Iontophoresis of vidarabine monophosphate into rabbit eyes. JAMES M. HILL, NO-HEE PARK,* LOUIS P. GANGAROSA, DAVID S. HULL, CAROL L. TUGGLE, KAREN BOWMAN, AND KEITH GREEN.

In order to investigate the efficacy of iontophoresis for increasing the penetration of vidarabine monophosphate into the eye, tritium-labeled vidarabine monophosphate was applied to rabbit eyes by topical and iontophoretic application, and the penetration of the compound into the eye, and its subsequent metabolism, were studied. At 20 min after treatment, the ratios of radioactivity for cathodal iontophoresis compared to topical application alone were cornes 8.6, aqueous humor 4.8, and iris 2.4, for 60 min the ratios were cornes 12.2, aqueous humor 17.5, and iris 2.5. In addition, the acid-soluble components were extracted from the cornea and aqueous humor. Vidarabine monophosphate, vidarabine, hypoxanthine arabinoside, adenosine, hypoxanthine, and adenine from the acid-soluble fraction were separated by thin-layer chromatography. The amount of vidarabine monophosphate and vidarabine in the cornea and aqueous humor from the iontophoretically treated group was six to 15 times higher than from the group that received topical application of the drug. It was concluded that cathodal iontophoresis resulted in significantly increased penetration of the antiviral drug vidarabine monophosphate into the anterior chamber of the eye. The effects of iontophoresis of vidarabine monophosphate on corneal epithelium, as observed by scanning electron micrographs, were equal to or less than those seen with the topical application of widely used preservatives in ophthalmic preparations.

5-Iodo-2'-deoxyuridine (IDU, idoxuridine) and 9-β-D-arabinofuranosyladenine (ara-A, vidarabine) are two drugs which can be used topically for the treatment of herpes simplex keratitis. Vidarabine and IDU have several disadvantages as antiviral agents. (1) The water solubility of both drugs is extremely low.4°–6° (2) They are rapidly metabolized to less effective or inactive compounds.4°–6° (3) When applied topically, there is only limited penetration of the drug into the aqueous humor. The use of vidarabine monophosphate (9-β-D-arabinofuranosyl-adenine-5'-monophosphate, Ara-AMP), the phosphorylated form of vidarabine, may eliminate certain of the disadvantages of IDU and vidarabine, since vidarabine monophosphate is a highly charged molecule and its water solubility is high.4°, 4°–6° Since Ara-AMP has a charged phosphate group, its transport across the cell membrane is limited. Iontophoresis was used in an attempt to enhance the penetration of this charged molecule into the anterior chamber of the eye.

Methods. Albino rabbits (3.5 kg body weight) were given intravenous urethane (1 to 2 gm/kg body weight) anesthesia. Prior to use in the experiments, the tritiated vidarabine monophosphate (spec. act. 5.0 Ci/mmol) was chromatographed with two different solvent systems,7°–9° and all the radioactivity present was found to be associated with the vidarabine monophosphate.

An eye cup was inserted with its periphery applied within the limits of the corneal limbus, and 0.7 ml of a 0.1% solution of vidarabine monophosphate (containing 5 μCi of tritiated vidarabine monophosphate) was applied inside the cup for 4 min of topical application. For cathodal (—) iontophoresis, the cathode was in contact with the drug solution, the return electrode (anode) was connected to the shaved right forelimb of the rabbit, and 0.5 mAMP of current was applied for 4 min. Immediately after completion of topical or iontophoretic administration of vidarabine monophosphate, the eyes were washed with Ringer’s solution. After either 20 or 60 min, an anterior chamber paracentesis was performed to obtain 0.15 ml of aqueous humor. After rewashing of the corneal surface, the cornea, iris, and lens were removed. These eye tissues were weighed and homogenized with a polytron in 0.5N HClO 4 (PCA) for preparation of the acid-soluble fraction. The aqueous humor was treated with 0.5N PCA. After centrifugation, the supernatant, designated as acid-soluble fraction, was collected, neutralized with KOH, and lyophilized. Total radioactivity was determined from an aliquot of each tissue sample. Also the radioactivities of the contralateral eye and blood were determined. From the neutralized and lyophilized acid-soluble fraction, vidarabine monophosphate, vidarabine, hypoxanthine arabinoside (ara-Hx, 9-β-D-arabinofuranosylhypoxanthine), adenine, hypoxanthine (Hx), and adenosine (Ado) were separated by thin-layer chromatography. A° An aliquot of the lyophilized sample was spotted on a 0.5 mm silica gel GF 254 (Brinkman chromatographic glass plate). Since the amounts of labeled compounds were so small, nonlabeled vidarabine monophosphate, vidarabine, adenine, ara-Hx, Ado, and Hx were applied at the origin. The plates were developed with the lower phase of a chloroform-containing solvent prepared

