Toward Improving Therapeutic Regimens for Bacillus Endophthalmitis

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**Purpose.** Bacillus cereus causes the most virulent and refractory form of endophthalmitis. The authors analyzed the effectiveness of early treatment with vancomycin or gatifloxacin, with or without dexamethasone, for experimental B. cereus endophthalmitis.

**Methods.** Rabbit eyes were injected intravitreally with 100 colony-forming units of B. cereus. At 2, 4, or 6 hours after injection, eyes were injected intravitreally with 0.1 ml gatifloxacin (0.5%), vancomycin (1.0%), either antibiotic plus dexamethasone, dexamethasone alone (1.0%), or PBS. Eyes were analyzed by electroretinography, bacterial quantitation, and antibiotic penetration analysis. Drug toxicity toward Müller cells, retinal pigment epithelium, and cones was also analyzed.

**Results.** Eyes treated at 2 hours with vancomycin or gatifloxacin, with or without dexamethasone, maintained higher ERG amplitudes than the dexamethasone alone and PBS control groups. Eyes treated with antibiotic plus dexamethasone at 6 hours had reduced retinal function compared to antibiotic treatment alone. With the exception of vancomycin with or without dexamethasone at 6 hours, all antibiotic treatments sterilized eyes. Only gatifloxacin reached aqueous concentrations greater than the minimal inhibitory concentration for B. cereus when measured at 8 hours. Neither gatifloxacin nor vancomycin was toxic to retinal cells in vitro.

**Conclusions.** Early intravitreal injection of vancomycin or gatifloxacin improved the therapeutic outcome of B. cereus endophthalmitis. The addition of dexamethasone to antibiotic treatment did not provide a therapeutic benefit over antibiotics alone and appeared to reduce the antibiotic efficacy of vancomycin 6 hours after infection. In this model, delay in treatment past 6 hours significantly reduced the potential for salvaging useful vision.

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**Materials and Methods**

**Bacteria and Drugs**

B. cereus strain ATCC 14579 (American Type Culture Collection, Manassas, VA) was used to infect rabbit eyes. This strain has previously been shown to cause rapid endophthalmitis in rabbits and mice, with clinical signs similar to those of human infections. A single colony
of B. cereus was used to inoculate 5 mL brain heart infusion media (BHI; Difco Laboratories, Detroit, MI). Cultures were incubated at 37°C overnight, subcultured, and grown to a concentration of \(1.0 \times 10^8\) colony-forming units (CFU)/mL. Cultures were serially diluted to 100 CFU/0.1 mL for intravitreal injections.

Drugs used in these studies were vancomycin (1.0%; Hospira, Lake Forest, IL), gatifloxacin (0.3% ophthalmic solution [Zymar]; Allergan, Irvine, CA), and dexamethasone (1.0%; American Regen Laboratories, Inc., Shirley, NY). Vancomycin powder and dexamethasone were resuspended in sterile phosphate-buffered saline (PBS; pH 7.4).

**Animals**

New Zealand White rabbits (body weight, 2–4 kg) were maintained in accordance with institutional guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Rabbits were anesthetized with intramuscular injection of ketamine (KetaVed, 35 mg/kg body weight; Phoenix Scientific Inc., St. Joseph, MO) and xylazine (Rompun, 5 mg/kg body weight; Bayer Corp., Shawnee Mission, KS) before intravitreal injection and electroretinography (ERG). Eyes were topically anesthetized with proparacaine HCl (Ophthetic, 0.5%; Allergan, New York, NY). Vancomycin (1% gatifloxacin with dexamethasone. At 8 hours after infection, rabbits were intravitreally injected with 0.1 mL drug(s) or vehicle. Rabbits were randomly divided into seven treatment groups as follows: no treatment, PBS, dexamethasone alone (1.0%), vancomycin alone (1.0%), gatifloxacin alone (0.3%), vancomycin with dexamethasone, or gatifloxacin with dexamethasone. At 8 hours after infection, rabbits were humanely killed, and eyes were harvested for analysis.

