₂. Subsequently, the swabs were squeezed against the tube wall and removed. Eagle's minimum essential medium with 7% fetal calf serum was added for pH adjustment and nutrition. The appearance of cytopathic effect consistent with HSV-1 infection was monitored for 14 days. HSV-1 sheddings in six eyes of Group D (Table 1) were detected by an eye-wash procedure that was described previously.²

Identification of Viral Isolates

Randomly selected viral isolates from ocular swab cultures were identified by a plaque-reduction assay on CV-1 monolayers using HSV-1 specific rabbit antiserum. This procedure is essentially the same as that described by Knotts et al.¹⁹ In all cases, the virus shed was identified as HSV-1.

Treatment of Rabbit Eyes

Iontophoresis: Rabbits were anesthetized by separate intramuscular injections of xylazine (4 mg/kg) and then ketamine (20 mg/kg). A sterile eye cup was centered with its periphery within the limits of the corneal limbus. Solutions of 6-HD were prepared at 1% and 0.1% immediately prior to administration by dissolving the powder in sterile deoxygenated distilled water with pH adjustment to 6.0 using sodium hydroxide. The appropriate 6-HD solution was added to the eye cup. The anode (+) made contact with the solution of 6-HD in the eye cup and the cathode (–) was attached to a shaved area of dorsum. Three iontophoretic conditions were employed: (1) 6-HD (1%) at 0.75 mA for 3 minutes; (2) 6-HD (0.1%) at 0.5 mA for 8 minutes; and (3) 6-HD (0.1%) at 0.5 mA for 4 minutes. Iontophoresis was performed only once at the initiation of treatment. A successful treatment was verified when pupillary dilation occurred within 30 minutes after iontophoresis.

Instillation of Epinephrine

Two drops (approximately 100 μ l) of a 2% epinephrine solution (1-epinephrine hydrochloride, Glaucon[®], Alcon Laboratories, Inc., TX) were instilled in eyes once on the first day and twice daily on days 2–5.

Iontophoretic Condition	P. I.	EYE	Days pre- and post-6-HD iontophoresis										
			-3	-2	-1	0	1	2	3	4	5	6	7
1% 6-HD 0.75mA 3 min	94	OD	-	-	-	-	+	+	+	+	N	N	N
	124	OD	-	-	-	-	-	+	+	C	-	-	-
	124	OS	-	-	-	+	-	+	+	+	-	-	-
	292	OD	-	-	-	-	-	C	C	+	+	+	-
	292	OS	C	-	-	-	-	C	+	+	+	-	-
147	OD	-	-	-	-	-	+	+	+	-	-	-	
0.1% 6-HD 0.5mA 8 min	87	OD	-	-	-	-	+	+	+	+	N	N	N
	87	OS	-	-	-	-	+	+	+	+	N	N	N
	153	OD	-	-	-	-	-	+	+	+	+	+	+
	167	OD	-	-	-	-	-	-	-	-	-	+	-
	108	OD	-	-	-	-	-	+	-	-	-	-	-
	66	OD	-	-	-	+	+	+	+	+	+	+	-
0.1% 6-HD 0.5mA 4 min	207	OS	-	-	-	-	+	+	-	+	N	N	N
	94	OS	-	-	-	-	-	+	+	-	C	-	-
	262	OD	-	-	-	-	-	+	+	+	+	-	-
	262	OS	-	-	-	-	-	+	+	+	-	-	-
	147	OS	-	-	-	-	C	+	+	+	-	-	-

Fig. 1. HSV-1 ocular shedding in rabbit eyes after 6-HD iontophoresis followed by topical epinephrine. (+) = HSV-1 ocular shedding; (-) = no shedding; N = not done; C = contamination ◀ = iontophoresis of 6-HD; (I) = topical epinephrine; and PI = postinoculation days. All eyes received iontophoresis of 6-HD only once. Epinephrine instillation was once on the first day and twice daily on days 2-5. Eye swabs were taken on the days indicated.

