Treatment of *Staphylococcus Epidermidis* Endophthalmitis with Intravitreal Moxifloxacin in a Rabbit Model

**Sitki Samet Ermis, Zafer Cetinkaya, Halil Kiyici and Faruk Ozturk**

*Department of Ophthalmology,* ¹ *Department of Microbiology, School of Medicine, University of Afyon Kocatepe, Afyon,* and *Department of Pathology, School of Medicine, University of Baskent, Konya, Turkey*

**Ermis, S.S., Cetinkaya, Z., Kiyici, H. and Ozturk, F. Treatment of Staphylococcus Epidermidis Endophthalmitis with Intravitreal Moxifloxacin in a Rabbit Model. Tohoku J. Exp. Med., 2005, 205 (3), 223-229** — *Staphylococcus epidermidis* is one of the most common causes of postoperative infectious endophthalmitis, which is a serious complication of ocular surgery and penetrating trauma. Moxifloxacin is a newly developed fluoroquinolone with a potent antimicrobial activity. Corticosteroids are used in endophthalmitis to suppress devastating intraocular inflammatory response. This study was designed to assess the efficacy of intravitreal moxifloxacin alone and in combination with intravitreal dexamethasone. To the best of our knowledge, there is no published report demonstrating the effect of intravitreal moxifloxacin on bacterial endophthalmitis. One eye of each rabbit (*n* = 24) was infected by inoculation of 10⁵ colony-forming units (CFU) of *S. epidermidis* into the vitreus cavity. Rabbits received intravitreal injection of moxifloxacin (50 μg) or a combination of moxifloxacin (50 μg) and dexamethasone (400 μg). No treatment was given to control group. Clinical and histopathological examination scores and microbiological analysis of vitreus aspirates were compared. In the treatment groups, the clinical and histopathological scores and mean CFU were significantly lower than those in the control group (*p* < 0.05) but showed no significant difference between the treatment groups. These results suggest that intravitreal injection of moxifloxacin is effective against *S. epidermidis* in this experimental rabbit model. Moxifloxacin may be a promising agent in the treatment of bacterial endophthalmitis. — *Staphylococcus epidermidis; endophthalmitis; moxifloxacin; dexamethasone*

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Bacterial endophthalmitis is an ocular inflammation resulting from the introduction of an infectious agent into the vitreus cavity; it is a serious complication of ocular surgery and penetrating eye trauma. Bacterial endophthalmitis remains one of the most challenging problems in ophthalmology despite to availability of wide spectrum antibiotics and vitreus surgery. Although antibacterial treatment is performed successfully through the use of intravitreal antibi-
otics, visual prognosis is generally poor due to irreversible damage in the delicate neurosensory retina and retinal pigment epithelium. Finally, the mean visual acuity reaches to 20 / 200 and approximately one third of the patients achieve a visual acuity of 20 / 40 or better (Endophthalmitis Vitrectomy Study Group 1995; Somani et al. 1997; Aaberg et al. 1998). The etiology of visual loss involves both primary bacterial toxicity and ocular inflammatory response (Maxwell et al. 1991; Callegan et al. 2002). Staphylococcus epidermidis is the most common isolated pathogen. The most common source of infection is patient’s own normal conjunctival and lid flora (Speaker et al. 1991). S. epidermidis has a remarkable ability to persist in a host and to adhere to materials such as plastic, glass and prolene (Quie and Belani 1987).

Quinolone antibiotics have been widely used in the treatment of bacterial endophthalmitis. They have good antimicrobial activity against gram-negative and gram-positive bacteria (Smith et al. 2001). Moxifloxacin is a fourth generation fluoroquinolone introduced to ophthalmology to counteract the resistance of ocular pathogens to antibiotics (Mather et al. 2002).

This study was designed to assess the efficacy of intravitreal moxifloxacin alone and in combination with intravitreal dexamethasone in the treatment of S. epidermidis endophthalmitis in an experimental rabbit model.

**MATERIALS AND METHODS**

The right eyes of 24 New Zealand rabbits weighing between 2 and 3 kg were used in this study. Animals were treated in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research. The rabbits were anesthetized before surgical procedures with intramuscular injection of a solution containing an equal mixture of xylocaine hydrochloride (10 mg/kg) and ketamine hydrochloride (30 mg/kg). Mydriasis was achieved with phenylephrine hydrochloride 2.5% (Mydrin, Fort Worth, TX, USA) and cyclopentolate hydrochloride 1% (Sikloplejim, Istanbul, Turkey). Topical anesthesia was achieved by proparacain 0.5% (Alcaine, Fort Worth, TX, USA). Anterior chamber paracentesis was performed to yield a 0.1 ml aqueous fluid to limit intraocular pressure increases before intravitreal injections. All intravitreal injections and aspirations were made using a 27-gauge needle attached to a 1-ml tuberculin syringe by introducing the needle into midvitreous cavity trough pars plana with the bevel of the needle facing anteriorly. Intravitreal injections were performed by slow injection under ophthalmoscopic observation.

