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Review

Acanthamoeba Keratitis, Pathology, Diagnosis and Treatment

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Abstract: Acanthamoeba keratitis is an unusual corneal infection that is recently increasing in frequency and is often contracted by contact lens wearers, someone who experienced recent eye trauma, or someone exposed to contaminated waters. Acanthamoeba survive in air, soil, dust, and water. Therefore, eye trauma and poor contact lens hygiene practices lead to the entrapment of debris and thus infection. Acanthamoeba keratitis results in severe eye pain, inflammation, and defects of the epithelium and stroma that can potentially result in vision loss if not diagnosed early and treated promptly. The disease can be diagnosed using corneal scrape/biopsy, polymerase chain reactions, impression cytology, or in vivo confocal microscopy. Once diagnosed, it is usually treated with an antimicrobial combination therapy of biguanide and aromatic diadine eye drops for several months. Advanced stages of the disease result in vision loss and the need for corneal transplants. Avoiding the risk factors and diagnosing the disease early are the most effective ways to combat Acanthamoeba keratitis.

Keywords: Acanthamoeba; keratitis; pathogenesis; diagnosis; therapy

1. Introduction

Acanthamoeba is a common free-living amoeba that is found in many different environmental niches and can be isolated from water bodies, drainages, sediments, surgical instruments, dialysis units, skin lesions, and to the point of this review, contact lenses [1]. These organisms are considered an emerging parasite primarily due to the difficulty of treating infections by these organisms [2]. Interestingly, these organisms can inhabit both in external environments as well as within the bodies of hosts they infect [3].

Acanthamoeba has the capacity to cause severe infections among immunocompetent as well as immunocompromised individuals [4]. The infections caused by Acanthamoeba include Acanthamoeba keratitis (AK) among healthy individuals, especially those wearing contact lenses, and a life-threatening Granulomatous amoebic encephalitis (GAE) infection.
18s rRNA gene typing [16]. Genotyping of Acanthamoeba is important because the different genotypes show variation in clinical presentation and response to medical therapy [17]. At least eight of the genotypic classes (T2, T3, T4, T5, T6, T10, T11, and T15) have been shown to cause AK, with the most common causative genotype being T4 [17,18]. The most common infectious species are Acanthamoeba castellani and Acanthamoeba polyphaga, both from the T4 genotype [16,19]. The pathogen is transmitted through corneal contact with a contaminated substance. Most cases seen in humans seem to be associated with contaminated water, soil, or trauma to the eye [20]. Once in the eye, the amoebae feed on the keratocytes with invasion and destruction of corneal stroma. Since AK is a relatively uncommon corneal infection, it often goes undiagnosed and untreated for long periods of time. This leads to a delayed institution of appropriate treatments with a greater ratio of unfavorable visual outcomes.

2. Pathogenesis

The progression of Acanthamoeba keratitis occurs in two main phases. An initial phase where infiltration is limited to the corneal epithelium, and a secondary phase where the parasite invades the underlying stroma. Once in the stroma, extensive damage to the collagen matrix occurs which provokes intense inflammation [21]. Treatment during the initial stages of pathogenesis is more successful than treatment during later stages of disease, which is why early diagnosis and treatment are essential.

The first step in the pathogenesis of AK is the adhesion of the microbe to the corneal surface. The process of adhesion is mediated by a number of proteins, the most important of which has been identified as a mannose-binding protein expressed by the amoeba [21,22]. The process continues with Acanthamoeba trophozoites breaking down the epithelial barrier by mechanisms of direct cytolysis, phagocytosis, and induction of apoptosis [22]. Following adhesion and breakdown of the corneal epithelium, trophozoites invade the underlying collagenous stroma. The process of stromal invasion is mediated by a number of products of the amoeba, including metalloproteinases and serine proteinases. These proteinases work to produce a potent cytotoxic effect that kills host cells and degrades the epithelial basement membrane as well as the stromal matrix to progress into deeper layers of the cornea [21,22]. Stromal involvement is typically seen late in the course of AK. Once in the stroma, the trophozoites feed on keratocytes and organic particles causing keratocyte depletion, induction of an intense inflammatory response, and finally stromal necrosis [21,22]. These latest stages of disease have potentially sight-threatening effects [23].

