Delivery of Gentamicin to the Rabbit Eye by Drug-Loaded Hydrogel Iontophoresis

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PURPOSE. To assess the corneal iontophoretic delivery of gentamicin by drug-loaded hydrogel probe, and to determine the resultant ocular disposition and elimination of the drug from the cornea and anterior chamber.

METHODS. Corneal iontophoresis of gentamicin sulfate was studied in healthy white rabbits by using drug-loaded disposable hydroxyethyl methacrylate (HEMA) hydrogel disk probes and a portable mini-ion device designed in the authors’ laboratory. The iontophoretic treatment was performed with a current intensity of 1 mA for 60 seconds only. Three control groups were used: mock iontophoresis (no current) for 60 seconds, topical eye drops of fortified gentamicin (1.4%) every 5 minutes for 1 hour, and subconjunctival injection of 0.25 mL of 40 mg/mL gentamicin solution. The animals in the iontophoretic experimental groups were killed at predetermined time points. The gentamicin concentrations in the cornea and aqueous humor were assayed with a fluorescence polarization immunoassay. Analysis of the gentamicin eye pharmacokinetics was performed with a modeling approach.

RESULTS. Peak gentamicin concentrations in the cornea (365.1 ± 127.3 µg/g) and in the aqueous humor (29.4 ± 17.4 µg/mL) were reached at 0 and 2 hours after the iontophoretic treatment, respectively. The peak gentamicin concentrations after a single iontophoresis treatment were 12 to 15 times higher than those obtained after gentamicin injection or after topical eye drop instillation, and much higher than in mock iontophoresis. The concentration versus time profile of gentamicin in the cornea and the anterior chamber after iontophoresis was appropriately described by applying a two-compartment pharmacokinetic model.

CONCLUSIONS. A short iontophoretic treatment using gentamicin-loaded hydrogels has potential clinical value in increasing drug penetration to the anterior segments of the eye and maintaining therapeutic drug levels in the cornea for more than 8 hours. (Invest Ophthalmol Vis Sci. 2004;45:2543–2548) DOI:10.1167/iovs.03-1294

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Ocular iontophoresis of gentamicin in experimental animals produced high levels of gentamicin in the cornea and aqueous humor and was associated with minimal toxicity.\(^5,^{12,13}\) However, the experimental protocol in these studies may not be suitable in clinical settings because of the long period of iontophoresis (10 minutes) and levels of electric current at approximately 2 mA. Moreover, iontophoresis of drug solution applied in these studies necessitates prolonged contact between the cup containing drug solution and the cornea, which is technically clumsy, may cause mechanical injury to the cornea, and demands sterilization of the solution and cup before each treatment. It has been proposed that most of these drawbacks could be prevented by applying semisolid drug-loaded probes for iontophoresis application, such as an agar-gentamicin probe.\(^14–16\)

Hydrogels are three-dimensional networks of hydrophilic polymers held together by association bonds or cohesive forces. We hypothesized that hydrogel probes would be suitable for gentamicin iontophoresis delivery because they can retain large quantities of gentamicin solution and can be directly applied to the cornea without need for sophisticated equipment. Hydrogels have been used for transdermal iontophoretic delivery of various drugs,\(^17,18\) and their ophthalmologic uses have been limited so far to application as contact lenses.\(^19\)

The objective of the present study was to assess the corneal iontophoresis delivery of gentamicin using drug-loaded hydrogel probes, and to determine the resultant ocular permeability and disposition of the drug in vivo. The study was performed in preclinical settings, using rabbits as a common model to study ocular drug pharmacokinetics\(^4,20\) and a novel mini-ion iontophoresis device.

**Materials and Methods**

Gentamicin sulfate was obtained from Sigma-Aldrich (St. Louis, MO). All the other materials and agents were purchased from Sigma-Aldrich (Rehovot, Israel). All solvents were HPLC grade from BioLab (Jerusalem, Israel).

**The Iontophoretic Device**

The iontophoretic device that was applied in this study is a portable mini-ion device (designed in our laboratory) that can be operated by battery or by an external electric source (Fig. 1). A probe, with a cylindrical well attached that is 5 mm in diameter and 3 mm in depth, is used for insertion of the drug-loaded hydrogel disk 5 mm in diameter and 5 mm in height. The mini-ion device applies a variable electrical current in the range of 0.1 to 1.0 mA for preset periods from 10 to 120 seconds.