Organisms were grown overnight at 37°C in tryptic soy broth. The cells were washed three times by centrifugation at 5,000 g for five minutes in 0.9% NaCl and bacteria were harvested. The turbidity of bacterial suspension was adjusted to give 0.08-0.1 absorbance at 625 nm wavelength. The bacterial suspension at this turbidity contains approximately 10⁸ viable bacteria per milliliter. The suspension was adjusted by serial dilution in sterile 0.9% NaCl to yield a final concentration of approximately 10⁵ viable bacteria per milliliter. All of the rabbits were infected by inoculation of 10⁷ colony-forming units (CFU) of S. epidermidis (American Type Culture Collection #12228) in 0.1 ml 0.9% NaCl. After confirming the presence of clinical signs of endophthalmitis such as moderate to severe conjunctival injection with vitreous haze which at least partially obscures retinal vasculature, the eyes were randomly assigned to one of the three groups equally. Moxifloxacin powder was supplied by the manufacturer (Bayer AG Leverkusen, Germany) and dissolved aseptically in balanced salt solution to a final concentration of 0.5 mg/ml. Twenty-four hours after the inoculation of S. epidermidis (day 2), one group received intravitreal 50 μg moxifloxacin in 0.1 ml balanced salt solution, another group received a combination of intravitreal 50 μg moxifloxacin and 400 μg dexamethasone in 0.1 ml balanced salt solution. In moxifloxacin + dexamethasone group the drugs were not combined in one syringe to avoid precipitation of the mixture. Untreated control group received 0.1 ml of balanced salt solution. The eyes were examined clinically on day 2 (before injection of drugs), day 3 and day 4 (respectively 24, 48 and 72 hours after the inoculation of bacteria) externally and by indirect ophthalmoscopy. Ophthalmological findings were graded clinically by a masked observer similar to the method adopted from Pleyer et al. (1992) (Table 1). After the last clinical examination on day 4, vitreus aspirates of 0.1 ml was obtained for microbiological analysis which were serially diluted and plated for quantification on blood agar and incubated at 35°C for 48 hours. After the incubation period, surface colonies were counted and identified as S.
**Results**

The mean colony counts of vitreal aspirates 72 hours after bacterial inoculation (day 4) was 110 ± 20, 102 ± 26 and 1,063 ± 355 CFU/ml in moxifloxacin, moxifloxacin + dexamethasone and control groups, respectively (Table 3). The difference among groups was statistically significant (p = 0.003, Kruskal-Wallis test). Control group had statistically higher number of CFU compared to moxifloxacin (p = 0.004) and moxifloxacin + dexamethasone groups (p = 0.004). There was no significant difference between the treatment groups (p = 0.631).

In moxifloxacin group clinical scores were 5.50 ± 1.05, 6.17 ± 0.75 and 6.33 ± 0.82, respectively on day 2, 3 and 4. In moxifloxacin + dexamethasone group clinical scores were 5.83 ± 0.75, 6.00 ± 0.89 and 6.83 ± 0.98; in control group they were 5.67 ± 0.82, 7.50 ± 1.05 and 8.17 ± 0.75 on day 2, 3 and 4, respectively. While there was no significant difference among the groups on day 2 (p = 0.814, Kruskal-Wallis test), the difference was significant on day 3 and 4 (p = 0.044 and p = 0.008 respectively, Kruskal-Wallis test). On day 3 and 4, control group had significantly higher

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**Table 1. Clinical grading scale**

<table>
<thead>
<tr>
<th>Score</th>
<th>Conjunctiva</th>
<th>Cornea</th>
<th>Iris</th>
<th>Vitreus</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Clear</td>
<td>Normal</td>
<td>Clear</td>
</tr>
<tr>
<td>1</td>
<td>Mild edema</td>
<td>Focal edema</td>
<td>Mild hyperemia</td>
<td>Areas of vitreus haze, some fundus details visible, good red reflex</td>
</tr>
<tr>
<td>2</td>
<td>Edema, mild hyperemia, slight exudate</td>
<td>Diffuse edema</td>
<td>Marked hyperemia</td>
<td>Moderate vitreus haze, fundus details not clear, partial red reflex</td>
</tr>
<tr>
<td>3</td>
<td>Edema, mild hyperemia, heavy exudate</td>
<td>Opaque</td>
<td>Marked hyperemia, engorged vessels, synechia, irregular pupil</td>
<td>No red reflex</td>
</tr>
</tbody>
</table>

