Pharmacokinetics and Safety of Transcorneal Iontophoresis of Tobramycin in the Rabbit

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Transcorneal iontophoresis of tobramycin in normal eyes of New Zealand white rabbits was compared to an eye cup control and application of fortified topical drops. Iontophoresis was performed with 25 mg/ml of tobramycin at 0.8 mAmps for 10 or 5 min. The eye cup with 25 mg/ml of tobramycin was placed on the eye for 10 min without current. Topical fortified drops (13.6 mg/ml) were applied every half hour for 4 hr. Epithelium, stroma, and aqueous humor were assayed separately at 1, 4, 8 and 16 hr after treatment. The eyes were examined using the slit-lamp biomicroscope before and immediately after the treatment, and prior to sacrifice. Two eyes were examined by light and scanning electron microscopy 5 and 10 min after iontophoresis. Iontophoresis yielded significantly higher tobramycin concentrations than the eye cup or fortified topical drops at 1 hr and 4 hr after treatment ($P = 0.001$).

In all treated eyes, iontophoresis resulted in epithelial edema and mucous discharge, which resolved by 24 hr after the treatment. Histologically there was evidence of epithelial disruption in the superficial layer after 5 min of iontophoresis and in all layers of the epithelium after 10 min of iontophoresis. Iontophoresis is a relatively safe, effective method to deliver medications to ocular tissues and may be useful alone or as an adjunct to current modes of antimicrobial chemotherapy. Invest Ophthalmol Vis Sci 29:1397–1401, 1988

Current modalities of ocular antibiotic delivery include commercially prepared antibiotic drops and ointments, fortified drops, subconjunctival injections and intraocular injections. Commercial drops are satisfactory when used to treat superficial infections; however, they are inadequate in delivering high concentrations of antibiotics to deeper corneal tissues as required in bacterial keratitis.1,2 Also, treatment of deeper infections such as bacterial keratitis often necessitates a hospital admission and extra nursing staff for frequent administration of fortified topical antibiotic drops. Subconjunctival injections are painful and result in nonuniform concentrations in the cornea.3,4 Experimental studies comparing topical drops to subconjunctival injections have yielded contradictory results.2,6–8

Iontophoresis is a procedure whereby polar (charged) medications can be driven across a semi-permeable membrane by the application of a low electrical current. It is capable of delivering high concentrations of medication rapidly and evenly to corneal tissues. Iontophoresis has been employed to administer medications to the eye both transcorneally and transsclerally and has been shown to be effective in reducing bacteria in experimental bacterial keratitis.9–12 No study to date has investigated the pharmacokinetics and safety of transcorneal iontophoresis of tobramycin in the rabbit.

The purpose of the present experiments was, therefore, to examine in rabbit eyes the effects of altering the length of time of each iontophoretic treatment with tobramycin and to determine the safety and toxicity of such administration.

Materials and Methods

New Zealand white rabbits weighing between 1.5 and 2 kg were employed. The rabbits were anesthetized by an intramuscular injection of 1 ml of a mixture of 10 ml ketamine (100 mg/ml) and 2 ml of xylazine (100 mg/ml). The care and maintenance of the rabbits used in these experiments conformed to the ARVO Resolution on the Use of Animals in Research.

