Intravitreal Dexamethasone Effect on Intravitreal Vancomycin Elimination in Endophthalmitis

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Objective: To determine whether intravitreal dexamethasone administration can alter the elimination of intravitreal vancomycin hydrochloride in rabbit eyes with experimental Streptococcus pneumoniae endophthalmitis.

Methods: Albin rabbits were infected with an intravitreal inoculum of S pneumoniae (2 \times 10^3 colony-forming units) and randomized after 24 hours to treatment with intravitreal vancomycin hydrochloride (1 mg), alone or in combination with intravitreal dexamethasone (400 µg). For comparison, uninfected eyes were similarly treated. All eyes were enucleated 24, 48, or 72 hours after treatment, and vitreous levels of vancomycin were quantitated using a fluorescence polarizing immunoassay.

Results: The half-life of intravitreal vancomycin in infected eyes was prolonged from 48 to 84 hours when eyes were treated with dexamethasone. Conversely, such treatment shortened the half-life in uninfected eyes from 56 to 42 hours.

Conclusions: Intravitreal dexamethasone administration reduces the elimination of intravitreal vancomycin in rabbit eyes with pneumococcal endophthalmitis, whereas an opposite effect is noted in uninfected eyes.

Clinical Relevance: In patients with eyes having endophthalmitis caused by virulent organisms, the elimination of intravitreal vancomycin may be reduced when intraocular inflammation is minimized with corticosteroid therapy. This may enhance the efficacy of intravitreal vancomycin therapy in treating the infection.


S TREPTOCOCCUS pneumoniae is a frequently encountered microorganism in endophthalmitis associated with a filtration bleb.1,2 Eyes with pneumococcal endophthalmitis generally have a poor outcome, despite prompt initiation of antimicrobial drug therapy, because of the destructive inflammatory response induced by the infection. In a previous study,3 we used a rabbit model of pneumococcal endophthalmitis to show that intravitreal dexamethasone therapy, when used in conjunction with intravitreal vancomycin administration, markedly reduced the inflammation and tissue destruction associated with the infection. A more recent study4 using a contrast-enhanced magnetic resonance imaging technique showed that intravitreal dexamethasone use also markedly reduced the associated blood-ocular barrier breakdown. Because the degree of breakdown correlates with the degree of intraocular accumulation of proteins and leukocytes from the blood,5-7 it is possible that a change in breakdown may affect the rate of elimination of a drug from the eye after intraocular administration. In this study, intravitreal vancomycin levels were measured in healthy eyes and in eyes with pneumococcal endophthalmitis to determine whether intravitreal dexamethasone therapy affects the elimination of vancomycin from the vitreous after intravitreal administration.

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RESULTS

Clinical signs of endophthalmitis were present after 24 hours in all rabbit eyes infected with S pneumoniae. Intravitreal vancomycin concentration 72 hours after treatment was significantly higher in eyes treated with intravitreal dexamethasone and vancomycin compared with those treated with vancomycin alone (P = .03) (Figure 1 and Table 1). Conversely, the intravitreal protein concentration, a marker of inflammation and breakdown of the blood-ocular barrier, was significantly...
MATERIALS AND METHODS

CULTURE OF BACTERIA

A strain of *S pneumoniae*, isolated from a patient’s corneal culture and used in a previous study of experimental pneumococcal endophthalmitis, was grown on *Brucella* agar containing 5% horse blood (BBL; Becton Dickinson Microbiology Systems, Cockeysville, Md) at 35°C as previously described. The cultured microorganisms were diluted in sterile saline solution to achieve a concentration of 2 × 10^8 colony-forming units (CFU)/mL. The suspension of bacteria was freshly made just before animal inoculation. A viable bacterial count of the suspension was confirmed by the growth on horse blood agar plates.

