Pharmacokinetics of Intravitreal Carbenicillin, Cefazolin, and Gentamicin in Rhesus Monkeys

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Although intravitreal injection of antibiotics is being used more widely in treatment of bacterial endophthalmitis, the pharmacokinetic principles that underlie such therapy have been derived exclusively from experiments in the rabbit. Therefore, we studied several representative antibiotics in normal eyes of rhesus monkeys. Carbenicillin (1,000 ng), cefazolin (1,000 ng), or gentamicin (100 μg) was injected intravitreally. Vitreous and aqueous humors were aspirated at intervals up to 96 hours after injection. The half-life values in the vitreous humor were: carbenicillin 10 hours, cefazolin 7 hours, and gentamicin 33 hours. Concomitant intraperitoneal administration of probenecid prolonged the vitreal half-life of carbenicillin to 20 hours and of cefazolin to 30 hours. The estimated vitreous volumes in these monkeys were approximately 3.0–4.0 ml.

These results are consistent with the hypothesis that, in primates as in rabbits, β-lactam antibiotics are eliminated via the retinal route and the aminoglycoside via the anterior route. This supports the applicability of the rabbit model to the treatment of endophthalmitis in humans. Invest Ophthalmol Vis Sci 24:1602–1606, 1983

Intravitreal injection of antibiotics is gaining increasing acceptance as a method of producing high vitreal concentrations of drugs that may be useful in the treatment of bacterial endophthalmitis.1–5 Toxicologic studies in rabbits6 and one investigation in primates6 have shown that many antibiotics may be administered intravitreally in potentially effective dosages with comparative safety. Although the pharmacokinetic behavior of intravitreal antibiotics has been examined in rabbits,2,3,5 comparable studies in primates are lacking. These would be helpful in supporting the hypothesis that data derived from animal models are applicable to the treatment of endophthalmitis in humans.

Experiments in rabbits have shown that the aminoglycoside antibiotics leave the vitreous humor via the “anterior route,” ie, the anterior chamber and the canal of Schlemm. These agents exhibit a relatively long half-life in the normal rabbit vitreous humor—about 20–24 hours; the half-life is reduced to about 10 hours in the inflamed eye.7 Aphakia accelerates the rate of clearance of gentamicin in the rabbit eye.8

In contrast, β-lactam antibiotics such as the penicillins and cephalosporins are transported actively across the retina (“posterior” or “retinal route”).3,8 The transport pump is inhibited by probenecid and by inflammation.10

Because of the lack of information concerning the pharmacokinetic behavior of intravitreal antibiotics in the primate eye, we have studied three representative agents: a penicillin (carbenicillin), a cephalosporin (cefoxolin), and an aminoglycoside (gentamicin) in the eyes of normal rhesus monkeys. We also have examined the effects of probenecid on the transport of the two β-lactam drugs.

Materials and Methods

Twelve, healthy, female “retired breeder” rhesus monkey, weighing 6–10 kg, were used in these experiments. Disodium carbenicillin was obtained as the commercial powder for injection from the Roerig Division of Pfizer Inc. (New York, NY); gentamicin sulfate was obtained as the injectable solution from Schering Corp. (Kenilworth, NJ); and sodium cefazolin was obtained as the commercial powder for injection from Eli Lilly and Company (Indianapolis, IN). The drugs were diluted in pyrogen-free normal saline to a concentration of 1 mg/ml for gentamicin and 10 mg/ml for carbenicillin and cefazolin. The dose for intravitreal injection was 100 μg for gentamicin, and 1,000 μg for carbenicillin and cefazolin, in a volume of 0.1 ml.

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The monkeys were tranquillized before all procedures by means of intramuscular ketamine hydrochloride (50 mg/kg) and acepromazine (0.25 mg/kg). In addition, one drop of proparacaine hydrochloride (0.5%) was applied topically. Before antibiotics were administered, aqueous paracentesis of approximately 0.1 ml was performed with a 25-gauge needle to decompress the globe. The antibiotic then was injected through a 30-gauge needle placed 7 mm from the limbus at the temporal aspect of the globe, angled posteriorly, and introduced to a depth of 10 mm. Specimens of aqueous humor (0.1 ml) and vitreous humor (0.1 ml) were aspirated at intervals of 3, 24, or 48 hours after intravitreal injection of cefazolin or carbenicillin, and 24, 48, or 96 hours after intravitreal injection of gentamicin. Each antibiotic was studied in at least four eyes at each interval.

