Coryneform Group A-4 Endophthalmitis

An Experimental Animal Model

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Diphtheroids, members of the coryneform family of bacteria, increasingly have been recognized as the cause of serious ocular diseases. After isolation of coryneform group A-4 from two patients with delayed endophthalmitis after cataract extraction and intraocular lens implantation, 10^7 organisms were injected into the vitreous of seven New Zealand white rabbits, producing endophthalmitis in all eyes inoculated. Coryneform group A-4 subsequently was isolated in six of seven eyes receiving 10^7 organisms, proving Koch’s postulates. Five of these seven eyes were treated with a single dose of intravitreal gentamicin, and three eyes remained culture positive. Eyes inoculated with 10^5 or 10^2 coryneform group A-4 organisms had transient anterior chamber and vitreal inflammation; all vitreous cultures were negative. These studies demonstrate that coryneform group A-4 endophthalmitis can be reproduced in an animal model and that gentamicin may not sterilize an eye infected with this organism. Future studies are needed to determine the optimum antibiotic regimen for treatment of this type of endophthalmitis. Invest Ophthalmol Vis Sci 32:2696–2699, 1991

The family of coryneform bacteria includes organisms from the genera Corynebacterium and Propionibacterium and other genera less commonly known to clinical ophthalmologists.1 Both *C. diphtheriae2 and *P. acnes3-8 are well-known causes of ocular disease. Diphtheroids, named because of microbiologic similarities to *C. diphtheriae, are defined as a group of gram-positive pleomorphic rods in the coryneform family. Ubiquitous in the environment, diphtheroids can be isolated readily from many sources including air, water, and soil. They colonize plants and animals9 and are part of the normal skin and mucous membrane flora.10 Previously dismissed by microbiology laboratories as contaminants, or by clinicians as non-pathogenic organisms, diphtheroids increasingly have been recognized as the cause of serious systemic9-12 and ocular13 diseases.

Although endophthalmitis caused by diphtheroids is rare,14 coryneform endophthalmitis has been reported after penetrating ocular trauma15 and keratoplasty.16 We recently reported two additional cases of patients who had coryneform group A-4 endophthalmitis after cataract extraction and anterior chamber intraocular lens implantation.17 We present an experimental animal model of coryneform group A-4 endophthalmitis in an attempt to define its pathogenesis and treatment further.

Materials and Methods

The two isolates recovered from human vitreous17 were grown in brain–heart infusion broth supplemented with 10% horse serum (SBHIB); this was diluted with sterile normal saline to provide inoculum sizes of 10^7, 10^5, and 10^2 organisms in 0.1 ml. The inoculum sizes were verified by colony counts. The control sample consisted 0.1 ml of SBHIB diluted ten-fold.

Nine New Zealand white rabbits (weight range, 5–7 pounds) were anesthetized with intramuscular ketamine hydrochloride 30 mg/kg and topical 0.5% proparacaine hydrochloride solution. All rabbits were treated in accordance with the ARVO Resolution on the Use of Animals in Research. A baseline slit-lamp and dilated fundus examination were done. Anterior chamber paracentesis was done under sterile conditions. 0.1 ml of the inoculum was injected through the pars plana into the midvitreous under direct visualization with a binocular operating microscope.

The animals were divided into two groups. One group of seven animals received 10^7 organisms in one eye and sterile diluted SBHIB in the other. The second group (two animals) received 10^5 organisms in one eye and 10^2 organisms of the same strain in the other eye. The animals were examined daily. The pu-
pils were dilated with topical 0.5% tropicamide and 2.5% phenylephrine hydrochloride solutions. Examination was done using both slit-lamp biomicroscopy and direct ophthalmoscopy.

At various times after inoculation (Table 1), the infected eyes underwent pars plana vitreous aspiration under ketamine and topical anesthesia using a 22-gauge needle directed into the opacity under a binocular operating microscope. An aliquot of 0.2–0.4 ml of vitreous was removed and inoculated into SBHIB and blood and chocolate agar. Control eyes had vitreous aspiration using the same techniques.

We also did a therapeutic study. Five of the seven animals receiving 10⁷ organisms in one eye also received 400 μg of gentamicin sulfate in 0.1 ml into the midvitreous of that eye after vitreous aspiration (Table 1). The eyes receiving intravitreal gentamicin had repeat vitreous aspiration for culture 2–10 days later.

