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Alkylphosphocholines and Quaternary Ammonium Compounds against Acanthamoeba Keratitis

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Abstract

Acanthamoeba keratitis (AK) is a sight threatening infection caused by the free-living amoeba Acanthamoeba. This infection is largely associated with contact lens wear and the recent increase in AK incidences highlights the ineffectiveness of existing curative and preventative treatments. Current curative and protective treatments being active in part, only against the infective trophozoites and often inducing their conversion to the protective cysts is a major issue, particularly when the latter are the main cause of disease resurgences and relapses. These point to the need for the discovery of new drugs for curative and preventive treatments. Two structurally similar chemical classes, alkylphosphocholines (APCs) and quaternary ammonium compounds (QACs) that address these issues will be discussed in this review.

Introduction

Acanthamoeba are free-living single celled eukaryotes, existing in two forms; an infective trophozoite and a dormant cyst. They are ubiquitously found in both natural and man-made environments and are of clinical relevance due to their opportunistic parasitic activities in humans. There are several Acanthamoeba associated infections in humans, but this review will focus on the sight threatening disease of the eye Acanthamoeba keratitis (AK) and the use of two promising compounds as curative and preventive treatments that will validate its incorporation into contact lens solutions. Curative treatment is the use of systemic oral drugs and topical eye drops while preventive treatment employs medical devices e.g. contact lens solutions to sterilise contaminated lenses and prevent contact lens to eye transmission.

AK is largely associated with improper contact lens hygiene and the limited activity of contact lens solutions against Acanthamoeba. The attachment of Acanthamoeba to contact lenses allows transmission to, and infection of the cornea arising from the feeding activities of trophozoites in the eye. Incidence rates of AK have been increasing globally since the start of the 21st century. On average, the annual number of AK cases within the Moorfields eye hospital, England increased from 12.8 to 48.6 between 2000-2008 and 2009-2016, and 16 to 49 in the Netherlands between 2009 and 2015. Similar reports are documented in Australia and the USA with rising incidences, the Sydney Eye Hospital reported the annual average cases of AK to be 2.6 and 3.6 before and after 2007, while cases in Iowa, USA increased in average annual cases from 2.9 to 6.5 between 2002-2009 and 2010-2017 respectively. The reasons for this spatio-temporal increase are not readily apparent but improved diagnostic
techniques and ineffective contact lens cleansing solutions are thought to be key factors.

Existing curative and preventive treatments for AK mainly target trophozoites but become inactive if they induce their conversion to cysts via the process of encystation. The cellulose cell walls of cysts protect the parasite against drug activity and the reduction of drug concentrations in the eye to sub-lethal levels initiates the reversion of cysts to the trophozoite stage, ultimately leading to disease resurgence.

The inability of current topical and oral drugs and many widely used contact lens solutions to produce death at low concentrations against cysts, the requirement of a prolonged treatment regime to ensure complete clearance from the eye and the ability to induce encystation are factors that have made AK treatment and prevention difficult. In some instances, frequent application of drugs topically for up to a year is required, causing corneal damage and very often patients require surgical intervention e.g. corneal transplant to repair the damaged cornea. Preventive protocols with commonly used contact lens solutions such as one-step hydrogen peroxide solutions are similarly ineffective with even compliant users at risk.

Two structurally similar compounds named alkylphosphocholines (APCs) and quaternary ammonium compounds (QACs) have received attention for their activity against several protozoan pathogens, including Acanthamoeba. Work by Walochnik and colleagues on their anti-amoebic properties for combating Acanthamoeba keratitis concluded that APCs are promising drug candidates that might prove useful against AK. This minireview provides an update and the state of the art of this chemical class and an analogue called QACs, extensively used as preventative strategy against AK.

Alkylphosphocholines against Acanthamoeba Keratitis

Structure of alkylphosphocholines

APCs are zwitterionic molecules comprised of a head made from a trimethylamine moiety containing a positively charged nitrogen atom and joined by a two alkyl-carbon linker to a negatively charged phosphoryl group. The tail, made of varied number of alkyl-carbons, is attached to the phosphoryl group (Figure 1).

