Acanthamoeba keratitis: early diagnosis, rational drug intervention and prevention

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Summary

Recognition of the protean clinical manifestations of *Acanthamoeba* keratitis is required by ophthalmologists in the Far East, due to the emergence of this protozoan as an ocular pathogen in both wearers and non-wearers of contact lenses in Asia. If unrecognized, progressive keratitis occurs that is not only extremely painful but also sight-threatening. A treatment regimen has been pioneered which comprises topical delivery of the combination of chlorhexidine as the digluconate salt (0.02% w/v) and propamidine isethionate (0.1% w/v), that has been successful in eradicating the corneal infection, especially if the diagnosis is made early in the course of the disease. Prevention of *Acanthamoeba* keratitis must be emphasized for soft contact lens wearers, especially as this modality of correcting myopia is rapidly increasing in Asia. This involves avoiding the use of domestic tap water in the lens storage case, regularly changing storage cases on a monthly basis, maintaining storage cases dry at all times when the lenses are worn and use of an effective acanthamoebicidal disinfectant such as a proprietary solution of 3% (v/v) hydrogen peroxide.

Introduction

Although considered as an infection which occurs infrequently, *Acanthamoeba*-associated keratitis nevertheless appears to be emerging in the Far East as a distinct disease entity.[1] The reasons for this are complex. There appears, however, to be a distinct relationship with use of cosmetic contact lenses (CL). In this context, there is now unequivocal evidence to support the contention[2] that the protozoan is carried from an environmental source, usually home tap water, into a CL storage case, and thence to the external eye,[3] where the amoebae can, under conditions of compromise to the ocular surface, be involved in the perceived keratitis process.

Acanthamoeba, which is the primary cause of amoebic keratitis, is ubiquitous in nature, being found especially in fresh water.[5] It is cosmopolitan in distribution.[5] In many circumstances, especially in rural areas, the amoebae appear to enter the eye accidentally as 'passengers' following trauma injury to the ocular surface by vegetable matter or mud-splashing.[6]

There appears to be an increase in the frequency of detection of CL-associated *Acanthamoeba* in the Far East,[7-9] and this appears to be associated with increasing use of contact lenses for cosmetic purposes. This group of presentations represents about half the cases.The limitations inherent in eliminating potentially-pathogenic microbes, including *Acanthamoeba*, from the CL storage case almost certainly represent the major risk factor for subsequent corneal infection of the CL wearer. Thus, as the number of CL wearers increases in the Far East[10-12] it is likely that there will be a concurrent rise in the reported cases of *Acanthamoeba* keratitis. This is a pattern which is reported in a number of countries, including the UK.[13,15]

It is of considerable importance, therefore, that ophthalmologists in the Far East are made aware of the optimal strategies which are available for early clinical diagnosis and drug treatment of *Acanthamoeba* keratitis. It has been established that early detection of the infection, followed by rapid implementation of effective and safe anti-*Acanthamoeba* drugs is prerequisite for successful medical outcome [16,17] of this relatively rare but potentially sight-threatening and extremely distressing corneal infection.

This paper provides current thinking of how this goal may be achieved in practice by: identifying landmarks necessary for diagnosis of the infection in both CL and non-CL wearers, making recommendations for optimal chemotherapy, and describing how to prevent the infection in CL wearers.

Clinical diagnosis

Acanthamoeba keratitis was recognized initially as a distinct disease entity at a late stage in the infection process when there was observed

