Recent Advances in the Treatment of Acanthamoeba Keratitis

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Infection of the eye caused by Acanthamoeba species constitutes a burgeoning and unsolved problem. Of individuals with Acanthamoeba keratitis, 85% wear contact lenses; abrasion of the cornea is implicated. Corneal infection often can be prevented by good lens care and hygiene. Severe Acanthamoeba keratitis often can be very difficult to treat; surgery can be less than successful and may lead to further problems. The encysted stage in the life cycle of Acanthamoeba species appears to cause the most problems; many biocides are ineffective in killing the highly resistant cysts. Combination therapy—that is, use of 2 or 3 biocides, sometimes with antibacterial antibiotics—appears to work best. Recurrence is common if treatment is stopped prematurely. Immunologic methods are being investigated as a form of prevention, and oral immunization of animals recently has been successful in the prevention of Acanthamoeba keratitis by inducing immunity before infection occurs. Immunization thus may eventually become the best approach for reduction of the incidence of amebic infection in humans.

The first case of Acanthamoeba keratitis, an amebic infection of the eye that involved Acanthamoeba polyphaga, was reported when a Texas rancher splashed tap water from a contaminated river source into his eye [1]. Acanthamoeba keratitis is now estimated to affect 1 in 250,000 individuals in the United States, and, in the United Kingdom, there have been a total of ~400 cases diagnosed since 1957.

Acanthamoeba organisms were characterized by Visvesvera [2] as amebae with pointed spindles at mitosis, double-walled cysts, and an irregular outer layer. Page [3] has pointed out that Acanthamoeba and Hartmannella species have nothing in common except for certain mitotic patterns that are nonspecific and that can also be seen in other amebae; these findings have been accepted by Singh [4] and others and have led to the further classification of the 2 organisms. Organisms of the genus Acanthamoeba are described as small, filose, free-living amebae with a cyst stage and oistioles [2]. The organisms typically are uninucleated trophozoites. They possess fine acanthapodia (protoplasmic projections), and they enter a cyst stage when in difficult environmental conditions [2]. The trophozoite itself can show some morphological changes depending on its surroundings; for example, it becomes larger when in the presence of bacteria. Bacteria and fungi sometimes coat the surface of the trophozoite. In the presence of bacteria in broth culture, the trophozoites clump together in groups of 10–30 cells [5, 6].

Acanthamoeba species have been isolated from many different sources, such as freshwater, seawater, chlorinated water from swimming pools, dental treatment units, and contact lens cases. Most of the strains found are not pathogenic. Some pathogenic forms are known to survive for extended periods in freshwater [7]. Protozoa, in general, become airborne when encysted. The presence of pathogenic Acanthamoeba organisms in the atmosphere is an important factor in the prevalence of
Acanthamoeba keratitis, although this is not its main cause [8]. Patients with Acanthamoeba keratitis usually are users of daily-wear disposable, soft contact lenses. The use of tap water to rinse contact lenses allows deposits of lime scale to accumulate, and this lime scale often contains pathogenic Acanthamoeba species [9]. Lenses create a corneal abrasion, facilitating entry of Acanthamoeba [10]. The organism can survive in contact lens cases and solutions.

HISTORY OF ACANTHAMOEBA KERATITIS

Acanthamoeba keratitis was extremely rare before the widespread use of contact lenses. The number of cases started to increase dramatically beginning in 1984, and, by 1985, an association with the use of contact lenses was established [11], especially among individuals who swim while wearing their contact lenses and those who use homemade saline.

CLINICAL SYMPTOMS OF ACANTHAMOEBA KERATITIS

The clinical features of the disease were reviewed in a study of 11 patients with culture-proven Acanthamoeba keratitis who were assessed during a 7-year period [12]. The combination of clinical signs includes excessive pain, radial keratoneuritis (figure 1a), and, in a later phase, a stromal ring infiltrate (figure 1b and 1c). Patients often have a history of contact lens use or contact with polluted water [14]. Acanthamoeba keratitis can occur in 2 separate forms. In the first form, the pathogen is restricted to the epithelium, and there is a good chance of recovery. In the second form, the parasite has entered the stroma, where it causes necrosis (figure 1d) and intense inflammation [15, 16].

PATHOGENESIS OF ACANTHAMOEBA ORGANISMS

The contact lens, when placed on the eye, can introduce the pathogen through an abrasion previously caused by the contact lens [10]. It is almost certain that this trauma contributes to the onset and development of an amebic infection [17], although this mode of infection is not solely responsible for Acanthamoeba keratitis, because persons who do not wear contact lenses have also been affected [18].

