The effect of topical corticosteroids on the susceptibility of immune animals to reinoculation with *Herpes simplex*

Michael Easterbrook,* Jack Wilkie,* Virginia Coleman, and Chandler R. Dawson

To evaluate the effect of topical corticosteroid treatment in recurrent herpetic corneal disease in rabbits, primary dendritic lesions were allowed to heal. After an interval of eight weeks the animals were then treated for 10 days with either dexamethasone ointment or ointment base with no recurrence of virus shedding or corneal herpetic disease; following reinoculation with the same strain of herpes virus and continuation of therapy, more animals with dendrites and virus isolations were found in the steroid-treated group. In a second experiment, the eyes of rabbits treated with dexamethasone and IDU ointments developed dendritic keratitis and had virus reisolated significantly less than those treated with the steroid alone. This animal model then would provide a means to compare new antiviral drugs with IDU in the immune host. Since most herpetic keratitis in man occurs in immune subjects, such a comparison might offer a model of herpetic keratitis more analogous to the human disease than the primary infection of rabbits' eyes.

Key words: *Herpes simplex* keratitis, viral chemotherapy, corticosteroid, animal model.

The deleterious effect of corticosteroids on herpes simplex infections of the eye in man has been supported by a number of experimental studies in animals. These animal studies have shown that, in primary ocular herpetic infections, corticosteroid pretreatment produces a more severe keratitis and an enhancement of virus multiplication. In previously infected (i.e., immune) animals, however, subconjunctivally or topically applied corticosteroids do not appear to induce dendritic keratitis or virus proliferation, although epinephrine has been successfully used in reactivations of herpes infections in immune rabbits.
In order to elucidate corticosteroid-induced changes in susceptibility of rabbits with previous ocular herpetic infection, we reinoculated animals with virus during treatment with steroid, and evaluated results in terms of clinical disease and recovery of virus. Using this model, we then studied the prophylactic effect of low doses of idoxuridine (IDU) in preventing recurrent herpetic keratitis.

Materials and methods

New Zealand albino male rabbits, weighing three pounds, individually caged, were used throughout the study. The 20 per cent mouse brain suspension in skim milk of PH strain Herpesvirus hominis, Type I, which was used had a titer of $4 \times 10^5$ PFU per milliliter. After initial examination of each animal with the slit lamp, 0.05 ml. of PH virus was dropped into the upper limbus without abrading the cornea, and the eyelids were held together for 30 seconds. During the experiments all animals were examined five or six days a week with a slit lamp, following staining with fluorescein. Only typical dendritic figures were considered as evidence of clinical herpetic disease.

To obtain specimens for virus cultures, a sterile cotton swab was inserted into the upper fornix, gently rotated across the cornea into the lower fornix where it was further rotated for another 5 to 10 seconds, then immediately placed in a tissue culture tube containing Eagles-minimal essential media (MEM) supplemented with 2 per cent fetal calf serum and nonessential amino acids. The appearance of the corneal epithelium was not affected by the technique. The content of the tube was inoculated into monolayers of VERO cells (African green monkey kidney). Cell sheets developing characteristic cytopathic effects within two weeks were considered to be positive for herpes virus.

Experimental design.

Primary inoculations. Among the 36 animals infected for the first time, 39 of 70 eyes (56 per cent) developed dendritic keratitis. Eyes without keratitis were inoculated at weekly intervals until both eyes of every animal had at least one episode of dendritic keratitis.

Reinoculation after steroid treatment. Sixty days after the last virus inoculation, 12 rabbits (24 eyes) were treated with topical dexamethasone ointment and 11 animals (20 eyes) with sterile ointment base. In the previous experiment 10 rabbits had received corticosteroid and 10 placebos. In two animals there were pre-existing keratitis in the steroid-IDU-treated eyes, and these eyes are excluded from the analysis of data. Treatment was carried out twice daily for 22 days. On the fourteenth day both eyes of all rabbits were reinoculated with herpes virus. Treatment was carried out twice daily for 17 days. On the eleventh day of treatment the animals were reinoculated with herpes virus. Specimens for virus isolation studies were taken on days 4, 7, 10, 14, and 17 of treatment.

Reinoculation after steroid and idoxuridine treatment. Twenty animals from the previous experiment were then treated in both eyes with dexamethasone ointment, and in the right eye with 0.5 per cent IDU ointment and in the left eye with ointment base. In the previous experiment 10 rabbits had received corticosteroid and 10 placebos. In two animals there were pre-existing keratitis in the steroid-IDU-treated eyes, and these eyes are excluded from the analysis of data. Treatment was carried out twice daily for 22 days. On the fourteenth day both eyes of all rabbits were reinoculated with herpes virus. Virus cultures were obtained on days 1, 4, 8, 11, 14, 17, and 22.

Results

In previously infected animals before virus reinoculation, 11 of 24 eyes given steroid and 12 of 20 eyes given the placebo had linear epithelial changes that appeared to be more severe than simple punctate staining but did not meet the criteria of unquestionable dendrites with branching (Table I). Virus cultures, moreover, were negative in all instances, so corticosteroids in this dosage did not increase the recurrence rate of herpetic keratitis or virus shedding.

