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 derivatives7-19 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 microbiologic20-22 and immunologic research also necessitates study of ocular drug penetration in this animal.

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 X 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

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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 serum and normal and in-
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 and Gibaldi & Perrier. The apparent absorption coefficient of aqueous humor, B, is given by:

\[ B = \frac{m_0k_c}{BaV_pA} \]  

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 \( t_{ep} \), the ratio of the concentration in aqueous humor, \( C_a \), to total plasma concentration, \( C_p \), when \( C_p \) is maximum at time \( t_{max} \). The calculation of A as above permits the evaluation of \( k_0 \) following IV injection:

\[ k_0 = \frac{A}{1 - r_{ap}C_p/C_a} \]  

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:

\[ k_i = k_0r_{ap} \]

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. 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-
contraction 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 drug flow occurred through the anterior chamber when corneal infection was present.

As shown in Figure 2, infection of the cornea with \( P. \text{aeruginosa} \) 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 \( k_c \)). However, the effect on rate of drug uptake (\( B \) and \( k_c \)) could not be determined algebraically due to the low value of \( g_{c,a} \) 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 \( t_{\text{max}} \) is taken at 32 minutes, the presence of infection lifts \( ra_p \) from approximately 2.5% to 3.9%. The table also shows that the rates of drug flow into (\( k_i \)) and out of (\( k_o \)) 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 \( P. \text{aeruginosa} \) 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. \( Ba \), \( ra_p \), and the rate constants were similar for aqueous humor and infected eyes. Human and rabbit values for \( ra_p \) in uninfamed eyes are shown in Table 1.

**Discussion**

Inoculation of the rat cornea with \( S. \text{aureus} \) or \( P. \text{aeruginosa} \), the two most common bacterial species isolated from keratitis patients in temperate climates, resulted in infections with symptoms and severity resembling those found in human cases. Twenty-four
hours after inoculation, the rat corneas have the appearance of established corneal infections in human patients; *S. aureus* induced a localized 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
Table 2. Pharmacokinetic data for antibiotic penetration into the rat eye following intravenous administration.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Normal Eye</th>
<th>Inflamed Eye</th>
<th>Normal Eye</th>
<th>Inflamed Eye</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>cornea</td>
<td>aqueous humor</td>
<td>cornea</td>
<td>aqueous humor</td>
</tr>
<tr>
<td>Cephradine</td>
<td>0.57</td>
<td>0.47</td>
<td>1.04</td>
<td>0.91</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.16</td>
<td>0.47</td>
<td>0.84</td>
<td>0.84</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Ba (mg-min/ml)</th>
<th>( t_{max} ) (min)</th>
<th>( r_a ) (%)</th>
<th>( k_a ) (hr(^{-1}))</th>
<th>( k_i \times 10^{-2} ) (hr(^{-1}))</th>
</tr>
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<tbody>
<tr>
<td>Cephradine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Eye</td>
<td>0.57</td>
<td>0.47</td>
<td>1.04</td>
<td>0.91</td>
</tr>
<tr>
<td>cornea</td>
<td></td>
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</tr>
<tr>
<td>aqueous humor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflamed Eye*</td>
<td>1.04</td>
<td>0.91</td>
<td>3.9</td>
<td>0.9</td>
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<tr>
<td>normal eye</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>aqueous humor</td>
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</tr>
<tr>
<td>Gentamicin</td>
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<td>0.47</td>
<td>3.8</td>
<td>0.4</td>
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<tr>
<td>sulfate</td>
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<tr>
<td>Normal Eye</td>
<td>0.16</td>
<td>0.47</td>
<td>3.8</td>
<td>0.4</td>
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<tr>
<td>cornea</td>
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<td>aqueous humor</td>
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<td>Inflamed Eye†</td>
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<td>0.52</td>
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<tr>
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\( Ba \), bioavailability; \( t_{max} \), time after drug application at which the concentration in aqueous humor is maximum; \( r_a \), ratio of concentration in aqueous humor to total plasma concentration at \( t_{max} \); \( k_a \), coefficient of loss from aqueous humor to blood; \( k_i \), coefficient of entry into the anterior chamber from blood.

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

† P. aeruginosa keratitis.

A high concentration of antibiotic in an infected cornea, eg 83 \( \mu g/ml \) 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 \( \mu g/ml \), 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 \( \mu g/g \)) and aqueous humor (in \( \mu g/ml \)) 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, 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 and humans. 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 10⁻³ cm/hr than is that of the rabbit (10⁻⁵ 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 r₃p for this drug in the rat, 3.8%, compares with the human value of 4%, while r₃p 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


