OriginalArticle

Comparing Lethal Dose of Povidone-iodine and Virkon® to the *Acanthamoeba* cyst: *In vitro* Study

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ABSTRACT

Objective: To compare the least concentration of Povidone-iodine and Virkon®, phenolic-based compound with accelerated hydrogen peroxide and potassium peroxymonosulfate, which can cause a lethal effect to the cyst form of the *Acanthamoeba* spp.

Methods: *Acanthamoeba* spp. was isolated from the keratitis patient and was cultured using amonoxenic medium supplement with heat killed *Escherichia coli* and incubated for up to seven days for the production of mature cysts. *Acanthamoeba* cysts on the culture plates were mixed with several dilutions of Povidone-iodine to compare to several dilutions of Virkon®. After incubation for 1 hour, they were washed and centrifuged to remove the chemical supernatant. The pellets of the mature cyst were viewed by a light microscope for seven days, and further recultured on the monoxenic medium to confirm that the cysts were all died (cysticidal) for seven days.

Results: Povidone-iodine 0.04% and Virkon® 0.25% solutions were the least concentrations which could cause a lethal effect to the *Acanthamoeba* cyst.

Conclusion: Our results demonstrated that both the Povidone-iodine and Virkon® showed antiamoebic activity. The further study should be done to determine whether Povidone-iodine and Virkon® can be used as a disinfecting solution for contact len cases.

Keywords: *Acanthamoeba*, Povidone-iodine, Virkon®

Siriraj Med J 2008;60:187-189
E-journal: http://www.sirirajmedj.com

Infective keratitis is an ocular emergency that requires prompt and appropiate management to ensure the best visual outcome for the patients. Without adequate treatment, corneal infection leads to blindness through corneal scarring and endophthalmitis.[1] To minimize ocular morbidity, timely antimicrobial treatment must be initiated on the basis of clinical and microbiological evaluation.[2,3] A clinical diagnosis of infective keratitis does not give an unequivocal indication of the causative organisms because a wide range of organisms can produce a similar clinical picture.[4-6]

Small free-living amebae of the genus *Acanthamoeba* have been repeatedly recovered and isolated worldwide from a variety of environmental niches.[7] Along with two other species, *Naegleria fowleri* and *Balamuthia mandrillaris*, *Acanthamoeba* can cause severe infections in humans. Besides causing an opportunistic granulomatous encephalitis, sporadically in immunocompromised hosts *Acanthamoeba* spp., are recognized as a cause of severe keratitis, which may result in blindness if it is not diagnosed and correctly treated.[8] Rarely reported in studies for many years, *Acanthamoeba* keratitis has been increasingly identified in contact lens wearers and, to date, several hundreds of human cases have been reported worldwide.[9-10] The severity of the disease is due to its misdiagnosis as herpes simplex keratitis or to a scarcity of effective topical and systemic drugs. However, clearance of this amoebic ocular pathology has been reported when the disease is treated with systemic azolic compounds associated with topical drugs (neomycin plus polymyxin B plus bacitracin,

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TABLE 1. Virkon® at the concentration of ≥ 0.25% killed *Acanthamoeba* cyst within 1 hour.

Final concentration	1%	0.5%	0.25%	0.125%	0.0625%	0.03125%	0.0156%	Control
Well 1	-	-	-	+	+	+	+	+
Well 2	-	-	-	-	+	+	+	+
NNA	-	-	-	-	-	+	+	+

- = Negative growth of trophozoite
+ = Positive growth of trophozoite

NNA = Non-nutrient agar over layed with heat killed *E. coli*

propamidine isethionate, polyhexamethylene biguanide, chlorhexidine, hexamidine etcetera).[11] Nevertheless, the study of other chemotherapeutic agents with low corneal toxicities and high amebicidal activity is required. The aim of this study was the comparative evaluation of the *in vitro* efficacy of povidone-iodine (PVP-I or Betadine) and Virkon® on the cystic forms of *Acanthamoeba* spp. isolated from patients with amoebic keratitis.