Activity of oral miltefosine against Acanthamoeba keratitis

The APC, miltefosine was first developed as an anti-cancer drug but was subsequently shown to have antifungal and anti-protozoal properties. Systemic use of miltefosine was licensed in the USA as a treatment of AK in 2016 and when incorporated into treatment regimens at 50mg/ml, given three times daily (TD) for up to two months has proved to be an effective oral systemic treatment against Acanthamoeba mainly in combination. The use of miltefosine solely as an oral administration is yet to be tested, but in combination with topical applications e.g. chlorhexidine (0.06%) and propamidine (0.1%) has demonstrated success. Hirabayashi et al. reported the use of miltefosine in the treatment of a patient suffering with progressively worsening AK. For this 17 yr old female patient, earlier treatments with topical polyhexamethylene biguanide (PHMB, 0.02%), chlorhexidine (0.02%), moxifloxacin (0.5%), cyclopentolate (1%) and oral administration of both 500mg valacyclovir and 200mg voriconazole twice daily (BD) were unsuccessful. The addition of miltefosine (50mg, TD, orally) in particularly with topical chlorhexidine (0.06%) and propamidine isethionate (0.1%) for five weeks reduced the infection and accompanying symptoms, pain improved conjunctival injection and corneal opacity were reduced and improved respectively. No resurgence was observed one-year post treatment. Similar results have been described by Dewan et al., again a 44yr old female who did not respond to treatment with topical chlorhexidine (0.02%), PHMB (0.02%), gatifloxacin (0.3%) and voriconazole (0.5mg/ml) and oral voriconazole (200mg, BD) was responsive to oral miltefosine (50mg, TD) after 11 months of treatment. Resurgence was not evident thirty-month post treatment. The efficacy of miltefosine is reproducible and has been replicated in part by Naranjo et al.
### Table 1: Oral miltefosine against Acanthamoeba keratitis

