INTRAVITREAL CLEARANCE OF MOXIFLOXACIN

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ABSTRACT

Purpose: To study the clearance of a single dose of intravitreally injected moxifloxacin in rabbits.

Methods: Intravitreal injections of 200 µg/0.1 mL of moxifloxacin were performed in rabbits. Four eyes per time interval after injection (1, 6, 12, 24, 36 hours) and three eyes at 48 hours were enucleated, immediately frozen, and placed at –80ºC. Ocular dissection and isolation of frozen vitreous were performed. Antibiotic assays were performed with use of 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 ± 49.23 µg/mL 1 hour after injection, which declined to 20.23 ± 5.85 µg/mL at 6 hours and 1.06 ± 0.81 µg/mL at 12 hours. The aqueous levels of moxifloxacin showed an exponential decay from 10 µg/mL at 1 hour after intravitreal injection to undetectable levels by 12 hours after injection.

Conclusions: Moxifloxacin clearance from the vitreous is rapid and consistent with previous clearance studies of ciprofloxacin. Given that the injected dose corresponds to several times the minimum inhibitory concentration at which 90% of isolates are inhibited (MIC_{90}) of organisms commonly involved in endophthalmitis, and that therapeutic levels are present up to 12 hours after injection, intravitreal moxifloxacin may have a role in the treatment of endophthalmitis.

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INTRODUCTION

Intravitreal antibiotics are a mainstay of treatment for bacterial endophthalmitis. The antibiotics most frequently used include vancomycin for gram-positive coverage and ceftazidime 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*, streptococcal spp , and enterococcal spp.[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 endophthalmitis management. Histopathologic and electroretinographic studies conducted in our laboratory have shown that intravitreal injections of 0.1 mL of 200 µg/0.1 mL moxifloxacin are not toxic to the retina in rabbits (unpublished data). The purpose of this study was to determine the clearance of a single dose of intravitreally injected moxifloxacin, and thereby the clinical relevance of intravitreal moxifloxacin in the management of bacterial endophthalmitis.

METHODS

Moxifloxacin (Avelox®, Bayer Pharmaceuticals Corporation, West Haven, Connecticut) was obtained in 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 this study. The experiments were conducted in accordance with Association for Research in Vision and Ophthalmology principles of animal maintenance, and the protocol was approved by the Institutional Review Board of Baylor College of Medicine, Houston, Texas.

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 one drop of tropicamide 1% and phenylephrine 2.5%. An anterior chamber paracentesis was performed, and 0.1 mL of 200 µg/0.1 mL moxifloxacin was injected into the vitreous cavity of both eyes of 12 rabbits with 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 sacrificing the animals. Two rabbits per time interval after injection (1 hour, 6 hours, 12 hours, 24 hours, 36 hours, and 48 hours) were sacrificed by using a lethal cardiac injection of pentobarbital sodium and phenytoin sodium (Beuthanasia-D, Schering Animal Health, Kenilworth, New Jersey). Anterior chamber samples were obtained prior to enucleation of eyes. Four eyes per time interval up to 36 hours and three eyes at 48 hours following injections were enucleated and immediately frozen in liquid nitrogen and placed at –80ºC.

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Bold type indicates AOS member.

Ocular dissection and isolation of the entire frozen vitreous were performed by using the technique described by Abel and Boyle.[10] Antibiotic assays were performed with use of high-performance liquid chromatography (HPLC). An additional control rabbit was sacrificed and the vitreous isolated as described above for performing standardization curves for HPLC analyses.

HPLC ANALYSIS

High-performance liquid chromatography analysis of the samples was carried out 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 a TL-100 ultracentrifuge (Beckman Coulter Inc, Fullerton, California) 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 in TL-100 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 by using a Waters dual-pump gradient HPLC system 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/minute. A 25-µL volume of each sample was injected onto a C18 column (VyDac, 4.6 mm ID × 250 mm) preequilibrated with 20% buffer B and washed with 5 mL of 20% buffer B; moxifloxacin was eluted with linear gradient of acetonitrile (20% to 50% containing 0.1% TFA). Moxifloxacin was monitored by the absorbance at 293 nm by using a Shimadzu detector interfaced to a computer running Beckman 32 Karat software. 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 by using an SLM-4800 spectrofluorimeter with upgraded electronics and software. All of the vitreous samples were analyzed in duplicate. The standard curve was linear to above 2 µg (correlation coefficient = 0.99958 for the range 0.0625 to 2 µg), and the detection limit using these methods was estimated to be approximately 6.5 ng (signal-to-noise ratio greater than 2).