**FIGURE 1.** The mini-ion device used for delivery of gentamicin by iontophoresis. The device is composed of a cylindrical well for the insertion of a disposable hydrogel, two electrodes, and a control panel for time and current control.

**Preparation of HEMA Gel Disks**

Hydroxyethyl methacrylate (HEMA; 97% pure), ethylene glycol dimethacrylate (EDGMA, 98% pure), and deionized water (2.0, 0.04, and 6.5 mL, respectively) were used to produce a clear solution, under a stream of dry nitrogen. The following initiator solutions were added: 2% sodium persulfate Na2S2O8 (0.05 mL), 2% sodium metabisulfite Na2S2O10 (0.05 mL), and 2% ammonium ferrous sulfate Fe(NH4)2(SO4)2 (0.025 mL). The solution was rapidly mixed and divided into a Teflon tray with wells (5 mm height, 6 mm diameter) and was left to polymerize overnight at room temperature, under a nitrogen blanket in the dark. The obtained disks were immersed in 0.5 L of water with stirring, to clean the hydrogels of unreacted monomers, catalysts, and low-molecular-weight by-products. The purified hydrogels were frozen with liquid nitrogen and were dehydrated by lyophilization overnight to form sponge cylinders that were of a size similar to that of the wet cylinders. Two hours before use, the dried hydrogels were immersed in a 10% (wt/vol) gentamicin sulfate solution. The drug-loaded hydrogels were solid but soft, and had a cylindrical shape with dimensions of 5 × 5 mm. The hydrogels were weighed before and after immersion in the gentamicin solution, to evaluate the drug load of the hydrogel before use.

**Animals**

Healthy New Zealand White male rabbits (n = 58) weighing 2.0 to 3.0 kg were used in the study. The study conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Drug Administration**

The animals were anesthetized by injection of ketamine and xylazine solution (intramuscularly, 25 and 2.5 mg/kg, respectively). Five groups of eight rabbits each underwent corneal iontophoresis with the mini-ion system with a hydrogel disk probe containing an average amount of 26.11 mg gentamicin sulfate (one eye per rabbit). Before the electrode was placed on the ocular surface, the eye was topically anesthetized with 0.4% benoxinate eye drops (Localin, Dr. Fischer Laboratories, Ltd., Bnei-Barak, Israel). The gentamicin-loaded hydrogel disk was inserted into the cylindrical opening edge of the iontophoretic device and placed onto the cornea. The complementary electrode was attached to the ear of the rabbit. Iontophoresis was performed on one eye by using a current intensity of 1.0 mA (5.1 mA/cm²) for 60 seconds. The experimental settings included three control groups of six rabbits each. One control group (group 6) was treated with mock iontophoresis, in the same way as described earlier, with the exception that no current was applied. Group 7 received topical eye drops of fortified gentamicin (1.4% wt/vol in water) every 5 minutes for 1 hour, and group 8 received a subconjunctival injection of 10 mg gentamicin (0.25 mL of 40 mg/mL gentamicin; Teva, Kfar-Saba, Israel) into the subconjunctival space.

**Sample Collection**

The animals in the experimental groups were killed immediately after the iontophoretic treatment and at 1, 2, 4, and 8 hours after treatment (groups 1–5, respectively) by an intravenous injection of a lethal dose of 2% pentobarbital. The animals in the control groups were killed immediately after the end of the drug administration period.

The aqueous humor was collected by aspiration, and the corneas were excised. The volume of the aqueous humor and weight of the cornea were measured after their collection from the animals.

**Gentamicin Assay**

**Cornea.** After excision, the specimen was rinsed in 5 mL 0.9% NaCl solution, cut with a scalpel, and weighed. Each sample of the specimen was transferred into the microcentrifuge tube and 1.0 mL of 0.01 M phosphate-buffered saline (pH 7.4; Sigma-Aldrich) was added. The tubes were incubated for 18 hours at 37°C with oscillation at 100 rpm followed by centrifugation at 2000 rpm for 10 minutes. The

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supernatant was collected (200 μL) and was assayed for gentamicin concentration using a fluorescence polarization immunoassay (TDx System Analyzer, Abbott Laboratories, Abbott Park, IL).

