Optic Nerve Involvement in Viral Spread in Herpes Simplex Virus Type 1 Retinitis

Marc E. Dosem,* Ryan Harris,* and Sally S. Atherton†

Colchicine was used to block fast axonal transport of the optic nerve of the uninjected eye after unioocular anterior chamber inoculation of herpes simplex virus type 1 (HSV-1). The results of these experiments suggest that second-wave virus is transported to the retina of the uninjected contralateral eye through the optic nerve of the uninjected eye, since intravitreal treatment with colchicine reduced the viral titer and prevalence of animals with histopathologic evidence of retinitis. The chemical block to viral entry provided by colchicine was both dose- and time-dependent, with administration of colchicine that was timed to interfere with the second viral wave being the most effective. Colchicine did not appear to be directly inhibitory to viral replication or toxic to the virus, and colchicine did not cause clinical or microscopic evidence of retinal inflammation. Intravitreal injection of colchicine did not alter the timing or recovery of the first wave of virus.

After unioocular anterior chamber inoculation of herpes simplex virus type 1 (HSV-1), retinitis followed by retinal necrosis develops 10–14 days later in the uninoculated contralateral eye.1 The virus reaches the fellow eye in two waves: (1) an early low-titer wave within 24 hours of inoculation and (2) a later, higher-titer wave which begins on day 7, reaches a peak titer on day 10 after inoculation, and coincides with the acute phase of retinitis.2 Not surprisingly, only contralateral eyes with high viral titers (average, 5.57 log10 plaque-forming units [PFU]/ml) develop retinitis.3 Results of investigations conducted in this and other laboratories suggest that one route by which second-wave virus reaches the contralateral eye involves sequentially the optic nerve of the ipsilateral eye, the brain (presumably through the optic tracts and projections), and the optic nerve of the contralateral eye.

The virus has been shown to reach the central nervous system after unioocular uniconal anterior chamber inoculation of HSV-1.2,4,5 Investigations to trace the viral path from the central nervous system to the uninoculated eye via the optic nerve have relied on isolation of infectious virus or viral DNA from the optic nerve2 or on immunoperoxidase staining to detect viral antigens in the nerve.6 It appears that most of the virus associated with development of retinitis arrives as part of the second wave. The first evidence of the second wave is expression of viral antigens on the ganglion cells of the retina, a finding which further supports the hypothesis that the contralateral optic nerve transmits the second wave of virus6,7 (Dix and Atherton, unpublished observations).

One way to demonstrate that the optic nerve is involved in transport of the second wave of virus would be to sever the optic nerve of the uninjected eye and demonstrate that this eye has lower titers of virus or a decreased incidence of retinitis. However, it is difficult to transect the optic nerve of a mouse and leave the blood supply to the retina intact. An alternative strategy would be to block axonal transport with a chemical and demonstrate that treated eyes had lower viral titers and a decreased incidence of retinitis. Colchicine has been shown to block fast axonal transport of the optic nerve after intravitreal injection.8

We took advantage of the axonal-transport-blocking capabilities of colchicine to examine the role of the optic nerve in viral transport to the uninoculated eye after anterior chamber inoculation of HSV-1. Our results demonstrate that blocking the optic nerve with colchicine prevents entry of only second-wave virus without affecting first-wave virus and support the hypothesis that second-wave virus reaches the contralateral eye from the central nervous system via the optic nerve. Because such treatment lowered viral titers and resulted in a lower incidence of retinitis in colchicine-treated eyes, these results confirm that second-wave virus accounts for the high titers of virus.
Materials and Methods

Virus

The KOS strain of HSV-1 was used throughout these experiments. Viral stocks were propagated in Vero cells grown in complete growth medium (CGM) consisting of Dulbecco’s minimal essential medium and containing 5% calf serum and antibiotics. An aliquot of each viral stock was titrated in six-well plates (Costar, Cambridge, MA) using standard plaque assay methods, and the titer was calculated in PFU/ml. Viral stocks were frozen in small amounts and stored at −70°C. A new aliquot of titered stock virus was thawed and used for each experiment.