RESULTS

Indirect ophthalmoscopy of the rabbit eyes immediately after injections and prior to enucleation revealed no retinal whitening, hemorrhages, or detachment in any eye following intravitreal injection of 200 µg/0.1 mL moxifloxacin. The moxifloxacin concentrations in the vitreous of uninflamed, phakic rabbit eyes at the various time intervals following intravitreal injection are shown in Table 1.

TABLE 1. VITREOUS CONCENTRATIONS OF MOXIFLOXACIN AT DIFFERENT TIME INTERVALS FOLLOWING INTRAVITREAL INJECTION OF 200 µg/0.1 ML OF MOXIFLOXACIN IN RABBITS

TIME (HR)	NO. OF SAMPLES	VITREOUS CONCENTRATION, MEAN ± SD (µg/ML)
1	4	120.49 ± 49.23
6	4	20.23 ± 5.85
12	4	1.06 ± 0.81
24	4	0.30 ± 0.46
36	4	0.18 ± 0.36
48	3	0.00 ± 0.00

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. 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 (Figure 1).

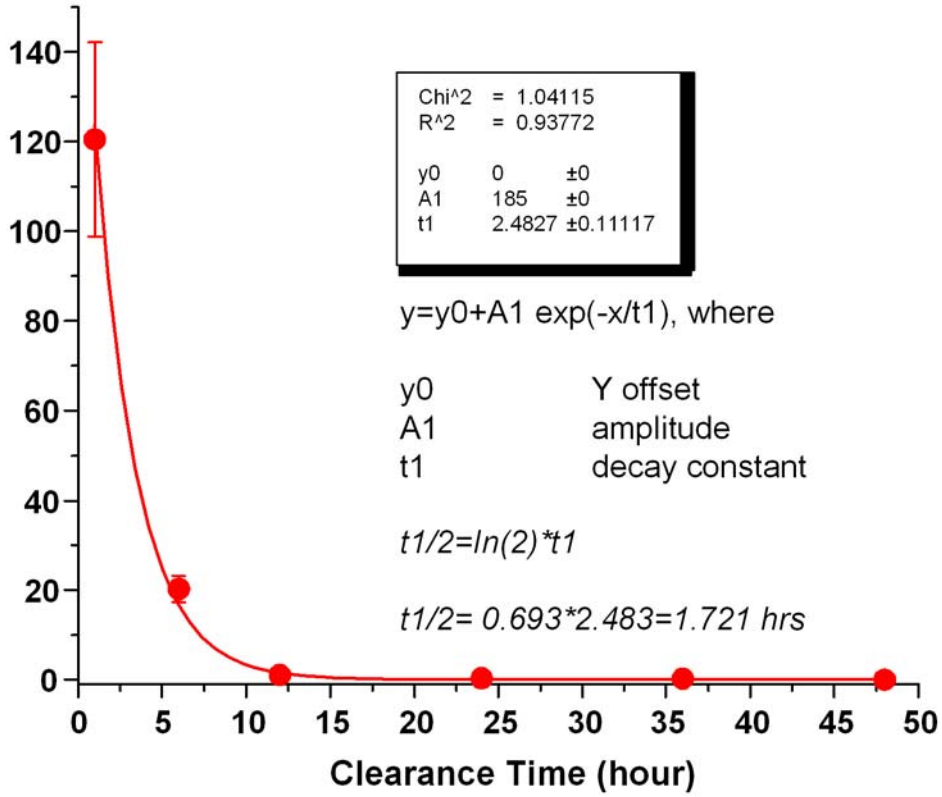

FIGURE 1

Moxifloxacin concentrations in the vitreous following intravitreal injection of 200 μg/0.1 mL of moxifloxacin.

