Evaluation of Therapeutic Measures for Treating Endophthalmitis Caused by Isogenic Toxin-Producing and Toxin-Nonproducing Enterococcus faecalis Strains

Bradley D. Jett,*† Harold G. Jensen,† Rajeshwari V. Atkuri,‡ and Michael S. Gilmore§

Purpose. Management of endophthalmitis typically includes antibiotic combinations to arrest bacterial growth and antiinflammatory agents to limit inflammatory damage to sensitive tissues. Little research has been reported that systematically evaluates the contribution of each therapeutic component for treating infections caused by organisms of varying virulence. The authors determined the relative value of the antiinflammatory corticosteroid, dexamethasone, as an intravitreal therapeutic adjunct for the treatment of infection caused by either Enterococcus faecalis expressing a cytolytic toxin previously shown to contribute to the course and severity of infection, or an otherwise identical strain of E. faecalis specifically attenuated in expression of the cytolytic toxin.

Methods. Endophthalmitis in rabbits was monitored using electroretinography (ERG). Eyes were infected with 100 colony forming units of either the cytolytic or the noncytolytic E. faecalis strain. Intravitreal ampicillin and gentamicin were administered at postinfection day 1, and intravitreal dexamethasone was either omitted or administered at day −1, 1, or 1.5.

Results. ERG B-wave amplitude declined precipitously throughout the course of infection with cytolytic toxin-producing E. faecalis, despite the administration of antibiotics and regardless of the time of dexamethasone administration. In fact, the ultimate course of infection caused by cytolytic E. faecalis did not differ from the course in untreated controls. In contrast, infections caused by specifically attenuated, noncytolytic strains of E. faecalis responded well to antibiotics augmented by antiinflammatory therapy when the latter was administered either 1 or 1.5 days after the initiation of infection. In these cases, no loss in ERG B-wave response was observed.

Conclusions. These results underscore the importance of bacterial toxins in infectious diseases of the eye and their contribution to treatment failures. These results further suggest that in cases of endophthalmitis caused by toxin producing bacteria, significant improvement in clinical outcome will require specific therapeutic targeting of the toxins involved. Invest Ophthalmol Vis Sci. 1995;36:9–15.

Endophthalmitis is a devastating complication of intraocular surgery or penetrating ocular injury. The most common etiologic agent of bacterial endophthalmitis is Staphylococcus epidermidis, and these infections generally respond well to therapeutic measures. However, endophthalmitis resulting from infection by other common causes, including Staphylococcus aureus, Streptococcus spp, Pseudomonas spp, and Bacillus spp, is associated with a much poorer visual outcome. In a previous study, we used transposon insertion to inactivate the gene encoding a cytolytic toxin expressed by some strains of Enterococcus faecalis. Derivation of such mutants allowed us to demonstrate directly that the cytolytic toxin makes a major contribution to the course and severity of enterococcal endophthalmitis. Similar direct tests have yet to be performed on the toxins expressed by other bacteria associated with fulminant or destructive endophthalmitis, but it has been speculated that toxin production contributes to the poor prognosis observed for other bacteria as well.

In addition to bacterial toxins, the host inflamma-
tory response makes a measurable contribution to the
damage that results from bacterial endophthalmitis.
Intraocular fibrocellular proliferation, membrane for-
mation, and traction retinal detachment have all been
described as secondary complications of the host re-
sponse that requires aggressive management to limit
visual loss.11 Dexamethasone has been found to be a
safe and effective adjunct to broad-spectrum antibiot-
cs for suppressing the inflammatory response during
treatment for endophthalmitis.3-7 Despite aggressive
therapy with synergistic antibiotic combinations
augmented with antiinflammatory agents, endoph-
thalmitis remains a sight-threatening condition with
frequently poor outcome.3-7 To define the basis for
endophthalmitis treatment failure, we systematically
analyzed the value of intravitreal antiinflammatory
therapy in treating infections by isogenic toxin-pro-
ducing and nontoxicogenic bacteria. The results of this
study highlight the importance of devising new thera-
pies that directly target contributory toxins expressed
by toxigenic organisms if the prognosis for endoph-
thalmitis caused by the latter is to be improved.

