Available online at www.sciencedirect.com
SCIENCE DIRECT®
Contact Lens & Anterior Eye 27 (2004) 209–212
Contact Lens & Anterior Eye
www.elsevier.com/locate/clae

Antimicrobial efficacy of a povidone iodine (PI) and a one-step hydrogen peroxide contact lens disinfection system

Simon Kilvington*

Department of Infection, Immunity and Inflammation, University of Leicester, Medical Sciences Building, P.O. Box 138, University Road, Leicester LE19HN, UK

Abstract

The antimicrobial efficacy of a novel povidone iodine (PI) contact lens disinfection system (Clencide) was compared with a one-step hydrogen peroxide system (AoSept). The PI system showed rapid killing of organisms with a 4.5–6.0 log reduction in bacteria, yeast, mould and *Acanthamoeba* trophozoites within 5 min and a 2.8–3.6 log kill of *Acanthamoeba* cysts after 2–4 h. The one-step peroxide gave a 4.0–6.0 log kill of bacteria in 0.5–1 h, 2.0–5.0 log for yeast after 2–6 h and 1.8 log for mould at 6 h. *Acanthamoeba polyphaga* trophozoites were reduced by 3.6 log at 1 h but cysts by only 1.2 log after 6 h. The study demonstrates that the PI system is an effective disinfection method for contact lenses.

Keywords: Povidone iodine; Hydrogen peroxide; Contact lens disinfection; *Acanthamoeba*

1. Introduction

Contact lens wear is a predisposing factor in some 50% of reported cases of microbial keratitis [1–3,12]. Bacterial keratitis is the most common form, with staphylococci, *Pseudomonas aeruginosa* and *Serratia* spp. the usual infectious organisms [1,2,12,22]. Although less common, keratitis due to the free-living amoeba *Acanthamoeba* is almost exclusively associated with contact lens wear, which accounts for 90% of reported cases, with an incidence in the UK some 15 times that of the USA and 3 times the rest of Europe [17–19,21,23].

It has long been recognised that poor contact lens hygiene enables microorganisms to colonise the lens storage case [13]. This can then result in biofilm production that protects potentially pathogenic microbes from disinfectant action [14]. Such organisms can then adhere to the contact lenses for inoculation on to the cornea [3,5,16]. Protein deposits also interfere with oxygen diffusion across the lens, and other debris on the lens surface may cause corneal abrasions resulting in discomfort or initiation of infection [9–11,15,16] thus, the regular cleaning and disinfection of contact lenses is fundamental to both the comfort and the safety of the wearer.

Currently, contact lens disinfection is achieved through the use of either multipurpose solutions (MPS) or hydrogen peroxide systems. The MPS utilise a single solution for disinfecting, rinsing and storing the lenses; with most MPS having an inherent cleaning system.

Hydrogen peroxide (3%) is a powerful disinfectant and has been shown to be effective against the highly resistant cyst stage of *Acanthamoeba*, giving a 3 log reduction in viability provided an exposure time of at least 4–6 h is allowed prior to neutralisation which is essential before lens wear to avoid pronounced discomfort and possible corneal damage [7]. To avoid these problems and simplify use, one-step hydrogen peroxide systems are available which do not require separate neutralisation. Here, neutralisation is achieved in the storage case during disinfection using a platinum-coated disc or soluble catalase tablet which catalyses the decomposition of hydrogen peroxide to water and oxygen [7].

Recently, a novel povidone iodine (PI) contact lens disinfection and cleaning system has been introduced to the market. The system comprises a disinfectant/proteolytic enzyme stage with simultaneous neutralisation of the PI

* Tel.: +44 116 252 2950; fax: +44 116 252 5030.
E-mail address: sk46@le.ac.uk.

1367-0484/$ – see front matter
doi:10.1016/j.clae.2004.09.001

through the addition of a separate reagent. Disinfection and neutralisation are complete within 10 min but the lenses are left in the solution for at least 4 h to effect the enzymatic cleaning. In this present study, the efficacy of the system in comparison with a one-step hydrogen peroxide contact lens disinfectant was evaluated for known ocular pathogenic bacteria, fungi and *Acanthamoeba*.

2. Materials and methods

2.1. Test organisms

The bacteria, fungi and *Acanthamoeba* spp. tested were: *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Serratia marcescens* (NCTC 10211), *Candida albicans* (ATCC 10231), *Fusarium solani* (ATCC 36031) and *Acanthamoeba polyphaga* (Ros), the latter isolated from a case of Acanthamoeba keratitis which occurred in the United Kingdom. The bacteria and fungi were cultured according to recommended protocols for contact lens disinfectant efficacy testing [8]. The *F. solani* conidia were prepared as described previously [6]. *A. polyphaga* (Ros) trophozoites were maintained in axenic culture as described previously [7]. Cysts were produced from the trophozoite cultures using Neff's constant pH encystment medium as described previously [7].

