Pharmacokinetics of Intravitreal Injection
Assessment of a Gentamicin Model by Ocular Dialysis

J. Ben-Nun,* D. A. Joyce,† R. L. Cooper,‡ S. J. Cringle,§ and I. J. Constable¶

Intravitreal drug administration is the treatment of choice for bacterial endophthalmitis, but improved knowledge of vitreal pharmacokinetics is essential for the development of optimal antibiotic regimes. We used our recently developed sampling device to estimate vitreal gentamicin concentrations for up to 30 hr after an intravitreal bolus injection of gentamicin. The device is based on the principle of dialysis, whereby a constant flow rate of dialysate through a loop of dialysis fiber in the vitreous attains a gentamicin concentration proportional to the intravitreal gentamicin level around the fiber. The dialysate is continuously recovered and the collected samples then assayed for gentamicin. Normal cat eyes and those with induced bacterial endophthalmitis formed the two groups tested. Concentration-time data fitted well to an open single compartment pharmacokinetic model that incorporated the processes of transfer of drug from the injection site to the sampling site (a function of diffusion within the vitreous), and the elimination from the sampling site (a function of elimination from the vitreous). The initial phase of transfer between the injection and sampling site was rapid and rates were comparable in the two groups. Elimination rate constants were uniformly greater in infected eyes than in controls (0.107 hr⁻¹ compared to 0.055 hr⁻¹). Aqueous humor gentamicin concentrations in control eyes varied between 3 and 6 times those found in fellow infected eyes at the end of each experiment. Accelerated elimination of gentamicin from the vitreous body of eyes with endophthalmitis may be explained by increased permeability of the blood—retinal barrier.

Materials and Methods

Surgical Procedure
Adult domestic cats of weight 2.5 to 5 kg were used. Anesthesia was induced by an initial intravenous injection of 2 ml Saffan (alfaxalone 9 mg/ml, alfadalone acetate 3 mg/ml). After endotracheal intubation the sympathetic trunk was severed bilaterally. The cats were ventilated at 33 strokes/min, with a mixture of 20% oxygen and 80% nitrogen. After an initial dose of 80 mg Flaxedil (gallamine triethiodide) to achieve paralysis, a constant intravenous infusion of Flaxedil (5 mg/kg/hr) and Saffan (2 ml/hr) produced stable anesthesia and paralysis. EKG was monitored continuously during each experiment, each of which lasted from 7 to 9 hr. Both pupils were dilated with drops of tropicamide 0.5% and phenylephrine 10%.

Ocular Procedure
Lateral canthotomy was performed in both eyes, and the area of the upper temporal part of the sclera was exposed. The superior rectus muscle was divided...
and a small piece of cotton wool was inserted into the gap between the posterior sclera and the superior margin of the orbit in order to stop bleeding. A drop of cyanoacrylate glue was applied to the cotton wool, which established good fixation of the eye. A disk of rubber 5 mm in diameter and 1 mm thick was glued to the sclera over the pars plana region in the supero-temporal quadrant of each eye. A 1 mm diameter hole was made by a diathermy probe through the rubber disks without penetrating the eye. A 20 gauge needle was then passed into the eye through each hole using direct ophthalmoscopic visualization. A sampling catheter for ocular dialysis was passed into the sclerotomy site in each eye and immediately fixed in place by a drop of cyanoacrylate glue, followed by a small drop of rapid-setting epoxy adhesive.

**Ocular Dialysis**

Vitreous and brain sampling by dialysis has been described elsewhere.4-20 Briefly, the ocular dialysis catheters, one for each eye, were connected in parallel to a double-barrel Harvard pump (Fig. 1) and flushed through for 3 min at 100 μl/min with normal saline solution, during which time the system was checked for leakage. No gentamicin was detected in samples of this fast-flow perfusate. Perfusion was reduced to 3.5 μl/min. Gentamicin was administered by intravitreal injection to a site away from the selected sampling point and perfusate was collected as detailed below. The recovery index, that is, the fraction of gentamicin recovered in the perfusate, expressed as a percentage of the concentration of gentamicin in the vitreous was 5% ± 0.5% (standard deviation, SD) for this sampling catheter.4

