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Review article

Acanthamoeba in the eye, can the parasite hide even more? Latest developments on the disease

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ABSTRACT

Acanthamoeba spp. is a free living protozoan in the environment, but can cause serious diseases. *Acanthamoeba* keratitis (AK), a severe and painful eye infection, must be treated as soon as possible to prevent ulceration of the cornea, loss of visual acuity, and eventually blindness or enucleation. Although the disease affects principally contact lens (CLs) wearers, it is recognized nowadays as a cause of keratitis also in non-CLs wearers. Although the number of infections caused by these amoebae is low, AK is an emerging disease presenting an extended number of cases each year worldwide mostly due to the increasing use of CLs, but also to better diagnostic methods and awareness.

There are two principal causes that lead to severe outcomes: misdiagnosis or late diagnosis of the causal agent, and lack of a fully effective therapy due to the existence of a highly resistant cyst stage of *Acanthamoeba*.

Recent studies have reported different genotypes that have not been previously associated with this disease. In addition, *Acanthamoeba* can act as a reservoir for phylogenetically diverse microorganisms. In this regard, recently giant viruses called Pandoravirus have been found within genotypes producing keratitis. What potential risk this poses is not yet known.

This review focuses on an overview of the present status and future prospects of this re-emerging pathology, including features of the parasite, epidemiology, clinical aspects, diagnosis, and treatment.

1. Introduction

Numerous free-living amoebae cause opportunistic infection in humans. *Acanthamoeba* genus is found in the air, soil, and fresh or brackish waters. Some strains of *Acanthamoeba* are responsible for causing human infections [1].

AK is an infiltrative corneal ulceration caused by some *Acanthamoeba* strains. It has been recognized as a potentially blinding disease, often only diagnosed at a late stage. The clinical presentation is sometimes confused with other infectious keratitis, particularly those of herpetic and fungal origin [2].

The causal agent exists in both active (trophozoite) and dormant (cyst) forms. The cysts are able to survive for long periods of time in hostile environments, including chlorinated swimming pools, hot tubs, and subfreezing temperatures in fresh water lakes, turning into trophozoites when environmental conditions are appropriate. The trophozoites produce a variety of enzymes that aid in tissue penetration and destruction [3]. Both trophozoites and cysts can adhere to the surface of unworn soft or rigid CLs [4], and then a break in the corneal epithelium may allow them to invade the eye tissues.

Most of *Acanthamoeba* infections are associated with CLs wear [5], and the expected incidence in developed countries is one to 33 cases per million CLs wearers [6]. Since CLs users numbers are growing every year worldwide and awareness and better diagnostics are available, the disease will become increasingly important over time [7,8].

AK is known to be difficult to diagnose and treat, despite advances in pharmacotherapy. Most patients are initially wrongly treated for viral, fungal, or bacterial keratitis before the diagnosis of *Acanthamoeba*

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is made. The need therefore, is to make a quick and accurate diagnosis to ensure effective treatment, and a good prognosis. When AK is suspected, a provisional diagnosis can be made through clinical features and confocal microscopy. However, a definitive diagnosis is only possible by culture, histology, or identification by polymerase chain reaction (PCR) [9].

The aim of this review is to highlight current information about the disease including general characteristics, epidemiology, clinical aspects, diagnosis, and treatment, focusing in recent discoveries in the biology of the parasite that involve changes in the prognosis and treatment of the AK.

2. *Acanthamoeba*'s free life in the environment

The life cycle of *Acanthamoeba* consists of two stages: a vegetative active trophozoite stage (25–40 μm) and a dormant protective cyst stage (13–20 μm) [10]. During the trophozoite stage *Acanthamoeba* actively feeds on bacteria, algae, yeasts or small organic particles and divides mitotically under optimal environmental conditions. Exposure to harsh conditions, such as lack of food, hyper- or hypo-osmolarity and extreme pH or temperature, results in cellular differentiation into a double-walled cyst form [11], in which *Acanthamoeba* can survive *in vitro* for more than 20 years [12].

Acanthamoeba is remarkably tolerant to a wide range of environmental conditions, being probably the most common protozoon found in soil, water, and in air samples [13,14], in a wide variety of habitats, from tropical to arctic regions. Recently, pathogenic genotypes were isolated from soils and water resources in Pakistan [15,16], from public thermal baths in Hungary [17], and from reservoirs of drinking water in Taiwan [18].

The major incidence factor in the distribution of *Acanthamoeba* in the environment is the presence of an available food supply. *Acanthamoeba* can take in bacteria via phagocytosis, after which it lyses them in the phagolysosomes [19]. However, some bacteria have established a stable symbiotic relationship with amoebae, a situation which may serve as reservoir for maintaining and dispersing pathogenic bacteria in the environment. It is puzzling that *Acanthamoeba* can host bacteria protecting them from the adverse environment and, at the same time, feeds on them to cover its nutritional requirements [19].

3. *Acanthamoeba* genotypes in AK

Species of *Acanthamoeba* were originally classified into three groups (I–III) based in differences in cyst morphology [20]. However, as culture conditions affect morphology, identifications are nowadays based on rRNA gene sequences and the genus is divided into 20 different evolutionary lines or clades (T1-T20) [21,22]. In the world, most of the clinical isolates from both keratitis and non-keratitis samples, have been typed as T4 genotype [23]. Other genotypes that have also been found associated to AK include T2a, T3, T5, T6, T10, T11, and T15 [[23],24]. Grun et al. [25] have recently reported the genotype T13 as an etiological agent of keratitis. Although T4 is the most frequently isolated genotype [26], non-T4 genotypes produce worse symptoms and have poorer response to medical therapy [27].

4. Pathogens' reservoir

Acanthamoeba trophozoites and cysts have the ability to harbor a variety of microorganisms [19]. Several pathogenic bacterial species were isolated from the inside of *Acanthamoeba* species, including *Salmonella enterica* [28], *Pseudomonas* spp. [29–31], *Mycobacterium* spp. [32,33], *Legionella pneumophila* [30], *Helicobacter pylori* [34], *Campylobacter jejuni* [35], *Listeria* spp., and *Vibrio* spp. [36]. In this regard, the presence of *P. aeruginosa* enhances the attachment of *Acanthamoeba* trophozoite to hydrogel CLs [37], but not to silicone ones [38], and Dini et al., 2000 [39] reported a case of AK where both agents were present. More work is needed to understand this relationship as inhibition of *Acanthamoeba* growth by *Pseudomonas* has also been observed [40].

Other protozoa, including *Toxoplasma gondii* [41]; fungi, as *Cryptococcus*, *Blastomyces*, *Sporothrix*, *Histoplasma*, *Exophiala*; and virus, as mimivirus, coxsackieviruses, adenoviruses [42], poliovirus, echovirus, enterovirus, vesicular stomatitis virus [19] have also been found as *Acanthamoeba* endosymbionts. Recently, a giant virus found inside *Acanthamoeba* strains producing keratitis was identified as a member of the emerging Pandoravirus family [43] and later its whole genome was sequenced [44]. On the other hand, studies on *Acanthamoeba polyphaga* mimivirus showed an inhibition of the amoebal encystment [45], which may represent an advantage in AK treatment.

5. AK epidemiology

Despite its wide distribution, diseases caused by *Acanthamoeba* are relatively uncommon. Among the infections, AK is the most frequent, although other types can be produced in immunocompromised hosts [46,47].

