Effects of Intravitreal Corticosteroids in the Treatment of Bacillus cereus Endophthalmitis

Samuel M. Liu, MD, PhD; Tin Way, MD; Merlyn Rodrigues, MD; Scott M. Steidl, MD, DMA

Objective: To investigate whether intravitreal corticosteroid therapy reduces the extent of inflammatory intraocular tissue damage caused by Bacillus cereus endophthalmitis.

Methods: New Zealand white rabbits were inoculated with 1 × 10^6 B cereus organisms and randomized to receive no treatment (control eyes; n=14), intravitreal vancomycin hydrochloride (n=13), or a combination of intravitreal vancomycin and dexamethasone sodium phosphate (n=13) after 24 hours. The eyes were examined and graded for clinical signs of infection and inflammation on days 7 and 14, followed by enucleation for histopathologic analysis.

Results: Both treated groups had significantly less clinical sequelae than controls on day 7. By day 14, eyes given combination treatment had significantly less clinically graded corneal (P=0.03) and conjunctival (P=0.007) inflammation than eyes treated with vancomycin. Histopathologic analysis revealed a significant decrease in inflammatory changes between all treated eyes and controls at day 14. The only statistically significant difference between eyes given combination treatment and eyes given vancomycin alone was in the retina (P=.03).

Conclusions: Intravitreal corticosteroids may enhance the recovery from B cereus endophthalmitis when given in conjunction with intravitreal antibiotics. The beneficial effect of corticosteroids is noted clinically, but not histologically, by day 14 after single-dose treatment in rabbits.

Clinical Relevance: This study provides evidence that the use of intravitreal corticosteroids with antibiotics for the treatment of B cereus endophthalmitis may lead to an improvement compared with the use of antibiotics alone.

Arch Ophthalmol. 2000;118:803-806
MATERIALS AND METHODS

The protocol for use and care of animals was approved by the Institutional Animal Care and Use Committee of the University of Maryland, Baltimore. Forty New Zealand white rabbits weighing 2.0 to 2.5 kg were anesthetized with a 50/50 mixture of ketamine hydrochloride (50 mg/mL) and xylazine hydrochloride (10 mg/mL) via intramuscular injection. Topical proparacaine hydrochloride was placed on the experimental left eye of each animal. Rabbits were inoculated with 1 x 10^6 colony-forming units of *B. cereus*. The source of the *Bacillus* organisms was a live culture originally obtained from the blood of a human patient with sepsis. The organisms were prepared from *B. cereus* cells cultured on agar for 24 hours by washing in isotonic sodium chloride solution and resuspended to an optical density of 0.098 (10^9 cells/mL). This was diluted to create a suspension of 10^5 colony-forming units/0.1 mL. Viable cell counts of each suspension were plated on blood agar dishes to quantitate the number of viable cells present. To ensure maximal accuracy of delivered volume, each 30-gauge syringe was preloaded with a total volume of 0.3 mL, which was reduced to 0.1 mL through ejection of the excess volume before inoculation of the eye. The inoculation of 1 x 10^6 organisms was delivered via an injection through the pars plana, approximately 2 to 3 mm posterior to the limbus. A 25-µg fentanyl citrate patch (Duragesic [fentanyl transdermal system]; Janssen Pharmaceutica Inc, Titusville, NJ) was then applied to a 5-cm shaved region on the backs of the rabbits for analgesic relief.

The eyes were then randomly assigned in a masked fashion to 1 of the following 3 groups: no treatment, intravitreal vancomycin alone, or intravitreal vancomycin and dexamethasone sodium phosphate injections. On day 0, the eyes were examined. This was approximately 24 hours after the initial inoculation with *B. cereus*. All eyes showed similar amounts of clinical endophthalmitis, which was defined as eyes having moderate conjunctival chemosis and hyperemia in the presence of vitreous haze or corneal edema obscuring view of the retina. The animals were then anesthetized using intramuscular injection. Those eyes randomized to receive antibiotic treatment alone received an intraocular injection of vancomycin hydrochloride, 1 mg in 0.1 mL isotonic sodium chloride solution. Eyes receiving combined treatment were injected with vancomycin hydrochloride, 1 mg, and dexamethasone sodium phosphate, 400 µg in 0.1 mL isotonic sodium chloride. Control eyes received injections of 0.1 mL isotonic sodium chloride solution alone.

With the use of portable slitlamp biomicroscopy and indirect ophthalmoscopy, clinical grading was performed by masked observers (S.M.L. and T.W.) on days 7 and 14 after institution of treatment. The severity of endophthalmitis was graded according to a modified scheme of Peyman et al.13 (Table 1). On day 14, the eyes were enucleated for histopathologic study. Whole eyes were fixed in 10% buffered formalin and embedded in paraffin. Representative 5-µm sections were stained with hematoxylin-eosin and examined by an ophthalmic pathologist (M.R.) who was unaware of the treatment each eye received. Intraocular inflammatory changes of endophthalmitis were graded according to the criteria listed in Table 2. The clinical and histopathologic grades of treated eyes were compared with those of untreated eyes by means of the Wilcoxon rank sum test. A statistically significant difference was defined as P<.05 when the groups were compared.

