Effect of Inflammation on Antibiotic Penetration Into the Anterior Segment of the Rat Eye

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Bacterial infections were established in the right cornea of rats. Animals infected with *Staphylococcus aureus* were given cephradine intravenously (IV) (40 mg/kg) or topically (50 mg/ml) to both eyes. Animals infected with *Pseudomonas aeruginosa* were given gentamicin sulfate IV (40 mg/kg) or topically (10 mg/ml). Antibiotic concentrations in cornea and aqueous humor were measured for 4 hrs following dosing using bioassay and radioimmunoassay. In general, infection significantly increased the concentrations obtained soon after dosing. Topically applied cephradine passed through infected eyes more quickly than through normal eyes. Of the pharmacokinetic parameters derived, the permeability of the corneal epithelium to gentamicin in the rat more closely agrees with reported human values than does the rabbit, while the coefficient of elimination from aqueous in the rat is considerably greater than that for either humans or rabbits. This suggests that there are both advantages and disadvantages in using the rat for therapeutic studies of ocular disease. Invest Ophthalmol Vis Sci 27:958–965, 1986

The penetration of antimicrobial agents into the anterior segment of the uninflamed human eye has been investigated by a number of workers. In the last five years alone, the agents tested for their ability to pass into the anterior chamber have included gentamicin,[1] tobramycin,[2] amikacin,[3-4] piperacillin,[5] doxycycline,[6] and many of the new cephalosporin derivatives[7-15] and antivirals.[16-17] Data for penetration into the inflamed human eye are less readily available. This can be attributed to the relatively small number of suitable cases presenting at an institution, the diverse nature of anterior segment infection, and the usual urgency associated with these patients. In studies of antibiotic penetration into inflamed eyes, subjects have included patients with chronic anterior uveitis and endophthalmitis,[18] and also a range of undefined conditions.[19] Until more human data become available, extrapolation of concepts from animal models will be important.

We report antibiotic concentrations obtained from topical and iv administration in the normal and inflamed rat cornea and aqueous humor, determine pharmacokinetic parameters for comparison with rabbit and human values, and present observations on the nature of antibiotic penetration which may deserve consideration in clinical practice. The antibiotics chosen for study here are an aminoglycoside and a cephalosporin commonly used to treat human corneal suppuration. The increasing use of the rat in anterior segment microbiologic[20-22] and immunologic[23] research also necessitates study of ocular drug penetration in this animal.

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Materials and Methods

Corneal Inoculation

Adult male outbred Porton rats were used. These are nonpigmented. Bacterial strains, isolated from human corneal infections, were cultured to log phase growth in brain-heart infusion broth and diluted in saline immediately before inoculation. Animals were anaesthetized by halothane inhalation. The right cornea of each rat was injected with approximately 5×10^3 colony-forming units of *Staphylococcus aureus* (phage type 94/96) or *Pseudomonas aeruginosa* (pyocin type 1) using methods described previously.[24] The uniformity of the infections 24 hours after inoculation were tested on groups of eight rats using a digital pachymeter (model CS1000, Storz Instrument Co., St. Louis, MO). The rats were handled in accordance with the ARVO Resolution on the Use of Research Animals.

Antibiotic Delivery

Twenty-four hours after inoculation, antibiotics were given in a single drop by the topical route or in a single injection by the IV route. Rats infected with *S. aureus* received cephradine sodium and rats infected with *P. aeruginosa* received gentamicin sulfate. Topically, 25

μl of 50 mg/ml cephradine or 10 mg/ml gentamicin in 5 mg/ml hydroxypropyl methylcellulose were given to both eyes using a Finn pipette. This volume exceeds the capacity of the rat conjunctival sac, and therefore portrays the usual situation when drops are given to humans. For IV injection, the animals were lightly anaesthetized with chloroform and given 40 mg/kg cephradine or gentamicin in isotonic saline via the tail vein.

Specimen Collection

The concentrations of antibiotics were followed in aqueous humor, cornea, and serum for 4 hours after dosing. By this time, tissue levels had generally fallen below the minimum inhibitory concentrations. For collection of ocular specimens at each time point, rats were killed with intrathoracic injection of pentobarbital sodium. Aqueous humor was collected using a 27-gauge needle and 1-ml syringe. Corneas were excised to within 1 mm of the limbus, weighed, and homogenized for 1 minute in 0.2 ml of cold phosphate-buffered saline using a glass tissue grinder. Blood was taken from the tails of lightly anaesthetized animals. Tissue was obtained from four to twelve animals for each time point.