<table>
<thead>
<tr>
<th>Participant specifics</th>
<th>Treatment regimen prior to miltefosine usage</th>
<th>Treatment regimen with miltefosine</th>
<th>Remarks</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-year old female</td>
<td></td>
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<td>15</td>
</tr>
<tr>
<td></td>
<td>T: Several Months - Antivirals and topical corticosteroids administered for several months.</td>
<td>T: 1 week - Topical; PHMB (0.02%; q1h)/Chlorhexidine (0.02%; QD), Moxifloxacin (0.5%; QD), cyclopentolate (1%; OD), Oral; miltefosine (50mg; TD)</td>
<td>- Cultures were negative for <em>Acanthamoeba</em> after T1.</td>
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<tr>
<td></td>
<td>T: 2 weeks - Topical; PHMB (0.02%; q1h)/Chlorhexidine (0.02%; QD), Moxifloxacin (0.5%; TD), cyclopentolate (1%; OD), Oral; valacyclovir (500mg; OD)</td>
<td>T: Undisclosed time - Topical; PHMB (0.02%; QD)/Chlorhexidine (0.02%), Oral; miltefosine (50mg; TD), doxycycline (50mg; BD), OD Vitamin C (1000mg; OD)</td>
<td>- Pain, conjunctival injection and cornea opacity decreased significantly after five weeks treatment with miltefosine (50mg; TD oral), chlorhexidine (0.06%; q1h topical), propamidine isethionate (0.01%; q2h topical), doxycycline (50mg; BD oral) and Vitamin C (1000mg; OD oral) (See T3).</td>
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<td></td>
<td>T: 6 weeks - Oral; Valacyclovir discontinued and replaced voriconazole (200mg; BD)</td>
<td>T: 5 weeks - Topical; PHMB was discontinued while treatment in T5 updated with Chlorhexidine (0.06%; q1h) and propamidine isethionate (0.1%; q2h)</td>
<td>- No recurrence was observed one year post treatment with miltefosine (see T6)</td>
<td></td>
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<td>44-year old female</td>
<td>T: 4 weeks (inconsistently) - Topical; prednisolone acetate (1%; q1h), dexamethasone sodium phosphate ointment (0.1%; OD), homatropine (2%; BD)</td>
<td>T: 3 months - Topical; Voriconazole (0.5mg) discontinued, chlorhexidine (0.02%; q1h)/PHMB (0.02%; q1h), gatifloxacin (0.3%; QD)</td>
<td>- Cultures were native for <em>Acanthamoeba</em> after T2.</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>T: 2 weeks - Topical; Tobramycin (0.3%; OD), dexamethasone ophthalmic ointment (0.1%; OD), gatifloxacin (0.3%; QD)</td>
<td>T: 3 months - Topical; prednisolone acetate (1%; q2h), chlorhexidine (0.02%; q2h), PHMB (0.02%; q2h) Oral; Miltefosine (50mg; BD), Voriconazole (200mg; BD)</td>
<td>- Standard treatments for AK (T1 to T4) were unsuccessful, but the addition of miltefosine in T5 to T6 cleared the infection (T7).</td>
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<tr>
<td></td>
<td>T: Undisclosed time - Topical; chlorhexidine (0.02%; q1h)/PHMB (0.02%; q1h), gatifloxacin (0.3%; OD)</td>
<td>T: 3 months - Topical; prednisolone acetate (1%; OD), Voriconazole (0.5mg ml; q1h) added, Oral; voriconazole (200mg; BD)</td>
<td>- No disease resurgence observed 30 months post treatment (T9).</td>
<td></td>
</tr>
<tr>
<td>29-year old female</td>
<td>T: 2 days - Topical; Ganciclovir gel (n/a), erythromycin ointment (n/a), Oral; Valacyclovir (n/a)</td>
<td>T: 15 days - Topical; Chlorhexidine (n/a), PHMB (n/a), Polymyxin/Trimethoprim (n/a) Oral; Moxifloxacin (50mg)</td>
<td>- Cultures were native for <em>Acanthamoeba</em> after T5.</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>T: 5 days - Topical; Empiric fortified vancomycin (n/a), Tobramycin eye drops (n/a)</td>
<td>T: 13 days - Topical; PHMB (n/a), prednisolone acetate (1%) propamidine (n/a) Oral; see T3.</td>
<td>- Patients symptoms worsened after 6 days treatment with miltefosine, predicted to be immune related (T8).</td>
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<td>T: 2 weeks - Topical; fortified vancomycin discontinued, chlorhexidine (n/a), PHMB (n/a), Polymyxin/Trimethoprim (n/a) added</td>
<td>T: 15 days - Topical; Chlorhexidine (n/a), Oral; Moxifloxacin (50mg; TD)</td>
<td>- Cultures returned negative for <em>Acanthamoeba</em>, only one cyst was identified in histopathological analysis (T6).</td>
<td></td>
</tr>
<tr>
<td>53-year old female</td>
<td>T: Undisclosed time - Topical; Ganciclovir ophthalmic gel (0.15%), prednisolone acetate (1%), Oral; Valacyclovir (n/a)</td>
<td>T: 20 days - Topical; Chlorhexidine (n/a), PHMB (n/a), Oral; see T3.</td>
<td>- No disease recurrence noted 12 months post treatment (T8).</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>T: Several months - Topical; Chlorhexidine (n/a), Prednisolone (n/a)</td>
<td>T: 15 days - Topical; Chlorhexidine (n/a), Oral; Moxifloxacin (50mg; TD)</td>
<td>- Culture were negative for <em>Acanthamoeba</em> after T4.</td>
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<td>T: 20 days - Topical; Chlorhexidine (n/a), PHMB (n/a), Oral; see T3.</td>
<td>- Symptoms worsened after 15 days treatment with miltefosine, presumed immune related (T6).</td>
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<td></td>
<td>- No disease recurrence noted 5 months post treatment (T9).</td>
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<tr>
<td>Age Group</td>
<td>Treatment Intervention Sequence</td>