The rabbits received one of four treatments. Both eyes were treated. Group one received iontophoresis of tobramycin for 10 min at 0.8 mAmps. Group two received iontophoresis of tobramycin for 5 min at 0.8 mAmps. Group three (eye cup control) had mock...
Iontophoresis performed for 10 min with no current applied. In these three groups, the treatment was performed by placing a cylindrical eye cup with an internal diameter of 11 mm at the corneoscleral limbus. The cup was filled with the solution (25 mg tobramycin/ml of sterile deionized, distilled water) (Alcon, Inc., Fort Worth, TX). In the first two groups, the anode (+) was attached to a platinum electrode in contact with the drug solution while the cathode was attached over a saline soaked gauze pad to the ipsilateral ear of the rabbit. Direct current was supplied by a transformer (Med-therm, Huntsville, AL). In group three, a cylindrical eye cup filled with 25 mg tobramycin/ml was placed on the eye without current for 10 min. The eyes were rinsed with sterile phosphate buffered saline (PBS), pH 7.4, at the end of the iontophoretic and eye cup treatments. Group four received fortified topical tobramycin drops prepared by adding 2 ml of injectable tobramycin (40 mg/ml, Distal Products, Indianapolis, IN) to a commercial bottle of Tobrex® drops (Alcon, Fort Worth, TX) to give a final concentration of 13.6 mg/ml. One drop was instilled in both eyes every 30 min for a total of 4 hr. The eyes in this group were rinsed with PBS prior to removal of the tissues.

All rabbits were sacrificed by an overdose of pentobarbital at 1, 4, 8 or 16 hr after the termination of treatment. The epithelium was removed by scraping with a number 15 Bard-Parker® blade (Rutherford, NJ). The aqueous humor was aspirated using a 27-gauge needle attached to a 1 ml tuberculin syringe. The cornea was excised and minced into small pieces with a razor blade. Samples were placed in pre-weighed vials and weighed to determine wet weight of the samples. PBS was added: 300 μl to the epithelial and 1 ml to the stromal samples. Corneal stroma was homogenized for 15 seconds using an Ultra Turrax tissue homogenizer (Tekmar Co., Cincinnati, OH). Epithelial and stromal specimens were sonicated at a setting of 5 for 1 min using a Sonifier (Branson Ultrasonics Corp., Stamford, CT), and centrifuged for 5 min at a setting of 5 in an IEC clinical centrifuge (International Equipment Co., Needham Heights, MA). The supernatant was analyzed for tobramycin concentration by Smith Kline Bioscience Laboratories, New Orleans, LA using the Emit® Tobramycin Immunoassay (Syva Co., Palo Alto, CA) which measures tobramycin concentration by monitoring spectrophotometric change following reaction with an antibody-linked enzyme. Preliminary experiments validated the accuracy of the immunoassay in the presence of ocular tissue samples. The lower limit of the assay was 1.0 μg/ml. There were at least four determinations at each time point. Tissue from each eye was analyzed separately and the values averaged. Results were expressed as the mean value ± the standard error of the mean in microgram/gram tissue for the epithelium and stroma or in microgram/milliliter for aqueous samples. Results were analyzed using the Statistical Analysis Systems (SAS). An analysis of variance was performed on the values obtained at 1, 4 and 8 hr. Following the finding of a significant analysis of variance, t-tests between the means from each treatment group were conducted.

To study the effects of tobramycin iontophoresis on the eye, all eyes were examined with the slit-lamp biomicroscope prior to treatment, immediately following the treatment, and prior to sacrificing the rabbits.

**Light and Electron Microscopy**

One rabbit received iontophoresis of tobramycin (25 mg/ml) for 10 min at 0.8 mAmps in the right eye and for 5 min in the left. The rabbit was sacrificed and the eyes enucleated immediately after treatment. The cornea was excised and fixed for 2 hr in 2.5% gluteraldehyde, 1% paraformaldehyde, and 0.1 M cacodylate buffer, pH 7.4, at room temperature. The specimens were transferred to cacodylate buffer, and osmicated in 2% OsO4 for 2 hr, followed by either ethanolic dehydration and embedding in epon for light microscopy or critical point drying using CO2 as the medium for scanning electron microscopy (SEM). Thick 1 μm sections were cut for orientation and stained with toluidine blue and basic fuchsin stain. Endothelium was examined by SEM with the Zeiss DSM 950 scanning electron microscope.

