SCIENTIFIC CORRESPONDENCE

Viability of *Acanthamoeba* after exposure to a multipurpose disinfecting contact lens solution and two hydrogen peroxide systems

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Background/aim: Contact lens cases contaminated with *Acanthamoeba* are a major risk factor for an infection of the eye. In this study the anti-*Acanthamoeba* activity of three different contact lens storage solutions was tested.
Methods: A new multipurpose contact lens storage solution (Meni Care Plus) and a two step (Titmus H_2O_2) and one step (Oxysept Comfort) hydrogen peroxide system were tested for their effects on trophozoites and cysts of three different *Acanthamoeba* species: *A castellanii*, *A hatchetti*, and *A lenticulata*.
Results: After a soaking time of 8 hours (overnight soaking of contact lenses) the Titmus H_2O_2 0.6% solution showed very good amoebicidal effects, while Oxysept Comfort 3% H_2O_2 could not effectively destroy the cysts of any of the three tested species. Viable cysts of the species *A lenticulata* and *A hatchetti* were still present after exposure to Meni Care Plus (0.0005% PHMB) for 8 hours.
Conclusion: Not all of the three tested contact lens storage solutions have sufficient amoebicidal effects. The two step peroxide system Titmus H_2O_2 is a very effective disinfectant contact lens solution in order to avoid a possible *Acanthamoeba* infection of the eye.

Contact lens wear is the most prevalent risk factor for *Acanthamoeba* keratitis.[1] Infection can occur in association with any type of contact lenses, but soft lens wearers are at enhanced risk of an infection.[2 3] The infection is painful and medical treatment is difficult, prolonged, and the disease can result in penetrating keratoplasty or blindness of the affected eye.[4–7]

Inadequate contact lens hygiene of lens storage systems is seen as the main risk factor for an infection of the eye.[2 3 8] Contact lens storage cases contaminated with micro-organisms like bacteria or fungi are an excellent culture medium for *Acanthamoeba*, since they feed on micro-organisms and multiply.[9 10] Possible sources for *Acanthamoeba* contamination of contact lens cases are the use of contaminated tap water or lens solutions when rinsing or cleansing the contact lens or storage case, contaminated bathroom dust, and improper contact lens hygiene.[3 11 12] As cysts, as well as active trophozoites, attach to the surface of contact lenses, they can easily be transmitted from the storage case onto the eye.[13] Therefore, the use of contact lens storage solutions that effectively kill *Acanthamoeba* is essential for the prevention of a possible infection of the eye. Since *Acanthamoeba* keratitis emerged as an epidemic in the mid-1980s, the contact lens industry has made efforts to produce disinfectants for contact lens storage solutions with anti-*Acanthamoeba* activity. While *Acanthamoeba* trophozoites are quite sensitive to many disinfecting solutions, cysts show a high degree of resistance.[8 14]

In our study, the effectiveness of a new multipurpose disinfecting solution was tested in comparison with a 3% hydrogen peroxide one step and a 0.6% hydrogen peroxide two step system against cysts and trophozoites of different strains of *Acanthamoeba.*

MATERIALS AND METHODS

Contact lens solutions

All tested contact lens storage solutions and their respective contents are listed in Table 1. All tested solutions were taken from their original wrappings and were used before their stated expiry date. According to the manufacturer's instructions for Titmus H_2O_2 the neutralisation of the hydrogen peroxide is achieved by soaking the contact lenses in a second solution (catalase 170 IU/ml) for 10 minutes before wearing. When applying Oxysept Comfort a catalytic tablet is put into the storage case together with the 3% hydrogen peroxide solution. A minimum contact lens soaking time of 6 hours is required.