The results of in vitro binding studies have shown that abrasions increased the binding of the parasite to the cornea. The first step in the infection involves adhesion of the trophozoite to the corneal epithelium. The trophozoites are species specific; they appear to bind only to corneal epithelial cells from humans, pigs, rabbits, and Chinese hamsters, whereas the majority of corneas in the animal kingdom are resistant to infection [19]. Pathogenic strains of Acanthamoeba castellani produce a variety of proteases, termed Acanthamoeba plasminogen activators; corneal invasion is probably thereby facilitated [20–22]. The stromal disease occurs later. Intrastromal injection of pure A. castellani cultures into the corneas of rats causes ring infiltrates that mimic those found in persons with Acanthamoeba keratitis [23].

FACTORS THAT AFFECT THE ADHERENCE OF ACANTHAMOEBA ORGANISMS

Adherence of Acanthamoeba organisms to contact lenses. To further understand Acanthamoeba keratitis and methods of its prevention, the mechanisms of adherence of Acanthamoeba trophozoites to the contact lens [24] were investigated by use of 50 unworn polymacon soft contact lenses with 38% hydration. Both cysts and trophozoites immediately adhered to the lens segments. More cysts and trophozoites adhered to the unwashed lenses than to the washed lenses.

Trophozoites that adhered to the lenses had surface projections (acanthapodia, filopodia, and lobopodia). The adherent cysts had wrinkled ectocysts, as revealed by scanning electron microscopy. These rough surfaces may provide the means by which cysts adhere to the lens surface, because surface projections are visible only in trophozoites.

Both cysts and trophozoites of A. castellani also adhered to worn, extended-wear soft contact lenses. Strong interaction was demonstrated when washed lenses continued to have attached cysts and trophozoites [24, 25]. Cytoplasmic microfilaments were concentrated in the region of the attachment of the cyst to the lens [24]; factors other than the age of the contact lens are involved with this adherence to the lens. Perkovich et al. [26] found that both new and previously worn contact lenses (used for 8–21 months) acted as surfaces for adherence of trophozoites and cysts.

Adherence of genetically different strains of Acanthamoeba (e.g., strain 1 A. castellani, strain 9 A. polyphaga, and strain 11 Acanthamoeba culbertsoni Lilly A-1) was studied by use of different exposure periods and temperatures [27]. Strain 1 was used as the relatively nonpathogenic control. After 120 min, strain 9 was the most adherent, followed by strain 11 and then strain 1; this correlated with pathogenicity.

Mannose-binding receptors. Mannose-binding receptors on the surface of Acanthamoeba organisms bind to mannosylated proteins on the corneal epithelial cell [28]. Studies by both McCully et al. [17] and Cao et al. [29] showed that the presence of mannose in incubates of corneal epithelial cells prevented this adhesion. It is suggested that mannose-containing glycoproteins near the surface of the cornea may be exposed
during a subtle corneal injury and that this makes the cornea susceptible to infection [30].

Serine proteinases are released from the organism, resulting in parasite-mediated cytolysis of the cornea [17, 31]. The secretion of cytotoxic proteinases that induce cytopathic effect is prevented by N-acetyl-d-glucosamine, which does not affect the binding of the receptor to the cell [29].

**Adherence of Acanthamoeba organisms to corneal epithelial cells.** Is the adherence of the organism to the contact lens the main risk factor, or do other factors contribute to the onset of the infection [24]? Sharma et al. [32] showed that the median percentages of cells to which Acanthamoeba organisms adhered were equal, regardless of whether they were from lens wearers or non-lens wearers.

**Interaction of biofilms, hydrogel lenses, and Acanthamoeba keratitis.** Free-living amebae can grow successfully as commensals and parasites, particularly with gram-negative bacteria (e.g., *Escherichia coli*) that naturally exist as part of the external eye flora. Acanthamoeba organisms are also known to be part of the natural eye flora in non-contact lens wearers [33, 34]. Contact lenses act to increase the number of amebic trophozoites and cysts in the eye [33]. Could other naturally occurring bacteria produce a symbiotic relationship that could favor amebic infection?

Simmons et al. [35] found that hydrogel contact lenses were particularly suitable for supporting the growth of biofilms of *Pseudomonas aeruginosa*; this biofilm, in turn, increased the adsorption of Acanthamoeba organisms to the lens. This finding suggested that contact lenses that are already contaminated with a bacterial biofilm provide an increased chance of development of Acanthamoeba keratitis [35–37]. Further work by Tomlinson et al. [38] showed that sodium salicylate reduced the risk of infection and affected the attachment of Acanthamoeba species to biofilms produced by *P. aeruginosa*.