Additional studies were performed in which animals were given probenecid intraperitoneally, 1.0 g twice daily, beginning 60–120 minutes before the intravitreal injection of cefazolin or carbenicillin and continuing until the last sample was obtained. Aqueous and vitreous humor were aspirated 3, 24, or 48 hours after injection of carbenicillin, and 3 or 48 hours after injection of cefazolin.

Each monkey was studied on more than one occasion as shown in Table 1. The number of experiments with a given eye ranged from one to nine. Experiments involving concomitant administration of probenecid were done last so that animals receiving this agent generally had undergone the greatest number of previous experiments. The shortest interval between successive experiments on the same animal was 14 days (two monkeys treated with carbenicillin) and in all other instances exceeded 21 days. The eyes were examined before each experiment with a direct ophthalmoscope to ascertain that the retina was intact.

Samples of aqueous and vitreous humors containing carbenicillin or cefazolin were diluted in phosphate-buffered saline and assayed by the standard agar-diffusion bioassay using *Bacillus subtilis* ATCC 6633 as the test organism. Gentamicin samples were assayed by radioimmunoassay (New England Nuclear Corporation, Boston, MA).

### Results

Figures 1–3 show the means and standard errors of concentrations of each antibiotic in the vitreous and aqueous humors at sequential intervals after intravitreal injection. In addition, the effect of concomitant probenecid is displayed (Figs. 2, 3). The half-life of each antibiotic in these ocular fluids, calculated by linear regression, is shown in Table 2. By extrapolation to time zero, the maximum initial concentration was estimated. This value was divided into the dose administered to calculate the volume of the vitreous humor (Table 2).

#### Table 1. Rhesus monkeys used in studies of intravitreal injections

<table>
<thead>
<tr>
<th>Gentamicin ± probenecid</th>
<th>Carbenicillin ± probenecid</th>
<th>Cefazolin ± probenecid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals used</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Number of eyes studied at each interval</td>
<td>4</td>
<td>4-8</td>
</tr>
<tr>
<td>Number of previous experiments in animals used for this study</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1*</td>
<td>0</td>
</tr>
<tr>
<td>6-8</td>
<td>1*</td>
<td>3</td>
</tr>
<tr>
<td>Shortest interval between studies on a single eye</td>
<td>≥26 days</td>
<td>14 days (2 animals)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥21 days (remainder)</td>
</tr>
</tbody>
</table>

* Animals studied to determine the effects of repeated experimentation on ocular concentrations on gentamicin. Values not included in determination of mean levels in Figure 1.

![Gentamicin](image_url)

**Fig. 1.** Concentrations of gentamicin in vitreous and aqueous humor of normal rhesus monkeys 24, 48, and 96 hours after intravitreal injection of 100 μg. Each point shows the mean and standard error of results in four eyes.
Fig. 2. Concentrations of carbenicillin in vitreous and aqueous humor of normal rhesus monkeys 3, 24, and 48 hours after intravitreal injection of 1,000 μg. Animals given probenecid concomitantly by the intraperitoneal route were studied at the same intervals. Each point shows the mean and standard error of results in four to eight eyes.

The vitreal half-life values of gentamicin, carbenicillin, and cefazolin were 33, 10, and 7 hours, respectively. Concomitant administration of probenecid prolonged the half-life of carbenicillin and cefazolin to 20 and 30 hours, respectively. The aqueous:vitreous concentration ratio 24 hours after injection was somewhat higher for gentamicin (0.57) than for carbenicillin (0.30) or cefazolin (0.28). Taken together, the half-life values, effect of probenecid, and aqueous:vitreous concentration ratios support the hypothesis that gentamicin is eliminated from the rhesus monkey eye via the anterior route, whereas the two β-lactam drugs are eliminated by active transport via the retinal route.

We noted a slight difference in the pharmacokinetic behavior of the drugs in eyes studied after multiple as opposed to few previous aspirations. Although we were not able to examine the effect systematically, we compared the levels of gentamicin in the eyes of one monkey 24 hours after one and six previous experiments, and another monkey 48 hours after zero and five previous experiments. Aqueous humor concentrations of gentamicin increased by 33–100% (mean 78%) and vitreous humor concentrations increased by 13–47% (mean 30.1%) after repeated experimentation. Thus, although the eyes appeared clinically normal, there was some decrease in the rate of egress of gentamicin. The situation with respect to carbenicillin was different. The mean vitreous level 48 hours after injection was 12.2 ± 1.4 (SE) μg/ml in eight eyes that had been studied in zero to one previous experiments and was 7.2 ± 1.4 μg/ml in seven eyes that had been studied in three to eight previous experiments. Thus, carbenicillin vitreal levels were slightly lower in eyes that had been injected repeatedly. However, the difference was not statistically significant by Student's t-test. Although