<td>Duration</td>
<td>Description</td>
<td>Drug Details</td>
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<tr>
<td>-----------</td>
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<tr>
<td>24-year old female</td>
<td><strong>T1</strong>: 3 weeks - <strong>Topical</strong>: ganciclovir ophthalmic gel (0.15%)</td>
<td>3 weeks</td>
<td>-</td>
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<td><strong>T2</strong>: 2 weeks - <strong>Topical</strong>: ganciclovir ophthalmic gel (0.15%) prednisolone (n/a), Oral; valacyclovir (n/a)</td>
<td>2 weeks</td>
<td>-</td>
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<td><strong>T3</strong>: 20 days - <strong>Topical</strong>: PHMB (n/a; q1h) added, Oral; see T3</td>
<td>20 days</td>
<td>-</td>
<td>-</td>
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<tr>
<td>25-year old female</td>
<td><strong>T4</strong>: Several weeks - <strong>Topical</strong>: ganciclovir ophthalmic gel (n/a) ofloxacin drops (n/a), Oral; famciclovir (n/a)</td>
<td>Several weeks</td>
<td>-</td>
<td>-</td>
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<td><strong>T5</strong>: 40 days - <strong>Topical</strong>: propamidine isethionate (n/a), PHMB (n/a), polymyxin/trimetoprim (n/a) Oral; valacyclovir (n/a)</td>
<td>40 days</td>
<td>-</td>
<td>-</td>
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<tr>
<td>16-year old female</td>
<td><strong>T6</strong>: Several weeks - <strong>Topical</strong>: prednisolone acetate (n/a), Oral; acyclovir (n/a)</td>
<td>Several weeks</td>
<td>-</td>
<td>-</td>
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<td><strong>T7</strong>: 1 month - <strong>Topical</strong>: PHMB (n/a), chlorhexidine (n/a), Oral; see T7</td>
<td>1 month</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td><strong>T8</strong>: 2 months - <strong>Topical</strong>: chlorhexidine discontinued, propamidine isethionate (n/a) added Oral; see T7</td>
<td>2 months</td>
<td>-</td>
<td>-</td>
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<tr>
<td>55-year old female</td>
<td><strong>T9</strong>: Undisclosed time - <strong>Topical</strong>: erythromycin (n/a), besifloxacin (n/a), valacyclovir (n/a), and difluprednate (n/a)</td>
<td>Undisclosed time</td>
<td>-</td>
<td>-</td>
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<td><strong>T10</strong>: Undisclosed time - <strong>Topical</strong>: moxifloxacin (n/a), bacitracin (n/a), polymyxin B (n/a)</td>
<td>Undisclosed time</td>
<td>-</td>
<td>-</td>
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<td><strong>T11</strong>: 5 weeks - <strong>Topical</strong>: moxifloxacin (n/a), bacitracin (n/a), polymyxin B (n/a) discontinued, PHMB (n/a), propamidine isethionate (n/a), cyclopentolate eye drops (n/a) added</td>
<td>5 weeks</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>59-year old male</td>
<td><strong>T12</strong>: 1 month - <strong>Topical</strong>: ofloxacin (n/a)</td>
<td>1 month</td>
<td>-</td>
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<td></td>
<td><strong>T13</strong>: 2 weeks - <strong>Topical</strong>: PHMB (0.02%; q1h), Oral; voriconazole (400mg; BD), reduced (200mg and eventually discontinued within this time</td>
<td>2 weeks</td>
<td>-</td>
<td>-</td>
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<td></td>
<td><strong>T14</strong>: 1 week - <strong>Topical</strong>: PHMB (0.02%; q2h), dexamethasone (0.1%)</td>
<td>1 week</td>
<td>-</td>
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<td><strong>T15</strong>: 2 weeks - <strong>Topical</strong>: PHMB (0.06%; q1h), hexamidine (0.1%; q1h), levofloxacin (0.5%; q1h)</td>
<td>2 weeks</td>
<td>-</td>
<td>-</td>
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<td><strong>T16</strong>: 4 weeks - <strong>Topical</strong>: hexamidine (0.1%), levofloxacin (0.5%) discontinued, PHMB (0.06%; QD), prednisolone (0.5; QD) added</td>
<td>4 weeks</td>
<td>-</td>
<td>-</td>
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<td></td>
<td><strong>T17</strong>: 2 months - <strong>Topical</strong>: QD PHMB (0.02%), QD dexamethasone (0.1%)</td>
<td>2 months</td>
<td>-</td>
<td>-</td>
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<td><strong>T18</strong>: 2 months - <strong>Topical</strong>: q1h PHMB (0.02%), q1h chlorhexidine (0.02%), q1h hexamidine (0.1%), q1h cefuroxime (5%), q1h gentamicin (1.5%)</td>
<td>2 months</td>
<td>-</td>
<td>-</td>
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<td></td>
<td><strong>T19</strong>: Undisclosed time - <strong>Topical</strong>: q1h chlorhexidine (0.2%), q1h imidazole (1%), QD cefuroxime (5%), QD gentamicin (1.5%), QD predforte (1%) Oral; BD posaconazole (300mg), BD tacrolimus (1mg), Intravenous; methylprednisolone (1g), Intracameral; voriconazole (0.5mg/ml)</td>
<td>Undisclosed time</td>
<td>-</td>
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<td><strong>T20</strong>: 2 weeks - <strong>Topical</strong>: chlorhexidine (0.2%; q1h), imidazole (1%; q1h), cefuroxime (5%; QD), gentamicin (1.5%; QD), predforte (1% QD) Oral; QD tacrolimus (1mg) discontinued, Miltefosine (50mg; TD), posaconazole (300mg; BD),</td>
<td>2 weeks</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