**Experimental Method**

A standing culture of *Staphylococcus aureus* (FDA 209P, NCTC 7447) was grown for 18 hr at 37°C in 10 ml of Brain-Heart-Infusion-Broth. Dilutions of 1/10, 1/20, 1/30 through 1/100 were made in saline for measurement of absorbance with a spectrophotometer, (Spectronic 20, Bausch and Lomb, Rochester, NY) at a wavelength of 420 nm using a saline blank. Further serial ten-fold dilutions of these samples were plated out on Brain-Heart-Infusion agar plates for colony counting. The number of colony forming units per milliliter (CFU/mL) was plotted against absorbance at 420 nm in order to calculate the appropriate dilution of the organism for injection into the vitreous humor (5000 CFU/ml).

A volume of 0.1 ml saline containing 500 CFU of *Staphylococcus aureus* was injected into the vitreous of the right eye of 12 cats via the pars plana using a 27 gauge needle (Table 1). Forty-eight hours later, 100 ± 20 μg (SD) of gentamicin was injected into the vitreous humor of both eyes of cats 1 through 8 (eyes 1 through 16), through the pars plana with a 27 gauge needle, except for eyes 17 through 24, in which 400 ± 80 μg (SD) was injected (Table 1). In eyes 1 through 12 the ocular dialysis catheter was implanted prior to the gentamicin injection so that intravitreal levels of gentamicin could be measured immediately following the injection. Perfusate was collected over 30 min periods for 3 hr and then hourly to 8 hr. In eyes 13 through 16, (cats 7 and 8), hourly collection of perfusate was continued for 8 hr.

**Table 1. Summary of experimental protocol**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Endophthalmitis</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right eyes</td>
<td>Left eyes</td>
</tr>
<tr>
<td>1. Time zero:</td>
<td>Injection <em>S. aureus</em> all right eyes</td>
<td>Injection of gentamicin 100 μg both eyes. Simultaneous ocular dialysis of both eyes of each cat for 8 hr: eyes 1 through 12.</td>
</tr>
<tr>
<td>2. Time zero:</td>
<td>Injection <em>S. aureus</em> all right eyes</td>
<td>Injection of gentamicin 100 μg both eyes. Time 54 hours: (8 hr after gentamicin) Simultaneous ocular dialysis of both eyes of each cat for 8 hr: eyes 1 through 16.</td>
</tr>
<tr>
<td>3. Time zero:</td>
<td>Injection <em>S. aureus</em> all right eyes</td>
<td>Injection of gentamicin 400 μg both eyes. Time 72 hours: (24 hr after gentamicin) Simultaneous ocular dialysis of both eyes of each cat for 6 hr: eyes 1 through 24.</td>
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</tbody>
</table>

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No. 6 VITREAL PHARMACOKINETICS AND OCULAR DIALYSIS / Den-Nun et al 1057

ate from the ocular dialysis catheter was started at 8 hr after the intravitreal injection of gentamicin and continued for the next 8 hr. In eyes 17 through 24 catheters were implanted 24 hr after the intravitreal injection of gentamicin and perfusate was collected over 30 min periods for 6 hr (Table 1). At the end of each experiment 150 μl of aqueous humor was withdrawn from the center of the anterior chamber, using a 1 ml syringe with a 26 gauge needle, for gentamicin assay. Fluorescence Polarisation Immunoassay (TDX analyzer, Abbott Diagnostics, Irving, TX) was used for the gentamicin assays, with a minimum detection level of 0.2 mg/l. All procedures adhered to the ARVO Resolution on the Use of Animals in Research.

