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Research Article

The Threshold of Ultraviolet Light Energy to Eliminate Acanthamoeba

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Abstract

Purpose: *Acanthamoeba* keratitis is a contact lens-related infection that may result in significant visual impairment. Treatment of *Acanthamoeba* keratitis can be difficult due to a lack of good medical therapy. *Acanthamoeba* can survive in contact lens storage cases and on the surface of contact lenses for extended periods of time. The aim of this study was to determine the threshold of Ultraviolet energy (UVE) to eliminate *Acanthamoeba* from contact lens and cases. We hypothesize that UVE with and without riboflavin will eradicate *Acanthamoeba* while maintaining the clarity of the lens.

Methods: The Stratagene UV Stratalinker 2400 Crosslinker™ was used to administer UVE in pulses to eliminate *Acanthamoeba castellanii*. Multiple modes of energy delivery were administered in a dose-dependent manner in microjoules/cm^2 x 100. Three groups were tested: 1) *Acanthamoeba castellanii* (10^6 cysts in saline) exposed to UVE alone; 2) *Acanthamoeba castellanii* (10^6 cysts in saline) plus soft contact lens exposed to UVE alone; 3) *Acanthamoeba castellanii* (10^6 cysts) plus soft contact lens exposed to UVE in a saline solution of 0.01% Riboflavin. *Acanthamoeba castellanii* viability was monitored for growth using non-nutrient agar with *Enterobacter aerogenes* overlay. The soft contact lenses were for evaluated for clarity by spectrophotometry.

Results: A total UVE of 89,991 microjoules x 100/cm^2 for a total of 180 min (3 hours) were required to eliminate *Acanthamoeba castellanii* solutions. A total of 99990 microjoules x 100/cm^2 for a total of 200 minutes was required to eliminate *Acanthamoeba castellanii* from solutions with contact lenses with and without riboflavin. One large dose in this amount of UVE did not effect the clarity of soft contact lenses in the time-frame of this study.

Conclusion: A very large dose of UVE had the potential to eliminate *Acanthamoeba* from solutions. Even more energy is required to kill *Acanthamoeba* with contact lens in solution. A one time exposure of contact lens to 89,991 - 99990 microjoules x 100/cm^2 UVE may produce minimal damage to soft contact lens.

Keywords: *Acanthamoeba; Total Kill Assay; Riboflavin; UV Light Exposure*

Abbreviations

UVE: Ultraviolet Light Energy; ACA: *Acanthamoeba*

Introduction

Acanthamoeba keratitis can be an extended infection with significant morbidity [1]. Soft contact lens appears to be a key vector for *Acanthamoeba* keratitis. Even early manifestations of the disease are difficult to treat once contracted [2,3]. Though successful medical therapy hinges on early recognition and aggressive therapy, patients are left with visually significant corneal scarring with a poor visual prognosis [4]. An obvious approach to decrease the incidence of *Acanthamoeba* keratitis is to prevent access of *Acanthamoeba* in contact lens paraphernalia. *Acanthamoeba* exists in both a trophozoite and cyst form with cysts being more resistant to chemical therapy. Trophozoites infect the cornea and are more susceptible to medical therapy, thus it is important to kill cysts before they transform to trophozoites [5].

Multiple attempts have been made to prove eradication of *Acanthamoeba* from a contact lens using thermal, hydrogen peroxide, and quaternary ammonium disinfection. Whereas thermal methodologies were presumed successful, this treatment severely compromised the integrity and functionality of the lenses [3,6]. Although the PuriLens System™ has been identified as a current device used to eliminate many microbial infections from contact lenses, [7] this device has been proven to be unsuccessful at eliminating *Acanthamoeba* [8]. Finally, ultraviolet light energy (UVE) has been shown to reduce the number of cysts significantly, however total elimination of activity has not been demonstrated [8,9]. Multiple publications have reported the germicidial effects of UVE effects based on reports of lower numbers of *Acanthamoeba* colony counts and cyst production. No publications have reported the total amount of UVE to produce a total kill and absence of cyst activity [10].

Aim of the Study

The major goal of this work was to evaluate the role of UVE to completely kill *Acanthamoeba* cysts.