**Results**

**Tobramycin Concentrations**

Iontophoresis of 10 min produced significantly higher concentrations of tobramycin in the stroma and aqueous humor 1 hr after treatment, compared with iontophoresis for 5 min ($P = 0.006$) (Table 1). Iontophoresis for either 5 or 10 min produced significantly higher concentrations of tobramycin in the epithelium, stroma, and aqueous humor 1 hr after treatment, compared with the eye cup controls (mock iontophoresis with no current) and with treatment by topical application of fortified drops ($P = 0.0004$).

Four hours after treatment, samples of stroma and aqueous humor from eyes that had undergone 10 min of iontophoresis still contained significantly higher concentration of drug, compared with samples from eyes that had had only 5 minutes of iontophoresis ($P = 0.0004$). Samples from eyes that had undergone 5 min of iontophoresis had significantly higher drug concentrations than the eye cup controls ($P = 0.04$).
Table 1. Tobramycin concentration in anterior segment (µg/gm or µg/ml) following iontophoresis, eye cup, and fortified topical drops

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tissue</th>
<th>1 hr</th>
<th>4 hr</th>
<th>8 hr</th>
<th>16 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>ION</td>
<td>Epithelium</td>
<td>539.1 ± 114.3 (6)</td>
<td>168.7 ± 30.8 (5)</td>
<td>103.2 ± 56.6 (6)</td>
<td>ND (6)</td>
</tr>
<tr>
<td>25 mg/ml</td>
<td>Stroma</td>
<td>880.2 ± 45.4 (6)</td>
<td>382.2 ± 61.5 (5)</td>
<td>20.7 ± 3.8 (6)</td>
<td>6.3 ± 1.5 (6)</td>
</tr>
<tr>
<td>10 min</td>
<td>Aqueous</td>
<td>312.8 ± 34.2 (5)</td>
<td>163.5 ± 15.2 (5)</td>
<td>5.9 ± 1.7 (6)</td>
<td>ND</td>
</tr>
<tr>
<td>0.8 mAmps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ION</td>
<td>Epithelium</td>
<td>1370 ± 202 (4)</td>
<td>133.5 ± 4.8 (4)</td>
<td>26.3 ± 4.1 (4)</td>
<td>—</td>
</tr>
<tr>
<td>25 mg/ml</td>
<td>Stroma</td>
<td>607.6 ± 99.8 (4)</td>
<td>75.4 ± 16.2 (4)</td>
<td>13.0 ± 3.0 (4)</td>
<td>—</td>
</tr>
<tr>
<td>5 min</td>
<td>Aqueous</td>
<td>145.3 ± 25.8 (4)</td>
<td>48.7 ± 9.7 (4)</td>
<td>3.1 ± 0.8 (4)</td>
<td>—</td>
</tr>
<tr>
<td>0.8 mAmps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye Cup</td>
<td>Epithelium</td>
<td>96.4 ± 29.7 (4)</td>
<td>15.5 ± 9.0 (4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>25 mg/ml</td>
<td>Stroma</td>
<td>168.7 ± 21.1 (4)</td>
<td>5.8 ± 5.8 (4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10 min</td>
<td>Aqueous</td>
<td>42.3 ± 4.0 (4)</td>
<td>9.5 ± 1.6 (4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Drops*</td>
<td>Epithelium</td>
<td>ND (4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>13.6 mg/ml</td>
<td>Stroma</td>
<td>ND (4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Every 1/2 h</td>
<td>Aqueous</td>
<td>0.4 ± 0.4 (4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Concentrations calculated 1 hr after the last drop administration.
ND = None detected.
— = Not done.
ION = Iontophoresis.

Eight hours after treatment, there were no significant differences between the samples from eyes that had undergone either 5 or 10 min of iontophoresis (P = 0.2).

Slit-Lamp Examination

None of the eyes had preexisting corneal pathology as determined by slit-lamp biomicroscopy. Following iontophoresis, all the eyes had developed corneal epithelial edema. This edema had nearly resolved by 16 hr after iontophoresis. In one eye that was examined at 24 hr, the edema was completely resolved. Of the 29 treated eyes, four developed epithelial defects. All eyes had a small amount of mucous discharge following the procedure. There was no evidence of iris abnormality or lenticular opacities in any of the rabbits.

