Cefazolin Levels After Intravitreal Injection

Effects of Inflammation and Surgery

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Cefazolin (2.25 mg) was injected into the vitreous cavity of phakic, aphakic, and aphakic/vitrectomized rabbits; inflamed eyes were compared to controls. Vitreous levels of cefazolin were determined at selected times from 2 to 48 hr, and the half-life was calculated. The effect of inflammation was to increase the half-life or to reduce the rate of elimination of cefazolin from the vitreous cavity. The drug was cleared substantially faster from aphakic/vitrectomized eyes than from phakic or aphakic eyes. Vitreous levels of cefazolin were above the MIC for most common gram-positive organisms causing endophthalmitis in all study groups at 24 hr, but in only the phakic inflamed eyes and in the aphakic eyes with intact vitreous at 48 hr. Invest Ophthalmol Vis Sci 31:502–505, 1990

Cefazolin is a first-generation cephalosporin commonly used in the treatment of endophthalmitis. It is highly effective against strains of S. aureus, S. epidermidis, streptococcal species (except enterococi) and other gram-positive organisms known to cause endophthalmitis. Direct intravitreal injection of the maximum nontoxic dose of antibiotic provides intraocular levels of drug substantially higher than can be obtained by systemic administration. Toxicity studies in animal models have established a maximum safe intravitreal dose of 2.25 mg cefazolin.1

The rate of elimination, or the half-life, of cefazolin from the vitreous cavity in normal primate eyes is 7 hr and may be increased 4-fold by the concomitant administration of probenecid.2 Cefazolin is believed to be removed from the vitreous cavity by a posterior or retinal route by an active pump mechanism.2 Little additional information is available, however, regarding the vitreous levels or half-life of cefazolin injected under various surgical conditions.

Clinical endophthalmitis occurs most frequently in the aphakic eye. Invariably, by the time an intravitreal injection is given, the eye is inflamed. The injections are administered to eyes either with an intact vitreous body or immediately after vitrectomy.

We determined the half-life of intravitreal cefazolin under the following study conditions, which simulate commonly seen clinical settings: 1) phakic eyes, 2) aphakic eyes, and 3) aphakic/vitrectomized eyes. Both inflamed and noninflamed eyes were studied.

Materials and Methods

Three groups of white New Zealand albino rabbits, weighing 2–3 kg, were studied: group 1, phakic eyes (control and inflamed); group 2, aphakic eyes (control and inflamed); and group 3, aphakic/vitrectomized eyes (control and inflamed).

Several weeks prior to drug administration, surgery was performed for groups 2 and 3. Anesthesia was introduced by intramuscular injection of a 50:50 mixture of ketamine (30 mg/kg) and Xylazine (10 mg/kg). Pupils were dilated with cyclopentolate 1% and phenylephrine 10%. For group 2, lensectomy was performed bilaterally through the pars plana by ultrasonication of the nucleus and aspiration of the lens cortex. The anterior lens capsule was left intact. For group 3, the lens and capsule were removed and vitrectomy was performed, with the removal of as much clear vitreous as possible with a cutting-suction instrument. Balanced salt solution (BSS+; Alcon Pharmaceutical) was used for irrigation. Surgery was performed on both eyes of groups 2 and 3. The eyes were allowed to become quiet for several weeks prior to drug administration and determination of vitreous cefazolin levels.

Twenty-four hours prior to drug administration, standardized intraocular inflammation was induced in the right eye of each rabbit under direct ophthalmoscopic control by a midvitreous injection as follows: groups 1 and 2 received 10⁹ heat-killed S. epi...
Table 1. Measured cefazolin levels

<table>
<thead>
<tr>
<th>Hr postinjection</th>
<th>Group 1: phakic</th>
<th>Group 2: aphakic</th>
<th>Group 3: aphakic/vitrectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Inflamed</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>1169.8 ± 323.6</td>
<td>1154.0 ± 146.3</td>
<td>893.8 ± 102.6</td>
</tr>
<tr>
<td>3</td>
<td>707.5 ± 38.4</td>
<td>977.5 ± 103.0</td>
<td>673.3 ± 181.5</td>
</tr>
<tr>
<td>8</td>
<td>147.25 ± 42.9</td>
<td>340.0 ± 43.7</td>
<td>183.3 ± 125.8</td>
</tr>
<tr>
<td>24</td>
<td>8.97 ± 3.5</td>
<td>57.4 ± 15.3</td>
<td>17.7 ± 17.8</td>
</tr>
<tr>
<td>48</td>
<td></td>
<td></td>
<td>6.5</td>
</tr>
</tbody>
</table>

Half-life (hr) 6.5 10.4 8.3 9.0 6.0 6.7

Concentrations of cefazolin are given in μg/ml, mean ± standard deviation.

dermidis organisms in 0.1 ml BSS; group 3 received $10^7$ heat-killed organisms. In a previous study, this was shown to produce equivalent clinical signs of inflammation at 24 hr. The left eye of each rabbit remained uninjected, as the control eye.

