Contact Lens and Anterior Eye xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Contact Lens and Anterior Eye

journal homepage: www.elsevier.com/locate/clae

Short communication

The use of dimethyl sulfoxide in contact lens disinfectants is a potential preventative strategy against contracting *Acanthamoeba* keratitis

Ruqaiyyah Siddiqui[a], Yousuf Aqeel[b], Naveed Ahmed Khan[a,*]

[a] Department of Biological Sciences, Faculty of Science and Technology, Sunway University, Malaysia
[b] Department of Molecular and Cell Biology, School of Medicine, Boston University, USA

ARTICLE INFO

Article history:
Received 15 January 2016
Received in revised form 11 April 2016
Accepted 17 April 2016

Keywords:
A. castellanii
Dimethyl sulfoxide (DMSO)
Encystation
Excystation

ABSTRACT

Acanthamoeba castellanii is the causative agent of blinding keratitis. Though reported in non-contact lens wearers, it is most frequently associated with improper use of contact lens. For contact lens wearers, amoebae attachment to the lens is a critical first step, followed by amoebae binding to the corneal epithelial cells during extended lens wear. *Acanthamoeba* attachment to surfaces (biological or inert) and migration is an active process and occurs during the trophozoite stage. Thus retaining amoebae in the cyst stage (dormant form) offers an added preventative measure in impeding parasite traversal from the contact lens onto the cornea. Here, we showed that as low as 3% DMSO, abolished *A. castellanii* excystation. Based on the findings, it is proposed that DMSO should be included in the contact lens disinfectants as an added preventative strategy against contracting *Acanthamoeba* keratitis.

1. Introduction

Acanthamoeba castellanii of the T4 genotype is an opportunistic protist pathogen that is widely distributed in the environment. The life cycle of *Acanthamoeba* consists of two stages: an actively feeding trophozoite stage during which it divides mitotically under favourable conditions but differentiates into a double-walled dormant cyst form under harsh conditions. *A. castellanii* is a well-known causative agent of a painful, sight-threatening keratitis and a fatal brain infection, called granulomatous amoebic encephalitis [1–4]. *Acanthamoeba* keratitis is frequently observed in contact lens (CL) wearers [1–4]. Poor hygiene in CL care, such as use of homemade saline for CL disinfection, is an important risk factor in contracting *Acanthamoeba* keratitis [5]. Amoebae transmigration from the environment onto the cornea involves amoebae attachment to CL, followed by their migration to the corneal epithelial cells during extended CL wear [1–4]. The extended wear allows parasite sufficient time to migrate onto the corneal surface. *Acanthamoeba* attachment to surfaces (biological or inert) and migration is an active process and transpires during the trophozoite stage of *Acanthamoeba*. Thus retaining amoebae in the cyst stage offers an important strategy in impeding parasite traversal, from the CL onto the cornea. Several studies have shown that the present marketed CL disinfectants target parasite viability with varied efficacy [1–6]. The agents that support trophozoite conversion into the cyst stage offer an important preventative strategy. Previous studies showed that exposure to dimethyl sulfoxide (DMSO) results in pseudocyst formation in *Acanthamoeba* [7]. In this study, we report that as low as 3% DMSO produced more than 90% encystation in *A. castellanii* and more importantly, 3% DMSO abolished *A. castellanii* excystation. Dimethyl sulfoxide is an aprotic solvent that can dissolve polar and non-polar solvents whereas it is also miscible in organic solvents and water [8]. Here, we describe the role of DMSO as an encystation trigger and its potential as an additive CL disinfectant.