### Table 1. Clinical Grading of Endophthalmitis Severity

<table>
<thead>
<tr>
<th>Structure, Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conjunctiva</strong></td>
<td>Normal</td>
</tr>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Mild chemosis</td>
</tr>
<tr>
<td>2</td>
<td>Moderate chemosis, mild to moderate hyperemia, slight exudate</td>
</tr>
<tr>
<td>3</td>
<td>Severe chemosis, marked hyperemia, heavy exudate</td>
</tr>
<tr>
<td><strong>Cornea</strong></td>
<td>Clear</td>
</tr>
<tr>
<td>0</td>
<td>Clear</td>
</tr>
<tr>
<td>1</td>
<td>Focal edema</td>
</tr>
<tr>
<td>2</td>
<td>Diffuse edema (iris visible)</td>
</tr>
<tr>
<td>3</td>
<td>Opaque (no view of iris)</td>
</tr>
<tr>
<td><strong>Iris</strong></td>
<td>Normal</td>
</tr>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Mild hyperemia</td>
</tr>
<tr>
<td>2</td>
<td>Moderate hyperemia</td>
</tr>
<tr>
<td>3</td>
<td>Marked hyperemia</td>
</tr>
<tr>
<td><strong>Vitreous</strong></td>
<td>Clear</td>
</tr>
<tr>
<td>0</td>
<td>Clear</td>
</tr>
<tr>
<td>1</td>
<td>Mild vitreous haze, visible fundus, good red reflex</td>
</tr>
<tr>
<td>2</td>
<td>Moderate vitreous haze, poor fundus view, partial red reflex</td>
</tr>
<tr>
<td>3</td>
<td>Marked vitreous haze or opacity, no red reflex</td>
</tr>
</tbody>
</table>

And dexamethasone sodium phosphate injections. On day 0, the eyes were examined. This was approximately 24 hours after the initial inoculation with *B. cereus*. All eyes showed similar amounts of clinical endophthalmitis, which was defined as eyes having moderate conjunctival chemosis and hyperemia in the presence of vitreous haze or corneal edema obscuring view of the retina. The animals were then anesthetized using intramuscular injection. Those eyes randomized to receive antibiotic treatment alone received an intraocular injection of vancomycin hydrochloride, 1 mg in 0.1 mL isotonic sodium chloride solution. Eyes receiving combined treatment were injected with vancomycin hydrochloride, 1 mg, and dexamethasone sodium phosphate, 400 µg in 0.1 mL isotonic sodium chloride. Control eyes received injections of 0.1 mL isotonic sodium chloride solution alone.

With the use of portable slitlamp biomicroscopy and indirect ophthalmoscopy, clinical grading was performed by masked observers (S.M.L. and T.W.) on days 7 and 14 after institution of treatment. The severity of endophthalmitis was graded according to a modified scheme of Peyman et al13 (Table 1). On day 14, the eyes were enucleated for histopathologic study. Whole eyes were fixed in 10% buffered formalin and embedded in paraffin. Representative 5-µm sections were stained with hematoxylin-eosin and examined by an ophthalmic pathologist (M.R.) who was unaware of the treatment each eye received. Intraocular inflammatory changes of endophthalmitis were graded according to the criteria listed in Table 2. The clinical and histopathologic grades of treated eyes were compared with those of untreated eyes by means of the Wilcoxon rank sum test. A statistically significant difference was defined as P<.05 when the groups were compared.

### Table 3

On day 14, the eyes of treated eyes were compared with those of untreated eyes by means of the Wilcoxon rank sum test. A statistically significant difference was defined as P<.05 when the groups were compared. The average histologic grade of 7 intraocular tissues for each eye was determined by an ophthalmic pathologist (M.R.) who was unaware of the study randomization. These eyes were graded and analyzed according to the

Histopathologic Examination

The average histologic grade of 7 intraocular tissues for each eye was determined by an ophthalmic pathologist (M.R.) who was unaware of the study randomization. These eyes were graded and analyzed according to the
scheme in Table 2. On day 14, eyes that were treated with antibiotic, with or without corticosteroids, were found to have significantly less intraocular inflammation than controls (Table 4; \( P < .05 \)). Fewer of the treated eyes had mild conjunctival inflammatory changes or increased corneal thickness compared with controls. Eyes given combination treatment also had a marked decrease in the amount of retinal necrosis compared with controls. Although eyes that received corticosteroids and antibiotics had lower overall cornea, iris, lens, choroid, and retina scores compared with eyes that received vancomycin alone, there was no statistically significant difference among the groups. There was no statistically significant difference in the histopathologic vitreous or choroid scores among any of the 3 experimental groups.

