Herpes simplex keratitis

An experimental study

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The incidence of complicated herpes simplex keratitis appears to have increased and the important factor seems to be associated with the use of topical corticosteroid hormone. A good experimental model exists for the study of herpetic keratitis. The disease corresponds to the primary infection in man. The unfavorable effects of corticosteroid hormone on experimental herpetic keratitis is reported.

Herpes simplex virus infection of the cornea is a serious eye disease, perhaps the most important corneal disease today. A review of the literature seems to indicate that herpetic keratitis has become a major eye problem in the last 15 years, and clinicians who have been in practice for the past 30 years state that they have noted this definite trend. They agree that probably the most significant factor causing this has been the development and use of corticosteroid hormones locally in the eye. They also note that other manifestations of herpes simplex, including labial, visceral, and cutaneous have not changed during this period.

Experimental study of herpes simplex keratitis is facilitated by having a good experimental model in the rabbit. The rabbit is not a natural host for the herpes simplex virus, but when the virus is inoculated into rabbits they develop a primary infection and react similarly to man. The experimental eye disease is an acute keratoconjunctivitis characterized by dendritic keratitis and conjunctivitis. The disease heals completely in 10 to 14 days without treatment.

Local corticosteroid treatment of experimental herpetic keratitis can produce all types of complicated herpetic lesions of the cornea—such as disciform keratitis, chronic herpetic ulcers, secondary bacterial infections, uveitis, and even corneal perforation.

As with any experimental work the interpretation of the results in rabbits is subject to definite limitations. However, the similarity of the herpetic corneal disease in man and rabbits is so striking that the results of the animal experiments are probably quite significant.

Experimental herpes simplex keratoconjunctivitis

*Primary infection.* When herpes simplex virus is scratched on the rabbit cornea a
Table I. Similarity of clinical and experimental primary infections with herpes simplex virus

1. The dendritic figure is identical morphologically
2. Epithelial scrapings of the ulcer show giant cells
3. Virus can be cultured from epithelial scrapings
4. Uncomplicated cases run a self-limited course
5. Local corticosteroid therapy makes the infection worse
   a. Prolongation of course
   b. Production of uveitis

Dendritic ulcer usually develops on the scratch in from 24 to 48 hours. There is an associated mild conjunctivitis with a slight mucous discharge. The eye is slightly inflamed, and, on close examination, a dendritic ulcer can be identified. If the virus is inoculated into Tenon's space, the virus then spreads to the cornea, probably by way of the corneal nerves. Many dendritic figures form diffusely over the whole cornea. The disease subsides in 11 to 14 days.

In the rabbit it is very similar to the primary keratoconjunctivitis in human beings although in the latter the disease is more serious. Table I summarizes the similarities.

Recurrences. There is no experimental model of latent and recurrent herpetic keratitis available in laboratory animals at this time. The only manifestation of herpes simplex virus that has been reactivated experimentally is encephalitis. Schmidt and Rasmussen reported activation of latent herpes simplex encephalitis in rabbits by intramuscular injection of Adrenalin into partially immunized rabbits. In human beings, of course, the recurrent disease is most important since the recurrences of herpetic keratitis result in corneal scarring with loss of vision.

Primary herpetic keratoconjunctivitis in rabbits treated with corticosteroids. The treatment of experimental herpetic keratoconjunctivitis with corticosteroids results in a more severe disease with a prolonged course and in many instances the rabbits die of encephalitis.

It is not known how the corticosteroids cause this worsening of the disease. We thought that it might be due to an increased multiplication of the virus because corticosteroids have been shown to have this effect on other viruses, such as those of influenza and poliomyelitis. Jawetz, Okumoto, and Sonne in 1959, however, were not able to show any increased multiplication of the virus in rabbit corneas. This suggested that the effect is not due to suppression of antibody formation by the steroid. We attempted to demonstrate a change in the mucopolysaccharide or the collagen of the cornea by the corticosteroid drug, but were unsuccessful.

Experimental study. The present study was designed to observe the histologic characteristics of herpetic keratitides and test the effect of corticosteroid hormone on experimental keratitis.

Methods and materials

Animals. Thirty black Dutch rabbits which weighed approximately 4 pounds were obtained from a commercial breeder and 3 series of 10

Table II. Clinical picture of experimental herpetic keratitis

<table>
<thead>
<tr>
<th>Days post inoculation</th>
<th>Steroids</th>
<th>Vehicle control</th>
<th>Saline control</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 to 5</td>
<td>Keratoconjunctivitis, mild</td>
<td>Keratitis, slight</td>
<td>Keratitis, slight</td>
</tr>
<tr>
<td>8 to 9</td>
<td>Keratoconjunctivitis, severe</td>
<td>Keratoconjunctivitis, mild Severe</td>
<td>Keratoconjunctivitis, mild Severe</td>
</tr>
<tr>
<td>12 to 13</td>
<td>Keratoconjunctivitis (disiform), severe Uveitis</td>
<td>Keratoconjunctivitis, mild Severe</td>
<td>Keratoconjunctivitis, mild Severe</td>
</tr>
<tr>
<td>19</td>
<td>Chronic keratoconjunctivitis, severe Uveitis</td>
<td>1 Uveitis</td>
<td>Corneas healed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Uveitis</td>
<td></td>
</tr>
</tbody>
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rabbits each were inoculated with virus suspension beneath Tenon's capsule.