MATERIALS AND METHODS

Bacterial Strains and Media

E. faecalis strain JH2SS harboring transposon Tn917
insertional mutations in the cytolysin-encoding plas-
mid pAD1 was used in this study.3 JH2SS(pAM771)
harboring Tn917 insertion in the cytolysin-encoding
region of pAD1 was selected as the noncytolytic mu-
ant. JH2SS(pAM714) contains Tn917 insertion in a
region of pAD1 not affecting cytolysin function and
was chosen as the isogenic, cytolysin-producing strain
(see ref. 9 for physical map of pAD1 and phenotype
of strains used in this study). E. faecalis strains were
routinely propagated overnight at 37°C in brain–heart
infusion broth (BHI; Difco, Detroit, MI) supple-
mented with streptomycin (500 μg/ml), spectino-
mycin (500 μg/ml), and erythromycin (10 μg/ml)
(Sigma, St. Louis, MO). Before intraocular inocula-
tion, organisms were harvested by centrifugation,
washed twice in sterile balanced salt solution (BSS;
Dey Laboratories, Napa, CA), and resuspended in BSS
at a final concentration of approximately 100 colony
forming units (cfu)/0.1 ml, as previously described.9,13
Viable organisms were enumerated at the conclusion
of the experiments by plating vitreal contents on BHI
agar (1.5% agar) supplemented with 5% human eryth-
rocytes, as previously described.9,13

Vertebrate Animals

New Zealand White rabbits (each weighing 2 to 4 kg)
were housed and cared for at the Dean A. McGee Eye
Institute (Oklahoma City, OK) animal care facility in
accordance with the ARVO Statement for the Use of
Animals in Ophthalmic and Vision Research.

Intraocular Injections

Intravitreal inoculation of anesthetized animals was
performed as previously described.9,13 General anes-
thesia consisted of intramuscular ketamine and xylaz-
ine. Proparacaine was used as topical anesthesia.
Briefly, pupils of anesthetized rabbits were dilated,
and approximately 0.1 ml of aqueous humor was aspi-
rated with a tuberculin syringe to relieve intraocular
pressure. Approximately 100 cfu (0.1 ml) of E. faecalis
were introduced intravitreally through the pars plana
approximately 3 mm from the limbus with a 25-gauge
needle and a 1-ml syringe. Experimental day 0 was
defined as the time of injection of 100 cfu E. faecalis
JH2SS(pAM771) or JH2SS(pAM714). Combination
antimicrobial therapy consisting of ampicillin (1 mg/
0.1 ml) and gentamicin (200 μg/0.1 ml) was adminis-
tered intraocularly on experimental day 1 (24 hours
after infection) in all animals. This antimicrobial com-
bination has been described as effective therapy for a
variety of infections caused by beta-lactam–aminogly-
coside-sensitive enterococci.14 Dexamethasone (400
μg/0.1 ml) was either omitted or injected intra-
ocularly at experimental day −1 (24 hours before infec-
tion), +1 (24 hours after infection), or +1.5 (36 hours
after infection). Eyes receiving no injections served as
absolute controls, whereas surgical control eyes re-
ceived 0.1 ml sterile BSS.

Electroretinography

The course of infection was monitored using scotopic
electroretinography (ERG), an objective measure that
was shown previously to parallel clinical observa-
tions.9,13 ERG was performed, as previously de-
scribed,9,13 on experimental days −1, +1, +3, and +5.
B-wave amplitude measurements were expressed as
mean percent of retained baseline (pre-experimental)
B-wave amplitude: [(experimental B-wave amplitude
baseline B-wave amplitude) × 100] ± standard error
of the mean. Immediately after the final ERG, anesthe-
itized animals were killed with pentobarbital and phe-
nytoin, and eyes were enucleated for bacterial quanti-
tation as described above.