Light and Electron Microscopy

Two corneas, one that received iontophoresis for 5 min and one for 10 min, were examined by light and scanning electron microscopy. In the 5 min eye, light microscopy revealed evidence of minimal disruption of the surface epithelial cells. In the 10 min eye, there were focal areas of epithelial edema and cell disruption at all levels of the epithelium. The stroma and endothelium were normal (see Fig. 1A, B). By scanning electron microscopy, both corneas had a normal appearing endothelial cell layer with intact cell membranes and intercellular junctions. No evidence of irreversible cell damage was observed.

Discussion

Iontophoresis has been investigated as a technique of drug delivery in ophthalmology since the 1940s.13-17 The technique has been used to deliver gentamicin transcorneally to the vitreous cavity in aphakic rabbits and has been adapted to deliver antibiotics transsclerally.9-11 To date, no investigations have employed tobramycin and compared different iontophoretic treatment times with an eye cup control and topical fortified drops. Additionally, in the present study, the corneal epithelium was analyzed separately to determine how much of the drug was found in the superficial cornea. At 1 hr after treatment, iontophoresis for 5 or 10 min treatment periods was significantly better than both the eye cup control and topical fortified drops (13.6 mg/ml every 1/2 hr for 4 hr). Iontophoresis for 10 min gave significantly higher concentrations of tobramycin in the stroma and aqueous humor when compared with iontophoresis for 5 min. The epithelial levels were higher after 5 min of iontophoresis. This may be due to increased variability produced when collecting a small sample such as epithelium. This difference was not apparent in the epithelium at 4 hr and in the epithelium, stroma, and aqueous humor by 8 hr after treatment. By 8 hr the forces of diffusion may have eliminated much of the tobramycin despite initial high levels thus obscuring the difference between the treatments.

The eye cup control, performed by placing a cylindrical eye cup on the eye with tobramycin solution in it, was significantly different from iontophoresis. Al-
through the drug concentrations were lower than iontophoresis, they were significant in terms of bactericidal levels, which are usually less than 5 μg/ml with sensitive organisms. The concentrations obtained with the eye cup were significantly better than fortified topical drops (13.6 mg/ml) instilled every 1/2 hr for 4 hr. In our experiments, topical drops resulted in very low concentrations of drug in the epithelium, stroma, and aqueous humor. Our results obtained with fortified topical drops are consistent with those of Gilbert et al.,1 who also employed uninfected New Zealand white rabbits and found low corneal and aqueous humor (1.7 μg/ml and 0 respectively) concentrations of tobramycin 1 hr after one drop of fortified tobramycin (1.1%) levels as we did. Our corneal levels after topical fortified drops were lower than his. We rinsed the eyes of our rabbits with PBS after sacrifice prior to harvesting the tissues. In addition, we did not solubilize the corneas in NaOH. We used a different assay, a modified enzyme linked immunosassay, whereas Gilbert et al1 used a biological assay. Perhaps these methodological differences could explain the slightly higher levels in corneal tissue obtained by Gilbert et al.1

The iontophoretic treatment, while effective in delivering high concentrations of tobramycin, was not without side effects. All of the rabbits in this study developed epithelial edema following the treatment. This edema was evident on histological sections, though we studied only two corneas and more would be desirable to validate our findings. Since the treatment would probably be employed in eyes with severe damage, the edema may not be clinically significant.

In conclusion, iontophoresis has been shown to be effective in delivering high concentrations of tobramycin to corneal tissues and aqueous in the normal rabbit eye. These concentrations are higher than those obtained with frequent topical fortified drops. Iontophoresis could be useful as a drug delivery system to treat bacterial keratitis and other difficult corneal infections by delivering high concentrations of antibiotics rapidly and evenly to corneal tissues.

Key words: iontophoresis, ocular drug delivery, pharmacokinetics, rabbit, tobramycin

Acknowledgments

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