Tolerability, Kinetics, and Efficacy of Subconjunctival Pefloxacin in Pigmented Rabbits
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Pefloxacin has been shown to have good intraocular penetration when given systemically. In order to extend its clinical use, we have assessed the tolerability, kinetics, and efficacy of subconjunctival pefloxacin in phakic pigmented rabbits. The tolerability of a single subconjunctival injection of pefloxacin (0.8, 1.6, 8, or 16 mg in 0.2 ml) in the right eyes of eight rabbits was evaluated by clinical and histopathological examination. The 0.8-mg dose of pefloxacin was well tolerated. The kinetics was evaluated after a single subconjunctival injection of 0.8 mg in 18 rabbits. Animals were sacrificed at 1, 3, 5, 7, 12, or 18 h postinjection. Drug concentrations were measured by high-performance liquid chromatography. Pefloxacin was found in the cornea (maximum concentration, 18.13 µg/ml; half-life, 3.92 h) and in the aqueous humor (maximum concentration, 3.40 µg/ml; half-life, 2.14 h). Pefloxacin did not penetrate into the vitreous humor by this route. The efficacy was evaluated in an experimental model of staphylococcal corneal ulcers in eight rabbits which received two subconjunctival injections of 0.8 mg of pefloxacin at 16 and 24 h after intrastromal inoculation. The results (expressed as mean log_{10} CFU per milliliter ± standard deviation) showed that pefloxacin significantly (P < 0.001) reduced the bacterial counts (4.39 ± 0.97) compared with those in control eyes (6.46 ± 0.69). For phakic eyes, subconjunctival pefloxacin might be of value for the treatment of corneal ulcers. Further studies are required to determine its penetration into the vitreous humor of aphakic eyes.

MATERIALS AND METHODS

We used 34 healthy pigmented (Fauve de Bourgogne) female rabbits, 10 to 15 weeks old and weighing 2 to 3 kg. They were housed in individual cages with a natural light-dark cycle, in accordance with French legislation on animal experimentation. The toxicity, kinetics, and efficacy studies involved 8, 18, and 8 animals, respectively.

Before subconjunctival injection, the rabbits were anesthetized by intramuscular injection of ketamine (15 mg/kg) and by local instillations of 0.4% oxyprocaine hydrochloride. The required pefloxacin concentrations were obtained by serial dilution, in sterile normal saline, of a commercially available preparation for intravenous use (5-ml vials containing 400 mg of pefloxacin mesilate [Peflacin; Laboratoires Roger-Bellon, Neuilly-sur-Seine, France]). The injection was performed with a 23-gauge needle mounted on a tuberculin syringe. The needle was introduced under the conjunctiva in the superior quadrant, 2 to 3 mm posterior to the corneoscleral limbus, inducing a subconjunctival bleb. The volumes of all subconjunctival injections were 0.2 ml.

Toxicity. Single subconjunctival injections of pefloxacin at concentrations of 0.8, 1.6, 8, and 16 mg/0.2 ml were made in the right eyes of eight phakic rabbits (two eyes for each concentration). These doses were chosen according to the results of a preliminary experiment with a very large range of doses and according to the systemic dose compared with those for other antibiotics. The left eyes served as controls and were injected with 0.2 ml of sterile normal saline.

Daily clinical examinations of both the anterior and posterior segments were carried out for 7 days. The fundus was observed by means of indirect ophthalmoscopy after pupillary dilatation with topical tropicamide, and photographs were taken. The rabbits were killed on day 7 by injection of 2 ml of sodium pentobarbital into the marginal vein of the ear. The eyes were immediately enucleated and placed in 10% phosphate buffered formalin for 24 h. After overnight fixation, the eyes were dehydrated in a graded alcohol series, embedded in paraffin, and cut with a microtome. Sections were stained with hematoxylin, eosin, and safran and examined under a light microscope by an observer who was unaware of the treatments.