Acanthamoeba also have two stages to their life cycle, a vegetative trophozoite stage and a dormant cystic stage. Acanthamoeba trophozoites can undergo encystment into the dormant form through the influence of host resting macrophages [22]. These Acanthamoeba cysts pose a serious risk of recurrence in patients who have been previously treated for AK. These two forms of the amoeba have important implications for management of the disease.
lens hygiene can prevent unexpected infection. However, most people do not adhere to contact lens hygiene properly. For this reason, multipurpose contact lens solutions should be used when storing and washing contacts. Hydrogen-peroxide based systems have the greatest efficacy toward killing *Acanthamoeba* and are significantly more effective than non-sterile saline solutions or chlorine-based solutions [27]. Efficacy of hydrogen-peroxide based systems may still be limited, as they are not always effective in killing all *Acanthamoeba* cysts [24]. AK is less frequently encountered by those who do not wear contact lenses. If the infection presents itself in a person who does not wear contact lenses, it is often due to minor corneal trauma of the eye associated with infectious debris abrading the cornea. This is the common mechanism seen in developing countries where the prevalence of wearing contacts is not as high [19]. The prevalence of AK is also higher in men than women, most likely due to a less strict hygiene regimen or more outdoor activities. AK is more often seen among younger adults, once again most likely due to hygiene habits or more outdoor exposures. Nonetheless, AK is also more seen among those older than 53, likely associated with corneal changes due to aging [20].

4. Signs/Symptoms

Diagnosing AK based on clinical presentation can be difficult because initial signs and symptoms resemble other corneal conditions. Similar to other corneal infections, the initial symptoms of AK are relatively nonspecific. The affected patient may only have minor ocular irritations, tearing or blurred vision. However, severe ocular or periocular pain is often the hallmark of AK associated with the progression of the infection and intense stromal inflammation [28,29].

The signs of *Acanthamoeba* keratitis are unilateral most of the time and progress slowly, beginning superficially at the epithelium and eventually affecting the stroma. Clinical findings of this condition are illustrated in Figures 1 and 2. Figure 1A,B is from a 40 year-old male contact user with unilateral, coarse superficial punctate epithelial erosions. Figure 2 is from a 74 year-old woman contact lens wearer with a dense ring stromal infiltrate. These figures reiterate the fact that as already described, this disease typically takes place in contact lens wearers. When one considers what typically occurs during this disease, the first signs begin to appear as a diffuse superficial keratopathy [26] as illustrated in Figure 1. Within the first two weeks of infection, the eye can undergo chamelecon-like epithelial changes referred to as “dirty epithelium” [30]. These changes include a pseudo-dendritiform epitheliopathy with grey epithelial opacities [30]. This dendritic keratitis can be misdiagnosed as viral keratitis caused by herpes simplex or herpes zoster. However, pseudodendrites caused by AK are characteristically different because its epithelial defects have no involvement of the endothelium and lack the widening terminal knots (known as epithelial dendrites), both of which are commonly seen in herpes sim-plex keratitis [26,31]. Furthermore, the patient with herpetic keratitis may experience significantly decreased corneal sensation [32]. Unusual infiltrates along radiating corneal nerves (known as radial keratoneuiritis) may present as the disease progresses, with a
Figure 1. A 40-year-old male contact lens user developed severe ocular irritations after using home-made saline solution for cleansing contact lenses. (A) Slit lamp photo showed diffuse, coarse superficial punctate epithelial erosions and mild anterior stromal haze without epithelial defect or stromal infiltrates. (B) Slit lamp photo with retro-illumination of the same cornea readily showed the coarse epithelial erosions and relatively clear stroma. (C) Impression cytology of the central cornea from the same eye showed multiple double-walled *Acanthamoeba* cysts (triangles) and occasional trophozoites (arrows) scattered among the epithelial cells in the superficial corneal epithelial sheet.
5. Diagnosis

When it comes to *Acanthamoeba* keratitis, high index of clinical suspicion and early diagnosis are essential to avoid untoward health outcomes for patients. The first step to diagnosing AK is having a clinical suspicion of the disease. Since AK is uncommon compared to the other causes of keratitis, it is often overlooked as a differential diagnosis. AK should be taken into consideration in anyone exhibiting the risk factors as described above, especially contact lenses wearers, or anyone who is demonstrating severe ocular pain. To complicate the matters, there are also many reports of AK in mixed form with viral, bacterial, or fungal pathogens also present [34,35]. These forms of mixed keratitis have important implications for diagnosis and management of disease. Early confirmation of the infectious agents leads to swift and effective treatment with earlier recovery before occurrence of any serious damage to the cornea and vision. There are multiple options available that can assist in the diagnosis of AK, and often multiple techniques are used to ensure proper diagnosis.

5.1. Corneal Scraping

Performing a corneal scraping for microbial culture to identify the causative pathogen(s) is generally considered as the gold standard clinically to confirm AK. Although culture tests carry a high specificity (100%), they generally lack strength regarding sensitivity, which ranges from 7–66.7% depending upon culture techniques using agar culturing plates with or without overlay of feeding bacteria [36,37]. This makes the test statistically weak for determining AK as a diagnosis. The test, however, is easy to perform and can be cost effective compared to other options. Another aspect to consider is the time that is required to get results back from a culture. Amoebic culture takes, on average, 10 days to demonstrate a positive result [36]. This delay allows clinical morbidities to further progress, making AK more difficult to treat. The procedure can also be invasive if corneal biopsy should be needed to acquire adequate tissue sample for better culture yields.

