Intravitreal injection of gentamicin in rabbits

Effect of inflammation and pigmentation on half-life and ocular distribution

Anne Kane, Michael Barza, and Jules Baum

Most regimens for intravitreal injection of antibiotics are based on studies in normal albino rabbits. We examined the effect of two variables, infection and pigmentation, on the ocular distribution of an intravitreal injection of 50 μg of gentamicin in rabbit eyes. The half-life of gentamicin in the vitreous of normal pigmented rabbits after intravitreal administration was 24 hr. Antibiotic levels in cornea and aqueous reached a peak 18 hr after injection; terminal half-lives in these sites were similar to those in vitreous. With inflammation, the half-life in the vitreous was decreased to 10 hr, and there was no accumulation of drug in anterior sites. To the extent that these data are applicable to humans, they suggest that the treatment of bacterial endophthalmitis by the intravitreal route may require more frequent injections than had been recommended on the basis of studies in normal eyes. Normal pigmented and albino rabbits showed similar levels of gentamicin in the cornea, aqueous, and vitreous; however, the drug was barely detectable in iris, choroid-retina, and sclera of pigmented animals, presumably on account of an interaction with melanin-containing tissues. This effect of pigment may explain the differences in the reported thresholds for toxicity of gentamicin with intravitreal injection.

Key words: gentamicin, inflammation, endophthalmitis, intravitreal injection, pigmentation, toxicity, vitreous, aqueous, aminoglycoside
Fig. 1. Time course of gentamicin in vitreous, aqueous, and cornea of normal and infected pigmented rabbit eyes after an intravitreal infection of 50 μg. The values presented are the mean ± S.E. of at least eight eyes at each interval. Gentamicin levels in the cornea of infected animals were undetectable.

Materials and methods

Intravitreal injections of gentamicin, 50 μg, were given bilaterally to Dutch belted (pigmented) and New Zealand white (albino) rabbits in the following manner. Animals were anesthetized with 0.6 ml of ketamine hydrochloride intramuscularly. Proparacaine hydrochloride was applied topically 1 to 5 min before the intravitreal injections. The eyes were propptosed, and approximately 0.1 ml of aqueous humor was removed through a 25-gauge, ½-inch needle. Although we recognize that proptosis and paracentesis may have some effect on the aqueous humor dynamics, we believed that the procedure was justified to avoid an excessive increase in ocular pressure upon injection of antibiotic. The removal of aqueous fluid prior to intravitreal injection is a standard procedure of other investigators. Commercial gentamicin sulfate (Schering Corp.) was diluted to a concentration of 500 μg/ml in normal saline; 0.1 ml of this solution was injected through a 27-gauge needle introduced 2 to 3 mm posterior to the equator.

The kinetics of gentamicin were studied in both normal and infected eyes of pigmented rabbits. Bilateral endophthalmitis was produced by intravitreal injection of approximately 500 cfu of S. aureus 209P. Antibiotic was administered after clear indication of the onset of infection, including aqueous flare, red ring, conjunctival edema, bogginess of iris, and loss of red reflex. These changes occurred within 48 hr of bacterial inoculation.

Pigmented rabbits were killed 3, 6, 18, 24, and 48 hr after gentamicin delivery; albino rabbits were killed at 3 and 18 hr only. At least four rabbits (eight eyes) were examined at each interval. Both eyes were enucleated. Immediately after aspiration of aqueous sample, the globes were frozen in a Dry Ice-acetone bath. Tissues were dissected while the eyes were frozen in order to minimize the postmortem diffusion of antibiotic. Concentrations of gentamicin in tissues (cornea, iris, sclera, choroid-retina) were determined by the modified trephine-disc bioassay method. Levels in aqueous and vitreous were measured by radioimmunoassay (RIANEN [131I]gentamicin, New England Nuclear). The lowest concentration of gentamicin detectable in aqueous and vitreous humor was 0.4 μg/ml; in ocular tissues the lowest measurable concentration was 1.5 μg/ml.

Results

The concentrations of gentamicin in the vitreous, aqueous, and cornea of normal and infected eyes of pigmented rabbits are shown in Fig. 1. The half-life of gentamicin in the
Intravitreal gentamicin in infected eyes

Table I. Concentrations of gentamicin in ocular sites of albino and pigmented rabbits after intravitreal injection of a 50 μg dose in normal eyes

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration (μg/ml or μg/gm) at indicated time after injection*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 hr</td>
</tr>
<tr>
<td></td>
<td>Albino n = 8     Pigmented n = 8</td>
</tr>
<tr>
<td>Vitreous</td>
<td>34.1 ± 1.8       36.0 ± 3.1</td>
</tr>
<tr>
<td>Aqueous</td>
<td>5.0 ± 0.6        4.0 ± 0.8</td>
</tr>
<tr>
<td>Cornea</td>
<td>2.1 ± 0.2        1.6 ± 0.2</td>
</tr>
<tr>
<td>Iris</td>
<td>4.4 ± 0.9        Not detectable</td>
</tr>
<tr>
<td>Choroid-retina</td>
<td>11.0 ± 1.4      Not detectable</td>
</tr>
<tr>
<td>Sclera</td>
<td>4.4 ± 0.5        Trace</td>
</tr>
</tbody>
</table>

*Values represent mean ± S.E.