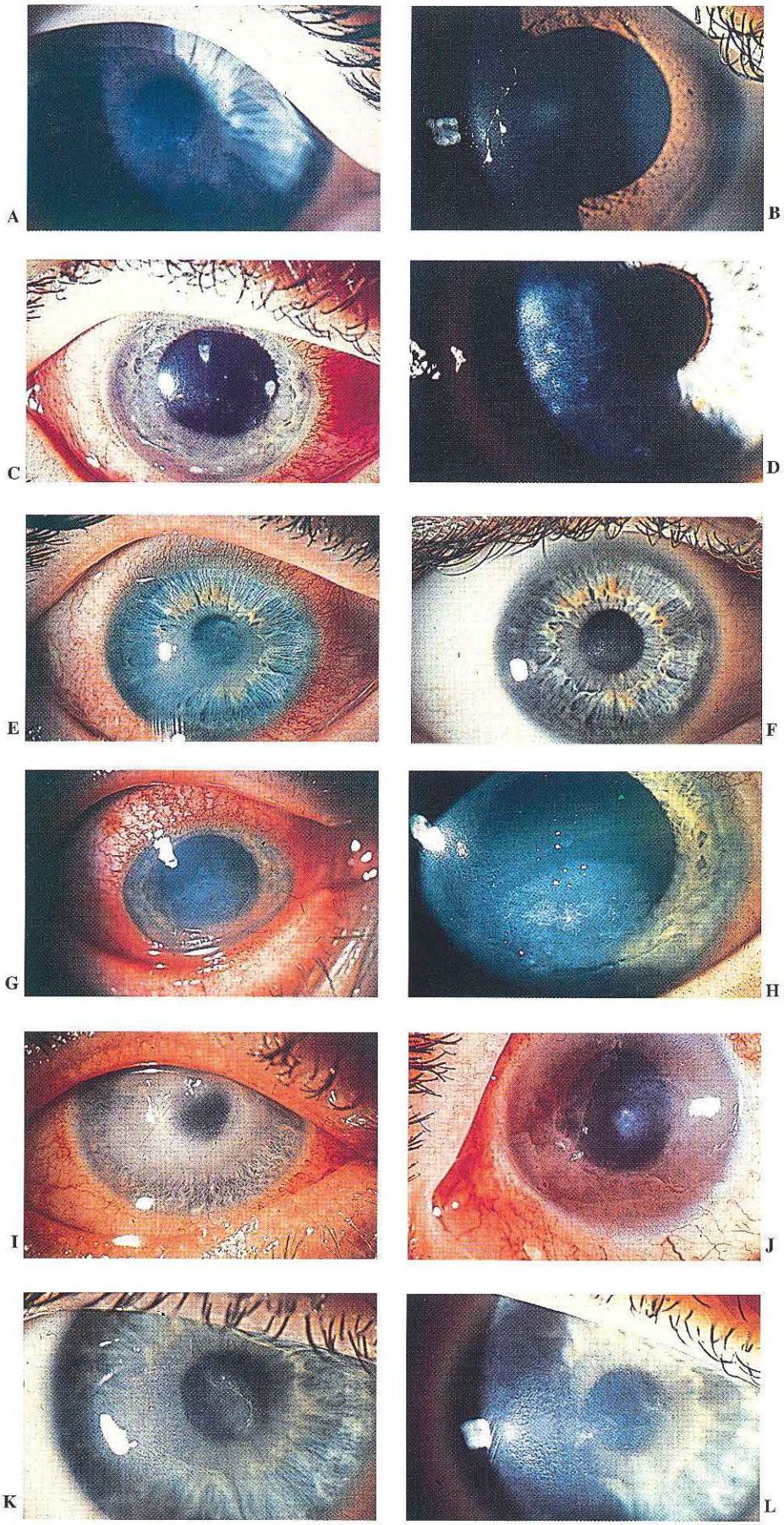


Figure 1.

A. Epithelial infiltration and oedema ('snowstorm' effect), with early linear nerve infiltrate, of Acanthamoeba infection in the central cornea of a soft contact lens wearer with 1 week of symptoms

B. Localised epithelial infiltrate of early Acanthamoeba infection with 1 week of symptoms

C. Ring of micro-abscesses with epithelial infiltrate, oedema and early linear nerve infiltrate of Acanthamoeba griffini infection in a soft contact lens wearer with 4 weeks of symptoms

D. Satisfactory early resolution of the infection in (C), after 3 weeks of chlorhexidine and propamidine therapy showing dissolution of the lesions

E. Anterior stromal infiltrate, limbitis and episcleritis of Acanthamoeba infection in a soft contact lens wearer with 8 weeks of symptoms

F. Satisfactory resolution of the infection in (E), after 8 weeks of chlorhexidine and propamidine therapy, leaving a significant scar in the central corneal visual axis

G. Severe ulcerative keratitis with limbitis and episcleritis of Acanthamoeba infection in a soft contact lens wearer with 12 weeks of symptoms

