Therapy of experimental herpes simplex keratitis

Herbert E. Kaufman and George M. Howard

Topical therapy with trypsin, chymotrypsin, and monoxychloroserase, and corneal scrub with iodine, ether, and silver nitrate did not demonstrably reduce the incidence of a positive virus culture from rabbit corneas infected with herpes simplex. It is suggested that the virucidal properties of such substances as measured by their effect on cell-free virus suspensions is not pertinent to the abolition of infection from primary experimental dendritic keratitis, and may not be an important consideration in the therapy of this condition.

Gundersen in 1936 described the value of iodine scrub in patients with herpes simplex keratitis and popularized the use of a scrub with virucidal agents in its therapy. Since that time many articles have appeared advocating the use of one agent or another for scrub. More recently, largely on the basis of the in vitro effect of these substances on the extracellular virus, some agents have been recommended over others.

Virucidal agents, such as iodine, silver nitrate, and ether, are protoplasmic poisons and do not enter live cells. These denaturing agents facilitate the removal of epithelium and may, in removing a focus of necrotic, antigenic, and perhaps toxic tissue, be beneficial. It seems possible, however, that all infected cells cannot be destroyed by such denaturing agents and that those agents which cannot act on the virus in live cells cannot obliterate the infection without destroying the eye. If these agents do not obliterate infection but merely aid in removal of the epithelium, perhaps the virucidal properties are inconsequential and our efforts should be directed toward methods of removing the majority of infected tissue with minimal trauma to the eye and little attention to extracellular virucidal properties.

This communication reports upon a study in which rabbit corneas infected with herpes simplex were treated by various means and then cultured for virus to determine whether such treatment eliminated the virus infection.

Materials and methods

Animals. New Zealand white rabbits weighing from 3 to 4 pounds were infected with the Virtue strain of the herpes simplex virus isolated from a patient with dendritic keratitis, passed 5 times in primary monolayer rabbit kidney tissue culture and stored at -70° C. The suspending medium consisted of 50 per cent Hank's balanced salt solution containing 2 per cent lactalbumin hydrolysate and 2 to 5 per cent calf serum, and 50 per cent skim milk. When suspended in this medium and stored at -70° C, the virus had an infectivity titer for rabbit kidney tissue culture of 10^-7.8.

To produce a reasonably standardized and central infection, rabbits were immobilized in a harness. The eyes were anesthetized with topical 0.5 per cent proparacaine and proptosed. Three

From the Massachusetts Eye and Ear Infirmary, Boston, Mass.
*Present address, J. Hillis Miller Health Center, University of Florida, Gainesville, Fla.
Interlocking wounds were made on the central corneas with a 5 mm. trephine set to a depth of approximately 0.05 mm. The eyes were then replaced in the orbits. Two drops of freshly thawed virus suspension were dropped on the corneas, and the lids were rubbed over the eyes.

Slit-lamp examination was performed the third day to confirm the presence of dendritic figures. In the study of scrubs, 7 per cent tincture of iodine, ether, and 0.5 per cent silver nitrate were used. Chemical cautery was carried out in all cases on the third day after inoculation. After topical anesthesia, the eye was proptosed. A cotton swab was soaked in the cauterizing agent and rubbed very vigorously over the entire cornea, including several millimeters of limbal conjunctiva, for 90 seconds. After cautery with iodine, topical cocaine was applied, and the animals were replaced in their cages.

In the study of topical therapy without scrub, trypsin, monoxylorosolene, and chymotrypsin were applied topically for 3 to 5 minutes by inverting over the cornea a 16 mm. test tube containing 5 ml. of the solution. Treatment was continued for 3 days. Simultaneously infected, randomly selected rabbits were used as controls in all experiments and received identical treatment with normal saline.

Method of culture. On the third day after therapy (sixth day after inoculation), each rabbit eye was anesthetized with topical Ophthaine and proptosed. The entire corneal epithelium was scraped off with a scalpel blade and inoculated immediately into rabbit kidney tissue culture. Fresh sterile instillments were used for each eye. The tissue culture tubes were examined daily for specific cytopathogenic effect. In case of doubt, collodion membranes of the cells were prepared, stained, and examined under oil, and the supernatant fluid was subinoculated into fresh tubes.

Results

Topical therapy. Trypsin. Since trypsin has recently been recommended in the treatment of herpetic keratitis,8 9 8 eyes of 4 rabbits were treated with trypsin8 9 The solution in a test tube of 16 mm. diameter was inverted over the cornea 5 minutes a day for 3 days; the results were most dramatic. Trypsin in this concentration caused a purulent conjunctivitis and iritis and dissolved the stroma. Six of the 8 eyes perforated either spontaneously or during the attempt to proptose the eye at the time of culture. Despite this vigorous therapy and the relatively high concentration of enzymes which apparently penetrated the stroma, the virus was recovered from the epithelium of all 8 eyes.

Chymotrypsin. Because of enthusiastic case reports and recent suggestions that chymotrypsin may inactivate the extracellular herpes simplex virus, this substance was studied.10 11

Alpha chymotripsin 1:5,000 was freshly prepared before each use and was applied in a manner similar to the trypsin, 3 minutes a day for 3 days. Under this therapy the cornea was well preserved and corneal damage was minimal, but the virus was recovered from all 8 treated eyes.