Aqueous Humor. Aliquots of 100 μL of the collected samples were diluted with 100 μL normal saline, and gentamicin concentrations were determined (TDx; Abbott Laboratories).

The TDx system analyzer is being used routinely by the Hadassah Hospital in Jerusalem for drug level detection in patient’s serum. For gentamicin, the detection limit of the TDx system is 0.1 μg/mL, at a 95% confidence level and average recovery of 102.6% with precision of less than a 3% coefficient of variation (CV). The instrument was calibrated before each measurement according to the authorized gentamicin standard in the kit.

The concentration of gentamicin in the cornea was calculated as follows

$$C_{sample} = (W_{sample} + V_{buffer}) \cdot C_{buffer}/W_{sample}$$  (1)

where $C_{sample}$ is the gentamicin concentration in the sample (micrograms per gram); $C_{buffer}$ is gentamicin concentration in the buffer in micrograms per gram; $W_{sample}$ is the weight of the sample in grams; and $V_{buffer}$ is the volume of buffer in milliliters.

Pharmacokinetic Analysis

Analysis of the pharmacokinetics of gentamicin after corneal iontophoresis was performed by using a modeling approach. The applied model consisted of two compartments attributed to the cornea and the anterior chamber (Fig. 2). The gentamicin transport between the compartments and elimination through the posterior segments of the eye was assumed to occur according to the first-order kinetic processes. The differential equations used to describe the pharmacokinetic model were as follows:

$$dX_1/dt = -k_{12} \cdot X_1 + k_{11} \cdot X_2$$  (2)

$$dX_2/dt = k_{12} \cdot X_1 - (k_{21} + k_{20}) \cdot X_2$$  (3)

where $X_1$ and $X_2$ are the gentamicin amounts in the cornea and the anterior chamber, respectively, and the rate constants are $k_{12}$ transport from the cornea to the anterior chamber, $k_{21}$ transport from the anterior chamber to the cornea, and $k_{20}$ elimination through the anterior chamber to the posterior segments of the eye.

The modeling was performed on computer (WinNonlin, ver. 4.0.1; Pharsight Corp., Mountain View, CA) applying the Gauss-Newton algorithm and 1/2 weighting. Both sets of concentration versus time data were analyzed simultaneously. The fitness of the applied model to the experimental data was assessed using Akaike (AIC) and Schwarz (SC) criteria.

Statistical Analysis

The Kruskal-Wallis test with the Dunn post hoc test was used for statistical evaluation of the gentamicin concentration obtained in the studied groups. $P < 0.05$ was considered significant. All results are expressed as the mean ± 1SD.

### DISCUSSION

Various approaches have been applied to achieving therapeutic concentrations of antibiotics in the cornea, aqueous humor, and vitreous humor of the eye. Subconjunctival, retrobulbar, intravenous, and intramuscular injections and topical drugs have been applied, but they do not produce adequate drug concentrations and are associated with a relatively high risk of complications and adverse effects.

Iontophoresis is a noninvasive technique for drug administration to the eye that has been sporadically reported in ophthalmology, mainly for administration of antibiotic drugs for the treatment of bacterial infections. Gentamicin is suitable for iontophoresis, because it is charged at physiologic pH (three primary amines per molecule), possesses a low molecular weight, and is effective against a wide range of bacterial species.
pathogenic Gram-negative and -positive bacteria. Gentamicin does not reach therapeutic concentrations in the eye tissue after regular topical administration (eye drops), because of its highly hydrophilicity and charge, leading to low permeability through biological membranes.

In our experiments, a short-term transcorneal iontophoresis of gentamicin produced drug concentrations in the cornea that were several times higher than those obtained in the control groups after mock iontophoresis, prolonged application of gentamicin eye drops, or gentamicin subconjunctival injection (Table 1). Despite the fact that the animals in the control groups were killed only at one time point (immediately after the end of the drug administration period.

