EFFICACY OF CONTACT LENS SOLUTIONS AGAINST THAI CLINICAL ISOLATES OF *ACANTHAMOEBA*

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Abstract. Three clinical isolates of *Acanthamoeba* (*A. castellanii*, *A. polyphaga*, and *A. mauritaniensis*) were used to evaluate the cysticidal activity of four different contact lens multi-purpose solutions (Complete Protec, ReNu MultiPlus, Solocare Aqua, and Opti-free Aldox). Enumeration of amoebic was carried out with the control and test samples at 0, 2, 4, 6, 8, 10, and 24 hours after being added to the solutions using the most probable number (MPN) technique. A contact lens solution which achieved a 3-log reduction of *Acanthamoeba* during the manufacturer's minimum recommended disinfection time (MMRDT) was considered an effective solution. The studied contact lens solutions demonstrated decreasing number of amoebic with increasing exposure times, but were not effective against *Acanthamoeba* cysts during the MMRDT. Solocare Aqua gave the greatest reduction in *A. castellanii* (0.70-log reduction) and *A. mauritaniensis* (0.33-log reduction) by 4 hours. Only Solocare Aqua caused a 3-log reduction in *A. castellanii* (3.02-log reduction) by 24 hours. Opti-free Aldox showed the greatest cysticidal activity against *A. polyphaga* (0.32-log reduction) by 6 hours, and gave the greatest reduction in *A. mauritaniensis* by 8, 10, and 24 hours.

INTRODUCTION

Acanthamoebic keratitis is a severe ocular infection resulting in permanent vision loss in more than 15% of patients. The disease is difficult to treat successfully due to the high resistance of the cyst stage to most antimicrobial agents. The wearing of contact lenses is a significant risk factor in up to 93% of cases (Radford *et al*, 1998). The estimated incidence of contact lenses related acanthamoebic keratitis was 1.36 per million in the United States and 3.06 per million in the Netherlands. A much higher incidence of amoebic keratitis (149 per million contact lenses wearers) is estimated in western Scotland. In England and Wales, the annual incidence of acanthamoebic keratitis had been estimated to be 1.13 to 1.26 per million adults but increased sharply to 17.53 to 21.14 per million contact lens wearers (Radford *et al*, 2002). In Thailand, acanthamoebic keratitis accounts for 5% of cases of microbial keratitis in contact lenses wearers (Preechawat *et al*, 2007).

Acanthamoebic keratitis can be clinically misdiagnosed as herpes simplex keratitis or fungal keratitis. The frequent occurrence of secondary bacterial infections may

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This study was presented as poster presentation at the XVII[th] International Congress for Tropical Medicine and Malaria, Jeju Island, Korea, October 2-3, 2008.

confound the diagnosis (Marciano-Cabral and Cabral, 2003). These may lead to delayed diagnosis and an ultimate worsening of the disease. The outcomes of therapy in Acanthamoebic keratitis are effected by the difficulty and delay in diagnosis and limited effective antiamoebic drugs. Corneal transplantation is often required in patients with extensive damage or failure to respond to medication; enucleation of the eye may be necessary in severe cases (Kosrirukvongs *et al*, 1999; Marciano-Cabral and Cabral, 2003). The use of contact lens solutions (CLSs) effective against *Acanthamoeba* with adequate disinfection times is a fundamental method in preventing corneal infection. Most previous studies demonstrated the efficacy of CLSs against *Acanthamoeba* strains in western countries (Niszl and Markus, 1998; Kilvington and Anger, 2001; Hiti *et al*, 2002; Beattie *et al*, 2003; Hughes *et al*, 2003; Borazjani and Kilvington, 2005; Hiti *et al*, 2006) In Thailand, the lack of data regarding amoebicidal activity of ophthalmic solutions against Thai clinical isolates of *Acanthamoeba* prompted us to evaluate the efficacy of commercially available CLSs. Information regarding the susceptibility of *Acanthamoeba* to commercial CLSs is essential for reducing the risk of infection in regional contact lens wearers.