Materials and Methods

Study design

The aim of the study was to determine the total amount of UVE that can eliminate *Acanthamoeba* from soft contact case and lenses. The Stratagene UV Stratalinker 2400 Crosslinker™ (Stratagene; La Jolla, CA) was used to administer multiple modes of UVE as pulses in microjoules/cm^2 x 100 in a dose-dependent manner. Three groups were tested: 1) *Acanthamoeba castellanii* (10^6 cysts in saline) exposed to UVE alone; 2) *Acanthamoeba castellanii* (10^6 cysts in saline) plus soft contact lens exposed to UVE alone; 3) *Acanthamoeba castellanii* (10^6 cysts) plus soft contact lens exposed to UVE in a saline solution of 0.01% riboflavin. *Acanthamoeba castellanii* viability was monitored for growth using non-nutrient agar with *Enterobacter aerogenes* overlay. The soft contact lenses were evaluated for clarity by spectrophotometry (Thermo Scientific; Waltham, MA).

Experimental protocol

1. Each of the three groups was seeded with 10^6 cysts of *Acanthamoeba*. The *Acanthamoeba* cysts were propagated from seven day lawns of *Enterobacter aerogenes* inoculated with an ATTC isolate of *Acanthamoeba castellanii* (30010).
2. Groups were placed into the Statagene UV 2400 Crosslinker Machine™ (Stratagene, La Jolla, CA) (administers UV 254 nm Germicidal pulsed UV light, and a power level of 4000 μwatts/cm^2) with varying doses of energy administered from 0 - 9999 microjoules/cm^2 × 100 to determine the energy input required to kill the cysts. The inability of 9999 microjoules/cm^2 × 100 to kill with a single dose would require multiple exposures at this energy level to eventually kill the cysts. The instruction book of the Statagene UV 2400 Crosslinker Machine™ warns not to operate the Stratalinker UV crosslinker with the door open. This would emit dangerous UV energy. One should avoid looking directly at the UV bulbs while the Stratalinker UV crosslinker is in use. Serious eye injury could result from overexposure to ultraviolet light. (https://ipmb.sinica.edu.tw/microarray/index.files/Stratalinker%20UV%20Crosslinker.pdf (accessed 1/25/2021).

3. Group 3 was treated with riboflavin, 0.01 % in saline solution, 30 minutes before being treated with UVE.
4. After UVE, samples were plated and monitored for *Acanthamoeba* viability to evaluate the killing of *Acanthamoeba* cysts.
5. The soft contact lenses from groups 3 and 4 were evaluated for transmittance of visuable light using a spectrophotometer. All lenses from the experiment had the same dioptric power (plano). In previous studies, light wavelengths longer than 400nm approximated a 0.905 transmittance through soft contact lenses [11]. This standard was confirmed using a soft contact lens in blank solution as a reference in our experiments [11].
6. All experiments were performed in triplicate.

Results

A single dose of UVE from 1 to 9999 microjoules × 100 was unsuccessful in killing *Acanthamoeba* cysts in all 3 groups. Nine doses of 9999 microjoules × 100 was required to kill all *Acanthamoeba* cysts in the solutions (average 100%). This totaled 89,991 microjoules x 100/cm^2 for a total of 180 minutes (3 hours) (Figure 1). Ten doses of 9999 microjoules x 100 was required to kill *Acanthamoeba* in solutions with contact lenses with (77.3% ± 19.6) and without riboflavin (88.6% ± 19.5) (Figure 1). Finally, soft contact lenses exposed to UVE (9 - 10 doses) with and without riboflavin demonstrated no effect on contact lens clarity at the 69,993 - 99990 microjoules x 100/cm^2 (Table 1).

