The difficulty of determining the route of intraocular penetration of gentamicin after subconjunctival injection in the rabbit

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In small animals such as the rabbit the ratio of eye size to body size is much larger than it is in humans. Accordingly, periocular injection of antibiotics in this animal model results in significantly higher serum concentrations than does a comparable dose in a human. To assess the effect of the systemic drug component on ocular penetration, we compared the levels of gentamicin in ocular tissues and fluids of the rabbit following injection of 20 mg by subconjunctival or intramuscular routes. Blood levels of gentamicin were similar with the two routes of administration. In normal rabbit eyes, no antibiotic was detectable in the vitreous humor for the first 3 hr after either subconjunctival or intramuscular injection. By 6 hr, low vitreous levels of drug were detectable after subconjunctival, but not after intramuscular, injection. Because these concentrations were so close to the threshold of sensitivity of the assay, it was not clear that the difference between the routes was significant in normal eyes. In infected eyes, the concentrations of gentamicin in the vitreous were similar after subconjunctival and intramuscular injection. These data indicate that the penetration of gentamicin into the infected vitreous humor of rabbits after subconjunctival injection could be attributed as well to hematogenous carriage as to direct penetration. The problem in distinguishing systemic from direct transport with periocular injections may be inherent in any small animal model in which the ratio of eye size to body size is high.

Key words: antibiotic, intramuscular, subconjunctival, gentamicin, ocular penetration, animal model, vitreous, endophthalmitis, aminoglycoside

In small animals the ratio of eye size to body size is generally much greater than in adult humans. For example, a 2 kg rabbit weighs about one-thirtieth as much as a grown person, but its eye size is about one-third as great. As a result of this 10-fold difference in ratios, periocular injections in the rabbit produce much higher systemic concentrations of drugs than do similar doses in humans. The systemic levels may be sufficiently great as to constitute a major source of antibiotic for ocular penetration, and this could confound the interpretation of studies of drugs administered by periocular routes. Recently we found evidence of such a phenomenon in experiments with subconjunctivally administered oxacillin, in which there was a striking correlation between serum levels and vitreous concentration; although we could not prove that the systemic circulation was the major route of entry into the vitreous, the results were consistent with that possibility.

The purpose of the present study was to compare the intraocular penetration of gen-
Gentamicin in rabbits given an identical dose by subconjunctival or intramuscular injection. In this manner we hoped to evaluate the potential contribution of the hematogenous route to the penetration of gentamicin into the eye after periorcular injection.

Materials and methods

Pigmented (Dutch Belted) rabbits, 1.5 to 2.5 kg, were used throughout the study. Endophthalmitis was induced by bilateral intravitreal injection of 500 cfu of Staphylococcus aureus 209P. Penetration studies were initiated after loss of red reflex, usually within 48 hr of inoculation.

Ninety-one animals (49 infected, 42 normal) received 20 mg of gentamicin either intramuscularly or subconjunctivally 2 to 3 mm from the corneoscleral limbus in the superior quadrant of the left eye. Serum samples were drawn ¼, ½, 1, 2, 3, and 6 hr after the injection. Animals were sacrificed 1, 3, or 6 hr after injection. Because of the results obtained, additional groups of five to eight animals with normal eyes were sacrificed 12 and 18 hr after subconjunctival injection, and similar groups with infected eyes at ¼, ½, and 9 hr after either subconjunctival or intramuscular injection.

Gentamicin levels in ocular tissues and fluids were measured in both eyes. Tissues from those eyes receiving the subconjunctival injection were assayed by the collagenase digest method. Three specimens of cornea (superior, inferonasal, and inferotemporal), four of sclera (superior, nasal, temporal, and inferior), two of choroid-retina (anterior and posterior), and the iris were examined. Tissues from the contralateral eye of rabbits receiving subconjunctival injection and both eyes of rabbits receiving drug intramuscularly were assayed by the trephine disc method. This technique was used because lower concentrations of drug can be detected in tissues by this assay than by the collagenase digest method. The sampling pattern was essentially the same as above, with the exception that one central and two peripheral specimens were trephined from the cornea. Aqueous, vitreous, and serum were assayed in all instances by standard agar-diffusion bioassay techniques. Statistical comparisons were made by unpaired t test.

Results

Fig. 1 shows the serum concentrations of gentamicin (mean ± S.E.) at various intervals after a 20 mg dose administered by intramuscular or subconjunctival injection to infected rabbits. There was no significant difference in serum levels between the two routes of administration (unpaired t test). Serum levels in normal rabbits were not significantly different from those in infected rabbits.

The concentrations of gentamicin in aqueous, cornea, and vitreous of normal eyes after injection by the two routes are displayed in Fig. 2. Penetration of both cornea and aqueous was strikingly greater by the subconjunctival than by the intramuscular route. Levels in the cornea and aqueous of the contralateral eye were similar to those of animals given the antibiotic intramuscularly. No gentamicin was detectable in the vitreous of animals receiving gentamicin intramuscularly or in the contralateral vitreous of animals treated subconjunctivally. However, in the vitreous of the subconjunctivally injected
Fig. 2. Gentamicin levels in cornea, aqueous, and vitreous of normal eyes after a 20 mg dose administered either subconjunctivally (S/C) in the left eye or intramuscularly. S/C contralateral, contralateral eyes of animals receiving the subcutaneous injection. Each point is the mean ± S.E. of at least five eyes. Corneal levels for the eyes receiving subconjunctival injection are from the inferotemporal sample, i.e., a minimum value; for the contralateral eyes and eyes of rabbits receiving intramuscular injection, the levels are from a peripheral sample, i.e., a maximum value. Arrowheads signify that levels became undetectable at the next time of sampling.

eyes, gentamicin was detectable 6 hr after administration and was measurable in four of five eyes 18 hr after delivery. It was not possible to compare the groups statistically because the levels were undetectable in the majority of eyes.