Experimental Design

Experiment 1: HSV-1 latently infected rabbits that had at least one positive spontaneous HSV-1 ocular shedding from each eye were selected and utilized in Experiment 1. Rabbits were divided into four groups and used between 41-292 days postinoculation (PI): Group A contained 17 eyes in 10 rabbits and were used between 66 to 292 days PI (mean, 159 days); Group A eyes were treated with topical epinephrine after iontophoresis of 6-HD. Group B contained 12 eyes in seven rabbits and were used between 66 to 228 days PI (mean, 160 days); Group B eyes received only iontophoresis of 6-HD. Group C contained 10 eyes of seven rabbits used between 41 to 207 days PI (mean, 85 days); Group C eyes received only 2% epinephrine instillation without iontophoresis of 6-HD. Group D contained 16 eyes of eight rabbits used between 76 to 287 days PI (mean, 175 days). Group D eyes received only debridement of the corneal epithelium, which allowed assessment of the corneal debridement on HSV-1 shedding. Corneal epithelial defects that occurred on approximately 25% of the corneal surface were observed after 6-HD iontophoresis plus epinephrine instillation. The debridement was done by removing approximately 25% of the central corneal epithelium by scraping with a Gill corneal knife and verified using a slit lamp and fluorescein stain. Ocular sheddings were determined for designated days before treatment and daily for seven consecutive days after treatment.

Experiment 2: In Experiment 2, previously infected rabbit eyes, which had not shed virus spontaneously during more than 25 days of swabbing, were utilized. The rabbits were divided randomly into three groups and used between 59-185 days PI. Group E contained nine eyes of five rabbits used between 93 to 164 days PI (mean, 132 days); Group E eyes received topical epinephrine after iontophoresis of 6-HD. Group F contained nine eyes of five rabbits used between 59 to 185 days PI (mean, 107 days); Group F eyes received only iontophoresis of 6-HD. Group G contained 10 eyes of five rabbits used between 129 to 136 days PI (mean, 134 days); Group G eyes received only 2% epinephrine instillation. The determination of ocular sheddings was the same as in Experiment 1.

Results

Figure 1 shows the results of the combined treatment (6-HD + epinephrine) on 17 eyes (10 rabbits) during 66 to 292 days PI. HSV-1 ocular shedding was detected in every eye (17/17) within 6 days after the initiation of the combined treatment (2 days after the last epinephrine instillation). The initiation of HSV-1 ocular shedding was first detected on day 1 or 2 after the iontophoresis for 14/17 eyes. Two eyes that had contaminated swabs on day 2 subsequently had positive swabs on days 3 or 4, and only one eye remained negative until the sixth day. The highest frequency of shedding was 93% (14/15) on day 2. The frequency of shedding on days 3 or 4 after the initiation of treat-

ment was 81% (13/16). The mean duration of shedding for all eyes was 3.2 ± 0.34 days (arithmetic mean \pm standard error of the mean).

Figure 2 displays the appearance of HSV-1 ocular shedding following iontophoresis of 6-HD (without topical application of epinephrine). Iontophoresis of 6-HD to 12 eyes between 66 to 228 days PI resulted in 50% ocular sheddings at least once within 7 days after iontophoresis. Only 25% (1/4) of the eyes shed virus when 1% 6-HD, 0.75 mA, 3 minutes was used. When 0.1% 6-HD, 0.5 mA, 8 minutes was used, 62% (5/8) of the eyes shed virus. The difference between the two subgroups of Group B eyes was not statistically significant ($P > 0.2$ by χ^2 test). The initiation of HSV-1 shedding was detected from the third to the sixth day after the treatment. The mean duration of shedding for all eyes was 0.9 ± 0.41 days, ranging from zero to five days. The highest daily frequency of shedding (50%, 4/8) occurred on day 5 after the initiation of treatment.

Figure 3 indicates the recovery of HSV-1 in the tear film following topical epinephrine once on the first day and twice daily on days 2-5. The ocular shedding frequency of the eyes was 60% (6/10) within 7 days after the initiation of treatment. The rabbits whose opposite eyes received iontophoresis of 6-HD plus epinephrine topically shed virus with a frequency of 75% (3/4) during the seven consecutive days of swabbing, while the rabbits receiving only topical epinephrine in both eyes shed virus with a frequency of 50% (3/6) during the seven consecutive days of swabbing. There was no statistical difference between these frequencies ($P > 0.4$). The initiation of HSV-1 ocular shedding was detected on the second to the fifth day after the first epinephrine instillation. The mean duration of shedding for all eyes was 1.4 ± 0.47 days, ranging from zero to four days. The highest daily frequency of shedding (57%, 4/7) was on day 5.

