Susceptibility of Acanthamoeba to Soft Contact Lens Disinfection Systems

Irene H. Ludwig,* David M. Meisler,† Isabel Rutherford,‡ Frank E. Bican,‡ Roger H. S. Langston,* and G. S. Visvesvara§

Members of the genus Acanthamoeba are increasingly recognized as agents of indolent, chronic, infectious keratitis. Recently, Acanthamoeba corneal infection has been reported in some persons who wear soft contact lenses. In this study, three "heat" and three "cold" soft contact lens disinfection systems were tested according to the manufacturers' instructions against Acanthamoeba castellanii and Acanthamoeba polyphaga in separate trials, and with appropriate controls. Suspensions of Acanthamoeba cysts or trophozoites of each species were tested individually. Each of the three heat disinfection units killed all Acanthamoeba in one cycle in all trials. A chlorhexidine 0.005%/thimerosal 0.001% solution killed A. castellanii trophozoites and cysts, but those of A. polyphaga survived. Trophozoites and cysts of both species survived an alkyl triethanol ammonium chloride 0.013%/thimerosal 0.002% solution and a hydrogen peroxide 3% preparation. Heat disinfection overall appears to be more effective in killing Acanthamoeba trophozoites and cysts as compared to cold disinfection methods. Invest Ophthalmol Vis Sci 27: 626–628, 1986

Acanthamoeba are small, free-living protozoans and are ubiquitous in nature.¹ The Acanthamoeba organism exists in two forms: the motile trophozoite and the double-walled cyst. In the encysted state they are protected from unfavorable environmental conditions, and are resistant to killing by freezing,² desiccation,³ and numerous antimicrobial agents.⁴

Members of the genus Acanthamoeba are increasingly recognized as agents of indolent, chronic, infectious keratitis. Recently, Acanthamoeba keratitis has occurred in persons who wear soft contact lenses.⁵⁻¹⁰ In this study, Acanthamoeba recovered from corneal infections in soft contact lens wearers were subjected to "heat" (thermal) and "cold" (chemical) soft contact lens disinfection systems to assess the efficacy of these systems against the organisms.

Materials and Methods. Organisms: The cultures tested were two clinical isolates, Acanthamoeba castellanii and Acanthamoeba polyphaga identified by one of us (G.S.V.) at the Centers for Disease Control. Stocks of Acanthamoeba cysts maintained at —70°C were slowly thawed and plated on nonnutrient agar with an Escherichia coli overlay for one to two days to obtain motile trophozoites. Axenically cultured organisms were prepared by transfer of the trophozoites to peptone-yeast extract-glucose (PYG) broth containing penicillin and streptomycin, and then subcultured to antibiotic-free PYG broth. Suspensions of Acanthamoeba trophozoites were obtained from one- to two-day subcultures at 35°C. Suspensions of Acanthamoeba cysts were obtained from 20- to 27-day subcultures at the same temperature. Suspensions of approximately 5 × 10³ trophozoites and suspensions of 5 × 10² cysts (determined by hemocytometer counts) of each species were prepared by centrifugation of the respective PYG broth suspension at 2500 RPM in an IEC Clinical Centrifuge for two minutes and decanting the PYG broth to leave a pellet of trophozoites or cysts.

Thermal disinfection: Three thermal disinfection units were tested; BoiniSoak** (Alcon Laboratories, Inc.; Fort Worth, TX), Mini-therm** (American Optical Corporation; Southbridge, MA), Allergan Heat Disinfection Unit** (Safeway Products Inc.; Middletown, CT). U.S. Food and Drug Administration guidelines require all thermal disinfection units to reach 80°C for at least 10 min. The pellet of trophozoites or cysts of each species was placed aseptically into the chamber of a clean contact lens case and suspended in 1.5 ml of standard preserved saline solution (sodium chloride 0.7%, sodium borate, boric acid, thimerosal 0.001%, EDTA disodium 0.1%). The suspensions were subjected to one complete thermal disinfection cycle. The cells were then washed three times, each washing consisting of centrifugation at 2500 RPM for 2 min, removal of the supernatant, and replacement with 10 ml of Page's saline. After washing, the cells were plated on nonnutrient agar with an E. coli overlay, incubated at 35°C, and inspected daily over a 14-day period for the presence of motile trophozoites, which represent organism viability. This procedure was carried out for each thermal disinfection unit. Suspensions of trophozoites and cysts of each Acanthamoeba species subjected to these same procedures, but omitting the heating cycle, served as controls.