The epidemiological features of infectious keratitis may vary among different geographic regions, climate conditions, and living environments. Traditionally, predisposing factors of AK included corneal trauma associated with exposure to contaminated water, soil or vegetation [48,49]. Nevertheless most cases of AK were reported in association with CLs wear [50–55]. However, in Asian countries the majority of the AK occurred in non-CLs wearers [56–59], being secondary to corneal injury. This scenario has been identified as the major risk factor for infectious keratitis in rural areas [60–62].

A recent review of case-control studies showed that the use of CLs increased by 10 times the risk of suffering AK [5]. It was found that *A. castellanii* trophozoites and cysts adhere not only to soft, but also to rigid CLs [[64],63]. Infections related to CLs are often associated with improper wear such as overuse, poor cleaning and sleeping or swimming with them. Exposure of CLs to water seems to be a significant risk factor for AK [64]. According to research conducted by the Center for Disease Control (CDC) in the US (Table 1), topping off and storing lenses in water were associated with more than four-fold increases in risk, closely followed by shorter duration of use, handling lenses with wet hands and rinsing cases with water before storage [65]. The fact that the risk is higher in patients with fewer years of use would indicate that the experience in handling could play a role in prevention of AK.

Some researchers suggested that municipal water supply and its treatment may play a role in the development of AK. In the UK the AK incidence was 0–42 cases per million inhabitants, this variability being associated with the distribution of hard and soft water around the country. It has been hypothesized that hard water, that leads to limescale deposits in house water tanks, provides a favorable environment for amoebae and reduces the efficiency of chorine disinfection systems [66]. The presence of *Acanthamoeba* spp. in swimming pools may

Table 1
Hygiene risk factors in CLs-related *Acanthamoeba* keratitis.
Source: USA CDC 2014 Multivariable analysis (Cope JR; 2014).

	Cases n/N (%)	Control n/N (%)	mOR[a]	(95% CI)
Topping off	69/101 (68)	48/144 (33)	4.46	(2.19–9.81)
CLW ≤ 5 yrs	35/109 (32)	26/157 (17)	2.98	(1.47–6.35)
Storing lenses in water	25/102 (25)	5/145 (3)	4.38	(1.47–15.88)
Handling with wet hands	66/109 (61)	82/155 (53)	2.19	(1.10–4.59)
Rinsing case before store	55/97 (57)	51/138 (37)	2.10	(1.06–4.32)

CLW = CLs wearing.
[a] mOR: m Odds ratio adjusted for age, sex, and CLs type using exact conditional logistic regression.

explain AK peaks during summer months [52,55].

In association with CLs use, *Acanthamoeba* adhesion increases with the water content of the lens, while it is minimized with proper cleaning procedures [67]. Previous studies have focused on the impact of the hygiene of lens, care solution, and storage case and environmental factors on the incidence of AK [68–70]. It seems that propylene glycol, commonly used as ophthalmic demulcent, could induce pseudoencystment, increasing the potential risk of infection due to resistance of acanthamoebae to disinfecting agents [71]. A study conducted in 2014 [72] indicated that many of the multi-purpose solutions (MPS) available in the Egyptian market were not effective against clinical and tap water *Acanthamoeba* strains. Assessment of anti-*Acanthamoeba* solution efficiency was limited by a lack of standardized testing methods, which is crucial because the susceptibility to biocides also varies with growth media, strain, and methods for encystation [73].

The Ophthalmic Devices Panel of the Food and Drug Administration (FDA) Medical Devices Advisory Committee recommends adding *Acanthamoeba* spp. as a challenge organism for testing CLs solutions [74]. A novel method was proposed to compare environmental *Acanthamoeba* susceptibility to MPS, being genotypes T3, T5, and T11 more resistant than the T4 [75].

Acanthamoeba has been found to produce co-infections with fungi, viruses, chlamydia, and bacteria [76–78].]. Thus, the possibility must be considered that ophthalmic solutions contaminated with amoebae, may also contain other microorganisms that synergistically invade along with amoebae corneal injury [79,80].

AK has also been reported after invasive corneal surgery or radial keratoplasty and after laser *in situ* keratomileusis (LASIK), where more serious injuries may occur in patients mainly due to delay in diagnosis and treatment [77,81,82]. Even within the past year, a case of AK in a young girl without risk factors was reported in the literature [83].

6. Clinical aspects

An interesting feature of AK, compared to most other forms of microbial keratitis, is that the disease usually progresses slowly [84]. The onset of symptoms can take a few days to several weeks, depending on the inoculum size and/or the corneal trauma [85]. The clinical picture of AK is remarkable for its variability. In most cases symptoms include pain, photophobia, and tearing, usually in one eye. CLs users can present with bilateral involvement [86]. In early AK a severe pain may be manifested disproportionately to the clinical signs, and there is usually a reduction in corneal sensation [9].

The earliest signs may be non-specific [86]. Within the first month, the disease can manifest as a mild conjunctival injection and epitheliopathy including a punctate keratopathy, pseudo dendrites (often mistaken for herpes simplex keratitis), epithelial or subepithelial infiltrates, and perineural infiltrates, with ring infiltrates in less than 20% of patients [9]. *Acanthamoeba* trophozoites often cluster around corneal nerves, producing radial keratoneuritis virtually pathognomonic for AK, being present in up to 63% of cases diagnosed within 6 weeks. Extreme pain is common in these cases [[9],87]. A careful slit-lamp examination may be necessary to identify these infiltrates, as only one or two nerves may be affected. The appearance of perineural infiltration is not yet fully understood, although it was suggested that the parasites may move more easily through the course of corneal nerves, or that they may preferentially damage nervous tissue [84].

The generation of the ring-shaped stromal infiltrates and corneal lesions can be related to the production of a collagenolytic enzyme [88]. In long standing cases a central defect, which is often associated with stromal thinning, may occur. The ring may be incomplete, or occasionally double and concentric [84]. Limbitis is a common finding in both early and late disease [9].

After a month, the disease is characterized by ring infiltrates, marked ulceration, and a secondary sterile anterior uveitis, sometimes with hypopyon. Some patients may present with corneal edema caused by endothelial plaques or a disciform reaction. Perineural infiltrates are less frequent in advanced disease [9]. Vascularization may occur, but it is not usually marked unless secondary bacterial infection has taken place [84]. The inflammation may involve the sclera, but evidence of direct scleral invasion by amoebae has often been elusive, leading to the conclusion that scleritis is a secondary immunologic reaction [84]. AK rarely progresses beyond the corneal endothelium to produce intraocular infection and endophthalmitis [88].

Further evolution to severe forms is more common in late-presenting disease and it may include abscess formation, keratic precipitates, anterior chamber cells, hypopyon, scleritis, extensive scleral ectasia occurring after prolonged scleritis, glaucoma, cataract, corneal melt, corneal perforation, and posterior segment inflammation, optic nerve edema, optic neuropathy and optic atrophy, retinal detachment, choroidal inflammation, and formation of a macular scar [9,84].

At this point it has to be remembered that non-T4 genotypes produce worse symptoms than genotype T4 [27].

7. Diagnosis

AK remains one of the more challenging clinical entities in corneal disease to be diagnosed [55]. It is usually misdiagnosed since its symptoms are similar to keratitis produced by other agents such as bacteria, viruses and fungi [55,89]. A good prognosis depends on the diagnosis being early and a prompt access to the appropriate medical therapy. If this is delayed for three weeks or more, the prognosis worsens [9]. To avoid this situation, AK should be considered whenever there is corneal trauma associated with exposure to contaminated water or soil, or CL wear. The disease must be also taken into account when there is a failure in the response to first line therapy for bacterial or herpes simplex virus keratitis [9].

