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Short Communication

Effects of acyclovir therapy during simultaneous reactivation of latent HSV-1 in rabbits

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Summary

Acyclovir (ACV) therapy with a simultaneous reactivation of latent HSV-1 was evaluated in HSV-1 infected rabbit eyes. When the latently infected rabbits received epinephrine iontophoresis into corneas without ACV therapy 100% of eyes shed virus into tear film. The shedding was initiated on the second day of the epinephrine iontophoresis and lasted for an average of 4.6 days. When the rabbits received ACV (60 mg/kg body weight) intravenously once daily and topically (5% ACV ointment) twice daily for 6 consecutive days while a 0.01% epinephrine solution was iontophoresed into cornea for the first 3 consecutive days, 33% (2/6) of eyes showed detectable HSV-1 in the tear film only after terminating the ACV therapy, and the duration of shedding was for only one day. The average quantity of virus detectable in the tear film was decreased 14-fold in the latter group compared to the epinephrine iontophoresis group without ACV therapy. Four days after the last ACV therapy the titer of HSV-1 in the cell-free homogenates of the trigeminal and superior cervical ganglia was determined. For the epinephrine iontophoresed group, 67% of ganglia (8/12) were HSV-1 positive, while only 33% (4/12) of the ganglia from the combined treatment group were HSV-1 positive. The difference was statistically significant ($p=0.034$). Furthermore, the titer of virus detectable in the cell-free homogenates of the virus-positive ganglia from the combined treatment group was less than that from the ganglia of the epinephrine iontophoresed group or untreated group. This suggests a reduction in the total number of latent foci for the combined treatment group.

HSV-1; Latency; Reactivation; ACV

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Acyclovir (ACV) is phosphorylated preferentially by herpes simplex virus (HSV) encoded thymidine kinase (TK) to its monophosphate form [2]. The ACV-monophosphate, in turn, is further phosphorylated by other kinases to its triphosphate form which is an inhibitor of the HSV-specific DNA polymerase [1]. Since the preferential phosphorylation of ACV by HSV-1 specific TK is the crucial step for its antiviral activity ACV will be effective against latent HSV only after the HSV-TK expression. One approach to eradication of latent HSV infection is to reactivate all the latent foci in neural tissues and at the same time destroy the virus or prevent its reinfection of other neurons. We have previously observed that epinephrine iontophoresis induces reproducibly HSV-1 shedding in the tear film in latently infected rabbits [5,6]. We have also known that the shedding resulted from the reactivation of latent HSV-1 in the neural tissues [3]. Therefore, we investigated the effect of ACV therapy with the simultaenous epinephrine iontophoresis on ocular HSV-1 shedding and on the amount of virus recovered from the neural tissues of latently infected rabbits. HSV-1, McKrae strain was inoculated into New Zealand albino rabbits as described previously [6]. Nine HSV-1 latently infected rabbits were chosen for this experiment. All eyes of the rabbits had at least one spontaneous shedding episode during post-inoculation (PI) days 40–80. The rabbits were randomly divided into 3 groups; group 1 received epinephrine iontophoresis only, group 2 received epinephrine iontophoresis plus ACV therapy and group 3 received no treatment.

Beginning on PI day 90, group I and group 2 received bilateral epinephrine iontophoresis [5] once a day for 3 consecutive days. Group 2 was treated with ACV for 6 consecutive days in addition to epinephrine iontophoresis. Therefore, ACV therapy and epinephrine iontophoresis in group 2 overlapped for the first 3 days, followed by 3 more days of ACV therapy alone. ACV was administered topically (5% ACV ointment) twice daily at 12 h intervals and intravenously (60 mg/kg body weight) once daily immediately after the first topical application. We employed topical treatment with intravenous administration to prevent or minimize reinfection of ganglia following virus replication on the cornea after reactivation, as indicated by Nesburn et al. [7]. The topical application was started 6 h prior to the bilateral epinephrine iontophoresis. Eye washes [6] were done immediately before ACV ointment twice daily at 12 h intervals for the determination of HSV titer shed into tear film from PI day 90 to PI day 100. On PI day 100 all 9 rabbits were sacrificed, and their trigeminal ganglia (TG) and superior cervical ganglia (SCG) were removed. The titer of HSV-1 in the neural tissue homogenates [3] was determined.