H. Close-up of (G) after 5 weeks of chlorhexidine and propamidine therapy complicated by secondary streptococcal crystalline keratopathy

I. Classical ring abscess of Acanthamoeba keratitis in a soft contact lens wearer with 8 weeks of symptoms

J. 'Refractory' ulcerative keratitis, due to Acanthamoeba infection, in a Middle Eastern non-contact lens wearer

K. Immuno-inflammatory infiltrate in the scar of treated Acanthamoeba keratitis 6 months previously (Eye 1996;10:413-21)

L. Rapid resolution of (K) with topical steroid therapy

the now 'classical' appearance of the typical ring abscess lesion (Figure 1). Typically, the patient would have had the infection for about 6 to 12 weeks prior to this clinical presentation. *Acanthamoeba* keratitis as it presents in the early and intermediate stages is illustrated in Figure 1. At 2 weeks post-infection there is evidence of perineuritis of the corneal nerves, which can be readily observed using the slit lamp (Figure 1,A). This sign is presently pathognomonic for *Acanthamoeba* infection of the cornea. The neural involvement may also explain the intense pain experienced at an early stage of infection, which accompanies other symptoms such as photophobia and lid swelling. The pain experienced by the patient is often much more severe than would be expected on cursory examination of the cornea. *Acanthamoeba* infection can also present as epithelial infiltration, which manifests with a 'snow-storm' appearance (Figure 1, A) with both punctate and diffuse infiltration. If this stage goes unrecognized, or is misdiagnosed (as is often the case) as *Herpes simplex* keratitis[18] or adenovirus keratitis[19] especially if a dendriform lesion is present or punctate staining is seen on fluorescein staining, then the keratitis can be expected progressively to worsen. At approximately two months post-infection, there is usually an episcleritis, and in some patients, a scleritis, limbitis, and more deeply infiltrated stromal lesion, without this necessarily having the 'classical' ring abscess (Figure 1, E, G). These clinical presentations always require corneal scraping to be performed using a hypodermic needle or sterile scalpel blade, with tissue being placed into a maximum of 1 ml of sterile isotonic saline in a sterile centrifuge tube.

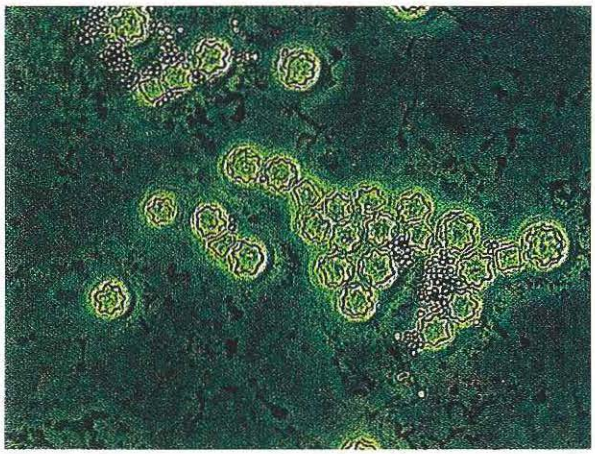


Figure 2. Cysts of *Acanthamoeba* from a culture plate observed using phase-contrast microscopy with a green filter.

Direct microscopy of an unstained wet film preparation[20] from the base of the gently spun tube, or with lactophenol cotton blue staining[21] can be very useful for rapid confirmatory diagnosis and should always be performed. This technique requires some protozoological skill to differentiate the amoebae from corneal epithelial cells and intrinsic inflammatory cells which may be present. This is followed by culturing of the sample for *Acanthamoeba* on the center of a non-nutrient plain agar plate, best made up with enriched amoebal saline[22] when the amoebae will begin to replicate without addition of bacteria. Otherwise, plates can be used after spreading the surface with heat-killed Gram-negative non-sporing bacteria (e.g. *Klebsiella spp*). Plates should be incubated at 25° and 32°C, not higher, for at least 4 weeks. *Acanthamoeba* will be seen within one week as trophozoites, spreading out from the centre of the plate towards the periphery. The characteristic double-walled cysts, containing the often stellate internalized amoeba (Figure 2) may be observed within a further week. The internalized amoeba emerges as a new trophozoite from the cyst ostiole, seen in Figure 2 at the tips of the stellate structure. The agar plate should be sealed with tape, after which it can be stored for many months and even years in a dark place. The amoebae will be recoverable when placed into a fluid medium or wet plate. The amoebae can also be cryopreserved.