Animals

Adult, female BALB/c mice, 6–12 weeks of age (Taconic, Germantown, NY), were used in these experiments. Mice were kept in a standard laboratory environment and given unrestricted access to food and water. All animal procedures were done using pentobarbital (0.65 mg/10 g body weight) as the anesthetic agent and were performed in accordance with the ARVO Resolution on the Use of Animals in Research.

Colchicine

Colchicine (Sigma, St. Louis, MO) was used throughout these experiments. Colchicine (molecular weight, 399.45) was dissolved in CGM at a concentration of 0.1 M (40 mg/ml). A 2-μl injection of this solution contained 80 μg of colchicine. The solution of colchicine was frozen in small amounts and stored at −70°C. A new aliquot of colchicine solution was used for each experiment. Lower doses of colchicine were prepared by making either a 1:10 (8 μg/2 μl) or a 1:100 (0.8 μg/2 μl) dilution of an aliquot of the 0.1 M stock solution.

Virus Inoculation

The anterior chamber of the right eye was inoculated using previously described methods. The eye was proptosed, and the aqueous was removed by paracentesis. The anterior chamber of the right eye of each mouse received an inoculum of 1–2 × 10⁴ PFU of HSV-1 contained in a total volume of 4 μl.

Colchicine Inoculation

The vitreous chamber of the left eye was inoculated by propoting the eye of the mouse and piercing the sclera. Intravitreal inoculations were placed laterally into the vitreous body behind the lens as described previously. The vitreous of the eye received 2 μl in all inoculations, but the dose of colchicine was varied as described above. This method of inoculation was also used for control experiments in which tissue culture medium was injected into the vitreous of the left eye.

Preparation of Tissues for Histopathology or Recovery of Infectious Virus

After clinical examination, the animals were killed. The left eyes were removed and fixed in phosphate-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Eyes were considered positive for retinitis if the microscopic architecture of the retina of the uninoculated eye was destroyed by an inflammatory process characterized by the presence of a large number of inflammatory cells, cellular infiltrates, and fibrinous exudate as described previously.

On day 10 after the inoculation (the day on which the maximum viral titer is recovered from the uninoculated eye in mice injected with virus via the anterior chamber route), ocular tissues for viral recovery were collected and stored at −70°C. Tissues to be titrated were thawed and homogenized in CGM using a 1.0-ml tissue homogenizer. Because of the uniform size of the eyes, each ocular sample was homogenized in 1.0 ml of CGM. Aliquots of 0.1 ml of the homogenized tissues (either neat or diluted serially in CGM) were adsorbed to monolayer cultures of Vero cells at 37°C for 1–2 hr. After adsorption, the plates of cells were overlaid with CGM containing 0.5% Sea-Plaque agarose (FMC Corporation, Rockland, ME). After 5 days, the plates were fixed with buffered formalin and stained with 0.13% crystal violet. The plaques were counted, and the titer was expressed as PFU/ml of homogenized tissue. Viral-recovery results were analyzed for significance using student’s t-test.

Results

Effect of Dose of Colchicine on Day-10 Viral Titers

Since colchicine has been demonstrated to block fast axonal transport and since previous results suggested that the optic nerve is involved in viral transport from the central nervous system to the uninoculated eye after anterior chamber inoculation, we attempted to determine if intravitreal injection of colchicine would prevent the virus from reaching the uninoculated eye. Groups of at least four mice were injected with varying doses of colchicine (2 μl containing 80 μg, 8 μg, or 0.8 μg) in the vitreous of the left eye.
day-10 titers of HSV-1 in the left eye for each group of animals. When colchicine was injected on or before day 7, the viral titer in the left eye on day 10 after inoculation was reduced significantly from that recovered from the left eyes of the HSV-1 injected, but not colchicine-treated controls (P < 0.05 or better).