Fig. 1 shows the effect of ACV therapy on HSV-1 ocular shedding induced by epinephrine iontophoresis. HSV-1 shedding was detected in 100% of eyes of group 1 rabbits. The shedding was initiated on day 2 after the first epinephrine iontophoresis and lasted for an average of 4.6 days until day 8. The highest titer obtained was 9×10^3 PFU/wash. In contrast, HSV-1 was not detectable in group 2 eyes until terminating ACV admnistration. Two out of six eyes (33%) shed virus after terminating the ACV administration. One shed virus 2 days after, while the other, 3 days after the last ACV treatment. The duration of shedding was for only

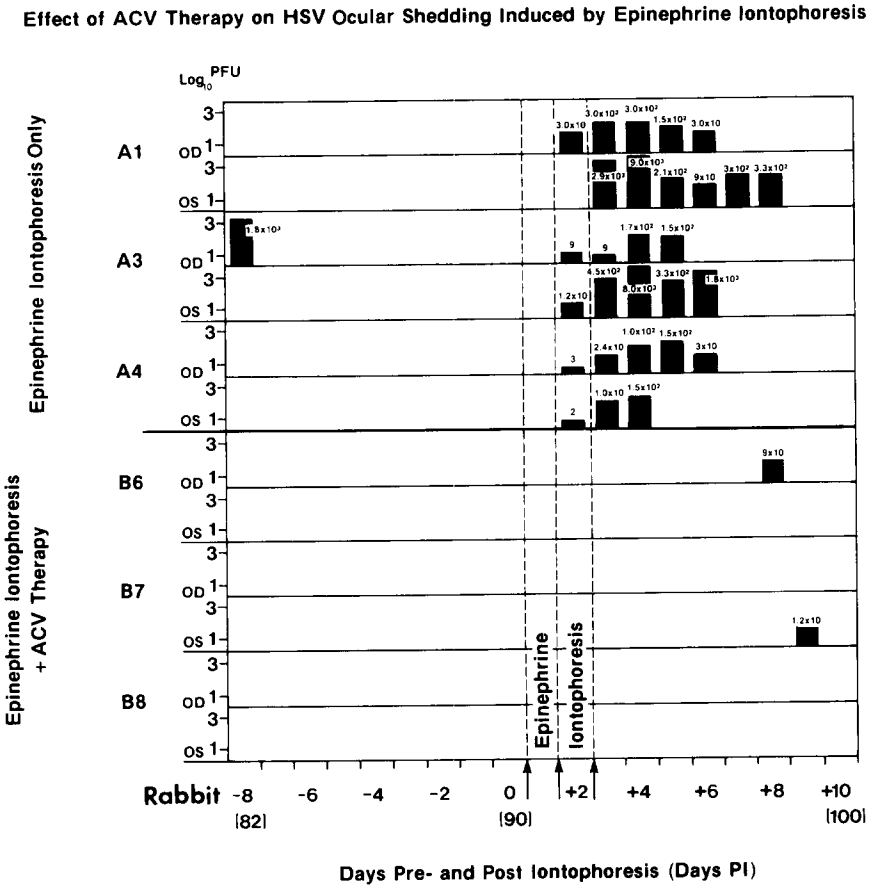

Fig. 1. Effect of ACV therapy on HSV ocular shedding induced by epinephrine iontophoresis ↑ ; days of epinephrine iontophoresis. For epinephrine iontophoresis the rabbits were anesthesized with 4 mg/kg of xylazine and 20 mg/kg of ketamine intramuscularly. An eye cup was inserted with its periphery within the limit of the corneal limbus. A 0.01% epinephrine solution was added to the eye cup. The cathode was attached to a shaved area of the trunk, and the anode made a wet contact with the epinephrine solution through a cotton wick. 0.8 mA was applied for 8 min. Eye washes were taken from PI day 80 to PI day 100 twice daily. Titers of virus were determined for each eye wash. To take the eye wash, a 0.1 ml of phosphate-buffered saline containing 2% bovine serum albumin was instilled into each eye. The closed eyelids were massaged against the eyeball to help break the tear film. The wash medium (0.5 ml) was again instilled onto cornea. The upper and lower cul-de-sacs were rinsed and the medium collected. This procedure was repeated 3 times using 1.5 ml of wash medium. A 0.2 ml aliquot of the wash media was used to determine HSV-1 titer on PRK monolayer. ACV therapy was performed for 6 consecutive days from PI day 90 to PI day 95. Each rabbit of group 2 (B6, B7 and B8) received 60 mg/kg of ACV once daily intravenously and 5% ACV ointment twice daily topically at 12 h intervals. A spontaneous viral shedding was observed on PI day 82 in the right eye of rabbit A3.

TABLE 1

Average titer[a] of HSV-1 recovered from tear film (PFU/wash).

Treatment	Days after 1st epinephrine iontophoresis										Average PFU from positive
	1	2	3	4	5	6	7	8	9	10	
Epinephrine iontophoresis (Group 1)	0	3.3×10	6.2×10^2	9.0×10^3	2.0×10^2	4.9×10^2	3.0×10^2	3.3×10^2	0	0	7.1×10^2