Data Analysis
Data from each eye were analysed by nonlinear, extended least squares regression, using the MK-Model program (Elsevier-Biosoft, Cambridge, UK) running on an Epson AX computer equipped with an 80287 coprocessor. A single compartment model that incorporated a pathway for transfer of gentamicin from the administration site to the sampling compartment (surrounding the sampling device) and a single pathway for elimination was used (Fig. 2). Estimates of the volume of the sampling compartment (V), the rate constant for transfer of the drug from the bolus to the sampling compartment (K_a), the rate constant for transfer out of the sampling compartment (K_g) and the clearance from the sampling compartment (Cl) and the time lag from administration to first appearance in the sampling compartment (Tlag) were obtained for eyes 1 through 12, for the first 8 hr of sampling. The transfer of gentamicin from the administration site to the sampling site (calculated from the rate constant obtained during fitting of data from eyes 1-12) was negligible after 8 hr, so this pathway was neglected in modelling data from eyes 13 to 24. Data from eyes 1 through 16 were also fitted to models which incorporated an additional peripheral compartment in order to model equilibration between the sampling compartment and the remainder of the vitreous soon after injection. The use of more complex models, however, did not improve the quality of data fitting. The amount of drug removed by the dialysis apparatus is negligible, so no compensation was made for this loss, which is included in the overall clearance. The concentrations of gentamicin in the sampling region were calculated from the concentrations in the dialysate and the known transfer characteristics of the apparatus. Results are expressed as mean ± SEM and statistical comparisons for eyes 1 through 12 were made using the Wilcoxon matched pairs test. P < 0.05 was taken as significant.

Fig. 2. Model for fitting data. A_o = Amount of bolus injection; V = volume of sampling compartment; C = concentration within sampling compartment; K_a and K_g = rate constants for transfer of drug into and out of sampling compartment.

Results
Forty-eight hours after inoculation of the bacteria a fulminant endophthalmitis was seen in all right eyes. No red reflex could be seen and the vitreous was opaque.

Eyes 1 through 12 (Sampling between 0 and 8 Hours after Gentamicin Administration)
A phase of transfer of drug into the sampling compartment was seen in each eye, followed in some cases by the beginning of an elimination phase. Peak concentrations occurred at approximately 5 hr (Figs. 3, 4). Satisfactory data fitting to the model was obtained for each eye (Fig. 5). The estimates of volume of the sampling compartment (V) were not significantly different between the control (1.64 ± 0.05 ml) and infected (2.70 ± 1.42 ml) eyes. Estimates of Tlag, K_a, K_g and Cl were also not significantly different in the two groups (Table 2). Estimates of elimination rate constants and clearances were variable within the groups, since elimination phases were not seen in all eyes, but the mean rates did not exceed those found in eyes 17 to 24, which were sampled during the terminal elimination phase.

Eyes 13 through 16 (Sampling between 8 and 16 Hours after Gentamicin Administration)
There was no evidence of continuing transfer of the drug into the sampling compartment after 9 hr (Fig. 6). Semilogarithmic data plots showed a single elimination phase, indicating that drug distribution within the vitreous was also complete by 9 hr (Fig. 7). Estimates of K_g and Cl were not greater than those found between 24 and 30 hr, confirming the absence of an additional rapid elimination (ie, distribution) phase.

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CONTROLS

Fig. 3. Plot of mean vitreal gentamicin concentrations in normal eyes for the first 8 hr after injection of 100 μg of gentamicin. Each point represents the concentration measured over the preceding time period, i.e., the value at 0.5 hr represents the concentration of the sample collected over the first 30 min from time zero. Error bars are ± standard error of the mean (N = 6). The increase in gentamicin concentration at the sampling site over the first few hours indicates the initial diffusion phase of gentamicin through the vitreous.

Cl and K, were greater in the infected than in the control eyes in both animals (Table 2) and concentrations of gentamicin were consequently lower.

Eyes 17 through 24 (Sampling between 24 and 30 Hours after Gentamicin Administration)

A single phase of elimination was present in the semilogarithmic plots of gentamicin concentration over time (Fig. 8). The model provided a satisfactory basis for describing the data in all cases (Figs. 9, 10). V was estimated as 2.39 ± 0.21 ml for normal eyes and 2.22 ± 0.22 ml for the endophthalmitic eyes. Cl and K, were greater in each of the endophthalmitic eyes than in the controls (Table 2).

Aqueous Humor

Terminal concentrations of gentamicin in the aqueous humor in infected eyes were lower than controls both in absolute terms and also as a percentage of the final intravitreal concentration (Table 3).

ENDOPHTHALMITIS

Fig. 4. Plot of mean vitreal gentamicin concentrations in infected eyes, for the first 8 hr after injection of 100 μg of gentamicin. Control eyes were the fellow eyes (left), the results from which are plotted in Figure 3. Error bars are ± standard error of the mean (N = 6).

