Clearance of Intravitreal Moxifloxacin

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PURPOSE. To study the clearance of moxifloxacin after intravitreal injection in rabbits.

METHODS. Intravitreal injections of 200 μg/0.1 mL of moxifloxacin were administered to rabbits. Four eyes per time point after injection (1 hour and 6, 12, 24, and 36 hours) and three eyes at 48 hours, respectively, were enucleated and immediately frozen and stored at −80°C. Ocular dissection and isolation of frozen vitreous was performed. Vitreous samples were acquired at the various time intervals after injection. Antibiotic assays were performed with high performance liquid chromatography.

RESULTS. The concentration of intravitreal moxifloxacin showed an exponential decay with a half-life of 1.72 hours. The mean vitreous concentration was 120.49 ± 49.25 μg/mL 1 hour after injection, and declined to 20.25 ± 5.85 μg/mL at 6 hours and 1.06 ± 0.81 μg/mL at 12 hours, respectively.

CONCLUSIONS. The vitreous concentrations achieved were several orders of magnitude greater than the MIC90 of organisms commonly involved in bacterial endophthalmitis, and therapeutic levels were maintained at 12 hours in uninflected, phakic rabbit eyes. The pharmacokinetic data suggest that intravitreal moxifloxacin may have a role in the treatment of bacterial endophthalmitis. (Invest Ophthalmol Vis Sci. 2006;47:317–319) DOI:10.1167/ iovs.05-1124

Intravitreal antibiotics are a mainstay of treatment for bacterial endophthalmitis. The antibiotics most frequently used include vancomycin for Gram-positive coverage and cefazolin, amikacin, or dimeth or amikacin for Gram-negative coverage.1,2 The most common organisms encountered in bacterial endophthalmitis are Gram-positive isolates, including coagulase-negative cocci, Staphylococcus aureus, Streptococcus species, and Enterococcus species.3 Gram-negative isolates, including Proteus mirabilis, Pseudomonas species, Enterobacter species, and Haemophilus influenzae account for 6% to 20% of all endophthalmitis cases.4,5 Moxifloxacin is a fourth-generation fluoroquinolone with broad-spectrum coverage that encompasses organisms commonly encountered in bacterial endophthalmitis.6–9 Intravitreal administration of fourth-generation fluoroquinolones may have a role in the management of endophthalmitis. Histopathologic and electroretinographic studies conducted in our laboratory have demonstrated the retinal safety of intravitreal injections of 200 μg moxifloxacin in rabbits (Gao H, et al. IOVS 2005;46:ARVO E-Abstract 5553). Khan et al. (IOVS 2005;46:ARVO E-Abstract 5574) found no signs of retinal toxicity by indirect ophthalmoscopy, light microscopy, or electroretinography in rabbit eyes after intravitreal injections of 160 μg moxifloxacin. The purpose of this study was to determine the clearance of moxifloxacin after intravitreal injection, and thereby the clinical relevance of intravitreal moxifloxacin in the management of bacterial endophthalmitis.

MATERIALS AND METHODS

Moxifloxacin (Avelox; Bayer Pharmaceuticals Corp., West Haven, CT) was obtained in pure powder form and reconstituted with sterile water to obtain a concentration of 200 μg/0.1 mL. Thirteen Dutch belted rabbits weighing 2 to 2.5 kg were used in the study. The experiments were conducted in accordance with ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the protocol was approved by the Institutional Review Board of the Baylor College of Medicine (Houston, TX).

The rabbits were anesthetized with an intramuscular injection of 0.5 mL/kg body weight of a mixture containing 42.8 mg/mL ketamine, 8.6 mg/mL xylazine and 1.4 mg/mL acepromazine. Mydriasis was achieved with 1 drop of tropicamide 1% and phenylephrine 2.5%. An anterior chamber paracentesis was performed followed by injection of 0.1 mL of 200 μg/0.1 mL moxifloxacin into the vitreous cavity of both eyes of 12 rabbits using a 27-gauge needle at a site 2 mm from the limbus superiorly. A cotton tip applicator was applied to the injection site immediately after removal of the needle, to prevent vitreous reflux from the injection site. The rabbits were examined with indirect ophthalmoscopy before and immediately after injections and at the time of the death of the animals. Two rabbits per time interval after injection (1 hour and 6, 12, 24, 36, and 48 hours) were killed with a lethal cardiac injection of pentobarbital sodium and phenytoin sodium (Beuthanasia-D; Schering Animal Health, Kenilworth, NJ). Anterior chamber samples were obtained before enucleation of eyes. Four eyes per time interval up to 36 hours and three eyes at 48 hours after injections were enucleated and immediately frozen in liquid nitrogen and placed at −80°C. Ocular dissection and isolation of the entire frozen vitreous was performed according to the technique described by Abel and Boyle.10 Antibiotic assays were performed with high-performance liquid chromatography (HPLC). An additional control rabbit was killed, and the vitreous was isolated as just described, to obtain standardization curves for HPLC analyses.