Key: Tn – Treatment intervention sequence where n is number of intervention, OD - once daily, BD - twice daily, TD - three times daily, QD - four times daily, qxh - taken every x hours, where x is the duration.

- Cultures were negative for Acanthamoeba after T1
- Symptoms worsened after 12 days treatment with miltefosine, presumed immune related (T4)
- No disease recurrence noted 9 months post treatment (T4)

- Cultures were negative for Acanthamoeba after T5
- Symptoms improved after 8 days treatment with miltefosine (T5)
- No disease recurrence noted 9 months post treatment (T5)

- Cultures were negative for Acanthamoeba after T7
- Symptoms improved after 20 days treatment with miltefosine (T7)
- No disease recurrence noted 9 months post treatment (T7)

- Cultures were negative for Acanthamoeba after T9
- Symptoms worsened after 21 days treatment with miltefosine, presumed immune related (T9)
- No disease recurrence noted 8 weeks post treatment (T9)

- Cultures were negative for Acanthamoeba after T11
- Miltefosine administered prior to surgery to prevent the spread of Acanthamoeba to the CNS (T11)
- Amoeba were not cleared but no spread was documented (T11)
- Amoeba present in the vitreous but further spread to the retina, choroid and optic nerve was prevented, not clear if this is a result of miltefosine treatment however (T11)
In vitro activity of APCs against Acanthamoeba

There are several in vitro studies describing the efficacy of APC analogues against Acanthamoeba with most showing miltefosine (hexadecylphosphocholine) to have optimal activity against Acanthamoeba trophozoites (Table 3). In a structure activity relationship study undertaken by Mooney and colleagues, a series of APCs with different alkyl-carbon chain length ranging from 8-18 carbons, demonstrated that miltefosine was cytotoxic at 46µM and cytostatic below this dosage against trophozoites. Another study showed that 39mM to 78mM of miltefosine was toxic to cysts. Similar results reported elsewhere for miltefosine have shown that the activity of miltefosine against Acanthamoeba is influenced by the life form, the Acanthamoeba species, the strain, and the duration of the drug in contact with the protist. Nevertheless, they have shown that approximately 3000-fold lower concentrations of miltefosine was required for in vitro treatment than for topical and oral application. The activity of APCs against Acanthamoeba species is of sufficient concentration to cause a good case for their incorporation into contact lens solutions as a preventive strategy. Studies show that human tissues such as the eye, organotypic skin models and mammalian breast cancer cells are refractory to miltefosine concentrations 160µM, 50µM and 150mM respectively which suggest that it could be safe to use as a preventive strategy and eye toxicity would not be an issue.

Quaternary Ammonium Compounds Against Acanthamoeba

Structure and classification of quaternary ammonium compounds

QACs lack the negatively charged phosphoryl group of APCs resulting in a net positive charge. They are cationic molecules containing a positively charged central nitrogen atom attached to four substituents. Attachments to aliphatic and aromatic functional groups have produced two main types of QACs. In this review, they will be referred to as benzylated and non-benzylated if the compound contains an aromatic benzyl ring (e.g. benzalkonium chloride and benzethonium chloride) or lacks one (e.g. tetraethylammonium bromide and dodecyltrimethylammonium bromide) attached to the nitrogen atom respectively (Figure 2).

Activity of quaternary ammonium compounds against Acanthamoeba

Broad antimicrobial properties against virus, bacteria and protozoans have been reported in 1,866 studies since 2015. The activity of both QACs against Acanthamoeba has also been reported recently. Benzylated QACs such as benzalkonium chloride (BAC) have widespread clinical applications, commonly used in ophthalmic
**Table 2:** Topical miltefosine against Acanthamoeba keratitis

