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Toward Improving Therapeutic Regimens for *Bacillus* Endophthalmitis

B.J. Wiskur^{A,1}, M.L. Robinson^{B,1}, A.J. Farrand^{C,1}, B.D. Novosad^{D,1,2}, and M.C. Callegan^{C,1,D,1,2,*}

A Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Oklahoma City, OK

B College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK

C Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK

D Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK

1 University of Oklahoma Health Sciences Center, Oklahoma City, OK

2 Dean A. McGee Eye Institute, Oklahoma City, OK

Abstract

Purpose—*Bacillus cereus* causes the most virulent and refractory form of endophthalmitis. We 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 CFU of *B. cereus*. At 2, 4, or 6 h postinfection, eyes were injected intravitreally with 0.1 ml of gatifloxacin (0.3%), 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 h with vancomycin or gatifloxacin, with or without dexamethasone, maintained higher ERG amplitudes compared to the dexamethasone alone and PBS control groups. Eyes treated with antibiotic plus dexamethasone at 6 h had reduced retinal function compared to antibiotic treatment alone. With the exception of vancomycin with or without dexamethasone at 6 h, all antibiotic treatments sterilized eyes. Only gatifloxacin reached aqueous concentrations greater than the MIC for *B. cereus* when measured at 8 h. 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 at 6 h postinfection. In this model, delay in treatment past 6 h significantly reduced the potential for salvaging useful vision.

Keywords

Bacillus; endophthalmitis; antibiotics; dexamethasone; rabbit

*Corresponding author: Department of Ophthalmology, 608 Stanton L. Young Blvd. DMEI 418, Oklahoma City, OK 73104. Phone: (405) 271-3674, Fax: (405) 271-8781, Email: michelle-callegan@ouhsc.edu.
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INTRODUCTION

Bacterial endophthalmitis can occur as a result of intraocular surgery, a penetrating eye injury or metastasis of bacteria to the eye from a distant point of infection. Penetrating eye injuries have the highest rate, 3 to 17%, of developing bacterial endophthalmitis.^{1,2} Staphylococci are the most frequent cause of post-traumatic bacterial endophthalmitis followed by the more virulent *Bacillus cereus*.³

B. cereus is a Gram-positive, aerobic, spore forming bacterium commonly found in ground soil, and as such, is often considered a pathogen of minimal clinical relevance. Inside the eye, however, *B. cereus* causes an explosive infection that causes rapid vision loss in 1–2 days and is usually refractory to treatment.⁴ Clinical symptoms of *B. cereus* endophthalmitis include severe pain, chemosis, periorbital swelling, extreme proptosis, fever and polymorphonuclear leukocytosis.^{4–6} A comprehensive review of post-traumatic cases of endophthalmitis caused by *B. cereus* reported that less than 30% of patients retained useful vision, and only 9% retained 20/70 vision or better.⁴ Despite therapeutic and surgical intervention, 48% of *B. cereus* and other *Bacillus* species infections required evisceration or enucleation of the eye.⁴

Current treatment recommendations for bacterial endophthalmitis include intravitreal injections of vancomycin (1.0 mg/ml) for Gram-positive bacteria and amikacin (0.4 mg/ml) or ceftazidime (2.2 mg/ml) for Gram-negative bacteria.^{7–9} Vancomycin has been reported to have 100% effectiveness against the most common Gram-positive and Gram-negative endophthalmitis pathogens, including *B. cereus*.^{3,7–9} Gatifloxacin, a fourth-generation quinolone, has been shown to penetrate the blood-ocular barrier and reach concentrations above the MIC for *B. cereus*, but only when administered systemically.^{10,11} Oral fluoroquinolones are not considered a mainstay for the treatment of endophthalmitis. However, intravitreal injections of gatifloxacin may provide clinicians with an alternative. Gatifloxacin solutions are readily available in rural areas, while vancomycin solution must be prepared from injectable powder.