**The rinsing procedure.** Cases of Acanthamoeba keratitis have been linked to such contaminated sources as water from hot tubs and areas used for water activities but, mainly, to contaminated saline [39]. Rinsing lenses in tap water before disinfection is often a cause of problems rather than a preventive measure [36, 40]. New 1-step disinfection solutions have been shown to be efficient in preventing bacterial growth; tap water is not required to rinse the lens [41].

Contamination of contact lenses appears to be rare, according to the findings of a study at Queen’s Medical Centre, Nottingham, United Kingdom [42]. The general population were asked, during their checkup visit, to exchange their old contact lenses for new ones. The lenses were placed in Acanthamoeba culture medium, but no growth of Acanthamoeba organisms was found on the 102 used contact lenses obtained [42].
AN EPIDEMIC CAUSED BY REGIONAL FLOODING

In 1998, Meier et al. [43] conducted a controlled investigation of the effects of regional flooding on the incidence of Acanthamoeba keratitis. The flooding affected the water supplies, and it was thought that this may have led to seasonally increased infection due to contamination of lenses after rinsing in tap water [43].

PENETRATING KERATOPLASTY

In the past, the symptoms of Acanthamoeba keratitis often led to an incorrect diagnosis of herpetic keratitis. Rapid diagnosis of the disease is paramount in lowering the number of patients who require penetrating keratoplasty [42], which often is the only form of rehabilitation. For example, 6 patients with uncontrollable Acanthamoeba keratitis were evaluated during therapy [44]; they underwent a total of 10 keratoplasties during a period of 10 years. Five of the 6 patients were fully rehabilitated. The sixth patient developed blindness in the infected eye after the fourth keratoplasty, when the eye became affected by secondary glaucoma. Keratoplasty was effective in eliminating the infectious pathogen, and it also prevented the recurrence of the same infection in most cases.

However, in some rare cases, keratoplasty proves unsuccessful. The alternative type of treatment, combination drug therapy, has been shown to have a high success rate only if the disease is diagnosed early enough.

BIOCIDAL RESISTANCE AND TREATMENT

Biocidal resistance of Acanthamoeba species. Srikanth and Berk [45] found that amebae, like other microorganisms, have the ability to adapt to the most unimaginably harsh environments. Amebae from cooling towers were discovered to become more resistant to biocides (even after just 72 h) than were ameba from natural habitats. Cross-resistance—that is, acquisition of resistance to one biocide causing resistance to others—was also found.

Pathogenic Acanthamoeba strains have been found in biocidal solutions for lens care [46, 47]. The cysts that are implicated have been known to resist desiccation for months to years; they can tolerate 2.0% HCl, peroxides, and chlorine [3]. Manufacturers should state the killing times required by contact lens solution for all pathogenic Acanthamoeba isolates [48].

Mechanisms of biocide action have been extensively studied recently [49–54]. It is thought that resistance that begins to increase after 8 h is caused by the development of an acid-insoluble encyst wall that contains protein and acts as a non-permeable barrier [55]. Alkali-insoluble residues, including cellulose, were detected after 16 h. This coincided with the emergence of complete resistance to all biocides, except hydrochloric acid and chlorhexidine diacetate, for which resistance was evident after 2 and 24 h, respectively.

Trials determining the efficacy of biocides as lens disinfectants and eye treatment. Polyhexamethylene biguanide (PHMB), chlorhexidine, and propamidine isethionate (Brolene; Rhone-Poulenc [Dagenham, United Kingdom]) in concentrations of 0.1% and 0.02% were tested against 5 different Acanthamoeba strains. Chlorhexidine was the only biocide that was effective against all 5 strains at lower concentrations. The cysts of all 5 strains were destroyed only when the 0.1% concentration was used [56]. Chlorhexidine was found to destroy cysts and trophozoites from many different strains in vitro more effectively than did PHMB [57, 58]. PHMB was shown to be more destructive to trophozoites (as determined by microscopy) than was chlorhexidine at the same concentration, even though a clear difference was not apparent in successive cultures [59].

Gatti et al. [60] compared the effectiveness of chlorhexidine to povidone iodine (Betadine). The latter agent is known to have broad antibacterial and antiviral properties. The results showed that concentrations of 0.5%–2.5% povidone iodine worked better on both trophozoites and cysts than did chlorhexidine.