When *Acanthamoeba* is isolated, and the clinical diagnosis is confirmed, treatment should be commenced with 2 hourly chlorhexidine (0.02% w/v, as the digluconate salt) and propamidine (0.1% w/v, as Brolene) day and night for three days, followed by 2 hourly by day for two months and continuing 4 hourly by day for 3 months.[18] It can also be beneficial to include a non-steroidal anti-inflammatory drug such as Froben that also acts as an analgesic. Preferably corticosteroids should not be used since they suppress macrophage activity, required to phagocytose the amoebae[23] and thus eliminate the infectious agent. The successful outcome with this regimen of chlorhexidine and propamidine is usually apparent within 2 months, and often sooner (Figure 1).

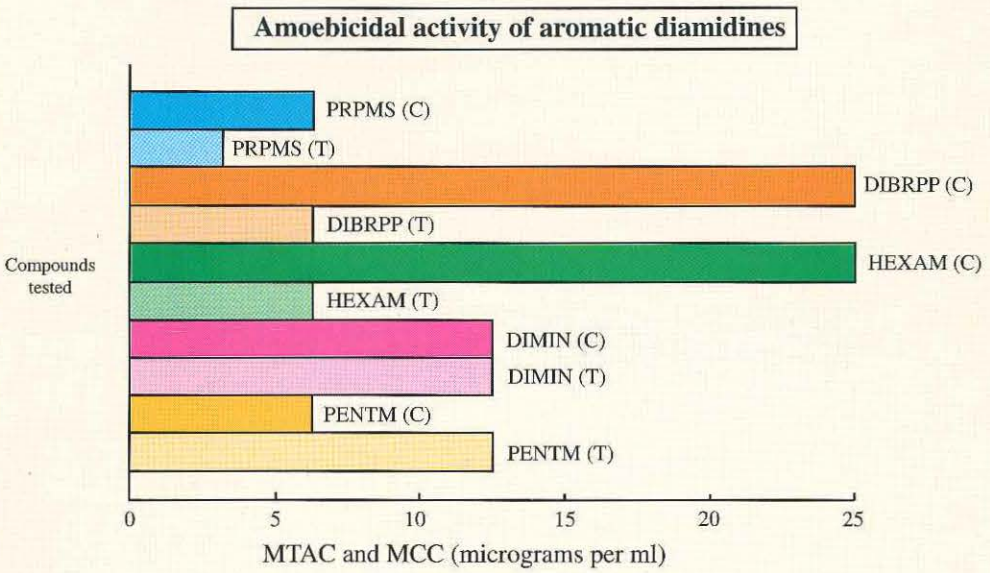
Despite details of early clinical signs and symptoms of *Acanthamoeba* keratitis being available,[16,17] late diagnosis may still occur (Figure 1). In contact lens wearers a clue is often the contact lens itself, which should also be examined microbiologically for amoebae. These presentations may require a biopsy of the deep ring abscess for the amoebae to be detected.[22,24] Treatment should be started with intensive chlorhexidine and propamidine,[18] as above. This may require 3 or even 6 months treatment until satisfactory medical control is gained. The late lesion can present instead as a large epithelial ulcer, with an infiltrated stromal base, severe episcleritis, severe pain and photophobia; the classic ring abscess need not be present (Figure 1, G). Despite treatment, these patients may have a persistently large ulcer for a number of weeks. During this time, secondary streptococcal infection may occur, which is perceived as a crystalline keratopathy.[25] It was initially considered that such an infection was unique to corneal grafts. It has now been shown that this can occur in the chronically-infected, *Acanthamoeba*-associated, open ulcer (Figure 1, H). Crystalline keratopathy is very difficult to treat,[25] and often, but not always, fails to respond to intensive antibiotic therapy with penicillin or vancomycin. A lamellar, or full-graft, then becomes necessary. It should be noted, however, that it is better practice to obtain medical control of the infection prior to surgery. This will enhance the likelihood of graft survival.

