of the minimum oxygen level needed to avoid edema may explain why even the present generation of highly oxygen-permeable lenses still induce significant corneal edema.12

Key words: cornea, minimum oxygen tension, edema, individual variation, contact lenses

Acknowledgments. The authors thank Bruce Cook for his helpful comments and Stuart Ingham for assistance with the manuscript preparation.

From the Cornea and Contact Lens Research Unit, School of Optometry, University of New South Wales, Sydney, N.S.W., Australia, and the Department of Ophthalmology, Medical School, University of Otago, Dunedin, New Zealand. Supported by Optometric Vision Research Foundation Grant 82/03. Submitted for publication: February 22, 1983. Reprint requests: Brien A. Holden, PhD, Cornea and Contact Lens Research Unit, School of Optometry, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033, Sydney, Australia.

References

Human InterferonαA or αD and Trifluridine Treatment for Herpetic Keratitis in Rabbits

Melvin D. Trousdale and Anthony B. Nesburn

Recombinant human interferon (IFN) αA and αD combined with 1% trifluridine ophthalmic solution gave beneficial results when applied topically at a dose of 1 x 10^6 U per eye four times a day commencing 4 hr after eyes were inoculated with herpes simplex virus (HSV-1). Acute herpetic keratitis was suppressed by trifluridine alone and the combined therapies, but the high-titered interferon preparations, alone, had little effect. Duration of HSV-1 shedding into tear film during topical treatment for acute herpetic keratitis was reduced slightly by combined therapy with either IFNαA or IFNαD with trifluridine. Invest Ophthalmol Vis Sci 25:480–483, 1984

Recent advances in genetic engineering technology have resulted in the availability of large quantities of recombinant IFN of very high potency. Smolin et al4 reported that topical treatment of herpetic keratitis with cloned IFN A was effective in suppressing epithelial damage. This report describes the topical use of purified, high-titered, recombinant-derived human IFNα preparations of both IFNαA and IFNαD for treatment of acute herpetic keratitis in rabbits. Each was employed alone and in combination with Viroptic, 1% Trifluridine (TFT), ophthalmic solution.

Materials and Methods. Ocular Infection: New Zealand white male rabbits, weighing 3 to 4 kg, conforming to the ARVO Resolution on the Use of Animals in Research were used for all experiments. Both eyes of all rabbits were examined with a slit-lamp biomicroscope to confirm the absence of any external pathology prior to inoculation with HSV-1 (McKrae strain, 10^6 PFU/eye).

Antiviral Treatment: Lyophilized recombinant IFNαA and recombinant IFNαD were reconstituted
in a normal serum albumin solution (1 mg/ml) at 5 μg/ml (equivalent to approximately 1 × 10⁶ units/ml for IFNαA). Beginning 4 hr after inoculation, both eyes were treated with one drop (0.05 ml) four times per day (8 AM, 10 noon, 4 PM, and 10 PM) of the appropriate substances for 14 consecutive days. When combination therapies were given, the two treatments were given approximately 15 min apart. Treatment groups of five to six animals (10 to 12 eyes) were as follows: IFNαA; IFNαD; Viroptic ophthalmic solution, 1% TFT; TFT plus IFNαA; TFT plus IFNαD; and placebo (normal serum albumin solution, 1 mg/ml).

Ocular Examination: Eyes were examined daily in a masked fashion by the same observer using a 0 (normal) to 4+ (most severe) grading system as previously described. The daily scores (day 0–13) represent an average score for eyes in each treatment group.

One-way analysis of variance was performed for each clinical parameter at each clinical time point to detect differences due to treatment regimens. If significant differences (P < .05) were found, then Newman-Keuls multiple comparisons were performed to isolate which means were the ones that produced the significant result.
Isolation of HSV from Tear Film and Tissue: Tear film specimens for virus isolation were collected each day before the first treatment using the swab method. Trigeminal ganglia were removed aseptically and processed for virus isolation as previously described. All degenerating, suspicious, and positive cultures were passed in secondary rabbit kidney cells and virus identity confirmed by serum neutralization studies.