Tirado-Angel et al. [59] conducted a study to determine the combined efficacy of PHMB and chlorhexidine. Chlorhexidine was slightly better as a disinfectant than was PHMB, although both were very good. The combination of the 2 biocides gave a slight further improvement in amebicidal action; time dependence of the combined therapy was demonstrated [59, 61].

The efficacy of biocide treatment in clinical trials. In vivo studies by Kosrirukvongs et al. [62] determined chlorhexidine to be an effective amebicidal agent. Chlorhexidine was applied to the eye every hour. As symptoms were reduced, the frequency of the applications was reduced. Of the 5 patients in the study, 4 showed improved visual acuity within 1 week; 1 patient with a perforated ulcer developed glaucoma. Chlorhexidine appeared to act successfully and rapidly, but bacterial coinfection occurred in 1 of the treated eyes [62].

Three cases of Acanthamoeba keratitis complicated by fungal infections were diagnosed at Kaohsiung Medical University, Taiwan. Treatment with PHMB and fluconazole achieved good results without any recurrence of infection [63]. Larkin et al. [64] and many others have reported their success in treating Acanthamoeba keratitis with PHMB in various combinations with different drugs. PHMB used alone was always less effective than PHMB used in combination therapy [65]; PHMB interacts with the cytoplasmic membrane, causing leakage of components and inhibition of the respiratory enzymes, resulting in the greatest cysticidal action. There are 3 possible explanations for the failure of treatment with PHMB alone: PHMB may have poorly penetrated the corneal stroma, the duration for which...
the PHMB was applied may have been inadequate, or PHMB may not be effective at the late stage of fulminating ring infiltration.

In a trial at Moorfields Eye Hospital (London), Duguid et al. [66] studied the clinical outcome of *Acanthamoeba* keratitis after treatment with PHMB and propamidine isethionate. From September 1992 through February 1995, a total of 105 patients, 92% of whom wore contact lenses, were treated with combination therapy with 0.02% PHMB and 0.1% propamidine isethionate every hour for 3 days. The treatment was reduced as the symptoms reduced. The main measure of efficacy of treatment was the improvement of visual acuity. The majority of patients showed improved visual acuity, and very few found the drug toxic; those who did were not seriously affected. Keratoplasty, which indicated no improvement at all, was necessary in only 10 cases. Overall, combined treatment with PHMB and propamidine isethionate was effective and well tolerated, although, in certain cases, reduction in therapy sometimes caused a relapse [66]. Many factors complicated the matter, such as duration of the disease before diagnosis; ocular complications are likely to be avoided if treatment is implemented early [40].

A similar trial of PHMB and propamidine isethionate was conducted in Thailand by Kosrirukvongs et al. [62]. Broad-spectrum antibiotics also were used in combination with the biocidal drugs; all subjects showed considerable improvement within 2–3 weeks. There also were no relapses (a possible outcome of cyst dormancy) of the condition during treatment [62, 67].

Hargrave et al. [68] evaluated the safety and efficacy of neomycin–polymyxin B–gramicidin ophthalmic solution (Neotra; Cilag) when it was administered with 0.1% propamidine isethionate. A total of 87 eyes affected by *Acanthamoeba* keratitis were studied; treatment was successful for 60% of those treated, whereas noncompliant patients showed exacerbation of the disease during the maintenance phase of treatment.

**COMPLICATIONS ASSOCIATED WITH TREATMENT**

Inoue et al. [69] at Osaka University Medical School found a case of amebic keratitis from which both *Acanthamoeba* and *Hartmannella* species were isolated from the same lesion. The isolated pathogen responded to treatment in vitro but not in vivo. Thus, Inoue et al. [69] concluded that organisms other than *Acanthamoeba* species (e.g., *Hartmannella* species) could cause keratitis. De Jonckheere and Brown [70] suggested that (1) further investigation of the pathogenicity of *Hartmannella* organisms is required by use of animal models, and (2) until their pathogenicity is proven in this way, *Hartmannella* species should be regarded as harmless [70].

Cases of *Acanthamoeba* keratitis have also been identified in non–contact lens wearers [71]. A 70-year-old patient had severe pain in the left eye after undergoing cataract surgery 40 days earlier. Despite the methods used, infection with *Acanthamoeba* species was not confirmed for 6 weeks. This was too late, because topical amebicidal drugs did not help; the keratitis progressed into sclerokeratitis, in which uveal tissue clearly could be seen through the sclera. The disease got worse before it eventually responded to treatment. By 6 months, the disease had resolved, but the eye was severely scarred [71].