HPLC Analysis

HPLC analysis of the samples was performed in a masked fashion by the HPLC operator. The rabbit vitreous samples and moxifloxacin standard (150 μL) were each mixed with 600 μL of 100% acetonitrile and vortexed for 1 minute at room temperature. The extract was centrifuged in an ultracentrifuge (TL-100; Beckman Coulter, Inc, Fullerton, CA) at 45,000 rpm for 30 minutes at 4°C. The supernatant was transferred to a clean tube and dried within a centrifugal vacuum system. The samples for injection were redissolved with 200 μL of 20% acetonitrile containing 0.1% trifluoroacetic acid (TFA) and vortexed for...
1 minute. Insoluble particles were removed by ultracentrifugation at 45,000 rpm for 30 minutes at 4°C.

The samples of aqueous humor (30 μL) were extracted with 150 μL of 100% acetonitrile. After drying, the samples were redissolved in 40 μL of 20% acetonitrile containing 0.1% TFA.

The samples were analyzed with a dual-pump gradient HPLC system (Waters Chromatography Division, Milford, MA) and a system of 0.1% TFA (buffer A) versus 0.1% TFA in acetonitrile (buffer B) with a flow rate of 1.0 mL/min. A 25-μL volume of each sample was injected onto a C18 column (4.6 mm inner diameter × 250 mm; VyDac, Hesperia, CA), prequillibrated with 20% buffer B, and washed with 5 mL of 20% buffer B. Moxifloxacin was eluted with a linear gradient of acetonitrile (20%-50% containing 0.1% TFA). Moxifloxacin was monitored by the absorbance at 293 nm using a detector (Shimadzu, Kyoto, Japan) interfaced to a computer (32 Karat software; Beckman Coulter).

The area of the moxifloxacin peak after baseline subtraction was calculated and compared with the area versus mass curve for the standard, to quantify the amounts of moxifloxacin in the samples. To verify that the 293-nm absorbance at the correct elution position for moxifloxacin was due to authentic moxifloxacin, fluorescence spectra were measured with a spectrophotometer (SLM-8000; Olis, Inc., Bogart, GA) with upgraded electronics and software. All the vitreous samples were analyzed in duplicate. The standard curve was linear to more than 2 μg (correlation coefficient = 0.99958 for the range 0.0625–2 μg), and the detection limit using these methods was estimated to be approximately 6.5 ng (signal-to-noise ratio >2).

**RESULTS**

Indirect ophthalmoscopy of the rabbit eyes immediately after injection and before enucleation revealed no retinal whitening, hemorrhages, or detachment in any eye after intravitreal injection of 200 μg/0.1 mL moxifloxacin. The moxifloxacin concentrations in the vitreous of uninfamed, phakic rabbit eyes at the various time points after intravitreal injection are shown in Table 1. The vitreous concentration was noted to decline rapidly with time. The mean vitreous concentration was 120.49 ± 49.23 μg/mL 1 hour after injection and declined to 20.23 ± 5.85 μg/mL at 6 hours and 1.06 ± 0.81 μg/mL at 12 hours, respectively. An exponential decay model was used to fit the data and a least-squares regression analysis was performed. The vitreous moxifloxacin concentration showed an exponential decay with a half-life of 1.72 hours. The mean aqueous concentrations were much lower and showed a rapid decline from 10.14 μg/mL at 1 hour after intravitreal injection to undetectable levels by 12 hours after injection.

**DISCUSSION**

Endophthalmitis is a serious complication of intraocular surgeries and penetrating ocular trauma. In the Endophthalmitis Vitrectomy Study (EVS), only 89.5% of Gram-negative isolates were sensitive to amikacin or ceftazidime. Although the Gram-positive isolates in the EVS were susceptible to vancomycin, emerging resistance to vancomycin is of concern. A recent study of preoperative normally encountered conjunctival bacteria revealed that the surface bacteria were susceptible to the fourth-generation fluoroquinolones, with the exception of 2% of the coagulase-negative staphylococci. These concerns, along with the need for an antibiotic with better Gram-negative coverage, led us to seek a possible alternative intravitreal antibiotic regimen for the management of endophthalmitis. The in vitro minimum inhibitory concentration to inhibit 90% of organisms (MIC90) of moxifloxacin for organisms commonly encountered in postoperative, posttraumatic, and bleb-associated endophthalmitis is in the range of 0.25 to 2.5 μg/mL, making it a promising candidate for treating ocular infections.