<table>
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<th>Experimental details</th>
<th>Preparation</th>
<th>Experimental treatments</th>
<th>Key Findings</th>
<th>Ref.</th>
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</thead>
</table>
| AK eye model from 40 male Syrian hamsters divided into 3 groups | Miltefosine prepared at 2mM dissolved in 5% ethanol | Group 1 - Miltefosine (160μM)  
Group 2 - Propamidine isethionate (0.1%) and PHMB (0.02%) combination  
Infected Control group - Ethanol (0.05%) in PBS  
Each regime was used to treat Acanthamoeba-infected eyes for 28 days | **Group 1** – 85% of eyes were normal with cultures of excised post-treatment tissues negative for *Acanthamoeba*  
**Group 2** – 65% of eyes were normal with 5% cultures of excised post-infection tissues positive for *Acanthamoeba*.  
**Infected Control group** – 5% of eyes were normal with 6% of culture of excised tissues positive for *Acanthamoeba*.  
**Conclusion:** Topical miltefosine was more effective in treating AK than propamidine isethionate and PHMB combinations often used in treating AK. | 33 |

| AK eye model from 63 male Wistar rats divided into 7 groups. | 2mM of Miltefosine prepared at 2mM in 5% ethanol | Group 1 - Miltefosine (160μM)  
Group 2 – chlorhexidine gluconate (0.02%)  
Group 3 – PHMB (0.02%)  
Group 4 – propamidine isethionate (0.1%)  
Group 5 – Miltefosine (160μM) and chlorhexidine gluconate (0.02%) combination  
Group 6 – Miltefosine (160μM) and PHMB (0.02%) combination  
Group 7 – Miltefosine (160μM) and propamidine isethionate (0.1%) combination  
Infected Control group - Ethanol in PBS (0.05%)  
Each regime was used to treat Acanthamoeba-infected eyes for 28 days | **Group 1** – 14.2% and 50% of eyes were normal or almost normal respectively with 28.5% cultures of excised tissues positive for *Acanthamoeba*.  
**Group 2** – 7.1% and 50% of eyes were normal or almost normal respectively with 28.5% of cultures of excised tissues positive for *Acanthamoeba*.  
**Group 3** – 7.1% and 42.8% of eyes were normal or almost normal respectively with 21.4% cultures of excised tissues positive for *Acanthamoeba*.  
**Group 4** – 7.1% and 35.5% of eyes were normal or almost normal with 28.5% cultures of excised tissue positive for *Acanthamoeba*.  
**Group 5** –14.2% and 57.1% of eyes were normal or almost normal respectively with 21.4% cultures of excised tissues positive for *Acanthamoeba*.  
**Group 6** – 28.4% and 64.2% of eyes were normal or almost normal respectively with 14.2% cultures of excised tissue positive for *Acanthamoeba*.  
**Group 7** – 7.1% and 35.5% of eyes were normal or almost normal respectively with 21.4% of cultures of excised tissues positive for *Acanthamoeba*.  
**Infected Control group** - 0% and 21.4% of eyes were normal or almost normal respectively with 71.4% cultures of excised tissues positive for *Acanthamoeba*.  
**Conclusion:** Topical treatments of miltefosine combined with PHMB or chlorhexidine were synergistic; while combinations with propamidine isethionate were additive. PHMB-miltefosine combination is an effective treatment for AK. | 34 |

**Figure 2:** General structures of benzylated QACs. The molecules contain a positively charged nitrogen group attached to (a) two methyl groups and a benzyl ring (benzylated) or (b) three methyl groups (non-benzylated). “n” indicates the variable alkyl-carbon tail.
### Table 3: In vitro APCs and QACs against Acanthamoeba