The preferred diagnostic technique is confocal microscopy (CFM), which shows specificity and sensitivity greater than 90% [90]. *Acanthamoeba* is confirmed positive by CFM when highly reflective round or ovoid structures (10–25 μm of diameter) are observed, or if double-walled structures compatible with *Acanthamoeba* cysts are visualized [90]. Tu et al. [91] compared *in vivo* tandem-scanning CFM with superficial corneal smear and superficial corneal culture, and analyzed the results for validity against microbiologic and clinical standards of AK, observing a sensitivity of 91% and a specificity of 100% for CFM. Specular microscopy, where the organisms appear as refractile granular opacities, has been also used to demonstrate the presence of *Acanthamoeba in vivo* within the cornea [84].

Although a presumptive diagnosis can be made clinically or with CFM, a definitive diagnosis of AK can only be made on the basis of culture or histology, or by the identification of amoebic DNA by PCR [92]. The identification using culture techniques consists of the direct inoculation on non-nutrient agar plates. On receipt in the laboratory, the inoculated area of agar is excised from the plate and inverted onto an *Escherichia coli*–seeded non-nutrient agar plate and cultured at 30 °C for periods as long as 6 days, with further stages at room temperature to ensure growth of some isolates [93]. Cultures may occasionally take three weeks to grow using this protocol and specimens should not be discarded until the end of this period [9]. However, culture results are inconsistent, with sensitivities ranging from 7 to 52% and have the further disadvantage of requiring long incubation times [94].

Smears immunostaining with immunoperoxidase using a polyclonal antibody for *Acanthamoeba* could become a useful method, but the antibody is not yet commercially available [84].

PCR is potentially a useful technique for AK diagnose, where *Acanthamoeba* DNA can be directly amplified from corneal scrapings [55,95,96]. Identification of *Acanthamoeba* by PCR showed 84% sensitivity and 100% specificity [55], but it has not yet been standardized or become widely available. Moreover, one recent report suggested that culture and PCR were statistically equivalent for detecting *Acanthamoeba* from ocular samples [97].

8. Current therapy

AK raises an extremely challenging clinical management problem with the potential for treatment failure. If diagnosed early, the prospect for complete recovery of vision is higher. However, the diagnosis methods currently used are invasive, requiring corneal culture and stromal biopsy, and are often postponed until there is high suspicion of the disease and/or no response to treatments for bacterial, viral and/or fungal keratitis [90].

There are no drugs specifically approved for AK by the FDA. Aggressive medical therapy is initiated using multiple antiamoebic, antibacterial, and antifungal agents. Different antimicrobial agents are used in combination to improve the likelihood of a successful treatment [98]. Medications have to be used for a long period of time after clinical resolution of infection to prevent relapses, because the drugs are less effective against the cystic forms. In this regard, Kumar and Lloyd [99] pointed out that the encysted stage in the life cycle of *Acanthamoeba* species appears to cause intractable problems, and that many biocides are ineffective in killing the highly resistant cysts.

Two classes of antimicrobial agents are currently used to treat most *Acanthamoeba* infections, biguanides and diamidines [100]. The biguanides, cationic antiseptics acting at membrane level, include polyhexamethylene biguanide (PHMB) and chlorhexidine (bis-biguanide), and among the diamidines, which inhibit DNA synthesis, the most frequently used are propamidine, hexamidine, and pentamidine [101]. These antimicrobial agents present different amoebicidal activities, which may vary due to the different degrees of pathogenicity and virulence among *Acanthamoeba* species or strains [27,102]. PHMB is generally the preferred agent, either alone or in combination [9,103,104]. Although chlorhexidine has also been used as a monotherapy, it does not appear to be as effective as when used in combination with propamidine [105].

The first 2 or 3 days of treatment the therapeutic agent is usually administered hourly day and night as an eye drop solution. After this period, the administration is reduced in frequency to hourly daytime for the next days, and then to four times a day [106]. The treatment could be prolonged for several months and requires regular controls until clinical evidence of disease resolution.

Lim et al. [107] compared the outcomes when using PHMB 0.02% and chlorhexidine 0.02% as monotherapy agents [107] in 56 eyes. They found that treatment produced a favorable clinical response within the first two weeks in 78% and 85.7% of the cases for PHMB and chlorhexidine, respectively. They also observed a recovery of the visual acuity in 56.5% and 71.4% and only 3/23 and 2/28 required penetraiting keratoplasty, when treated with PHMB and chlorhexidine, respectively. The results allowed them to conclude that the outcomes were similar for both agents in treating AK.

Biguanides are usually chosen since they have the lowest minimum cysticidal concentrations and are generally more effective against *Acanthamoeba* cysts [108]. In fact, diamidines are not recommended as monotherapy agents since they favor resistance development [104]. This is also the reason why propamidine is used in combination with PHMB, or chlorhexidine. However, some clinicians preferred the use of PHMB in combination with hexamidine, because they believe that this diamidine is more effective and less toxic than propamidine [104].

A combination of propamidine isethionate 0.1% (Brolene®) and 0.02% PHMB in drops was found to be well tolerated, non-toxic and largely effective against *Acanthamoeba* species [8]. Alternatively, a combination of Brolene® and fluoroquinolone with chlorhexidine may give good results [99]. Topical steroids can be used to control persistent inflammation but their use should finish before cessation of antiamoeba therapy [109].

Ultraviolet-A light and riboflavin therapy is an alternative therapeutic option in non-responsive cases to topical agents [110,111]. Khan et al. [110] evaluated this therapy in 3 cases of AK unresponsive to medical treatment [110]. After two treatment sessions involving topical application of 0.1% riboflavin solution and 30 min of Ultraviolet-A irradiation, the ulcers in all patients were closed within 3 to 7 weeks of the first application.

Recalcitrant chronic *Acanthamoeba* stromal keratitis may be treated with extended systemic voriconazole with good preservation of vision. Tu et al. [112] reported the successful use of this agent in three eyes of two patients unresponsive to traditional therapies, achieving clinical resolution in a period of 7 to 11 months [112].

Flurbiprofen is used orally as adjunctive therapy providing anti-inflammatory and analgesic properties [104]. Also imidazoles 1% (e.g., ketoconazole), used as additive (never as primary therapy), are effective against trophozoites but not against cysts [113,114].

Despite scraping is usually necessary for diagnosis, it may also have a therapeutic benefit if carried out during the first stages when the disease is intraepithelial [115].

Although penetrating keratoplasty for visual recovery should be delayed as possible until a medical cure has been achieved, it should be considered as a therapeutic tool when the infectious process spreads to the Para central corneal stroma despite an aggressive antiamoebic therapy [8]. In these cases, this procedure may allow total removal of the organisms by excising the clinically involved tissue and a rim of clear surrounding cornea. Penetrating keratoplasty may also be required when therapeutic failure in cases of severe AK. However, graft survival is poor; postoperative glaucoma is frequent and is associated with shorter graft survival [116].

With respect to prognosis after AK treatment, it has been shown that a late diagnosis decreases the likelihood of a good visual result. A study conducted by Cardine et al. [117] found an average delay of two months between first symptoms and diagnosis [117], which was associated to a poor visual outcome in 10 from 25 eyes. In all cases the treatment included topical antiparasitic eye drops, and in nine of them surgical treatment was required.