Chemical disinfection: Three different chemical disinfection systems were tested according to the manufacturers' instructions. These included, with their respective disinfectant component, (1) Flex-care** (Alcon Laboratories, Inc.; Fort Worth, TX), chlorhexidine gluconate 0.005%/thimerosal 0.001%, (2) Allergan Soft Contact Lens disinfection solution** (Allergan Pharmaceuticals, Inc.; Irvine, CA), alkyl triethanol ammonium chloride 0.013%/thimerosal 0.002%, and (3) Lensept** (American Optical Corporation; Framingham, MA), hydrogen peroxide 3%. For both the

* Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Dept. of Health and Human Services.
chlorhexidine and alkyl triethanol ammonium chloride disinfection systems, trophozoites were subjected to one trial and cysts were subjected to two. The pellet of trophozoites or cysts of each species was suspended in 2 ml of the disinfection solution for 4 hr at room temperature. For the hydrogen peroxide system, a pellet of trophozoites or cysts of each species was suspended in 7.0 ml of the disinfection solution for 10 min at room temperature. Additionally, a pellet of trophozoites or cysts of each species was subjected to a 30-min exposure. After disinfection, the suspensions were centrifuged, and the organisms were washed, plated, and inspected daily as previously described. Pellets of trophozoites and cysts of each Acanthamoeba species, suspended in standard preserved saline instead of disinfection solutions and subjected to the same conditions, served as controls.

**Results.** Thermal disinfection: For each thermal disinfection unit tested, and for all trials of trophozoites and cysts of each species, no motile trophozoites were observed by day 14 of incubation (see Table 1). Motile trophozoites were observed in all control trials by day 1.

Chemical disinfection: In the chlorhexidine trials with A. castellanii, no motile trophozoites were observed by day 14. In one trial of the A. polyphaga trophozoites and cysts, motile trophozoites were observed by day 6. In a second trial of A. polyphaga cysts, motile trophozoites were observed by day 1. For the other two chemical disinfection systems, in all trials of trophozoites and cysts of each species, motile trophozoites were observed by day 1. Motile trophozoites were observed by day 1 in all controls.

**Discussion.** Infectious keratitis is a serious complication of soft contact lens wear. Most reported cases have been attributed to bacterial and, to a lesser extent, fungal organisms. Recently, however, protozoa of the genus Acanthamoeba have been demonstrated histologically and recovered in cultures from corneal infections in soft contact lens wearers. Although the direct cause and effect relationship between soft contact lens wear and this unusual infection is as yet unproved, several factors should be considered that support this association. Most cases of Acanthamoeba keratitis have been associated with antecedent trauma. It is possible that mechanical or hypoxic epithelial trauma and damage induced by soft contact lens wear may provide a portal or foothold for the Acanthamoeba organism. If the soft contact lens itself provides a vector for the introduction of the Acanthamoeba organism to the cornea, possible sources of contamination include contact lens cases and solutions. Bacteria and fungus have been recovered from soft contact lens and solutions of patients with corneal ulcers; however, confirmation of Acanthamoeba contamination has been elusive. This, in part, may be attributed to failure to identify or to culture the Acanthamoeba organism. Also, contact lens paraphernalia is often discarded during the delay in diagnosis of this unusual infectious keratitis. However, Acanthamoeba organisms were demonstrated by cytology to be present in a contact lens case of a patient with Acanthamoeba keratitis (personal communication: Cobo LM, Proia A, and Klintworth GK). Also, Acanthamoeba organisms were recovered by culture from a contact lens case of a patient with Acanthamoeba keratitis whom we treated (unpublished data) and from the contact lens solution of another patient with Acanthamoeba corneal infection.

