Intravitreal Moxifloxacin: Retinal Safety Study with Electroretinography and Histopathology in Animal Models

Hua Gao,1 Mark E. Pennesi,2 Xiaoxi Qiao,1 Moban N. Iyer,2 Samuel M. Wu,2 Eric R. Holz,2 and William F. Mieler5

PURPOSE. To determine whether moxifloxacin can be used safely as an intravitreal antibiotic, retinal safety of intravitreal moxifloxacin was studied with electroretinography (ERG) and histopathology in animal models.

METHODS. Moxifloxacin was injected into mouse eyes at intravitreal concentrations of 5 to 500 μg/mL and into rabbit eyes at 150 μg/mL. As the control, the vehicle was injected into the fellow eyes of each animal. Four weeks after injection, ERG recordings were performed, and animal eyes were processed for histologic examination.

RESULTS. ERG studies showed no significant difference between control and moxifloxacin-injected eyes at any dose in either the mouse or rabbit model. Histologic examination revealed no retinal abnormality in mice at 5 to 100 μg/mL or in rabbits at 150 μg/mL intravitreal moxifloxacin. In mice at 500 μg/mL, occasional focal retinal necroses were observed, suggesting isolated retinal toxicity at this concentration of moxifloxacin.

CONCLUSIONS. Intravitreal moxifloxacin, up to 100 μg/mL in mice or 150 μg/mL in rabbits, caused no ERG or retinal histologic abnormality. These results indicate that moxifloxacin is a safe intravitreal antibiotic in mouse and rabbit animal models. If proven safe and efficacious by further study in humans, intravitreal injection of moxifloxacin could be considered as an alternative to currently used antibiotics in selected patients with resistance or allergy to the more traditional antibiotics.

(B)acterial endophthalmitis is a devastating ocular disease that can lead to permanent blindness in a short time if not treated properly and promptly. The Endophthalmitis Vitrectomy Study (EVS) showed that after intraocular surgery such as cataract extraction, 94% of culture-proven incidences of endophthalmitis are vancomycin for Gram-positive bacteria and amikacin or tobramycin for Gram-negative bacteria.2 Today, most cases of endophthalmitis are treated with vancomycin and ceftazidime, because amikacin and tobramycin have been reported to cause retinal infarction, even at therapeutic concentrations.3 It would be much more beneficial for the patients and more convenient for ophthalmologists if only one antibiotic instead of two could be used to treat both Gram-positive and Gram-negative bacterial endophthalmitis.

Fluoroquinolone has been widely used in ophthalmology, because of its broad Gram-positive and Gram-negative cover-age.4–17 However, significant bacterial resistance to second- and third-generation fluoroquinolone emerged in the mid-1990s. For example, resistant strains causing bacterial keratitis increase from 5% to 35% over a period of 4 years.8,9 The in vitro susceptibility rates of Staphylococcus aureus, isolated from patients with endophthalmitis, to ciprofloxacin, ofloxacin, and levofloxacin decreased from 100% in 1993 to 0% in 2001.10

Moxifloxacin (Avelox; Bayer Pharmaceuticals Corp., West Haven, CT), a fourth-generation fluoroquinolone, was initially developed to treat Gram-positive bacterial respiratory infec-tions.11–14 Unlike the second- and third-generation fluoroquinolones, which bind to only one of the two key enzymes involved in bacterial DNA replication, moxifloxacin binds both enzymes: bacterial DNA gyrase and topoisomerase IV.15,16 More extensive binding provides more effective bacterial killing and less bacterial mutation into resistant organisms. Thus, moxifloxacin provides superior coverage against those Gram-positive bacteria that were already resistant to the second- and third-generation fluoroquinolones, and it also maintains excellent coverage for Gram-negative bacteria.17 It has been demonstrated that moxifloxacin’s minimum inhibitory concentration (MIC) and MIC90 (the concentration of drug causing a 90% growth inhibition of organisms) are the lowest among all genera-tions of fluoroquinolones, and its antibacterial activity is the highest against the most common Gram-positive pathogens in the EVS, such as coagulase negative Staphylococcus, S. aureus, and Streptococcus pneumoniae.1

This experimental study was designed to examine whether moxifloxacin can be safely used as an intravitreal agent in the treatment of bacterial endophthalmitis in animal models. Intravitreal injection of moxifloxacin was performed on mice and rabbits, and retinal function and morphology were subsequently examined using electroretinography (ERG) and histology studies.