Monoxylorosolene. Monoxylorosolene is a stable derivative of hypochlorous acid which is said to liberate nascent chlorine and is thereby claimed to be virucidal.12 13 For this study a 0.2 per cent solution in distilled water was applied, as before, in a bath, 3 minutes a day for 3 days. The epithelium became gelatinous and was easily scraped off, and the stroma developed gross hill and valley irregularities similar to a lunar landscape. In all 10 treated eyes the virus was recovered following therapy.

The results of the topical therapy are summarized in Table I, and it is clear that none abolished virus from the cornea.

Scrub. Iodine. A very vigorous scrub of the whole cornea and limbal conjunctiva with iodine caused severe ocular inflammation and stromal infiltration. After the profound stromal damage caused by penetration of the stroma by iodine, epithelial regrowth was inhibited so that it was minimal by the time of culture. Some corneas had almost no visible epithelial regrowth by the time of culture, others had only occasional patches of cells, and no cornea had a complete sheet of epithelium. It was remarkable that with so

*Armour, Tryptar containing 50,000 units per cubic centimeter.

Armour, Chymur.

*Armour, Tryptar containing 50,000 units per cubic centimeter.

Since this paper was submitted, studies by Chung14 have confirmed the inefficacy of chymotrypsin in the treatment of herpes simplex keratitis.

1Dorsey Laboratories, Chlorpactin.
Table I. Topical therapy

<table>
<thead>
<tr>
<th>Topical agent</th>
<th>Virus positive (No.)</th>
<th>Virus negative (No.)</th>
<th>Total (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Monooxychloroserine</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Saline</td>
<td>14</td>
<td>2</td>
<td>16</td>
</tr>
</tbody>
</table>

Table II. Chemical cautery

<table>
<thead>
<tr>
<th>Chemical agent</th>
<th>Virus positive (No.)</th>
<th>Virus negative (No.)</th>
<th>Total (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine 7%</td>
<td>5</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Ether</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Silver nitrate 0.5%</td>
<td>3</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>19</td>
<td>32</td>
</tr>
<tr>
<td>Saline scrub</td>
<td>8</td>
<td>5</td>
<td>13</td>
</tr>
</tbody>
</table>

Table III. Contamination control

<table>
<thead>
<tr>
<th>Eye</th>
<th>Virus positive (No.)</th>
<th>Virus negative (No.)</th>
<th>Total (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>19</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Left</td>
<td>0</td>
<td>19</td>
<td>19</td>
</tr>
</tbody>
</table>

little epithelium to culture any positive culture could be obtained, but 5 of 16 eyes revealed culturable virus. Corneal scrapings from 8 other iodine-scrubbed eyes were stained by fluorescein-labeled antibody and an intracellular virus was found in 3.

Ether and silver nitrate. The inhibition of epithelial regrowth was less after ether and silver nitrate scrubs than after the iodine treatment. After scrub with ether, the virus was present in 5 of the 8 eyes, and after silver nitrate scrub, virus was cultured from 3 of the 8 eyes.

Saline. As might be expected, saline scrub inhibited epithelial healing less than scrub by the denaturing agents. After saline scrub, virus was cultured from 8 of 13 eyes, a minimally though not significantly higher rate of recovery than from eyes treated with denaturing agents, but lower than those treated only with topical solutions. It seemed likely that these differences, if real, mirrored difficulty in obtaining adequate cultures from eyes with poor epithelial regrowth rather than any true difference in ability to abolish virus.

Contamination control. Since recovery of virus from the treated animals was so common and regular, it seemed desirable to study the possibility of reinfection after treatment or contamination of the cultures by the nonocular laboratory virus. During one regular experiment, therefore, 20 additional rabbits were infected with herpes simplex in the right eye. The following day the left eye was vigorously scarified with a 25 gauge needle so that the epithelium was well macerated and largely denuded. The rabbits were then placed 2 in each cage, and 3 days later both eyes were cultured for virus. The results are summarized in Table III and indicate that the virus was isolated only from those eyes intentionally infected. None of the scarified but noninfected rabbits later became infected, and no contamination or reinfection could be demonstrated.

Discussion

The interpretation of results such as those cited must be made with caution. It seems most likely that the small differences in recovery of the virus after scrub are due primarily to difficulties in culturing poorly epithelized cornea. Although in this study the differences between saline and specifically treated corneas are not statistically significant, we cannot completely rule out the possibility of some difference attributable to the choice of therapy. There is little question, however, that under the conditions of the experiment no agent regularly rendered the cornea free of virus.

When the epithelium is scrubbed off, where is the virus? There is insufficient evidence that, despite the absence of Bowman's membrane in the rabbit, the virus can be recovered with any regularity from the corneal stroma; indeed, virus cannot generally be cultured from the stroma of the infected rabbit cornea. In addition, trypsin, as well as all agents used for
scrubbing the cornea, appeared to penetrate throughout the stroma causing superficial and deep opacification and vascularization. The limbal conjunctiva was scrubbed and no effort was made to protect the other areas of conjunctiva from the denaturing agents. It seems possible, however, that conjunctiva, lachrymal apparatus, or epithelial cells which survive to regenerate the corneal epithelium may all harbor the virus.

Despite the species differences, we suspect that these results in experimental herpes simplex keratitis also apply to primary human dendritic keratitis. In these studies, the virucidal action (as determined on the extracellular virus) of the several substances investigated does not seem important in the abolition of infection from tissues. It is suggested that, if removing infected tissue is worthwhile, such virucidal action should not be a consideration in selecting the method.

REFERENCES