Iontophoretic condition	P.I.	EYE	Days post-6-HD iontophoresis							
			0	1	2	3	4	5	6	7
1% 6-HD 0.75mA 3 min	143	OD	-	-	-	-	-	N	-	-
	143	OS	-	-	-	-	-	N	-	-
	102	OD	-	-	-	-	-	+	-	-
	102	OS	-	-	-	-	-	-	-	C
0.1% 6-HD 0.5mA 8 min	228	OD	-	-	-	C	-	N	-	-
	228	OS	-	-	C	-	-	N	-	-
	167	OS	-	-	C	+	-	-	-	-
	66	OS	-	-	-	-	-	-	-	C
	202	OD	-	-	-	-	+	+	+	+
	202	OS	-	-	-	-	-	-	+	-
	174	OD	-	-	+	+	+	+	+	+
	174	OS	-	-	-	C	-	+	-	-

Fig. 2. HSV-1 ocular shedding in rabbit eyes after 6-HD iontophoresis. See the legend for Figure 1 for symbol identification. All eyes received iontophoresis of 6-HD only once. Eye swabs were taken once immediately before and daily for seven days after treatment. The shedding frequency showed no significant difference between the two different iontophoretic conditions ($P > 0.2$ by χ^2 test).

P.I.	EYE	Days after the initiation of treatment								
		0	1	2	3	4	5	6	7	
207	OD*	-	-	-	-	-	N	N	N	
94	OS*	-	-	+	+	+	N	N	N	
153	OS*	-	-	+	+	+	-	-	-	
94	OD*	-	-	-	+	-	C	-	-	
55	OD	-	-	-	-	-	-	C	N	
55	OS	-	-	-	C	-	+	+	N	
55	OD	-	-	-	-	-	-	-	C	N
55	OS	-	-	-	+	+	+	+	N	
41	OD	-	-	-	-	-	-	-	N	
41	OS	-	-	-	-	-	+	C	N	

Fig. 3. HSV-1 ocular shedding after topical application of epinephrine (2%). See the legend for Figure 1 for symbol identification. All eyes received only epinephrine instillation once on the first day and twice daily on days 2-5. The procedure of eye swabs were the same as for Figure 2. The shedding frequency showed no significant difference between the groups of eyes with or without 6-HD iontophoresis in the contralateral eye ($P > 0.4$ by χ^2 test). * = The opposite eye received iontophoresis of 6-HD.

The ocular shedding frequency of the rabbit eyes in Group D (Table 1) was only 12% (2/16) during seven days following corneal debridement. In both positive cases, the initiation of HSV-1 ocular shedding was detected on the sixth day post-debridement. No recovery of HSV-1 in the tear film was detected for five consecutive days before corneal debridement.

The treatments of rabbit eyes in Group E (Table 1) are identical to those in Figure 1 (Group A). The combined treatment resulted in ocular sheddings in only two eyes (22%, 2/9) during seven consecutive days after the first treatment. The initiation of HSV-1 ocular shedding was detected on the second day after 6-HD iontophoresis.

All rabbits in Groups E, F, and G (Table 1) contained eyes from previously infected rabbits that had never shed virus spontaneously as determined by 25 days of swabbing prior to any experimental treatment. Group F (only 6-HD iontophoresis) and G (only topical epinephrine) shed virus in 0% and 20% of the eyes, respectively. Each of three iontophoretic conditions was employed for three eyes in Group F. HSV-1 ocular shedding was detected once in two eyes on either the second or fourth day after the first epinephrine instillation in Group G.

Table 1 shows a summary of the recovery of HSV-1 from the tear film in all treatment groups. The shedding frequency of Group A (combined treatment) was significantly higher than that of Groups B ($P < 0.001$), C ($P < 0.005$), D ($P < 0.001$), E ($P < 0.001$), F ($P < 0.001$), and G ($P < 0.001$). The shedding frequency of Group B (6-HD iontophoresis in shedders) was significantly higher ($P < 0.05$) than that of Group F (6-HD iontophoresis in nonshedders), but there was no difference statistically between Group C (topical epinephrine in shedders) and G (topical epinephrine in

nonshedders). The viral shedding frequency of the identical treatment groups (except topical epinephrine) showed a significant difference between the two types of previously infected rabbits (spontaneous viral shedders or nonshedders).

Discussion

Epinephrine instillation following 6-HD iontophoresis to the cornea induced HSV-1 ocular shedding (100%, 17/17) from rabbits used between 66 to 292 days PI (mean, 159 days). Previously, epinephrine iontophoresis alone performed on three consecutive days induced 100% viral shedding in rabbits 60 days PI,¹ 95% in rabbits 90 days PI,² and 75% in rabbits 170–365 days PI.^{1,3} These results, plus data on spontaneous shedding,¹ suggest that the frequency of detection of induced or spontaneous HSV-1 shedding is related to the time from the acute or primary infection. The initiation of HSV-1 shedding and the date of occurrence of the highest frequency of shedding were 24 hours earlier for the combined treatment (6-HD + epinephrine) in the present experiments compared with the previous experiments with only epinephrine iontophoresis.^{1–3} The mean duration of consecutive HSV-1 sheddings was 3.2 days in the present experiments, while it was 2.1 days in the previous experiments using rabbits 60 days PI,¹ and 1.5 days using rabbits 170 to 365 days PI.¹ However, the mean duration of consecutive HSV-1 sheddings detected by the eye-wash method was 3.6 days in the epinephrine iontophoresis experiments using rabbits 90 days PI,² and also it was 2.7 days in all eyes in experiments using rabbits 220 to 280 days PI.³ In the present experiments, some eye swabs were contaminated and some were not taken during all seven days; therefore, the mean duration of shedding could have been higher than detected under these conditions.