ANIMAL STUDY

All animals were maintained and cared for in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research. All animal experiments were conducted at the University of Texas Southwestern Medical Center, Dallas, in accordance with the study protocol approved by the institutional animal care and research advisory board. New Zealand albino rabbits weighing 2.0 to 2.5 kg were infected with an intravitreal inoculum of *S pneumoniae* (2000 CFU) as previously described (n = 7 per data point per group, N = 42 rabbits). Briefly, the rabbits were anesthetized with a 1-mL intramuscular dose of a solution containing an equal mixture of ketamine hydrochloride (100 mg/mL; Parke-Davis Pharmaceutical Research, Morris Plains, NJ) and xylazine hydrochloride (20 mg/mL; Mobay Corp, Shawnee, Kan). Topical anesthesia was achieved with 0.5% proparacaine hydrochloride ophthalmic solution (Allergan Inc, Irvine, Calif). When adequate anesthesia was achieved, anterior chamber paracentesis was performed in the right eye with a 30-gauge needle to yield about 0.1 mL of aqueous fluid. The right eye was infected with a direct intravitreal inoculum of live bacteria—a 0.1-mL suspension containing 2000 CFU—delivered through the pars plana into the vitreous chamber paracentesis was performed in the right eye with a 30-gauge needle. Infected eyes were examined by indirect ophthalmoscopy 24 hours after inoculation to confirm the presence of clinical signs of endophthalmitis, ie, moderate to severe conjunctival injection with vitreous haze resulting in at least partial obscuration of the retinal and choroidal vasculature. The eyes were randomized to treatment with intravitreal vancomycin hydrochloride (1 mg in 0.1 mL of solution), alone or in combination with intravitreal dexamethasone (400 µg in 0.1 mL of solution). For comparison, uninfected rabbit eyes were similarly randomized to treatment (n = 7 per data point per group).

The rabbits were euthanized 24, 48, or 72 hours after treatment using an intracardiac dose of pentobarbital sodium (120 mg/kg). The treated eyes were enucleated and promptly frozen in liquid nitrogen. The frozen eyes were dissected and the vitreous was obtained after removing the cornea, iris, lens, and retina-choroid-sclera layer. The vitreous concentration of vancomycin was measured using a fluorescence polarization immunoassay as previously described (TDX; Abbott Laboratories, North Chicago, Ill). The vitreous concentration of protein was measured with a commercially available protein assay that uses bovine serum albumin as a standard (BCA protein assay; Pierce Chemical Co, Rockford, Ill).

PHARMACOKINETIC AND STATISTICAL ANALYSIS

Pharmacokinetic analysis of the relationship between intravitreal vancomycin concentration and time was performed using a nonlinear least squares regression analysis as previously described. The vitreous concentration of vancomycin at time zero, estimated vitreous volume, and vitreous half-life of vancomycin were calculated based on observed intravitreal vancomycin concentrations. Based on the model selection criteria and coefficients of determination, the concentrations of intravitreal vancomycin over time were best fitted to a 1-compartment model, consistent with a previous report.

Statistical analysis was performed for comparison of means using an unpaired, 2-tailed t test. Statistical significance was defined as *P* ≤ .05.

To determine the effect of infection on the elimination of intravitreal vancomycin, intravitreal vancomycin and protein concentrations were compared between infected and uninfected eyes treated with vancomycin alone. Mean ± SEM intravitreal vancomycin concentration was significantly lower in the infected eyes 72 hours after treatment (78 ± 41 vs 120 ± 22 µg/mL; *P* = .04). Mean ± SEM intravitreal protein concentration was significantly higher in infected eyes 72 hours after treatment compared with uninfected eyes (24.3 ± 8.5 vs 6.1 ± 3.8 mg/mL, *P* < .001). On pharmacokinetic analysis, the infection shortened the vitreous half-life of intravitreal vancomycin from 56 to 48 hours (Table 2).

The possible benefits of intravitreal or systemic corticosteroid therapy to treat infectious endophthalmitis is an area of ongoing controversy and has yet to be shown in a prospective randomized clinical trial. However, several re-
ports of animal studies show that it reduces inflammation associated with the infection. In the case of pneumococcal endophthalmitis, it was previously shown histologically in a rabbit model that a single intravitreal injection of dexamethasone, in conjunction with vancomycin treatment, can dramatically reduce inflammation and tissue destruction associated with the infection.

In this study, we investigated whether intravitreal dexamethasone therapy may also alter the elimination of intravitreally administered antibiotic medication from the eye. Our results show that vancomycin is eliminated less readily in eyes with pneumococcal endophthalmitis when concurrently treated with dexamethasone. This corticosteroid therapy effect is correlated temporally with a reduction in intravitreal protein concentration, which is a marker of intraocular inflammation and blood-ocular barrier breakdown. Based on this observation, we speculate that the degree of breakdown of the blood-ocular barrier associated with the infection may affect the rate of vancomycin elimination from the eye. Consistent with this hypothesis, we found that, in the absence of corticosteroid therapy, the rate of elimination of intravitreal vancomycin was faster in eyes with pneumococcal endophthalmitis compared with uninfected eyes (Figure 1 and Table 2).