MATERIALS AND METHODS

Animals

C57BL/6j mice 4 weeks of age (~25 g) were obtained from the Charles River Laboratories (Wilmington, MA). Mice were fed ad libitum with laboratory chow (Purina, Richmond, IN) and water. Dutch Belted rabbits weighing 2 to 2.5 kg were obtained from the Myrtle’s Rabbity, Inc. (Thompson Station, TN). The animals were housed in room light-
ing with a 12-hour light–dark cycle. The experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the protocol was approved by the Institutional Review Board of Baylor College of Medicine.

Intravitreal Injection

Mice were anesthetized by intraperitoneal injection of a solution containing ketamine (95 mg/mL) and xylazine (5 mg/mL) at a dose of 0.2 mL/100 g body weight. Proparacaine hydrochloride 0.5% (Alcon Laboratories, Inc., Fort Worth, TX) was used for additional topical anesthesia. Ofloxacin ophthalmic solution 0.3% (Allergan Inc, Irvine, CA) was applied to the ocular surface before injection, and bacitracin ophthalmic ointment (E. Fougera & Co., Melville, NY) was applied after injection, to prevent infection. Moxifloxacin, a white hyophilized powder, was kindly provided by the Bayer Pharmaceuticals Corp. (West Haven, CT). The minimum inhibitory concentration (MIC) of moxifloxacin to bacterial keratitis isolates was found to be 0.016 to 4.0 µg/mL. Because this is a retinal toxicity study, we chose to use 5 µg/mL as a baseline concentration. Moxifloxacin solutions were serially diluted with sterile water as instructed by the manufacturer so that the final intravitreal concentrations were 5, 25, 50, 100, and 500 µg/mL based on an estimated adult mouse vitreous volume of 20 µL. Serially diluted moxifloxacin solutions of 2 µL were injected intravitreally into mouse eyes under a dissecting microscope using a micro-injector (Hamilton Co., Reno, NV). Five mice were used for each concentration. A 30-gauge needle was first used to make a punch incision 0.5 mm posterior to the temporal limbus, and the microinjection needle was then inserted through the incision, approximately 1.5 mm deep, angled toward the optic nerve until the tip of needle was visualized in the center of the vitreous. Sterile water of the same volume (2 µL) was injected into the fellow eyes of each mouse as the control. After injection, animals were kept in ambient light on a 12-hour light–dark schedule. Four weeks after injection, animals were processed for electroretinogram (ERG) recordings and subsequent retinal histology examinations.

Dutch Belted rabbits were also used, to examine the interspecies difference and to confirm the experimental results in the mice. The rabbits were anesthetized with an intramuscular injection of 0.5 mL/kg body weight of a solution containing ketamine 42.8 mg, xylazine 8.6 mg, and acepromazine 1.4 mg/mL. Topical proparacaine hydrochloride was used for additional anesthesia, and ofloxacin and bacitracin were also used to prevent infection. After anterior chamber paracentesis, 200 µg moxifloxacin in a volume of 0.1 mL was injected intravitreally into the left eye of each rabbit. The injection was performed with a 27-gauge needle through a site 2 mm posterior to the superior limbus. A previous report showed that rabbit vitreous volume was 1.5 mL, but we measured vitreous volume in 27 adult rabbits and found it to be 1.2 mL. Based on this measurement, the final intravitreal concentration of moxifloxacin was approximately 150 µg/mL, with the concentration confirmed by HPLC (high pressure liquid chromatography). Five rabbits were used. The fellow eye of each rabbit was injected with 0.1 mL drug vehicle (water) as the control. After the injection, the animals were kept for 4 weeks in ambient light on a 12-hour light/dark schedule. The rabbits were examined with indirect ophthalmoscopy, and no retinal changes or detachments were noted in any eye 4 weeks after the injections. ERGs were then performed with subsequent retinal histology examinations.