Table 1 Contact lens solutions tested

Trade name	Manufacturer	System	Disinfectant	Lens type
Meni Care Plus	Menicon Pharma F-67400 Illkirch Grafenstaden	Multipurpose solution	Polyhexamethylene biguanide 0.0005%	Rigid gas permeable
Titmus H_2O_2	Ciba Vision Corp, Duluth, GA 30097, USA	Two step hydrogen peroxide	0.6% H_2O_2	Soft and rigid gas permeable
Oxysept Comfort	Pharm Allergan GmbH, D-76260, Ettlingen	One step hydrogen peroxide	3% H_2O_2	Soft

Table 2 Viability of *Acanthamoeba* cysts after 30 minutes of exposure time

	A castellanii (4CL)			*A hatchetti (11DS)*			*A lenticulata (72/2)*		
	10^3	10^4	10^5	10^3	10^4	10^5	10^3	10^4	10^5
Meni Care Plus									
1	+	+	+	+	+	+	+	+	+
1:1	+	+	+	+	+	+	+	+	+
1:2	+	+	+	+	+	+	+	+	+
1:3	+	+	+	+	+	+	+	+	+
Oxysept Comfort									
1	+	+	+	+	+	+	+	+	+
1:1	+	+	+	+	+	+	+	+	+
1:2	+	+	+	+	+	+	+	+	+
1:3	+	+	+	+	+	+	+	+	+
Titmus H_2O_2									
1	–	–	–	–	–	+	+	+	+
1:1	+	+	+	+	+	+	+	+	+
1:2	+	+	+	+	+	+	+	+	+
1:3	+	+	+	+	+	+	+	+	+

Amoebae

Three different strains of *Acanthamoeba* spp, including two strains of morphological group II (*A hatchetti* 11DS, *A castellanii* 4CL)[15 16] and one strain of morphological group III isolated from the brain of an experimentally infected mouse (*A lenticulata* strain 72/2)[17] were used in this study. Tests were performed on both cysts and trophozoites of each strain.

The amoebae were cultured in the absence of any other organisms in 150 cm^2 tissue culture flasks in 36 ml of PYG (proteose peptone-yeast extract-glucose). Trophozoites and cysts were harvested from liquid cultures by centrifugation (500 *g* for 7 minutes) and the pellet resuspended in sterile 0.9% NaCl. The amoebae were counted in a Bürker-Türk haemocytometer. Trophozoites and cysts of each strain at a concentration of 10^3, 10^4, and 10^5 cells/ml were used in the tests.

Performance of the tests

Tests were performed in 24 well microtitre plates. One ml contact lens solution per well was applied. Oxysept Comfort tests were performed in 15 ml Falcon tubes using 6 ml solution. According to the manufacturer's instructions the neutralising tablet (catalase 1 mg per tablet) was added at the same time as the 3% hydrogen peroxide solution. Further, three dilutions (1:1, 1:2, 1:4) from each of the solutions were tested for their amoebicidal effects.

After a soaking time of 30 minutes and 8 hours (overnight soaking of contact lenses), respectively, 100 µl samples were inoculated onto non-nutrient agar plates covered with a lawn of *Escherichia coli*. The plate cultures were sealed and incubated at 30°C for 14 days. All experiments were carried out in triplicate. The control groups were performed with sterile 0.9% NaCl. Amoebic growth was observed daily by phase contrast microscopy.

RESULTS

The effect of the three contact lens storage solutions on the different *Acanthamoeba* strains subject to the concentration of cysts, soaking time, and dilution is shown in Tables 2 and 3. As expected, the sensitivity of *Acanthamoeba* trophozoites to the applied solutions was higher than that of the cysts. After a soaking time of 8 hours trophozoites of all strains were killed by all tested disinfectant solutions.

After a soaking time of 8 hours the two step hydrogen peroxide system Titmus H_2O_2 0.6% showed the best amoebicidal effects. The cysts of the morphological group II (*A hatchetti, A castellanii*) were totally destroyed. Only the cysts of the morphological group III, *A lenticulata*, at a concentration of 10^5 showed viability. Even in a dilution of 1:1 all cysts of morphological group II in all concentrations were completely destroyed. Cysts of *A castellanii* and *A hatchetti* were destroyed after exposure to this solution for 30 minutes (Table 2)