MATERIALS AND METHODS

Acanthamoeba strains

Cysts of three strains of *Acanthamoeba* species [*Acanthamoeba castellanii* (Ac0296), *Acanthamoeba polyphaga* (Ac0496), and *Acanthamoeba mauritaniensis* (Ac0196)] were used in the study since these were previously isolated from Thai patients with ulcerative keratitis in 1996, then identified by Dr Govinda S Visvesvara of the Centers for Disease Control and Prevention, Atlanta, Georgia, USA (Kosrirukvongs *et al*, 1999). The organisms were maintained in non-nutrient agar plates covered with Page's amoebic saline (PAS) medium at 37ºC. The amoebic were transferred to and grown in an axexic medium comprised of proteose peptone glucose broth (PPG). The organisms were harvested as described previously (Beattie *et al*, 2003). Briefly, the trophozoites were subcultured in a tissue culture flask containing 30 ml of PPG and incubated at 32ºC for 4 days. Subsequently, the PPG was substituted with Page's amoebic saline (PAS). The amoebic cells were gently removed from the flask with a sterile cell scraper, and concentrated by centrifugation at 3,000 rpm for 10 minutes. The amoebic pellets were resuspended in PAS and transferred to non-nutrient agar plates with no bacteria. The plates were incubated for 14 days at 32ºC. Mature cysts were harvested and washed in PAS. The amoebae were counted with a hemocytometer counting chamber (HBG, Germany) and the concentration was adjusted to 10^6 cells/ml.

Contact lens solutions

Four commonly available commercial contact lens multi-purpose solutions (Complete Protec, ReNu MultiPlus, Solocare Aqua, and Opti-free Aldox) were selected for study. Their formulations and the manufacturers' minimum recommended disinfection times (MMRDTs) are shown in Table 1. The studied CLSs were assessed before their stated expiratory dates and were taken from their original wrappings.

Procedure

All experiments were conducted separately in triplicate against three species of *Acanthamoeba*; the control group consisted of 0.9% PAS, according to the method of Beattie *et al* (2003) with modifications. Of the calibrated *Acanthamoeba* cyst suspension, 0.1 ml aliquot was added to 9.9 ml of each CLS and a control in sterile test tubes to provide an initial cell density of 10^4 cells/ml. The

Table 1
Formulations and details of tested contact lens multi-purpose solutions and their manufacturers' minimum recommended disinfection times.

Contact lens solution	Active ingredients	Other agents	MMRDT	Manufacturer
Complete Protec, No-rub Formula	Polyhexamethylene biguanide 0.0001%	Phosphate buffer, potassium chloride, sodium chloride, edetate disodium, hydroxypropyl methylcellulose (HPMC), Poloxamer 237	6 hr	AMO Inc, Santa Ana, California, USA
ReNu MultiPlus, Triaction Formula	Polyaminopropyl biguanide 0.0001% (DYMED™)	Hydroxyalkylphosphonate (Hydranate), boric acid, edetate disodium, Poloxamine, sodium borate, sodium chloride	4 hr	Bausch & Lomb, Greenville, SC, USA
Solocare Aqua	Polyhexanide 0.0001%	Dexpanthenol and sorbitol (Hydrolock), sodium phosphate, tromethamine, polyxamer 407, disodium edetate	4 hr	CIBA Vision Canada Inc, Ontario, Canada
Opti-free Aldox	Myristamidopropyl dimethylamine 0.0005% (Aldox)	Sodium chloride, sorbitol, edetate disodium, boric acid, aminomethylpropanolol, citrate, Tetronic 1304 Poloxamine, Poluquad® (Polidronium chloride) 0.001%	6 hr	Alcon Laboratories, Inc, Fort Worth, Texas, USA

samples were homogenized by vortexing, then shaken with a rotary shaker at 80 rpm. At the exposure-time intervals (0, 2, 4, 6, 8, 10, and 24 hours), 1-ml aliquots were obtained from each CLS and the control solution and placed in 9 ml of Dey Engley (DE) broth (Difco, USA) for study. Subsequently 1:10 and 1:100 dilutions were prepared with PAS. Five aliquots each of 1, 0.1, and 0.01 ml were transferred onto non-nutrient agar plates overlaid with heat-killed *Escherichia coli*. One-millimeter aliquots of the homogenate were inoculated into the wells of flat-bottom 6-well tissue culture plates, and 0.1- and 0.01-ml aliquots were inoculated into the wells of 12-well plates. The plate cultures were sealed and incubated at 32ºC. The wells were observed microscopically for amoebic growth at 3 and 7 days of incubation.

Culture plates demonstrating *Acanthamoeba* growth were categorized as 1, and no growth was categorized as 0. The enumeration of ameoba cells was carried out using the most probable number (MPN) technique as described by Beattie *et al* (2003).