ERG Recordings

The mice were placed in dark adaptation overnight before ERG testing. In dim red light, mice were anesthetized with a solution of ketamine and xylazine, as described earlier. Pupils were dilated with a single drop of 0.5% tropicamide and 2.5% phenylephrine. A drop of 0.5% proparacaine hydrochloride was applied for corneal anesthesia. The mice were placed on a heating pad maintained at 35°C, inside a Ganzfeld dome coated with highly reflective white paint (Munsell Paint, New Windsor, NY). A small amount of 2.5% methylcellulose gel was applied to the eye, and a platinum electrode was placed in contact with the center of the cornea. Similar platinum reference and ground electrodes were placed in the forehead and tail, respectively. After placement in the dome, the mice were allowed to remain in complete darkness for 5 minutes before the ERG was started. Signals were amplified (P122 band-pass 0.1-1000 Hz; Grass-Telefactor, West Warwick, RI). Data were then acquired at a sampling rate of 10,000 Hz (DAQ board; National Instruments Laboratory; Austin, TX). Traces were averaged and analyzed with custom software (written in MatLab; The MathWorks, Natick, MA).

For ERGs in the rabbits, the test protocol was similar to that in the mice. The rabbits were dark adapted for 1 hour, and 1 drop of 1% tropicamide and 2.5% phenylephrine were instilled in the left eyes for dilation. After 1 drop of hydroxypropyl methylcellulose (Geniosol; Chiron Vision Ophthalmics) was instilled in the eye, a corneal contact electrode (a contact electrode with gold foil; JET; KLC Technologies, Gaithersburg, MD) was placed on the cornea. The reference electrode was placed near the lid, and the ground electrode, in the scruff of the neck. The ERG signals were amplified, and data were acquired as in the mouse ERG test. Before intravitreal moxifloxacin injection, ERG was performed as a baseline. Four weeks after the injection, ERG was repeated on the same eyes.

Flashes were calibrated in a manner similar to that described previously and are described in detail elsewhere.21 Flashes for scotopic measurements were generated by a photostimulator (model PS-35+; Grass-Telefactor). Light was spectrally filtered with a 500-nm interference filter (Edmund Industrial Optics, Barrington, NJ). A series of metal plates with holes of various diameters and glass neutral-density filters were used to attenuate the flash. Flashes varied in intensity from −3.85 to −0.76 log scotopic cd/s/m². The scotopic b-wave was digitally filtered using the “filtfilt” function in the software (low pass filter, Fc 60 Hz; MatLab; The MathWorks), to remove oscillatory potentials before fitting. The relationship between b-wave amplitude and flash intensity can be described by a saturating hyperbolic function (Naka-Rushton) with the formula:

\[
b = \frac{b_{\text{max,scot}} \cdot I}{b_{\text{max,scot}} + I_0.5}
\]

where \(b_{\text{max,scot}}\) is the saturated scotopic b-wave amplitude, and \(I_0.5\) is the intensity that provides half saturation. The baseline and peak of each filtered trace was measured, and the data for multiple intensities were fit to the equation.

For analysis of the a-wave and cone function, 1500-watt xenon flash lamps (Novatron, Dallas, TX) were used to provide intense illumination, approximately 2.92 log scotopic cd/s/m². To analyze the rod function, we used the following equation (Lamb-Pugh model) to fit a series of a-waves at increasing intensities:

\[
1 - a(t) = a_{\text{max}} \cdot \exp\left[-\frac{1}{2} \cdot \phi \cdot A \cdot (t - t_\text{eff})^2\right]
\]

where \(a(t)\) is the a-wave, \(a_{\text{max}}\) is its saturating amplitude, \(\phi\) is the number of photoisomerizations rod produced by the flash, \(A\) is the amplification factor, and \(t_\text{eff}\) is a brief delay. Cone-driven responses were recorded in the presence of a 540-nm rod-saturation background light that measured 40 scotopic cd/m² in intensity. The oscillatory potentials were removed before measurement by digitally filtering the cone driven b-wave with the filtfilt function in MatLab (low pass filter, \(F_c = 60\) Hz; MatLab; The MathWorks), to remove oscillatory potentials before fitting. The relationship between b-wave amplitude and flash intensity can be described by a saturating hyperbolic function (Naka-Rushton) with the formula:

\[
b = \frac{b_{\text{max,phot}} \cdot I}{b_{\text{max,phot}} + I_0.5}
\]

where \(b_{\text{max,phot}}\) is the saturated photopic b-wave amplitude, and \(I_0.5\) is the intensity that provides half saturation. The baseline and peak of each filtered trace was measured, and the data for multiple intensities were fit to the equation.