Drug treatment

Once confirmation has been obtained that *Acanthamoeba* is the etiological agent of the keratitis, then appropriate drug therapy should be instituted. The background to the development of the chlorhexidine and propamidine combination, which has been used to treat successfully the spectrum of *Acanthamoeba* presentations described above, is of considerable importance therapeutically, since often the clinician will obtain a satisfactory resolution of presumed (culture-negative) *Acanthamoeba* keratitis, and will thus presume that this therapy can be extrapolated into all patients with this infection. It must be emphasized that this approach is not to be recommended.

Examples of drugs which have provided anecdotal evidence of a successful outcome are: itraconazole plus miconazole;[26] clotrimazole;[27] ketoconazole;[2] neosporin with or without miconazole or ketoconazole;[9] pimarcin plus neodecodron (dexamethasone phosphate, neomycin sulphate); hydroxyuracil; rifampicin; and atropine.[29] Of importance, however, is that more consistent reports of successful outcome of *Acanthamoeba* keratitis has occurred using aromatic diamidines. The combination of dibromopropamidine, propamidine and neomycin,[30] as well as propamidine as monotherapy[31] and propamidine in combination with neomycin-polymyxin B- gramicidin and neosporin,[32] have

Figure 3. In vitro sensitivity, expressed as modal value, of a series of corneal isolates of Acanthamoeba to various aromatic diamidines.



each proven effective in many patients. Hexamidine has also been shown to provide a successful outcome in some patients.[33] Pentamidine,[30] hydroxy stilbamidine[34,35] and diminazine[22,30] have also been found to have *in vitro* activity against *Acanthamoeba* strains originally isolated from the cornea. Furthermore, the cationic surfactants chlorhexidine and polyhex-amethylene biguanide (PHMB) have also been shown to have a potent acanthamoebicidal effect against trophozoites and cysts both *in vitro* and *in vivo*.[22,36]

To establish the validity of selection of these drugs for treatment of *Acanthamoeba* keratitis, a series of compounds with chemical structures similar to those of the latter series, which comprised examples from the guanidine derivatives, aromatic diamidines and biguanides, a group which incorporates the biguanides, bis-biguanides and polymeric biguanides, was screened for *in vitro* activity against cornea-derived strains of *Acanthamoeba*. The method used for the drug screening is given below, and described in detail elsewhere.[22]

Briefly, 100µl containing approximately 2 x 10^4 trophozoites or cysts are instilled into each well of microtitre plates; 100µl of doubling dilutions (100 - 0.8µg/ml) of the cationic surfactants or other drugs are added. Sealed plates are mixed gently for 10 minutes on a plate rotator and incubated for 48 hrs in air at 32°C. After removal of residual drug and instillation of a defined medium,[37] plates are reincubated for a further 48 hours. During this time wells are inspected microscopically for the lowest concentration of drug that results in complete lysis or degeneration of trophozoites (minimum trophozoite amoebacidal concentration, MTAC) or, for cysts, the lowest concentration of test compound that results in no excystment (minimum cysticidal concentration, MCC).