Bioassay

Antibiotic concentrations were assayed by agar diffusion bioassay. *Micrococcus luteus* ATCC9341 was the test organism used for cephradine and *Bacillus subtilis* NCTC8236 was used for gentamicin. Petrie dishes containing seeded agar with 10 μl wells were prepared. Serum samples suspected of having high antibiotic concentrations (>50 μg/ml) were diluted in isotonic saline prior to assay. Samples were transferred to wells using a microsyringe. This procedure was randomized amongst dishes. Known antibiotic concentrations were prepared in serum and normal and infected rat aqueous humor and corneal homogenate. Plates were incubated overnight in 5% CO_2/air at 37°C. The linearity of the plots of zone sizes against concentrations of standards (in $\log_{10}$ μg/ml) was checked by regression analysis. The concentrations derived for test corneal suspensions were multiplied by the dilution factors to give the original corneal concentrations. The minimum concentration detectable in aqueous humor was 0.2 μg/ml for cephradine and 0.1 μg/ml for gentamicin. The minimum corneal concentration detectable was 1.8 μg/g for cephradine and 1.2 μg/g for gentamicin. The accuracy of the bioassay for gentamicin was checked on parallel samples using radioimmunoassay (Therapeutic Drug Monitoring System, Abbott Laboratories, North Chicago, IL). Untreated animals that had undergone identical anaesthetic and killing procedures had no detectable antibacterial activity in their ocular tissues. A total of 162 rats were used for the ocular drug determinations, and 36 rats for serum drug determinations.

Data Analyses

Antibiotic concentrations were plotted against time. The calculation, use, and abbreviation of pharmacokinetic parameters were based on the work of Maurice and Mishima[25] and Nagataki and Mishima.[26] When the logarithm to base 10 of the concentration in ocular samples was plotted against time, the points approximated linear (first order) pharmacokinetic behaviour in the first three hours after dosing.

For topical data, regression lines were drawn with respect to points between the high point and the point preceding the 3-hour mark, a period of decreasing concentrations in both cornea and aqueous humor. The slope of this line in an aqueous humor graph gave A, the apparent elimination coefficient of aqueous humor. Corneal and aqueous concentrations declined approximately in parallel; their ratio, g_{ca}, was determined at the mean point of the aqueous humor regression line. The volume of aqueous humor in the Porton rat eye, V_a, was about 25 μl. Corneal volume, V_c, in the normal eye was estimated to be 10.1 μl. V_c of *S. aureus*-infected eyes was 12.2 μl and for *P. aeruginosa*-infected eyes 19.2 μl. These volumes were determined from the weights of collected tissue. The coefficient of loss from aqueous humor to blood, k_0, may be calculated:

$$k_0 = A(1 + g_{ca}V_c/V_a) \quad (1)$$

The ratio of drug concentration in cornea to that in aqueous humor at steady state, r_{ca}, was taken as 0.75 on the assumption that the drug is in simple solution in stromal tissue fluid. The transfer coefficient between cornea and aqueous humor, k_c, is given by:

$$k_c = \frac{A}{1 - r_{ca}/g_{ca}} \quad (2)$$

The corneal area, Q, of the rat eye is about 0.28 cm^2. The initial tear concentration after instillation, C_0, may be assumed to be equal to the concentration instilled. The turnover rate in tears, α, was assumed to be about 15% per minute. This is the estimate given for both human and rabbit eyes,[25] and may depend more on state of lacrimation than species or viscosity of the vehicle.[27] An estimate of the amount of drug initially absorbed by the cornea, m_0, was obtained from the regression line on a corneal concentration graph when time equals zero. The permeability of corneal epithelium, K, may then be found:

$$K = \frac{m_0\alpha}{QC_0} \quad (3)$$

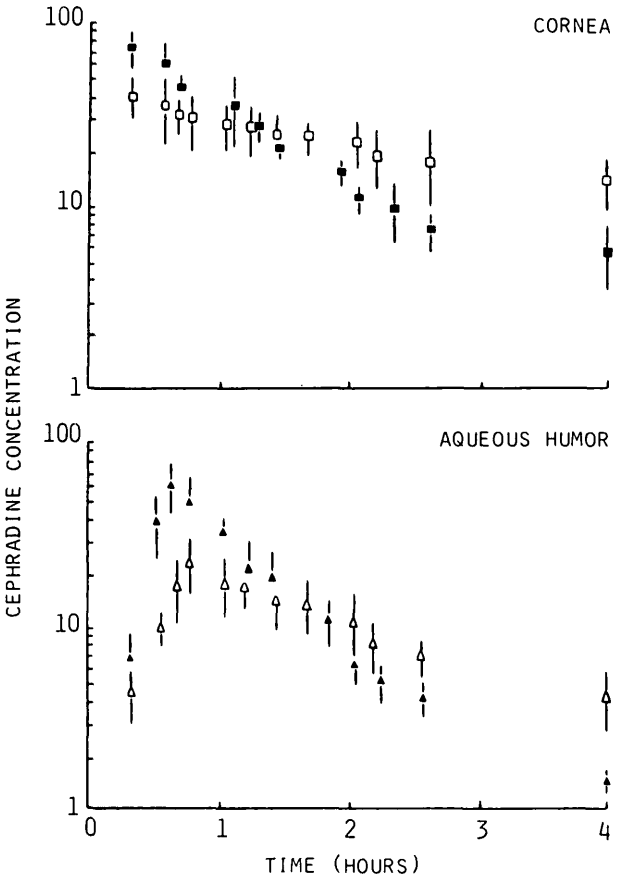

Fig. 1. Concentration of cephradine in the cornea (in μg/g) and aqueous humor (in μg/ml) of rat eyes following one drop (25 μl) of 50 mg/ml cephradine in 5 mg/ml hydroxypropyl methylcellulose. Open symbols represent normal eyes and shaded symbols represent eyes with *S. aureus* keratitis. Each point is the mean ± SD of 4 to 12 measurements.