2.2. Test solutions

0.05 mg/8 ml = 0.625 mg/ 100 ml =6.25 mg/L = 6.25 ppm

The PI contact lens disinfectant system (Clencide) was supplied from the manufacturer (Ophtecs Corporation, Osaka, Japan). The system comprises a sachet of disinfectant/proteolytic enzyme containing the PI (0.05 mg) and *Bacillus subtilis* protease (8 mg), a neutralisation tablet (sodium sulfite 2.4 mg) and a diluting/rinsing solution (sodium borate, sodium chloride and EDTA). The diluting/rinsing solution (8 ml) is added to the contact lens storage case and the disinfectant/proteolytic enzyme granules and neutralising tablet added. The lenses are then placed inside the case. The disinfection and neutralisation process is complete within 10 min (a colour indicator in the system changes from orange to clear to show that the disinfection stage is complete) but the lenses are left in the solution for at least 4 h to effect the enzymatic cleaning. The lenses are then removed from the case and rinsed with the remaining diluting/rinsing solution before wearing.

The one-step hydrogen peroxide contact lens disinfectant (AoSept, Ciba Vision, Atlanta, USA) was obtained locally and comprises a 3% (v/v) hydrogen peroxide solution and a contact lens storage case containing a platinum coated neutralising disk. As with the PI system, the disinfection and neutralisation process occurs simultaneously and the lenses can be worn after 6 h.

Phosphate buffered saline (PBS) was used in place of the disinfectant solutions in control studies. Dey–Engley Neutralising Broth (Difco, MI, USA) was used in the PI studies and 0.02% (w/v) bovine liver catalase (Sigma Chemical Company, Dorset, UK) for the hydrogen peroxide system.

2.3. Test methods

The systems were used exactly according to the manufacturers' recommendations and challenged with the test organisms in a volume $\leq$0.1% of the disinfectant solution volume. The challenge test assays for the bacteria and fungi were conducted exactly in accordance with recommended procedures [8].

The method for the *Acanthamoeba* trophozoites and cysts was as described previously [7]. Sample time-points for the PI system were after 0, 5 min and 1, 2, 3, 4 h exposure with PI, and 0, 0.5, 1, 2, 4 and 6 h with the hydrogen peroxide system. Experiments were conducted in triplicate.

In one experiment, the PI system was activated and left for 10 min before being challenged with *P. aeruginosa*. Viable bacterial counts were then conducted over a 4-h period.

3. Results

3.1. PI disinfection system

Activity of the PI system against the test organisms is shown in Table 1. Complete kill (5–6 log reduction)

Table 1
Efficacy of povidone iodine system against bacteria, fungi and *Acanthamoeba polyphaga*

Time	*P. aeruginosa* (ATCC 9027)	*S. marcescens* (NCTC 10211)	*S. aureus* (ATCC 6538)	*C. albicans* (ATCC 10231)	*F. solani* (ATCC 36031)	*A. polyphaga* (Ros)	
						Trophozoites	Cysts
Log organism kill							
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5 min	5.6 (0.4)[a]	5.8 (0.3)	6.0 (0.1)	4.7 (0.2)	4.8 (0.4)	4.5 (0.4)	1.0 (0.25)
1 h	–[b]	–	-	–	–	–	1.0 (0.3)
2 h	–	–	-	–	–	–	2.8 (0.4)
3 h	–	–	-	–	–	–	3.5 (0.3)
4 h	–	–	-	–	–	–	3. 6 (0.2)

[a] Mean (n = 3) log reduction in viable organisms with standard error of the mean in parenthesis.
[b] No viable organism detect at this time point.

occurred with all bacteria and fungi tested by the first time point of 5 min. With *A. polyphaga*, complete kill (4.5 log) of trophozoites occurred within 5 min. For the cysts, a 2.8 log reduction occurred after 2 h and 3.6 log by 4 h.

In the experiment where the system was challenged with *P. aeruginosa* following a delay of 10 min after combining the PI disinfectant, enzyme granules and neutralisation tablet, no killing of the bacterium was detected over a 4-h period; indicating that the disinfectant capacity of the system is fully neutralised within 10 min.

No loss in organism viability was found in control studies in which the test organisms were inoculated into PBS or the Dey–Engley Neutralising Broth (results not shown).