Iontophoresis ensures the penetration of the drugs into ocular tissues, thereby increasing the concentration and duration of their effects.^{15,16} However, epinephrine iontophoresis appears unnecessary after prior sensitization of the ocular adrenergic system by 6-HD iontophoresis, since the epinephrine applied topically induced HSV-1 ocular shedding in 100% of the eyes. As a result, the model using the combined treatment is simpler because the need for anesthetizing the rabbits on three consecutive days is eliminated. In addition, the combined treatment to the eye induced a higher frequency of shedding than systemic application of epinephrine.^{1,4,5} Iontophoresis of epinephrine was necessary to achieve a high drug concentration in the previous studies,^{1–3} but the same effect can be achieved with topical epinephrine when ocular supersensitivity is produced. These results suggest that chemical sympathectomy enhances the pharmacologic action of epi-

nephrine in the reactivation of HSV-1, and that adrenergic receptors at the peripheral site may be involved directly in the reactivation.

The combined treatment, involving both 6-HD iontophoresis and topical epinephrine, caused 100% reactivation, while iontophoresis of 6-HD alone induced viral shedding in only 50% of the eyes. Also, the mean duration of consecutive viral sheddings was 3.2 days in the former, and 0.9 days in the latter. The HSV-1 shedding in the rabbits receiving only 6-HD might be related to the release of endogenous catecholamines.¹⁰ However, iontophoresis of 6-HD alone induced viral sheddings at a significantly lower frequency than the combined treatment (Table 1).

Topical epinephrine without 6-HD iontophoresis elicited viral shedding in 60% of the eyes. The mean duration of shedding was 1.4 days. This result shows that topical epinephrine alone can induce HSV-1 ocular shedding. Rabbits were used from 41 to 207 days PI with a mean of 85 (Table 1, Fig. 3). The ability to induce viral shedding in this group of rabbits might be related to the days PI, since in previous experiments^{1–3} a lower PI resulted in greater frequency of shedding.

Corneal epithelial defects which occurred on approximately 25% of the whole corneal surface, were found with frequencies of 50% and 70%, respectively, by slit-lamp examination following the use of 6-HD iontophoresis and epinephrine instillation in uninfected and HSV-1 latently infected rabbits (data not shown). However, after debridement of corneal epithelium, HSV-1 was detected in only two out of 16 eyes, which is a significantly ($P < 0.05$) lower frequency than Groups A, B, and C (Table 1). When the shedding frequency of the debridement experiment was compared with the frequency of spontaneous sheddings,¹ there was no statistically significant difference. The total positive cultures per total cultures taken in spontaneous shedders at 81–180 days PI was 3.0%,¹ while it was 2.9% (3/101) after debridement of corneal epithelium (data not shown). Hill and Blyth²⁰ have shown that mild trauma to the ear skin can trigger HSV-1 reactivation in about 30% of the mice. These results suggest that the role of trauma as a trigger might be different at various peripheral sites and possibly species related.

Some possible reasons for the low response after combined treatment (6-HD + epinephrine) in the rabbits that had not spontaneously shed virus at the peripheral site may be related to the following factors: (1) the initial HSV-1 replication in the ocular tissues was so low that the trigeminal ganglion or superior cervical ganglion may not have been infected acutely; (2) the immune system might inactivate HSV-1 and prevent the establishment of latency or the spontaneous

and induced shedding; and (3) conduits for the movement of HSV-1 to the periphery, which might need to be established for spontaneous shedding to occur, are not operable and cannot be induced. Regardless of the mechanisms involved, the presence of spontaneous sheddings indicates that both HSV-1 latency and reactivation are operable. We suggest that the previous history of spontaneous shedding should be studied and recorded for animals used in any induced reactivation system.

Kibrick et al⁶ reported that topically applied 2% epinephrine ointment in eyes previously treated with topical steroid produced HSV shedding (Rodanus strain) in 75% of the eyes. Figure 3 shows 60% viral shedding after only topical application of epinephrine. However, Martin et al²¹ reported no HSV-1 shedding (RE strain) after only topical application of epinephrine and proposed that reactivated viral shedding might depend on the characteristics of the viral strain. Neither report^{6,21} cited whether or not the rabbit eyes had shed HSV spontaneously prior to the attempted reactivation of HSV with topical epinephrine. The differences might be related to pretreatment with steroids, lack of spontaneous shedding, or the viral strains used.