In a study by Tu et al. [118], the final visual acuity of 65 eyes affected by AK was evaluated, concluding that 40% achieved a final visual acuity of at least 20/25 [118]. They also associated deep stromal involvement or the presence of an annular infiltrate with worse visual results, and found that the duration of symptoms was not related to the final visual result. Keratoplasty was performed in 17% of the patients recruited.

Robaei et al. [119] performed an exhaustive analysis of the outcome of 196 patients with AK between1991 an 2012 [119]. They found that 25.5% required penetrating or anterior lamellar keratoplasty, and a 20% of them had repeat keratoplasty. From the total of the patients undergoing keratoplasty, 52% did it for therapeutic basis and 48% for visual rehabilitation. In the second group, 54.2% achieved a visual acuity of 20/30 compared to a 26.9% in the first one, showing that the prognosis is better when the keratoplasty is performed for visual recovery when the medical cure has been achieved. They also confirmed the importance of early diagnosis of *Acanthamoeba* in improving prognosis and avoiding keratoplasty.

9. Future prospects for treatment

Although many drugs can be safely delivered by eye drops, effective treatment has a strong dependence on patient compliance [120].

A major goal of pharmaco-therapeutics is the attainment of an effective drug concentration at the intended site of action for a desired length of time. Efficient delivery of a drug while minimizing its systemic and/or local side effects is the key to the treatment of ocular diseases. The design and development of drug delivery systems for ocular administration has to overcome the challenges posed by the physiological and anatomical characteristics of the eye; however, along with the understanding of the mechanisms of ocular drug absorption and disposition, the knowledge in this field rapidly increases. Systems may include different alternatives, varying from simple solutions to new delivery systems such as, corneal collagen shields, iontophoresis,

biodegradable polymeric systems, and viral and nonviral gene delivery systems [121].

Currently, vehicles and carriers including nanoparticles and substances with gelling properties, are being evaluated [122,123]. L-alpha-phosphatidylcholine liposomes and cholesterol or ergosterol have been tested to enhance the potency of pentamidine isethionate. It was observed that at a drug concentration of 10 μg ml^{-1}, the liposomal drug was 12 times more effective than the free drug preventing the binding of *Acanthamoeba* to human cells and reducing significantly the cytopathogenicity of human cells mediated by parasites [122].

In addition, the design of pro-drugs, which hydrolize within the eye, provides an alternative to achieve higher concentrations, reduced toxicity, and prolonged activity for topical medications. Many lipophilic ester prodrugs such as 15-acetyl, 15-pivaloyl, 1-isopropyl, 15-valeryl, 1,11-lactone, and 11,15-dipivaloyl esters were tested for transport and bioconversion. The ester conjugate can easily cross the corneal epithelium due to its lipophilic nature, but the stroma acts as a hydrophilic barrier. This layer forms a depot to such lipophilic drugs until hydrolysis to parent drug [124].

Drugs approved for other uses have also been tested for AK treatment. In this sense, Jha et al. [125] found that tigecycline, a third-generation tetracycline antibiotic which is commonly used to treat antibiotic resistant bacterial infections, significantly inhibited the growth of *Acanthamoeba* without affecting cell viability and induction of encystment. This same effect has not been observed with other tetracycline antibiotics groups such as tetracycline and doxycycline. It was observed that tigecycline decreased cellular adenosine triphosphate (ATP) over control and increased mitochondrial mass. These findings suggest that selective mitochondrial dysfunction and corresponding decrease in ATP production would be the potential mechanism by which tigecycline controls *Acanthamoeba* growth.

On the other hand, Deng et al. [126] demonstrated that artemether, an antimalarial agent, can be used as an inhibitor of phosphoglycerate dehydrogenase to control or block *Acanthamoeba* infections. The drug exhibited *in vitro* amoebicidal activity in a time- and dose-dependent manner, and induced ultrastructural modification and cellular apoptosis. Although artemether showed amoebicidal activity at relatively high concentrations, further studies are needed to test this agent in combination with other drugs as an approach to more effectively treat *Acanthamoeba* infections.

Immunological methods are also being investigated. Oral immunization of animals has been successfully achieved in the prevention of AK [80,99]. However, it is unlikely that immunization will be used to reduce the incidence of CLs induced *Acanthamoeba* infection in humans due to the small incidence of the disease worldwide.

In normal clinical practice, patients are advised not to wear CLs when they present with infections of any type. However, therapeutic CLs would sometimes be indicated, since their use could protect against possible perforations and/or continuously release a drug, either for pain treatment or to control the infectious agent. In these cases a daily check should be done, and the CLs should be placed and removed by a physician.

Certain requirements must be met by a therapeutic lens to make it ideal. It should minimize mechanical trauma, hypoxia and tear film disruption, and at the same time, it should stimulate recovery of the condition being treated. The transmissibility of oxygen must be maximized, particularly when a patient will continuously use the lens. Numerous attempts have been made related to loading drugs into CLs [127–129], including hydrogels to control drugs release [130], and using alternative processes to ensure a high load of active components [131]. Despite all the efforts made so far, progress towards introducing such a strategy into the usual ophthalmological practice for AK treatment has been limited.

10. Perspectives

AK is a re-emerging disease, and although much is known about the pathogenesis of corneal invasion, there is a lack of understanding of the causes of the severe, extra-corneal, non-infectious, inflammatory disorders that may be associated with the primary infection and that can result in blindness for some patients who have severe disease [9]. *Acanthamoeba* is able to host many more microorganisms than previously thought and the parasite includes genotypes that were not previously taken into account as keratitis producers, but which have come to light due to the current development of diagnostic methods. Future studies are needed to identify the genetic basis for virulence factors producing disease and because the parasite and host factors have been found to be equally important in the pathogenesis of *Acanthamoeba* infection, it is reasonable to predict that emerging genomic techniques will play a fundamental role in providing novel therapeutic strategies [7].

Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] H. Alizadeh, T. Tripathi, M. Abdi, A.D. Smith, Pathogenic strains of Acanthamoeba are recognized by TLR4 and initiated inflammatory responses in the cornea, PLoS One 9 (2014) e92375.
[2] T.J. Liesegang, Contact lens-related microbial keratitis: part I: epidemiology, Cornea 16 (1997) 125–131.
[3] R. Siddiqui, N.A. Khan, Biology and pathogenesis of *Acanthamoeba*, Parasit Vectors 5 (A) (2012) 1–13.
[4] L.D. Kelly, D. Long, D. Mitra, Quantitative comparison of *Acanthamoeba castellanii* adherence to rigid versus soft contact lenses, CLAO J 21 (1995) 111–113.
[5] E. Pacella, G. La Torre, M. De Giusti, C. Brillante, A.M. Lombardi, G. Smaldone, et al., Results of case-control studies support the association between contact lens use and *Acanthamoeba keratitis*, Clin Ophthalmol 7 (2013) 991–994.
[6] CDC, Parasites - Acanthamoeba - Granulomatous Amebic Encephalitis (GAE); Keratitis. Epidemiology & Risk Factors, in, CDC, https://www.cdc.gov/parasites/acanthamoeba/epi.html.
[7] N.A. Khan, Pathogenesis of *Acanthamoeba* infections, Microb Pathog 34 (2003) 277–285.
[8] J. Lorenzo-Morales, N.A. Khan, J. Walochnik, An update on *Acanthamoeba keratitis*: diagnosis, pathogenesis and treatment, Parasite 22 (2015) 10.
[9] J.K. Dart, V.P. Saw, S. Kilvington, *Acanthamoeba keratitis*: diagnosis and treatment update 2009, Am J Ophthalmol 148 (2009) 487–499 e482.
[10] F. Marciano-Cabral, G. Cabral, *Acanthamoeba* spp. as agents of disease in humans, Clin Microbiol Rev 16 (2003) 273–307.
[11] R. Siddiqui, R. Dudley, N.A. Khan, *Acanthamoeba* differentiation: a two-faced drama of Dr Jekyll and Mr Hyde, Parasitology 139 (2012) 826–834.
[12] R. Sriram, M. Shoff, G. Booton, P. Fuerst, G.S. Visvesvara, Survival of *Acanthamoeba* cysts after desiccation for more than 20 years, J Clin Microbiol 46 (2008) 4045–4048.
[13] F.C. Page, A New Key to Freshwater and Soil *Gymnamoebae* Ambleside, (1988) Cumbria U. K..
[14] S. Rodriguez-Zaragoza, Ecology of free-living amoebae, Crit Rev Microbiol 20 (1994) 225–241.
[15] T. Tanveer, A. Hameed, A. Gul, A. Matin, Quick survey for detection, identification and characterization of *Acanthamoeba* genotypes from some selected soil and water samples across Pakistan, Ann Agric Environ Med 22 (2015) 227–230.
[16] T. Tanveer, A. Hameed, A.G. Muazzam, S.Y. Jung, A. Gul, A. Matin, Isolation and molecular characterization of potentially pathogenic *Acanthamoeba* genotypes from diverse water resources including household drinking water from Khyber Pakhtunkhwa Pakistan, Parasitol Res 112 (2013) 2925–2932.
[17] C. Kiss, Z. Barna, M. Vargha, J.K. Torok, Incidence and molecular diversity of *Acanthamoeba* species isolated from public baths in Hungary, Parasitol Res 113 (2014) 2551–2557.
[18] P.M. Kao, B.M. Hsu, C.T. Chen, S.W. Huang, E.S. Kao, J.L. Chen, et al., Identification and quantification of the *Acanthamoeba* species and genotypes from reservoirs in Taiwan by molecular techniques, Acta Trop 132 (2014) 45–50.
[19] R. Siddiqui, N.A. Khan, War of the microbial worlds: who is the beneficiary in