**Treatment Regimens**

Eyes received intravitreal injections of approximately 100 CFU B. cereus/0.1 mL BHI into the mid-vitreous of one eye. The contralateral eye served as a noninjection control. At 2 hours, 4 hours, or 6 hours after infection, rabbits were anesthetized, underwent paracentesis, and were intravitreally injected with 0.1 mL drug(s) or vehicle. Rabbits were randomly divided into seven treatment groups as follows: no treatment, PBS, dexamethasone alone (1.0%), vancomycin alone (1.0%), gatifloxacin alone (0.3%), vancomycin with dexamethasone, or gatifloxacin with dexamethasone. At 8 hours after infection, rabbits were humanely killed, and eyes were harvested for analysis.

**Analysis of Therapeutic Efficacy**

**Bacterial Quantification.** At 8 hours after infection, viable bacteria were quantified as described previously. Briefly, the cornea, iris, and lens were removed aseptically, and the vitreal contents were homogenized (60 seconds, 5000 rpm; Mini-BeadBeater; Biospec Products, Bartlesville, OK). The homogenates were serially diluted in PBS and plated out in triplicate on BHI for quantitation.

**Retinal Function Analysis.** Retinal function was monitored by scotopic ERG, as described previously. Rabbit eyes were dilated with 10% phenylephrine hydrochloride ophthalmic solution (AK-Dilate; Akorn, Inc., Buffalo Grove, IL) and dark adapted for 10 minutes before ERG. The amplitude of the A-wave (a measure of photoreceptor cell activity) was measured from the prestimulus baseline to the A-wave trough. The amplitude of the B-wave (a measure of Müller, bipolar, and amacrine cell function) was measured from the A-wave trough to the B-wave peak. A-wave and B-wave amplitudes were recorded for each eye (EPIC2000 and UTAS3000; LKC Technologies, Inc., Gaithersburg, MD). The percentage of retinal function retained was calculated by using either of the following equations:

\[
\text{Retinal Function Retained} = \left(1 - \frac{\text{A-wave amplitude (normal)} - \text{A-wave amplitude (injured)}}{\text{A-wave amplitude (normal)}}\right) \times 100
\]

**Histology and Biomicroscopy.** Before harvest, eyes were visualized and photographed with an operating biomicroscope (Zeiss S7; Zeiss Inc., Thornwood, NY). Eyes were scored independently based on the degree of change in anterior and posterior segment inflammation and retinal architecture, based on a scale from 0 (no change) to 4 (+ significant inflammation and retinal architectural damage). For histology, eyes were enucleated and fixed in 10% formalin for 24 hours. The eyes were sectioned and stained with hematoxylin and eosin by standard procedures.

**Antibiotic Penetration into the Eye.** The concentration of antibiotics in the vitreous and aqueous humor was determined by an antibiotic diffusion assay. Assay plates were prepared with 30 mL antibiotic agar (no. 2 Oxoid Antibiotic Agar; Oxoid USA, Inc., Columbia, MD), and 10 CFU/mL indicator bacterial strain was aerobically grown on the agar surface. Staphylococcus aureus ATCC 25923 or Klebsiella pneumoniae ATCC 13883 were used as the indicator strains in vancomycin or gatifloxacin bioassays, respectively. Aqueous humor, vitreous, or antibiotic standards (10 μL) were transferred aseptically onto sterile filter discs (6 mm; Whatman, WVR, West Chester, PA), and the discs were transferred to the agar surface. Assays were incubated in a humidified chamber at 37°C for 24 hours, and zones of inhibition were measured. All samples and standards were analyzed in triplicate. Antibiotic concentrations were determined from a standard curve of zone size versus CFU/mL concentration, the slope of which was determined from a best-fit curve by the least-square mean method.