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Retinal Histology

After ERG tests, mice were euthanatized with an overdose of intraperitoneal ketamine and xylazine, and rabbits were killed with a lethal cardiac injection of Beuthanasia-D (Scherling Plough Animal Health, Omaha, NE). The animal eyes were then enucleated, a large full-
thickness incision was made in the cornea, and the eye was fixed immediately in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4). After 15 minutes in the fixative, the lens was removed and the eye was cut along the cornea-optic nerve axis into halves. Gross examinations of the tissues were performed. Tissues were further fixed overnight in 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Tissues were then embedded in paraffin, sectioned at a thickness of 6 μm, and stained with hematoxylin and eosin. A light microscope was used for histologic examinations.

**RESULTS**

**Electroretinograms**

ERG recordings from control and moxifloxacin-injected mouse eyes are shown in Figure 1. Figure 1A shows scotopic b-wave responses to increasing intensities of flashed light. The scotopic b-wave is a measurement of the extracellular field potential that primarily arises from rod bipolar cells in response to dim flashes of light. The relationship between scotopic b-wave amplitude and intensity can be modeled with a hyperbolic saturation (Naka-Rushton) function. This model yields two parameters, $b_{\text{max,scot}}$ and $I_{0.5}$, representing the maximum b-wave amplitude and the intensity that provides half saturation, respectively. As shown in Figure 1A, scotopic recordings from control eyes demonstrated a progressive increase in b-wave amplitude with increasing intensity of light. The maximum b-wave amplitude in control eyes was $530 \pm 55 \mu V$ and the half-saturation intensity measured $1.9 \pm 0.2 \mu V/rod$. The b-waves measured from eyes injected with various concentrations of moxifloxacin demonstrate similar morphology and amplitude. There was no statistically significant difference in $b_{\text{max,scot}}$ or $I_{0.5}$ between control eyes and any of the groups of moxifloxacin-injected eyes (Table 1).

To measure cone function, photopic ERG was recorded in the presence of a rod-saturation background (Fig. 1C). For control eyes, the saturated scotopic a-waves from control eyes and moxifloxacin-injected eyes show no significant difference. To measure cone function, photopic ERG was recorded in the presence of a rod-saturation background (C). Again, there was no significant difference in the photopic responses measured from control eyes and moxifloxacin-injected eyes (Table 1 for detailed data).

**TABLE 1.** ERG Data of Mouse Eyes with Various Intravitreal Concentrations of Moxifloxacin

<table>
<thead>
<tr>
<th>Dose</th>
<th>$b_{\text{max,scot}}$ (μV)</th>
<th>$I_{0.5}$ (μV/rod)</th>
<th>$a_{\text{max,scot}}$ (μV)</th>
<th>$b_{\text{max,phot}}$ (μV)</th>
<th>$a_{\text{max,phot}}$ (μV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=4)</td>
<td>530 ± 55</td>
<td>1.9 ± 0.2</td>
<td>690 ± 25</td>
<td>190 ± 40 (n=3)</td>
<td>75 ± 15</td>
</tr>
<tr>
<td>5 μg/mL (n=4)</td>
<td>450 ± 50</td>
<td>1.8 ± 0.1</td>
<td>550 ± 45</td>
<td>250 ± 10 (n=4)</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>50 μg/mL (n=4)</td>
<td>575 ± 35</td>
<td>2.6 ± 0.4</td>
<td>590 ± 20</td>
<td>210 ± 50 (n=3)</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>500 μg/mL (n=5)</td>
<td>505 ± 25</td>
<td>2.0 ± 0.01</td>
<td>650 ± 35</td>
<td>270 ± 25 (n=5)</td>
<td>55 ± 10</td>
</tr>
</tbody>
</table>

Concentration is the intravitreal concentration of moxifloxacin. Control is the eyes injected with the drug vehicle, which was water. $b_{\text{max,scot}}$ is the maximum b-wave amplitude in scotopic condition, $I_{0.5}$, the flash intensity that provides half saturation, and $a_{\text{max,scot}}$ the maximum a-wave amplitude. $b_{\text{max,phot}}$ and $a_{\text{max,phot}}$ are the maximum b-wave amplitude and maximum a-wave amplitude, respectively, in photopic stimulation.