Two of the seven animals receiving 10⁷ organisms had no treatment. Additionally, the two animals receiving 10⁵ and 10⁴ organisms were observed without treatment. All four animals (six eyes) had vitreous aspiration for culture (Table 1). After the final vitreous aspiration, the animals were killed with intravenous sodium pentobarbital, and their eyes were enucleated. The globes were fixed in formalin and processed for histologic examination in the usual manner. Hematoxylin-eosin and tissue Gram stains (Brown and Brenn method) were applied.

Results

Clinical Appearance

All seven eyes receiving 10⁷ organisms had small white midvitreal opacities interconnected with fibrin strands on days 2–4. At this time, the vitreous had 2+-3+ cells, the anterior chamber had 2+-3+ flare and cells with no hypopyon, and mild chemosis was present. Over the next few days, the vitreal opacities expanded into an irregular shape and increased in density. There was no change in the anterior chamber reaction, although posterior synechiae formed in some cases. By day 8, the anterior chamber reaction and chemosis had decreased to some extent. At this time, the vitreous was filled partly with a white opacity with multiple projections (Fig. 1). Over the next few days, until the animals were entered into the therapeutic study, the clinical appearance was unchanged. There was no difference in the behavior of the animals injected with the two different coryneform strains. Two animals did not receive any treatment and had persistent mild anterior chamber reaction and a very slowly expanding white vitreal opacity until they were killed on days 14 and 32, respectively. Five animals received intravitreal gentamicin with no change in clinical appearance; the anterior chamber reaction persisted, and the vitreal opacity was unchanged.

The eyes receiving 10⁵ organisms had a white vitreal opacity with 2+-3+ anterior chamber reaction without hypopyon by day 4. However, by day 7, the anterior chamber reaction and vitreal opacity were minimal. Over the next 14 days, the vitreal opacity resolved except for a few white specks, and the anterior chamber cleared.

The eyes receiving 10⁴ organisms had small white vitreal opacities and a 2+-3+ anterior chamber reaction between days 6–8. By day 10, the anterior chamber was clear, and the vitreal opacity resolved. The control eyes showed no vitreal reaction and had a clear anterior chamber.

Culture Results

Of the seven eyes receiving 10⁷ organisms, six had coryneform group A-4 isolated from the vitreous (Table 1). Five of these seven eyes then received intravitreal gentamicin; unfortunately, because we did not wait for vitreous culture results before injecting intra-

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>No. of organisms inoculated</th>
<th>Strain</th>
<th>Vitreal opacity (day)</th>
<th>Vitreous aspiration (day)</th>
<th>Culture result</th>
<th>Intravitreal gentamicin (day)</th>
<th>Vitreous aspiration (day)</th>
<th>Culture result</th>
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<td>II</td>
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<td>I</td>
<td>2</td>
<td>6</td>
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<td>I</td>
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Table 1. Culture results of rabbit eyes inoculated with coryneform group A-4

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vitreal gentamicin in some animals, one of the treated animals had a negative initial vitreous culture. Thus three of five treated eyes subsequently had positive vitreous cultures, and three of four documented culture-positive eyes were culture positive after treatment. The four eyes injected with $10^2$ or $10^3$ organisms and all control eyes were culture negative. The interval between development of the vitreal abscess and the vitreous aspiration was unrelated to the culture results.

Histopathologic Examination

Hematoxylin-eosin-stained sections of rabbit eyes inoculated with $10^7$ coryneform group A-4 organisms showed focal areas of vitreous inflammation containing red blood cells, polymorphonuclear leukocytes, mononuclear cells, and fibrin debris. In some sections, detached retina with a loss of normal retinal morphology was observed in conjunction with the areas of vitreous inflammation. Other areas of retina were normal, but the entire choroid was thickened with a mononuclear cell infiltrate. Tissue gram-stained sections contained pleomorphic gram-positive bacilli in the areas of vitreal inflammation.

Hematoxylin-eosin-stained sections showed less inflammation in the anterior segment; the cornea was not remarkable, the anterior chamber was clear, and the iris had engorged blood vessels and minimum cellular infiltration. Fibrin debris was present in the posterior chamber and on the anterior lens capsule. The control eyes showed no abnormalities.