**Antibiotic Toxicity Assays**

The following cell lines were used to analyze drug toxicity: human Muller cells (MIO-M1, a kind gift from Astrid Limb, Institute of Ophthalmology, Moorfields Eye Hospital, London, UK), retinal pigment epithelium cells (ARPE-19; ATCC), and cone photoreceptor cells (661W, a kind gift from Muayyad Al-Ubaidi, University of Oklahoma Health Sciences Center). Müller cells and cone cells were cultivated in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% glutamine. RPE cells were grown in a 1:1 dilution of DMEM and F-12 nutrient mixture (HAM) with 10% FBS and 1% glutamine. Cells were incubated at 37°C with 5% CO2. Lactate dehydrogenase (LDH) release assays were used to determine whether antibiotics or dexamethasone were toxic to retinal cells. Retinal cells were cultivated in the appropriate media, as described, on 24-well plates to 70% to 80% confluence. Vancomycin, gatifloxacin, or dexamethasone was added to the cells at the identical final concentration used for intravitreal injection. Supernatants were harvested at 0, 2, 4, 6, and 8 hours, and the percentage of LDH release was quantified (CytotoxOne kit; Promega, Madison, WI). Fluorescence was recorded with an excitation wavelength of 530 nm and an emission wavelength of 590 nm with a microplate fluorescence reader (FL600Bio-Tek, Winooski, VT). Values were calculated based on 100% lysis (freeze-thaw) controls.

**Checkerboard Assay**

Because our results suggested that treatment with vancomycin or vancomycin/dexamethasone at 6 hours resulted in inferior bacterial killing in vivo, we analyzed the efficacy of antibiotic/dexamethasone combinations against B. cereus using a checkerboard assay. MICs were determined for gatifloxacin (0.12 μg/mL) and vancomycin (1.95 μg/mL). Based on these results, MICs of gatifloxacin/dexamethasone and vancomycin/dexamethasone combinations were determined. Final antibiotic concentrations in combinations (0–1.0 mg/mL vancomycin or 0–300 μg/mL gatifloxacin) for each individual antibiotic with dexamethasone (0–1.0 mg/mL) were assayed, as were controls without dexamethasone and controls without drugs. B. cereus was cultured in Müller Hinton broth (VWR) at a concentration equivalent to that of 0.5 McFarland standard. B. cereus cultures were then added to an equal volume of antibiotic/dexamethasone, incubated for 24 hours at 37°C, and the absorbance (OD600) was determined by spectrophotometry.

**Statistical Analysis**

All values represent the mean ± SEM for four or more eyes per time point, unless otherwise specified. For LDH and antibiotic penetration assays, all values represented the mean ± SEM for three or more replicate samples per time point, unless otherwise specified. Descriptive statistics and two-tailed, two-sample t-tests assuming equal (n > 5) or unequal (n < 5) variance were used for statistical comparisons between groups. P ≤ 0.05 was considered significant.
RESULTS

Bacterial Killing

Bacterial quantitation of eyes infected with *B. cereus* is summarized in Figure 1. The gatifloxacin alone and gatifloxacin plus dexamethasone groups sterilized all infected eyes regardless of the time of treatment. Treatment with vancomycin alone or vancomycin plus dexamethasone at 2 hours and 4 hours after infection sterilized all infected eyes. However, treatment with vancomycin alone or vancomycin plus dexamethasone at 6 hours after infection did not sterilize infected eyes. The dexamethasone alone, PBS, and no treatment groups had similar CFU counts (6.5 – 7.0 log<sub>10</sub> CFU/mL; *P* > 0.05).

The CFU counts of nontreated infected eyes recovered at 2, 4, and 6 hours were 2.5 ± 0.5, 3.6 ± 0.2, and 5.2 ± 0.3 log<sub>10</sub> CFU/mL, respectively.