Fig. 3. Concentrations of cefazolin in vitreous and aqueous humor of normal rhesus monkeys 3, 24, and 48 hours after intravitreal injection of 1,000 μg. Animals given probenecid concomitantly were studied 3 and 48 hours after antibiotic administration. Each point shows the mean and standard error of results in four eyes.
Table 2. Half-life and estimated maximum concentrations in vitreous and aqueous humor of rhesus monkeys after intravitreal injection

<table>
<thead>
<tr>
<th></th>
<th>Gentamicin</th>
<th>Carbenicillin</th>
<th>Cefazolin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No probenecid</td>
<td>Probene</td>
<td>No probenecid</td>
</tr>
<tr>
<td><strong>Vitreous humor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-life (hours)</td>
<td>33</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Estimated maximum concentration (µg/ml)</td>
<td>18</td>
<td>270</td>
<td>400</td>
</tr>
<tr>
<td>Estimated vitreous volume (ml)</td>
<td>5.6</td>
<td>3.7</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Aqueous humor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-life (hours)</td>
<td>34</td>
<td>8</td>
<td>39</td>
</tr>
<tr>
<td>Estimated maximum concentration (µg/ml)</td>
<td>9.2</td>
<td>120</td>
<td>135</td>
</tr>
</tbody>
</table>

these changes were neither dramatic nor in a consistent direction, they suggest that eyes that have been repeatedly studied traumatized are not entirely normal for purposes of pharmacokinetic studies.

Discussion

There is abundant evidence in the rabbit that β-lactam antibiotics are eliminated from the vitreous humor by an active transport mechanism, located in the retina and capable of being inhibited by probenecid. Accordingly, these drugs have relatively short vitreal half-lives. Calculations from data in the normal rabbit eye yield values of five to six hours for penicillin G and methicillin, and five hours for carbenicillin. The half-life of carbenicillin in the rabbit eye was prolonged to 13 hours by the concomitant administration of probenecid. Although our calculation from the results of one study suggest that cefazolin has a vitreal half-life of 1.2 days (29 hours), this seems excessively long.

In contrast to the β-lactam drugs, the aminoglycoside antibiotics leave the vitreous humor via the anterior route, ie, the anterior chamber and canal of Schlemm. They manifest correspondingly long vitreal half-lives. Data from the normal rabbit eye afford values of 2013 and 24 hours for gentamicin, 16 hours for tobramycin, and 20 hours for streptomycin. The vitreal half-life of gentamicin is reduced by inflammation. All of these data are based on studies in the rabbit eye. Because we were unable to find comparable information in primates, we carried out the present set of experiments.

The results of this study in healthy rhesus monkeys support the hypothesis that the β-lactam drugs, carbenicillin, and cefazolin are eliminated by the retinal route. Evidence for this is the short vitreal half-life of the drugs (10 and 7 hours, respectively), the marked prolongation of the half-life when probenecid was given concomitantly (20 and 30 hours, respectively), and the relatively low aqueous:vitreous concentration ratios. In contrast, gentamicin exhibited a much longer half-life (33 hours) and a higher aqueous:vitreous concentration ratio, consistent with the supposition that it is eliminated via the anterior route.

The vitreous half-lives of these agents in the rhesus monkey were somewhat longer than those measured in the normal rabbit eye (eg, 10 vs 5 hours for carbenicillin, 33 vs 24 hours for gentamicin). This may be explained by the fact that there are larger diffusional distances in the eyes of rhesus monkeys, ie, vitreous volumes of 3-4 ml compared with about 1.4 ml in the rabbit eye.

For reasons of economy, we used each animal's eyes more than once in this study. Although we did not systematically explore the pharmacokinetic effects of repeated experimentation, there was a suggestion that gentamicin levels in the vitreous were slightly higher after five or six experiments than after no or one previous experiment; the reverse phenomenon seemed to occur with carbenicillin but was not statistically significant. Fortunately, despite the fact that relatively few animals were used in these studies, there was excellent linearity in the rate of fall of vitreous concentrations and the standard errors were very small (Figs. 1-3).

These studies indicate that the pharmacokinetics of intravitreal β-lactam antibiotics and aminoglycosides in the rhesus monkey eye are very similar to those in the rabbit eye. This further supports the belief that the general principles gleaned from these animal models are relevant in the treatment of infections in humans.

Key words: intravitreal, carbenicillin, cefazolin, gentamicin, probenecid, monkeys

Acknowledgment

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References