Concomitant administration of intravitreal steroids, such as dexamethasone, with antibiotics to minimize ocular inflammation and bystander damage caused by the immune system during endophthalmitis has produced mixed results.^{12–23} This study evaluated the therapeutic effectiveness of gatifloxacin ophthalmic solution, vancomycin, and a combination of each antibiotic with dexamethasone in a well-established *B. cereus* endophthalmitis rabbit model.^{24–27} In addition, the potential toxicity of gatifloxacin, vancomycin, and dexamethasone for Müller, cone photoreceptor, and retinal pigment epithelial (RPE) cells were analyzed *in vitro*.

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 that of human infections.^{27,28} A single colony of *B. cereus* was used to inoculate 5ml brain heart infusion media (BHI; Difco Laboratories, Detroit, MI). Cultures were incubated at 37°C overnight, subcultured, and grown to a concentration of 1.0×10^8 CFU/ml. Cultures were serially diluted to 100 cfu/0.1 ml for intravitreal injections.

The following drugs were used in these studies: 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 Association for Research in Vision and Ophthalmology Statement on the Use of Laboratory Animals in Ophthalmic Research. Rabbits were anesthetized with an intramuscular injection of ketamine (KetaVed™; Phoenix Scientific Inc., St. Joseph, MO; 35 mg/kg of body weight) and xylazine (Rompun™; Bayer Corp., Shawnee Mission, KS; 5mg/kg of body weight) prior to intravitreal injection and electroretinography (ERG). Eyes were topically anesthetized with proparacaine HCl (Ophthetic™; Allergan, Hormigueros, Puerto Rico; 0.5%) prior to intravitreal injection. Aqueous humor (0.1 ml) was removed by paracentesis prior to intravitreal injection.

Treatment Regimens

Eyes received intravitreal injections of approximately 100 CFU of *B. cereus*/0.1ml BHI into the mid-vitreous of one eye. The contralateral eye served as a non-injection control. At 2 h, 4 h, or 6 h postinfection, rabbits were anesthetized, underwent paracentesis, and were intravitreally injected with 0.1 ml of 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 h postinfection, rabbits were euthanized and eyes harvested for analysis as described below.

Analysis of Therapeutic Efficacy

Bacterial Quantification—At 8 h postinfection, viable bacteria were quantified as described previously.^{24–27} Briefly, the cornea, iris and lens were removed aseptically, and the vitreal contents were homogenized (60s, 5,000 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 electroretinography (ERG) as previously described.^{24,26,27} Rabbit eyes were dilated with AK-Dilate (10% Phenylephrine Hydrochloride Ophthalmic Solution; Akorn, Inc., Buffalo Grove, IL) and dark adapted for 10 min prior to ERG. The amplitude of the A-wave (a measure of photoreceptor cell activity) was measured from the pre-stimulus 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 the following equations: (i) $100 - \{[1 - (\text{experimental A-wave amplitude} / \text{absolute control A-wave amplitude})] \times 100\}$ or (ii) $100 - \{[1 - (\text{experimental B-wave amplitude} / \text{absolute control B-wave amplitude})] \times 100\}$.

Histology and Biomicroscopy—Prior to 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).²⁸ For histology, eyes were enucleated and fixed in 10% formalin for 24 h. The eyes were sectioned and stained with hematoxylin and eosin by standard procedures.³⁰

Antibiotic Penetration into the Eye—The concentration of antibiotics in the vitreous and aqueous humor was determined by an antibiotic diffusion assay.³¹ Assay plates were prepared with 30 ml No. 2 Oxoid Antibiotic Agar (Oxoid USA Inc., Columbia MD). 10^5 cfu/ml of an indicator bacterial strain was aseptically spread onto the agar surface. *S. aureus* ATCC 25923 or *K. pneumoniae* ATCC 13883 were used as the indicator strains in vancomycin or gatifloxacin bioassays, respectively. Aqueous humor, vitreous, or antibiotic standards (10 μ l) were transferred aseptically onto sterile filter discs (6mm Whatman, VWR, West Chester, PA), and the discs were transferred to the agar surface. Assays were incubated in a humidified chamber at 37°C for 24 h, 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 $\log_{10}$ concentration, the slope of which was determined from a best-fit curve by least-square means method.