**Table 1.** Concentrations of Gentamicin in Cornea and Aqueous Humor after Transcorneal Iontophoresis with the Mini-Ion System with Hydrogel Disk Probe, Compared with the Control Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sampling Time (h)</th>
<th>Gentamicin Concentration in the Cornea (µg/g)</th>
<th>Gentamicin Concentration in the Aqueous Humor (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Iontophoresis</td>
<td>0</td>
<td>363.1 ± 127.3*†‡</td>
<td>3.0 ± 2.9</td>
</tr>
<tr>
<td>2</td>
<td>Iontophoresis</td>
<td>1</td>
<td>122.1 ± 47.1*§</td>
<td>9.4 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>Iontophoresis</td>
<td>2</td>
<td>138.7 ± 119.6*</td>
<td>29.4 ± 17.4</td>
</tr>
<tr>
<td>4</td>
<td>Iontophoresis</td>
<td>4</td>
<td>47.8 ± 19.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Iontophoresis</td>
<td>8</td>
<td>24.9 ± 6.1</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>Mock Iontophoresis</td>
<td>—</td>
<td>2.7 ± 2.1</td>
<td>0.0§</td>
</tr>
<tr>
<td>7</td>
<td>Eye drops</td>
<td>—</td>
<td>22.7 ± 14.8</td>
<td>3.2 ± 2.1</td>
</tr>
<tr>
<td>8</td>
<td>Subconjunctival injection</td>
<td>—</td>
<td>30.0 ± 8.6</td>
<td>3.2 ± 1.6</td>
</tr>
</tbody>
</table>

Groups 1–5 (n = 8) received transcorneal iontophoresis with the mini-ion system with gentamicin-loaded hydrogel disk probe at current intensity of 1 mA (5.1 mA/cm²) for 60 seconds. Group 6 (n = 6) received transcorneal mock iontophoresis (no current) applying the Mini-ion system with gentamicin-loaded hydrogel disk probe for 60 seconds. Group 7 (n = 6) received topical fortified gentamicin drops (1.4% wt/vol in water) every 5 minutes for 1 hour. Group 8 (n = 6) received a subconjunctival injection of 0.25 mL containing 10 mg gentamicin. The animals in the experimental groups (groups 1–5) were killed at predetermined time points, and the animals in the control groups were killed immediately after the end of the drug administration period.

* Significantly higher compared to group 6 (mock iontophoresis), P < 0.001.
† Significantly higher compared to group 7 (eye drops), P < 0.001.
§ Significantly higher compared to group 8 (injection), P < 0.01.
‡ Significantly higher compared to group 7 (eye drops), P < 0.05.
¶ Significantly higher compared to group 6 (mock iontophoresis), P < 0.05.
§ Significantly higher compared to group 6 (mock iontophoresis), P < 0.05.

Below the detection limit of the TDx system.

Figure 3. The time course of the gentamicin concentrations in the cornea (circles) and the anterior chamber (triangles) after transcorneal iontophoresis of the drug. (A) Linear plot; (B) semilogarithmic plot. Data points are the observed data, and lines are the best fits according to the applied pharmacokinetic model (Fig. 2, and equations 1, 2). Gentamicin concentrations were obtained from five experimental groups of rabbits that underwent transcorneal iontophoresis with the mini-ion system with the drug-loaded hydrogel probe, at a current of 1 mA for 60 seconds. The rabbits in the experimental groups were killed immediately or at 1, 2, 4, or 8 hours after the iontophoretic treatment.
The estimated values were obtained by pharmacokinetic analysis of gentamicin concentrations following corneal iontophoresis using a two-compartment pharmacokinetic model (see Figure 2). The gentamicin transport between the compartments and elimination to the posterior segments of the eye was assumed to occur according to the 1st order kinetic processes.

The rate constants of gentamicin transport are: $k_{12}$ transport from the cornea to the anterior chamber, $k_{21}$ transport from the anterior chamber to the cornea, and $k_{20}$ elimination from the anterior chamber to the posterior segments of the eye.

$\dagger$Coefficient of variation.

