Penetration of Ocular Tissues and Fluids by Moxalactam in Rabbits with Staphylococcal Endophthalmitis

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Moxalactam was administered subconjunctivally in 100-mg doses to rabbits with infected eyes (Staphylococcus aureus endophthalmitis). High concentrations of drug were detected in the sclera, cornea, and choroid; much lower levels were found in the retina, whereas peak concentrations in the vitreous were about 6 μg/ml. Repeated intramuscular injections of 50 mg/kg every 4 h produced peak serum levels of about 100 μg/ml. A gradient between the choroid and the retina was again evident, and peak vitreous levels were about 6 μg/ml after six injections. These data are consistent with the concept of a blood-retina barrier analogous to the blood-brain barrier. Moxalactam appears to penetrate the eye somewhat better than do other β-lactams; however, the peak levels produced in the vitreous humor in this animal model were below the level required to inhibit most strains of Pseudomonas aeruginosa.

Moxalactam (LY127935) is a new oxα-β-lactam antibiotic with a wide spectrum of activity encompassing most strains of Enterobacteriaceae, Pseudomonas aeruginosa, and Bacteroides fragilis. The drug penetrates the cerebrospinal fluid of animals (7, 12) and humans (9) better than do most cephalosporins. Because the blood-ocular barrier shares many similarities with the blood-brain barrier (2), we hoped that moxalactam would penetrate the vitreous humor in concentrations which might be effective in the treatment of bacterial endophthalmitis. Accordingly, we examined the intraocular penetration of moxalactam into infected eyes of rabbits after a single subconjunctival injection. We also studied the intraocular levels of this agent after repeated intramuscular injections. These two routes were chosen because they are commonly used in the treatment of serious ocular infections in humans. To simulate the circumstances in which such therapy might be applied clinically, we employed a model of bacterial endophthalmitis which has been used previously to study the intraocular penetration of antibiotics (5).

MATERIALS AND METHODS

Induction of infection. Dutch-belted (pigmented) rabbits, 1.5 to 2.5 kg, were used throughout the study. We produced bilateral endophthalmitis by intravitreous inoculation of 500 colony-forming units of Staphylococcus aureus 209P. Antibiotic administration was begun 48 h after infection. At this time, there was conjunctival edema, ciliary flush, aqueous flare and cells, and loss of red reflex. Preliminary studies showed that at 48 h after infection, the vitreous contains approximately 10^3 colony-forming units of S. aureus per ml; microscopic examination of the vitreous demonstrated 5 to 10 polymorphonuclear leukocytes per high-power field.

Subconjunctival injection. Moxalactam (100 mg) was administered in the superior quadrant of the left eye 2 to 3 mm from the limbus. This dosage was chosen because it is in the range commonly used in the treatment of human ocular infections. Animals were killed 15, 30, 60, 180, 240, and 360 min later. At least five animals were killed at each interval. At these times and at 120 min after injection, samples of blood (for antibiotic assay) were obtained by aspiration through the marginal vein of the ear. The final blood sample was obtained immediately before sacrifice.

Both eyes were enucleated, cleansed, and rinsed. Samples of aqueous and vitreous humor were aspirated and immediately assayed by an agar diffusion bioassay technique, using Escherichia coli 10536 (supplied by Eli Lilly & Co.) as the test organism. Standard solutions were prepared in phosphate-buffered saline. Serum samples were assayed in the same manner, using standards prepared in normal rabbit serum. In our experience, this assay was reproducible to within ±15% on repeated testing.

Concentrations in the cornea (superior and inferior), iris (superior and inferior), sclera (superior, inferior, nasal, and temporal), choroid (anterior and posterior), and retina of the injected eye, in which levels were expected to be high, were examined by the collagenase digest bioassay method (4). Standards were prepared in similar collagenase solutions and maintained under the same conditions of temperature. To determine whether moxalactam was fully recoverable in the pres-
ence of tissue under the conditions of the collagenase digest assay, tissue samples were "spiked" with known amounts of antibiotic (final concentration, 10 and 50 \( \mu g/ml \)) and assayed after the usual overnight digestion. Recovery of activity was 85 to 100% of the expected values for all ocular tissues examined (cornea, sclera, and choroid-retina), with the exception of the iris, where activity loss was 15 to 25%. Samples from the contralateral eye were examined by the trephine disk method, which detects lower concentrations than does the collagenase digest method. Standards for this assay were prepared in phosphate-buffered saline (13).