The aqueous levels of moxifloxacin also showed an exponential decay from 10 μg/mL at 1 hour after intravitreal injection to undetectable levels by 12 hours after injection (Figure 2).

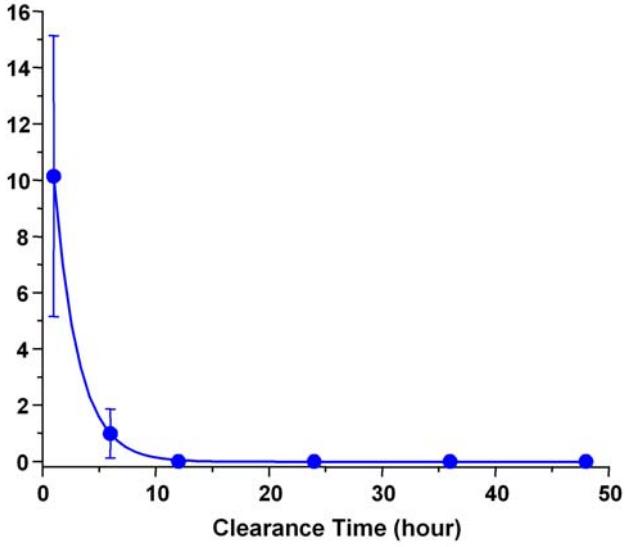

FIGURE 2

Moxifloxacin concentrations in the aqueous following intravitreal injection of 200 μg/0.1 mL moxifloxacin.

TABLE 2. IN VITRO SUSCEPTIBILITIES OF MOXIFLOXACIN SHOWING MINIMUM INHIBITORY CONCENTRATION AT WHICH 90% OF ISOLATES ARE INHIBITED (MIC_{90}) FOR SELECTED ORGANISMS

ORGANISM	MIC_{90} (µg/ML)*
Gram-positive	
Staphylococcus epidermidis	0.13 to 2.0
Staphylococcus aureus (MSSA)	0.06 to 0.12
Staphylococcus aureus (MRSA)	0.12 to 2.0
Streptococcus pneumoniae	0.06 to 0.25
Streptococcus pyogenes	0.25
Streptococcus viridans	0.73†
Bacillus cereus	0.13
Enterococcus faecalis	1.0
Gram-negative	
Proteus mirabilis	0.025
Pseudomonas aeroginosa	0.50 to 8.0
Haemophilus influenzae	0.03 to 0.06
Enterobacter species	0.06
Escherichia coli	0.06 to 1.0
Klebsiella pneumoniae	0.12 to 0.25
Neisseria gonorrhoeae	0.015
Anaerobic	
Bacteriodes fragilis	0.125 to 2.0
Clostridium species	0.50 to 1.0
Propionibacterium acnes	0.032 to 0.25

MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-sensitive *Staphylococcus aureus*.
*Data from references 8, 9, and 25.|
†Data from Callegan MC et al.[9]

DISCUSSION

Endophthalmitis is a serious complication of intraocular surgeries and penetrating ocular trauma. The organisms in postoperative endophthalmitis are usually gram-positive cocci and less commonly gram-negative organisms.[3-5] In the Endophthalmitis Vitrectomy Study (EVS),[3] only 89.5% of gram-negative isolates were sensitive to amikacin or ceftazidime. Whereas the gram-positive isolates in the EVS were susceptible to vancomycin, emerging resistance to vancomycin is of concern.[11-13] 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.[14] 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.

Moxifloxacin is a fourth-generation 8-methoxyfluoroquinolone that acts by inhibiting bacterial topoisomerase II (DNA gyrase) and topoisomerase IV. The fourth-generation fluoroquinolones require two genetic mutations for resistance to develop and are promising candidates for treating ocular infections.[6] In addition, the in vitro minimum inhibitory concentration to inhibit 90% of organisms (MIC_{90}) of moxifloxacin to organisms commonly encountered in postoperative, post-traumatic, and bleb-associated endophthalmitis is low (Table 2).