Acanthamoeba-bacterial interactions? Exp Parasitol 130 (B) (2012) 311–313.
[20] M. Pussard, R. Pons, Morphologie de la paroi kystique et taxonomie du genre *Acanthamoeba* (Protozoa, Amoebida), Protistologica 8 (1977) 557–598.
[21] P.A. Fuerst, Insights from the DNA databases: approaches to the phylogenetic structure of *Acanthamoeba*, Exp Parasitol 145 (Suppl) (2014) S39–S45.
[22] D. Corsaro, M. Kohsler, M. Montalbano Di Filippo, D. Venditti, R. Monno, D. Di Cave, et al., Update on *Acanthamoeba jacobsi* genotype T15, including full-length 18S rDNA molecular phylogeny, Parasitol Res 116 (2017) 1273–1284.
[23] G.S. Visvesvara, H. Moura, F.L. Schuster, Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*, FEMS Immunol Med Microbiol 50 (2007) 1–26.
[24] A. Risler, B. Coupat-Goutaland, M. Pelandakis, Genotyping and phylogenetic analysis of *Acanthamoeba* isolates associated with keratitis, Parasitol Res 112 (2013) 3807–3816.
[25] A.L. Grun, B. Stemplewitz, P. Scheid, First report of an *Acanthamoeba* genotype T13 isolate as etiological agent of a keratitis in humans, Parasitol Res 113 (2014) 2395–2400.
[26] S.K. Maciver, M. Asif, M.W. Simmen, J. Lorenzo-Morales, A systematic analysis of *Acanthamoeba* genotype frequency correlated with source and pathogenicity: t4 is confirmed as a pathogen-rich genotype, Eur J Protistol 49 (2013) 217–221.
[27] F. Arnalich-Montiel, B. Lumbreras-Fernandez, C.M. Martin-Navarro, B. Valladares, R. Lopez-Velez, R. Morcillo-Laiz, et al., Influence of *Acanthamoeba* genotype on clinical course and outcomes for patients with *Acanthamoeba keratitis* in Spain, J Clin Microbiol 52 (2014) 1213–1216.
[28] D. Tezcan-Merdol, M. Ljungstrom, J. Winiecka-Krusnell, E. Linder, L. Engstrand, M. Rhen, Uptake and replication of *Salmonella enterica* in *Acanthamoeba rhysodes*, Appl Environ Microbiol 70 (2004) 3706–3714.
[29] R. Michel, H. Burghardt, H. Bergmann, *Acanthamoeba* naturally intracellularly infected with *Pseudomonas aeruginosa*, after their isolation from a microbiologically contaminated drinking water system in a hospital, Zentralbl Hyg Umweltmed 196 (1995) 532–544.
[30] Z. Zeybek, A.R. Binay, Growth ability of Gram negative bacteria in free-living amoebae, Exp Parasitol 145 (Suppl) (2014) S121–S126.
[31] V. Jose Maschio, G. Corcao, M.B. Rott, Identification of *Pseudomonas* spp. as amoeba-resistant microorganisms in isolates of *Acanthamoeba*, Rev Inst Med Trop Sao Paulo 57 (2015) 81–83.
[32] M. Salgado, M. Alfaro, F. Salazar, X. Badilla, E. Troncoso, A. Zambrano, et al., Application of cattle slurry containing *Mycobacterium avium* subsp. paratuberculosis (MAP) to grassland soil and its effect on the relationship between MAP and free-living amoeba, Vet Microbiol 175 (2015) 26–34.
[33] M. Drancourt, Looking in amoebae as a source of mycobacteria, Microb Pathog 77 (2014) 119–124.
[34] N. Alipour, N. Gaeini, A. Taner, F. Yildiz, S. Masseret, P. Malfertheiner, Vacuoles of *Acanthamoeba castellanii* behave as a specialized shelter (host) for helicobacter pylori, Helicobacter 20 (6) (2015) 485–493.
[35] A. Vieira, A.M. Seddon, A.V. Karlyshev, *Campylobacter-Acanthamoeba* interactions, Microbiology 161 (2015) 933–947.
[36] J. Barker, M.R. Brown, Trojan horses of the microbial world: protozoa and the survival of bacterial pathogens in the environment, Microbiology 140 (Pt 6) (1994) 1253–1259.
[37] A. Tomlinson, P.A. Simmons, D.V. Seal, A.K. McFadyen, Salicylate inhibition of *Acanthamoeba* attachment to contact lenses: a model to reduce risk of infection, Ophthalmology 107 (2000) 112–117.
[38] T.K. Beattie, A. Tomlinson, A.K. McFadyen, D.V. Seal, A.M. Grimason, Enhanced attachment of *Acanthamoeba* to extended-wear silicone hydrogel contact lenses: a new risk factor for infection? Ophthalmology 110 (2003) 765–771.
[39] L.A. Dini, C. Cockinos, J.A. Frean, I.A. Niszl, M.B. Markus, Unusual case of *Acanthamoeba polyphaga* and *Pseudomonas aeruginosa keratitis* in a contact lens wearer from Gauteng South Africa, J Clin Microbiol 38 (2000) 826–829.
[40] M.N. Qureshi, A.A. Perez 2nd, R.M. Madayag, E.J. Bottone, Inhibition of *Acanthamoeba* species by *Pseudomonas aeruginosa*: rationale for their selective exclusion in corneal ulcers and contact lens care systems, J Clin Microbiol 31 (1993) 1908–1910.
[41] J. Winiecka-Krusnell, I. Dellacasa-Lindberg, J.P. Dubey, A. Barragan, *Toxoplasma gondii*: uptake and survival of oocysts in free-living amoebae, Exp Parasitol 121 (2009) 124–131.
[42] P. Scheid, R. Schwarzenberger, *Acanthamoeba* spp. as vehicle and reservoir of adenoviruses, Parasitol Res 111 (2012) 479–485.
[43] P. Scheid, C. Balczun, G.A. Schaub, Some secrets are revealed: parasitic keratitis amoebae as vectors of the scarcely described pandoraviruses to humans, Parasitol Res 113 (2014) 3759–3764.
[44] M.H. Antwerpen, E. Georgi, L. Zoeller, R. Woelfel, K. Stoecker, P. Scheid, Whole-genome sequencing of a pandoravirus isolated from keratitis-inducing *Acanthamoeba*, Genome Announc 3 (2015).
[45] P. Boratto, J.D. Albarnaz, G.M. Almeida, L. Botelho, A.C. Fontes, A.O. Costa, et al., *Acanthamoeba polyphaga* mimivirus prevents amoebal encystment-mediating serine proteinase expression and circumvents cell encystment, J Virol 89 (2015) 2962–2965.
[46] F.L. Schuster, G.S. Visvesvara, Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals, Int J Parasitol 34 (2004) 1001–1027.
[47] R.S. Bradbury, L.P. French, L. Blizzard, Prevalence of *Acanthamoeba* spp. in Tasmanian intensive care clinical specimens, J Hosp Infect 86 (2014) 178–181.
[48] X. Sun, Y. Zhang, R. Li, Z. Wang, S. Luo, M. Gao, et al., *Acanthamoeba keratitis*: clinical characteristics and management, Ophthalmology 113 (2006) 412–416.
[49] N.A. Khan, *Acanthamoeba*: biology and increasing importance in human health, FEMS Microbiol Rev 30 (2006) 564–595.