**Intramuscular injection.** Five rabbits were given intramuscular injections of moxalactam, 50 mg/kg every 4 h for six injections. This dosage was sufficient to produce peak serum levels in the range that might be used to treat human infections; furthermore, they were similar to the concentrations produced by subconjunctival injections, thereby facilitating comparisons between the two routes. Serum levels were measured 30 min (peak) and 120 and 240 min (trough) after the first injection, 30, 90, and 240 min after the second injection, 240 min after the fifth injection, and 30 and 90 min after the sixth injection. The animals were killed 90 min after the sixth injection. Both eyes were removed and cleaned of blood and conjunctiva, and the aqueous was aspirated. The globes were frozen in a dry ice-acetone bath to prevent diffusion of antibiotic (1). Tissues were dissected while still frozen and were assayed within 5 h of sacrifice by the trephine disk method.

**RESULTS**

**Subconjunctival injection.** The concentrations of antibiotic in ocular tissues and humor after subconjunctival injections are shown in Fig. 1. There were marked regional variations in the levels of drug in the sclera, the highest values (3,000 \( \mu g/g \)) being found in superior segments near the site of injection and the lowest values (1,300 \( \mu g/g \)) being found in the inferior segments. Regional variations in other tissues were much less striking: 26% in the cornea (superior versus inferior), 35% in the iris (superior versus inferior), and 50% in the choroid (anterior versus posterior). The tissue levels shown in Fig. 1 are for the segment nearest the site of subconjunctival injection.

The levels of antibiotic in ocular tissues attained a peak 15 to 30 min after injection and fell fairly steadily thereafter. Sclera reached the highest levels of any tissue; concentrations in the choroid were slightly lower. Of particular note was the 10-fold gradient in concentrations between the choroid and the retina. Levels in the vitreous rose slowly, reaching a peak of 5.7 \( \mu g/ml \) 240 min after injection. Retinal concentrations initially resembled those in the serum, but later paralleled those in the vitreous. The cornea contained maximum levels of about 800 \( \mu g/g \). Concentrations in the aqueous humor rose rapidly during the first 30 min and then remained constant at 30 to 40 \( \mu g/ml \).

Tissue levels in the contralateral (uninjected) eye showed no regional variations in concentration. Thus, the values for various segments were pooled (Fig. 2). Tissue levels peaked within 30 min of subconjunctival injection. By 180 min, levels in the sclera and choroid exceeded those in the serum. Concentrations in the cornea (data not shown) were similar to those in the aqueous.

There was a two- to threefold gradient between the choroid and the retina. Penetration into the aqueous and vitreous was good, reaching peak levels which were 38 and 54%, respectively, of those in the injected eye.

**Intramuscular injection.** Intramuscular injections of 50 mg/kg resulted in 30-min serum levels which were similar to those found after a subconjunctival dose of 100 mg (Fig. 3). Trough levels rose slightly from 2.4 \( \mu g/ml \) after the first injection to 4.0 \( \mu g/ml \) just before the final dose. Because there were no significant regional variations in tissue concentrations with this route, the data for the various segments of each tissue were pooled in Fig. 3. Tissue and vitreous levels of moxalactam measured 90 min after the sixth injection were similar to those on the extrapolated line between 60 and 180 min after subconjunctival administration. The concentration gradient between the choroid and the retina was approximately twofold.

**DISCUSSION**

The excellent penetration of moxalactam into the infected meninges of humans (9) and animals (7, 12) led us to examine its transport into the rabbit eye. Subconjunctival injections produced high concentrations in most ocular tissues. The corneal levels approached 1,000 \( \mu g/g \), which should be more than adequate to inhibit most strains of Enterobacteriaceae, as well as Pseudomonas aeruginosa, a finding which may be relevant in treating bacterial keratitis or corneal ulcer. Concentrations in the aqueous, iris, sclera, and choroid exceeded 16 \( \mu g/g \) or 16 \( \mu g/ml \) for at least 3 h after injection. At this level, 50% of the strains of P. aeruginosa and 90% of the strains of S. aureus and Enterobacteriaceae are inhibited (6, 10, 13). Intramuscular injection also produced levels in excess of 16 \( \mu g/ml \) in most ocular tissues. Presumably because of a rapid rate of fall off, however, the levels did not increase significantly with repeated injections.