Topical administration of moxifloxacin in humans has been reported to achieve aqueous concentrations that are greater than the MIC90 of the organisms commonly encountered in endophthalmitis but does not achieve therapeutic levels in the vitreous.[15] Vitreous penetration of orally administered gatifloxacin and moxifloxacin in humans has been studied in two separate reports; oral administration of two doses, 12 hours apart prior to elective pars plana vitrectomy surgery, was shown to achieve vitreous concentrations of 1.23 ± 0.28 µg/mL[16] and 1.34 + 0.66 µg/mL, respectively.[17] These concentrations provide excellent coverage

against the majority of organisms encountered in the postoperative, post-traumatic, bleb-related, and delayed-onset settings of infection.[3,16,17] Following intravenous administration of 5 mg/kg and 20 mg/kg moxifloxacin in infected rabbits, peak vitreous concentrations achieved have been reported to be 0.68 ± 028 µg/mL and 2.50 ± 0.67 µg/mL, respectively.[18]

Intravitreal antibiotics have remained the mainstay of treatment of postoperative endophthalmitis. Compared with other routes of antibiotic delivery, such as topical, subconjunctival, and systemic administration, intravitreal antibiotic injection achieves immediate therapeutic vitreous concentrations, provides a localized treatment for a localized infection, and avoids potential systemic side effects.

Studies of the safety and clearance of intravitreal moxifloxacin were undertaken to assess the clinical efficacy of such treatment. The tested dose of intravitreal moxifloxacin was noted to be safe in rabbit eyes on the basis of ophthalmoscopic, histopathologic, and electroretinographic studies in our laboratory, and results will be published separately. The intraocular clearance of intravitreal moxifloxacin in uninflamed, phakic eyes was rapid and consistent with a previous report of the clearance of ciprofloxacin.[19] Pearson and associates[19] found the half-life of intravitreal ciprofloxacin to be 2.2 hours in uninflamed, phakic rabbit eyes and 1.2 hours in uninflamed, aphakic, vitrectomized rabbit eyes. Ozturk and coworkers[20] found the elimination half-life of intravitreal ciprofloxacin in uninflamed, phakic rabbit eyes to be 6.02 hours, and a prolonged half-life of 15.06 hours in infected, traumatized rabbit eyes.

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 compared with cationic compounds and drugs such as vancomycin and gentamicin, which are primarily cleared by passive transport via the anterior chamber and aqueous humor.[21] In the inflamed and infected eye, the mechanism of active transport across the retina is compromised, and drugs such as cefazolin[21] 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.[22] Our data suggest that moxifloxacin, like other fluoroquinolones and unlike vancomycin and gentamicin, is eliminated primarily via a posterior route. In our study, the half-life of intravitreal moxifloxacin was 1.72 hours in uninflamed, phakic rabbit eyes. Intravitreal clearance of moxifloxacin would be expected to be more rapid in vitrectomized, aphakic rabbit eyes and less rapid in infected, inflamed eyes, according to similar trends in such eyes with other antibiotics.

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.[23] 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.[24] 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 if 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 measured vitreous volume of the rabbit eyes was approximately 1.06 ± 0.09 mL; thus the injected dose of 200 µg/0.1 mL results in a vitreous concentration of approximately 189 µg/mL. The peak vitreous levels achieved were thus approximately 75 to greater than 1,000 times the MIC_{90} of moxifloxacin to organisms commonly encountered in bacterial endophthalmitis (Table 2). 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 of moxifloxacin.[25] An in vitro time-kill study of moxifloxacin at four times the MIC_{90} concentrations showed a reduction of three $\log_{10}$ colony-forming units/mL in methicillin-resistant *S 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.[26] A significant reduction in bacterial colony counts is thus expected with our tested dose, which was several orders of magnitude greater than the MIC_{90} of organisms encountered in endophthalmitis.

Two methods of use of intravitreal moxifloxacin in endophthalmitis management can be envisioned: (1) as a single agent, and (2) in combination with vancomycin to achieve double coverage for gram-positive organisms and coverage for gram-negative organisms. Intravitreal moxifloxacin with its low MIC_{90} and coverage of organisms commonly encountered in bacterial endophthalmitis appears promising as a single agent in the management of endophthalmitis, although existing or emerging resistance to one agent is always a concern.[14] In addition, sustained vitreous levels may be potentially maintained by combining the intravitreal treatment with an oral fourth-generation fluoroquinolone. Current treatment regimens employ intravitreal vancomycin and either ceftazidime or amikacin. Amikacin is typically reserved for patients with allergy to cephalosporins because of concerns of retinal toxicity with aminoglycosides. Moxifloxacin offers an expanded gram-positive and gram-negative coverage when compared to ceftazidime and a favorable safety profile, and is thus a potential alternative to ceftazidime or amikacin. Further studies in animal endophthalmitis models are required to assess the efficacy and safety of combining moxifloxacin with vancomycin.