**Virus.** The PH ("O") strain of herpes simplex virus was used. The stock virus was prepared by injecting mouse brain and making a 20 per cent suspension of mouse brain in skimmed milk. We injected 0.03 ml. of this virus suspension with an L.D.50 titer of \(1 \times 10^{-5}\) in each eye.

**Steroids.** Prednisolone acetate (Meticortelone acetate*), 25 mg. per milliliter was used in the first series. Two days prior to the virus injection 0.2 ml. (5 mg.) of the suspension was injected subconjunctivally and it was repeated every other day for the length of the experiment.

**Steroid vehicle control.** The vehicle solution of Meticortelone acetate for the second series was prepared according to the formula issued with each multidose vial (phenylethyl alcohol 5 mg. per milliliter, benzalkonium chloride 0.1 mg. per milliliter, H-O.C.P.). This vehicle solution was injected subconjunctivally (0.2 ml.) into eyes of the control series.

**Saline.** Saline was used in the third series.

Rabbits from each series were sacrificed periodically starting from the fourth day post inoculation. Eyes were photographed prior to injecting air into the marginal ear vein.

**Results**

**Clinical.** Table II summarizes the difference in the clinical picture of the corticosteroid-treated rabbits and the controls. The treated animals developed a slight discharge and inflammation of the eyes as early as the second day after the virus was inoculated. By the fourth and fifth days the keratoconjunctivitis was moderately severe. The control rabbits required 6 to 7 days before the keratoconjunctivitis became evident clinically. The eyes of the control animals healed by the twelfth postinoculation day, but the condition in the treated controls persisted through the nineteenth postinoculation day, and all of the rabbits developed uveitis. Two eyes of the vehicle control developed uveitis by the nineteenth postinoculation day.

**Pathologic.** Sections stained with hematoxylin and eosin were studied.

**Fourth to fifth day post inoculation.** Corticosteroid-treated rabbits all developed a moderately severe keratoconjunctivitis. The rabbits inoculated with control fluids developed only a few corneal lesions, and, grossly, the eyes appeared normal.

Histologic sections showed typical epithelial lesions of herpes simplex in both the treated and the control series. The epithelial cells bordering the dendritic ulcer showed intranuclear inclusion bodies (Fig. 1) and viral type giant cells (Fig. 2). The
corticosteroid-treated lesions (Fig. 3) showed the presence of inflammatory cells earlier and the stroma showed the presence of edema 3 or 4 days earlier than in the control animals. Along with the edema there were increased numbers of corneal fibrocytes.

**Eighth to ninth day post inoculation.** The corneal lesions were more extensive in the treated animals as compared with the controls. Intranuclear inclusion bodies and giant cells were still present in both series. The stroma of the corticosteroid-treated rabbits showed more extensive infiltration with inflammatory cells (Fig. 4). Fig. 5 is a section of the eye of a control which shows only an epithelial lesion.

**Twelfth to thirteenth day post inoculation.** The treated eye remained actively inflamed with discharge, keratitis, and uveitis (Fig. 6). Fig. 7 shows the presence of inclusion bodies in corticosteroid-treated eyes 13 days after virus inoculation. The corneas of the control animals were completely healed and no intranuclear inclusion bodies were seen by the twelfth day. Giant cells were also not seen in the control animals by the twelfth day.

**Sixteenth day post inoculation.** The eyes of the control animals are completely healed and the sections show a fairly normal picture. The treated eyes still show an active keratoconjunctivitis (Fig. 8). The sections (Fig. 9) show a marked keratitis with edema and necrosis.

**Nineteenth day post inoculation.** The treated eyes remain chronically inflamed (Fig. 10), and, clinically, many of these corneas resembled a chronic herpetic keratitis in man. Inclusion bodies persisted although in diminishing numbers up to the nineteenth day. The cornea showed ex-
tensive necrosis of the superficial lamellae (Fig. 11). The picture is certainly similar to the chronic herpetic keratitis that is seen clinically in human beings who have been treated with steroids.

The chronic keratitis persisted for several weeks past the nineteenth day and healed with scarring of the cornea.

Comment

The weight of evidence seems to indicate strongly that corticosteroid hormones have an unfavorable effect on herpes simplex virus infection of the cornea in rabbits. By treating experimental herpetic keratitis with Meticortelone acetate, the course of the keratitis can be prolonged and invariably the eyes develop uveitis. This appears to indicate that the steroid enhances the spread of the virus.

Clinically, it is generally accepted that corticosteroid hormones are contraindicated in active herpetic keratitis. Besides the danger of prolonging the course and making the disease more severe, it has been well established that it promotes super-infections by fungi.

The mechanism by which steroids affect herpetic keratitis is still unknown.

REFERENCES