There was no significant reduction in day-10 viral titers when colchicine was administered intravitreally on day 8. In addition, the administration of colchicine immediately before killing on day 10 did not alter significantly the viral titer from that recovered from the uninjected eye of control mice on day 10. These results suggest that colchicine is effective only when administered immediately before or at the beginning of the second wave of virus. After the arrival of the second wave of virus, administration of colchicine does not affect the day-10 viral titers significantly. Our results of viral recovery from animals injected with colchicine on day 8 and from mice injected with colchicine immediately before death together suggest that the reduction in the day-10 titer observed in mice treated on or before day 7 is not likely to be due to direct killing of the virus by colchicine or by colchicine affecting the retina in a manner which prevents viral replication.

Effect of Intravitreal Injection of Colchicine on Retinal Architecture

To determine whether intravitreal injection of colchicine alone would produce changes in the microscopic architecture of the retina of the un inoculated eye, which might account for the lower viral titers in

### Table 1. Effect of dose of colchicine on day 10 virus titer

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Dose of colchicine</th>
<th>Day of intravitreal colchicine injection</th>
<th>Day 10 virus titer (log_{10} PFU/ml ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>0.8 μg</td>
<td>-1</td>
<td>5.29 ± 0.43</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>8.0 μg</td>
<td>-1</td>
<td>3.98 ± 0.83</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>8.0 μg</td>
<td>+5</td>
<td>4.36 ± 0.91</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>80.0 μg</td>
<td>-1</td>
<td>3.96 ± 0.41</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>80.0 μg</td>
<td>+5</td>
<td>2.65 ± 0.27*</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>4.90 ± 0.49†</td>
</tr>
</tbody>
</table>

* Significantly different from group 6 (untreated controls), P < 0.001.

† Not different from the average titer of virus recovered from the uninoculated eye of mice with retinitis (see ref. 3).

### Table 2. Effect of time of colchicine administration on day 10 virus titer

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Day of intravitreal colchicine injection</th>
<th>Day 10 virus titer (log_{10} PFU/ml ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>+3</td>
<td>2.94 ± 0.43*</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>+5</td>
<td>2.65 ± 0.27†</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>+7</td>
<td>2.55 ± 0.34†</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>+8</td>
<td>4.12 ± 0.39†</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>+10</td>
<td>4.60 ± 0.42†</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>—</td>
<td>4.90 ± 0.49†</td>
</tr>
</tbody>
</table>

* Significantly different than day 10 colchicine-treated mice, P < 0.05.
† Not significantly different than day 10 colchicine-treated mice, P < 0.005.
‡ No significant difference between these groups and group 6 (untreated day 10 controls).
Fig. 1. Photomicrograph of an eye of an uninfected BALB/c mouse 5 days after intravitreal injection of colchicine demonstrating that intravitreal injection did not cause retinal inflammation or retinal destruction (A). Focal disruption of the retina without accompanying inflammation was observed at the site of colchicine injection (B). Hematoxylin and eosin, X160.
this eye, the left eyes of mice were injected with colchicine intravitreally or, for comparison, with an equivalent volume of tissue culture medium. The right eye was not manipulated or injected with the virus. The colchicine- or tissue culture medium-injected eyes were harvested 5 days after intravitreal injection. After histopathologic preparation and staining, the eyes of both control and colchicine-treated mice were examined microscopically. As shown in Figure 1A, intravitreal injection of colchicine in the absence of virus did not result in retinal inflammation or in destruction of the retina. Examination of ocular sections that included the site of colchicine injection revealed focal retinal disruption that appeared to be the result of the intravitreal injection piercing the retina (Fig. 1B). This conclusion seems reasonable since similar focal disruption of the retina was also observed in the posterior segment of left eyes injected with tissue culture medium alone. Like the injection of colchicine alone, intravitreal inoculation of tissue culture medium did not result in retinal inflammation or destruction (data not shown). In no case did intravitreal inoculation of colchicine or tissue culture medium result in a pattern of inflammation resembling the destructive retinitis observed in the uninoculated eyes of mice injected with HSV-1 via the anterior chamber route.