On the day of drug administration (day 0), 2.25 mg cefazolin in 0.1 ml sterile water for injection, USP, was injected slowly into the midvitreous cavity of both eyes of all rabbits under indirect ophthalmoscopic control. The animals were sacrificed, and vitreous samples were taken according to the following schedule: group 1: 2, 8, 24, and 48 hr; group 2: 3, 8, 24, and 48 hr; and group 3: 2, 8, 24, and 48 hr. Animals were sacrificed by an intracardiac injection of a mixture of pentobarbital sodium and phenytoin sodium. Eyes were enucleated immediately, rapidly frozen in liquid nitrogen, and stored at -70°C. Vitreous samples were obtained by dissection of the frozen eyes as described by Abel and Boyle. Bioassay was performed by the Eli Lilly and Company Laboratories using E. coli M.B. 3804 or B. subtilis as the test organism.

Animals were maintained and used in accordance with the ARVO Resolution on the Use of Animals in Research.

Statistical Analysis

Cefazolin levels were determined in four to six eyes at each sampling time. The mean levels obtained were plotted against time. From the log of drug concentration versus time, the rate of elimination was calculated, and half-life (T 1/2) determined from the slope of the line. Initial concentrations were estimated by extrapolation of the line to time 0. The rates of elimination between study groups were compared by examining the rate of elimination, or slope of the line of log concentration versus time. Statistically significant differences among groups were evaluated by comparing the slopes of the regression lines with analysis of covariance.

Results

The mean cefazolin levels measured for each group at all sampling times are listed in Table 1 and are depicted in Figure 1. The slopes of the lines of log concentration versus time are depicted in Figure 2. Calculated half-lives, derived from the slope of the line of log concentration versus time (Figure 2), appear in Table 1.

In all three groups, cefazolin was eliminated more rapidly in the control (noninflamed) eyes than in the inflamed eyes. This difference was most pronounced in group 1 (phakic eyes), in which the rate of elimination of cefazolin in control eyes was significantly faster than in the inflamed eyes ($P = 0.003$). This difference between inflamed and noninflamed eyes was less pronounced in groups 2 and 3. In group 2, the aphakic control eyes cleared the drug only 8% faster than did the aphakic inflamed eyes; the aphakic/vitrectomized control eyes cleared the drug 10% faster than did the fellow inflamed eyes.

Drug half-life was shorter in aphakic/vitrectomized eyes than in phakic eyes, and far lower drug concentrations were found in the aphakic/vitrectomized at 24 and 48 hr. Inflamed aphakic/vitrectomized eyes cleared the drug significantly faster than did inflamed phakic eyes ($P = 0.04$); and aphakic/vitrecto-
mized noninflamed (control) eyes cleared the drug faster than did phakic noninflamed (control) eyes ($P = 0.04$).

Early drug concentrations were higher in phakic eyes than in aphakic or aphakic/vitrectomized eyes. This difference is explained partially by the fact that removal of the lens results in injection of the drug into a larger volume. Results indicate that early drug concentrations in phakic and aphakic eyes were higher than in aphakic/vitrectomized eyes. At 8 hr, vitreous levels of cefazolin were substantially lower in aphakic/vitrectomized eyes (group 3) than in phakic (group 1) or aphakic (group 2) eyes. At 24 hr, this difference remained true.

In the estimation of initial (time-0) drug concentrations, extrapolation of the line to the intersect (y axis) indicated that the initial drug concentrations in each group were not equal despite injections of an equal amounts of drug. The estimated initial concentrations of drug in group 3 (aphakic/vitrectomized eyes) were substantially lower than in groups 1 and 2 (phakic and aphakic eyes). This relationship was seen also in measured levels at 8, 24, and 48 hr (and between groups 1 and 3 at 2 hr).