As an alternative option to culture, corneal scrapes or corneal biopsy can be used in a direct smear with special stains for cytologic examination. Several studies have shown that using microscopy and analyzing a smear with special stains, especially Calcofluor white, has been an effective and rapid method for identification of *Acanthamoeba* cysts [38–40]. This method may still require the invasive nature of corneal biopsy.

5.2. Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a molecular technique that is growing as a diagnostic tool for corneal infections including AK. PCR is not used clinically as broadly as corneal culture; however, the diagnostic tool does improve upon statistical strength for diagnosis of AK. PCR demonstrates a high specificity (100%), as well as a moderately better sensitivity than corneal culture, which ranges from 66.7–100% depending on the DNA section used [36,37]. These statistical values trend toward the higher end if multiple PCR tests are run using different DNA segments. PCR also provides faster results, with
conditions of the eye. However, IVCM is not as readily available as some other diagnostic tools. In facilities with IVCM, it is considered to be the first-line method for diagnosis of AK. IVCM has great statistical value in diagnosing AK with a very high specificity (100%) and a high sensitivity, ranging from 85.3–100% [36,41]. This makes the technique very strong at both confirming and excluding AK as a diagnosis. IVCM can also help differentiate other types of keratitis from AK or identify the presence of mixed keratitis. The test also provides rapid results, especially when compared to other diagnostic tools. One limitation to this method is that only Acanthamoeba cysts can be recognized using this method, meaning that diagnosis can only be made at later stages of the disease [42]. The test requires proficient imaging expertise and is also not as widely available and may be more expensive than other options. A recent comparison of two of the confocal microscopes that have been used for this analysis, namely the Nidek Confoscan4 (NIDEK technologies, Fremont, CA, USA) and Heidelberg HRT3 Rostock Cornea Module (RCM) (Heidelberg Engineering, Heidelberg, Germany) have revealed a slight advantage to using the Heidelberg RCM [43]. This study also identified several additional limitations with this technique. The most important being the experience of the operator using these instruments to identify AK. Another set of limitations are the small area of the cornea in any particular scan, so it is possible scans were obtained in an area remote from the pathology. Furthermore, stromal inflammation can result in false negatives if the inflammatory cells and edema mask Acanthamoeba cysts, or false positives when macrophages are misidentified as Acanthamoeba cysts.

5.4. Impression Cytology

The least used diagnostic tool for diagnosing AK is impression cytology. The technique is used for dry eye diagnosis by obtaining superficial corneal epithelial cells with nitrocellulose filters and special stains [44]. As shown in Figure 1C, numerous double walled Acanthamoeba cysts interspersed can be seen in a sheet of corneal epithelial cells obtained by impression cytology. There is no reported analysis regarding the statistical prowess of impression cytology. While it is relatively non-invasive and highly specific for diagnosing AK, special stains and expertise in cytopathology are required. However, 4–6 h turn-over can be achieved in a well-experienced lab. Another limitation of this technique is that it only surveyed the superficial corneal epithelial tissues and cannot readily detect pathogens in the deeper stroma. It should be acknowledged that recent reports have indicated that cytological analysis is a much more common technique in other countries [45] and has resulted in very high rates of positivity when compared to culturing of amoeba [46].

6. Treatment

The Acanthamoeba can exist in an actively mobile trophozoite form or a dormant cyst form which is highly resistant to drugs. Biguanides and aromatic diamidines are effective antimicrobial agents for killing the pathogen but must be given together to overcome drug resistance. Neomycin, an antibiotic, is also beneficial but when given alongside other drugs
6.1. Biguanides

Biguanides are useful antimicrobial agents because they can kill both forms of *Acanthamoeba*, trophozoites and cysts. The positively charged molecules bind to and penetrate the amoebas and increase the cytoplasmic membrane permeability resulting in death of the pathogen [26]. The two biguanide compounds consistently proven effective in a drug treatment are polyhexamethylene biguanide (PHMB) and chlorhexidine. PHMB, a pool disinfectant, at a low concentration of 0.02% has a high cisticidal activity against multiple strains of the pathogen [50]. Chlorhexidine has a slightly lower cisticidal activity than PHMB but may still be a more effective alternative to PHMB because it is a smaller molecule that can invade the stroma more easily [51]. Another advantage of using PHMB and chlorhexidine is that these compounds seem to have less toxicity problems when compared to the aromatic diamidine propamidine [28]. Side effects of this treatment can involve an elevated intraocular pressure and toxic keratopathy [26]. Therefore, patients may have to be monitored for their intraocular pressure and the need for antiglaucoma medication alongside this treatment.