In this study, the intravitreal clearance of moxifloxacin was noted to be rapid but expected to be prolonged in inflamed rabbit eyes and in human eyes; the vitreous concentrations achieved were several orders of magnitude greater than the MIC_{90} of most organisms involved in bacterial endophthalmitis; and therapeutic levels were maintained at 12 hours in uninflamed, phakic rabbit eyes. Given that time-kill is concentration-dependent, an intravitreal injection of 200 µg/0.1 mL of moxifloxacin in rabbit eyes would be expected to sterilize an infection despite its rapid clearance. Additional animal studies in infected, aphakic, and vitrectomized eyes may help determine the clinical efficacy of the antibiotic in terms of its application as a single agent or in combination with other antibiotics.

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PEER DISCUSSION

DR M. GILBERT GRAND. Dr Mieler and colleagues have elegantly demonstrated that an intravitreal injection of moxifloxacin 200 mcg/0.1 ml into phakic, non-inflamed, non-infected, non-vitrectomized rabbit eyes resulted in vitreous concentrations of moxifloxacin

that, over at least six hours, greatly exceed the MIC_{90} of organisms that most commonly cause endophthalmitis. Dr Mieler reports that in his unpublished data this concentration of moxifloxacin is non-toxic to the rabbit retina. Because of the potential of vancomycin resistance among gram-positive organisms and less than complete coverage achieved by the combination of vancomycin and ceftazidime for the range of gram-negative organisms associated with endophthalmitis as reported in the Endophthalmitis Vitrectomy Study, the authors suggest the need to develop alternative antibiotic regimens for the treatment of endophthalmitis. They postulate that because of advantages in the spectrum of organisms covered, moxifloxacin is a potential alternative to ceftazidime or amikacin for the treatment of gram-negative endophthalmitis. The authors conclude that because of this wide spectrum of coverage for both gram-positive and gram-negative organisms, as well as a low MIC_{90}, intravitreal moxifloxacin appears promising as either a single agent or in combination with vancomycin in the management of endophthalmitis.

Before we embrace this concept, a number of issues should be evaluated. While it is true that there is reason for concern regarding potential emergent vancomycin resistance, at least at the time of the Endophthalmitis Vitrectomy Study, all gram-positive isolates from eyes with acute bacterial endophthalmitis were sensitive to vancomycin. In a study of preoperative conjunctival flora, de Kaspar found 124 strains of coagulase-negative Staphylococci. Of these, 100% were sensitive to vancomycin; 98% were sensitive to moxifloxacin; and 2% were resistant to moxifloxacin or gatifloxacin.[1] Recent data presented by Harper and Flynn (Harper T, ARVO 2005, Abstract) indicate that of 35 isolates of coagulase-negative Staphylococcus obtained from 1993 to 2004, 100% were sensitive to vancomycin. In contrast, however, only 76.5% of these isolates were sensitive to moxifloxacin. Since coagulase-negative Staphylococcus remains the principal organism responsible for acute postoperative bacterial endophthalmitis, these data suggest that the proposed replacement of vancomycin with moxifloxacin would result in potential failure of therapy and, therefore, the use of moxifloxacin as a single agent in the treatment of endophthalmitis would appear to be unwise.

The authors correctly address the issue of gram-negative coverage for patients with endophthalmitis. In the Endophthalmitis Vitrectomy Study, approximately 10% of isolates of gram-negative organisms were resistant to ceftazidime.[2] While moxifloxacin may have an expanded range of coverage compared to ceftazidime, in order to provide simultaneous coverage for gram-positive organisms it would necessitate being used in combination with vancomycin. While this is a plausible consideration, its implementation should be delayed to allow for further evaluation of the safety profile of this combination.