Antibiotic Toxicity Assays

The following cell lines were used to analyze drug toxicity: human Müller cells (MIO-M1, a kind gift from Dr. Astrid Limb, Institute of Ophthalmology, Moorfields Eye Hospital, London), retinal pigmented epithelium cells (ARPE-19, ATCC), and cone photoreceptor cells (661W, a kind gift from Dr. Muayyad Al-Ubaidi, OUHSC).^{32,33} Müller cells and cone cells were cultivated in Dulbecco's 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 above on 24-well plates to 70–80% confluency. 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 h, and percent LDH release was quantified using a CytotoxOne kit (Promega®, Madison, Wisconsin). Fluorescence was recorded with an excitation wavelength of 530 nm and an emission wavelength of 590 nm using the FL600 Microplate Fluorescence Reader (Bio-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 h resulted in inferior bacterial killing *in vivo*, we analyzed the efficacy of antibiotic/dexamethasone combinations against *B. cereus* using a checkerboard assay. Minimal inhibitory concentrations (MIC) were determined for gatifloxacin (0.12 μ g/ml) and vancomycin (1.95 μ g/ml). Based on these results, MICs of gatifloxacin/dexamethasone and vancomycin/dexamethasone combinations were determined. Final antibiotic concentrations in combinations (ranging from 0 to 1.0 mg/ml vancomycin or 0 to 300 μ g/ml gatifloxacin) for each individual antibiotic with dexamethasone (ranging from 0 to 1.0 mg/ml) were assayed, as well as controls without dexamethasone and controls without drugs. *B. cereus* was cultured in Müller Hinton broth (VWR) to a concentration equivalent to that of a 0.5 McFarland standard. *B. cereus* cultures were then added to an equal volume of antibiotic/dexamethasone, incubated for 24 h at 37°C, and the absorbance (OD₆₅₀) was determined by spectrophotometry.

Statistics

All values represent the mean $\pm$ standard error of the mean (SEM) for ≥ 4 eyes per time point, unless otherwise specified. For LDH and antibiotic penetration assays, all values represented the mean $\pm$ SEM for ≥ 3 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. A P-value of ≤ 0.05 was considered significant.

RESULTS

Bacterial Killing

Bacterial quantitation of eyes infected with *B. cereus* are 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 h and 4 h postinfection sterilized all infected eyes. However, treatment with vancomycin alone or vancomycin plus dexamethasone at 6 h postinfection did not sterilize infected eyes. The dexamethasone alone, PBS, and no treatment groups had similar CFU counts ($6.5 - 7.0 \log_{10}$ CFU/ml, $P > 0.05$). The CFU counts of non-treated infected eyes recovered at 2, 4, and 6 h were 2.5 ± 0.5 , 3.6 ± 0.2 and $5.2 \pm 0.3 \log_{10}$ 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 h postinfection retained approximately 100% of retinal function when evaluated at 8 h postinfection. In eyes treated with dexamethasone alone at 2 h postinfection, the mean retinal response declined to $<20\%$ for A-wave function and $<10\%$ for B-wave function by 8 h postinfection. B-wave function in eyes treated with dexamethasone alone at 2 h was similar to that of PBS-treated (2 h) and untreated eyes ($P > 0.05$). Although the addition of dexamethasone to gatifloxacin or vancomycin resulted in significantly greater amplitudes following the 2 h 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 gatifloxacin alone to gatifloxacin plus dexamethasone showed little clear advantage of using one therapeutic regimen over the other when drugs were administered at 2 h postinfection.

