Trifluridine Decreases Ocular HSV-1 Recovery, but Not Herpetic Lesions after Timolol Iontophoresis

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To determine the effect of a topically applied antiviral agent on shedding of herpes simplex virus type 1 (HSV-1) into the tear film and corneal epithelial lesions, ten rabbits latently infected with HSV-1 were subjected to transcorneal iontophoresis of 0.01% timolol once a day for 3 consecutive days to induce viral shedding and lesions. Iontophoretic induction was performed similarly in five uninfected rabbits as controls. Half of the infected rabbits and all of the uninfected controls received topical 1.0% trifluridine five times a day for 9 days, beginning the day after the first iontophoresis. All eyes were examined daily for 10 days by slit-lamp biomicroscopy and tear film samples collected on swabs were analyzed for virus. In the infected rabbits, the eyes treated with trifluridine had significantly fewer swabs positive for HSV-1 than the untreated eyes (P < 0.001); however, there was no significant difference in the numbers of lesions in the treated and untreated eyes. The uninfected controls had no positive swabs and developed no lesions. These results suggest that topical treatment with trifluridine may reduce recovery of HSV-1 from the tear film, but does not affect the incidence of iontophoretically induced corneal epithelial lesions. Invest Ophthalmol Vis Sci 30:678-683, 1989

Herpes simplex virus type 1 (HSV-1) causes an initial ocular infection followed by latency in the autonomic and sensory ganglia of the head and neck.1-3 Stimuli such as fever, UV radiation, stress and radial keratotomy are known to cause reactivation of viral shedding and often clinically detectable disease.4-6 Once the virus has achieved the latent state, chemotherapeutic strategies are ineffective in eliminating the virus from the host. Experimentally, Kaufman et al7 demonstrated no decrease in spontaneous HSV-1 recovery and detectable clinical disease with the concomitant administration of oral acyclovir or bromovinyl deoxyuridine. Nesburn et al8 showed a decrease in HSV-1 ocular recovery following induction with epinephrine iontophoresis and treatment with a combination of oral, intramuscular and topical acyclovir. Demangone et al9 demonstrated a decrease in induced HSV-1 recovery from tear film samples following epinephrine iontophoresis with concomitant intravenous and topical acyclovir, as well as a significant quantitative reduction in recovery of HSV-1 from the trigeminal ganglia. In contrast, Nesburn et al8 showed no significant qualitative reduction. In other words, although systemic and topical medications may have reduced the recovery of infectious HSV-1, there was no depletion of the neural reservoir of HSV-1. Kaufman et al7 examined the rabbits for spontaneous ocular recurrences and found no reduction in clinically detectable lesions, while Nesburn et al8 and Demangone et al9 reported no data on ocular lesions.

The question therefore remains, if elimination of the virus from the neural tissues is not possible due to its privileged site within the neuron, could induced recurrent disease be prevented with administration of topical antiviral medication? The current study was undertaken to determine whether the administration of topical TFT is effective in reducing HSV-1 ocular recovery and whether topical TFT is effective in reducing the development of recurrent ocular disease. We have previously demonstrated the effectiveness of iontophoresis of adrenergic agents in inducing both HSV-1 shedding in the tear film and the development of detectable corneal lesions.10-13 In this study rabbits latently infected with McKrae strain HSV-1 were induced to shed virus by three daily iontophoresis treatments with timolol. Eyes were concurrently treated with TFT daily for 9 consecutive days. Ocular recurrence was determined by daily examination with the slit-lamp biomicroscope.

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Materials and Methods

Virus

HSV-1 strain McKrae was propagated on primary rabbit kidney (PRK) cell monolayers and titered by plaque assay on green monkey kidney cell monolayers. The virus was partially purified from a freeze-thaw lysate of PRK. The virus was frozen in small aliquots at -70°C, and the same batch was used for inoculation of all rabbits.