Recovery of HSV has been documented in the superior cervical ganglion of humans.⁹ Martin et al²¹ suggested that the superior cervical ganglia might be involved in recurrent herpetic infections of the eye in the rabbit model. Mintsoulis et al²² detected herpetic eye disease, especially iritis and virus reisolation from the eye, after direct HSV inoculation of the rabbit superior cervical ganglia. These events might be consistent with our results. Indeed, 100% of the rabbits had iritis manifested by hyperemia of the iris at 24–48 hours after the initiation of our combined treatment (data not shown).

The supersensitivity produced by 6-HD related to reduction of intraocular pressure appears to involve both α and β receptors.^{23,24} If the action of epinephrine to reactivate HSV-1 harbored in the neural tissues were brought about via α and/or β receptors, specific adrenergic antagonists might inhibit the induced reactivation of HSV-1. Epinephrine iontophoresis altered the state of latent HSV-1 in the neural tissues,³ which suggests that the combined treatment might act by a similar mechanism. The adrenergic activation seems to suggest that the sympathetic superior cervical ganglion might be involved as the source of the virus. However, the sympathetic nerve endings also could stimulate the sensory fibers and serve as the trigger for trigeminal ganglion reactivation. Laties and Jacobowitz²⁵ suggested that the corneal adrenergic fibers might influence the sensory transmission. A third possibility is that both sympathetic and sensory ganglia might be triggered by an adrenergic mechanism.

Price¹⁷ reported that 6-HD given to mice intraperitoneally two days after HSV intraocular inoculation caused a decrease in the development of latency in the superior cervical ganglion. However, he found that more virus could be recovered from the superior cervical ganglion in the acute stage. To explain the concomitant increase in acute HSV replication and a decrease in latency, Price¹⁷ proposed that 6-HD-induced cell lysis in the ganglia during the productive infection that precluded a role for acutely infected cells as a viable reservoir for latent virus.

Our combined treatment (6-HD + epinephrine) might elicit continual viral shedding at the peripheral site if repeated at intervals after the first induced reactivation. Since epinephrine supersensitivity in the rabbit eye after 6-HD iontophoresis has been reported to return to normal after 3 weeks,¹² repeating the combined treatment (6-HD + epinephrine) might result in continual viral shedding. Perhaps, the combined treatment (6-HD + epinephrine) conducted continuously, or at repeated intervals, might eradicate or reduce the latent virus in the neural tissues, especially if combined with antiviral chemotherapy. Nesburn et al²⁶ recently have reported that intensive acyclovir treatment during epinephrine iontophoresis did not eradicate latent HSV infection from the trigeminal ganglia of rabbits.

The combined treatment induced viral shedding at the peripheral site within 24-hours after the initial treatment. Nesburn et al²⁷ detected HSV-1 shedding after the surgical stimulation of the rabbit trigeminal ganglia at 18 hours in 20% of the eyes, and within 24 hours in 70% of the eyes. HSV-1 sheddings in the precocular tear film of rabbits were initiated at 24 or 48 hours after the electrical stimulation of the trigeminal ganglion.²⁸ The onset of initial shedding is similar when these three different HSV-1 reactivation models are compared. However, the combined treatment (6-HD + epinephrine) produced the highest level of reactivation, probably by supersensitizing the ocular adrenergic nervous system.

The involvement of adrenergic mechanisms suggests that adrenergic agonists and antagonists should be studied. We currently are conducting such experiments in our laboratory. Preliminary experiments (unpublished data) indicate that iontophoresis of levo(–) epinephrine induces HSV-1 ocular shedding in latently infected rabbits with a higher frequency ($P < 0.05$) than that of dextro (+) epinephrine, which seems to be correlated to the receptor potency of these compounds.

In conclusion, iontophoresis of 6-HD combined with topical epinephrine produces HSV-1 ocular shedding in 100% of eyes. Since 6-HD produces a supersensitivity to adrenergic agonists, the data further implicate the

role of the adrenergic neural elements as a trigger for reactivation. This new model may be useful as a procedure for investigating adrenergic mechanisms involved in induction of HSV-1 ocular shedding and for elucidation of mechanisms of HSV latency, reactivation, and recurrence.

Key words: herpes simplex virus type 1, rabbit, eye, reactivation, 6-hydroxydopamine, epinephrine, iontophoresis, superior cervical ganglion

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