Kinetics. Eighteen phakic pigmented rabbits received a single subconjunctival injection of 0.8 mg of pefloxacin in each eye. The animals were killed 1, 3, 5, 7, 12, or 18 h postinjection with 2 ml of intravenous sodium pentobarbital, with six eyes harvested at each time point.

Blood samples were obtained prior to killing by direct intracardiac puncture. Aqueous and vitreous humors were immediately aspirated by puncture with a 23-gauge needle mounted on a tuberculin syringe. The eyes were removed and cleansed of conjunctival tissue and blood to avoid contamination. Samples were immediately stored at −80°C to minimize antibiotic diffusion until assay (1).

Pefloxacin was measured by means of high-performance liquid chromatography with UV detection at 280 nm (12), using a technique described elsewhere (5, 6). Individual tissues were obtained by dissection of the frozen eyes, weighed, and homogenized with an Ultra-Turrax mixer (IKA, Staufen, Germany) in 3 ml of 0.05 M sodium phosphate-citrate buffer (pH 5.8) containing the internal standard. After centrifugation, the supernatant was removed, and one-step liquid-liquid extraction was performed with chloroform. After agitation and centrifugation, the lower organic phase was evaporated to dryness; the dry residue was diluted in 0.1 ml of the mobile phase (pH 4.8), and an aliquot was injected into the chromatograph by a Wisp 710B automatic injector (Waters). Serum standards (with pefloxacin concentrations ranging from 1 to 10 or from 0.02 to 1 mg/liter, depending on the expected concentrations in the tissues) were used routinely in the measurement of pefloxacin concentrations in ocular tissues and humors. A linear regression analysis of the results confirmed the linearity of the method over the range of concentrations used (0.02 to 10 mg/liter; r > 0.995). The detection limit was 5 ng of pefloxacin. The within-run and between-day coefficients of variation were 2.8 and 4.6%, respectively, for the high-level pefloxacin control (2,000 ng) and 6.8 and 7.6%, respectively, for the low-level control (50 ng).

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Blood contamination in the vascularized tissues was evaluated by means of a hemoglobin microassay. Contamination was minor (1.9, 3.5, and 0.6% in the iris, chorioretina, and sclera, respectively), and its contribution to the antibiotic concentration was considered negligible.

Results obtained for six pooled eyes at the same kinetic time were expressed in milligrams per liter, assuming tissues specific gravities of 1. Areas under the concentration-time curves were calculated for the experimental period (0 to 18 h) by using the trapezoidal rule. Half-lives were calculated with nonlinear APIS pharmacokinetics software (8). The best fit for concentration-versus-time data was obtained with maximum-likelihood estimates by using a one-compartment model. When the slope did not differ from zero, the half-life could not be evaluated.

In vitro antibiotic susceptibility tests. The *Staphylococcus aureus* strain used for the experimental keratitis was isolated from a human ocular infection. The MIC and MBC of pefloxacin for the *S. aureus* strain were determined in Mueller-Hinton infusion broth (Diagnostic Pasteur, Paris, France) by the tube dilution method (13). Each tube contained twofold dilutions of antibiotic and a final bacterial density of 10^7 CFU/ml. The tubes were incubated for 18 h at 37°C. The MIC was defined as the lowest concentration of antibiotic that prevented turbidity in the test tube after incubation. The MBC was defined as the lowest concentration of antibiotic that killed at least 99.9% of the organisms after incubation, as determined by plating 0.1 ml from each clear broth tube onto Trypticase soy agar and incubating the plates for 18 h at 37°C.

Efficacy. *S. aureus* was grown overnight at 37°C on Trypticase soy agar. One colony was selected and seeded into 10 ml of Trypticase soy broth. The broth was then incubated for 18 h at 37°C. Bacteria were harvested by centrifugation, washed twice, and suspended in sterile normal saline. A suspension with a density of 10^7 CFU/ml was prepared by serial dilution and spectrophotometric estimation. The density was checked by plating 0.1 ml of each dilution on Trypticase soy agar.