Rabbits receiving antibiotics at 4 h postinfection retained significant A-wave function in all treatment groups but reduced B-wave function in 3 of 4 treatment groups when evaluated at 8 h postinfection. The vancomycin alone treatment group was the only group that maintained $>100\%$ B-wave function following treatment at 4 h postinfection. Vancomycin plus dexamethasone, however, maintained the least B-wave function (approximately 47%), compared to the other 4 h antibiotic treatment groups ($P < 0.05$). The gatifloxacin and gatifloxacin plus dexamethasone treatment groups retained a mean A-wave function of 80% and B-wave function of 60%. Retinal function comparisons of gatifloxacin alone to gatifloxacin plus dexamethasone or either gatifloxacin treatment group to vancomycin plus dexamethasone again showed no clear therapeutic advantage of treatment regimens when administered 4 h postinfection. The B-wave responses for eyes treated at 4 h 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 h with vancomycin alone or gatifloxacin alone ($P = 0.35$). Although the mean A-wave and B-wave responses were greater for eyes treated at 6 h 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 h showed minimal retention of retinal function. Overall, animals treated at 6 h postinfection with vancomycin alone was the only treatment group that maintained a mean A- and B-wave function $>60\%$. 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 B-wave function of approximately 15%. Animals treated with gatifloxacin retained a mean A-and B-wave function of approximately 40%. Animals treated with gatifloxacin plus dexamethasone retained a mean A-wave function of approximately 50% but >20% B-wave function. Comparison of gatifloxacin plus dexamethasone to 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 at 6 h postinfection ($P=0.005$ and 0.029 respectively).

Biomicroscopy and Histology

Biomicroscopic analysis of infected eyes treated at 2 h with vancomycin, gatifloxacin, or either antibiotic with dexamethasone revealed little or no inflammation in the cornea or anterior segment (score of 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 h were indistinguishable from those treated with antibiotics alone, and these eyes were similar in appearance to uninfected eyes throughout 8 h of analysis. Infected eyes that were injected with PBS or dexamethasone were similar in appearance to untreated infected eyes through 8 h of analysis.

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

For eyes treated at 6 h with vancomycin, gatifloxacin, or either antibiotic with dexamethasone, approximately 75% of eyes had minimal accumulation of fibrin in the anterior segment (score of 1+) and no corneal inflammation, but a moderate iritis (score of 2–2.5+) and moderate infiltration of cells and fibrin accumulation in the posterior segment (score of 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 h postinfection. The majority of these eyes had mild accumulation of fibrin in the anterior segment (score of 1–1.5+), minimal corneal inflammation (score of 1+), a significant iritis (score of 3+), and moderate infiltration of cells and fibrin accumulation in the posterior segment (score of 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 h postinfection, 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 that of our biomicroscopic analysis (Figure 3). Eyes treated with antibiotics with or without dexamethasone at 2 h postinfection were significantly less inflamed than those treated with the same regimens at 6 h postinfection. Notable differences between these groups that were not visible upon biomicroscopic evaluation were changes in the retina and extent of inflammatory cell influx in eyes treated at 6 h postinfection. While some retinas appeared to be detached in Figure 3, these detachments may have been histological artifacts. However, using a greater magnification, it was apparent that retinas of eyes treated at 6 h postinfection exhibited retinal folding with inflammatory cells visible beneath the retina (data

not shown), indicating that detachment may have occurred in these eyes (e.g. the antibiotic/dexamethasone 6 h-treated eyes in Figure 3). Significant numbers of inflammatory cells and fibrin accumulation were seen in the posterior segment, indicative of substantial inflammation. The degree of inflammation observed in all 6 h treatment groups was similar to that of eyes treated with PBS and dexamethasone alone.