Kunimoto et al. [18] performed the largest single-center study of microbial keratitis in Hyderabad, south India. The study backed up findings from other studies that suggested that elderly people were more susceptible to microbial keratitis, regardless of whether they used contact lenses. Only 2 cases of microbial infection were associated with contact lens wear. Elderly persons are more susceptible to microbial keratitis (not just that caused by *Acanthamoeba* species but, also, that caused by other microbes, such as *Staphylococcus epidermidis* and *Streptococcus pneumoniae*) because of the increased microbial occupancy of the eye that occurs with advancing age. Delays in diagnosis and treatment will also have influenced these results [18, 72].

**IMMUNOLOGY OF ACANTHAMOEBA SPECIES**

*Immune response to a corneal infection.* Between 50% and 100% of the population have antibodies to *Acanthamoeba* species; therefore, environmental exposure must be very common [73, 74]. It is still unknown whether corneal infection provokes a systemic immune response in humans, but, in animal studies, corneal infection with *Acanthamoeba* organisms failed to induce either serum IgG antibody to *Acanthamoeba* species or any delayed-type hypersensitivity (DTH) [75, 76]. Intramuscular immunization of the parasite antigen resulted in DTH and IgG production in an animal model.

*Langerhans cells.* When Langerhans cells were experimentally induced in the cornea before infection with *Acanthamoeba* organisms, parasite-specific DTH later developed in the central cornea. A stimulus, such as infection, minor surgery, or injection of IL-1, is required if the Langerhans cells are to migrate from the limbus to the central part of the cornea [77, 78].

It is thought that the lack of Langerhans cells at the central cornea is probably an adaptation to prevent the induction of the DTH response to antigens [79]. The prevention of such a response is important if the cornea is to retain its clarity and function correctly. The most common forms of infectious blindness are stromal keratitis due to herpes simplex virus and trachoma; in some geographic areas, onchocerciasis is a very prominent cause. A DTH reaction inflicts damage to the corneal tissue [80].

On the basis of animal studies, many had hypothesized that exposure of *Acanthamoeba* trophozoites would cause a DTH
response. When this was tested, the results were surprising. In nearly all cases, injection of IL-1 into the central cornea to stimulate the migration of Langerhans cells failed to cause the disease; mostly, resistance to the infection was elicited [81]. Further research into this topic will provide basic understanding of and better treatments for Acanthamoeba keratitis and other corneal infections [74].

The role of macrophages and neutrophils. Acanthamoeba organisms are attacked and killed by neutrophils and macrophages in vitro [82–84]. Both parasite-specific antibodies and IFN-γ increase the rate at which macrophages kill parasites in vitro. The role of neutrophils during the immune response is still unclear, but macrophages have been shown to provide a significant amount of protection in animal studies [85]. In situ studies of Chinese hamsters have demonstrated how, by removing macrophages from the cornea by use of a macrophagocidal drug called clodronate, the Acanthamoeba keratitis could be worsened. These findings suggested that macrophages are important in the first line of defense when the cornea initially becomes infected by the trophozoites [74, 86].

The role of antibodies in immunization. A high percentage of the population possesses serum IgG antibody, but there is very little evidence that this antibody reacts with the antigen of Acanthamoeba species. In animal models, the antibody can be induced by intramuscular immunization, yet the presence of these antibodies does not prevent the chronicity or severity of Acanthamoeba keratitis [75]. In vitro experiments show that specific antibodies cause complement-dependent lysis of the trophozoites. Complicating matters further is the fact that in vitro studies also show that the complement cascade can be active against Acanthamoeba trophozoites even when antibodies are not present [87, 88]. It is possible that complement-regulating proteins, such as CD55, which are present in vitro. The role of neutrophils during the immune response is still unclear, but macrophages have been shown to provide a significant amount of protection in animal studies [85]. In situ studies of Chinese hamsters have demonstrated how, by removing macrophages from the cornea by use of a macrophagocidal drug called clodronate, the Acanthamoeba keratitis could be worsened. These findings suggested that macrophages are important in the first line of defense when the cornea initially becomes infected by the trophozoites [74, 86].

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Tears contain humoral components, such as lactoferrin, lysozymes, and lactoperoxidase, and also IgG, IgM, and IgA [89, 90]. Cholera toxin is used in animal studies as an adjuvant and promotes the production of secretory IGA from the mucosal surface. At the time of oral immunization, the antigens produce a strong resistance to Acanthamoeba organisms [91, 92].

Thus, immunization may eventually become the best approach to reducing the incidence of human amebic infection.

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