equivalent to 1 gm./mm. on the average (Fig. 3A). This is similar to the threshold to stimulation with von Frey hairs in the rat. The discharge was characteristic of fibers responding to the onset of the stimulus, and their duration, 0.4 to 0.5 msec., suggests they corresponded to A fibers. No attempt was made to study conduction velocity in these experiments.

A spike that was elicited by stimuli just above threshold and which could be identified by its amplitude was used to map the receptor field. Such fields usually encompassed one fourth to one third of the corneal surface as described by Tower in the cat (Fig. 4, A). Occasional receptors with very restricted field, less than 1 mm.,2 were also found (Fig. 4, B). Since these are inherently less likely to be chosen for study, they may be more frequent than immediately apparent. Only preliminary investigations using thermal stimuli were carried out, but it was evident that raising or lowering the temperature of the jet a few degrees Celsius in comparison to that in the bath would elicit action potentials (Fig. 3, B). Responses to both mechanical and thermal stimuli were abolished shortly after the application of 0.5% proparacaine HCl through the jet.

In addition to the A fiber responses noted above, a larger number of smaller spikes were observed both discharging spontaneously and responding to nociceptive stimuli. Although a definite identification has not been made, their duration, 1 to 3 msec., suggests that these are C fibers. Recordings from such fibers in the cornea have already been noted.1

Mr. Gunther Kuhn fabricated the equipment used in these experiments.

From the Division of Ophthalmology, Stanford University School of Medicine, Stanford, Calif. This work was supported by NIH Grant EY 00431. Submitted for publication Dec. 29, 1976. Reprint requests: Dr. David Maurice, Division of Ophthalmology, Stanford University School of Medicine, Stanford, Calif. 94305.

Key words: cornea, sensory nerves, sensation, receptor fields.

REFERENCES


The endothelial surface of rabbit corneas was perfused with vidarabine monophosphate (with and without adenosine deaminase inhibitor), vidarabine (with and without adenosine deaminase inhibitor), and ara-Hx. In concentrations 10 times to 1,500 times higher than those that have been obtained in the aqueous humor following topical, subconjunctival, or systemic administration, none of the compounds had any effect on corneal endothelial cell function or ultrastructure for the duration of the experimental model.

Recent work has suggested that systemic administration of vidarabine (adenine arabinoside, 9-β-D-arabinofuranosyl adenine, ara-A; Vira-A [Parke, Davis & Co., Detroit, Mich.]) is of benefit in treating some patients with herpes simplex keratouveitis.1 Measurable aqueous humor levels of vidarabine and its metabolite ara-Hx (hypoxanthine arabinoside, 9-β-D-arabinofuranosyl hypoxanthine) have been demonstrated in these patients. However, the effect of vidarabine and related compounds on the corneal endothelium is not currently known. Since these drugs enter the eye, it is imperative to establish what dose levels are cytotoxic to the endothelium. This investigation was conducted to determine the effects of vidarabine and related compounds on corneal endothelial physiology and ultrastructure during in vitro perfusion.

Methods. Adult albino rabbits weighing about 3 kg. were killed with an overdose of intravenous sodium pentobarbital. The eyes were enucleated together with conjunctival sac and eyelids, after which the corneas were mounted in a speculum microscope.2-4 Using the speculum microscope allowed constant perfusion of the corneal endothelium with Ringer’s solution at a temperature of 37° C. and a pressure of 15 mm. Hg. Sequential observations of the corneal endothelium and measurements of corneal thickness were made every half hour. Silicone oil (Dow Chemical Co., Midland, Mich.) was placed on the epithelial surface. Corneal swelling rates were determined by linear regression analysis, and a comparison of experimental and control regression lines was made by an analysis of co-vari-

Fig. 1. Scanning electron micrograph of corneal endothelium after a 3 hr. perfusion with vidarabine, 500 μg/ml., shows normal endothelial mosaic. (Original magnification, ×500.)

ance. All control corneas were perfused with Krebs-Ringer bicarbonate with added reduced glutathione (0.092 gm./L.) and adenosine (0.134 gm./L.).

Experimental corneas were perfused for 1 hr. with control solution, and after the corneal thickness had stabilized, they were perfused for 3 hr. with the experimental antiviral agent dissolved in the Ringer’s solution. The perfusing solutions were collected from the efflux tubing of the specular microscope chambers, frozen, and sent to Parke, Davis & Co. for thin-layer chromatography and high-performance liquid chromatography. At the end of each experiment, the corneas were fixed in 2% glutaraldehyde in phosphate buffer and submitted for electron microscopy.

The corneas were put into five groups, with the nominal drug concentrations as follows:

Group I. Vidarabine monophosphate (adenine arabinoside monophosphate, 9-β-D-arabinofuranosyl adenine-5′-monophosphate), 100 μg/ml.

Group II. Vidarabine, 500 μg/ml.

Group III. Ara-Hx, 1,000 μg/ml.

Group IV. Vidarabine monophosphate, 1,000 μg/ml. plus coidarabine, an adenosine deaminase inhibitor—(R)-3-(2-deoxy-β-D-erythro-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-d] (1,3) diazepin-8-ol, 10 μg/ml.