Eight phakic pigmented rabbits were anesthetized by intramuscular injection of ketamine (15 mg/kg) and by local instillation of 0.4% oxyprocaine hydrochloride. Bilateral keratitis was induced by intrastromal injection of 10^5 CFU of *S. aureus* in 0.01 ml, via a 30-gauge needle attached to a 50-µl syringe (Hamilton Co., Reno, Nev.). At 16 and 24 h after corneal inoculation, each animal received a subconjunctival injection of 0.8 mg of pefloxacin in the right eye. The left eyes were injected with 0.2 ml of sterile normal saline and served as controls. Sixteen hours after the last injection, the rabbits were killed with 2 ml of intravenous sodium pentobarbital. The eyes were enucleated and rinsed with sterile normal saline. Corneas were removed aseptically by excision at the corneoscleral limbus and cut into small pieces. These were then ground with an Ultra-Turrax mixer in 3 ml of sterile normal saline. The supernatants were serially diluted in sterile normal saline, and 0.1 ml of each dilution was plated on Trypticase soy agar. Colonies were counted after 18 h of incubation at 37°C. The results were expressed as log_{10} CFU per milliliter, and means were compared by using Student’s paired t test. *P* values of 0.05 or less were considered significant.

**RESULTS**

**Toxicity.** The results of the toxicity studies are summarized in Table 1.

(i) **Clinical examination.** All of the eyes were clinically normal prior to the subconjunctival injection.

One hour after the injection, the eyes treated with the high dose (16 mg) of pefloxacin exhibited hemorrhagic chemosis and corneal ulceration. White crystalline precipitates were attached to the surrounding conjunctiva and cornea (Fig. 1). The eyes injected with 8 and 1.6 mg of pefloxacin showed hemorrhagic chemosis and punctuate hemorrhaging, respectively. The eyes injected with 0.8 mg exhibited minor punctuate hemorrhaging at the site of injection (Fig. 2).

On day 7, the eyes treated with 16 mg showed residual subconjunctival hemorrhages, but crystalline precipitates and chemosis were no longer present. In the eyes treated with 8 mg, only mild subconjunctival hemorrhages were noted. The eyes injected with 1.6 and 0.8 mg of pefloxacin were normal.

**FIG. 1.** Rabbit eye at 1 h after a subconjunctival injection of 16 mg of pefloxacin, showing hemorrhagic chemosis, white crystalline precipitates, and corneal ulceration.

**FIG. 2.** Rabbit eye at 1 h after a subconjunctival injection of 0.8 mg of pefloxacin, showing minor punctiform hemorrhaging at the site of injection.
No clinical toxicity for the lens, vitreous humor, or retina was noted at any dose.

All of the control eyes remained normal throughout the 7 days, except for two eyes with punctiform subconjunctival hemorrhaging at the site of injection after 1 h, which cleared rapidly.

(ii) Histopathologic studies. On day 7, full light microscopic examinations of both treated and control eyes disclosed no histopathologic abnormalities. All of the ocular tissues remained normal. No cataract formation was observed.

Kinetics. The results of the kinetics studies are summarized in Table 2.

After a single subconjunctival injection of 0.8 mg, pefloxacin showed good penetration into the anterior segment of normal phakic eyes, especially in the cornea, where the maximum concentration was 18.13 mg/liter. Levels in corneas remained above the MIC at which 90% of S. aureus isolates are inhibited for 18 h (Fig. 3). In the aqueous humor, pefloxacin penetration was lower than it was in the cornea, with a maximum concentration of 3.40 mg/liter.

Pefloxacin levels in the vitreous humor were near the detection limit after subconjunctival injection. Penetration into the lens was poor, with a maximum concentration of 0.17 mg/liter, which decreased very slowly.

Maximum concentrations in the pigmented tissues were high (97.84 and 87.96 mg/liter in the iris and chorioretina, respectively). The half-lives in these pigmented tissues were very long (14.09 and 20.90 h, respectively).