Epinephrine iontophoresis + ACV Therapy (group 2)	0	0	0	0	0	0	0	9×10	1.2×10	0	5.1×10

[a] An average titer indicates the mean HSV-1 titer among the shedding-positive eyes on the day indicated.

one day and the titer of virus in the tear film was only 1.2~9.0 × 10 PFU/wash. As shown in Table 1, the average titer of HSV-1 in tear film of group 1 increased gradually, reached peak on the third day of shedding and decreased thereafter. The average titer of HSV-1 in tear film was decreased 14-fold in group 2 compared with group 1. This result suggests that the reactivated HSV-1 was eliminated partly by ACV action. During the experimental period (PI day 90–100) one eye of group 3 rabbits shed virus. The shedding lasted for 3 days. The average titer of the spontaneous shedding was 1.1×10^3 PFU/wash.

On PI day 100 when HSV-1 was no longer detected from eye washes, TG and SCG of the 9 rabbits (groups 1, 2 and 3) were removed and incubated in Eagle's minimal essential medium (EMEM) containing 3% fetal bovine serum (FBS) at 37°C for 4 days. After incubation, the tissues were minced and sonicated with a Sonifier (Branson Ultrasonic Corp., Stamford, CT). TG was prepared in 1.5 ml EMEM (about 5% solution) and SCG in 1.0 ml EMEM (about 1% solution). Cell debris was removed by centrifugation at 5000 × *g* for 10 min at 4°C. A 0.5 ml aliquot of the supernatant of the tissue homogenate was inoculated onto a primary rabbit kidney cell (PRK) monolayer in a 60 mm Petri dish. The cultures were incubated for 2 h at 37°C in a CO_2 incubator and EMEM containing 7% FBS was added to the petri dishes for further incubation. The cells were observed for the appearance of cytopathic effect (CPE) consistent with HSV-1 infection for 7 days. The specimens which showed CPE were used to determine the viral titer employing the remaining portion of sample which had been stored at −70°C. As shown in Table 2, the percentage of HSV-1 positive ganglia was 67% (8/12) for both group 1 and group 3. Only 33% (4/12) of ganglia were positive for group 2 (epinephrine iontophoresis plus ACV therapy). The difference was statistically significant (p=0.034). A higher titer of HSV-1 was obtained from the group 1 (epinephrine iontophoresis only) when compared with the group 2 (150 vs. 10) or the group 3 (150 vs. 97). Titer of HSV-1 in group 2 tissues was also less than that in group 3 (97 vs. 10). These data suggest that ACV therapy with the simultaneous HSV-1 reactivation results in a reduction of the total number of latent foci.