Table 2. Pharmacokinetics of intravitreal gentamicin in normal and endophthalmitic eyes

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Endophthalmitis</th>
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</thead>
<tbody>
<tr>
<td>Eyes 1 to 12: sampling from 0 to 8 hr after injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K,</td>
<td>0.665 ± 0.222</td>
<td>0.562 ± 0.145 hr⁻¹</td>
</tr>
<tr>
<td>K,</td>
<td>0.036 ± 0.016</td>
<td>0.088 ± 0.046 hr⁻¹</td>
</tr>
<tr>
<td>Cl</td>
<td>0.048 ± 0.023</td>
<td>0.098 ± 0.043 ml/hr</td>
</tr>
<tr>
<td>T_{lag}</td>
<td>0.270 ± 0.057</td>
<td>0.332 ± 0.072 hr</td>
</tr>
<tr>
<td>Eyes 13 to 16: sampling from 8 to 16 hr after injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K,</td>
<td>0.070</td>
<td>0.100 hr⁻¹</td>
</tr>
<tr>
<td>Cl</td>
<td>0.065</td>
<td>0.148 ml/hr</td>
</tr>
<tr>
<td>Eyes 17 to 24: sampling from 24 to 30 hr after injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K,</td>
<td>0.055 ± 0.001</td>
<td>0.107 ± 0.009 hr⁻¹</td>
</tr>
<tr>
<td>Cl</td>
<td>0.133 ± 0.015</td>
<td>0.235 ± 0.033 ml/hr</td>
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Other Investigators

<table>
<thead>
<tr>
<th>References</th>
<th>Normal</th>
<th>Endophthalmitis</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>4, K,</td>
<td>0.020 hr⁻¹</td>
<td>—</td>
<td>Cat</td>
</tr>
<tr>
<td>15, K,</td>
<td>0.035 hr⁻¹</td>
<td>0.037 hr⁻¹</td>
<td>Rabbit</td>
</tr>
<tr>
<td>16, K,</td>
<td>0.022 hr⁻¹</td>
<td>0.069 hr⁻¹</td>
<td>Rabbit</td>
</tr>
<tr>
<td>17, K,</td>
<td>0.029 hr⁻¹</td>
<td>—</td>
<td>Rhesus monkey</td>
</tr>
<tr>
<td>21, K,</td>
<td>0.021 hr⁻¹</td>
<td>—</td>
<td></td>
</tr>
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</table>
Discussion

The technique of ocular dialysis was used to thoroughly examine the diffusion and elimination of gentamicin in normal cat eyes and those with induced endophthalmitis. Intravitreal gentamicin concentrations measured at a sampling site distant from the site of administration of a bolus injection could be fitted to a single compartment model of linear drug transfer and elimination. No phase of drug distribution could be discerned, either by visual inspection of semilogarithmic plots or by fitting data to models comprising an additional peripheral compartment. Distribution of gentamicin in the vitreous is therefore occurring within the first 8 hr after administration and the transfer of drug between the administration and sampling sites is a component of the overall distribution. Direct vitreal sampling after iontophoretic application of gentamicin to the sclera has also indicated that distribution is complete within 8 hr. Eyes 17 through 24 were sampled during part of the elimination phase (24 to 30 hr), after distribution within the vitreous humor was complete. The values for V derived from these data therefore estimate vitreous volumes in normal (2.39 ml) and infected (2.22 ml) eyes. The estimates agree with our previously calculated value of 2.37 ml. The estimates of V in eyes sampled for the first 8 hr were very similar to these, further indicating that distribution was complete during this time.
ENDOPHTHALMITIS

Table 3. Aqueous humor gentamicin concentration expressed as a percentage of vitreal concentration at the end of each time period

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Normal eyes</th>
<th>Infected eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–8 hr</td>
<td>13.93% ± 3.7%</td>
<td>2.24% ± 0.99%</td>
</tr>
<tr>
<td>8–16 hr</td>
<td>14.5%</td>
<td>3.30%</td>
</tr>
<tr>
<td>24–30 hr</td>
<td>11.75% ± 4.85%</td>
<td>4.53% ± 0.67%</td>
</tr>
</tbody>
</table>

Values predicted by Maurice and Mishima (see text)

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Normal eyes</th>
<th>Infected eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–8 hr</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>8–16 hr</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>24–30 hr</td>
<td>10%</td>
<td></td>
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</tbody>
</table>