Rabbits and Ocular HSV-1

The unscarified corneas of New Zealand white rabbits (1.5-2.5 kg) were inoculated with 25 µl of a suspension of HSV-1 strain McKrae (2-4 × 10⁶ PFU/ml). The lids were closed and the viral suspension was massaged on the corneal surface for 20-40 seconds with care being taken to avoid leakage of the suspension. The primary corneal infection (HSV-1 dendritic and geographic keratitis) was verified by slit-lamp biomicroscopic examination (SLE) on post-inoculation (PI) days 4-8. Spontaneous recurrent epithelial lesions during PI days 20-39 were documented by SLE. The lesions were characterized as deep punctate lesions, dendritic lesions or geographic epithelial defects. Spontaneous HSV-1 ocular shedding during PI days 20-39 was determined by the eye swab procedure. Animal care and treatment in this investigation was in compliance with the ARVO Resolution on the Use of Animals in Research.

Tear Film Swabs

Precorneal tear film was collected from the rabbit eyes on a sterile Dacron-tipped swab by gentle rotation of the swab in the upper cul-de-sac and then into the lower fornix, where the swab was allowed to absorb the tear film for 5 seconds. Care was taken to avoid swabbing the cornea to minimize damage to the corneal epithelium. The swabs were immediately placed in tubes containing tissue culture medium and confluent PRK cell monolayers and incubated for 18-24 hr at 37°C in a CO₂ incubator. Subsequently, the swabs were squeezed against the side of the tubes to remove excess medium and removed. Eagle’s minimum essential medium with 2% fetal bovine serum (1 ml) was added for nutrition and pH adjustment. The tubes were monitored daily for 7-9 days for the appearance of cytopathic effects indicative of HSV-1.

Timolol Iontophoresis

The rabbits were anesthetized by an intramuscular injection of 1-1.5 ml of a mixture of 10 ml of ketamine (100 mg/ml) and 2 ml xylazine (100 mg/ml). An eye cup filled with the timolol solution (0.01%) was centered within the limits of the corneoscleral limbus. The timolol solution was prepared each day by dilution of 0.5% Timoptic® (timolol maleate; 0.5%; Merck, Sharp and Dohme, West Point, PA) with sterile, deionized, distilled water. The anode (+) made contact with the timolol solution. The cathode (−) was attached to the ear over a saline-saturated gauze pad. Iontophoresis was performed at 0.8 mAmp for 8 min once daily for 3 consecutive days. After the timolol iontophoresis, the eyes were taped closed until the rabbits had recovered fully from the anesthetic.

Slit-Lamp Biomicroscopic Examination

Following swabbing to collect the tear film, all eyes had SLE performed after fluorescein staining to examine for HSV-1 corneal epithelial lesions beginning on the first day of iontophoresis, prior to treatment. The lesions were characterized as deep punctate lesions, dendritic lesions, or geographic epithelial defects. These induced lesions were similar to those observed to occur spontaneously in the rabbit. At the time of examination, the result of the eye swab was not known to the observer; thus all SLE data were obtained in a masked fashion with respect to eye swab data. SLE data from 48 hr after the last iontophoresis were used to compare lesion occurrence rates since in the control uninfected rabbits all corneas were judged normal by this time.

Experimental Design

Both eyes of all rabbits were used. In the latently infected rabbits, at least one eye of each rabbit developed typical HSV-1 dendritic epithelial keratitis during PI days 4-8. In the latently infected rabbits, at least one eye had a positive episode of spontaneous or induced ocular shedding during these experiments. Another group of five rabbits with no previous HSV-1 infection similarly received iontophoresis of timolol as a control. Both eyes received ocular iontophoresis of timolol once daily for 3 consecutive days. The PI day of first iontophoresis in the latently infected rabbits was day 68 in the untreated (non-TFT) rabbits and day 58 in the TFT-treated rabbits. All eyes were swabbed and had SLE performed once daily beginning on the day after the first iontophoresis and continuing for 10 consecutive days.

In five of the latently infected rabbits, one drop of 1.0% trifluridine, (Viroptic®, Burroughs Wellcome Co., Research Triangle Park, NC) was instilled in each eye five times per day at 7:00 AM, 9:30 AM, 12:30 PM, 3:30 PM, and 6:00 PM, beginning on the
31 day after the first iontophoretic treatment and continuing for 9 consecutive days. On days 6 and 7 after the first iontophoresis, four drops per day were instilled. Eye swabs and SLE in this group were performed immediately before the third drop application, 2.5 hr after the second trifluridine instillation.