### Table 2. Histopathologic Grading of Endophthalmitis Severity

<table>
<thead>
<tr>
<th>Structure</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctiva</td>
<td>Normal</td>
<td>Mild inflammation</td>
<td>Moderate inflammation</td>
<td>Marked inflammation</td>
</tr>
<tr>
<td>Cornea</td>
<td>Normal</td>
<td>Focal thickening</td>
<td>Diffuse thickening</td>
<td>Marked thickening</td>
</tr>
<tr>
<td>Iris</td>
<td>Clear</td>
<td>Few inflammatory cells</td>
<td>Mild cellular infiltrate</td>
<td>Severe iridocyclitis</td>
</tr>
<tr>
<td>Vitreous</td>
<td>Clear</td>
<td>Occasional cells</td>
<td>Moderate cells or early fibrin membranes</td>
<td>Marked cellular infiltrate, opacity</td>
</tr>
<tr>
<td>Choroid</td>
<td>Normal</td>
<td>Focal vascular changes, thickening</td>
<td>Moderate vasculitis, more diffuse changes</td>
<td>Marked fibrinous membranes, cells</td>
</tr>
<tr>
<td>Retina</td>
<td>Normal</td>
<td>Focal retinitis</td>
<td>Diffuse retinitis</td>
<td>Marked necrosis</td>
</tr>
</tbody>
</table>

Data are given as mean ± SEM histopathologic grade, described in Table 2.

### Table 3. Clinically Graded Endophthalmitis

<table>
<thead>
<tr>
<th>Grade, Treatment Group</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitreous</strong></td>
<td><strong>Control</strong></td>
<td><strong>Vancomycin Hydrochloride</strong></td>
<td><strong>Dexamethasone Sodium Phosphate and Vancomycin</strong></td>
</tr>
<tr>
<td><strong>Conjunctiva</strong></td>
<td>2.50 ± 0.52</td>
<td>2.23 ± 0.57</td>
<td>2.38 ± 0.59</td>
</tr>
<tr>
<td><strong>Cornea</strong></td>
<td>1.50 ± 0.70</td>
<td>1.46 ± 0.65</td>
<td>1.54 ± 0.63</td>
</tr>
<tr>
<td><strong>Iris</strong></td>
<td>1.57 ± 0.49</td>
<td>1.31 ± 0.58</td>
<td>1.54 ± 0.43</td>
</tr>
<tr>
<td><strong>Vitreous</strong></td>
<td>2.43 ± 0.57</td>
<td>2.31 ± 0.47</td>
<td>2.38 ± 0.53</td>
</tr>
</tbody>
</table>

Data are given as mean ± SEM clinical grade, described in Table 1. Treatment groups are described in the “Materials and Methods” section.

\( * P < .05 \), compared with controls and eyes receiving vancomycin alone.

### Table 4. Histopathologically Graded Endophthalmitis

<table>
<thead>
<tr>
<th>Structure</th>
<th>Control</th>
<th>Vancomycin Hydrochloride</th>
<th>Dexamethasone Sodium Phosphate and Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctiva</td>
<td>1.07 ± 0.35</td>
<td>0.46 ± 0.22†</td>
<td>0.46 ± 0.24†</td>
</tr>
<tr>
<td>Cornea</td>
<td>1.00 ± 0.23</td>
<td>0.46 ± 0.30†</td>
<td>0.38 ± 0.28†</td>
</tr>
<tr>
<td>Iris</td>
<td>1.57 ± 0.41</td>
<td>1.00 ± 0.53</td>
<td>0.69 ± 0.28†</td>
</tr>
<tr>
<td>Lens</td>
<td>1.77 ± 0.37</td>
<td>0.75 ± 0.44†</td>
<td>0.64 ± 0.50†</td>
</tr>
<tr>
<td>Vitreous</td>
<td>1.93 ± 0.65</td>
<td>1.38 ± 0.70</td>
<td>1.38 ± 0.65</td>
</tr>
<tr>
<td>Choroid</td>
<td>1.86 ± 0.66</td>
<td>1.23 ± 0.55</td>
<td>1.15 ± 0.59</td>
</tr>
<tr>
<td>Retina</td>
<td>1.71 ± 0.38</td>
<td>1.46 ± 0.23</td>
<td>0.85 ± 0.35†‡</td>
</tr>
</tbody>
</table>

Data are given as mean ± SEM histopathologic grade, described in Table 2. Treatment groups are described in the “Materials and Methods” section.

\( †P < .05 \), compared with controls.

\( ‡P < .05 \), compared with eyes receiving vancomycin alone.