The area under the concentration-time curve gives the overall bioavailability (Ba). This is strictly correct only with drugs and biological systems showing linear pharmacokinetic behaviour. The concept and mathematical treatment of Ba have been reviewed by Smolen[28] and Gibaldi & Perrier.[29] The apparent absorption coefficient of aqueous humor, B, is given by:

$$B = \frac{m_0 k_c}{Ba V_a A} \quad (4)$$

where Ba is bioavailability in aqueous humor.

For the IV data, Ba was determined as above. An indication of how well a drug will penetrate into an eye is given by r'_{ap}, the ratio of the concentration in aqueous humor, C_a, to total plasma concentration, C'_p, when C_a is maximum at time t_{max}. The calculation of A as above permits the evaluation of k_0 following IV injection:

$$k_0 = \frac{A}{1 - r'_{ap} C'_p / C_a} \quad (5)$$

for C'_p and C_a taken at the mean point of the aqueous humor regression line. The coefficient of entry into the anterior chamber from blood, k_i, is given by:

$$k_i = k_0 r'_{ap}$$

at t_{max}, if the one-compartment approximation is used in which the effect of the posterior chamber is ignored.

Differences between pairs of mean concentrations and bioavailabilities were tested by paired t-tests.

Results

Infection of the Rat Cornea

The appearance of the keratitis produced in rats by *S. aureus* and *P. aeruginosa* resembled that produced in humans. *S. aureus* gave rise to an ulcer localised at the site of inoculation and involving about 25% of the area of the cornea. *P. aeruginosa* caused edema and a large polymorphonuclear cell response involving the whole cornea, increasing its weight considerably above normal. The rat infections have been characterised in detail in a previous study.[21] From the pachymeter measurements, the average thickness at the center of the normal cornea of these outbred rats was 0.196 mm, with a range from 0.181 mm to 0.206 mm. For a cornea with a centrally located ulcer due to *S. aureus*, the average thickness was 0.296 mm, with a range from 0.271 mm to 0.327 mm. Pachymeter readings could not be obtained for *P. aeruginosa*-infected corneas. Perhaps the severe disruption to the stroma seen with this infection altered the ultrasonic characteristics of the tissue.

Topical Administration

Figure 1 shows the penetration of cephradine into the normal and infected rat eye from the topical route. Corneal concentrations peaked within minutes of drug instillation; the highest concentration measured in normal eyes was 41 ± 10 μg/g. The peak concentration in aqueous humor occurred later, about 40 minutes after instillation. With inflammation due to staphylococcal corneal infection, the cephradine concentration in cornea and aqueous humor at time points soon after instillation was significantly higher ($P < 0.05$) than in normal eyes. However, by 4 hours after dosing, the concentration in infected eyes had dropped significantly lower ($P < 0.05$) than in normal eyes, a "passing through" effect. The finding that cephradine moved through an infected cornea more rapidly is indicated by the data in Table 1. Ba, which depends on concen-

Table 1. Pharmacokinetic data for antibiotic penetration into the rat eye following topical administration

	Ba (mg-min/ml)	m_o (μg)	*K* (cm/hr)	*A* (hr^{-1})	g_{ca}	k_o (hr^{-1})	k_c (hr^{-1})	*B* (hr^{-1})
Cephradine								
Normal Eye:								
cornea	4.08	0.41	2.6×10^{-4}	—	—	—	—	—
aqueous humor	1.42	—	—	0.32	2.00	0.58	0.51	1.10
Inflamed Eye:*								
cornea	2.75	1.22	#	—	—	—	—	—
aqueous humor	2.26	—	—	0.76	1.50	1.32	1.52	2.59
Gentamicin sulfate								
Normal Eye:								
cornea	0.52	0.20	6.4×10^{-4} (10^{-5}[a], 10^{-3}[b])	—	—	—	—	—
aqueous humor	0.96	—	—	0.67 (0.15[c], 0.2[d])	0.65	0.85	high	high
Inflamed Eye:†								
cornea	1.89	2.61	‡	—	—	—	—	—
aqueous humor	2.42	—	—	0.72	1.36	1.47	1.61	5.79

Ba, bioavailability; m_0, mass of drug initially absorbed by the cornea; K, permeability of corneal epithelium; A, apparent elimination coefficient of aqueous humor; g_{ca}, ratio of corneal to aqueous humor concentration during phase of parallel decline; k_o, coefficient of loss from aqueous humor to blood; k_c, transfer coefficient between cornea and aqueous humor; B, apparent absorption coefficient of aqueous humor.

* *S. aureus* keratitis.

† *P. aeruginosa* keratitis.

‡ Epithelium not intact.

[a] Value for humans derived by D. Maurice and S. Mishima from Utermann et al[42] and Ellerhorst et al.[19]

[b] Value for rabbits derived from Bloomfield et al.[43]

[c] Value for humans derived from Uterman et al.[42]

[d] Value for rabbits derived from Baum et al.[40]

tration and time, was higher in aqueous humor from infected eyes than from normal eyes. However, despite an increase in m_0, Ba was lower in infected corneas than normal corneas. The rate constants A, B, k_0, and k_c determined for cephradine (Table 1) show that an increased rate of flow of antibiotic occurred through the anterior chamber when corneal infection was present.