Table 3 Viability of *Acanthamoeba* cysts after 8 hours of exposure time

	A castellanii (4CL)			*A hatchetti* (11DS)			*A lenticulata* (72/2)		
	10^3	10^4	10^5	10^3	10^4	10^5	10^3	10^4	10^5
Meni Care Plus									
1	–	–	–	–	–	+	+	+	+
1:1	–	–	+	–	+	+	+	+	+
1:2	+	+	+	+	+	+	+	+	+
1:3	+	+	+	+	+	+	+	+	+
Oxysept Comfort									
1	+	+	+	+	+	+	+	+	+
1:1	+	+	+	+	+	+	+	+	+
1:2	+	+	+	+	+	+	+	+	+
1:3	+	+	+	+	+	+	+	+	+
Titmus H_2O_2									
1	–	–	–	–	–	–	–	–	+
1:1	–	–	–	–	–	–	+	+	+
1:2	–	+	+	+	+	+	+	+	+
1:3	+	+	+	+	+	+	+	+	+

The multipurpose disinfecting solution Meni Care Plus also has amoebicide effects. However, cysts of the strain *A lenticulata* (morphological group III) and *A hatchetti* (morphological group II) at a concentration of 10^5 could not be completely destroyed.

One step Oxysept Comfort was less effective against *Acanthamoeba* cysts. After a soaking time of 8 hours cysts of all tested strains were still viable.

A lenticulata (72/2) belonging to morphological group III was found to be the most resistant strain in comparison with the other isolates tested.

DISCUSSION

We have shown that the two step hydrogen peroxide system 0.6% Titmus H_2O_2, after an exposure time of 8 hours (overnight soaking of contact lenses), is very effective against *Acanthamoeba*.

The disinfecting ability of hydrogen peroxide is directly proportional to its length of exposure to the micro-organism: an incubation of 2–3 hours of exposure time to 3% H_2O_2 is sufficient to kill bacteria, HIV, fungi, and *Acanthamoeba*.[18 19] However 3% H_2O_2 Oxysept Comfort with a neutralisation tablet (1 mg catalase per tablet) was not effective against cysts of all three species in the lowest concentration even after 8 hours of soaking time. Because of the toxic effects of hydrogen peroxide to the conjunctival and corneal epithelium it must be neutralised before contact lens wear. Therefore, one step systems often use a catalyst (platinum ring, tablet) to neutralise the H_2O_2, producing oxygen and water. Presumably, the catalytic tablet of the Oxysept Comfort neutralises the 3% hydrogen peroxide too quickly to guarantee sufficient soaking time and effective killing of the *Acanthamoeba* cysts. The cationic antiseptic agent polyhexamethylene biguanide (PHMB) is effective in killing *Acanthamoeba* cysts and trophozoites by inhibiting membrane function.[3 19] In 1992 Larkin *et al*[20] reported its successful clinical use at a concentration of 0.02% in treatment of *Acanthamoeba* keratitis. Meni Care Plus storage solution for rigid gas permeable contact lenses contains 0.0005% PHMB. Even after a soaking time of 8 hours Meni Care Plus was ineffective against cysts of the species *A lenticulata* and *A hatchetti* at a concentration of 10^5 cells/ml.

Cysts of *Acanthamoeba* are known to be highly resistant to extremes of pH and temperature and also to disinfectants of contact lens solutions.[3 21] Mazur *et al*[22] demonstrated the viability and invasiveness of *Acanthamoeba* cysts after they had been stored in water at 4°C for a period of 24 years. These capabilities may vary between the different species and morphological groups. Our tests have shown that *A lenticulata* belonging to morphological group III showed the greatest resistance against the three tested disinfectant solutions. However, most clinical isolates are representatives of morphological group II and so far *A lenticulata* has not been reported to cause *Acanthamoeba* keratitis.[3 21]

The main goal in prevention of *Acanthamoeba* keratitis is to keep the contact lens storage case free of micro-organisms, *Acanthamoeba*, and biofilm. Appropriate measures to prevent microbial contamination and the development of biofilm are frequent microwave treatment or boiling of the storage case (destroys *Acanthamoeba* cysts and trophozoites), mechanical cleansing and use of intensive cleaning solutions (removal of biofilm), avoidance of contact with contaminated water, frequent renewal of the storage case, use of effective solutions, and proper contact lens care.[3 13 19 23–26] However, the development and use of storage solutions which are effective against *Acanthamoeba* is of great importance. We can recommend the Titmus H_2O_2 two step system for overnight soaking of contact lenses as an effective disinfectant against *Acanthamoeba.*

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