The concentrations of moxalactam that can be achieved in the vitreous humor are relevant both because this site is the focal point of infec-
tion in bacterial endophthalmitis and because it is the most inaccessible part of the eye in terms of drug penetration. Although we are not aware of any data which directly relate the intraocular levels of antibiotic to the likelihood of therapeutic success, it is reasonable to assume that favorable characteristics of intraocular penetration, especially into the vitreous humor, offer therapeutic benefit, depending upon the intrinsic susceptibility of the infecting organism. The penetration of subconjunctivally administered moxalactam into the infected vitreous humor was markedly better than that for several other β-lactam antibiotics studied in our laboratory. For example, the peak levels of oxacillin, methicillin, and cefazolin after subconjunctival doses of 100 mg were less than 1.0 µg/ml (M. Barza, A. Kane, and J. Baum, submitted for publication). In contrast, the same dose of moxalactam produced peak vitreous levels of 5.7 µg/ml.

Levels of moxalactam in the vitreous after repeated intramuscular injections were approximately 6 µg/ml, i.e., about 5.2% of the peak serum level. The corresponding values (peak vitreous/peak serum level) after a single subconjunctival injection were 7.6% for the injected eye.
and 4.1% for the contralateral eye. The higher value for the injected than for the noninjected eye suggests that at least some of the drug entered the vitreous by direct diffusion from the subconjunctival site rather than by the hematogenous route. This is in contrast to the findings with gentamicin (M. Barza, A. Kane, and J. Baum, Invest. Ophthalmol., in press) and oxacillin (3), in which virtually all of the activity detected in the infected vitreous could be accounted for by hematogenous spread. The paucity of regional variations in concentration within the cornea also differs from the findings with other antibiotics (3, 11), suggesting that moxalactam may have a greater capacity than these other drugs to diffuse through tissues.

The maximal penetration ratio of moxalactam into the vitreous after intramuscular injection in this study (5.2%) exceeds that reported for oxacillin given by continuous infusion in the same rabbit model (2 to 3%) (5). Although the difference is not great, it is consistent with the comparatively good penetration of moxalactam into the cerebrospinal fluid. Augmented penetration of moxalactam could be due either to increased passage across the blood-ocular barriers or to a slower removal from the vitreous by the organic anion transport pump. Further study will be needed to elucidate these points.

There was a pronounced concentration gradient between the choroid and the retina in this study. It was as much as 10-fold in the eyes treated by subconjunctival injections and two- to threefold in contralateral eyes or those of animals given repeated intramuscular injections. This finding is consistent with the concept of a blood-retina barrier located in the retinal capillaries, the retinal pigment epithelium, or both (8).

Moxalactam penetrates the infected vitreous better than do other β-lactam antibiotics. Nonetheless, peak levels were still only 6 to 8 μg/ml with doses that would be considered fairly high in terms of peak serum levels in humans. Some
Fig. 3. Antibiotic levels in serum at various intervals in the course of a series of six intramuscular injections of moxalactam, 50 mg/kg every 4 h. Values on the right are moxalactam concentrations in various ocular tissues and fluids 90 min after the final injection. Each point represents the mean and standard error of samples from at least five eyes.

strains of S. aureus and most of P. aeruginosa are not inhibited by those concentrations. Thus, although the drug may be useful in many cases of bacterial endophthalmitis, especially those due to susceptible strains of Enterobacteriaceae, it cannot be regarded as adequate coverage for all common pathogens in this disease. Our data do not permit us to draw a conclusion as to the optimal route of delivery of moxalactam for the treatment of bacterial endophthalmitis. There appears to be moderate penetration by both the systemic and the subconjunctival routes. Further study will be needed to determine whether combining these routes will afford significantly better vitreous penetration than that achieved by either alone.

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LITERATURE CITED