Subconjunctival injection of 0.8 mg of pefloxacin did not lead to appreciable levels in the serum: the concentration was 0.18 mg/liter at 1 h, 0.02 mg/liter at 5 h, and below the detection limit thereafter.

Efficacy. The results of the efficacy studies are summarized in Table 3.

The MIC for the test strain of S. aureus was 0.5 mg/liter, and the MBC was 1 mg/liter.

Two subconjunctival injections of 0.8 mg of pefloxacin significantly ($P < 0.001$) reduced the mean bacterial counts (4.39 ± 0.97) relative to those in control eyes (6.46 ± 0.69).

**DISCUSSION**

Pefloxacin is a new fluoroquinolone antibiotic with a broad antibacterial spectrum and good penetration into tissues (7), including the cerebrospinal fluid (18) and brain (9). Previous studies have shown good penetration of pefloxacin into the aqueous humor when given systemically to patients undergoing cataract extraction (15). These findings have been confirmed for rabbits receiving a single intramuscular injection of 50 mg of pefloxacin per kg (5); pefloxacin penetrated into all ocular tissues and humors, with penetration ratios in the aqueous and vitreous humors of 0.24 and 0.45, respectively. This ability to

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**TABLE 2. Kinetics of pefloxacin in rabbit eyes after a single subconjunctival injection of 0.8 mg**

<table>
<thead>
<tr>
<th>Tissue or fluid</th>
<th>AUC (mg L⁻¹ h⁻¹)</th>
<th>$t_{1/2}$ (h)</th>
<th>$C_{max}$ (mg/liter)</th>
<th>$T_{max}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>0.40 ± 0.05</td>
<td>1.19 ± 0.08</td>
<td>0.18 ± 0.2</td>
<td>1</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>7.53 ± 2.08</td>
<td>2.14 ± 0.22</td>
<td>3.40 ± 0.62</td>
<td>1</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>—</td>
<td>—</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>Cornea</td>
<td>53 ± 10.38</td>
<td>3.92 ± 0.46</td>
<td>18.13 ± 5.49</td>
<td>1</td>
</tr>
<tr>
<td>Lens</td>
<td>1.84 ± 0.44</td>
<td>—</td>
<td>0.17 ± 0.07</td>
<td>3</td>
</tr>
<tr>
<td>Iris</td>
<td>1.027 ± 340</td>
<td>14.09 ± 4.99</td>
<td>97.84 ± 47.39</td>
<td>3</td>
</tr>
<tr>
<td>Chorioretina</td>
<td>1.185 ± 243</td>
<td>20.90 ± 7.81</td>
<td>87.96 ± 54.71</td>
<td>1</td>
</tr>
<tr>
<td>Sclera</td>
<td>214 ± 46</td>
<td>9.28 ± 1.83</td>
<td>31.49 ± 12.15</td>
<td>1</td>
</tr>
</tbody>
</table>

$^a$ AUC, area under the concentration-time curve; $t_{1/2}$, half-life; $C_{max}$, maximum concentration; $T_{max}$, time to maximum concentration; —, half-life too long to be measured during the course of the experiment; ND, not detectable.

$^b$ Expressed as mean ± standard deviation.

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**FIG. 3.** Concentrations of pefloxacin in the corneas and aqueous (Aq) humors of pigmented rabbits after a single subconjunctival injection of 0.8 mg. MIC<sub>90</sub>, MIC at which 90% of the isolates are inhibited.
cross the blood-ocular barriers could be related to the low molecular weight and high lipophilicity of pefloxacin. In order to extend the therapeutic applications of pefloxacin in eye infections, we investigated the tolerability, kinetics, and efficacy of this antibiotic when it is given by the subconjunctival route. Although subconjunctival injections are relatively traumatic, they are still used for long-term drug delivery when the hourly instillation of fortified drops is not possible. Subconjunctival injections also produce much higher concentrations in the cornea, which could be of clinical value in induction therapy or for relatively refractory corneal ulcers (3).