Following reinoculation, 20 of 24 eyes in the steroid-treated group developed dendrites compared to 12 of 20 eyes in the control group (Table II). Virus cultures yielded isolates in 19 of 24 eyes treated with steroids and in 9 of 20 eyes treated with the control medication (p < 0.025). Although typical dendritic keratitis occurred more frequently in the steroid-

<table>
<thead>
<tr>
<th>Keratitis (atypical)</th>
<th>Keratitis and virus isolate</th>
<th>Virus isolate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid (24)</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Placebo (20)</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Two animals in this group had a pre-existing epithelial ulcer in one eye, neither of which was included in the tabulation of results. None of the other animals had pre-existing epithelial defects.
Table II. Dendritic keratitis and virus recovery following reinoculation of immune rabbits receiving either steroid or placebo

<table>
<thead>
<tr>
<th></th>
<th>Keratitis only</th>
<th>Keratitis and virus isolate</th>
<th>Virus isolate only</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid (24)</td>
<td>1</td>
<td>19*</td>
<td>0</td>
<td>20/24</td>
</tr>
<tr>
<td>Placebo (20)</td>
<td>3</td>
<td>9*</td>
<td>0</td>
<td>12/20</td>
</tr>
</tbody>
</table>

*Chi square = 4.13, p < 0.05.

treated group (83 per cent vs. 60 per cent) the steroid-treated eyes showed no more severe disease than the control eyes. Indeed, while three eyes in the control group had small geographic ulcers, there were no geographic ulcers or stromal disease noted in the steroid group. The keratitis or reinfection was always less severe than on the initial infection and there was little associated conjunctivitis.

In the subsequent experiment 20 animals were treated with steroid and IDU in one eye and steroid and placebo in the other (Table III). Again virus was not recovered nor did any typical dendrites appear during the initial treatment. Following virus reinoculation, typical dendritic keratitis or virus isolation was found in 12 of 20 eyes in the steroid-placebo-treated group, but in only 2 of 18 eyes in the steroid-IDU group. The differences between the IDU- and placebo-treated eyes are highly significant (p < 0.01) if one compares virus culture results alone (11/20 vs. 2/18) or combines the clinical and virologic results (12/20 vs. 2/18). The one eye with dendritic keratitis but no virus isolate had manifest disease on only one examination 24 hours after inoculation.

In the two experiments, a total of 87 virus isolation attempts were negative before any topical steroid therapy to the rabbits who had been infected 60 to 175 days previously. Another 95 cultures failed to yield virus from eyes following corticosteroid treatment, but before reinoculation.

Discussion

Although herpetic encephalitis has been reactivated by anaphylactic shock\(^8\) or systemic epinephrine,\(^8\) it is more difficult to reactivate healed herpetic keratitis in rabbits. Anderson reactivated herpes simplex virus in healed rabbit corneas by evoking a local Arthus phenomenon.\(^9\) Systemic epinephrine induced reactivated virus in previously infected rabbit eyes.\(^12-14\) In the present study, during the 10 days of steroid treatment prior to reinoculation no dendritic ulcers appeared in either the dexamethasone or ointment base-treated group, nor were virus isolates obtained on the three occasions they were attempted. Like Laibson and Kibrick,\(^15\) we did not consider atypical epithelial lesions in the absence of positive cultures evidence of reactivation.

The failure to reisolate virus in animals with or without steroid treatment contrasts with previous studies in which up to 65 per cent of rabbits shed virus spontaneously;\(^11,13\) the lack of spontaneous virus shedding from the animals in this study may have been due to the strain of virus used or may be due to the relatively small number (182 in all) of isolation attempts prior to reinfection.

In previously infected rabbits spontaneous recurrences of episodes of virus shedding are more frequent than dendritic figures.\(^12,15\) Similarly it has been suggested that in humans, virus can be isolated in tears before the appearance of recurrent clinical disease.\(^15\) In our study the exogenous reinoculation of herpes simplex was intended to simulate such spontaneous recurrences of virus shedding. In this
modification of the natural recurrent herpetic infection, virus proliferation was detected more frequently in association with clinical dendritic keratitis although some animals yielded virus in the absence of disease. The reinoculation of previously infected animals, then, may be regarded as a model of clinically manifest recurrent dendritic keratitis in man where disease and virus proliferation occur in a previously infected host.

Steroid treatment prior to and after reinoculation did increase slightly the susceptibility of the cornea to recurrent dendritic keratitis and did produce a statistically significant increase in virus recovery rates. In animals pretreated with steroid, IDU markedly reduced the occurrence of dendritic ulcers and virus recovery following reinoculation (Table III). These results are similar to those found in the controlled study of Patterson and Jones, in which patients receiving topical corticosteroid and placebo for stromal herpetic keratitis had significantly more recurrent dendritic ulcers than patients treated with steroids and IDU. Thus this animal model of recurrent dendritic keratitis in man may offer a way to evaluate the prophylactic effect of antiviral drugs in the prevention of clinical episodes of herpetic keratitis.

REFERENCES