Antibiotic Penetration

Intraocular penetration of antibiotics was analyzed by measuring gatifloxacin and vancomycin concentrations in the vitreous and aqueous humor (Figure 4). In a comparison of gatifloxacin and vancomycin treatment alone, vancomycin achieved significantly higher concentrations in the vitreous at all time points compared to that of gatifloxacin regardless of the time of treatment. In addition, injection of gatifloxacin or vancomycin at 2 h achieved significantly greater initial vitreal concentrations than treatment at 4 h ($P=0.005$ and 0.002 respectively) or 6 h ($P=0.004$ and 0.003 respectively). Following the 2 h and 4 h treatments, gatifloxacin concentrations in the vitreous declined significantly ($P<0.05$). Vancomycin concentrations in the vitreous declined significantly following the 4 h 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 h ranged from $1.07 \pm 0.1 \mu\text{g/ml}$ to $1.53 \pm 0.9 \mu\text{g/ml}$ ($P>0.05$). Vancomycin was not detected in the aqueous humor.

Antibiotic concentrations in the vitreous following treatment combined with dexamethasone were also analyzed. Vitreous concentrations of vancomycin were 20-fold less in eyes treated with the vancomycin plus dexamethasone compared to eyes treated with vancomycin alone ($P<0.05$). Gatifloxacin concentrations in eyes treated with the gatifloxacin/dexamethasone combinations were similar to that 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 were significantly toxic ($\leq 12.1\%$ LDH release) to Müller cells, RPE, or cone photoreceptor cells *in vitro* (Figure 5). The highest percentages of LDH release occurred when Müller cells were treated with gatifloxacin ($12.04 \pm 2.41\%$). Treatment with dexamethasone alone resulted in the least %LDH release ($\leq 0.2\%$) 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 to that of vancomycin alone following injection at 6 h postinfection. 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

Post-traumatic cases of *B. cereus* endophthalmitis result in rapid vision loss with less than a 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.⁴ 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 post-traumatic models of infection.^{34,35} In a swine model of *B. cereus* endophthalmitis, clinical signs of inflammation were noted at 4 h postinfection. Infected eyes treated with vancomycin had less inflammation and tissue destruction than controls and ciprofloxacin-treated eyes.³⁵ 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 at 6 h postinfection was significantly less effective in preventing infection when compared to the same treatment administered at 1 h postinfection.³⁴ Our results concur with the authors' findings that intravitreal antibiotics were not as effective if treatment was delayed.

Recent clinical assessments of post-operative endophthalmitis report conflicting results in visual retention following combined intravitreal administration of antibiotics and dexamethasone.^{12–14,36} None of these clinical series specifically evaluated postoperative *Bacillus* endophthalmitis, except for a single series with 3 eyes with post-traumatic injury receiving such combined treatment, all of which became phthisical.¹²

Rabbit models of post-traumatic bacterial endophthalmitis have been used to analyze the therapeutic effectiveness of dexamethasone for *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* infections.^{15–22} The studies found that dexamethasone had a moderately beneficial effect,^{16–20} no effect^{21,22} or a detrimental effect¹⁵. These varying results are mirrored by varying clinical results.^{12–14,36}

The effectiveness of dexamethasone and antibiotics has been analyzed in a *B. cereus* sterile endophthalmitis model.²³ 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 h postinfection in an experimental *B. cereus* endophthalmitis rabbit model were measured.¹⁶ The author concluded 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 h of infection.⁸ 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 when compared to other well-described *B. cereus* strains tested *in vivo*^{24–28,37}, suggesting that the strain used may 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 response was not analyzed.