RESULTS

As a first step in assessing the efficacy of standard
regimens for treatment of endophthalmitis, two pa-
rameters were systematically analyzed, the administra-
tion of the antiinflammatory corticosteroid dexameth-
asone and the timing of administration and the viru-
ulence of the organism causing endophthalmitis. The
first set of experiments examined the value of dexa-
methasone as an adjunct to antimicrobial therapy in
Dexamethasone and *E. faecalis* Endophthalmitis

The data presented in Figure 1 show that, irrespective of treatment group, eyes infected with attenuated, noncytolytic *E. faecalis* JH2SS(pAM771) exhibited no significant loss in ERG B-wave amplitude 24 hours after infection. These findings are consistent with previous observations on the kinetics of untreated endophthalmitis caused by noncytolytic *E. faecalis*.9,13 Treatment with the antibiotic regimen that included 1 mg ampicillin and 200 μg gentamicin 24 hours after infection did little to alter the ensuing precipitous decline in ERG responsiveness. As shown in Figure 1 (curves 1 and 2), ERG loss at day 3 in eyes treated with combined antibiotics without the antiinflammatory agent was virtually identical to that observed for eyes that received no treatment at all. In contrast, eyes that received combined antibiotic therapy augmented by simultaneous administration of 400 μg of dexamethasone exhibited no significant loss in ERG at 3 or 5 days after infection when compared to surgical and absolute controls (day 1, *P* > 0.5; day 3, *P* > 0.1; Student's *t* test). These results clearly illustrate the value of the inclusion of antiinflammatory agents in the therapeutic regimen for treatment of organisms that are deficient in production of a cytolytic virulence factor.

Repeating this line of experimentation with isogenic *E. faecalis* strain JH2SS(pAM714) that expresses wild-type levels of the cytolsin yielded a strikingly different result. As shown in Figure 2, a substantial reduction in ERG B-wave amplitude for all groups occurred by 24 hours after infection with the cytolsin expressing *E. faecalis* strain, as has been described previously.15 After the administration of combined antibiotic therapy alone (that is, without dexamethasone), ERG responsiveness was lost at a rate that was not significantly different from eyes receiving no treatment (Fig. 2, curves 1 and 2; *P* > 0.5). In contrast to the above results with attenuated organisms, eyes infected with the cytolytic strain and treated with combined antibiotics and dexamethasone at 24 hours after infection showed no moderation in the loss of ERG B-wave amplitude. This result indicates that although dexamethasone was completely effective in limiting loss of retinal function in eyes infected with an attenuated strain, it was of surprisingly little value in the treatment of infections caused by the isogenic, cytolsin-producing strain. Moreover, there was no difference in outcome whether or not any therapy was provided.

The timing of dexamethasone administration was varied to determine whether treatment could be optimized to salvage residual ERG responsiveness in eyes infected with cytolytic *E. faecalis*. A recommendation has been made previously that antiinflammatory therapy for infectious endophthalmitis be postponed for 12 hours after antibiotic administration to allow for efficient killing of the offending organism.15 It was, therefore, of interest to determine whether postponing treatment of endophthalmitis caused by a specifically attenuated, noncytolytic strain of *E. faecalis*, JH2SS(pAM771) was administered as described on graph. No Dex = ampicillin/gentamicin alone at postinfection day 1. Cyl+ = noncytolytic *E. faecalis*. Absolute controls = no injection.

Surgical controls = saline injection only. Numbers in boldface brackets are referred to in text as curves 1, 2, 3, and so on. Absolute and surgical control data are included only in Figure 1 for clarity of graphs.

**FIGURE 1.** B-wave amplitude retention in eyes infected with noncytolytic *Enterococcus faecalis*. Amp (ampicillin, 1000 μg) and Gen (gentamicin, 200 μg) were administered at postinfection day 1 except untreated animals, absolute control animals, and surgical control animals. Dex (dexamethasone, 400 μg) was administered as described on graph. No Dex = ampicillin/gentamicin alone at postinfection day 1. Cyl+ = noncytolytic *E. faecalis*. Absolute controls = no injection. Surgical controls = saline injection only. Numbers in boldface brackets are referred to in text as curves 1, 2, 3, and so on. Absolute and surgical control data are included only in Figure 1 for clarity of graphs.