Group V. Vidarabine (ara-A), 250 μg/ml., plus covidarabine, 2.5 μg/ml.

Results. None of the corneas perfused with any of the antiviral agents showed a change in corneal thickness during the perfusion that was statistically significantly greater than that shown by the controls (Table I). Electron microscopy showed preservation of the endothelium during perfusion by the antivirals whether or not they were administered with covidarabine (Figs. 1 to 3).

Results of chromatographic analysis of the perfusion medium with their respective antiviral agents after passing through the specular microscope chambers are also shown in Table I. Perfusion of corneal endothelium with vidarabine monophosphate (group I) showed only the monophosphate in the effluent solution. When covidarabine was added to the monophosphate (group IV), about 1% to 3% vidarabine was present in the effluent solutions, and the rest was the monophosphate. Perfusion with vidarabine both with and without covidarabine (groups II and V) showed only vidarabine in the effluent solution. As expected, perfusion with ara-Hx (group III) showed only ara-Hx in the effluent solution, since ara-Hx is the final product of the metabolic pathway.

Discussion. It is well known that some of the antiviral agents are capable of producing a punctate epithelial keratitis when administered topically. It was the purpose of this investigation to determine if vidarabine and related metabolic compounds would be damaging to the corneal...
Fig. 2. Transmission electron micrograph of corneal endothelium following a 3 hr. perfusion with vidarabine, 500 μg/ml., shows preservation of cell membranes and intracellular organelles. (Original magnification, ×16,800.)

Table I. Changes in corneal thickness and mean concentration of antiviral agents and metabolites in the effluent solution

<table>
<thead>
<tr>
<th>Group</th>
<th>Antiviral (concentrations) in perfusing solution</th>
<th>N</th>
<th>Change in corneal thickness (μ/hr.) ± 95% confidence limits*</th>
<th>Concentration of antiviral in effluent solutions (μg/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vidarabine monophosphate</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>16</td>
<td>+2.2 ± 2.1†</td>
<td>87</td>
</tr>
<tr>
<td>I</td>
<td>Vidarabine monophosphate (100 μg/ml.)</td>
<td>3</td>
<td>+4.6 ± 4.1†</td>
<td>—</td>
</tr>
<tr>
<td>II</td>
<td>Vidarabine (500 μg/ml.)</td>
<td>3</td>
<td>-1.2 ± 4.0†</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>Ara-Hx (1,000 μg/ml.)</td>
<td>4</td>
<td>+1.8 ± 2.8†</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>Vidarabine monophosphate (1,000 μg/ml.) +</td>
<td>3</td>
<td>-4.1 ± 6.6†</td>
<td>1,337</td>
</tr>
<tr>
<td></td>
<td>Covidarabine (10 μg/ml.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Vidarabine (250 μg/ml.) + Covidarabine (2.5 μg/ml.)</td>
<td>3</td>
<td>-4.4 ± 2.1†</td>
<td>0</td>
</tr>
</tbody>
</table>

*In the regression equation y = a + bx, b = change in corneal thickness (μ/hr.) and the 95% confidence limit is determined by t_{a}, e, 5.2 (see ref. 5).

†No experimental cornea swelled at a rate faster than controls—p >0.1.

endothelium at the concentrations likely to be reached in the anterior chamber, when given topically, subconjunctivally, or systemically.

The nucleotide vidarabine monophosphate is dephosphorylated in the living organism by a phosphomonoesterase to the nucleoside vidarabine, which is further deaminated to ara-Hx. The deaminase causing the last reaction can be blocked by the deaminase inhibitor covidarabine. The lack of any apparent metabolism to ara-Hx (groups I and II) indicates that the deaminase may be lacking in the corneal endothelium or that the adenosine (0.134 gm./L.) in the perfusion solution was competing with vidarabine as a substrate for the enzyme. This bears further investigation.

The presence of vidarabine (1.6% of the vidarabine monophosphate) in the effluent solution of corneas perfused with vidarabine monophosphate in the presence of covidarabine, and not in the experiments when the monophosphate was used alone, probably reflects the higher concentration of the monophosphate (1,000 μg/ml.) in the experiments in which covidarabine was present than in those when the monophosphate (100 μg/ml.) was used alone. The dephosphoryla-
Fig. 3. Scanning electron micrograph of corneal endothelium after a 3 hr. perfusion with ara-Hx, 1,000 μg/ml., shows normal endothelial mosaic. (Original magnification, ×500.)

It is known that humans given vidarabine intravenously at 20 mg./kg./day had maximum aqueous humor concentrations of 1.3 μg/ml.1 Ara-Hx concentrations (the metabolite of vidarabine) in this same group of patients reached a maximum of 11.2 μg/ml. When vidarabine monophosphate was given intravenously to rabbits at 100 mg./kg., the highest aqueous humor level of vidarabine monophosphate was 5.8 μg/ml., the highest level of vidarabine was 0.3 μg/ml., and highest level of ara-Hx was about 100 μg/ml.9 The concentrations used in the perfusion chambers in this experiment were many times higher than those found in the aqueous humor in the above experiments (Table I). At these high concentrations and for the duration of the experiment none of the compounds studied had any effect on corneal endothelial cell function or ultrastructure. What delayed toxic effects could occur with these compounds is currently not known.