Effect of Intravitreal Colchicine on Retinitis in the Uninoculated Eye

The results from viral-recovery studies of the left eyes of mice treated with colchicine suggest that intravitreal treatment of the uninoculated eye with colchicine blocked the second wave of virus. Previous results from this laboratory linked high viral titers with the second wave and the development of retinitis. Since only low viral titers were recovered from the eyes of colchicine-treated animals, we hypothesized that mice treated with colchicine intravitreally would not have retinitis in the contralateral eye. To address this question, we injected a group of ten mice with HSV-1 in the anterior chamber of the right eye on day 0 and then injected colchicine intravitreally in the left eye on day 5 after inoculation. A control group of 12 mice was injected with HSV-1 alone. On day 10, all of the uninoculated eyes were examined clinically for retinitis characterized by obliteration of the retinal vasculature, hemorrhage, and loss of retinal transparency. The incidence of retinitis was 30% (3 of 10) in the colchicine-treated group compared with 91.6% (11 of 12) in the untreated control group (P < 0.006). After clinical examination, all animals were killed. The left eyes were removed, and the clinical results were confirmed by microscopic examination of sections of the uninoculated eyes or by viral plaque assay. Any eye with a titer ≥ 5.0 log_{10} PFU/ml was considered to be positive for retinitis. The rate of retinitis in the control group was comparable to that reported previously in this model.

Effect of Colchicine on First-Wave Virus

Injection of colchicine prevented entry of the second viral wave into the uninoculated eye when the colchicine was administered immediately before (day 5) or coincident with (day 7) the arrival of the second wave of virus. Since these results suggested that the optic nerve was an important route of spread of second-wave virus from the central nervous system to the uninoculated eye, we hypothesized that if early (within the first 24 hours) viral spread from the injected eye to the uninjected eye was also through the optic nerve, then injection of colchicine before day 1 might affect the titer of first-wave virus. To answer this question, two panels of six mice were used. One group of animals was injected intravitreally with colchicine in the left eye one day before (day −1) inoculation of HSV-1 into the right eye. Another group of six mice was given tissue culture medium intravitreally in the left eye on day −1. All mice were injected with HSV-1 in the anterior chamber of the right eye on day 0. All animals were killed 24 hours after inoculation when first-wave virus is recovered routinely from the uninjected eye of animals inoculated with HSV-1 via the anterior chamber. Eyes were cultured for viral recovery as described. As Table 3 shows, there was no significant difference in the day-1, first-wave viral titers in the left eye between the tissue culture medium-treated and the colchicine-treated mice. These results suggest that the optic nerve of the contralateral eye is not involved in the passage of first-wave virus into the uninjected eye.

Discussion

Our results present further evidence which supports the hypothesis that the optic nerve is involved in transmission of virus into the contralateral eye from the central nervous system after uniocular anterior chamber inoculation of HSV-1. Several points deserve special emphasis. Although we and others

Table 3. Intravitreal colchicine does not affect titer of first wave virus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals</th>
<th>Day 1 virus titer (log_{10} PFU/ml ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue culture</td>
<td>6</td>
<td>2.35 ± 0.29</td>
</tr>
<tr>
<td>Colchicine</td>
<td>6</td>
<td>2.01 ± 0.11</td>
</tr>
</tbody>
</table>


have suggested previously that, in the mouse, the optic nerve is involved in the transport of HSV-1 to the uninoculated eye from the brain, it has been difficult to implicate this nerve directly. Olson and coworkers demonstrated in the rabbit that, after anterior chamber inoculation of HSV-1, an intact optic nerve was required for the development of retinitis in the uninoculated eye. They also showed that surgical transection of the optic nerve of the uninjected eye prevented retinitis. In larger species, such as the rabbit, transection of the optic nerve can be done fairly easily with an extracranial approach. However, in smaller species, such as the rat and mouse, a transection which spares the vascular supply of the retina can only be done with an intracranial approach with removal of a portion of the brain tissue. In the mouse, such surgery is difficult and traumatic, and mortality is high.