[50] D.V. Patel, S. Rayner, C.N. McGhee, Resurgence of *Acanthamoeba keratitis* in Auckland, New Zealand : a 7-year review of presentation and outcomes, Clin Exp Ophthalmol 38 (2010) 15–20 quiz 87.
[51] F.R. Carvalho, A.S. Foronda, M.J. Mannis, A.L. Hofling-Lima, R. de Freitas, D. Belfort Jr., Twenty years of *Acanthamoeba keratitis*, Cornea 28 (2009) 516–519.
[52] P. McAllum, I. Bahar, I. Kaiserman, S. Srinivasan, A. Slomovic, D. Rootman, Temporal and seasonal trends in *Acanthamoeba keratitis*, Cornea 28 (2009) 7–10.
[53] J.Y. Ku, F.M. Chan, P. Beckingsale, *Acanthamoeba keratitis* cluster: an increase in *Acanthamoeba keratitis* in Australia, Clin Exp Ophthalmol 37 (2009) 181–190.
[54] M.N. Fraser, Q. Wong, L. Shah, S.P. Holland, M. Morshed, J. Isaac-Renton, et al., Characteristics of an *Acanthamoeba keratitis* outbreak in British Columbia between 2003 and 2007, Ophthalmology 119 (2012) 1120–1125.
[55] M.A. Page, W.D. Mathers, *Acanthamoeba keratitis*: a 12-year experience covering a wide spectrum of presentations diagnoses, and outcomes, J Ophthalmol 2013 (2013) 6.
[56] K.G. Watt, H.A. Swarbrick, Trends in microbial keratitis associated with orthokeratology, Eye Contact Lens 33 (2007) 373–377 discussion 382.
[57] H. Ertabaklar, M. Turk, V. Dayanir, S. Ertug, J. Walochnik, *Acanthamoeba keratitis* due to *Acanthamoeba* genotype T4 in a non-contact-lens wearer in Turkey, Parasitol Res 100 (2007) 241–246.
[58] P. Manikandan, M. Bhaskar, R. Revathy, R.K. John, V. Narendran, K. Panneerselvam, *Acanthamoeba keratitis* – a six year epidemiological review from a tertiary care eye hospital in south India, Indian J Med Microbiol 22 (2004) 226–230.
[59] C.C. Buerano, A.D. Trinidad, L.S. Fajardo, I.Y. Cua, M.O. Baclig, F.F. Natividad, Isolation of *Acanthamoeba* genotype t4 from a non-contact lens wearer from the Philippines, Trop Med Health 42 (2014) 145–147.
[60] X.J. Pan, T. Jiang, H. Zhu, P.P. Liu, Z.Y. Zhou, A.J. Mao, Corneal infection in Shandong peninsula of China: a 10-year retrospective study on 578 cases, Int J Ophthalmol 9 (2016) 53–57.
[61] C. Jiang, X. Sun, Z. Wang, Y. Zhang, *Acanthamoeba keratitis*: clinical characteristics and management, Ocul Surf 13 (2015) 164–168.
[62] P. Lalitha, C.C. Lin, M. Srinivasan, J. Mascarenhas, N.V. Prajna, J.D. Keenan, et al., *Acanthamoeba keratitis* in South India: a longitudinal analysis of epidemics, Ophthalmic Epidemiol 19 (2012) 111–115.
[63] L.M. van Zyl, N. Andrew, M. Chehade, T.A. Sadlon, P.R. Badenoch, *Acanthamoeba lenticulata keratitis* in a hard contact lens wearer, Clin Exp Ophthalmol 41 (2013) 810–812.
[64] S. Kilvington, T. Gray, J. Dart, N. Morlet, J.R. Beeching, D.G. Frazer, et al., *Acanthamoeba keratitis*: the role of domestic tap water contamination in the United Kingdom, Invest Ophthalmol Vis Sci 45 (2004) 165–169.
[65] J.R. Cope, CDC's *Acanthamoeba keratitis* Investigations, 1985–2011 and Healthy Contact Lens Wear Initiative, in, FDA, (2014) http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/OphthalmicDevicesPanel/UCM397604. pdf.
[66] C.F. Radford, D.C. Minassian, J.K. Dart, *Acanthamoeba keratitis* in England and Wales: incidence, outcome, and risk factors, Br J Ophthalmol 86 (2002) 536–542.
[67] J.F. Reverey, R. Fromme, M. Leippe, C. Selhuber-Unkel, In vitro adhesion of *Acanthamoeba castellanii* to soft contact lenses depends on water content and disinfection procedure, Cont Lens Anterior Eye 37 (2014) 262–266.
[68] C.E. Joslin, E.Y. Tu, T.T. McMahon, D.J. Passaro, L.T. Stayner, J. Sugar, Epidemiological characteristics of a Chicago-area *Acanthamoeba keratitis* outbreak, Am J Ophthalmol 142 (2006) 212–217.
[69] Y.M. Por, J.S. Mehta, J.L. Chua, T.H. Koh, W.B. Khor, A.C. Fong, et al., *Acanthamoeba keratitis* associated with contact lens wear in Singapore, Am J Ophthalmol 148 (2009) 7–12 e12.
[70] N. Thebpatiphat, K.M. Hammersmith, F.N. Rocha, C.J. Rapuano, B.D. Ayres, P.R. Laibson, et al., *Acanthamoeba keratitis*: a parasite on the rise, Cornea 26 (2007) 701–706.
[71] J. Kliescikova, J. Kulda, E. Nohynkova, Propylene glycol and contact-lens solutions containing this diol induce pseudocyst formation in *Acanthamoebae*, Exp Parasitol 127 (2011) 326–328.
[72] Y.H. Alam-Eldin, H.A. Aminou, The efficacy of different commercial contact lens solutions on different concentrations of *Acanthamoeba* spp. trophozoites and cysts in Egypt, Parasitol United J 7 (2014) 122–128.
[73] M.E. Shoff, M.B. Eydelman, Strategies to optimize conditions for testing multipurpose contact lens solution efficacy against *Acanthamoeba*, Eye Contact Lens 38 (2012) 363–367.
[74] N. Carnt, F. Stapleton, Strategies for the prevention of contact lens-related *Acanthamoeba keratitis*: a review, Ophthalmic Physiol Opt 36 (2016) 77–92.
[75] M. Shoff, A. Rogerson, S. Schatz, D. Seal, Variable responses of *Acanthamoeba* strains to three multipurpose lens cleaning solutions, Optom Vis Sci 84 (2007) 202–207.
[76] H.C. Lin, C.H. Hsiao, D.H. Ma, L.K. Yeh, H.Y. Tan, M.Y. Lin, et al., Medical treatment for combined *Fusarium* and *Acanthamoeba keratitis*, Acta Ophthalmol 87 (2009) 199–203.
[77] F. Arnalich-Montiel, A. Almendral, F. Arnalich, B. Valladares, J. Lorenzo-Morales, Mixed *Acanthamoeba* and multidrug-resistant *Achromobacter xyloxidans* in late-onset keratitis after laser in situ keratomileusis, J Cataract Refract Surg 38 (2012) 1853–1856.
[78] E.Y. Tu, C.E. Joslin, L.M. Nijm, R.S. Feder, S. Jain, M.E. Shoff, Polymicrobial keratitis: *Acanthamoeba* and infectious crystalline keratopathy, Am J Ophthalmol 148 (2009) 13–19 e12.
[79] E.J. Bottone, R.M. Madayag, M.N. Qureshi, *Acanthamoeba keratitis*: synergy between amebic and bacterial cocontaminants in contact lens care systems as a

