Dissemination of corneal herpes simplex

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Some strains of herpesvirus have wider dissemination than others and may penetrate into the eye and multiply in the endothelium without virus persisting in the stroma. In evaluating chemotherapeutic response, both the strain of the virus and the frequency of drug administration are important; less frequent drug administration gives a poorer therapeutic response. The drug in ointment is more effective than the drug in saline or in dimethyl sulfoxide.

There are conflicting reports as to the degree to which idoxuridine (IDU) is effective in experimental herpetic keratitis, and some clinical cases do not respond to IDU therapy. Two possible reasons for these differences will be investigated in this study: (1) the effect of different therapeutic regimens used by the different investigators, and (2) the possibility that some virus stains may spread to tissues relatively inaccessible to the drug, and, in the process, cause varied signs and symptoms.

Methods

In order to study the effect of different dosages of IDU, the corneas of New Zealand rabbits were infected with the HF 378 strain of herpesvirus, as previously described. Three days after infection, treatment was begun. IDU drops, 0.1 per cent were used either every 2 hours around the clock or every 2 hours from 9 A.M. to 9 P.M.; 0.5 per cent IDU ointment was used five times during the day only. The epithelial ulcers were evaluated after 4 days of treatment on a double-blind basis and graded from 1 to 4.

To determine whether herpesvirus could multiply in the endothelium, 0.1 ml. of McKrae strain herpesvirus was injected into the anterior chambers of 12 eyes as a control. At various times after the injection, whole corneas were removed, frozen, and stained with tetrazolium in order to visualize the endothelium. This stain for NADH diaphorase has been previously described by Kaufman, Capella, and Robbins. Fluorescent antibody studies of endothelial scrapings and virus cultures were also made.

In order to determine whether virus infection of the epithelium would penetrate through the cornea to the endothelium and into the aqueous, rabbit eyes were infected in our usual manner. Two days after the infection, one half of the animals were treated with a subconjunctival injection of 4 mg. repository methylprednisolone acetate (Depo-Medrol*) plus 0.5 per cent hydrocortisone drops for 2 days to determine whether steroids aided in the dissemination of the virus. Four days after infection, the animals were put to death and the eyes examined. The endothelium and aqueous from one third of the eyes were cultured for the presence of virus by means of rabbit kidney tissue cultures for the McKrae strain of virus and chick embryo cultures for the HF 378 virus.

Frozen sections were made of one third of the eyes. These were stained with antiherpes fluorescein-conjugated human antibody. The remaining third of the eyes was graded on a double-blind basis for epithelial ulcers. The whole corneas

*The Upjohn Company.
were then removed, frozen, and stained for NADH diaphorase and the loss of endothelial cells was evaluated.

**Results**

The results of treating herpetic keratitis with different IDU dosages are shown in Table I. The best results were obtained by treating the animals with 0.5 per cent IDU ointment only 5 times a day. The results with IDU solutions were clearly better when treating around the clock than during the day only. It has previously been shown that it is possible to relate the score of the treated eyes to that of the untreated controls and obtain a linear-response relationship. This applies not only to the severity of ulcers but also to the amount of virus that can be recovered from the epithelium. This present study shows that decreasing the frequency of treatment has the same effect as lowering the concentration of the drug used.

When live herpesvirus was injected into the aqueous of the rabbit, endothelial loss could be seen by 12 hours. This loss progressed until the endothelium was completely destroyed 48 hours after injection. No changes were seen when ether-killed virus was injected.

Three strains of herpesvirus were studied for their dissemination through the cornea —the McKrae strain, the HF 378 strain, and the Deese strain. Two different passages of the McKrae strain were studied —the tenth passage which produces encephalitis in all our rabbits and the eighth passage which seldom produces encephalitis. The Deese strain was studied in both immune and nonimmune animals. The results of the virus isolations from the endothelium and the aqueous are shown in Table II. No virus was isolated either from the steroid-treated or the untreated animals infected with the HF 378 virus, and no virus was isolated from the endothelium or aqueous of animals infected with the Deese strain. The virus was isolated from 2 of 10 endothelia and 2 of 10 aqueous from the untreated animals infected with the milder eighth passage of the McKrae virus and from 3 of 10 endothelia and 1 of 10 aqueous of steroid-treated animals. When the animals were infected with the encephalitogenic tenth passage, the virus was isolated from all endothelium and aqueous in both the steroid and the untreated groups.