3.2. One-step peroxide system

The efficacy of the hydrogen peroxide system against the bacteria, fungi and *A. polyphaga* is shown in Table 2. The system gave a 6 log kill of *P. aeruginosa* and *S. marcescens* by 0.5 h (total kill for these bacteria) and a 4 log kill of *S. aureus* at 1 h. With *C. albicans*, a 1 log kill was obtained after 0.5 h, 2 log by 1 h and 5 log at 6 h. For *F. solani* conidia only a 1.8 log kill was obtained by 6 h. *A. polyphaga* trophozoites were reduced by 3.6 log (total kill) at 1 h but only a 1.2 log reduction in cyst viability was found at 6 h.

Again, no change in organism viability was found in control studies in which the test organisms were inoculated into PBS or catalase neutraliser (results not shown).

4. Discussion

The suitability of contact lens disinfectant systems for human use is determined by the Food and Drug Administration (FDA) and the International Organization for Standardization [18]. In the "Stand Alone Test" (ISO 14729) employed here, a disinfectant must be capable of reducing the viability of specified bacterial and fungal species by 3 log (99.9%) and 1 log (90%), respectively within the disinfection time recommended by the product manufacturer [8]. However, there is no requirement to demonstrate activity against *Acanthamoeba* [8].

In this study, we compared the disinfectant efficacy of a novel PI contact lens disinfectant system with that of a one-step hydrogen peroxide method. Although both products surpassed the requirements of the "Stand Alone Test" for bacteria and fungi, the PI system showed greater efficacy in the rate of disinfection and activity against *Acanthamoeba* cysts.

In the PI system the disinfectant is mixed with the proteolytic cleaning granules in the lens storage case. A neutralising tablet is added. Although the manufacturer recommends a 4-h contact time, this is to enable the proteolytic cleaner to work; it was found that the disinfectant stage is complete within 10 min. Under these conditions, the PI system produced at least a 5 log reduction in bacteria and 4 log reduction in fungi by the first sample point of 5 min. The system also demonstrated good activity against *Acanthamoeba*, giving a 4.5 log trophozoite kill by 5 min and a 2.8 or 3.6 log kill of cysts within 2 and 4 h, respectively. It is not clear why killing of the cysts continues, even after PI neutralisation is complete. Possibly, the cysts take up the PI during the disinfection process with lethal effect on the trophozoite within and that this process continues progressively after the external disinfectant has been neutralised. The efficacy of PI against *Acanthamoeba* spp. has been reported elsewhere and is confirmed by the findings of this study [4].

The one-step hydrogen peroxide system studied here employs a platinum coated disc as part of the storage case lens basket, to catalyse the breakdown of the disinfectant into water and oxygen. Such a process offers the convenience of a single disinfection-neutralisation step and eliminates the painful consequence of inserting non-neutralised lenses into the eye that can occur with two-step systems. However, as has been previously described, the rapid neutralisation of the peroxide results in decreased efficacy against *Acanthamoeba* cysts in comparison with a two-step system [7]. The system was also found to be less effective than PI against fungi, giving only a 1.2 log and 1.8 log kill of *F. solani* conidia after 4 and 6 h exposure.

In conclusion, the PI system represents a new and effective contact lens disinfectant system efficacious against ocular pathogenic bacteria, fungi and *Acanthamoeba*. However, unlike multipurpose disinfectant systems, once

Table 2
Efficacy of one-step hydrogen peroxide system against bacteria, fungi and *Acanthamoeba polyphaga*

Time	*P. aeruginosa* (ATCC 9027)	*S. marcescens* (NCTC 10211)	*S. aureus* (ATCC 6538)	*C. albicans* (ATCC 10231)	*F. solani* (ATCC 36031)	*A. polyphaga* (Ros)	
						Trophozoites	Cysts
Log organism kill							
0	0	0	0	0	0	0	0
0.5 h	6.4 (0.2)[a]	5.9 (0.3)	2.8 (0.3)	1.4 (0.2)	0	2.8 (0.3)	0
1 h	–[b]	–	4.4 (0.2)	1.9 (0.4)	0.7 (0.2)	3.6 (0.1)	0.9 (0.3)
2 h	–	–	4.7 (0.2)	2.1 (0.4)	0.95 (0.1)	–	0.9 (0.4)
4 h	–	–	–	2.5 (0.2)	1.2 (0.3)	–	0.7 (0.3)
6 h	–	–	–	5.5 (0.4)	1.8 (0.2)	–	1.2 (0.25)

[a] Mean (n = 3) log reduction in viable organisms with standard error of the mean in parenthesis.
[b] No viable organism detect at this time point.

neutralisation is complete there is no residual disinfectant activity for continued antimicrobial protection against organisms that may have survived the disinfection process or been introduced from the environment on opening the storage case [20]. Accordingly, lenses should be re-disinfected before wearing if they have been stored for more than 24 h when using the PI system.