In the five control uninfected rabbits, swabs were taken at the same time as in the latently infected rabbits, placed directly in 3 ml of culture media (no cells present), and placed into a CO2 incubator at 37°C. The culture media contained 2% fetal bovine serum. Swabs were removed from the culture tubes 24 hr after elution of any absorbed TFT from the swabs. Confluent microwell (96 wells/microwell plate II) cultures of CV-1 cells were infected by adding 50 μl of virus solution (500 PFU HSV-1 strain McKrae). Following incubation at 37°C for 45 min, residual virus was removed. Aliquots consisting of 200 μl of media from the eyes of each rabbit on each of 9 successive days were added to the wells. A total of 90 samples were tested. The plates were incubated for 48 hr at 37°C in the CO2 incubator and then examined for characteristic cytopathic effect.

In Vitro Drug Susceptibility of McKrae Strain HSV-1

The in vitro drug susceptibility of trifluridine was determined. Confluent microwell cultures of CV-1 cells were infected by adding 50 μl of HSV-1 strain McKrae (500 PFU). Following incubation at 37°C for 45 min, residual virus was removed. Two hundred microliters of 1% trifluridine serially diluted in Eagles minimal essential medium (E-MEM) with 2% fetal calf serum (400 μg/ml to 0.07 μg/ml, 2-fold dilutions) was added to quadruplicate wells. For each drug dilution uninfected cell controls, virus infected, and drug free controls were prepared simultaneously. The plates were incubated for 48 hr at 37°C in a CO2 incubator. At the end of the incubation time, the drug concentration which protected the cells from virus challenge was determined to be the minimum inhibitory concentration of the drug.

Results

Table 1 shows the history of acute HSV-1 dendritic epithelial keratitis, spontaneous HSV-1 ocular shedding, spontaneous recurrent epithelial lesions and the results of timolol iontophoretic induction of HSV-1 ocular shedding and corneal epithelial lesions. During PI days 20–39 spontaneous ocular shedding was determined by eye swabs. In all rabbits, at least one eye of each rabbit had at least one positive episode of spontaneous HSV-1 ocular shedding.
In the TFT-treated group (Table 2), 10/10 eyes shed HSV-1 spontaneously during PI days 20-39. In the untreated group, 9/10 eyes shed spontaneously. By chi-square analysis, there was no significant difference between the TFT-treated and the untreated groups (P > 0.05). In the TFT-treated group, 8/10 eyes developed recurrent HSV-1 corneal lesions spontaneously during PI days 20-39. In the untreated group, 6/10 eyes developed corneal lesions spontaneously. By chi-square analysis, there was no significant difference in the development of spontaneous recurrent HSV-1 corneal epithelial lesions between the TFT-treated and the untreated groups (P > 0.05) during PI days 20-39.

Eye swabs were performed daily for 10 days beginning on the day after the first iontophoresis in the TFT-treated group and for 9 days beginning on the second day after the first iontophoresis in the untreated group. In the TFT-treated group, the ratio of swabs positive for HSV to total swabs was 6/91 (6.6%) compared with 32/89 (36%) in the untreated group. By chi-square analysis, there was a significant difference between the two groups (P < 0.001). In the TFT-treated group there were 46 induced lesions per 70 days of observation compared with 37/70 in the untreated group. By chi-square analysis, there was no significant difference between the two groups (P > 0.05). In the uninfected control eyes, there were no lesions detected 48 hr after the last iontophoretic treatment. When only geographic lesions were analyzed, there were 42 lesions per 70 days of observation in the TFT group and 9/70 in the untreated group. By chi-square analysis, the difference was significant (P < 0.05).

The eluent from the uninfected rabbit tear swabs which were treated with TFT was not inhibitory for CV-1 incubated for 48 hr at 37°C. The minimal inhibitory concentration of TFT for the McKrae strain HSV-1 was determined to be 12.5 ng/ml.