**FIGURE 2.** B-wave amplitude retention in eyes infected with cytolytic *Enterococcus faecalis*. Amp (ampicillin, 1000 μg) and Gen (gentamicin, 200 μg) at postinfection day 1 except untreated animals, absolute control animals, and surgical control animals. Dex (dexamethasone, 400 μg) was administered as described on graph. No Dex = ampicillin/gentamicin alone at postinfection day 1. Cyl+ = cytolsin-producing *E. faecalis*. Numbers in boldface brackets are referred to in the text as curves 1, 2, 3, and so on. Absolute and surgical control data are included only in Figure 1 for clarity of graphs.
Previous reports on the nature of endophthalmitis caused by cytolytic strains of *Enterococcus faecalis* showed that a loss in ERG responsiveness was easily observed by 24 hours, an observation confirmed in the present study. To determine whether prophylactic antiinflammatory therapy would delay the initial rapid loss of ERG function or would otherwise enhance retention of retinal function, especially in infections of the cytolytic strain, the following experiment was performed. Two groups of animals were treated 24 hours postinfection with dexamethasone. One group was infected with the cytolytic strain JH2SS(pAM714), and the other group was infected with the isogenic, attenuated noncytolytic strain JH2SS(pAM771). Both groups were then treated with combined antibiotic therapy at 24 hours postinfection, and the course of infection was followed by ERG. As shown in Figure 4, eyes infected with the attenuated, noncytolytic strain JH2SS(pAM771) and treated preemptively with dexamethasone lost ERG responsiveness (curve 2) to a significantly greater degree than eyes similarly infected with the attenuated strain but treated simultaneously at 24 hours after infection with antibiotics and dexamethasone (curve 1; *P* < 0.04, days 3 and 5). In this case, predadministration of dexamethasone before infection provided no benefit because the outcome was not significantly different from that observed for eyes that were infected with the attenuated strain and received no therapy or that received antibiotics alone (*P* > 0.3). Significant differences in ERG B-wave amplitudes were observed at postinfection day 1 for dexamethasone-pretreated eyes infected with the cytolytic strain JH2SS(pAM714) (curve 3; *P* = 0.009). However, this initial enhancement of ERG responsiveness did not persist, and no significant differences were noted between treatment groups infected with the cytolytic organism at day 5 or 3 (Fig. 4; *P* > 0.16). Vitreous was examined for the presence of viable organisms at postinfection day 5. Vitreous from a single eye, belonging to the group infected with the attenuated strain JH2SS(pAM771) and receiving dexamethasone 24 hours before infection, grew a low num-

![FIGURE 3. B-wave amplitude retention in eyes infected with cytolytic or noncytolytic *Enterococcus faecalis*. Amp (ampicillin, 1000 µg) and Gen (gentamicin, 200 µg) were administered at post infection day 1 except absolute control and surgical control animals. Dex (dexamethasone, 400 µg) was administered as described on graph. Cyl+ = cytolytic producing *E. faecalis*. Cyl— = noncytolytic *E. faecalis*. Numbers in boldface brackets are referred to in text as curves 1, 2, 3, and so on. Absolute control and surgical control data are included only in Figure 1 for clarity of graphs.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933181/)
Dexamethasone and *E. faecalis* Endophthalmitis

ber of organisms at day 5 (fewer than $10^2$ cfu/ml). All other eyes receiving antibiotics and dexamethasone in this study were sterile at postinfection day 5.

**DISCUSSION**

To identify the basis for treatment failure and associated catastrophic loss of vision, the value of intravitreal antibiotic and antiinflammatory therapies was compared systematically for treatment of endophthalmitis caused by isogenic strains of *E. faecalis*. To our knowledge, this is the first report comparing the efficacy of therapeutic regimens for the treatment of eye infections caused by bacteria specifically attenuated in expression of a virulence factor, in this case the cytolysin, using molecular biologic techniques. The existence of strains identical genetically, except in the production of cytolysin, allowed us to determine the specific contribution of this virulence factor to treatment failure.