Although we did not determine the ACV concentration of tear film we felt that the failure to detect tear film HSV during the therapy was not due to the residual ACV in the tear films. Eye washes were done immediately before each topical application. This corresponds to 12 h after topical and 12 or 24 h after intravenous administration of ACV. The tear film was further diluted into 1.5 ml of wash medium, 0.2 ml of which was employed to determine HSV titer. When this amount of the sample was spread over 60 mm Petri dish, the amount of ACV which each cell received is probably negligible. Furthermore, Nesburn et al. [7] reported that when known amounts of HSV were mixed with tear film samples from ACV- and placebo-treated eyes, no difference in antiviral activity was observed.

It has been shown that ACV therapy has no effect on an established HSV latency using systemic [8] or chronic oral administration [4] of ACV or on the recurrence of viral shedding or clinical disease course. Nesburn et al. [7] reported that when topical, oral and intramuscular administration of ACV was combined with concurrent reactivation by bilaterial epinephrine iontophoresis, the incidence

TABLE 2

Effect of ACV therapy with simultaneous epinephrine iontophoresis on the recovery of HSV from rabbit TG and SCG.

Group	Rabbit[a] Number	Treatment	Detection[b] frequency (HSV positives/total)	Titer[c] of HSV from ganglia				Average titer from positives (PFU/TG or SSG)
				TG		SCG		
				Right	Left	Right	Left	
	A1	epinephrine[d]		++	++	++	–	
1	A3	iontophoresis	67% (8/12)	–	++	+++	–	150
	A4	only		++	+++	–	+	
	B6	epinephrine[d]		+	–	–	–	
2	B7	iontophoresis	33% (4/12)[e]	–	+	–	–	10
	B8	+ ACV therapy						
	H9			++	++	–	+	
3	H10	no treatment	67% (8/12)	–	–	++	++	97
	H11			++	–	++	+	

[a] All the rabbits had been previously infected with HSV-1. No apparent ocular lesions remained as determined with a slit lamp biomicroscope. Every eye spontaneously shed virus at least once between PI days 40 and 80. The treatment was initiated at PI day 90.

[b] Ten days after initiation of treatment (PI day 90), the rabbits were sacrificed and TG and SCG were obtained. The tissues were incubated in EMEM and 3% FBS at 37°C for 4 days. At the end of the incubation time, the tissues were minced into fine pieces and sonicated. The supernatants were employed for plaque assay on PRK cell monolayers.

[c] Virus titers were expressed as follows: – = no virus detected; + = 1 to 50 :PFU/ganglion; ++ = 51 to 250 PFU/ganglian; +++ = 251 to 500 PFU/ganglion.

[d] Epinephrine iontophoresis was performed once a day for 3 consecutive day (PI days 90–92) at 0.8 mA for 8 min. ACV was given intravenously (60 mg/kg body weight) once a day and topically (5% ACV ointment) twice a day for 6 consecutive days (PI day 90–95). Therefore, the first 3 days overlapped between reactivation stimuli and antiviral therapy.

[e] The detection frequency of HSV-1 from group 2 ganglia was significantly less than that from group I or group 3 (p=0.034, chi-square analysis).

of HSV shedding was reduced but the treatment had no effect on the incidence of viral recovery from trigeminal ganglia. Our results are similar to those of Nesburn et al. [7] in showing reduction of HSV shedding but we differ in demonstrating less HSV recovery from ganglia. The disparity might be attributable to a difference in treatment method since they did not administer ACV through the intravenous route. Another consideration is that Nesburn et al. [7] began to treat the latently infected rabbits 36 days after the HSV inoculation while we started 90 days after HSV inoculation when the frequency of spontaneous shedding episode was reduced. Spontaneous HSV shedding decreases with time and stabilizes at a certain point after the inoculation.

In the present study we evaluated the effect of ACV therapy concomitant with simultaneous reactivation of latent HSV-1 in rabbits. The rationale of this approach was two-fold; (1) the epinephrine iontophoresis creates a maximum environment for the action of the antiviral drug ACV by inducing replication and HSV-

1 specific enzymes, including TK which activates ACV, and (2) metabolically altered ACV then inhibits HSV-1 replication and prevents HSV-1 from infecting other neurons.

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