As shown in Figure 2, infection of the cornea with *P. aeruginosa* increased the gentamicin sulphate concentration about fourfold in cornea and twofold in aqueous humor over that in normal eyes after topical dosing. Also, Ba of gentamicin in the cornea and aqueous humor of infected eyes was considerably increased (Table 1). Aqueous humor Ba was greater than corneal Ba for this antibiotic. As seen with cephradine, the presence of inflammation increased the flow of gentamicin out of the aqueous humor (rate constants A and k_o). However, the effect on rate of drug uptake (B and k_c) could not be determined algebraically due to the low value of g_{ca} for gentamicin in normal rat aqueous humor. Human and rabbit values for A and K in uninflamed eyes are shown in Table 1.

Intravenous Administration

Antibiotic concentrations measured in rat serum for 4 hours after iv dosing are shown in Figure 3. The penetration of cephradine into the rat eye from blood is shown in Figure 4. The plot indicates that the peak concentrations in cornea and aqueous humor occurred within 32 minutes of injection. For concentrations measured in the first 90 minutes, the presence of staphylococcal keratitis increased aqueous humor cephradine levels significantly over those in normal eyes ($P < 0.05$). For the cornea, this difference was not significant. Ba increased in both compartments in infected eyes (Table 2). If t_{max} is taken at 32 minutes, the presence of infection lifts r'_{ap} from approximately 2.5% to 3.9%. The table also shows that the rates of drug flow into (k_i) and out of (k_0) aqueous humor were somewhat increased with inflammation.

As with topical dosing, iv gentamicin (Fig. 5) resulted in significantly higher concentrations ($P < 0.05$) in corneas infected with *P. aeruginosa* than in normal corneas for each time point over 4 hours. Ba markedly increased from 0.16 to 0.84 mg-min/ml (Table 2). In contrast, the aqueous humor concentrations were unaffected by the presence of inflammation. Ba, r'_{ap}, and the rate constants were similar for aqueous humor and infected eyes. Human and rabbit values for r'_{ap} in uninflamed eyes are shown in Table 1.

Discussion

Inoculation of the rat cornea with *S. aureus* or *P. aeruginosa*, the two most common bacterial species isolated from keratitis patients in temperate climates,[30] resulted in infections with symptoms and severity resembling those found in human cases. Twenty-four

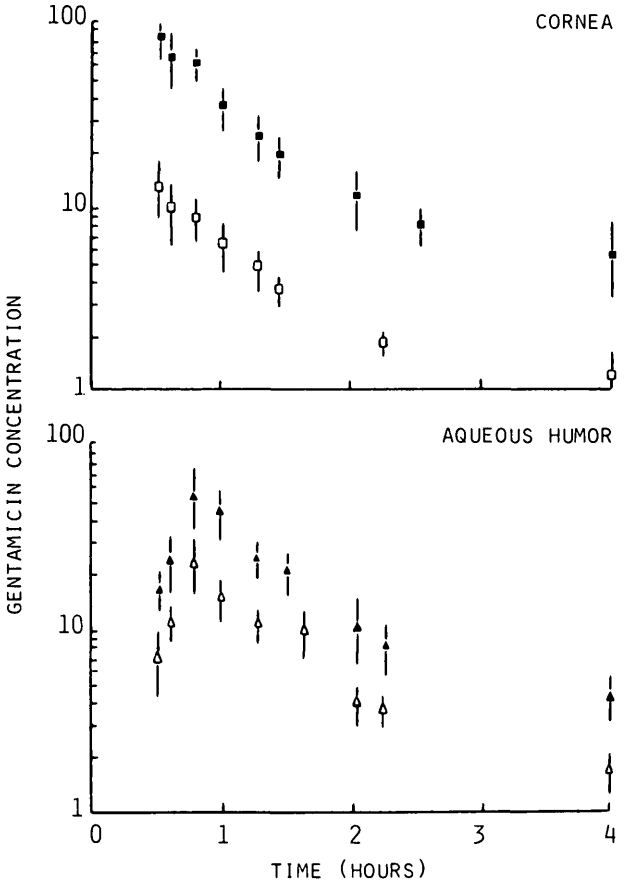

Fig. 2. Concentration of gentamicin sulfate in the cornea (in μg/g) and aqueous humor (in μg/ml) of rat eyes following one drop (25 μl) of 10 mg/ml gentamicin in 5 mg/ml hydroxypropyl methylcellulose. Open symbols represent normal eyes and shaded symbols represent eyes with *P. aeruginosa* keratitis. Each point is the mean ± SD of 4 to 12 measurements.

hours after inoculation, the rat corneas have the appearance of established corneal infections in human patients; *S. aureus* induced a localised ulcer involving about 25% of the corneal area, and *P. aeruginosa* induced opacity involving the whole tissue. Therefore, a study of the effect of inflammation on the penetration of antibiotics using the rat model of bacterial keratitis was believed to be relevant to human disease.