Vitreous of normal eyes was 24 hr. Extrapolating the levels back to time zero gave an apparent vitreous volume of 1.4 ml. Antibiotic concentrations in the aqueous humor remained fairly constant at 4 to 5 μg/ml for 18 hr; thereafter the clearance of drug from the aqueous proceeded at the same rate as from the vitreous, the terminal half-life being 25 hr. The maximum ratio of aqueous/vitreous levels was 0.28, which occurred 18 hr after injection. Corneal levels were initially much lower than those in the aqueous; however, concentrations in the two sites equilibrated by 18 hr. The terminal half-life in the cornea was 33 hr.

Inflammation dramatically decreased the half-life of gentamicin in the vitreous to 10 hr. Aqueous levels also declined rapidly and were undetectable by 18 hr. Antibiotic was irregularly detectable in the cornea at 3 hr and was not measurable thereafter.

Table I shows the antibiotic concentrations in normal eyes of albino and pigmented animals 3 and 18 hr after injection. There was no difference between the two strains of rabbits with regard to levels in the vitreous, aqueous, and cornea. In contrast, gentamicin levels in the iris, choroid-retina, and sclera were strikingly lower in pigmented than in albino strains. Indeed, the drug was undetectable in the iris and choroid-retina and barely detectable in the sclera of normal pigmented animals (Table I). Likewise, although the data are not shown, gentamicin was undetectable in the iris, choroid-retina, and sclera of infected pigmented eyes; albino rabbits were not studied in the infected state.

Discussion

There has been increasing interest recently in the use in intravitreal injections for the therapy of bacterial endophthalmitis. Most of the suggested therapeutic regimens are based on studies in rabbits. There is little information concerning the concentrations achieved in sites other than the vitreous humor, the half-life of drug in infected as opposed to normal eyes, and the effect of pigmentation on these levels. The purpose of this study was to examine the influence of these variables on the intraocular kinetics of gentamicin after intravitreal infection in rabbits.

There are differences in the literature regarding the toxic dose of gentamicin in normal rabbit eyes. It is interesting to note that Peyman et al., who studied albino rabbits, found a higher threshold for toxicity than did Zachary and Forster, who worked with pigmented animals. We have shown previously that gentamicin binds irreversibly to melanin; in addition, Lindquist has demonstrated binding of streptomycin to pigment granules. Whether drug binding and retinal toxicity are related cannot be determined from our data.

In order to minimize the likelihood of retinal damage, we used a dose severalfold lower than that considered safe by Zachary and Forster. Although it is conceivable that the kinetics of distribution may be concentra-
tion-dependent, our value for the half-life of gentamicin in the normal rabbit vitreous is in agreement with that reported for a dose 10 times as great. Therefore it appears likely that the kinetics observed in this study are applicable to the range of clinically desirable levels of gentamicin.

Gentamicin is among those substances that are believed to be cleared almost exclusively via the "anterior route," i.e., the anterior chamber and canal of Schlemm. For such molecules, Maurice11 was able to construct a theoretical plot of half-life in the vitreous vs. the ratio of concentrations in the aqueous/vitreous. Our values for gentamicin in the normal rabbit eye fall almost precisely upon the predicted line. In addition, our estimated vitreous volume is in close agreement with published values.12

The slow but progressive rise in concentration of antibiotic in aqueous humor and cornea is consistent with anterior route exit. The similarity between concentrations in aqueous and cornea suggests a lack of diffusional barriers between these sites. In contrast, a marked gradient was observed between the vitreous and sclera. The tight junctions in the pigmented retinal epithelium13 may comprise the barrier to egress of intravitreal gentamicin, which is reinforced in pigmented animals by the aminoglycoside-binding capabilities of the pigmented tissues.

The rate of decline in gentamicin levels was dramatically accelerated in infected eyes. One possible explanation of this observation would be inactivation of the antibiotic. The S. aureus used to produce the infection was sensitive to gentamicin and therefore not likely to produce inactivating enzymes. A reduction in activity due to pus or a difference in pH would be expected to occur rapidly.14, 15 Yet, in a separate study in our laboratory, gentamicin was incubated in aspirated vitreous of S. aureus–infected eyes for up to 24 hr at 37° C without loss of activity (unpublished observations). Thus the differences in concentrations in normal and infected eyes appear to represent a real difference in egress of drug from the eye.

The concentrations of drug in the aqueous of infected eyes were lower than those in normal ones. This could be due either to an increased rate of aqueous turnover or to "leakiness" of the pigmented retinal epithelial junctions permitting loss across the retinal surface. Hyaluronidase, an enzyme produced by S. aureus, has been shown to increase aqueous outflow.16 However, it is also well established that inflammation is accompanied by breakdown of retinal barriers, which would provide a posterior egress route for gentamicin. Our data do not permit us to distinguish between these two possibilities.

Several points of potential clinical importance emerge from this comparison of infected and normal eyes. First, the ocular distribution of antibiotic is more limited in infected eyes. Although vitreous concentrations are high, levels in anterior sites are low and fleeting. Second, the antibiotic half-life predicted by studies in normal eyes may overestimate by more than twofold the half-life in infected eyes. This observation is not intended to militate against the usefulness of intravitreal therapy, which appears to be the most reliable method for the attainment of therapeutically effective levels in the vitreous humor. Rather, it is intended to suggest that intravitreal injections may need to be repeated at intervals shorter than those suggested on the basis of studies in normal eyes.

REFERENCES
6. Young P, Barza M, Kane A, and Baum J: Radioactive and bioassay of intracocular antibiotics: double-assay technique to compare penicillin G, cefamandole,