Previous studies on *B. cereus* endophthalmitis in experimental models show complete loss of both A- and B-wave function within 12 h of infection.^{24,28,37} Our control data correlates precisely with previous studies. The addition of dexamethasone to vancomycin resulted in additional retinal function loss when administered at 4 h and 6 h postinfection. Our data shows that rabbits treated at 2 h postinfection with gatifloxacin with dexamethasone or vancomycin with or without dexamethasone maintained greater than 100% A- and B-wave amplitudes at 8 h postinfection. 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 at 6 h postinfection, all treatment groups completely sterilized the eyes by 8 h postinfection. Our results showed that vancomycin or vancomycin plus dexamethasone may not sterilize the eye within two 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 *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 to vancomycin alone, the addition of dexamethasone to vancomycin resulted in reduced intravitreal concentrations of vancomycin. Our results are similar to a previous report of reduced concentrations of intravitreal vancomycin in the presence of intravitreal dexamethasone in a *Staphylococcus epidermidis* endophthalmitis rabbit model.³⁸ Our findings 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^{13,39}. Previous studies have shown that the decrease in vancomycin concentrations in the presence of dexamethasone was not due to drug inactivation or precipitation.^{38,40} Our *in vitro* checkerboard data and *in vivo* bacterial killing data following treatment at 2 and 4 h postinfection support this conclusion. The reasons for reduced *in vivo* concentrations of vancomycin in the presence of dexamethasone are not clear. Gan *et al.*³⁶ 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.*³⁹ in which dexamethasone reduced the elimination of intravitreal vancomycin in a *Streptococcus pneumoniae* endophthalmitis rabbit model. In this same study, vancomycin elimination was increased in uninfected eyes treated with dexamethasone.³⁹ Gan *et al.* suggested that the trabecular meshwork may have been blocked during severe inflammation in the streptococcal model, thus slowing the elimination of vancomycin.³⁶ In the *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.^{41,42} Previous studies have expressed concerns regarding possible toxicity in the intraocular administration of fluoroquinolones.^{43,44} Our *in vitro* studies showed that gatifloxacin (0.3%), vancomycin (1.0%) and dexamethasone (1.0%) were not overtly 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 via 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 *Pseudomonas* endophthalmitis, cultures from eyes treated with dexamethasone and ciprofloxacin at 12 h postinfection were positive, whereas cultures from eyes treated with ciprofloxacin alone were negative.²² However, studies comparing intravitreal moxifloxacin treatment with or without dexamethasone reported no difference in clinical outcome between groups.^{21,45}

In terms of antibiotic penetration during infection, gatifloxacin was able to penetrate into the aqueous humor within 8 h postinfection, but vancomycin did not. Aqueous humor gatifloxacin concentrations were approximately 10-fold higher than the MIC for *B. cereus* ATCC 14579. For *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 as well as those that may migrate into 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, *IOVS*, 2006, 47, ARVO E-Abstract, 1903; Mah FS, *IOVS*, 2006, 47, ARVO E-Abstract, 1905). In a pilot study, we intravitreally injected 100 µl of a solution of BAK (0.005%) in PBS at 6 h postinfection to mimic the concentration found in gatifloxacin formulation used in the present study. Preliminary data demonstrated that at 12 h postinfection, BAK alone reduced the number of *B. cereus* in the vitreous from 10⁸ CFU/ml to 6×10⁴ 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 h.

Our results reinforce the necessity of early intravitreal injection of antibiotics when treating *B. cereus* endophthalmitis. Early treatment (i.e. at 2 h or 4 h) likely kill bacteria before significant levels of toxins are present, limiting retinal damage and preserving vision. Treatment delays beyond 6 h postinfection significantly reduced the retention of retinal function. Our results identified both strengths and weaknesses in using either antibiotic against *B. cereus* endophthalmitis. Strengths of gatifloxacin included its greater penetration into the aqueous humor and its killing ability at 6 h postinfection. Gatifloxacin ophthalmic solution is also readily available for use in the clinical setting. Weaknesses of gatifloxacin included the lower B-wave retention following treatment at 4 h. Strengths of vancomycin included a trend toward higher retinal function retention and sufficient killing at 2 h and 4 h postinfection. Weaknesses of vancomycin included its inability to penetrate into the aqueous humor within 6 h following treatment. Vancomycin must also be formulated from injectable powder prior to use. We also demonstrated that therapeutic outcomes were not improved by the addition of dexamethasone to the treatment regimen, which may correlate with a very moderate or lack of clinical value of adjunct dexamethasone and antibiotic treatment in *B. cereus* endophthalmitis patients.⁴⁶ 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 differing treatments, and the value of vitrectomy during the later stages of infection in order to identify regimens designed to preserve vision following *B. cereus* endophthalmitis.