The presence of infection significantly increased the peak concentration of topically applied antibiotic measured in the rat cornea and aqueous humor. The concentration of gentamicin in *Pseudomonas*-infected eyes remained elevated during the 4 hours. However, *S. aureus* keratitis, a less severe infection, did not enhance the penetration of cephradine to the same degree. A likely reason for this difference was found in preliminary histologic studies. With *P. aeruginosa* keratitis in rats (and humans), there is almost total corneal epithelial degeneration; while in *S. aureus* keratitis, most of the epithelium remains intact. The importance of

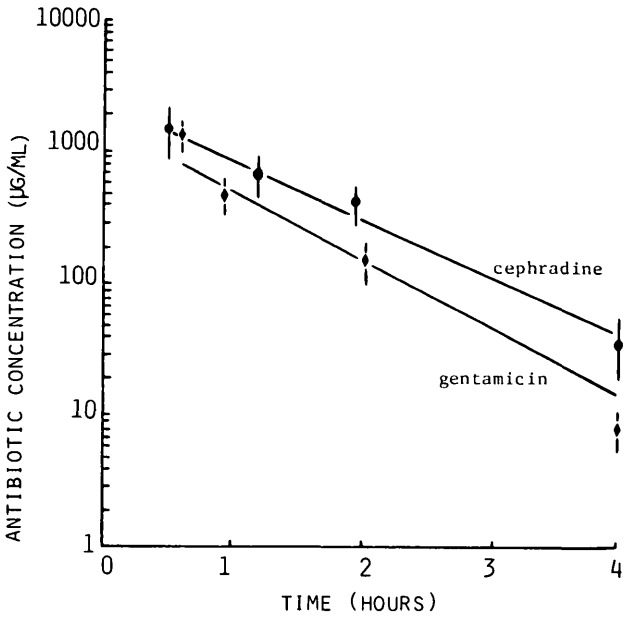

Fig. 3. Serum concentration of antibiotic in rats following one intravenous injection (40 mg/kg). Each point is the mean ± SD of eight measurements.

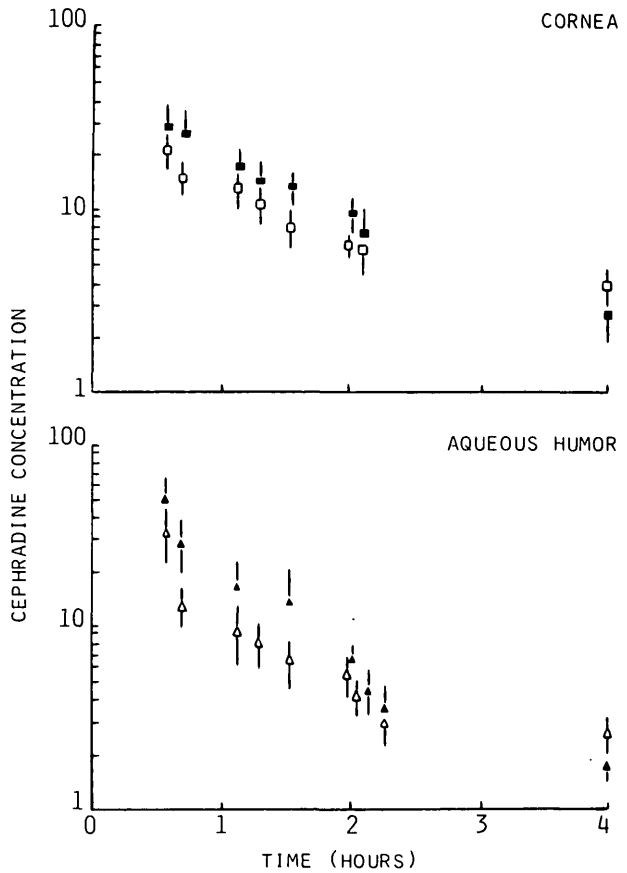

Fig. 4. Concentration of cephradine in the cornea (in μg/g) and aqueous humor (in μg/ml) of rat eyes following one intravenous injection (40 mg/kg). Open symbols represent normal eyes and shaded symbols represent eyes with *S. aureus* keratitis. Each point is the mean ± SD of 4 to 12 measurements.

Table 2. Pharmacokinetic data for antibiotic penetration into the rat eye following intravenous administration.

	Ba(mg-min/ml)	t_{max}*(min)*	r'_{ap} *(%)*	$k_o(hr^{-1})$	$k_i \times 10^{-2}\ (hr^{-1})$
Cephradine					
Normal Eye:					
cornea	0.57	—	—	—	—
aqueous humor	0.47	32	2.5	0.8	1.9
Inflamed Eye:*					
cornea	1.04	—	—	—	—
aqueous humor	0.91	32	3.9	0.9	3.3
Gentamicin sulfate					
Normal Eye:					
cornea	0.16	—	—	—	—
aqueous humor	0.47	36	3.8 (4[a], 7[b])	0.4	1.3
Inflamed Eye:†					
cornea	0.84	—	—	—	—
aqueous humor	0.52	38	3.4	0.4	1.2

Ba, bioavailability; t_{max}, time after drug application at which the concentration in aqueous humor is maximum; r'_{ap}, ratio of concentration in aqueous humor to total plasma concentration at t_{max}; k_o, coefficient of loss from aqueous humor to blood; k_i, coefficient of entry into the anterior chamber from blood.