**Discussion**

Our studies demonstrate that the surgical status of the eye and the presence of inflammation significantly alter the kinetics of cefazolin after intravitreal injection. The consistent effect of inflammation in our study groups was to cause the drug to be eliminated more slowly, and so to increase the half-life values. It has been proposed that inflammation prolongs the half-life of penicillins and ß-lactam antibiotics in the vitreous.\textsuperscript{2,4} These drugs are believed to be eliminated from the vitreous by a retinal pump mechanism similar to that in the central nervous system.\textsuperscript{2,4} Inflammation has the dual effect of increasing the "leakiness" of the blood–retina barrier (permitting greater penetration across the blood–retina barrier into the eye) and of decreasing the rate of elimination from the vitreous cavity by interfering or damaging the mechanisms of the retinal pump that eliminates these drugs.\textsuperscript{2} Aminoglycoside antibiotics, in contrast, are believed to be eliminated by an anterior route after intravitreal injection.\textsuperscript{5,6} Inflammation has the opposite effect on clearance of these antibiotics.\textsuperscript{5,6}

The half-life of gentamicin in the primate eye is decreased from 33 hr to 10 hr when inflammation is present.\textsuperscript{5}

Despite the intravitreal injection of the same doses into the eyes, the clinical situations of phakia, aphakia, and aphakia/vitrectomy create different effects on the drug levels and on the clearance of the drug from the vitreous cavity. Drug clearance was faster in inflamed aphakia/vitrectomized eyes (group 3) than in inflamed phakic and aphakic eyes (groups 1 and 2). A similar, more rapid clearance of drug in vitrectomized eyes has been demonstrated also for amphotericin, fluorouracil, and vancomycin.\textsuperscript{7-9} It is not clear whether this phenomenon reflects enhanced drug binding, the physical limitation of drug movement by the vitreous reducing active or passive elimination; or some indirect effect of surgery.

Assuming that the injection of the initial 2.25-mg dose was accurate, examination of the data and extrapolation to initial concentrations suggest that the apparent volumes in which the dose is distributed is unequal in these three clinical settings. In the control phakic rabbit eye, we found initial concentrations comparable to those reported by Barza et al in the primate, allowing for the differences in the size of the initial dose and the difference in the size of the vitreous cavity.\textsuperscript{2} Assuming the normal volume of the vitreous in the rabbit to be 1.4 ml, a substantially higher apparent vitreous volume was found in both eyes of the aphakic/vitrectomized animals (group 3). Initial concentrations in group 2 (aphakic) were also found to be lower than in group 1 (phakic), suggesting that
the surgical procedures of lensectomy and vitrectomy result in lower initial drug concentrations. This result was expected, since the rabbit lens is approximately 0.35 ml in volume; therefore, the volume into which the drug was injected in aphakic eyes was approximately 25% greater than in phakic eyes. It is not clear why removal of the vitreous creates an even greater apparent initial volume than does lensectomy alone; this greater apparent volume may have been due to enhanced initial elimination rates or changes in the intraocular distribution of the drug (for example, the drug could now penetrate the anterior chamber, since the capsule had been removed).

Little difference in drug levels was present between groups 1 and 2 at 24 and 48 hr, but drug levels were substantially lower in group 3. Correlation of our measured drug levels to the standard inhibitory concentration (MIC) of cefazolin was complex. In our laboratories, organisms termed sensitive to cefazolin were inhibited by 10 μg or less per ml, although many were inhibited by 1 μg. Levels greater than 10 μg/ml of cefazolin were achieved in every study group at 24 hr, but not at 48 hr in the phakic control and the aphakic vitrectomized groups.

Applications of the standard laboratory test procedures which create in vitro killing effects are not necessarily applicable to the avascular vitreous cavity of the eye. The minimal bactericidal concentration (MBC) is increasingly advocated as a more useful index of drug potency in avascular spaces. The MIC may be an inadequate guide to drug levels required in endophthalmitis to eradicate the infecting organism. In some instances, the MIC and MBC levels may be similar, but for other drugs or bacteria, the MBC may be significantly higher than the MIC. Furthermore, drug levels 10–30 times the MBC are advocated to kill organisms effectively in infections of the cerebrospinal fluid (CSF).10–15 These models of meningitis may be more applicable to intraocular infections than previously believed, and support the necessity of intravitreal injections to achieve adequate drug levels in the vitreous cavity. Since the required levels of the drug may be much greater than previously believed, studies of the postinjection kinetics of the drugs assume increasing importance.

Key words: cefazolin, pharmacokinetics, intravitreal injection, inflammation, endophthalmitis

References