The MPN of *Acanthamoeba* per millimeter of the tested CLSs and control groups were determined. The CLS that achieved a 3-log reduction of amoeba during the MMRDT was considered as an effective disinfectant (Beattie *et al*, 2003). ANOVA with Dunnett Multiple Comparisons was used for

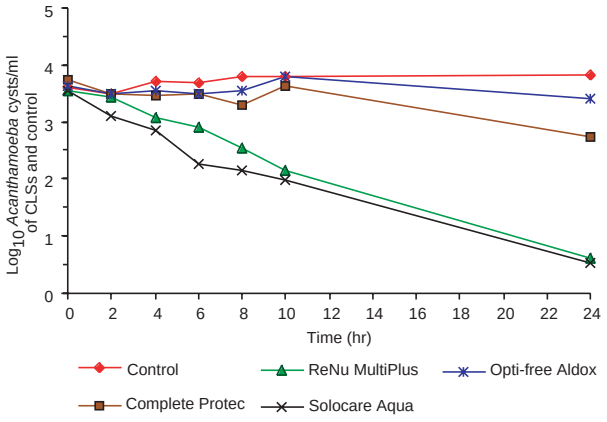

Fig 1–Average amoebicidal effects of CLSs against *Acanthamoeba castellanii* cysts (n = 3).

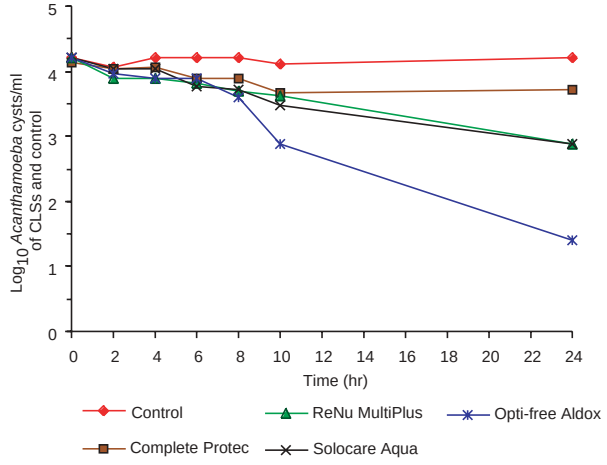

Fig 2–Average amoebicidal effects of CLSs against *Acanthamoeba polyphaga* cysts (n = 3).

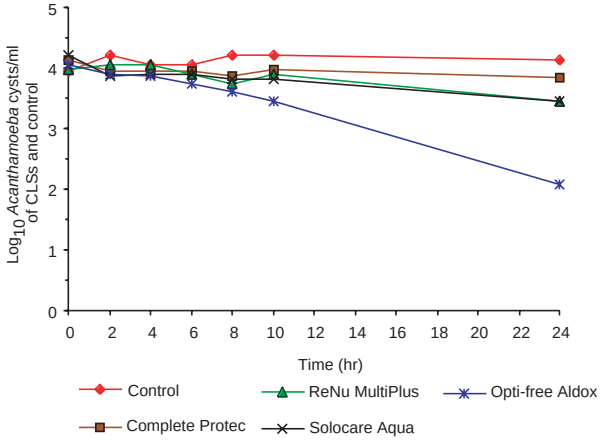

Fig 3–Average amoebicidal effects of CLSs against *Acanthamoeba mauritaniensis* cysts (n = 3).

statistical analysis of the data.

RESULTS

All CLS demonstrated some cysticidal activity against the three clinical isolates of *Acanthamoeba* after the MMRDT, with the exception of ReNu MultiPlus against *A. mauritaniensis* (-0.08-log reduction) (Table 2). Although, most CLS demonstrated decreasing numbers of amoeba with increasing exposure times (Figs 1-3), none of the CLS achieved a 3-log reduction against mature amoebic cysts after the MMRDT. Solocare Aqua gave the greatest reduction in *A. castellanii* (0.70-log reduction) and *A. mauritaniensis* (0.33-log reduction) after 4 hours. Opti-free Aldox had the greatest amoebicidal activity against *A. polyphaga* cysts (0.32-log reduction) after 6 hours.

DISCUSSION

Acanthamoeba cysts are markedly more resistant than trophozoites to CLS; immature cysts are more sensitive to CLS than mature cysts (Kilvington and Anger, 2001; Beattie *et al*, 2003). No minimum infective number of *Acanthamoeba* for infection has been documented; the presence of any *Acanthamoeba* within the contact lens case may cause amoebic keratitis (Beattie *et al*, 2003). The amoebicidal activity of CLS against mature *Acanthamoeba* cysts was the focus of our study. *A. castellanii* and *A. polyphaga* are the two most frequently diagnosed pathogens in cases of *Acanthamoeba* keratitis. The efficacy of CLS against these pathogens has been studied previously (Niszl and Markus, 1998; Kilvington and Anger, 2001; Beattie *et al*, 2003; Hughes *et al*, 2003; Borazjani and Kilvington, 2005). *A. mauritaniensis* is

Table 2
Efficacy of contact lens solutions against 3 strains of clinical isolates of *Acanthamoeba* cysts.