* *S. aureus* keratitis.

† *P. aeruginosa* keratitis.

[a] Value for humans derived by D. Maurice and S. Mishima from Utermann *et al.*[42]

[b] Value for rabbits derived from Golden and Coppel[44] and Kuming and Tonkin.[45]

an intact epithelium as a barrier to penetration of hydrophilic drugs has been demonstrated.[31] From histology, it was also seen that neither bacterial species spread to the anterior chamber within 28 hours of inoculation, Descemet's membrane remaining an effective barrier.

Both antibiotics penetrated well into rat aqueous humor when applied topically. Indeed, the Ba of gentamicin in aqueous humor of both normal and inflamed eyes was higher than in the cornea. This unusual result may be due to a very low level of gentamicin binding to stromal tissue, giving small g_{ca} and r_{ca} values. It is known that the fraction of gentamicin bound to protein in rat serum is low compared to that of cephradine (5% versus 25%).[32,33]

A high concentration of antibiotic in an infected cornea, eg 83 μg/ml of gentamicin obtained 30 minutes after topical application to a *Pseudomonas*-infected eye, may not be to the host's advantage. As the minimum bactericidal concentration (MBC) of gentamicin for most *P. aeruginosa* strains is 4 μg/ml, and assuming an antibiotic is as effective in the cornea as in vitro, there is a surplus of drug in the cornea at this time that may interfere with wound healing.[34,35] A peak antibiotic concentration that is many times above the MBC of the infecting organism is an advantage over a peak concentration just above the MBC only if it prolongs the time that the concentration remains above the MBC.[36] However, it is clearly preferable to have too much antibiotic in the cornea rather than too little.

When applied topically, cephradine diffused through an infected eye more quickly than through a normal

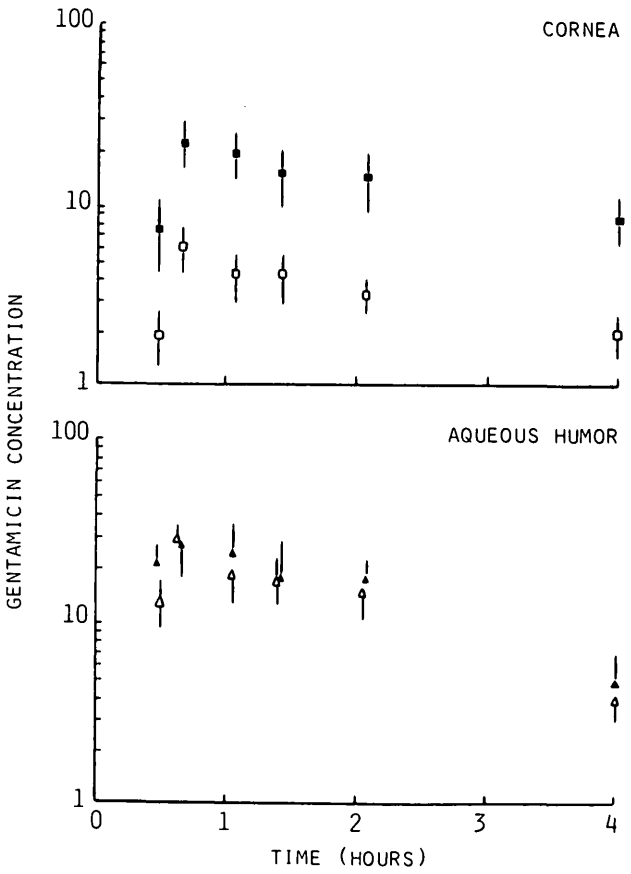

Fig. 5. Concentration of gentamicin sulfate in the cornea (in μg/g) and aqueous humor (in μg/ml) of rat eyes following one intravenous injection (40 mg/kg). Open symbols represent normal eyes and shaded symbols represent eyes with *P. aeruginosa* keratitis. Each point is the mean ± SD of 4 to 12 measurements.

eye. This effect depended on the antibiotic used and not on the type of infection, for in a further experiment in this laboratory, in which cephradine was placed on to a *Pseudomonas*-infected eye, a similar, though more pronounced, phenomenon was observed. Since antibiotic penetration studies with humans have nearly all been performed on uninflamed eyes, drugs such as cephradine may have to be applied more frequently than previously believed to maintain effective concentrations in the clinical situation.

The effect of inflammation on antibiotic penetration into rat corneas from the iv route followed the trend of the topical dosing studies; *P. aeruginosa* infection increased gentamicin concentrations markedly compared to normal corneas, while *S. aureus* infection had a smaller effect on cephradine concentrations. However, the *Pseudomonas* infection had no observed effect on penetration of gentamicin from blood to aqueous humor. This surprising result requires further experimentation, but might be related to the reluctance of this hydrophilic drug to pass the blood-aqueous barrier into a chamber with a high pus content. The result cannot be explained by binding of gentamicin to accumulated leukocytes, since the radioimmunoassay reagent mixture contained surfactant, nor can it be explained by binding to uveal melanin in these nonpigmented rats.