Results. When HSV-1 (McKrae) was inoculated into New Zealand white male rabbit eyes without scarification, acute herpetic keratitis was apparent within 3 days. Placebo-treated eyes (open circles) demonstrate the typical pattern of corneal epithelial involvement (Fig. 1a), conjunctivitis (Fig. 1b) and iritis (Fig. 1c) progressing to maximum severity about 5 to 7 days postinoculation. Maximum corneal云d (stromal keratitis and edema) developed slightly slower (Fig. 1d). Pathological appearance of the eyes slowly abated during the period of 8 to 14 days after inoculation; except for the presence of some conjunctivitis, eyes appeared normal by day 14. Eyes treated with topical IFNaD appeared slightly better but not significantly different (P > .05) from those treated with IFNaA or placebo. Eyes treated topically with TFT, TFT/IFNaA, or TFT/IFNaD (solid symbols) did not develop detectable corneal epithelial involvement (Fig. 1a), conjunctivitis (Fig. 1b), iritis (Fig. 1c), or corneal clouding (Fig. 1d).

Fifty percent or more of the tear film samples from eyes treated with IFNaA, IFNaD or placebo were negative for infectious HSV by day 9 (Table 1). The duration of virus shedding in tear film samples was reduced by TFT and even more by combination therapy with TFT/IFNaA or TFT/IFNaD (≥50% virus-negative tear films on days 8, 7, and 5, respectively). All eyes receiving the combination therapies stopped shedding detectable virus by day 7 or 8 following inoculation. The median day until an eye became negative for virus shedding was found to be significantly different (P < .001) for the combined treatment compared with all other treatment groups.

When rabbits were killed 21 to 23 days postinoculation, HSV-1 was isolated from trigeminal ganglia regardless of the type of topical therapy employed (Table 2). There was no significant difference between the treatment groups (P > .40).

Discussion. In the rabbit ocular model, we found, as did Smolin et al (unpublished data), that IFNaD was slightly better than IFNaA, although our findings were not significantly different (P > .05). Past studies in the eye also show human IFNa to be more efficacious when used in combination with other treatment regimens. The recombinant IFN tested in this study appeared to give beneficial effects with TFT, similar to the natural human IFN preparations used by Sundmacher et al. Our results with TFT alone and in combination with either IFNaA or IFNaD prevented the development of obvious corneal epithelial involvement, iritis or corneal clouding.

Duration of HSV shedding into tear film was shorter for animals receiving either combined therapy. Also, the number of HSV positive trigeminal ganglia was significantly less after either combined therapy. Although the number of animals that died during the study (4 of 31) was very small, fewer deaths were observed in animals receiving TFT, IFN, or combined therapies as compared with those in the placebo group. Furthermore, when deaths occurred, they were delayed in groups receiving TFT, IFNaA, or IFNaD therapy. Under our experimental conditions, it was not possible to determine what portion of the observed beneficial therapeutic effect was due to TFT and what was due

Table 1. Duration of HSV shedding into tear film during topical treatment for acute herpetic keratitis

<table>
<thead>
<tr>
<th>No. animals</th>
<th>Treatment</th>
<th>Day postinoculation</th>
<th>Day postinoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≥50% eyes negative</td>
<td>100% eyes negative</td>
</tr>
<tr>
<td>6</td>
<td>IFNaA</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>IFNaD</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>TFT</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>TFT/IFNaA*</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>TFT/IFNaD*</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Placebo</td>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>

* The median day until an eye becomes negative for virus shedding was compared across the six treatment groups using the Kruskal-Wallis nonparametric analysis of variance procedure. The combined treatments were significantly different (P < .001) from the placebo and IFNaA ad IFNaD alone.