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[References and details omitted for brevity]
Table I. Total radioactivity in acid-soluble fraction of rabbit eye after administration of tritiated vidarabine monophosphate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Min</th>
<th>Cornea</th>
<th>Aqueous humor</th>
<th>Iris</th>
<th>Lens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical application</td>
<td>20</td>
<td>9,262 ± 3,477</td>
<td>2,344 ± 850</td>
<td>240 ± 33</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5,206 ± 1,543</td>
<td>906 ± 443</td>
<td>250 ± 41</td>
<td>0</td>
</tr>
<tr>
<td>Cathodal (—) iontophoresis</td>
<td>20</td>
<td>79,571 ± 10,211*</td>
<td>11,303 ± 1,466*</td>
<td>332 ± 13*</td>
<td>199 ± 12*</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>63,711 ± 3,923f</td>
<td>15,697 ± 2,899f</td>
<td>618 ± 42f</td>
<td>644 ± 273f</td>
</tr>
</tbody>
</table>

Each group contains data from 4 eyes.

*Significantly different (p < 0.01) from topical application (20 min) (t test).

fSignificantly different (p < 0.01) from topical application (60 min) (t test).

Table II. Amount of vidarabine monophosphate and its metabolites in acid-soluble fraction of rabbit cornea after topical or iontophoretic administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Min</th>
<th>Vidarabine monophosphate</th>
<th>Vidarabine</th>
<th>Ara-Hx</th>
<th>Adenine</th>
<th>Hx and Ado</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical application</td>
<td>20</td>
<td>110 ± 29</td>
<td>130 ± 16</td>
<td>150 ± 24</td>
<td>90 ± 11</td>
<td>240 ± 5</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>50 ± 2</td>
<td>50 ± 15</td>
<td>80 ± 5</td>
<td>50 ± 7</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>Cathodal (—) iontophoresis</td>
<td>20</td>
<td>1,560 ± 347*</td>
<td>880 ± 275*</td>
<td>1,640 ± 317*</td>
<td>1,100 ± 166*</td>
<td>1,650 ± 292*</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>400 ± 541</td>
<td>190 ± 101</td>
<td>370 ± 681</td>
<td>480 ± 91</td>
<td>410 ± 501</td>
</tr>
</tbody>
</table>

Each value is a mean of 4 corneas. The concentrations of vidarabine monophosphate and its metabolites were calculated from the radioactivity determined after chromatography and the specific activity of the tritiated vidarabine monophosphate (5 μCi/700 μg).

*Significantly different (p < 0.01) from topical application (20 min) (t test).

fSignificantly different (p < 0.01) from topical application (60 min) (t test).

Table III. Amount of vidarabine monophosphate and its metabolites in aqueous humor of rabbit eye after topical or iontophoretic administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Min</th>
<th>Vidarabine monophosphate</th>
<th>Vidarabine</th>
<th>Ara-Hx</th>
<th>Adenine</th>
<th>Hx and Ado</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical application</td>
<td>20</td>
<td>5 ± 1.6</td>
<td>9 ± 3.6</td>
<td>29 ± 6.6</td>
<td>8 ± 1.7</td>
<td>2 ± 0.9</td>
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<tr>
<td></td>
<td>60</td>
<td>4 ± 1.1</td>
<td>11 ± 1.7</td>
<td>45 ± 4.9</td>
<td>10 ± 2.0</td>
<td>2 ± 1.2</td>
</tr>
<tr>
<td>Cathodal (—) iontophoresis</td>
<td>20</td>
<td>44 ± 4.2*</td>
<td>38 ± 3.5*</td>
<td>87 ± 2.0*</td>
<td>23 ± 2.0*</td>
<td>20 ± 2.3*</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>45 ± 7.71</td>
<td>28 ± 2.71</td>
<td>195 ± 33.21</td>
<td>25 ± 4.71</td>
<td>10 ± 1.91</td>
</tr>
</tbody>
</table>

The concentrations of vidarabine monophosphate and its metabolites were calculated from the radioactivity determined after chromatography and the specific activity of the tritiated vidarabine monophosphate (5 μCi/700 μg).

*Significantly different (p < 0.01) from topical application (20 min) (t test).

fSignificantly different (p < 0.01) from topical application (60 min) (t test).

Results. Table I shows the total radioactivity present in the cornea, aqueous humor, iris, and lens 20 and 60 min after treatment. In all these eye tissues, the highest level of radioactivity was obtained after cathodal iontophoresis of tritiated vidarabine monophosphate. At 20 min after treatment, the ratios of total radioactivity for cathodal iontophoresis compared to topical application were cornea 8.6, aqueous humor 4.8, iris 2.4; at 60 min after treatment the ratios were cornea 12.2, aqueous humor 17.5, and iris 2.5. No radioactivity was detected in the lenses of eyes receiving topical application of tritiated vidarabine monophosphate. In eyes receiving cathodal iontophoresis of the drug, a significant level of radioactivity was present in the lens at 20 and 60 min after treatment. In
Fig. 1. Scanning electron micrograph of corneal epithelial surface 4 min after cathodal iontophoresis of vidarabine monophosphate. A few superficial cells are damaged and some are desquamating. (X200.)

the aqueous humor, iris, and lens, the radioactivity was higher 60 min after iontophoresis than at 20 min, but in the cornea the levels were lower at 60 min when compared to levels at 20 minutes. No radioactivity was detected in the contralateral eye or the blood.