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Figure 1. Quantitation of CFU in the vitreous following treatment of experimental *B. cereus* endophthalmitis

Rabbit eyes were injected with 100 CFU *B. cereus*. Eyes were injected intravitreally with gatifloxacin, vancomycin, those antibiotics with dexamethasone, PBS, or were left untreated. Vitreal CFU counts were determined at 8 h postinfection following intravitreal treatment at either 2, 4, or 6 h postinfection. Eyes treated with dexamethasone or PBS were analyzed at 8 h postinfection following intravitreal treatment at 2 h only. Values represent the mean $\pm$ SEM of $N \geq 4$ eyes per group, * $P > 0.05$.

Figure 2. Retinal function analysis following treatment of experimental *B. cereus* endophthalmitis Rabbit eyes were injected with 100 CFU *B. cereus*. Eyes were injected intravitreally with gatifloxacin, vancomycin, those antibiotics with dexamethasone, PBS, or were left untreated. Scotopic ERGs were performed and A- and B-wave amplitudes recorded at 8 h postinfection following treatment at either 2, 4, or 6 h postinfection. Eyes treated with dexamethasone or PBS were analyzed at 8 h postinfection following intravitreal treatment at 2 h only. Values represent the mean $\pm$ SEM of $N \geq 4$ eyes per group, * $P < 0.05$.

Figure 3. Histology following treatment of experimental *B. cereus* endophthalmitis

Rabbit eyes were injected with 100 CFU *B. cereus*. Eyes were injected intravitreally with gatifloxacin (Gati), vancomycin (Vanc), those antibiotics with dexamethasone (Dex), PBS, or were left untreated (Uninj). Eyes treated with dexamethasone or PBS were analyzed at 8 h postinfection following intravitreal treatment at 2 h only. Eyes were enucleated and fixed in 10% formalin for 24 h. The eyes were sectioned and stained with hematoxylin and eosin by standard procedures.

Figure 4. Intraocular antibiotic penetration following treatment of *B. cereus* endophthalmitis Rabbit eyes were injected with 100 CFU *B. cereus*. Eyes were injected intravitreally with gatifloxacin (Gati), vancomycin (Vanc), those antibiotics with dexamethasone (Dex), PBS, or were left untreated. [A] For eyes treated with gatifloxacin or vancomycin alone at 2, 4, or 6 h postinfection, antibiotic concentrations were analyzed at 4, 6, or 8 h postinfection. * $P < 0.05$ compared with the initial time point in each treatment group, ** $P < 0.05$ compared with initial time point of treatment at 2 h postinfection. [B] Eyes were treated with antibiotic/dexamethasone combinations at 2 h, and antibiotic concentrations in the vitreous and aqueous humor were quantified at 8 h postinfection. * $P < 0.05$ compared with vancomycin treatment alone at 2 h postinfection. Values represent the mean $\pm$ SEM of $N \geq 4$ eyes per group.

Figure 5. Retinal cell drug toxicity

The effect of gatifloxacin, vancomycin, and dexamethasone on Müller cells, cone photoreceptors, and RPE cells was analyzed *in vitro*. Antibiotics or dexamethasone was added to confluent cell monolayers at the identical final concentrations used for intravitreal injection. LDH was measured in culture supernatants at 0, 2, 4, 6 and 8 h. Percent LDH release was calculated based on 100% lysis (freeze-thaw) controls. Values represent the mean $\pm$ SEM of N=6 per group.