**Table 1. Vitreous Vancomycin Concentration After Intravitreal Injection in Rabbit Eyes With Pneumococcal Endophthalmitis**

<table>
<thead>
<tr>
<th>Time After Injection, h</th>
<th>Vitreous Vancomycin Concentration, Mean ± SEM, µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vancomycin Hydrochloride Treatment</td>
</tr>
<tr>
<td>24</td>
<td>218 ± 109</td>
</tr>
<tr>
<td>48</td>
<td>145 ± 24</td>
</tr>
<tr>
<td>72</td>
<td>76 ± 41</td>
</tr>
</tbody>
</table>

* Statistically significant difference from vancomycin treatment alone based on the t test (P < .05).

**Figure 1. Intravitreal vancomycin levels 24 to 72 hours after vancomycin hydrochloride injection (1 mg) (n = 7 per data point; values presented as mean ± SEM). A, Infected eye treated with vancomycin (1 mg) alone. B, Infected eye treated with vancomycin (1 mg) and dexamethasone (400 µg). C, Uninfected eye treated with vancomycin (1 mg) alone. D, Uninfected eye treated with vancomycin (1 mg) and dexamethasone (400 µg).**

**Figure 2. Intravitreal protein concentrations in eyes with pneumococcal endophthalmitis after treatment with vancomycin hydrochloride, alone or combined with dexamethasone (n = 7 per data point; values presented as mean ± SEM). Asterisk indicates a statistically significant difference from eyes not treated with intravitreal corticosteroid drugs, based on an unpaired t test (P < .05).**
Schlemm, as is the case with intravitreally administered vancomycin.18

Whether this decrease in the elimination of intravitreal vancomycin by corticosteroid therapy in infected eyes alters the bactericidal activity of the antibiotic drug is unknown. We did not address this question because the previous report17 using this animal model showed that rabbit eyes infected with pneumococcus become sterile within 7 days of infection, regardless of whether the eye was treated with antibiotic drugs. However, the observations made in this study are noteworthy since repeated intravitreal injection of antibiotic is frequently advocated in eyes with endophthalmitis that show minimal response to the initial antibiotic injection due to the relative rapid elimination of intravitreally injected antibiotics from the eye.19 Unfortunately, these second injections are associated with toxic adverse effects on the retina.20,21

The second observation of this study is that the corticosteroid effect on intravitreal vancomycin elimination noted in eyes with pneumococcal endophthalmitis contrasts with that seen in uninfected eyes. As shown in Figure 1 and Table 2, an opposite effect of dexamethasone on intravitreal protein concentration (Figure 3), and is unlikely to be secondary to an increase in intraocular inflammation resulting from additional trauma of a second intravitreal pharmacological injection. Although the exact mechanism for this observed corticosteroid therapy effect in uninfected eyes is unclear, it is similar to that reported previously12 in an animal model of Staphylococcus epidermidis endophthalmitis. In that study,12 eyes treated with intravitreal dexamethasone and vancomycin had lower vancomycin concentration than eyes treated with vancomycin alone, regardless of whether the eye was infected. The authors speculate that, because the normal route of elimination of vancomycin is anteriorly through the canal of Schlemm,18 intravitreal dexamethasone administration may facilitate this elimination in uninfected or minimally inflamed eyes. We speculate that, in eyes associated with severe inflammation due to infection with severe breakdown in the blood-aqueous barrier, both anteriorly and posteriorly—as is the case in eyes with pneumococcal endophthalmitis5—the effect of intravitreal corticosteroid in reducing the blood-aqueous barrier breakdown may have a more pronounced effect on the rate of elimination of intravitreal vancomycin from the eye than the possible direct effect of intravitreal corticosteroid in enhancing the elimination of vancomycin through the canal of Schlemm.

In conclusion, results of this study show that intravitreal dexamethasone treatment decreases the rate of elimination of intravitreal vancomycin from eyes with pneumococcal endophthalmitis, and enhances this rate in uninfected eyes. This observation further supports the use of intravitreal dexamethasone in the short-term management of infectious endophthalmitis that is characterized by marked inflammation on initial examination, especially when more virulent organisms are suspected to be involved, such as in cases of posttraumatic or postinfiltration bleb endophthalmitis. When endophthalmitis follows cataract surgery with mild or moderate inflammation at initial examination and less virulent microorganisms may be involved, the possible effect of intravitreal dexamethasone use to enhance the rate of elimination of intravitreal antibiotic drugs from the eye should be considered.

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