Drug levels in the aqueous, cornea, and vitreous of infected eyes are shown in Fig. 3. Aqueous concentrations in subconjunctivally injected eyes were similar to those in normal animals. However, inflammation caused a marked increase in gentamicin levels in the cornea and aqueous of contralateral eyes and those treated by the intramuscular route. Gentamicin could be detected in the vitreous in all three groups of infected eyes. Drug levels were measurable as early as 30 min after injection and increased to >21% of the concentration in aqueous by 6 hr. Although modest disparities existed among the groups at ½ and 1 hr after injection, there was no significant difference in vitreous levels among the three groups by 3 hr.

Subconjunctival injection resulted in higher concentrations of gentamicin in ocular tissues than did intramuscular injection. Indeed, the drug was detectable in iris and choroid-retina only in those eyes receiving subconjunctival injection, though only at the 1 hr interval. There were marked variations in the concentration of drug within cornea and sclera after subconjunctival injection, the highest levels in each tissue being nearest the site of injection. In the contralateral eye of
Fig. 3. Gentamicin levels in cornea, aqueous, and vitreous of infected eyes after a 20 mg dose administered either subconjunctivally (S/C) or intramuscularly. See legend for Fig. 2.

Discussion

This study supports the widespread belief that subconjunctivally administered antibiotic penetrates the anterior ocular segment, particularly the cornea, much more readily than systemically administered drug. Levels in cornea and sclera were highest near the site of subconjunctival injection, as has been shown previously. The fact that antibiotic was detectable in choroid-retina only in subconjunctivally treated eyes and only at the 1 hr interval is presumably related to binding to melanin in pigmented tissues. Our results also support the notion that ocular inflammation enhances the penetration of systemically administered drug as shown by the increase in levels of gentamicin in cornea, aqueous, and vitreous in inflamed as opposed to normal eyes after intramuscular injection. This would account for our finding that the discrepancy in aqueous levels between subconjunctival and intramuscular injection was less pronounced in inflamed than in normal eyes. Litwack et al. reported similar results in a study of aqueous penetration of gentamicin after subconjunctival and intramuscular delivery; levels after injection by the two routes were similar in secondary, but not in primary, aqueous. Paradoxically, inflammation seemed to reduce slightly the concentrations in ocular tissues after subconjunctival injection, a phenomenon that we have noted previously.
This could be related to enhanced systemic absorption of drugs from inflamed eyes.

A principal finding of this study was that vitreal levels in inflamed eyes were identical after subconjunctival or intramuscular injection. The most obvious explanation is that drug enters the inflamed vitreous primarily via the systemic route whether it is given subconjunctivally or intramuscularly. This interpretation is supported not only by the equivalence of vitreal concentrations with the routes but also by the similarity in levels between the injected and contralateral eye.

Recently, Davis et al. studied tobramycin in guinea pigs with Pseudomonas keratitis. Like us, they found that levels of antibiotic in the aqueous were similar after subconjunctival or intramuscular injection; however, in contrast to us, they found lesser penetration of the vitreous with the intramuscular route. The difference between the two studies may reflect the fact that their model of ocular inflammation (corneal ulcer) would be less likely than ours to produce a breakdown in blood-retinal barriers. In normal eyes we did in fact find less penetration of vitreous with the intramuscular route.

Although our data suggest that the systemic circulation is the major route of entry of subconjunctival gentamicin into the infected vitreous of rabbit eyes, another explanation is possible. It may be that subconjunctival drug enters the vitreous and the systemic circulation simultaneously but independently. In that event, the apparent relation between the concentrations in the two sites would be a fortuitous result of the eye-to-body ratio of the rabbit and the anatomy of the eye. It is difficult to test this hypothesis because there is no convenient way to lower serum levels in the rabbit after subconjunctival injection short of performing hemodialysis or of disrupting the circulation of the eye. We attempted to study large and small rabbits (1 to 4 kg) in the hope that the eye-to-body ratio would be significantly different; however, this did not prove to be the case (unpublished data). That only subconjunctivally injected eyes had detectable choroid-retina levels may be construed to suggest that direct penetration of choroid-retina does occur; however, this must be transient (not evident in 3 hr subconjunctival injected eyes) and, in any event, does not prove the route of entry of antibiotic for the vitreous. Examination of the time-course of penetration into the vitreous of infected eyes also failed to shed light upon the mode of entry. Although we cannot demonstrate conclusively that subconjunctivally injected drug enters the vitreous via the systemic circulation, our data indicate that the serum levels produced in this small animal were sufficient in and of themselves to produce the observed vitreous concentrations without periocular injection.

The results of this study should serve as a caution that at least in the rabbit, penetration of drug into the inflamed vitreous after subconjunctival injection may be attributable to systemic absorption. A similar problem may exist in the aqueous of the inflamed eyes. Our data are inconclusive regarding the route of penetration into the normal vitreous because, although the drug was evident only in subconjunctivally injected eyes, the levels were close to the threshold of sensitivity of the assay and statistical comparisons were not possible. One means to monitor the possibility that this phenomenon is occurring is simply to compare the injected eye with the contralateral eye after periocular administration. It is tempting to try to calculate the contribution of direct transport after periocular administration by subtracting levels in the contralateral eye from those in the injected one; however, this expedient may not be valid, since there could be a pharmacokinetic interaction (e.g., competition for uptake) if two routes were operating simultaneously.

REFERENCES
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