Because of these difficulties, we chose colchicine as a less traumatic alternative strategy to block viral transport in the optic nerve. Such an approach has been used in other systems to impede fast axonal transport of the proteins of the retinal ganglion cells. As has been shown, HSV enters or leaves sites of infection such as the eye by axonal transport of the nerves supplying the area of infection. Therefore, using colchicine to block or reduce axonal transport of HSV-1 into the left eye after viral inoculation of the anterior chamber of the right eye provided a reasonable and relatively nontraumatic approach to studying viral transport into the contralateral eye by the optic nerve. By itself, colchicine injection into the vitreous of an uninfected mouse does not result in retinitis or in any pattern of retinal pathology resembling that seen in mice with retinitis and subsequent retinal necrosis. There is little microscopic evidence of the relatively acute (within 5 days) effect of intravitreal colchicine injection in the mouse eye. The inhibitory effect of colchicine on microtubule formation and synthesis of ganglion cell proteins is well documented. It is possible that these effects would prevent HSV-1 from replicating in the retina once virus has entered the uninjected eye or that these effects would be directly toxic to the virus. However, such direct effects on the virus seem to be unlikely, since treatment of eyes with colchicine on day 8 after inoculation did not reduce significantly the viral titer recovered on day 10. In addition, the viral titers recovered from mice injected intravitreally with colchicine a few hours before killing on day 10 were not reduced from those recovered from untreated mice on day 10. These results favor the hypothesis that colchicine prevents viral entry into the eye from the central nervous system rather than the hypothesis that colchicine is inhibitory to the virus once the virus has reached the eye.

When taken together with our previous results and those of Whittum-Hudson and Pepose, the results of these experiments provide further evidence to support the hypothesis that, in the mouse, the optic nerve is the route by which the second wave of virus reaches the uninoculated eye and causes retinitis. These results are also supported by the findings of Olson and coworkers who showed that, in the rabbit, an intact optic nerve was required for the development of retinitis in the uninoculated eye after anterior chamber inoculation of HSV-1. In our experiments, it is not surprising that most of the mice treated with intravitreal colchicine did not have microscopic evidence of retinitis in the colchicine-treated eye because only low viral titers were recovered from comparable groups of mice whose eyes were cultured for viral recovery. We recently showed a correlation between the clinical observation of retinitis and high titers of HSV-1 in the uninoculated eye.

There are at least three reasons why low viral titers are still recovered on day 10 after inoculation from the eyes treated with intravitreal colchicine on day 5: (1) first-wave virus may persist and be recovered as a low viral titer on day 10; (2) other nerves transmit a small part of the second viral wave and are unaffected by intravitreal colchicine; or (3) colchicine does not completely block fast axonal transport. The first explanation is supported by our earlier finding that, in the absence of a second viral wave, a low viral titer was still recovered on day 10. However, the second and third explanations may also be applicable. Although intravitreal inoculation of colchicine would be expected to block mainly fast axonal transport in the optic nerve, it is possible that the other nerves which supply the eye (trigeminal, trochlear, and the somatic and the visceral motor components of the oculomotor) are unaffected by intravitreal colchicine treatment and transport a low viral titer to the uninoculated eye. It has also been shown previously that colchicine does not block all fast axonal transport, so the low viral titer recovered from colchicine-treated eyes on day 10 may result from a small amount of virus that enters the uninoculated eye because fast axonal transport in the optic nerve is incompletely blocked. Without complete interruption of axonal transport in the optic nerve (such as would be accomplished by surgical transection), it is not possible to differentiate among these possibilities and to identify the source(s) of the low viral titer recovered on day 10 from colchicine-treated eyes.

The results of these experiments also suggest that
the optic nerve is not involved in transmission of first-wave virus from the injected to the uninjected eye. They also confirm our earlier finding that virus or viral DNA could not be recovered from the optic nerve of the uninjected eye until day 5. This time corresponds to the arrival of the second wave of virus. Therefore, the route of the first wave of virus into the uninjected eye is likely to be by a non-optic nerve pathway, but identification of the route of the first viral wave awaits further study.

**Key words:** HSV-1, retinitis, mouse, optic nerve, colchicine

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**References**