**FIGURE 1.** ERG recordings from mouse eyes injected with various concentrations of intravitreal moxifloxacin (5, 50, and 500 μg/mL) or drug vehicle as the control. (A) Scotopic recordings demonstrated a progressive increase in b-wave amplitude with increasing intensity of light. b-Waves measured from the control eyes and moxifloxacin-injected eyes showed very similar morphology and amplitude. (B) ERG response to an intense flash that saturates the rod photoreceptors. The saturated scotopic a-waves from control eyes and moxifloxacin-injected eyes show no significant difference. To measure cone function, photopic ERG was recorded in the presence of a rod-saturation background (C). Again, there was no significant difference in the photopic responses measured from control eyes and moxifloxacin-injected eyes (see Table 1 for detailed data).
Similarly, ERG recordings were performed in rabbit eyes before and 4 weeks after moxifloxacin injection. Scotopic b-wave responses to increasing intensities of flashed light (Fig. 2A), saturated scotopic a-wave (Fig. 2B), and photopic recordings (Fig. 2C) were measured before (left) and after (right) moxifloxacin injection. Scotopic $b_{\text{max}}$ was 397 ± 54 and 408 ± 23 μV, and $I_{0.5}$ was 9.4 ± 0.6 and 9.1 ± 0.7 log rod, before and after moxifloxacin injection, respectively. Scotopic $a_{\text{max}}$ was 199 ± 17 and 177 ± 54 μV; photopic $b_{\text{max}}$ was 192 ± 12 and 177 ± 13 μV, and photopic $a_{\text{max}}$ was −94 ± 5 and −85 ± 5 μV, before and after moxifloxacin injection, respectively. Again, statistical analysis showed no significant difference between pre- and postinjection groups in any of the scotopic or photopic recordings (Fig. 2). Thus, ERG studies show no evidence of retinal functional change in either the mouse or rabbit model after intravitreal moxifloxacin injection of any concentration groups tested in this study.

**Discusion**

This study shows that moxifloxacin did not display retinal toxicity when examined by ERG or histology, when the intra-

**Figure 2.** ERG recordings from rabbit eyes before (left) and 4 weeks after (right) moxifloxacin injection. (A) Scotopic b-wave responses to increasing intensities of flashed light and (B) saturated scotopic a-wave and (C) photopic recordings. Statistical analysis shows no significant difference between pre- and postinjection groups in any of these either scotopic or photopic recordings.

**Figure 3.** Retinal histology of mouse eyes with intravitreal injection of moxifloxacin. (A) In the mouse eyes with intravitreal moxifloxacin from 5.0 to 100 μg/mL, no retinal abnormality was observed in any retinal area. The retinal detachment is an artifact of tissue processing. (B) In the eyes injected with 500 μg/mL intravitreal moxifloxacin, very small focal retinal necroses were occasionally noted in the retina. In these necrotic areas, the photoreceptor layer was disorganized. Photoreceptor degeneration was evident, and photoreceptor inner and outer segments were absent. The inner nuclear layer also showed a mild degenerative change. The ganglion cell layer appeared intact. In the areas where focal necrosis was not observed, the retina was normal in light microscopy examination. In the rabbit eyes with 150 μg/mL intravitreal moxifloxacin, histologic examination showed completely normal retinas with no evidence of retina necrosis in any area (Fig. 4).
vitrectomy, intravitreal moxifloxacin. Histologic examination showed a completely normal retina, with no evidence of retina necrosis in any area. Abbreviations as in Figure 3. Scale bar, 100 μm.

Thus, if proven safe and efficacious by further study in humans, intravitreal injection of moxifloxacin could be considered an alternative to currently used antibiotics in selected patients with resistant bacterial infection or allergy to the more traditional antibiotics.

References