CLS	MMRDT	Log_{10} reduction over MMRDT			Log_{10} reduction over 24 hr		
		A. castellanii	*A. polyphaga*	*A. mauritaniensis*	*A. castellanii*	*A. polyphaga*	*A. mauritaniensis*
Control	6 hr	-0.08	0.00	-0.08	-0.22	0.00	-0.16
Complete Protec	6 hr	0.24	0.26	0.16	0.99[a]	0.41[a]	0.28
ReNu MultiPlus	4 hr	0.48	0.31	-0.08	2.94[a]	1.34[a]	0.50
Solocare Aqua	4 hr	0.70	0.18	0.33	3.02[a]	1.36[a]	0.76[a]
Opti-free Aldox	6 hr	0.14	0.32	0.31	0.22	2.80[a]	1.97[a]

CLS, Contact lens solution; MMRDT, Manufacturers' minimum recommended disinfection time
[a]Statistical difference

less frequently documented as a cause of disease (Krosrirukvongs *et al*, 1999; Ledee *et al*, 2003). Data concerning the susceptibility of *A. mauritaniensis* to antimicrobial agents are limited. A human corneal isolate of *A. mauritaniensis* was used to assess the acanthamoebicidal activity of povidone-iodine (Gatti *et al*, 1998). Thus, *A. castellanii*, *A. polyphaga* and *A. mauritaniensis* were selected for evaluation of CLS in our experiments.

In our study *A. castellanii* cysts were more sensitive to Solocare Aqua and ReNu MultiPlus, while *A. polyphaga* and *A. mauritaniensis* cysts were more sensitive to Opti-free Aldox. None of the CLS achieved a 3-log reduction in amoeba by the MMRDT. A similar study also found CLS (Complete, ReNu MultiPlus, Solo-care soft, and Optifree Express) were not amoebicidal against mature cysts of *A. castellanii* during the MMRDT (Beattie *et al*, 2003). In a prior study, Borazjani and Kilvington (2005) showed that ReNu with MoistureLoc successfully killed both *A. castellanii* and *A. polyphaga* cysts (>3-log reduction) within the MMRDT, but Complete MoisturePlus, Solo-care Plus, and Optifree Express did not. ReNu with MoistureLoc contains Alexidine 0.00045%, whereas ReNu MultiPlus contains polyaminopropyl biguanide 0.0001%. Although the relevant ingredients in CLS in our study were not exactly the same as those used in the previous studies, they contained the same active agents against *Acanthamoeba* in equal concentrations and demonstrated concordant results, except for ReNu with MoistureLoc and ReNu MultiPlus which contain different active agents. The concentration of active ingredients of the relevant CLS (except for ReNu with MoistureLoc) was lower than the effective concentration against mature cysts of *Acanthamoeba*. The cysticidal activity of contact lens solutions was directly proportional to the soaking time of the organisms in the solution. Hence, an appropriate concentration of active amoebicidal ingredients in CLS and an adequate exposure time are necessary for effective killing of *Acanthamoeba*. There was a recent recall of Complete MoisturePlus (not Complete Protec) and ReNu with MoistureLoc (not ReNu MultiPlus) from sale due to increased risk for acanthamoebic keratitis and *Fusarium* keratitis, respectively (US FDA 2006, 2007; Chang *et al*, 2006; Saw *et al*, 2007; Sansanayudh *et al*, 2008).

The MPN technique used in the experiments provided a rapid simple and reliable method for enumeration of *Acanthamoeba*. Compared with direct counting using a hemocytometer, the MPN technique is less laborious and time consuming. The MPN

technique is based on the presence or absence of growth on culture plates inoculated with serial dilutions of organisms. Theoretically, if at least one organism exists in that aliquot, visible growth should be seen. The estimation of the number of organisms relies on mathematical calculations. Computerized MPN tables provide better accuracy with enumeration (Beattie *et al*, 2003).

Acanthamoebic keratitis is one of the most devastating diseases associated with contact lens use (Marciano-Cabral and Cabral, 2003). Diagnosis and treatment of the disease is difficult because the clinical manifestations are similar to other kinds of microbial keratitis, unfamiliarity of clinicians due to the low prevalence of the disease, and the limitations of therapeutic alternatives. Good lens hygiene is extremely important for contact lens users to prevent ocular infections which can lead to serious microbial keratitis. Contact lens wearers should strictly follow the proper lens care guidelines and wearing instructions provided by their eye care professional. The efficacy of commonly available CLS in use should be communicated to users to help prevent *Acanthamoeba* infection of the cornea. The manufacturers' minimum recommended disinfection times for the products for killing *Acanthamoeba* must be reconsidered. CLS effective against *Acanthamoeba* should be investigated and adequate exposure times determined and communicated to contact lens users.

ACKNOWLEDGEMENTS

This study was supported by the Siriraj Routine to Research Management Fund, Faculty of Medicine Siriraj Hospital, Mahidol University.

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