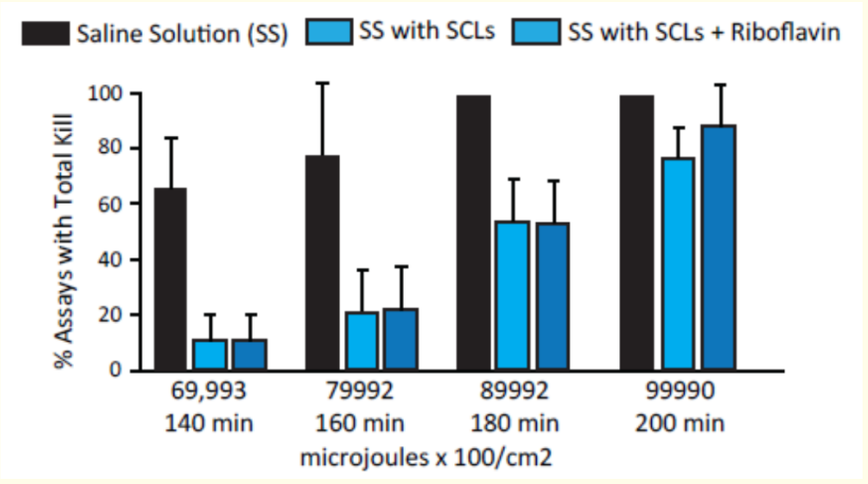

Figure 1: *The effect of ultraviolet light on ACA in saline solution (SS) [black], SS with soft contact lenses (SCLs), and SS with SCLs plus riboflavin.*

Experimental Condition	Exp 1 (λ 400 nm)	Exp 2 (λ 400 nm)	Exp 3 (λ 400 nm)
blank	0.362	0.362	0.342
Media	0.778	0.765	0.771
Media and SCLs	0.874	0.869	0.876
SCL pulsed 7x with UV light	0.894	0.901	0.888
Media with Riboflavin	0.465	0.453	0.465
SCL pulsed 8x with UV light and Riboflavin	0.842	0.853	0.875
SCL pulsed 9x with UV light and Riboflavin	0.851	0.849	0.852
SCL pulsed 10x with UV light and Riboflavin	0.851	0.857	0.863

Table 1*: Total transmittance in contact lenses exposed to riboflavin and UVC pulsed therapy at a UV wavelength of 400 nm.*

Discussion

ACA is a hardy parasite able to live for long periods of time in contact lens solution [12]. The focus of this study was to determine the total amount of UVE needed to eradicate ACA from solutions. In the literature, multiple modalities (thermal, chemical, and mechanical) of energy have been used to kill microbial infection [3,6,8]. The Purilens™, a system that delivers UVE, is well known for its ability to eliminate bacterial infections from contact lenses, however multiple studies have shown that it is ineffective at treating ACA [7,13].

This investigation indicates that UVE, in an experimental model, can produce a total kill of trophozoites and cysts using *Acanthamoeba castellanii*. The presence of contact lenses in solution required more energy to kill *Acanthamoeba*, however a total kill was obtained. The presence of riboflavin with contact lenses in solution did not effect the ability of the UVE to produce a total kill at a lower energy, but required more energy. Exposure to UVE with or without riboflavin did not effect contact lens clarity in our study. We clearly demonstrated that the total UVE required to completely kill *Acanthamoeba* in solutions is 89,991 microjoules x 100/cm^2.

Many publications have used thermal (such as heat), chemical (such as hydrogen peroxide and quaternary ammonium) and mechanical energy (sonication) to kill *Acanthamoeba*. Future directions would ideally involve the use of UVE combined with thermal, chemical, and mechanical energies to eliminate *Acanthamoeba*. Presumably, combining these modalities should allow less power to be used, for a shorter duration of time. Less energy could potentially minimize damaged to contact lenses with repeated doses. Although a one time dose of 69,993 - 89,991 microjoules x 100/cm^2 had no effect on the contact lens clarity, it is not clear how this amount of UV light administered multiple times (for a similar duration) would effect contact lenses over a longer period of time.

Finally, contact lenses in solutions required more energy to kill *Acanthamoeba* (99,990 microjoules x 100/cm^2) than the solutions alone. Contact lenses appear to confer some protection to *Acanthamoeba* in solutions. This observation supports the use of multiple modalities (thermal, chemical, and mechanical) to add to the efficacy of UVE.

Conclusion

A very large dose of UVE had the potential to eliminate *Acanthamoeba* from solutions. Even more energy is required to kill *Acanthamoeba* with contact lens in solution. A one time exposure of contact lens to 89,991 - 99990 microjoules x 100/cm^2 UVE may produce minimal damage to soft contact lens.

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Disclosures

The authors have no "Conflict of Interest" for the completion of this study.

Author Contributions

AGR: Study concept and design; data acquisition, analysis and interpretation; manuscript and figure preparation.

RPK: Study concept and design; data interpretation; funding support; manuscript and figure preparation.

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