Clinical endophthalmitis may affect the elimination half-life of a drug, depending on whether the drug is eliminated via an anterior route by passage into the aqueous or a posterior route by active transport across the retina. Zwitterions such as ciprofloxacin and other fluoroquinolones are eliminated by the posterior route and have shorter elimination half-lives than do cationic compounds and drugs such as vancomycin and gentamicin, which are primarily cleared by passive transport via the anterior chamber and aqueous humor. In the inflamed and infected eye, the mechanism of active transport across the retina is compromised, and drugs such as cefazolin that are cleared via the posterior route have an increased half-life in the vitreous in phakic, nonvitrectomized eyes. However, this increase in half-life with inflammation was not noted in aphakic, vitrectomized rabbit eyes, presumably because of greater clearance via the anterior route in aphakic eyes and decreased drug retention by the vitreous gel in vitrectomized eyes.

Given the low aqueous concentrations achieved, our data suggest that moxifloxacin, like other fluoroquinolones, is eliminated primarily via a posterior route.

Furthermore, drug elimination has been noted to be more rapid in rabbits and rats than in humans. Elimination of moxifloxacin from the serum has been reported to have a half-life of 1 to 2 hours in rabbits versus 12 to 15 hours in humans. Similarly, moxifloxacin elimination was reported to be more rapid in rats and to have parallel concentration-time courses in plasma and lung tissue, with half-lives of approximately 1.5 hours. Our study yielded a vitreous elimination rate in the range of the previously reported serum elimination rate in rabbits. Further studies are needed to determine whether the vitreous elimination rate in humans parallels the serum elimination rate, in which case a prolonged vitreous elimination half-life would be expected in humans.

The injected dose of 200 μg/0.1 mL in rabbit eye results in a vitreous concentration of approximately 150 μg/mL. The peak vitreous levels achieved were thus approximately 75 to greater than 1000 times the MIC90 of moxifloxacin to organisms commonly encountered in bacterial endophthalmitis (range, 0.06–2.0). A significant reduction in colony counts of Staphylococcus epidermidis in the vitreous was recently shown in a rabbit endophthalmitis model 3 days after intravitreal injection of 50 μg moxifloxacin. An in vitro time-kill study of moxifloxacin at four times the MIC90 concentrations showed a reduction of 3 log10 colony-forming units/mL in methicillin-resistant Staphylococcus aureus at 4 hours, penicillin-sensitive Streptococcus pneumoniae at 1.5 hours, penicillin-resistant S. pneumoniae at 3 hours, Streptococcus pyogenes at 10 hours, and Enterococcus faecalis at 7 hours, respectively. A significant reduction in bacterial colony counts is thus expected with our tested dose, which was several orders of magnitude greater than the MIC90 of organisms encountered in endophthalmitis.

**Table 1. Vitreous and Aqueous Concentrations of Moxifloxacin at Different Time Intervals after Intravitreal Injection of 200 μg/0.1 mL in Rabbits**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Aqueous Concentration</th>
<th>Vitreous Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.14 ± 7.06 (2)</td>
<td>120.49 ± 49.23 (4)</td>
</tr>
<tr>
<td>6</td>
<td>0.98 ± 1.73 (4)</td>
<td>20.23 ± 5.85 (4)</td>
</tr>
<tr>
<td>12</td>
<td>0.00 ± 0.00 (2)</td>
<td>1.06 ± 0.81 (4)</td>
</tr>
<tr>
<td>24</td>
<td>0.00 ± 0.00 (5)</td>
<td>0.30 ± 0.46 (4)</td>
</tr>
<tr>
<td>36</td>
<td>0.00 ± 0.00 (4)</td>
<td>0.18 ± 0.36 (4)</td>
</tr>
<tr>
<td>48</td>
<td>0.00 ± 0.00 (4)</td>
<td>0.00 ± 0.00 (3)</td>
</tr>
</tbody>
</table>

Data are expressed as the mean concentration in micrograms per milliliter (μg/mL).
In summary, the clearance of moxifloxacin after intravitreal injection was determined to have a half-life of 1.72 hours, the vitreous concentrations achieved were one to several orders of magnitude greater than the MIC90 of most organisms involved in endophthalmitis, and therapeutic levels were maintained at 12 hours in uninflamed, phakic rabbit eyes. Based on retinal safety data (Gao H, et al. IOVS 2005;46:ARVO E-Abstract 5553; Kahn PNS, et al. IOVS 2005;46:ARVO E-Abstract 5574) and the pharmacokinetic data in this study, intravitreal moxifloxacin at a dose of 400 µg in humans, which will result in peak vitreous concentration of 100 µg/mL, may have a role in the treatment of bacterial endophthalmitis.

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References