<table>
<thead>
<tr>
<th>Class</th>
<th>Compound name</th>
<th>Experimental conditions</th>
<th>Key Findings</th>
<th>Ref.</th>
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<tr>
<td>APCs</td>
<td>Hexadecyl-PC (Miltefosine), Octadecyl-PC, Eicosanoyl-PC, (Z)-12-heneicosanoyl-PC, (Z)-13-docosanoyl-PC, (Z)-10-docosanoyl-PC, (Z,Z)-6,12-eicosenoyl-PC, (Z,Z)-6,15-tetracosadienoyl-PC</td>
<td>A. castellanii, A. polyphaga, A. lenticulata trophozoites and cysts treated with miltefosine at concentration ranging from 5-160μM for 72 hours.</td>
<td><strong>Findings:</strong> Miltefosine was most effective against A. castellanii and A. polyphaga trophozoites (MIC, 40μM) and A. lenticulata trophozoites 80μM. These concentrations killed 60%-80% of cysts of all strains. <strong>Conclusion:</strong> Miltefosine had species-specific activity against Acanthamoeba at low micromolar concentrations in vitro.</td>
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<tr>
<td>APC:</td>
<td>Hexadecyl-PC (Miltefosine)</td>
<td>Trophozoites of A. castellanii, A. polyphaga (2 strains), Unknown Acanthamoeba sp. treated with miltefosine at concentrations ranging from 10-80μM for 1 week</td>
<td><strong>Findings:</strong> Miltefosine killed Acanthamoeba spp. at concentrations above 40μM with recovery within 1-2 weeks below this concentration. <strong>Conclusion:</strong> Miltefosine had concentration-dependent cytostatic and cytotoxic activity.</td>
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<tr>
<td>APC:</td>
<td>Hexadecyl-PC (Miltefosine)</td>
<td>Cysts of three environmental isolates within T3, T4 and T5 genotypes were treated with miltefosine at concentrations ranging from 2.42-77.44μM for 1 week</td>
<td><strong>Findings:</strong> minimal cysticidal concentration for cysts of the T4 and T5 genotypes were killed after 1 day at 38.72μM while for T3 cysts, it was 77.44μM. At 7 days it decreased 9.68μM, 4.84μM and 4.84μM for the T3, T4 and T5 genotypes respectively. <strong>Conclusion:</strong> The species- and life form-specific activity of miltefosine against Acanthamoeba was time dependent. Activity on environmental isolates was significantly higher than lab-derived strains.</td>
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<tr>
<td>APC:</td>
<td>Hexadecyl-PC (Miltefosine), octadecyl-PC, elaedyl-PC, eru-cyl-PC, edelfosine</td>
<td>Trophozoites of A. castellanii, A. polyphaga incubated with miltefosine at concentrations ranging from 7.8-1000μM for 96 hours.</td>
<td><strong>Findings:</strong> The order of activity against Acanthamoe- ba spp was Hexadecyl-PC &gt; octadecyl-PC albeit with A. castellanii more susceptible than A. polyphaga. MIC for hexadecyl-PC was 62.5μM and 125μM for A. castellanii and A. polyphaga respectively. <strong>Conclusion:</strong> Species-specific activity of miltefosine against Acanthamoeba was highlighted, vital to inform a preventative strategy.</td>
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<tr>
<td>QACs</td>
<td>Polyquad-1</td>
<td>Cysts of A. castellanii, A. polyphaga, A. hatchetti treated with 0.001% Polyquad-1 formulated in the contact lens solutions Alcon Opti-Clean II, Alcon Opti-Free Express, Alcon Opti-Free Replenish for 24 hours</td>
<td><strong>Findings:</strong> Viable cysts were observed for all 3 Acanthamoeba spp. after 24 hours incubation in all Polyquad-1-containing contact lens formulations <strong>Conclusion:</strong> Polyquad-1 concentrations in contact lens solutions are inactive against Acanthamoeba cysts</td>
<td>11</td>
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<tr>
<td>nbQAC</td>
<td>Benzalkonium chloride (BAC)</td>
<td>Trophozoites and cysts of A. castellanii, A. polyphaga treated with BAC at concentrations ranging from 2.7-134μM (0.005-0.02%) for 48 hours in the BAC containing solutions Levofloxacin (Oftaquix; 0.005% BAC), trifluorothymidine ( 0.02% BAC)</td>
<td><strong>Findings:</strong> BAC monotherapy was effective in killing trophozoites at concentrations of 0.02μM and 0.005μM and cysts at concentrations of 0.04μM and 0.02μM for A.castellanii and A.polyphaga respectively. The Levofloxacin (Oftaquix) ophthalmic solution with 0.005% BAC has a minimal amoebicidal concentration of 156μg/ml and 312μg/ml and cysticidal concentration of 625μg/ml and 625μg/ml for A.castellanii and A. polyphaga respectively. For TMT containing 0.02% BAC these same respective concentrations were 625μg/ml and 1250μg/ml for trophozoites and 5000μg/ml and 2500μg/ml for cysts. <strong>Conclusion:</strong> BAC is highly potent to Acanthamoeba trophozoites and cysts and addition to contact lens solutions significantly increases efficacy.</td>
<td>42</td>
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</tbody>
</table>
Acanthamoeba (DTAB) with 12 alkyl-carbon atoms was non-toxic against benzylated QAC, dodecyltrimethylammonium bromide being 107.52μM compounds to produce death by micelle formation35. This structural variation is linked to the ability of the forms to produce death at concentrations ranging from 2.69μM to 20.97M solutions as topical and preventive strategies, and can cause death at concentrations ranging from 2.69μM to 20.97M and 20.97μM to 41.93μM against trophozoites and cysts respectively46, far lower than that in ophthalmic solutions.