The authors discuss the potential advantage of fourth generation fluoroquinolones such as moxifloxacin since, because of their structure, two independent mutations are required for an organism to develop resistance. It should be remembered that virtually every newly introduced antibiotic has eventually been limited in its effectiveness by the development of strains of resistant organisms. It is of concern that Miller and colleagues (Miller D, ARVO 2005, Abstract) have recently documented emerging resistance of common ocular isolates of Streptococci and Pseudomonas aeruginosa to moxifloxacin.

In the current study, the intravitreal clearance of moxifloxacin was in fact quite rapid. The data reported were obtained from phakic, non-inflamed and non-infected, non-vitrectomized rabbit eyes. In the management of human endophthalmitis, eyes may be phakic or pseudophakic but are virtually universally inflamed and infected. Furthermore, current clinical management often involves vitrectomy in combination with antibiotics, as opposed to injection of antibiotics alone. Whether the injection of moxifloxacin into inflamed, infected, pseudophakic, vitrectomized eyes would result in delayed or more rapid clearance of moxifloxacin is a complex issue that is yet to be addressed or resolved. Since time kill does depend on concentration of antibiotic, studies of vitreous concentration and clearance under these varied circumstances will be of critical importance.

The authors are to be commended for this very important contribution to our literature. Clearly, further studies of vitreous concentrations and clearance in primate models involving eyes that are phakic or pseudophakic, infected, and with or without concomitant vitrectomy will provide critically important additional data. Finally, additional information is required regarding the safety profile of intravitreally-injected moxifloxacin or the combination of moxifloxacin with vancomycin.

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DR DAVID J. WILSON. The antibiogram at our institution indicates that moxifloxacin is effective against 60-70% of coagulation negative staphylococcus organisms. Is there a difference in the way those sensitivities are reported since they do not usually take into account the MIC_{90}? They just report resistant or sensitive.

DR TRAVIS A. MEREDITH. Studies need to be performed that demonstrate that the given antibiotic actually cures an infection. In the infectious disease literature, there is little data to indicate how long bacteria have to be exposed to an antibiotic *in vivo* for most of the diseases that are treated. Therefore most treatments are empirical. The next step is to test this in an experimental infection to determine the effectiveness. A paper by Davey and colleagues (Davey PG, Barza M, Stuart M. Dose response of experimental Pseudomonas endophthalmitis to ciprofloxacin, gentamicin, and imipenem: evidence of resistance to "late" treatment of infections. *J Infect Dis* 1987; 155: 518-523) about 15 years ago showed that pseudomonas infections that had been established in the eye for 48 hours or longer were virtually resistant to almost every antibiotic that was put into the eye, regardless of what the sensitivities were. The next step here is to determine in an *in vivo* animal model whether it really works.

DR WILLIAM F. MIELER. Addressing Dr Gil Grand's comments, we certainly share the concern of utilizing moxifloxacin as a single agent. With respect to the concerns of both Drs David Wilson and Travis Meredith regarding resistance, we would most likely recommend employing this antibiotic in combination with another antibiotic, most likely vancomycin. Of course there also are potentially adverse reactions related to these antibiotics inside the eye and we do not have combination data at the present time to answer these concerns.

The resistance question is a difficult one to fully address. A manuscript recently published by Mino de Kaspar and colleagues looking at pre-operative conjunctival cultures showed only a 2% resistance of coagulase-negative staphylococci to the fourth generation fluoroquinolones (Mino de Kaspar H, Koss MJ, He L, et al. Antibiotic susceptibility of preoperative normal conjunctival bacteria. *Am J Ophthalmol* 2005:123:39-44). Dr Harry Flynn's unpublished data presented at the 2005 annual ARVO meeting, indicated that among 35 isolates, only 3/4ths of coagulase-negative Staphylococcal organisms were sensitive to the fourth generation fluoroquinolones. Why there is such a discrepancy between these two studies is not known.

Concerning Dr David Wilson's comments regarding how drug resistance is reported, MIC_{90} levels versus just sensitive or resistant, I do not have the answer for that.

Certainly more work is needed to ensure that moxifloxacin is a safe and effective combination therapy before it's employed intravitreally on a regular basis in humans.