6.2. Aromatic Diamidines

Aromatic diamidines such as propamidine and hexamidine are often used to treat AK in combination with biguanides to prevent drug resistance to diamidines. One of the mechanisms of action of aromatic diamidines probably involves binding to the parasite’s DNA which would result in inhibition of its growth [51]. Propamidine was one of the first treatments discovered for treating AK and has therefore been a part of many different combination therapies. Propamidine has also been successfully administered for AK with antifungal medications such as topical miconazole 1% and oral itraconazole [26,28,52]. Propamidine is found to be the most effective aromatic diamidine, however there are many reports of *Acanthamoeba* developing resistance to propamidine which is why the preferred treatment is to take it alongside a biguanide like chlorhexidine [46,53,54].

6.3. Antibiotics

Neomycin can eliminate the trophozoite form of *Acanthamoeba* but does not have a high cisticidal activity like other previously mentioned drugs. This drug cannot be used alone because along with cysts being resistant, neomycin can promote hypersensitivity to itself and cause the development of neomycin-resistant temperature-sensitive mutants [52]. Using neomycin in a “triple therapy” such as with propamidine and dibromopropamidine has effectively treated many AK patients [55,56]. This is because neomycin has an indirect effect on *Acanthamoeba* by decreasing bacterial food for trophozoites and preventing bacterial superinfection [31].

6.4. Steroids

The use of corticosteroids to decrease the intense inflammation that occurs in AK is controversial because of the disadvantage of potentially promoting encystment and extending the duration of the disease [57]. Steroids can also cause keratic precipitates, corneal edema, and deep stromal infiltrates. Therefore, steroids are generally avoided in the treatment of AK, except in exceptional cases when other treatments fail [31].
6.5. Surgery

If the recommended treatment of biguanides and aromatic diamidines fail [28,53], and the infection has progressed to an advanced stage, then a therapeutic corneal transplantation or various keratoplasties can be the last resort of treatment. A variation of keratoplasty called Deep Anterior Lamellar Keratoplasty has been suggested as a better surgical option for AK patients for prevention of intraocular invasion by pathogens due to its non-penetrating nature [28]. Corneal cryotherapy and amniotic membrane transplantation are other options if topical treatments fail [31]. Keratoplasty is most useful for patients who are experiencing vision loss due to either the advanced stromal destruction induced by uncontrolled infection or corneal scarring after medical eradication of the infection.

6.6. New Treatment Approaches

While chlorhexidine, PMHB, and propamidine are currently the recommended agents of AK treatment [26,51], a few reports have employed collagen cross-linking using riboflavin and UV lights as a successful adjunct strategy for the conventional topical therapy [29]. It is possible that the photochemical reactions stabilize the collagen and prevent further tissue damage with prevention of pathogen reproduction [29].

Plants are also potential therapeutic agents. Many species are advantageous sources of amoebicidal agents because along with having high anti-amoebic activity, they have a much lower toxicity than the drugs currently used to treat AK [47]. There are reports of at least ten different medicinal plants having high trophozoite and cysticidal activity with no toxicity to human keratocytes [47]. Tea tree oil demonstrated 100% effectiveness against both trophozoite and cyst forms of Acanthamoeba in vitro [48]. Although further research is required, this semi-in vivo study demonstrates that tea tree oil can potentially destroy amoebae in the cornea through the form of eye drops. Tea tree oil can penetrate tissues therefore, it would be able to attack both shallow and deep layers of the cornea [48]. The oil did not damage the eyes of any of the mice therefore demonstrating no toxicity [48]. An aqueous extract of the nigella sativa plant was also been studied as a potential therapy for AK patients. Nigella sativa has antioxidant activity along with phenolic, alkaloid, and saponin constituents that enhance opsonization and phagocytosis of Acanthamoeba [49]. This plant also inhibits bacterial biofilm, and therefore could result in disrupting Acanthamoeba's binding to the cornea [49]. Experiments have proven both of these plants to be non-toxic but studying the effects in a larger sample size is the next step to take for future studies.

Regarding surgical approaches for the resultant corneal scarring, photorefractive keratectomy (PRK) has been reported to result in vision improvement with no disease recurrence in a few patients [26].

7. Prevention

General prevention paradigm for AK should start with the avoidance of common risk factors. Avoiding contaminated waters and corneal trauma will help minimize risk for the disease.
a more reliable laboratory test for AK. This disease can be effectively treated with aromatic diamidines and biguanides in a combination therapy. As there is an increasing prevalence of contact lens usage in the developed world and currently no efficacious monotherapy for AK, further search for newer therapeutic agents or strategies are warranted. However, one needs to be bear in mind that Acanthamoebas are phyllogenetically similar to humans and therefore make it challenging to find an agent that selectively harms the parasite without harming its host [26]. Taking future steps in augmenting consumer education and public awareness should prevent the occurrence and improve the outcome of AK.

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