Retinal Function

ERG results of eyes infected with *B. cereus* are summarized in Figure 2. Animals treated with gatifloxacin with or without dexamethasone or vancomycin with or without dexamethasone at 2 hours after infection retained approximately 100% of retinal function when evaluated at 8 hours after infection. In eyes treated with dexamethasone alone at 2 hours after infection, the mean retinal response declined to less than 20% for A-wave function and less than 10% for B-wave function by 8 hours. The A- and B-wave amplitudes were recorded at 8 hours after infection following treatment at either 2, 4, or 6 hours after infection. Eyes treated with dexamethasone or PBS were analyzed at 8 hours after infection following intravitreal treatment at 2 hours only. Values represent the mean ± SEM of four or more eyes per group. *P* < 0.05.
hours after infection. B-wave function in eyes treated with dexamethasone alone at 2 hours was similar to that of PBS-treated (2 hours) and untreated eyes (P > 0.05). Although the addition of dexamethasone to gatifloxacin or vancomycin resulted in significantly greater amplitudes after the 2-hour treatment, these amplitude changes resulted in supernormal scotopic ERG responses, potentially indicative of changes in the retinal cells responsible for the A- and B-wave. Therefore, comparison of the efficacy of vancomycin alone to vancomycin plus dexamethasone or of gatifloxacin alone to gatifloxacin plus dexamethasone showed little clear advantage of using one therapeutic regimen over the other when drugs were administered 2 hours after infection.

Rabbits receiving antibiotics 4 hours after infection retained significant A-wave function in all treatment groups but reduced B-wave function in 3 of 4 treatment groups when evaluated at 8 hours after infection. The vancomycin alone treatment group was the only group that maintained more than 100% B-wave function after treatment 4 hours after infection. Vancomycin plus dexamethasone, however, maintained the least B-wave function (approximately 47%) compared with the other 4-hour antibiotic treatment groups (P < 0.05). The gatifloxacin and gatifloxacin plus dexamethasone treatment groups retained a mean A-wave function of 80% and a B-wave function of 60%. Retinal function comparisons of gatifloxacin alone to gatifloxacin plus dexamethasone or of either gatifloxacin treatment group to vancomycin plus dexamethasone again showed no clear therapeutic advantage of treatment regimens when administered 4 hours after infection. The B-wave responses for eyes treated at 4 hours with vancomycin alone were significantly higher than those of eyes treated with gatifloxacin alone (P = 0.005). There was no significant difference in A-wave retention between eyes treated at 4 hours with vancomycin alone or gatifloxacin alone (P = 0.55). Although the mean A-wave and B-wave responses were greater for eyes treated at 6 hours with vancomycin alone than with gatifloxacin alone, there was no significant statistical difference in B-wave responses (P = 0.055) or A-wave responses (P = 0.17) between these groups.

Rabbits that received treatment at 6 hours showed minimal retention of retinal function. Overall, animals treated 6 hours after infection with vancomycin alone constituted the only treatment group that maintained mean A- and B-wave function greater than 60%. However, the addition of dexamethasone to vancomycin resulted in the least retinal function retained of the antibiotic treatment groups analyzed, maintaining a mean A-wave function of approximately 30% and a mean B-wave function of approximately 15%. Animals treated with gatifloxacin retained mean A-wave and B-wave function of approximately 40%. Animals treated with gatifloxacin plus dexamethasone retained mean A-wave function of approximately 50% but a mean B-wave function of greater than 20%. Comparison of gatifloxacin plus dexamethasone with vancomycin plus dexamethasone showed no significant difference in the overall retention of retinal function (P = 0.54). The addition of dexamethasone to either vancomycin or gatifloxacin showed a significantly reduced retention of B-wave function when administered 6 hours after infection (P = 0.005 and P = 0.029, respectively).

**Biomicroscopy and Histology**

Biomicroscopic analysis of infected eyes treated at 2 hours with vancomycin, gatifloxacin, or either antibiotic with dexamethasone revealed little or no inflammation in the cornea or anterior segment (score, 0–0.5+), no iritis, and no visible posterior segment inflammation. These eyes also had no conjunctival injection or edema. Eyes treated with antibiotics plus dexamethasone at 2 hours were indistinguishable from those treated with antibiotics alone, and these eyes were similar in appearance to uninfected eyes throughout 8 hours of analysis. Infected eyes that were injected with PBS or dexamethasone were similar in appearance to untreated infected eyes through 8 hours of analysis.