There are both advantages and disadvantages in using rats rather than rabbits or guinea pigs in studies of this nature. Rats are cheap, easy to handle, and blink about 3.7 times per minute in the laboratory (personal observation). Blinking rate is a major factor in penetration of topically applied drugs,[37] and the rat rate is nearer the human rate than that of rabbits (four per hour) or guinea pigs, which do not seem to blink at all.

The antibiotic concentrations achieved in the rat eye from a single topical dose were high compared to what might be expected from previous studies with rabbits[19,38–40] and humans.[19,41] However, in a current project we have measured levels averaging 20 μg/g in uninflamed corneas from patients given one drop of 1% gentamicin in hydroxypropyl methylcellulose 1 hour before keratoplasty. Inflamed corneas have approximately double this concentration. Perhaps the levels reported in the present study seem high because we used 5% cephradine and 1% (instead of 0.3%) gentamicin in a viscous vehicle. The assay technique or the pharmacokinetic characteristics of the rat eye itself may also play a role. The permeability of the rat corneal epithelium for gentamicin, 6.4×10^{-4} cm/hr, is nearer the reported human value of 10^{-5} cm/hr than is that of the rabbit (10^{-3} cm/hr). Conversely, the rate of elimination of gentamicin from aqueous humor, A, in the uninflamed rabbit eye was 0.2 hr^{-1}, much nearer the reported human value of 0.15 hr^{-1} than in the rat (0.67 hr^{-1}). The estimate of r'_{ap} for this drug in the rat, 3.8%, compares with the human value of 4%, while r'_{ap} in the rabbit is 7%. These data suggest that the rat is a useful alternative laboratory animal to the rabbit in therapeutic studies of ocular disease, although a good deal of work remains to be done to further substantiate this claim.

Key words: rat, anterior segment, *Staphylococcus aureus, Pseudomonas aeruginosa,* antibiotics

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References

1. Jain MR and Bansal VD: Gentamicin penetration in human aqueous after subconjunctival injection. Indian J Ophthalmol 29:307, 1981.
2. Gorden TB and Cunningham RD: Tobramycin levels in aqueous humor after subconjunctival injection in humans. Am J Ophthalmol 93:107, 1982.
3. Eiferman RA and Stagner JI: Intraocular penetration of amikacin. Arch Ophthalmol 100:1817, 1982.
4. Wingfield DL, McDougal RL, Roy FH, and Hannah C: Ocular penetration of amikacin following intramuscular injection. Arch Ophthalmol 101:117, 1983.
5. Woo FL, Johnson AP, Caldwell DR, Lertora JJL, and George WJ: Piperacillin levels in human tears and aqueous humor. Am J Ophthalmol 98:17, 1984.
6. Kulshrestha OP, Kurian, and Keshwan HH: Penetration of doxycycline in aqueous humor (After oral administration in humans). Indian J Ophthalmol 29:251, 1981.
7. Axelrod JL and Kochman RS: Cefoxitin levels in human aqueous humor. Am J Ophthalmol 90:388, 1980.
8. Axelrod JL and Kochman RS: Cefaclor levels in human aqueous humor. Arch Ophthalmol 98:740, 1980.
9. Axelrod JL and Kochman RS: Cephradine levels in human aqueous humor. Arch Ophthalmol 99:2034, 1981.
10. Axelrod JL and Kochman RS: Cefoperazone concentrations in human aqueous humor after intravenous administration. Am J Ophthalmol 94:103, 1982.
11. Uwaydah MM, Jabbour N, Fahd S, Jaafar M, and Faris BM: Penetration of intravenous moxalactam into human aqueous humor. Arch Ophthalmol 100:329, 1982.
12. Axelrod JL and Kochman RS: Moxalactam concentration in human aqueous humor after intravenous administration. Arch Ophthalmol 100:1334, 1982.
13. Axelrod JL and Kochman RS: Cefonicid concentrations in human aqueous humor. Arch Ophthalmol 102:433, 1984.
14. Axelrod JL, Kochman RS, Horowitz MA, and Youngworth L: Ceftazidime concentrations in human aqueous humor. Arch Ophthalmol 102:923, 1984.
15. Rubinstein E, Avni I, Tuizer H, Treister G, and Blumenthal M: Cefsulodin levels in the human aqueous humor. Arch Ophthalmol 103:426, 1985.
16. Poirier RH, Kingham JD, de Miranda P, and Annel M: Intraocular antiviral penetration. Arch Ophthalmol 100:1965, 1982.
17. Hung SO, Patterson A, and Rees PJ: Pharmacokinetics of oral acyclovir (Zovirax) in the eye. Br J Ophthalmol 68:192, 1984.