**Table 2. Histopathological grading scale**

<table>
<thead>
<tr>
<th>Score</th>
<th>Cornea and limbus</th>
<th>Anterior chamber</th>
<th>Ciliary body</th>
<th>Vitreus</th>
<th>Choroid</th>
<th>Retina</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>No detachment</td>
</tr>
<tr>
<td>1</td>
<td>Mild inflammation</td>
<td>Fibrin, exudates, mild inflammation</td>
<td>Minimal inflammation</td>
<td>Only fibrin and exudates</td>
<td>Mild inflammation</td>
<td>Partially detachment</td>
</tr>
<tr>
<td>2</td>
<td>Moderate inflammation</td>
<td>Fibrin, exudates, moderate inflammation</td>
<td>Moderate inflammation</td>
<td>Partially filled with infiltrate</td>
<td>Moderate inflammation</td>
<td>Segmental detachment</td>
</tr>
<tr>
<td>3</td>
<td>Severe inflammation</td>
<td>Severe inflammation</td>
<td>Severe inflammation</td>
<td>Completely filled with infiltrate</td>
<td>Severe inflammation</td>
<td>Total detachment</td>
</tr>
</tbody>
</table>
scores compared to moxifloxacin and moxifloxacin + dexamethasone groups (on day 3: \( p = 0.038 \) and \( p = 0.032 \), on day 4: \( p = 0.007 \) and \( p = 0.022 \), respectively).

The mean histopathological scores were 4.33 ± 1.51, 4.67 ± 1.03 and 7.50 ± 1.05 in moxifloxacin, moxifloxacin + dexamethasone and control groups, respectively (Table 3). The difference among groups was statistically significant (\( p = 0.006 \), Kruskal-Wallis test). Control group had significantly higher scores compared to moxifloxacin and moxifloxacin + dexamethasone groups (\( p = 0.009 \) and \( p = 0.02 \), respectively). There was no significant difference between the treatment groups (\( p = 0.457 \)). A moderate inflammation of ciliary body and vitreus in moxifloxacin + dexamethasone group is shown in Fig. 1.

**DISCUSSION**

This study was designed using an experimental rabbit model of *S. epidermidis* endophthalmitis to investigate the bacteriological, clinical and histopathological effects of intravitreal moxifloxacin alone and in combination with dexamethasone. To the best of our knowledge this is the first study to demonstrate the effect of intravitreal moxifloxacin on the bacterial endophthalmitis. *S. epidermidis* has relatively low virulence and

**Table 3. Summary of bacteriological, clinical and histopathological findings**

<table>
<thead>
<tr>
<th>Group</th>
<th>CFU/ml day 3 ≤</th>
<th>Clinical scores</th>
<th>Histopathological scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 2</td>
<td>Day 3</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>110 ± 20</td>
<td>5.50 ± 1.05</td>
<td>6.17 ± 0.75</td>
</tr>
<tr>
<td>Moxifloxacin + dexamethasone</td>
<td>102 ± 26</td>
<td>5.83 ± 0.75</td>
<td>6.00 ± 0.89</td>
</tr>
<tr>
<td>Control</td>
<td>1063 ± 355</td>
<td>5.67 ± 0.82</td>
<td>7.50 ± 1.05</td>
</tr>
</tbody>
</table>

\( a \) The difference among groups is statistically significant (\( p = 0.003 \), Kruskal-Wallis test).

\( b \) The difference among groups is statistically significant (\( p = 0.044 \), Kruskal-Wallis test).

\( c \) The difference among groups is statistically significant (\( p = 0.008 \), Kruskal-Wallis test).

\( d \) The difference among groups is statistically significant (\( p = 0.006 \), Kruskal-Wallis test).

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Fig. 1. Moderate mixed inflammation of ciliary body and vitreus (Hematoxylin & Eosin, × 200). Short arrows, ciliary processes of ciliary body; long arrow, vitreus.
causes slow progression (Maxwell et al. 1993). Endophthalmitis was observed in all eyes after the inoculation of $10^5$ CFU of *S. epidermidis* in our study. Different numbers of inoculum size have been used to produce endophthalmitis in experimental models (Forster 1992; Maxwell et al. 1993). Different strains may have different growth patterns (Maxwell et al. 1993). In our experimental model, eyes were treated for 24 hours after the inoculation of bacteria in order to mimic clinical setting, since usually the disease is discovered and therapy is given after the examination on first postoperative day (Aguilar et al. 1996).