Acknowledgements

The technical assistance of Reanne Hughes and James Lonnen is gratefully acknowledged. This study was supported, in part, by a donation from Ophtecs Euro Ltd., Limerick, Ireland.

References

[1] Bourcier T, Thomas F, Borderie V, et al. Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. Br J Ophthalmol 2003;87:834–8.
[2] Cheng KH, Leung SL, Hoekman HW, et al. Incidence of contact-lens-associated microbial keratitis and its related morbidity. Lancet 1999;354:181–5.
[3] Dart JK, Stapleton F, Minassian D. Contact lenses and other risk factors in microbial keratitis. Lancet 1991;338:650–3.
[4] Gatti S, Cevini C, Bruno A, et al. In vitro effectiveness of povidone-iodine on *Acanthamoeba* isolates from human cornea. Antimicrob Agents Chemother 1998;42:2232–4.
[5] Gorlin AI, Gabriel MM, Wilson LA, et al. Binding of *Acanthamoeba* to hydrogel contact lenses. Curr Eye Res 1996;15:151–5.
[6] Hughes R, Dart J, Kilvington S. Activity of the amidoamine myristamidopropyl dimethylamine against keratitis pathogens. J Antimicrob Chemother 2003;51:1415–8.
[7] Hughes R, Kilvington S. Comparison of hydrogen peroxide contact lens disinfection systems and solutions against *Acanthamoeba polyphaga*. Antimicrob Agents Chemother 2001;45:2038–43.
[8] International Organization for Standardization: ISO 14729. Ophthalmic optics–contact lens care products–microbiological requirements and test methods for products and regimens for hygienic management of contact lenses. 2001.
[9] John T, Desai D, Sahm D. Adherence of *Acanthamoeba castellanii* cysts and trophozoites to unworn soft contact lenses. Am J Ophthalmol 1989;108:658–64.
[10] Kilvington S, Larkin DF, White DG, et al. Laboratory investigation of Acanthamoeba keratitis. J Clin Microbiol 1990;28:2722–5.
[11] Kilvington S, Larkin DFP. *Acanthamoeba* adherence to contact lenses and removal by cleaning agents. Eye 1990;4:589–93.
[12] Lam DS, Houang E, Fan DS, et al. Incidence and risk factors for microbial keratitis in Hong Kong: comparison with Europe and North America. Eye 2002;16:608–18.
[13] Larkin DF, Kilvington S, Easty DL. Contamination of contact lens storage cases by *Acanthamoeba* and bacteria. Br J Ophthalmol 1990;74:133–5.
[14] McLaughlin-Borlace L, Stapleton F, Matheson M, et al. Bacterial biofilm on contact lenses and lens storage cases in wearers with microbial keratitis. J Appl Microbiol 1998;84:827–38.
[15] Miller MJ, Ahearn DG. Adherence of *Pseudomonas aeruginosa* to hydrophilic contact lenses and other substrata. J Clin Microbiol 1987;25:1392–7.
[16] Miller MJ, Wilson LA, Ahearn DG. Effects of protein, mucin, and human tears on adherence of *Pseudomonas aeruginosa* to hydrophilic contact lenses. J Clin Microbiol 1988;26:513–7.
[17] Morlet N, Duguid G, Radford C, et al. Incidence of Acanthamoeba keratitis associated with contact lens wear. Lancet 1997;350:414–6.
[18] Radford CF, Lehmann OJ, Dart JK. National Acanthamoeba Keratitis Study Group. Acanthamoeba keratitis: multicentre survey in England 1992–6. Br J Ophthalmol 1998;82:1387–92.
[19] Radford CF, Minassian DC, Dart JK. Acanthamoeba keratitis in England and Wales: incidence, outcome, and risk factors. Br J Ophthalmol 2002;86:536–42.
[20] Rosenthal RA, Stein JM, McAnally CL, et al. A comparative study of the microbiologic effectiveness of chemical disinfectants and peroxide-neutralizer systems. CLAO J 1995;21:99–110.
[21] Seal DV, Beattie TK, Tomlinson A, et al. Acanthamoeba keratitis. Br J Ophthalmol 2003;87:516–7.
[22] Stapleton F, Dart JK, Seal DV, et al. Epidemiology of *Pseudomonas aeruginosa* keratitis in contact lens wearers. Epidemiol Infect 1995;114:395–402.
[23] Stehr-Green JK, Bailey TM, Brandt FH, et al. Acanthamoeba keratitis in soft contact lens wearers. A case-control study. J Am Med Assoc 1987;258:57–60.