18. Kanski JJ, Young JH, and Vogel R: Penetration of intravenously administered cefoxitin into the aqueous humor of inflamed human eyes. Am J Ophthalmol 94:516, 1982.
19. Ellerhorst B, Golden B, and Jarudi N: Ocular penetration of topically applied gentamicin. Arch Ophthalmol 93:371, 1975.
20. Sutphin JE, Wilhelmus KR, Jones DB, and Osato MS: A quantitative assay of filamentous fungal replication in the cornea. ARVO Abstracts. Invest Ophthalmol Vis Sci 25:(Suppl)187, 1984.
21. Badenoch PR, Hay GJ, McDonald PJ, and Coster DJ: A rat model of bacterial keratitis: Effects of antibiotics and corticosteroid. Arch Ophthalmol 103:718, 1985.
22. Osato M, Wade C, and Wilhelmus K: Use of sodium clavulanate in experimental penicillinase-producing staphylococcal keratitis. ARVO Abstracts. Invest Ophthalmol Vis Sci 25:(Suppl)188, 1984.
23. Williams KA and Coster DJ: Penetrating corneal transplantation in the inbred rat: A new model. Invest Ophthalmol Vis Sci 26: 23, 1985.
24. Badenoch PR, Finlay-Jones JJ, and Coster DJ: Enzymatic disaggregation of the infected rat cornea. Invest Ophthalmol Vis Sci 24:253, 1983.
25. Maurice DM and Mishima S: Ocular pharmacokinetics. *In* Handbook of Experimental Pharmacology; Volume 69: Pharmacology of the Eye, Sears ML, editor. Berlin, Springer-Verlag, 1984, pp. 19–116.
26. Nagataki S and Mishima S: Pharmacokinetics of instilled drugs in the human eye. *In* International Ophthalmology Clinics. Clinical Pharmacology of the Anterior Segment, Holly FJ, editor. Boston, Little, Brown and Co., 1980, pp. 33–49.
27. Mishima S: Clinical pharmacokinetics of the eye. Invest Ophthalmol Vis Sci 21:504, 1981.
28. Smolen VF: Bioavailability and pharmacokinetic analysis of drug responding systems. Ann Rev Pharmacol Toxicol 18:495, 1978.
29. Gibaldi M and Perrier D: *In* Drugs and the Pharmaceutical Sciences; Volume 1: Pharmacokinetics, Swarbrick J, editor. New York, Dekker, 1975, pp. 129–174.
30. Jones DB: A plan for antimicrobial therapy in bacterial keratitis. Trans Am Acad Ophthalmol Otolaryngol 79:95, 1975.
31. Kleinberg J, Dea FJ, Anderson JA, and Leopold IH: Intraocular penetration of topically applied lincomycin hydrochloride in rabbits. Arch Ophthalmol 97:933, 1979.
32. Trottier S, Bergeron MG, and Gauvreau L: Intrarenal concentration of netilmicin and gentamicin. *In* Current Chemotherapy: Proceeding of the 10th International Congress of Chemotherapy, Siegenthaler W and Luthy R, editors. Washington DC, American Society for Microbiology, 1977, pp. 953–955.
33. Meinardi G, Monti G, Grasso S, Tamassia V, and De Carneri I: Penetration of cephalosporins into extravascular fluids: Role of protein binding and elimination rate. *In* Current Chemotherapy: Proceedings of the 10th International Congress of Chemotherapy, Siegenthaler W and Luthy R, editors. Washington DC, American Society for Microbiology, 1977, pp. 379–381.
34. Stern GA, Schemmer GB, Farber RD, and Gorovoy MS: Effect of topical antibiotic solutions on corneal epithelial wound healing. Arch Ophthalmol 101:644, 1983.
35. Petroutsos G, Guimaraes R, Giraud J, and Pouliquen Y: Antibiotics and corneal epithelial wound healing. Arch Ophthalmol 101:1775, 1983.
36. Eagle H, Fleischman R, and Musselman AD: Effect of schedule of administration of the therapeutic efficacy of penicillin. Importance of the aggregate time penicillin remains at effectively bactericidal levels. Am J Med 9:280, 1950.
37. Maurice DM: Structures and fluids involved in the penetration of topically applied drugs. *In* International Ophthalmology Clinics. Clinical Pharmacology of the Anterior Segment, Holly FJ, editor. Boston, Little, Brown and Co., 1980, pp. 7–20.
38. Furgiuele FP: Ocular penetration and tolerance of gentamicin. Am J Ophthalmol 64:421, 1967.
39. Sloan SH, Pettit TH, and Litwack KD: Gentamycin penetration in the aqueous humor of eyes with corneal ulcers. Am J Ophthalmol 73:750, 1972.
40. Baum JL, Barza M, Shushan D, and Weinstein L: Concentration of gentamicin in experimental corneal ulcers. Arch Ophthalmol 92:315, 1974.
41. Hillman JS, Jacobs SI, Garnett AJ, and Kheskani MB: Gentamicin penetration and decay in the human aqueous. Br J Ophthalmol 62:794, 1979.
42. Utermann D, Matz K, and Meyer K: Gentamicinspiegel im Kammerwasser des Menschennach parenteraler, subkonjunktivaler und lokaler Applikation. Klin Monatsbl Augenheilkd 171: 579, 1977.
43. Bloomfield SE, Miyata T, Dunn MW, Bueser N, Stenzel KH, and Rubin AL: Soluble gentamicin ophthalmic inserts as a drug delivery system. Arch Ophthalmol 96:885, 1978.
44. Golden B and Coppel SP: Ocular tissue absorption of gentamicin. Arch Ophthalmol 84:792, 1970.
45. Kuming BS and Tonkin M: Use of gentamicin sulphate in ophthalmology. I. Absorption of gentamicin into the rabbit aqueous. Br J Ophthalmol 58:609, 1974.