Table 2. Isolation of HSV-1 from rabbit trigeminal ganglia following topical therapy for acute herpetic keratitis

<table>
<thead>
<tr>
<th>No. animals*</th>
<th>Treatment*</th>
<th>No. HSV positive TG%</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>IFNaA</td>
<td>5/12</td>
<td>42</td>
</tr>
<tr>
<td>6</td>
<td>IFNaD</td>
<td>5/12</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>TFT</td>
<td>6/10</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>TFT/IFNaA</td>
<td>5/10</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>TFT/IFNaD</td>
<td>4/12</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>Placebo</td>
<td>5/6</td>
<td>83</td>
</tr>
</tbody>
</table>

* Two placebo (day 10), 1 IFNaD (day 11), and 1 TFT/IFNaA (day 13) treated rabbits died and trigeminal ganglia (TG) were not examined.
  † Treatments were given bilaterally four times per day for 14 days beginning on day 0 as follows: IFNaA (5 μg/ml or 1 x 10^6 U/ml; IFNaD (1 x 10^6 U/ml); TFT (1%); TFT/IFNaA; TFT/IFNaD and placebo (1 mg/ml normal serum albumin). Rabbits were killed on day 21 to 23 postinoculation and trigeminal ganglia were removed aseptically and cultured for HSV as described in "Materials and Methods."
‡ Chi-square analysis was performed to test the rate of virus isolation across the six treatment groups. No statistical differences were found (χ² = 5.01 df = 5, P < 0.40).
to the combined use of IFN (either αA or αD). However, it was apparent that the most efficacious response was achieved when TFT and IFN were used in combination.

**Key words:** Herpes simplex keratitis, recombinant interferon, trifluridine, rabbit, combination antiviral therapy, reactivation

**Acknowledgments.** The authors wish to thank Ann Fredal, Anita Avery, and Emmanuel Bato for their technical assistance. We are also grateful to Martha Hernandez for her secretarial assistance. The animals used in this study were maintained in animal care facilities fully accredited by the American Association of Laboratory Animal Science.

From the Department of Ophthalmology, University of Southern California School of Medicine and the Estelle Doheny Eye Foundation, Los Angeles, California. Supported in part by Research Grants EY-00858, EY-02957, and EY-03040 from the National Eye Institute and in part by the Discovery Fund. Submitted for publication: April 25, 1983. Reprint requests: Melvin D. Trousdale, PhD, Estelle Doheny Eye Foundation, 1355 San Pablo St., Los Angeles, CA 90033.

**References**


**Complement Inhibitors in Normal Cornea and Aqueous Humor**

Dortly J. Mondino and Humphrey Sumner

C1 inhibitor, beta 1H and C3b inactivator are important inhibitory proteins that regulate the complement system. These inhibitor proteins were detected by gel double diffusion in eluates from normal corneas, but not in normal aqueous humor. Functional tests of C1 inhibitor and C3b inactivator showed low-to-absent levels of these inhibitory proteins in normal aqueous humor. On the other hand, the mean activities of C1 inhibitor and C3 inactivator in corneas were nearly as high as those in sera, and there were no statistically significant differences between the values. The lower molecular weights of complement inhibitors, relative to other complement components, may account for their higher levels in normal cornea and may tip the balance in favor of inhibition of complement activation in the noninflamed cornea. Invest Ophthalmol Vis Sci 25:483-486, 1984.

The complement system is not only a fundamental element of our normal host defense, but also is involved in autoimmune tissue damage. Inhibitory proteins play an important role in regulating the complement system. C1 inhibitor (C1INH) is a serum alpha-2 globulin that stoichiometrically unites with C1, blocks its activity and thereby serves to regulate complement activation at the first step of the classical pathway. C1INH also inhibits the enzymatic action of plasma kallikrein and plasmin. The absence of this inhibitor in patients with hereditary angioedema leads to localized swelling of tissues because of the excessive release of C2-derived kinin after trauma or without apparent cause that may have lethal consequences. Beta 1H, a beta-globulin which inhibits the alternative pathway, acts by blocking access of Factor B to C3b and by dissociating the Bb fragment of Factor B from C3b, thus inactivating the alternative C3 convertase and rendering C3b vulnerable to cleavage by C3b inactivator (C3bINA), a proteolytic beta-globulin which degrades C3b. C3bINA deficiency results in depletion of C3 and Factor B and is associated with severe bacterial infections.

There have been no studies of these important inhibitory proteins of the complement system in the