Among eyes treated at 4 hours with vancomycin, gatifloxacin, or either antibiotic with dexamethasone, approximately half had minimal accumulation of fibrin in the anterior segment (score, 1+), mild iritis (score, 1+), and mild cellular infiltration and fibrin accumulation in the posterior segment (score, 1–1.5+). The remaining eyes in these groups were similar in appearance to uninfected eyes. No eyes in these groups had conjunctival injection or edema. Eyes treated with antibiotics plus dexamethasone were indistinguishable from those treated with antibiotics alone.

Among eyes treated at 6 hours with vancomycin, gatifloxacin, or either antibiotic with dexamethasone, approximately 75% had minimal accumulation of fibrin in the anterior segment (score, 1+) and no corneal inflammation but moderate iritis (score, 2–2.5+) and moderate infiltration of cells and fibrin accumulation in the posterior segment (score, 2–2.5+). Some of the more inflamed eyes also had mild edema and injection of the conjunctiva (score, 0.5–1+). Again, eyes treated with antibiotics plus dexamethasone were indistinguishable from those treated with antibiotics alone.

Sham (PBS)-injected eyes and eyes injected with dexamethasone alone were moderately inflamed at 8 hours after infection. Most of these eyes had mild accumulation of fibrin in the anterior segment (score, 1–1.5+), minor corneal inflammation (score, 1+), significant iritis (score, 3+), and moderate infiltration of cells and fibrin accumulation in the posterior segment (score, 2.5+). These eyes also had mild to moderate edema and injection of the conjunctiva (score, 1.5–2+). In a comparison of the sham and dexamethasone-treated eyes with those treated with all antibiotic regimens at 6 hours after infection, those treated with antibiotics appeared to be slightly less inflamed in terms of conjunctival and anterior segment inflammation than those injected with PBS or dexamethasone alone (P < 0.05).

Histologic findings paralleled those of our biomicroscopic analysis (Fig. 3). Eyes treated with antibiotics with or without dexamethasone at 2 hours after infection were significantly less inflamed than those treated with the same regimens at 6 hours after infection. Notable differences between these groups that were not visible on biomicroscopic evaluation were changes in the retina and extent of inflammatory cell influx in eyes treated at 6 hours after infection. Although some retinas appeared to be detached in Figure 3, these detachments might have been histologic artifacts. However, using a greater magnification, it was apparent that retinas of eyes treated at 6 hours after infection exhibited retinal folding, with inflamma
tory cells visible beneath the retina (data not shown), indicat
ing that detachment might have occurred in these eyes (e.g., the antibiotic/dexamethasone 6-hour-treated eyes in Fig. 3). Significant numbers of inflammatory cells and fibrin accumulation were seen in the posterior segment, indicative of substan
tial inflammation. The degree of inflammation observed in all 6-hour treatment groups was similar to that of eyes treated with PBS and dexamethasone alone.

**Antibiotic Penetration**

Intraocular penetration of antibiotics was analyzed by measur
gatifloxacin and vancomycin concentrations in the vitreous and aqueous humor (Fig. 4). In a comparison of gatifloxacin and vancomycin treatment alone, vancomycin achieved signific
tically higher concentrations in the vitreous at all time points.
compared with that of gatifloxacin, regardless of the time of treatment. In addition, injection of gatifloxacin or vancomycin at 2 hours achieved significantly greater initial vitreal concentrations than treatment at 4 hours ($P < 0.005$ and $P = 0.002$, respectively) or 6 hours ($P = 0.004$ and $P = 0.003$, respectively). After the 2-hour and 4-hour treatment, gatifloxacin concentrations in the vitreous declined significantly ($P < 0.05$). Vancomycin concentrations in the vitreous declined significantly after the 4-hour treatment only ($P < 0.002$). Concentrations of vancomycin and gatifloxacin in the vitreous were well above the MIC for *B. cereus* regardless of the time of injection or recovery. Aqueous humor gatifloxacin concentrations from eyes treated at 2, 4, or 6 hours ranged from $1.07 \pm 0.1 \mu g/mL$ to $1.53 \pm 0.9 \mu g/mL$ ($P > 0.05$). Vancomycin was not detected in the aqueous humor.