Previous studies characterizing the natural course of endophthalmitis demonstrated that the growth rate of cytolytic and noncytolytic *E. faecalis* strains in vivo is comparable, achieving numbers of approximately $10^6$ cfu per gram of vitreous. Histopathologic examination of tissues from these infections revealed that retinas from infections of the cytolytic strain exhibited substantial disorganization and lysis of cells in all retinal layers. In contrast, retinas from infections of the attenuated, noncytolytic isogenic *E. faecalis* strain were structurally intact, with the primary finding a substantial infiltration of immune cells into the vitreous. These findings suggested that ERG loss in eyes infected with the wild-type cytolytic strain was the result of toxin-mediated destruction of the retina, whereas reduction in ERG in eyes infected with the noncytolytic strain was attributable to the inflammatory reaction, perhaps opacification of the vitreous, as previously reported. Both deductions are supported by the findings of the present study.

The administration of the corticosteroid dexamethasone as an adjunct to antimicrobial therapy, consisting of a combination of ampicillin and gentamicin, was highly effective in preserving ERG response in eyes infected with the attenuated, noncytolytic strain of *E. faecalis*. The efficacy of dexamethasone was independent of the time of administration at the two times tested—24 or 36 hours after infection (simultaneously with or 12 hours after antibiotic administration, respectively). Prophylactic administration of dexamethasone 24 hours before infection provided no enhancement in ERG retention over control experiments where dexamethasone therapy was omitted.

The impact of the expression of a single virulence factor dramatically altered the therapeutic responsiveness of endophthalmitis in the model tested. When eyes were infected with *E. faecalis* strains differing only in the expression of the *E. faecalis* cytolysin (recognizable clinically by the occurrence of zones of hemolysis surrounding *E. faecalis* colonies cultured on agar containing erythrocytes from a source other than sheep), the resultant endophthalmitis was refractory to antibiotic treatment with or without coadministered dexamethasone. Manipulation of the timing of dexamethasone administration did not significantly affect the negative treatment outcome. However, a measurable, though short-lived, benefit was observed when cytolytic infections were pretreated with dexamethasone, arguing strongly against a delay in antiinflammatory therapy. With cytolytic infections, the observation that the ultimate outcome was not enhanced by any therapy over the results of untreated infection emphasizes the importance of characterizing the offending toxin and targeting it for therapeutic intervention.

Dexamethasone has been shown to be safe and efficacious in the treatment of human intraocular infections and in animal models. Its use as an adjunct in the treatment of endophthalmitis was recently reviewed. Graham and Peyman observed a significant reduction in inflammatory response in the anterior and posterior chambers, vitreous, retina, and choroid in experimental *Pseudomonas* endophthalmitis when dexamethasone and gentamicin were administered within 5 hours of infection. More severe inflammatory reactions were seen in eyes treated with gentamicin alone or eyes in which the administration of the antibiotic–corticosteroid combination was delayed until 10 hours after infection. Although broadly similar in experimental design, the experiments of Graham and Peyman differ from the present study in several respects. First, earlier studies used a considerably larger inoculum (20,000 cfu *Pseudomonas* versus 100 cfu *E. faecalis*), which may have accelerated the course of infection. Second, treatment of gram-negative endophthalmitis with antibiotics alone can result in the release of inflammatory endotoxin, thereby exacerbating inflammation-mediated ocular damage.

In the present study, the effects of dexamethasone administered 24 hours prior to infection were minimal. This loss of protective activity may result from dexamethasone clearance before infection. The intravitreal half-life of dexamethasone has been reported to be approximately 3 hours, with an approximate 500-fold decrease in intravitreal concentration at 24 hours.

The value of subconjunctival gentamicin or combination gentamicin–dexamethasone, when administered prophylactically, has been studied in a rabbit model of *Staphylococcus aureus* endophthalmitis. Although the coadministration of dexamethasone did not adversely affect the outcome of gentamicin prophylaxis, it provided no observable benefit over prophylactic gentamicin alone. Meredith et al used a...
rabbit model of *Staphylococcus epidermidis* endophthalmitis to show that intramuscular corticosteroid provided a level of antiinflammatory activity that was comparable to the effect of intravitreal administration. However, intravitreal injection appears to be an effective route of administration. It is unknown whether intramuscular corticosteroid would have been effective in the present study because intramuscular administration was not tested. Although corticosteroids appear to be a safe adjunct to endophthalmitis therapy, retinal toxicity has been observed with high dose (800 to 4000 μg) intravitreal dexamethasone.