### APC and QAC

<table>
<thead>
<tr>
<th>QAC</th>
<th>Activity</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>bQAC: Benzalkonium chloride (BAC)</td>
<td>Trophozoites and cysts of A. castellanii (2 strains), A. polyphaga (2 strains), A. mauritaniensis (6 strains)</td>
<td>0.004% BAC</td>
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<tr>
<td>nbQAC: Polyquad-1</td>
<td>Trophozoites and cysts of A. castellanii at concentrations ranging from 0.5-400μM for 96 hours.</td>
<td>0.004% BAC</td>
</tr>
<tr>
<td>APC: Dodecyl-TMAB, Tetradecyl-TMAB, Octadecyl-TMAB</td>
<td>Trophozoites and cysts of A. castellanii at concentrations ranging from 0.98-500μM for 48 hours.</td>
<td>0.004% BAC</td>
</tr>
<tr>
<td>nbQAC: Dodecyl-PC, Tetradecyl-PC, Hexadecyl-PC (Miltefosine)</td>
<td>Octadeyl-TMAB and miltefosine (hexadecyl-PC) were most effective QAC and APCs against Acanthamoeba trophozoites respectively, with the nbQACs, Octadeyl-TMAB cytotoxic at low concentrations and demonstrated cysticidal activity after 24 hours. Dodecyl-TMAB and miltefosine was synergistic.</td>
<td>0.004% BAC</td>
</tr>
<tr>
<td>APC: Dodecyl-PC, Tetradecyl-PC, Hexadecyl-PC (Miltefosine)</td>
<td>QACs can rapidly reverse the net negative charge of the plasma membrane to produce shock due to the opposing charge of the molecule. It has been shown that toxicity to human cells can be an issue, but currently available data only investigates higher concentrations than those recorded as toxic against Acanthamoeba. For example, octadeyltrimethylammonium bromide causes severe eye irritation at concentrations above 510mM, while ~1200-fold (42μM) lower doses are required for activity against Acanthamoeba. Nevertheless, studies are required to validate QACs as a preventative strategy. Should corneal toxicity be an issue, neutralising agents such as β-cyclodextrin could provide a second step during contact lens cleansing. Two-step methods have been effectively used for hydrogen peroxide based contact lens solutions.</td>
<td>0.004% BAC</td>
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</table>

### Key:
- bQAC – benzylated QAC, nbQAC – non-benzylated QAC, -TMAB – trimethyl ammonium bromide, -PC - Phosphocholine
- APCs are more effective against Acanthamoeba trophozoites than QACs.
- The activity of benzylated QACs against different Acanthamoeba species and strain.
- QACs formulated in medical devices with longer incubation times in cytotoxicity assays have increased efficacy against trophozoites and cysts at concentrations ranging from 0.5-400μM for 96 hours.

### Findings:
- Octadeyl-TMAB and miltefosine (hexadecyl-PC) were most effective QAC and APCs against Acanthamoeba trophozoites respectively, with the nbQACs, Octadeyl-TMAB cytotoxic at low concentrations and demonstrated cysticidal activity after 24 hours. Dodecyl-TMAB and miltefosine was synergistic.
- QACs are more effective against Acanthamoeba trophozoites than APCs.

**Conclusion**

The impact of APCs in AK treatments, even amongst the ‘difficult to treat’ immuno-suppressed patients is promising. Despite the clinically observed effect, little

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Acanthamoeba spp with comparable MICs, 15.6μM.**
is known about its clinical pharmacodynamics, mainly because good quantitative markers of parasite load and treatment response are not available for AK. Disease-specific pharmacokinetics for miltefosine alone and in combination are available for other anti-parasitic infections e.g. Leishmaniasis but are yet to be available for AK. Further clinical research is required for these compounds use against AK. The studies highlighted in this review suggest that miltefosine may reduce current disease resurgence, common with current curative and preventative treatments. Similarly, pharmacodynamics and pharmacokinetic for QACs are scarce and are perhaps hampered by their extensive use as preservatives. The strong efficacy of QACs against *Acanthamoeba* is a framework for development as a treatment or preventative for AK, if corneal toxicity issues are addressed.

**References**