<table>
<thead>
<tr>
<th>Rate Constant $^*$</th>
<th>Estimated Value</th>
<th>%CV $^\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{12}(h)$</td>
<td>0.651</td>
<td>26.0</td>
</tr>
<tr>
<td>$k_{21}(h)$</td>
<td>0.256</td>
<td>57.5</td>
</tr>
<tr>
<td>$k_{20}(h)$</td>
<td>0.334</td>
<td>13.0</td>
</tr>
</tbody>
</table>

The time course of gentamicin concentration in the anterior segments of the eye after corneal iontophoresis was successfully described by the two-compartment pharmacokinetic model (Figs. 2, 3). More complex models, with additional (reservoir) compartments and/or drug transport processes, that were applied previously to determine the disposition in the eye of different drugs did not provide a better description of the experimental data observed for gentamicin. According to the modeling results, the elimination of gentamicin from the cornea occurs predominantly through the anterior chamber, and the contribution of other possible elimination pathways (through the sclera, back to the precorneal area, or enzymatic metabolism) is negligible. The values of the calculated pharmacokinetic parameters (Table 2) indicate that the rate-limiting step of elimination of gentamicin from the anterior segments of the eye is by drug transport from the anterior chamber to the posterior segments of the eye (with $k_{20}$ rate constant; Table 2, Fig. 2).

Gentamicin’s $t_{1/2}$ in the anterior chamber after an iontophoretic treatment of 1 mA for 60 seconds (2.07 hours) was found to be similar to its $t_{1/2}$ after topical eye drop administration (1.9 hours), as reported by Schoenwald.22 However, gentamicin’s $t_{1/2}$ was higher than the $t_{1/2}$ of aqueous humor turnover ($t_{1/2} = 46.2$ minutes),22 and gentamicin’s clearance from the anterior chamber (CL = 1.75 µL/min) was less than aqueous humor’s clearance by bulk flow (CL = 4.67 µL/min).22 These data indicate that gentamicin’s molecules may be bound, to a certain degree, to the eye tissues and are characterized by prolonged retention in the anterior segments of the eye.

Aminoglycoside antibiotics are characterized by a concentration-dependent bactericidal effect over a wide range of concentrations. In addition, exposure to aminoglycosides has a prolonged effect on bacteria (the postantibiotic effect) that persists for several hours, despite the decline of the drug concentrations below the MIC.24,25 Thus, higher concentrations of gentamicin, compared with the MIC, are expected to produce rapid and potent bactericidal effects.

It has been determined that the MIC of gentamicin against the Pseudomonas aeruginosa species is approximately 3 µg/mL.12 and the MICs in 94% of 1142 isolated bacteria strains were 10 µg/mL gentamicin or less.26 Because gentamicin concentrations in the cornea and the aqueous humor observed after iontophoresis in the present study (Table 1) were mostly higher than those MICs, potent bactericidal effects in these tissues are probable. In contrast, gentamicin eye drops produced lower concentrations and was thought to produce limited pharmacodynamic effects, despite a higher total dose of the drug. In addition, higher local concentrations of gentamicin, with a lesser extent of systemic exposure to the drug, is thought to provide additional beneficial effects, particularly during prolonged therapy, including fewer systemic adverse effects27 and a reduced rate of emergence of drug-resistant mutants.28 The efficacy of delivering gentamicin by iontophoresis, as described in the current study, was demonstrated in rabbits infected with Pseudomonas aeruginosa (Frucht-Pery J, Raiskup F, Mechoulam H, Shapiro M, Eljarrat-Binstock E, Domb AJ. Iontophoretic treatment of experimental Pseudomonas keratitis in rabbit eye using gentamicin-loaded hydrogel. Submitted).

As a result of a better understanding of the unique pharmacodynamic properties of gentamicin and the mechanisms responsible for its toxicity, a new method of once-daily doses is administered in various hospitals for systemic treatment of infections. The iontophoretic treatment can promote the use of this once-daily method in therapy for eye infections.29 With respect to the clinical applicability of the hydrogel-based iontophoresis, the results presented in this report demonstrate the ability of iontophoresis to deliver doses of charged drugs to different segments of the eye by using a convenient, easy-to-operate device with a disposable drug-loaded hydrogel. Other complementary studies (to be published) have shown high effectiveness in treating eye infections, and a preliminary toxicity study revealed no permanent damage to the cornea.30

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References