2. Methods

2.1. A. castellanii *cultures*

All chemicals were purchased from Sigma (Poole, Dorset, England) unless otherwise stated. *A. castellanii* belonging to the T4 genotype (originally isolated from the keratitis patient) was purchased from American Type Culture Centre (ATCC 50492). Amoebae were grown routinely as described previously [9]. Briefly cultures were grown in T–75 cm^2 tissue culture flasks suspended in 10 mL PYG medium [proteose peptone 0.75% (w/v), yeast extract

* Corresponding address at: Department of Biological Sciences, Faculty of Science and Technology, Sunway University, Selangor, 47500, Malaysia.
E-mail address: naveed5438@gmail.com (N.A. Khan).

http://dx.doi.org/10.1016/j.clae.2016.04.004
1367-0484/

0.75% (w/v) and glucose 1.5% (w/v)] without shaking at 37 °C. The medium was refreshed 24 h prior to experiments. Amoebae adherent to flask represented the trophozoite form and were used in all subsequent assays.

2.2. Encystation assays

Encystation assays were performed as described previously [10] but with modifications. Briefly, 5×10^5 *A. castellanii* were inoculated in PYG (proteose peptone, yeast extract and glucose), HBMEC's and Huh-7 in the presence or absence of 3% DMSO in 24-well tissue culture plates at 37 °C. After 24 h, *A. castellanii* viability was determined using Trypan blue exclusion assay. Next, sodium dodecyl sulfate (SDS, 0.5% final concentration) was added and plates incubated at room temperature for 10 min to solubilize all *A. castellanii* trophozoites, HBMEC's and Huh-7 cells and cysts were enumerated using a haemocytometer (cysts are resistant to 0.5% SDS). The percentage encystation was determined as follows: No. of *A. castellanii* post-SDS treatment/total number of *A. castellanii* $\times 100$ = percent encystation. Data are presented as the mean ± standard error of at least three independent experiments performed in duplicate.

2.3. Excystation assays

Excystation assays were performed as described previously [11]. Briefly *A. castellanii* cysts were treated with 0.75% Triton-X 100 for 60 min to lyse remaining trophozoites. The cells were pelleted after centrifugation at 1500*g* for 10 min. After this, amoebae were washed twice with PBS to remove remaining traces of the detergent and 3×10^5 cysts were inoculated in PYG. The plate was incubated without shaking at 30 °C for 24, 48 and 72 h. Following this incubation, *A. castellanii* trophozoites were counted using a haemocytometer. Data are represented as the mean ± standard error of at least three independent experiments performed in duplicate.

2.4. Calcofluor white staining

A. castellanii cysts were prepared using 3% DMSO as described above. Additionally, *A. castellanii* cysts were prepared using non-nutrient agar plates (3% Oxoid Agar technical using distilled water) as previously described [10]. Briefly, *A. castellanii* trophozoites (10^6 amoebae) were inoculated on non-nutrient agar plates and incubated at 30 °C for up to 14 days to allow trophozoite differentiation into cysts. Following this incubation, no trophozoites were observed under a phase contrast inverted microscope. Next, 15 mL of PBS was poured on non-nutrient agar plates and placed on a shaker for 30 min at room temperature. After this incubation, cysts were gently scraped off the agar surface using a cell scraper. Cysts were collected in a 50 mL tube, followed by centrifugation at 1500*g* for 10 min. The supernatant was discarded and the pellet resuspended in 10 mL PBS. This process was repeated 3× to wash *A. castellanii* cysts and counted using a haemocytometer. Cysts from both assays were resuspended in 2.5% Calcofluor white stain and incubated at 30 °C for 2 h. Next, cysts were collected by centrifugation at 1500*g* and resuspended in PBS. This process was repeated twice to remove excess Calcofluor white stain, and cysts were resuspended in PBS. Finally, 30 μL suspensions was applied to glass slides and wet mounts of each slide were examined under Olympus, BX-41 fluorescent microscope (excitation 405 nm and emission band pass 420–480 nm). Previous studies have shown that Calcofluor white does not stain the trophozoite stage of amoebae [12], hence *A. castellanii* trophozoites were used as negative controls.