The results of staining with fluorescent antibody showed virus antigen in the endothelium of all eyes, untreated and steroid treated, infected with the HF 378 virus.

**Table I.** Treatment of experimental herpetic keratitis using various IDU regimens

<table>
<thead>
<tr>
<th>Grade severity of epithelial ulcers</th>
<th>2 days' treatment</th>
<th>4 days' treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% IDU, every 2 hours day and night</td>
<td>0.70 ±0.11</td>
<td>1.06 ±0.32</td>
</tr>
<tr>
<td>0.1% IDU, every 2 hours day only</td>
<td>1.02 ±0.31</td>
<td>1.19 ±0.38</td>
</tr>
<tr>
<td>0.5% IDU, 5 times a day</td>
<td>0.29 ±0.10</td>
<td>0.38 ±0.14</td>
</tr>
<tr>
<td>Saline control</td>
<td>1.94 ±0.34</td>
<td>3.00 ±0</td>
</tr>
</tbody>
</table>

**Table II.** Virus isolations

<table>
<thead>
<tr>
<th>McKrae 8th passage</th>
<th>McKrae 10th passage</th>
<th>HF 378 Herpes</th>
<th>Deese immune</th>
<th>Deese non-immune</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated endothelium</td>
<td>No. of eyes</td>
<td>Positive cultures</td>
<td>No. of eyes</td>
<td>Positive cultures</td>
</tr>
<tr>
<td>Aqueous</td>
<td>10</td>
<td>2</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Steroid-treated endothelium</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Aqueous</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
and the tenth passage of McKrae virus. Only four untreated eyes and 5 steroid-treated eyes infected with the eighth passage of McKrae showed positive staining in the endothelium. Staining was not seen in any of the animals, immune or non-immune, steroid treated or untreated, infected with the Deese strain.

Despite the frequent findings of virus in the endothelium by culture and fluorescent antibody staining, examination of the stroma by fluorescent antibody techniques did not reveal virus antigen.

A comparison of the double-blind evaluation of the epithelial ulcers in this study showed no difference between the untreated and steroid-treated groups in either immune or nonimmune animals, and no significant difference between these groups in the loss of endothelial cells. There were marked differences between strain, however, in the endothelial damage observed. Severe loss of endothelium occurred in the animals infected with the tenth passage of the McKrae virus, moderate loss in those infected with the HF 378 virus, and only slight loss with the eighth passage of McKrae.

The spread of virus is not limited to the globe but occurs in the conjunctiva as well. By staining the eyes with a fluorescein–methylene blue solution, as suggested by Sery, rather than fluorescein alone, dendritic figures were found on the conjunctiva of the animals infected with the HF 378 virus. It had previously been reported by us that the virus could be isolated from conjunctiva washed in .5 percent silver nitrate, and more recently Kimura confirmed this with fluorescent antibody staining.

Summary and Conclusions

Although it has not been possible to show that steroids enhance the dissemination of herpesvirus through the rabbit cornea by means of any of the strains in our laboratory, it has been possible to show that the virus frequently penetrates from the epithelium to the endothelium and aqueous, and also causes conjunctival ulcers. Byvoet has found that IDU penetrates poorly to the endothelium and its efficacy on mucous membrane lesions has not been demonstrated. These factors may be responsible for some resistance to IDU therapy.

Some virus strains have wider dissemination and produce greater loss of endothelial cells than others. This destruction of endothelium may, in part, account for the finding of Williams and colleagues that some virulent strains of virus produce a disciform lesion (corneal swelling) in previously nonimmunized animals, whereas other strains only produce disciform lesions in immune animals. In addition, these results suggest that superficial infection may penetrate into the eye without the virus persisting in the stroma. Similar penetration is suggested by the work of Pettit and associates.

There is wide variability in the strains of herpesvirus in terms of steroid sensitivity. This suggests that conclusions from these studies in which the clinical conditions were nearly the same in both the steroid-treated and control groups, and penetration was also similar, may not apply to virus which is more steroid sensitive.

In evaluating chemotherapeutic responses, therefore, it is essential to consider both the biologic behavior and the distribution of the particular strain and the passage of virus and the dose-response relationships of the drug. Decreasing the frequency of drug administration is similar to decreasing its concentration and its effect, and the vehicle also plays an important role. With IDU, the drug in ointment appears much more effective than in saline; whereas a dimethyl sulfoxide solution appears less effective than saline.

REFERENCES