The outcome of bacterial endophthalmitis depends on multiple factors such as virulence of responsible organism, the duration between onset of infection and institution of treatment, the therapy chosen, the condition of the eye and the patient’s age (Foster et al. 1996). Although several antibiotics have been used for treatment via several routes, the recommended administration is direct injection of the agent into the vitreus cavity (Baum et al. 1982; Endophthalmitis Vitrectomy Study Group 1995). Potent antibiotics such as vancomycin and aminoglycosides do not penetrate into vitreus due to blood-ocular barrier after systemic administration. Although intraocular inflammation increases permeability of blood-ocular barrier, intravitreal antibiotic levels vary substantially and fall below therapeutic levels for many bacteria (Ferencz et al. 1999; Callegan et al. 2002). Intravitreal administration of antibiotics has been reported to be a key component in the treatment of bacterial endophthalmitis since intravitreal levels can be directly and immediately achieved and remain above minimum inhibitory concentration for causative organisms (Callegan et al. 2002).

Fluoroquinolones have broad spectrum antibacterial activity and were introduced for the treatment of corneal and conjunctival infections. They are widely used for prophylaxis of bacterial endophthalmitis (Mather et al. 2002). In humans orally administered moxifloxacin was reported to achieve an inhibitory concentration in the aqueous for most of the frequently isolated gram-positive and gram-negative bacteria responsible for the disease. It was concluded that oral moxifloxacin could be considered in the prophylaxis and adjunctive therapy of bacterial endophthalmitis (Garcia-Saenz et al. 2001). Topical application of moxifloxacin was shown to reach sufficient intraocular levels to prevent endophthalmitis in an animal model and surgical prophylaxis with topical moxifloxacin was suggested to be a valuable adjunct for the prevention of endophthalmitis (Kowalski et al. 2004). A fourth generation fluoroquinolone moxifloxacin was reported to be more potent than second and third generation fluoroquinolones in invitro studies testing endophthalmitis isolates. Additionally moxifloxacin reported to cover second and third generation fluoroquinolone resistance among *Staphylococcus* isolates (Mather et al. 2002). Susceptible gram-positive bacteria were reported to be less likely resistant to moxifloxacin than other fluoroquinolones. Although a single mutation to the bacterial topoisomerase intravenously conveys bacterial resistance to older fluoroquinolones, it requires a double mutation to the DNA gyrase and topoisomerase intravenously to convey bacterial resistance to moxifloxacin (Ruiz 2003). Additionally because of its chemical structure, moxifloxacin is less affected by bacterial efflux mechanism that pumps antibiotic out of the cell (Scheld 2003).

Bacterial endophthalmitis is an ocular destructive condition resulting from the host inflammatory response in addition to direct effects caused by bacterial organism (Peyman et al. 1978). Meredith et al. (1990b) suggested that the host inflammatory response is more important than previously recognized in models of infectious endophthalmitis which become culture negative despite continued intraocular inflammation and subsequent tissue destruction. It was stated that successful management of bacterial endophthalmitis necessitates the elimination of infecting organism and the control of host inflammatory responses in the shortest possible time (Driebe et al. 1986). *S. epidermidis* endophthalmitis can cause loss of integrity of the intraocular contents as a result of toxins and enzymes released by both the organism and by the host immune system.
which can result in the complete loss of vision (Hassan 1994). Suppression of the inflammatory response may minimize retinal damage and preserve retinal architecture (Smith et al. 1986). Contradictory results have been reported on the benefits of intravitreal corticosteroid administration. Concomitant administration of dexamethasone was reported to be beneficial (Maxwell et al. 1991; Yoshizumi et al. 1998), had no effect (Jett et al. 1995; Aguilar et al. 1996) or was detrimental (Meredith et al. 1996) to outcome in experimental models of bacterial endophthalmitis. Corticosteroids can suppress the ocular inflammatory response to gram-positive organisms induced by growing organism as well as metabolically inactive organisms, whole cell walls and cell wall components (Merino et al. 1998; Callegan et al. 1999; Chen et al. 1999). It was also shown that intravitreal dexamethasone use markedly reduced blood-ocular breakdown in bacterial endophthalmitis (Vallar et al. 1995).

In summary, moxifloxacin (50 μg) administered intravitreally is effective in the treatment of 

S. epidermidis endophthalmitis. A novel fourth generation fluoroquinolone may be a promising agent in the treatment of bacterial endophthalmitis. The combination of dexamethasone to the antibacterial treatment does not affect the bacteriological, clinical and pathological outcome. Further ocular pharmacokinetic and experimental research is necessary for the investigation of retinal toxicity and dose-response relation.

References
Intravitreal Moxifloxacin in Treatment of Endophthalmitis