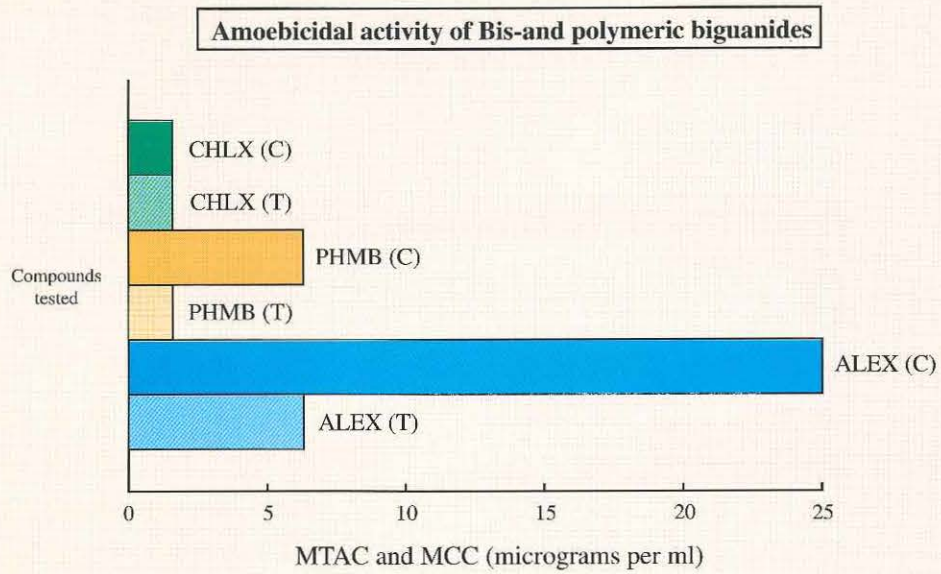
Only those compounds categorized within the aromatic diamidines or bis-and polymeric-biguanides, showed consistent anti-acanthamoebicidal effects, and so were worthy of investigation as candidate drugs for treatment of *Acanthamoeba* infection. Of the aromatic diamidines (Figure 2), propamidine was consistently the most effective. Diminizene and pentamidine were the next most useful drugs, followed by dibromopropamidine and hexamidine which were less effective than the other drugs tested in this category, against the cyst form of *Acanthamoeba*.

Chlorhexidine was the most effective of the other major category of drugs (Figure 3). This was generally comparable in effect to PHMB. Both compounds were more effective than alexidine. These findings confirm the preliminary selection of propamidine and chlorhexidine as drugs which are effective against both the trophozoite and cyst form of *Acanthamoeba*, and establish that the combination which has been used for the successful treatment of the infection[17] has a rational basis, using as a criterion of effectiveness of the drugs their acanthamoebicidal activity in sensitivity studies. The protocol recommended for clinical use[18] has been provided in the 'clinical diagnosis' section of this paper.

Reasons why treatment failure can occur include:

1. non-compliance with the rigorous and strict treatment regimen (which is why recognized 'non-compliant' patients are treated in hospital);
2. decreased bio-availability of drug at the target site, which can be due to non-specific corneal tissue binding, a more likely problem with PHMB than with chlorhexidine, because of the larger relative molecular mass and increased ratio of cationic guanidine groups, although mole-for-mole PHMB has greater activity; and,
3. development of resistance which is recognized for propamidine[38] but has not yet been recorded for the bis- and polymeric-biguanides, chlorhexidine and PHMB, used for successfully treating *Acanthamoeba* keratitis.

Figure 4. In vitro sensitivity, expressed as modal value, of a series of corneal isolates of Acanthamoeba to selected bis- and polymeric biguanides.



Discussion

Acanthamoeba was first identified as a potential human pathogen by Culbertson in 1956. There was contamination of tissue culture cell lines being used to prepare the first commercial batch of live attenuated polio virus vaccine which involved injecting the trial vaccine into the spinal fluid of monkeys. The contaminated batch led to fatal amoebic encephalitis[39] and this isolate was named *A culbertsonii.*

Acanthamoeba was first recognized as an ocular pathogen in 1974 in the UK[40] and the USA.[41] These patients presented with progressive keratitis which was refractory to treatment with conventional antibiotics. *Acanthamoeba* was isolated from the cornea. This form of keratitis was subsequently recognized in CL wearers. In all such patients, treatment proved difficult, with some progressing to bilateral blindness. Many drug treatments were tried but with minimal or only anecdotal success. The first successfully recorded treatment was with the combination of topical anti-*Acanthamoeba* chemotherapy, propamidine and neomycin.[30] Use of this treatment, however, did not provide the panacea that was initially predicted, and corneal strains of *Acanthamoeba* proved resistant to either or both drugs.[38] Furthermore, cases of *Acanthamoeba* keratitis proved refractory to the therapy. *Acanthamoeba* keratitis was then usually recognized only at a late stage where there was a typical ring abscess lesion (Figure 1, I) which became apparent 6 to 12 weeks post-initial infection. Medical experience at this time suggested that a patient with *Acanthamoeba* keratitis required topical treatment with the combination of propamidine (as Brolene) and neomycin for at least one year, with there always being the likelihood of a protracted course, with recurrence, which was complicated by concurrent corticosteroid therapy, included in the treatment regimen to suppress the intense inflammation associated with the infection. Half of the patients being treated with this combination failed to respond, and these required corneal grafts. In the absence of adequate medical therapy of the infection, however, these grafts became reinfected with *Acanthamoeba* which recrudesced from the host corneal rim, so that only 20% of grafts survived for 2 years.[42] In one patient with bilateral infection involving the whole cornea, the amoebae developed resistance to propamidine, while the patient developed a delayed hypersensitivity to neomycin.[38] Repeated bilateral grafts were required in this case, with the second just 1 month after the first. A surgical cure was eventually achieved, but this resulted in secondary complication,s including severe glaucoma.