In this study, soft contact lens disinfection systems were tested against two Acanthamoeba species recovered from corneal infections in soft contact lens wearers. Thermal disinfection killed all trophozoites and cysts of A. castellanii and A. polyphaga. The alkyl triethanol ammonium chloride/thimerosal and hydrogen peroxide chemical disinfection systems were ineffective against Acanthamoeba. The chlorhexidine/thimerosal solution killed all A. castellanii trophozoites and cysts,

### Table 1. Results of disinfection trials

<table>
<thead>
<tr>
<th>Disinfection system</th>
<th>A. castellanii</th>
<th>A. polyphaga</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trophs</td>
<td>Cysts</td>
<td>Trophs</td>
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<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>Trial 1</td>
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<tr>
<td>Each thermal disinfection unit</td>
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<td>§</td>
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<tr>
<td>Chlorhexidine (0.005%)</td>
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<tr>
<td>Alkyl triethanol ammonium chloride (0.013%)</td>
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<td>Hydrogen peroxide (3%)</td>
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<tr>
<td>(10 min.)</td>
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<tr>
<td>(30 min.)</td>
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* No motile trophozoites observed by 14 days.
† Motile trophozoites observed on first day of inspection.
§ Not done.

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but only delayed excystation of *A. polyphaga* organisms. The reason for the apparent greater effectiveness of this system over the other two chemical disinfection systems is not clear. It is also unclear whether different strains of *Acanthamoeba* species would be variably susceptible to these disinfection systems. However, results of this study indicate that heat disinfection was more effective overall in killing *Acanthamoeba* trophozoites and cysts as compared to cold disinfection systems.

**Key words:** *Acanthamoeba*, keratitis, soft contact lens, disinfection

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**References**


**Bioavailability and Corneal Anti-Inflammatory Effect of Topical Suprofen**

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The bioavailability in rabbit cornea and aqueous humor of an ophthalmic formulation of suprofen, a nonsteroidal anti-inflammatory drug, was evaluated following topical administration of a single dose to the eye. The drug penetrated rapidly into the uninflamed cornea with intact epithelium; highest levels occurred during the first 30 to 45 min after instillation and decreased thereafter. The bioavailability of suprofen in cornea and aqueous humor following administration of a 1.0% concentration was twice that produced by a 0.5% concentration of the drug. Topical application of multiple doses of suprofen failed to suppress polymorphonuclear leukocyte invasion of the cornea if treatment was started after the induction of inflammation. Suprofen therapy initiated prior to the induction of corneal inflammation and maintained into the post-inflammation period did produce a significant (*P* < 0.01) decrease in the numbers of PMNs that invaded the inflamed cornea. There was no significant difference (*P* > 0.05) in the corneal anti-inflammatory effect achieved by the 0.5% and 1.0% concentrations of suprofen when administered according to this regimen. Invest Ophthalmol Vis Sci 27:628–631, 1986

Locally administered corticosteroids effectively suppress corneal inflammation, but their use carries the risk of several ocular complications, including cataract, glaucoma, and the enhancement of actively replicating herpes simplex virus. This has prompted the search for other effective but potentially less toxic compounds. We have studied suprofen, a nonsteroidal anti-inflammatory agent, and report here our data on its bioavailability and anti-inflammatory effectiveness in the cornea following topical administration to the rabbit eye.

**Materials and Methods.** Bioavailability studies: Sodium thiamylal was administered intravenously to New Zealand albino rabbits (1.8–2.4 kg), producing light