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From the Department of Ophthalmology and Physiology, Medical College of Georgia, Augusta. Supported by a grant from Parke, Davis & Co., Inc., by a grant from the National Eye Institute (EY-01654, Dr. Hull), in part by a grant from the National Eye Institute (EY-01412, Dr. Green), and by the Lions Clubs of Augusta, Georgia. Submitted for publication Oct. 21, 1976. Reprint requests: David S. Hull, M.D., Department of Ophthalmology, Medical College of Georgia, Augusta, Ga. 30902.

Key words: Vidarabine monophosphate, vidarabine, hypoxanthine arabinoside, covidarabine, corneal endothelium, specular microscope.

REFERENCES
4. McCarey, B. E., Edelhauser H. F., and Van Horn, D. L.: Functional and structural changes in the corneal endothelium during in...
Resistance to reinfection with a chlamydial agent (guinea pig inclusion conjunctivitis agent). AHMAD AHMAD, CHANDLER R. DAWSON, CHIEKO YONEDA, BIRGITTA TOGNI, AND JULIUS SCHACHTER.

Although most chlamydial infections are chronic or recurrent, infection of the guinea pig's eye with guinea pig inclusion conjunctivitis (GPIC) agent induces a marked resistance to reinfection. To characterize this resistance to GPIC agent, we compared the disease and infection in previously infected guinea pigs with that in animals infected for the first time. In animals experiencing primary infection, even the lowest dose (10 egg-lethal doses [ELD₅₀]) produced the disease and chlamydial inclusions in conjunctival smears, but the incubation period became progressively shorter with the highest inocula (10⁴ and 10⁵ ELD₅₀). In animals with previous infection only these two highest inocula produced disease and infection, but the disease was short-lived, and replication of the agent was severely limited. The mechanism of this resistance may be due to secretory antibody in the tears, cellular immunity, or other local factors.

Natural infections with chlamydial agents do not ordinarily induce solid immunity. As a result, chlamydial diseases such as trachoma and inclusion conjunctivitis are usually chronic and recurrent. In two host-parasite systems, however, a marked degree of resistance to chlamydial eye infection can be induced. (1) The ocular inoculation of owl monkeys (Aotus trivirgatus) with trachoma agent produces an acute conjunctivitis that subsides and leaves the animal resistant to challenge inoculation.³ (2) In the same way, the naturally occurring chlamydial disease, guinea pig inclusion conjunctivitis (GPIC), is followed by resistance to further infectious challenge with GPIC agent.³ Since neither systemic immunization nor passive transfer of antibody prevents eye disease, it has been suggested that secretory immunoglobulins are largely responsible for such local immunity.³–⁴ In order to characterize further this resistance to GPIC agent, we have determined the infectious dose necessary to infect and produce disease in the eyes of previously infected animals.

Materials and methods

Agent. A single pool of the ninth egg passage of a GPIC agent (gp 86) originally isolated in this laboratory was used in all these experiments. Individual aliquots of 50% yolk sac-grown agent were stored at -70° C. until used. The titer of this pool was about 10⁴ egg-lethal doses (ELD₅₀) per milliliter.

Animals and inoculation. We carried out the study with two groups of Hartley strain guinea pigs (19 animals in each group) that were known to be free of GPIC. To inoculate the eyes, 0.1 ml. of various concentrations of the agent was dropped on the anesthetized cornea through a plastic well, and the agent was kept in contact with the cornea for a period of 5 min. We examined the animals' eyes with a modified slit lamp before inoculation and at intervals after inoculation.

Smears. To quantitate the growth of the agent in the eye, we collected conjunctival smears for microscopic study at each examination. After Giemsa staining of the slides, we counted the number of chlamydial inclusions per 100 epithelial cells on each slide.

Light and electron microscopy. A portion of the cornea was fixed for electron microscopy in 2% glutaraldehyde with a cacodylate buffer, postfixed with osmium, and embedded in araldite plastic for transmission electron microscopy. Another portion was fixed in formalin for paraffin embedding, and sections were stained with Giemsa stain and with hematoxylin and eosin for light microscopy.

Experimental design. The first group of 19 guinea pigs was inoculated with 10¹ ELD₅₀ of GPIC agent, and all developed a typical keratoconjunctivitis that lasted for an average of 3 weeks, with inclusions in the conjunctival smears of all 19 animals. One month after inoculation, when the disease had totally subsided and the smears were negative, the same animals were rechallenged with serial 10-fold increasing doses of agent (10, 10², 10³, and 10⁴ ELD₅₀), and two guinea pigs received 10¹ yolk sac only. At the same time the other 19 guinea pigs that had not yet been infected (primary infection group) were also inoculated with the same serial 10-fold increasing doses of agent and with 10% yolk sac (Table I).