Tables II and III show the amounts of vidarabine monophosphate, vidarabine, Ara-Hx, adenosine, Hx, and Ado present in the cornea and aqueous humor 20 and 60 min after treatment. Cathodal iontophoresis of tritiated vidarabine monophosphate provides the highest concentrations of vidarabine monophosphate and its metabolites in the cornea and aqueous humor. In Table II note that the levels of tritiated vidarabine monophosphate and its metabolites in the cornea are lower at 60 min after application than at 20 min. However, in the aqueous humor (Table III), the levels of the drug and metabolites are generally about the same at 60 and 20 min after drug administration, with the exception of Ara-Hx which is significantly increased 60 min after drug administration.

Slit-lamp examination after fluorescein staining of the corneal surface revealed no difference in the staining of corneas treated by either the iontophoretic or topical application of vidarabine monophosphate. The corneal epithelium was examined by scanning electronmicroscopy (SEM). Immediately after topical or iontophoretic application of Ara-AMP, SEM of the cornea showed a very small amount of surface pitting, with limited exposure of cells underlying the superficial epithelium. Fig. 1 shows the scanning electron micrograph of the cornea after iontophoretic application of vidarabine monophosphate.

Discussion. Cathodal iontophoresis of Ara-AMP significantly increased its penetration into rabbit eyes as compared to topical application. Furthermore, iontophoresis resulted in high levels of the antiviral agent deep in the corneal stroma and aqueous humor. This may be of benefit in the treatment of deep stromal herpes keratitis and herpes uveitis. Whether there are potential dangers from the high concentrations of antiviral agents in the lens, e.g., cataract formation, is not known and warrants further investigation. Although the ocular penetration of vidarabine monophosphate was greatly enhanced by cathodal iontophoresis, no radioactivity was detected in the blood or contralateral eye. It appears that the tritiated vidarabine monophosphate and its radioactive metabolites do not diffuse into other tissues and are metabolized by known pathways.

Most of the vidarabine monophosphate is dephosphorylated to vidarabine; the vidarabine can be deaminated to Ara-Hx or cleaved to arabinose and adenine. Although the level of penetration of vidarabine monophosphate is significantly higher for iontophoresis than for topical application, the
metabolic degradation remains the same. In the cornea (Table I and II) vidarabine monophosphate appears to be more rapidly metabolized after topical application compared to iontophoretic application. In the aqueous humor (Table III), the amounts of vidarabine monophosphate and vidarabine are almost the same 20 and 60 min after iontophoretic and topical application. Therefore the catabolism in the aqueous humor must be slower, diffusion from the cornea higher, or some combination of the two in order to maintain the same amount of vidarabine monophosphate and vidarabine for 20 and 60 min.

Erlander used iontophoresis as a procedure for obtaining high levels of drugs in eye tissues. Although iontophoresis is an uncomplicated, safe, well-documented method for assuring penetration of charged drugs, the technique has not been widely used. Harris reviewed the uses of iontophoresis in medicine and dentistry and cited the advantages of iontophoresis in drug delivery to surface tissues, giving numerous examples. Iontophoresis offers the advantages of assuring penetration of the drug to the desired site, i.e., corneal stroma and aqueous humor, and is an alternative to topical application, systemic administration, or direct injection into the eye tissue.

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Key words: Iontophoresis, vidarabine, vidarabine monophosphate, hypoxanthine arabinoside, corneal epithelium, cornea, aqueous humor

REFERENCES


Histochemical demonstration of cyclic guanosine 3′,5′-monophosphate phosphodiesterase activity in retinal photoreceptor outer segments. Richard M. Robb.

A technique for the histochemical demonstration of cyclic guanosine monophosphate phosphodiesterase in retina is described. Enzyme activity was identified on photoreceptor outer segment lamellae, a finding in agreement with previous biochemical data on isolated outer segment preparations. The distribution of phosphodiesterase activity for cyclic guanosine monophosphate was similar to that found previously in rod outer segments for cyclic adenosine monophosphate, suggesting that the same enzyme may hydrolyze both nucleotides.

The cyclic nucleotide phosphodiesterase which hydrolyzes cyclic adenosine 3′,5′-monophosphate (cyclic AMP) to 5′-adenosine monophosphate has previously been demonstrated on photoreceptor outer segment lamellae by histochemical means. Although a similar enzyme hydrolyzing cyclic...