### Table 2. Spontaneous and timolol-induced HSV-1 ocular shedding and epithelial lesions (TFT-treated)

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>Eye</th>
<th>Acute dendritic keratitis</th>
<th>Spontaneous ocular HSV-1</th>
<th>Spontaneous recurrent lesion</th>
<th>Days after first iontophoresis of timolol</th>
<th>Totals days 4-10</th>
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* TFT five times per day for 10 consecutive days except on days 5 and 6, four times per day.

† For this and the rest of the columns through day 10, the notation is as follows: The numerator (to the left of the slash) represents the results of the swabbing; + indicates a positive swab, ie, the presence of ocular HSV-1 shedding, and - indicates a negative swab, ie, the absence of ocular HSV-1 shedding. C indicates a contaminated swab. The denominator (to the right of the slash) represents the results of slit lamp examination: + indicates a positive examination, ie, observation of a lesion, and - indicates a negative examination, ie, no observed lesion. The subscripts associated with the + indicate the type of lesion: D, dendritic lesion; G, geographic lesion; P, punctate lesion. 1 Indicates iontophoresis.
Table 3. Timolol iontophoresis (uninfected, normal eyes; TFT-treated)

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>Eye</th>
<th>Days after first iontophoresis of timolol</th>
<th>Days 1-10 totals</th>
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<td>OS</td>
<td>01* + 02* + 03* + 04* + 05* + 06* + 07*</td>
<td>0</td>
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<tr>
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<td></td>
<td>OS</td>
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<td>0</td>
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</tr>
<tr>
<td></td>
<td>OS</td>
<td>01* + 02* + 03* + 04* + 05* + 06* + 07*</td>
<td>0</td>
</tr>
</tbody>
</table>

* TFT 5 times per day for 10 consecutive days except on days 5 and 6, four times per day.

This table represents the results of slit-lamp examination; + indicates a positive examination, ie, observation of a lesion, and − indicates a negative examination, ie, no observed lesion. The subscript “G” associated with the + indicates a geographic lesion. No dendritic or punctate lesions were observed. I Indicates iontophoresis.

that adrenergic agents are effective in inducing viral shedding and disease led to the hope that adrenergic blockers would maintain the latent state. However, to date, investigations in this area have produced no inhibitors of HSV-1-induced reactivation and recurrence. In fact, we have reported that iontophoresis of timolol, a beta-blocking agent, is as effective as epinephrine in inducing viral shedding and recurrent HSV-1 specific corneal epithelial lesions.10,14,15

If the latent virus cannot be eliminated and if reactivation cannot be prevented, might it be possible to prevent the reactivated virus from causing detectable disease? Kaufman et al7 demonstrated that systemic acyclovir and BVDU were ineffective in this regard. The results of this study demonstrate that while viral recovery from the ocular surface was reduced by topical TFT, epithelial lesions were not reduced. These lesions may not be analogous to those recurring in human disease. Important to note, however, is that while HSV-1 was recovered less frequently in the TFT-treated group, rabbit corneal epithelial lesions were observed. This suggests that the topical treatment had an effect on viral replication on the ocular surface, but that the disease was probably produced proximal to this, at the site of HSV-1 viral release at the free nerve endings within the intracellular space in the epithelium and subepithelium, and not by viral invasion of the epithelium from virus released in the tear film. We have shown that corneal nerves are essential in adrenergic HSV-1 reactivation in rabbits.19 The reduction in HSV-1 recovery could possibly be the result of a direct antiviral effect of the TFT collected on the swab, although the swab was taken 2.5 hr after the previous drop application, by which time most of the drug would have been eliminated.

Our controls demonstrated that there was no antiviral activity of the swab eluate at the time of swabbing.

 Clinically, it may seem intuitively logical that long-term administration of antiviral drugs should prevent or at least ameliorate recurrent disease and reduce complications. These results, however, suggest that chronic antiviral coverage in patients with HSV ocular disease may have no effect on the frequency of recurrences, despite a reduction in virus recovery from the tear film. Further studies employing a model which does not include direct damage to the cornea, as iontophoresis does, are required to definitively answer this question.

Key words: HSV-1 reactivation, iontophoresis, rabbit, recurrent corneal epithelial lesions, trifluridine

References