Other investigators

<table>
<thead>
<tr>
<th>References</th>
<th>Normal</th>
<th>Aqueous/vitreous (%)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>4, Kc</td>
<td>0.020 hr</td>
<td>6%</td>
<td>Cat</td>
</tr>
<tr>
<td>15, Kc</td>
<td>0.033 hr</td>
<td>22%</td>
<td>Rabbit</td>
</tr>
<tr>
<td>16, Kc</td>
<td>0.22 hr</td>
<td>14%</td>
<td>Rabbit</td>
</tr>
<tr>
<td>17, Kc</td>
<td>0.029 hr</td>
<td>19% (11–40%)</td>
<td>Rabbit</td>
</tr>
<tr>
<td>21, Kc</td>
<td>0.021 hr</td>
<td>47% (57%)</td>
<td>Rhesus monkey</td>
</tr>
</tbody>
</table>

* Reported range of values.

This method of analysis provides estimates of both the rate of gentamicin diffusion within the vitreous (transfer from the administration site to the sampling site) and the rate of elimination from the vitreous. The rate of diffusion of injected gentamicin within the vitreous is rapid and is unaltered by endophthalmitis. The model, of course, does not cover circumstances where the homogeneity of the vitreous is lost, such as during loculation. Our values for Kc are similar in magnitude to those reported elsewhere (Table 2). The rate of elimination from the vitreous, however, is consistently greater in the presence of endophthalmitis than in normality. This presumably occurs as a consequence of breakdown of the blood-retinal barrier, and explains why intravitreal gentamicin concentrations are lower in endophthalmitic eyes. An increase in Kc in the presence of infection agrees with previous reports (refs. 16, 17; Table 2). The magnitude of this increase should be related to the diameter of the vitreous humor, but there are not enough data available among different species to test this prediction. Lower terminal concentrations of gentamicin in aqueous humor of infected eyes than in control eyes reflect lower intravitreal concentrations. Reduced ratios of aqueous humor concentrations to intravitreal concentrations are consistent with previous observations on xenobiotic elimination from infected eyes (ref. 14; Table 3). Assuming vitreal volumes of 1.4, 2.4 and 4 ml, aqueous flow rates of 3.6, 13 and 3.0 μl/minute, for rabbit, cat and rhesus monkey, respectively, and using values obtained for Kc in each case, the aqueous/vitreal humor gentamicin concentration ratios for normal eyes were calculated by the formula suggested by Maurice and Mishima. A good correlation between our findings and predicted values for normal feline eyes were obtained, suggesting that the blood–vitreal barrier was not affected by the ocular dialysis procedure (Table 3). Values calculated from similar studies in the literature for the rabbit and rhesus monkey are shown in the table for comparison. Apart from our results in an earlier paper, these correlate fairly well with theoretical values suggested for the normal rabbit eye. In the infected rabbit eye the ratio is approximately 5% compared to a mean of 3.3% in our study. This difference probably relates to differences in vitreous cavity dimensions between species.

In addition to confirming the applicability of ocular dialysis to studies of intravitreal pharmacokinetics, these experiments have provided information which may assist the clinical management of endophthalmitis. The aim of intravitreal therapy is to provide adequate bactericidal concentrations of gentamicin without retinal toxicity. Retinal toxicity is presumably related to excessive concentration of the drug at the retinal surface. It is probable that only transient exposure is necessary, such as may occur at the site of a juxta-retinal bolus injection. The technique of injection is therefore likely to be an important factor in preventing localized areas of retina being exposed to high concentrations of the injected drug.

Distribution of gentamicin within the vitreous is rapid, so that uniform concentrations are likely to occur throughout the vitreous within 8 hr of the injection. The rate of elimination of intraocular gentamicin is related to the presence, and possibly the severity, of infection. The appropriate amount and
timing of subsequent doses could be assessed by measuring the rate of elimination of the drug from the eye. Data on human intravitreal pharmacokinetics, with and without endophthalmitis, and with and without vitrectomy or lensectomy are required before clearer guidelines for optimal clinical dosage can be formulated. Refinement of the technique of ocular dialysis may provide the means by which these data can be obtained.

Key words: pharmacokinetics, vitreous humor, cat, gentamicin

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References