Bacillus cereus endophthalmitis is a devastating event for most affected patients. Subjects typically experience decreased visual acuity, pain, severe chemosis, and periorbital swelling. A corneal ring abscess develops in many. The infection can progress rapidly, affecting anterior and posterior segment components. In its worst manifestation, it may require enucleation in as little as 48 hours.

The role of corticosteroids in the treatment of endophthalmitis is controversial. To our knowledge, this is the first evaluation of their use in the primary treatment of B cereus endophthalmitis. Some clinicopathologic studies with other pathogens suggest that concurrent intraocular corticosteroid treatment in rabbit eyes with induced endophthalmitis led to significant benefits. In experimental S aureus endophthalmitis, early treatment with dexamethasone and vancomycin was found to be beneficial. Bacillus cereus infection of the eye may lend itself well to the study of corticosteroid effects in the treatment of endophthalmitis. Mounting evidence suggests that exotoxins are an important mediator of tissue damage caused by B cereus endophthalmitis. Multiple extracellular virulence factors, including hemolysin BL (hemolysin composed of binding component, B, and 2 lytic components, L1 and L2), have been implicated in contributing to the toxic effects of B cereus intraocular infections, through direct effects and active inflammation. If corticosteroid treatment can mili-
gate or decrease the effects of these toxins during the active phase of infection, the resulting tissue damage potentially could be reduced.

Our results indicate that concurrent use of vancomycin and dexamethasone in the treatment of *B. cereus* endophthalmitis leads to an improvement in the clinical picture of the affected rabbit eye by day 14 of treatment. These results are in agreement with those of other groups, who have found a beneficial corticosteroid effect in the treatment of infectious endophthalmitis. We postulate that the addition of corticosteroids to the treatment regimen for *B. cereus* endophthalmitis may lessen the severity of the inflammatory activity of the Bacillus exotoxins, particularly in the anterior segment. In our study, there was no beneficial effect on the posterior segment structures that could be identified clinically. However, cataracts developed by day 7 in a significant percentage (18/40 [45%]) of untreated and treated eyes, resulting in a poor view of posterior segment structures clinically. For this reason, our clinical data on vitreous inflammation may have been affected by the clarity of the lens in some cases.

Nevertheless, histopathologic analysis suggests that the addition of dexamethasone did have a beneficial effect on the retina. Microscopic examination of the eyes on day 14 revealed a significant decrease in graded retinal inflammatory changes in the eyes given combination therapy compared with controls and eyes receiving vancomycin alone. This suggests that the anti-inflammatory effect of dexamethasone may be acting to reduce the exotoxin-mediated damage to the retina. There was no definitive difference seen in the choroid. The clinical difference in the anterior segment scores between the eyes receiving the combination treatment and the eyes receiving vancomycin alone was suggested, but not definitively confirmed, by results of histopathologic analysis of the tissues. In fact, both treated groups showed similar decreases in the levels of graded histopathologic inflammatory damage in their anterior segment structures when compared with control eyes.

Our results also demonstrate that vancomycin treatment alone in *Bacillus* endophthalmitis led to improved clinical scores at days 7 and 14. Other groups have reported clinical improvement with vancomycin treatment of endophthalmitis involving other bacterial and fungal species, although another group reported no improvement with vancomycin alone in their study of *Streptococcus pneumoniae* endophthalmitis.

Adjuvant dexamethasone treatment with vancomycin decreases the severity of clinical anterior segment inflammation in experimental *B. cereus* endophthalmitis. It has a beneficial effect on the retina based on our histopathologic findings in this rabbit model. Our results also indicate that there is no increase in inflammatory tissue damage or exacerbation of the infection secondary to addition of dexamethasone to the treatment regimen. There was no clinical or histopathologic evidence of a secondary bacterial or fungal infection as a result of the dexamethasone addition. Culture of the vitreous and/or retina may be a more direct way of determining this possibility. Based on our results, it is possible that intravitreal administration of corticosteroids may play an important role in limiting the inflammatory damage of exotoxins in *Bacillus*-mediated endophthalmitis. Further studies examining the doses and time course of corticosteroid injection(s), and whether dexamethasone may interfere with the ability of the immune system to fight the infection, may help define an appropriate method to maximize the benefits of corticosteroids as an adjunctive treatment of inflammation due to *B. cereus* endophthalmitis.

Accepted for publication January 3, 2000.

We thank Bing-Jing Roberts for her technical assistance and Sadaf Quayumi, MS, Department of Microbiology, Veterans Affairs Hospital, Baltimore, Md, for providing the Bacillus cereus samples. Nancy Ellish, DrPH, provided statistical support.

Corresponding author: Scott M. Steidl, MD, DMA, Department of Ophthalmology, University of Maryland Medical Center, 22 S Greene St, Baltimore, MD 21201 (e-mail: ssteidl@som.umaryland.edu).

REFERENCES