prelude to infection, J Clin Microbiol 30 (1992) 2447–2450.
[80] H. Alizadeh, S. Neelam, M. Hurt, J.Y. Niederkorn, Role of contact lens wear, bacterial flora, and mannose-induced pathogenic protease in the pathogenesis of amoebic keratitis, Infect Immun 73 (2005) 1061–1068.
[81] D.P. Sharma, S. Sharma, M.R. Wilkins, Microbial keratitis after corneal laser refractive surgery, Future Microbiol 6 (2011) 819–831.
[82] P. Garg, S. Chaurasia, P.K. Vaddavalli, R. Muralidhar, V. Mittal, U. Gopinathan, Microbial keratitis after LASIK, J Refract Surg 26 (2010) 209–216.
[83] C. Stan, C. Vlăduţiu, M. Popovici, *Acanthamoeba keratitis* challenges a case report, Roman J Ophthalmol 60 (2016) 40–42.
[84] C.D. Illingworth, S.D. Cook, Acanthamoeba keratitis, Surv Ophthalmol 42 (1998) 493–508.
[85] N. Khan, Acanthamoeba, Biology and Pathogenesis, (2009) Norfolk UK.
[86] C.D. Illingworth, S.D. Cook, C.H. Karabatsas, D.L. Easty, *Acanthamoeba keratitis*: risk factors and outcome, Br J Ophthalmol 79 (1995) 1078–1082.
[87] N. Niyadurupola, C.D. Illingworth, *Acanthamoeba keratitis* associated with misuse of daily disposable contact lenses, Cont Lens Anterior Eye 29 (2006) 269–271.
[88] D.W. Clarke, J.Y. Niederkorn, The pathophysiology of *Acanthamoeba keratitis*, Trends Parasitol 22 (2006) 175–180.
[89] H. He, H. Liu, X. Chen, J. Wu, M. He, X. Zhong, Diagnosis and treatment of pythium insidiosum corneal ulcer in a Chinese child: a case report and literature review, Am J Case Rep 17 (2016) 982.
[90] K. Winchester, W.D. Mathers, J.E. Sutphin, T.E. Daley, Diagnosis of *Acanthamoeba keratitis* in vivo with confocal microscopy, Cornea 14 (1995) 10–17.
[91] E.Y. Tu, C.E. Joslin, J. Sugar, G.C. Booton, M.E. Shoff, P.A. Fuerst, The relative value of confocal microscopy and superficial corneal scrapings in the diagnosis of *Acanthamoeba keratitis*, Cornea 27 (2008) 764–772.
[92] N. Bouheraoua, A. Labbe, C. Chaumeil, Q. Liang, L. Laroche, V. Borderie, Kératites amibiennes, J Fr Ophtalmol 37 (8) (2014) 640–652.
[93] J.L. Duarte, C. Furst, D.R. Klisiowicz, G. Klassen, A.O. Costa, Morphological, genotypic, and physiological characterization of *Acanthamoeba* isolates from keratitis patients and the domestic environment in Vitoria, Espirito Santo, Brazil Exp Parasitol 135 (2013) 9–14.
[94] J. Kokot, D. Dobrowolski, A. Lyssek-Boron, M. Milka, A. Smedowski, L. Wojcik, et al., New approach to diagnosis and treatment of *Acanthamoeba keratitis*-systematic review of literature, Klin Oczna 114 (2012) 311–316.
[95] N.M. El-Sayed, M.S. Younis, A.M. Elhamshary, A.I. Abd-Elmaboud, S.M. Kishik, *Acanthamoeba* DNA can be directly amplified from corneal scrapings, Parasitol Res 113 (2014) 3267–3272.
[96] P.P. Thompson, R.P. Kowalski, R.M. Shanks, Y.J. Gordon, Validation of real-time PCR for laboratory diagnosis of *Acanthamoeba keratitis*, J Clin Microbiol 46 (2008) 3232–3236.
[97] R.P. Kowalski, M.A. Melan, L.M. Karenchak, A. Mammen, Comparison of validated polymerase chain reaction and culture isolation for the routine detection of *Acanthamoeba* from ocular samples, Eye Contact Lens 41 (6) (2015) 341–343.
[98] N.S. Gokhale, Medical management approach to infectious keratitis, Indian J Ophthalmol 56 (2008) 215–220.
[99] R. Kumar, D. Lloyd, Recent advances in the treatment of *Acanthamoeba keratitis*, Clin Infect Dis 35 (2002) 434–441.
[100] J.J. Perez-Santonja, S. Kilvington, R. Hughes, A. Tufail, M. Matheson, J.K. Dart, Persistently culture positive *Acanthamoeba keratitis*: in vivo resistance and in vitro sensitivity, Ophthalmology 110 (2003) 1593–1600.
[101] G. Brasseur, L. Favennec, D. Perrine, J.P. Chenu, P. Brasseur, Successful treatment of *Acanthamoeba keratitis* by hexamidine, Cornea 13 (1994) 459–462.
[102] J. Hay, C.M. Kirkness, D.V. Seal, P. Wright, Drug resistance and *Acanthamoeba keratitis*: the quest for alternative antiprotozoal chemotherapy, Eye (Lond) 8 (Pt 5) (1994) 555–563.
[103] A.S. Bacon, D.G. Frazer, J.K. Dart, M. Matheson, L.A. Ficker, P. Wright, A review of 72 consecutive cases of *Acanthamoeba keratitis* 1984–1992, Eye (Lond) 7 (Pt 6) (1993) 719–725.
[104] D.V. Seal, *Acanthamoeba keratitis* update-incidence, molecular epidemiology and new drugs for treatment, Eye (Lond) 17 (2003) 893–905.
[105] D.P. Yolton, S.P. Haesaert, Chapter 11 – anti-Infective drugs, in: J.D.B.D.J.S, R.G.F.R.H.L. Prokopich (Eds.), Clinical Ocular Pharmacology, 5th ed., Butterworth-Heinemann, Saint Louis, 2008, pp. 175–220.
[106] M. Yanoff, J.S. Duker, J.J. Augsburger, Ophthalmology, 2nd ed., Mosby St. Louis, MO, 2004.
[107] N. Lim, D. Goh, C. Bunce, W. Xing, G. Fraenkel, T.R.G. Poole, et al., Comparison of polyhexamethylene biguanide and chlorhexidine as monotherapy agents in the treatment of *Acanthamoeba keratitis*, Am J Ophthalmol 145 (2008) 130–135.
[108] A. Mattana, G. Biancu, L. Alberti, A. Accardo, G. Delogu, P.L. Fiori, et al., In vitro evaluation of the effectiveness of the macrolide rokitamycin and chlorpromazine against *Acanthamoeba castellanii*, Antimicrob Agents Chemother 48 (2004) 4520–4527.
[109] J. Kanski, Clinical Ophthalmology, Butterworth-Heinemann Publishers, Oxford, 2003.
[110] Y.A. Khan, R.T. Kashiwabuchi, S.A. Martins, J.M. Castro-Combs, S. Kalyani, P. Stanley, et al., Riboflavin and ultraviolet light a therapy as an adjuvant treatment for medically refractive *Acanthamoeba keratitis*: report of 3 cases, Ophthalmology 118 (2011) 324–331.
[111] A. Arance-Gil, A.R. Gutierrez-Ortega, C. Villa-Collar, A. Nieto-Bona, D. Lopes-Ferreira, J.M. Gonzalez-Meijome, Corneal cross-linking for *Acanthamoeba keratitis* in an orthokeratology patient after swimming in contaminated water, Cont Lens Anterior Eye 37 (2014) 224–227.
[112] E.Y. Tu, C.E. Joslin, M.E. Shoff, Successful treatment of chronic stromal *Acanthamoeba keratitis* with oral voriconazole monotherapy, Cornea 29 (2010) 1066–1068.
[113] M.J. Elder, S. Kilvington, J.K. Dart, A clinicopathologic study of in vitro sensitivity testing and *Acanthamoeba keratitis*, Invest Ophthalmol Vis Sci 35 (1994) 1059–1064.
[114] L. Ficker, D. Seal, D. Warhurst, P. Wright, *Acanthamoeba keratitis*–resistance to medical therapy, Eye (Lond) 4 (Pt 6) (1990) 835–838.
[115] N.J.R. Maycock, R. Jayaswal, Update on *Acanthamoeba keratitis*: diagnosis, treatment, and outcomes, Cornea 35 (2016) 713–720.
[116] R.T. Kashiwabuchi, D. de Freitas, L.S. Alvarenga, L. Vieira, P. Contarini, E. Sato, et al., Corneal graft survival after therapeutic keratoplasty for *Acanthamoeba keratitis*, Acta Ophthalmol 86 (2008) 666–669.
[117] S. Cardine, T. Bourcier, C. Chaumeil, O. Zamfir, V. Borderie, L. Laroche, [Clinical management and prognosis in *Acanthamoeba keratitis*: a retrospective study of 25 cases], J Fr Ophtalmol 25 (2002) 1007–1013.
[118] E.Y. Tu, C.E. Joslin, J. Sugar, M.E. Shoff, G.C. Booton, Prognostic factors affecting visual outcome in *Acanthamoeba keratitis*, Ophthalmology 115 (2008) 1998–2003.
[119] D. Robaei, N. Carnt, D.C. Minassian, J.K.G. Dart, Therapeutic and optical keratoplasty in the management of *Acanthamoeba keratitis*: risk factors, outcomes, and summary of the literature, Ophthalmology 122 (2015) 17–24.
[120] J. Cao, Y. Yang, W. Yang, R. Wu, X. Xiao, J. Yuan, et al., Prevalence of infectious keratitis in Central China, BMC Ophthalmol 14 (2014) 43.
[121] R. Gaudana, H.K. Ananthula, A. Parenky, A.K. Mitra, Ocular drug delivery, AAPS J 12 (2010) 348–360.
[122] R. Siddiqui, A. Syed, S. Tomas, J. Prieto-Garcia, N.A. Khan, Effect of free versus liposomal-complexed pentamidine isethionate on biological characteristics of *Acanthamoeba castellanii* in vitro, J Med Microbiol 58 (2009) 327–330.
[123] N. Nagai, Y. Ito, N. Okamoto, Y. Shimomura, A nanoparticle formulation reduces the corneal toxicity of indomethacin eye drops and enhances its corneal permeability, Toxicology 319 (2014) 53–62.
[124] K. Cholkar, S.P. Patel, A.D. Vadlapudi, A.K. Mitra, Novel strategies for anterior segment ocular drug delivery, J Ocul Pharmacol Ther 29 (2013) 106–123.
[125] B.K. Jha, I. Seo, H.H. Kong, S.I. Suh, M.H. Suh, W.K. Baek, Tigecycline inhibits proliferation of *Acanthamoeba castellanii*, Parasitol Res 114 (2015) 1189–1195.
[126] Y. Deng, W. Ran, S. Man, X. Li, H. Gao, W. Tang, et al., Artemether exhibits amoebicidal activity against *Acanthamoeba castellanii* through inhibition of the serine biosynthesis pathway, Antimicrob Agents Chemother 59 (2015) 4680–4688.
[127] D. Silva, L.F.V. Pinto, D. Bozukova, L.F. Santos, A.P. Serro, B. Saramago, Chitosan/alginate based multilayers to control drug release from ophthalmic lens, Colloids Surf B Biointerfaces 147 (2016) 81–89.
[128] C.C. Peng, M.T. Burke, B.E. Carbia, C. Plummer, A. Chauhan, Extended drug delivery by contact lenses for glaucoma therapy, J Control Release 162 (2012) 152–158.
[129] G. Qin, Z. Zhu, S. Li, A.M. McDermott, C. Cai, Development of ciprofloxacin-loaded contact lenses using fluorous chemistry, Biomaterials 124 (2017) 55–64.
[130] F.A. Maulvi, H.H. Choksi, A.R. Desai, A.S. Patel, K.M. Ranch, B.A. Vyas, et al., pH triggered controlled drug delivery from contact lenses: addressing the challenges of drug leaching during sterilization and storage, Colloids Surf B Biointerfaces 157 (2017) 72–82.
[131] V.C.P. Costa, M.E. Braga, J.P. Guerra, A. Duarte, C. Duarte, E.O.B. Leite, et al., Development of therapeutic contact lenses using a supercritical solvent impregnation method, (2010).