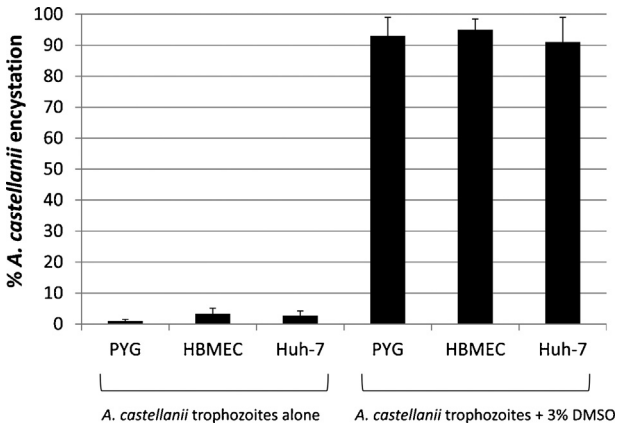

Fig. 1. DMSO induced *A. castellanii* encystation in growth medium. *A. castellanii* were inoculated in PYG in the presence or absence of 3% DMSO and incubated at 37 °C. After 24 h, sodium dodecyl sulfate (SDS, 0.5% final concentration) was added and plates incubated at room temperature for 10 min to solubilize *A. castellanii* trophozoites and amoebae cysts were counted using a haemocytometer. For cell culture experiments, *A. castellanii* were incubated with HBMEC and Huh-7 in the presence of 3% DMSO in 24 well-tissue culture plates at 37 °C in a 5% CO_2 incubator. After 24 h sodium dodecyl sulfate (SDS, 0.5% final concentration) was added and plates incubated at room temperature for 10 min to solubilize *A. castellanii* trophozoites, HBMEC's and Huh-7 cells and cysts were enumerated using a haemocytometer (cysts are resistant to 0.5% SDS). Data are represented as the mean ± standard error of at least three independent experiments performed in duplicate. Note that 3% DMSO induced more than 90% encystation even in the presence of PYG, HBMEC and Huh-7.

3. Results

3.1. DMSO-induced encystation in A. castellanii

Encystation assays were performed to determine whether DMSO induces encystation in *A. castellanii* even in the presence of growth triggers such as PYG, HBMEC and Huh-7. In the absence of 3% DMSO, amoebae remained in the trophozoites forms and the percent encystation was observed at 1% ± 0.5, 3.3%± 1.8 and 2.7% ± 1.5 in PYG, HBMEC and Huh-7 respectively (Fig. 1). In the presence of DMSO, the findings revealed that 3% DMSO induced amoebae encystation at 93% ± 6, 95% ± 3.5 and 91% ± 8 in PYG, HBMEC and Huh-7 respectively (Fig. 1). Conversely, the addition of 3% DMSO had no effect on the viability of *A. castellanii* as determined by Trypan blue dye exclusion test. To further confirm whether DMSO-induced cysts remain viable, cysts were washed 3× with PBS to remove any traces of DMSO and incubated with growth medium. When incubated with PYG, cysts re-emerged as viable trophozoites. The number of amoebae increased from to 3×10^5 to $5.7 \times 10^5 \pm 9.7 \times 10^4$, $7.92 \times 10^5 \pm 1.07 \times 10^5$, and $1.04 \times 10^6 \pm 1.145 \times 10^5$ after 24, 48 and 72 h, respectively.

3.2. Cysts prepared using DMSO or non-nutrient agar plates bind to calcofluor white stain

Calcofluor white is a fluorescent dye that specifically binds to β, 1–3 and β, 1–4 linked glycan polymers and has been used as a marker to differentiate *Acanthamoeba* cysts and trophozoites [12]. The findings revealed that Calcofluor white stained both DMSO-induced *A. castellanii* cysts as well as cysts prepared using non-nutrient agar (Fig. 2) exhibiting similar biochemical profiles. When visualized under fluorescent microscope, distinct layers were visible in cysts, prepared through both encystation methods.

Please cite this article in press as: R. Siddiqui, et al., The use of dimethyl sulfoxide in contact lens disinfectants is a potential preventative strategy against contracting *Acanthamoeba* keratitis, Contact Lens & Anterior Eye (2016), http://dx.doi.org/10.1016/j.clae.2016.04.004