The number of presentations of *Acanthamoeba* keratitis has increased world-wide. Most reported cases in Europe and the USA are associated with CL wear. In contrast those from the tropics especially South India are more often associated with minor trauma or mud splashing of the eye without associated CL wear.[6] This has permitted earlier recognition of the signs and symptoms of the infection. Earlier clinical recognition of *Acanthamoeba* infection,[16,17] when it is confined to the epithelium, allows for more rapid anti-protozoal intervention, and where the drugs can target the non-cystic amoebae prior to their migration into the stroma.[43] It is at this stage that there can be confusion with a herpetic type dendriform ulcer, a very rare presentation in the young CL wearer, or with the punctate type keratopathy of adenovirus infection.[19] The latter can occur in young CL wearers, but the stromal infiltration below the epithelium is usually observed in *Acanthamoeba* within 8 days,[3] as opposed to adenovirus infection, when the stromal infiltration occurs after 8 days[19] as a presumed immunological response to the presence of the virus.

In all of these situations, it is a pre-requisite to confirm protozoologically the diagnosis of 'presumed' *Acanthamoeba* keratitis, prior to intervention with drugs. This can, in most instances, be done by isolation of amoebae from a corneal scraping or biopsy,[24] the latter being first used to confirm *Acanthamoeba* keratitis in Taiwan in 1989. The corneal scraping should initially be examined microscopically, as a wet preparation, and cultured as described above. If possible, transmission electron microscopy should be performed on the corneal

biopsy specimen, when the amoebae, if present, can be recognized in the stroma.[44] This is a difficult procedure to interpret, however, due to confounding features of keratocytes and inflammatory cells. While isolation of *Acanthamoeba* from a biopsy is always pathognomonic of the infection, recent experience of culturing both CL as worn, and peripheral bacterial ulcers, has found isolation of *Acanthamoeba* to be possible on the corneal epithelial surface without active invasion of the epithelium.[45,46] This implies that *Acanthamoeba* can be present on the ocular surface as a 'transient' without any obvious invasive infection taking place. It is essential, therefore, to consider both the spectrum of clinical features of the condition and the isolation of *Acanthamoeba*, before concluding that the former is caused by the latter.

Once the diagnosis of *Acanthamoeba* keratitis has been confirmed, there is a need to institute a chemotherapeutic regimen. Due to the problems inherent in the use of propamidine and neomycin, it was considered that other more reliable drugs should be sought to overcome the potential problem of propamidine resistance. Preliminary studies[22] revealed that the combination of chlorhexidine and propamidine was effective both *in vitro* and *in vivo*. This observation led to a multicentere trial which showed the benefit of chlorhexidine and propamidine as first-line therapy for *Acanthamoeba* keratitis.[17]

This treatment, following the regimen described above,[18] proved effective in a range of clinical presentations representing early, intermediate and late stages of the infection.[17] Chlorhexidine is relatively non-toxic to the cornea when given topically and has a proven profile of use in humans for the last 40 years. The exception is neuroepithelium,[47] which must NOT be instilled into the anterior chamber. If chlorhexidine is used excessively by the patient by the topical route, however, the first signs of toxicity will be loss of the epithelium. The latter regenerates on withdrawal of the drug. This situation also occurs at certain other body sites, and is to be expected if the concentration on the surface exceeds 0.5% (w/v). Chlorhexidine is likely to be more advantageous than the polymeric biguanide, PHMB, for the treatment of *Acanthamoeba* keratitis as the smaller molecule is expected to penetrate better into the stroma.[48] It must be emphasized that pure chlorhexidine only should be used and NOT commercially-available products containing detergent or alcohol, since these will cause coagulative necrosis to the corneal cells. PHMB should be used only if chlorhexidine is unavailable. The trade product 'Cosmocil' should be used in preference to 'Bacquacil' or 'Vantocil' since it is the purer product. All three grades are produced as commercial biocides, however, and not as pharmaceutical drugs, which is the case for chlorhexidine.