Discussion

Our experimental data showed that the coryneform A-4 organisms (isolated in pure culture from two human patients with endophthalmitis after cataract extraction and anterior chamber intraocular lens implantation) inoculated into the vitreous of rabbit eyes produced a subacute endophthalmitis similar to that seen in patients, ie, a mild to moderate anterior chamber reaction associated with the development of white vitreal opacifications interconnected by fibrin strands. The organisms isolated in pure culture from the rabbit vitreous were identified as coryneform group A-4, proving Koch's postulates.

Our animal model showed a dose–response relationship between the number of organisms inoculated and the time of onset of symptoms and eventual clinical results. Eyes inoculated with $10^7$ organisms had progressive endophthalmitis with positive vitreous cultures; those inoculated with $10^3$ or less organisms had transient inflammation with negative cultures. The transient inflammation in eyes inoculated with $10^3$ or less organisms may have been secondary to an infection that was eradicated by the host immune response or to the antigenic load of bacteria with no actual infection. Because early vitreous cultures were not taken from these eyes, we cannot distinguish between these two possibilities. We are unsure why an apparent threshold inoculum size exists, but we believe this may be related to a lack of intrinsic virulence, especially in a healthy host. By comparison, others found that the vitreous was culture positive in 66–100% of rabbit eyes 48 hr after inoculation with 700 organisms of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, or *Escherichia coli*.

Although human and rabbit eyes may differ in their ability to eradicate inoculated bacteria, we think it is unlikely that either of our patients was inoculated with a large number of coryneform group A-4 organisms. Therefore other factors need to be considered that may have adversely affected the ability of the patient's eye to clear these organisms. One such possibility is the use of increasing amounts of topical corticosteroids, combined with the addition of subconjunctival corticosteroid injections without appropriate intravitreal antibiotic injection after unexpected postoperative inflammation began. In addition, iron may be an essential factor for growth of coryneform group A-4. Neither patient had endophthalmitis associated with a metallic intraocular foreign body. Because diphtheroids can be isolated from the conjunctiva of 21–39% of normal eyes, further studies are necessary to define the conditions that allow normal conjunctival bacterial flora to cause significant ocular disease.
Our studies show that gentamicin sulfate, a drug commonly used for the treatment of bacterial endophthalmitis, was ineffective in this experimental endophthalmitis. We believe several factors explain the treatment failure. The first factor was the high minimum bactericidal concentration (MBC) of gentamicin for these two isolates, 25 and 50 mg/L, respectively. This high MBC showed relative resistance to gentamicin, and high antibiotic concentrations, often difficult to achieve clinically, were necessary to eradicate the organism. The second factor was the relatively short half-life of gentamicin in inflamed rabbit eyes—10 hr. Intravitreal injection of 400 μg of gentamicin should provide a peak concentration of 285 mg/L, assuming a rabbit vitreous volume of 1.4 ml. However, the half-life of 10 hr caused the intravitreal gentamicin concentration to decline below the MBC of 50 mg/L in only 25 hr. Thus these two factors combined to give a short duration of bactericidal gentamicin levels in the vitreous. The third factor was the relatively long doubling time of coryneform group A-4, which we observed during preparation of the initial inoculum. This may have been lengthened in our animal model by attaining a plateau growth phase after 13–23 days' incubation in rabbit vitreous. We chose our treatment times to correspond to those of our patients, who had delayed endophthalmitis and treatment. Because gentamicin is more effective against actively dividing organisms, even though the in vitro MBC was exceeded for 25 hr, many organisms in vivo probably were not affected. Because the MBC was obtained using actively dividing organisms, the gentamicin concentration needed to sterilize our infected rabbit eyes probably was much higher. We found previous studies indicating that intravitreal injections of gentamicin of 400 μg may cause macular infarction. Therefore a smaller dose currently is recommended but would be even less effective in eradicating these organisms. Further studies are indicated to determine the optimum antibiotic regimen for patients with coryneform group A-4 endophthalmitis.

Key words: corynebacteria, coryneform group A-4, endophthalmitis, endophthalmitis, delayed, pseudophakic endophthalmitis

References