Antibiotic concentrations in the vitreous after treatment combined with dexamethasone were also analyzed. Vitreous concentrations of vancomycin were 20-fold lower in eyes treated with vancomycin plus dexamethasone compared with eyes treated with vancomycin alone ($P < 0.05$). Gatifloxacin concentrations in eyes treated with the gatifloxacin/dexamethasone combinations were similar to those of eyes treated with gatifloxacin alone ($P > 0.05$). When combined with dexamethasone, concentrations of vancomycin and gatifloxacin in the vitreous were again well above the MIC for *B. cereus*.

**Drug Toxicity**

Neither gatifloxacin, vancomycin, nor dexamethasone was significantly toxic (≤12.1% LDH release) to Müller cells, RPE, or cone photoreceptor cells in vitro (Fig. 5). The highest percentages of LDH release occurred when Müller cells were treated with gatifloxacin (12.04% ± 2.41%). Treatment with dexamethasone alone resulted in the least percentage of LDH release (≤0.2%) for all retinal cell types tested.
Drug Combinations and Killing Efficacy

Our ERG results suggested that the combination of vancomycin and dexamethasone resulted in a possible inhibitory pharmacokinetic response when compared with that of vancomycin alone after injection 6 hours after infection. We therefore analyzed the efficacy of antibiotic/dexamethasone combinations against B. cereus using a checkerboard assay. The addition of dexamethasone to either antibiotic did not result in reduced killing of B. cereus in vitro, regardless of the concentration of antibiotic tested.

**DISCUSSION**

Posttraumatic cases of B. cereus endophthalmitis result in rapid vision loss, with less than 30% chance of retaining useful vision. Nearly half of B. cereus and other Bacillus species infections resulted in the evisceration or enucleation of the eye. In the absence of an established treatment regimen for B. cereus endophthalmitis, it is unlikely that the high number of treatment failures will be reduced. We therefore sought to determine the effectiveness of early intravitreal antibiotic treatment with or without concomitant dexamethasone administration during experimental B. cereus endophthalmitis.

The effectiveness of antibiotics against B. cereus endophthalmitis has been analyzed in posttraumatic models of infection. In a swine model of B. cereus endophthalmitis, clinical signs of inflammation were noted at 4 hours after infection. Infected eyes treated with vancomycin had less inflammation and tissue destruction than controls and ciprofloxacin-treated eyes. These findings were similar to our results in which vancomycin treatment appeared to limit inflammation and retinal function loss when given early in the course of infection. In a rabbit model of B. cereus, intravitreal injection of ciprofloxacin 6 hours after infection was significantly less effective in preventing infection than the same treatment administered 1 hour after infection. Our results concur with that study’s findings that intravitreal antibiotics were not as effective if treatment was delayed.

Recent clinical assessments of postoperative endophthalmitis reported conflicting results in visual retention after combined intravitreal administration of antibiotics and dexamethasone. None of these clinical series specifically evaluated postoperative models of endophthalmitis, except for a single series with three eyes with posttraumatic injury receiving such combined treatment, all of which became phthisical. Rabbit models of posttraumatic bacterial endophthalmitis have been used to analyze the therapeutic effectiveness of dexamethasone for Staphylococcus aureus, Streptococcus pneumoniae, Staphylococcus epidermidis, and Pseudomonas aeruginosa infections. The studies found that dexamethasone had a moderately beneficial effect, no effect, or a detrimental effect. These varying results are mirrored by varying clinical results.

The effectiveness of dexamethasone and antibiotics has been analyzed in a B. cereus sterile endophthalmitis model. Dexamethasone was unable to attenuate the inflammatory response induced by purified Bacillus exotoxins injected intravitreally. During infection, B. cereus produces numerous toxins and other enzymes that alone are inflammogenic in the eye. In addition, sterile models of B. cereus endophthalmitis do not address bacterial growth or its migration throughout the eye. In a more recent study, the effects of combined vancomycin and dexamethasone treatment starting at 24 hours after infection in an experimental B. cereus endophthalmitis rabbit model were measured, and the conclusion was that dexamethasone use in conjunction with vancomycin led to an improved outcome. B. cereus carries genes that encode for multiple virulence factors, and ocular isolates have been shown to produce several toxins that have the potential to cause significant retinal damage within 24 hours of infection. The B. cereus strain used in that study was isolated from a patient with sepsis, and its toxin profile was not evaluated. The strain caused a significantly less virulent infection than other well-described B. cereus strains tested in vivo, suggesting that the strain used might not have been as virulent as typical B. cereus ocular isolates. Furthermore, the early therapeutic intervention necessary to prevent the significant retinal damage caused by the bacteria, bacterial toxins, and host immune responses was not analyzed.