The first step in validating this route was to determine the well-tolerated dose, which varies according to the physicochemical properties of a drug. For example, a subconjunctival injection of 100 mg of cefazidime induces only mild local irritation at the site of injection in rabbits (16), whereas injection of 100 mg of fusidic acid induces severe conjunctival necrosis and corneal edema (17). The well-tolerated dose of subconjunctival pefloxacin was 0.8 mg on the basis of clinical and histopathological criteria. The slight hemorrhaging at the site of injection noted on day 1 in animals treated with either 0.8 mg of pefloxacin or normal saline was due to perforation of the conjunctiva by the needle and resolved rapidly and spontaneously. The crystalline precipitates of pefloxacin observed on day 1 in the eyes treated with 16 mg disappeared spontaneously, a phenomenon previously described with topical ciprofloxacin (14). We considered that 0.8 mg was a well-tolerated dose with a wide safety margin, since no histopathological abnormalities of ocular tissues were observed with any of the doses (0.8 to 16 mg) tested in eight rabbits.

For the kinetics study, we used pooled data from a population of rabbits to determine the complete distribution of the drug in every eye structure. We thus could not obtain serial samples from individual rabbits, since this approach allows the determination of kinetics in the tumors but not in the tissues (11).

The kinetics of subconjunctival pefloxacin was evaluated with the well-tolerated dose of 0.8 mg. High concentrations of pefloxacin were reached in the cornea and, although to a lesser extent, in the aqueous humor. The half-life in the cornea was relatively long (3.92 h). The half-life of pefloxacin in the aqueous humor was shorter than that in the cornea, because of the turnover of the aqueous humor. It is noteworthy that levels of pefloxacin in the anterior segment were higher after subconjunctival injection of 0.8 mg than after intramuscular injection of 50 mg/kg (5).

Pefloxacin was undetectable in the vitreous humor after sub-

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Colony count (log_{10} CFU/ml) in:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (left) eye</td>
<td>Treated (right) eye</td>
</tr>
<tr>
<td>1</td>
<td>7.12</td>
<td>5.38</td>
</tr>
<tr>
<td>2</td>
<td>7.18</td>
<td>5.35</td>
</tr>
<tr>
<td>3</td>
<td>6.77</td>
<td>4.78</td>
</tr>
<tr>
<td>4</td>
<td>6.84</td>
<td>4.86</td>
</tr>
<tr>
<td>5</td>
<td>6.39</td>
<td>4.75</td>
</tr>
<tr>
<td>6</td>
<td>6.24</td>
<td>4.03</td>
</tr>
<tr>
<td>7</td>
<td>6.11</td>
<td>3.18</td>
</tr>
<tr>
<td>8</td>
<td>5.05</td>
<td>2.78</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.46 ± 0.69</td>
<td>4.39 ± 0.97</td>
</tr>
</tbody>
</table>

The efficacy of subconjunctival pefloxacin was assessed for *S. aureus* keratitis, one of the most common types of bacterial keratitis. In our experimental model, two subconjunctival injections of 0.8 mg of pefloxacin significantly reduced corneal bacterial counts. This was expected, since the concentration of pefloxacin in the cornea remained above the MIC for *S. aureus* for 18 h.

In human keratitis, local administration of antibiotics is often sufficient to treat bacterial infections. However, in severe cases with the risk of endophthalmitis, it seems that systemic administration is useful, provided that it induces high levels in the aqueous humor. Therefore, in these cases, the combination of subconjunctival and systemic pefloxacin could be more powerful than subconjunctival treatment alone.

Given its broad antibacterial spectrum, favorable kinetics, and good tolerability, subconjunctival pefloxacin could be useful in the treatment of severe corneal ulcers due to susceptible pathogens in noncompliant patients or as induction therapy.

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**REFERENCES**