Optimal antimicrobial combinations and route of administration for treatment of endophthalmitis are subjects of continuing debate. Stern et al observed that 5 of 7 culture-positive patients who were treated with a single antibiotic injection and no vitrectomy suffered either recurrence of their infection or did not respond to treatment. One patient who received repeated intravitreal antibiotic injections and all patients who received repeated intravitreal antibiotic injections in combination with vitrectomy experienced resolution of their infections. Forster et al recommended that intravitreal antibiotics be injected repeatedly at approximately 48- to 96-hour intervals in patients whose cultures are positive. However, Oum et al described toxic reactions in the retinas of rabbits, especially in the outer retina and retinal pigmented epithelium, after repeated intravitreal antibiotic injections. The present study found that a single injection of ampicillin-gentamicin was effective in sterilizing the vitreous of all but one eye by postinfection day 5.

The combination of ampicillin and gentamicin was chosen for the present study because this antimicrobial combination has been shown to be highly efficacious for the treatment of severe enterococcal infection. Although the laboratory *E. faecalis* strain JH2SS used in this study is susceptible to combined ampicillin and gentamicin, this combination therapy is effective in the treatment of fewer enterococcal isolates because of the increase in strains producing aminoglycoside-modifying enzymes. Because of the increase in gentamicin resistance and retinal toxicity, alternative combinations that include agents such as ceftazidime, amikacin, and vancomycin appear to be preferred over formerly recommended combinations for the initial treatment of endophthalmitis.

Patients with endophthalmitis often have a postoperative or posttraumatic emergency. Even though the incidence of endophthalmitis is low (0.12% to 0.16%) in the United States, numerous organisms are able to establish infection rapidly and to destroy intraocular tissue. Many organisms, such as *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus spp*, and *Pseudomonas spp* liberate potent toxins and tissue-damaging enzymes that may contribute to virulence in intraocular infections. The treatment of infections with such organisms is problematic because although the intraocular spaces may be sterilized with antibiotic infusions, significant amounts of bacterial debris and potentially toxic products remain. Aside from the direct demonstration of a role for *E. faecalis* cytolysin in contributing to the course and severity of endophthalmitis, the role of toxins expressed by other bacterial species remains speculative as the availability of control strains specifically deficient in production of the toxin of interest is limited. Based on the dramatic effect of cytolysin expression on treatment failure observed in the present study, however, it is likely that other bacterial toxins do play determinant roles in the outcome of endophthalmitis. We are actively testing this prospect for several of them.

Although many cases of postoperative endophthalmitis are caused by nontoxigenic, coagulase-negative staphylococci, most severe infections are caused by bacteria known to produce one or more toxins. Identifying toxins that contribute to the course, severity, and now therapeutic responsiveness of endophthalmitis caused by toxigenic organisms is critical if visual outcome in such cases is to be improved. Understanding at the molecular level of the structure and mechanism of the action of toxins found to be contributory will provide a basis for developing rational or information-based therapeutics. By comparing the structures and functions of several contributory toxins, it may be possible to derive new and general therapeutic principles that permit successful, empirical treatment of endophthalmitis even before the toxigenic nature of the offending organism is known. This analysis using isogenic strains of *E. faecalis* was facilitated by the fact that *E. faecalis* strains express, at most, a single recognized toxin, cytolysin. A similar analysis of virulence for *Staphylococcus aureus*, *Bacillus cereus*, or *Streptococcus pyogenes* will be much more formidable because each of these strains expresses a constellation of toxins.

**Key Words**

*Enterococcus faecalis*, endophthalmitis, dexamethasone, cytolysin, inflammation

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Dexamethasone and *E. faecalis* Endophthalmitis