Previous studies on B. cereus endophthalmitis in experimental models show complete loss of A- and B-wave function within 12 hours of infection. Our control data correlate precisely with previous studies. The addition of dexamethasone to vancomycin resulted in additional retinal function loss when administered 4 hours and 6 hours after infection. Our data show that rabbits treated 2 hours after infection with gatifloxacin with dexamethasone or vancomycin with or without dexamethasone maintained greater than 100% A- and B-wave amplitudes 8 hours after infection. Overall, the addition of dexamethasone to antibiotics had no beneficial effect on the retention of retinal function as measured by ERG. With the exception of the dexamethasone alone, vancomycin alone, and vancomycin plus dexamethasone groups 6 hours after infection, all treatment groups completely sterilized the eyes by 8
hours after infection. Our results showed that vancomycin or vancomycin plus dexamethasone may not sterilize the eye within 2 hours if the bacteria are at an intraocular concentration greater than $10^6$ CFU/mL in the vitreous. Gatifloxacin alone and gatifloxacin plus dexamethasone completely sterilized the eyes regardless of the intravitreal concentration of \textit{B. cereus} reached in this study.

Studies disagree on the potential for positive clinical outcomes for endophthalmitis treated with vancomycin/dexamethasone combinations. Our results show that dexamethasone did not affect the in vitro antibacterial effectiveness of gatifloxacin or vancomycin as measured by checkerboard assay, nor did it affect the in vivo effectiveness as measured by ERG, intraocular antibiotic concentration, or intravitreal CFU counts. When compared with vancomycin alone, the addition of dexamethasone to vancomycin resulted in reduced intravitreal concentrations of vancomycin. Our results are similar to those of a previous report of reduced concentrations of intravitreal vancomycin in the presence of intravitreal dexamethasone in a \textit{Staphylococcus epidermidis} endophthalmitis rabbit model.37,39 Our results differ from a report of enhanced efficacy of intravitreal vancomycin in the presence of dexamethasone in a postoperative bacterial endophthalmitis study and a pneumococcal endophthalmitis rabbit model.15,38 Previous studies have shown that the decrease in vancomycin concentrations in the presence of dexamethasone was not caused by drug inactivation or precipitation.57,59 Our in vitro checkerboard data and in vivo bacterial killing data after treatment 2 and 4 hours after infection support this conclusion. The reasons for reduced in vivo concentrations of vancomycin in the presence of dexamethasone are not clear. Gan et al.35 suggested that dexamethasone may facilitate an increase in the rate of vitreous flow through the trabecular meshwork, thereby reducing the concentration of vancomycin. This hypothesis contradicted the findings of Park et al.58 in which dexamethasone reduced the elimination of intravitreal vancomycin in a \textit{S. pneumoniae} endophthalmitis rabbit model. In this same study, vancomycin elimination was increased in uninfected eyes treated with dexamethasone.38 Gan et al. suggested that the trabecular meshwork might have been blocked during severe inflammation in the streptococcal model, thus slowing the elimination of vancomycin.55 In the \textit{B. cereus} endophthalmitis model, inflammation was minimal at the time of dexamethasone and antibiotic administration.