The *in vitro* effectiveness of the aromatic diamidines (Figure 3) and the biguanides (Figure 4) is confirmed. The mechanism(s) by which the drug combination provides its observed acanthamoebicidal effect is suggested by chlorhexidine binding to and disrupting the cell membrane of the trophozoite, or internalized amoeba, followed by coagulation of intracellular proteins. In the case of cysts, the drug may penetrate through apertures in the wall of the exocyst to target the endocyst membrane, or may penetrate via the ostioler plug, or both processes may occur simultaneously. The outcome of this effect is that chlorhexidine facilitates penetration of effective drug, whether this be chlorhexidine or propamidine or both. Propamidine, as an aromatic diamidine, probably binds non-intercalatively to DNA resulting in inhibition of growth of the amoeba, although other mechanisms are also likely to occur.[49] Monotherapy with this drug is not recommended,[50] because of the recognition of the development of resistance to it,[38] as well as the general decreased susceptibility of cysts.[22] Hexamidine (Desmodine) has been used successfully as monotherapy in France[33] and has been suggested as a substitute for propamidine, although our experience does not confirm this notion.

The source of *Acanthamoeba* keratitis in the CL wearer has been identified unequivocally in one study as tap water used to rinse the CL storage case or the lenses themselves.[3] This was based on molecular sequencing of 18srDNA of *Acanthamoeba* isolates from the cornea, storage case and domestic tap water. This confirms previous findings of *Acanthamoeba* in domestic water systems[51] and the presence of these amoebae in in-use CL storage cases.[52] These sources have been further confirmed in a recent keratitis study,[45] so that there is a need to emphasize to CL wearers that tap water must never be used for CL hygiene, and must never enter the storage case. Generally, the CL itself, storage case and all disinfecting solutions should be disposed of together, and all of these replaced, at least monthly to avoid their contamination with bacteria, fungi or amoebae and to obviate growth of biofilms. If the CL is worn as a non-disposable lens, then there is still need to change the storage case and solutions every month, in order to avoid any infection which may be associated with them.

Again, not all presentations of *Acanthamoeba* keratitis are CL related. In South India over 80 cases of non-CL associated *Acanthamoeba* infections have been recognized, with several being associated with CL wear.[6,21] In one case, a chronic ulcer developed in the cornea of a Middle-Eastern refugee who had infection within it with *Acanthamoeba* (Figure 1, J) associated with previous trachoma.[53] There was an initial differential diagnosis of a refractory keratitis, 'presumed' microbial, which was unresponsive to antibiotics. Such chronic ulcers should always be sampled for *Acanthamoeba*, which can be present without the classical ring infiltrate. The patient responded well to the combination of chlorhexidine and propamidine.

The arrival of CL-associated *Acanthamoeba* keratitis has been recorded in the Far East with 2 cases reported recently from Malaysia[7,54] together with several cases involving minor trauma, 2 from the Philippines with CL wear[8] and 3 published from China, together with 2 non-CL cases associated with minor trauma.[55] More cases of *Acanthamoeba* keratitis are known to the authors to have been diagnosed worldwide, but not as yet reported in the world literature. This experience reflects that of Europe, the USA and India, in that patients with *Acanthamoeba* keratitis are being recognized with positive cultures of *Acanthamoeba* from both CL and non-CL wearers.

It is our contention that ophthalmologists should be able to recognize the protean clinical ocular manifestations of this protozoal infection of the cornea and commence early effective chemotherapy. It is the expectation of these authors that there will be a steady increase in the numbers of patients presenting in the Far East with potentially sight-threatening *Acanthamoeba* keratitis, and that approximately half of these may be associated with the wear of soft contact lenses, unless appropriate hygiene measures, as discussed above,[1] can be introduced to primary care contact lens practice.

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