Retinal cells are extremely sensitive to chemical and physical insult. Antimicrobial agents, such as amikacin and gentamicin, used at doses necessary to sterilize the eye were found to have detrimental effects on visual retention.40,41 Previous studies have expressed concerns regarding possible toxicity in the intraocular administration of fluoroquinolones.42,43 Our in vitro studies showed that gatifloxacin (0.5%), vancomycin (1.0%), and dexamethasone (1.0%) were not overly toxic to Müller, cone, or RPE cells, as measured by LDH assay. Gatifloxacin, vancomycin, and dexamethasone did not significantly increase the amount of LDH released in any cell type when compared to the 100% lysis control, indicating that these drugs were not toxic to retinal cells in vitro at clinically administered concentrations. Further studies analyzing retinal cell-specific function by ERG can determine whether these drugs have any affect on retinal function in vivo.

Decreases in intraocular quinolone effectiveness in the presence of dexamethasone have been reported. In experimental \textit{Pseudomonas} endophthalmitis, cultures from eyes treated with dexamethasone and ciprofloxacin at 12 hours after infection were positive, whereas cultures from eyes treated with ciprofloxacin alone were negative.72 However, studies comparing intravitreal moxifloxacin treatment with or without dexamethasone reported no difference in clinical outcomes between groups.21,44

In terms of antibiotic penetration during infection, gatifloxacin was able to penetrate the aqueous humor within 8 hours after infection, but vancomycin was not. Aqueous humor gatifloxacin concentrations were approximately 10-fold higher than the MIC for \textit{B. cereus} ATCC 14579. For \textit{B. cereus} endophthalmitis, achieving adequate antibiotic concentration in the vitreous and aqueous is critical in terms of killing organisms that reside in the posterior segment and those that may migrate to the anterior segment.

The gatifloxacin ophthalmic formulation used in these studies contains the preservative benzalkonium chloride (BAK). Studies have shown that the addition of BAK may augment the antimicrobial efficacy of gatifloxacin, both in vitro and in vivo (Blondeau JM. \textit{IOVS} 2006;47:ARVO E-Abstract 1903; Mah FS. \textit{IOVS} 2006;47:ARVO E-Abstract 1905). In a pilot study, we intravitreally injected 100 μL BAK (0.005%) solution in PBS 6 hours after infection to mimic the concentration found in the gatifloxacin formulation used in the present study. Preliminary data demonstrated that at 12 hours after infection, BAK alone reduced the number of \textit{B. cereus} in the vitreous from $10^6$ CFU/mL to $6 \times 10^4$ CFU/mL, demonstrating its potential for antimicrobial activity in the eye. However, BAK alone was unable to sterilize any infected eyes, and retinal function was not detected in infected eyes treated with BAK alone at 6 hours.

Our results reinforce the necessity of early intravitreal injection of antibiotics when treating \textit{B. cereus} endophthalmitis. Early treatment (i.e., 2 or 4 hours) likely kills bacteria before significant levels of toxins are present, limiting retinal damage and preserving vision. Treatment delays beyond 6 hours after infection significantly reduced the retention of retinal function in our results. Our results identified both strengths and weaknesses in using either antibiotic against \textit{B. cereus} endophthalmitis. Strengths of gatifloxacin included its greater penetration into the aqueous humor and its killing ability 6 hours after infection. Gatifloxacin ophthalmic solution is also readily available for use in the clinical setting. Weaknesses of gatifloxacin included the lower B-wave retention after treatment at 4 hours. Strengths of vancomycin included a trend toward higher retinal function retention and sufficient killing at 2 hours and 4 hours after infection. Weaknesses of vancomycin included its inability to penetrate the aqueous humor within 6 hours of treatment. In addition, vancomycin must also be formulated from injectable powder before use. We also demonstrated that therapeutic outcomes were not improved by the addition of dexamethasone to the treatment regimen, which may correlate with a moderate or a lack of clinical value of adjunct dexamethasone and antibiotic treatment in patients with \textit{B. cereus} endophthalmitis.15 Future analysis will focus on determining the effectiveness of different anti-inflammatory drugs in combination with effective antibiotics, analyzing the inflammatory response in response to different treatments and the value of vitrectomy during the later stages of infection to identify regimens designed to preserve vision after \textit{B. cereus} endophthalmitis.

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\section*{References}

Improving Therapeutic Regimens for Endophthalmitis