MATERIALS AND METHODS

The cystic form of *Acanthamoeba* spp., was isolated from patient with amoebic keratitis which was further produced from cultures on 3% non-nutrient agar (NNA) plates seeded with heat killed *Escherichia coli* (NNA-*E. coli*) incubated at 30°C for 7 days. The cysts were harvested, washed in amoeba saline solution, and adjusted to a final concentration of 104 cysts/ml.

Pharmaceutical agents :

1. *PVP-I* (ASTA Medica Laboratories, Milan, Italy) is a solution of polyvinyl pyrrolidone and 10% iodine. PVP-I is an antiseptic agent that has proved to be effective against a wide spectrum of bacteria, yeasts and molds and some viruses. It was recently used as a prophylaxis against neonatal conjunctivitis in prospective trials in developing countries instead of treatment with silver nitrate or erythromycin because it is less toxic and less expensive.[12]

2. *Virkon®* (Antec International Limited England) has been successfully tested against numerous strains of virus, bacteria, yeast and mold. These include the use of phenol-based compounds, accelerated hydrogen peroxide and potassium peroxy-monosulfate. No organism tested to date has proved resistant to Virkon®.

In the cyst assay, the cystic forms adhere to the wells before and after drug exposure. The addition of a living *E. coli* suspension (McFarland standard No.1 = 300 x 10^6 bacteria/ml) to the wells of the microplate favors excystment and the multiplication of viable vegetative forms. Serial twofold dilutions of 50 µl of the drugs were made with amoeba saline solution in the well of a tissue – culture microtiter plate. Control wells of the two different tests recieved, respectively, 50 µl of amoeba saline solution. After one hour the wells were checked microscopically with the inverted microscope to detect viable cysts and photomicrographs were taken. The well solution was aspirated, after two washes with 150 µl of amoeba saline solution, and 50 µl of amoeba saline solution with living *E. coli* suspension was added to each well. The test was performed in triplicate. Two wells of each dilution were sealed, reincubated, and checked after 7 days for the growth of the trophozoites from the cysts. After gentle aspiration, the cysts were transferred from the third well of each dilution to NNA – *E. coli* plates and incubated at 30°C for 7 days in order to test the viability of the cysts and micrographs were again taken.

The minimum cysticidal concentration (MCC) was the lowest concentration that prevented the excystment after a week of incubation.

RESULTS

Effect of drugs on the *Acanthamoeba* cyst. The cysticidal action were found in both the PVP-I and Virkon®. A significant difference between the disinfecting solution efficacy on the cysts maintained in microtiter wells plate minimum cysticidal concentration (MCC) = 0.078%, and that on the NNA-*E. coli* subculture (MCC) = 0.04% was observed.

In the case of Virkon® as observed for the trophic growth, in the case of cystic forms, the MCCs for NNA plates were nonsignificantly lower than those in microwells. A 0.25% of Virkon® minimum concentration showed a cysticydal activity. A working concentra-

TABLE 2. Povidone iodine (PVI) at the concentration of ≥ 0.039% (0.04%) killed *Acanthamoeba* cyst within 1 hour.

Final concentration	5%	2.5%	1.25%	0.625%	0.3125%	0.15625%	0.078%	0.039%	0.0195%	0.0095%	0.0048%	0.0024%	Control
Well 1	-	-	-	-	-	-	-	-	-	+	+	+	+
Well 2	-	-	-	-	-	-	-	-	-	+	+	+	+
NNA	-	-	-	-	-	-	-	-	+	+	+	+	+

- = Negative growth of trophozoite
+ = Positive growth of trophozoite

NNA = Non-nutrient agar over layed with heat killed *E. coli*

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tion of Virkon® is 1% while cysticidal activity is 0.25%.

DISCUSSION

Acanthamoeba keratitis is a severe disease related to the use of soft contact lenses.[10,13] Over the years various therapeutic regimens have been proposed, but none has shown constant effectiveness in achieving clinical and parasitological cure. An important factor that might influence treatment is the identification and culture of the *Acanthamoeba* strains and a subsequent *in vitro* assay for known antiparasitic agents.[14] This experiment would aid the clinician in planning the therapeutic regimen in order to obtain the best possible outcome. The effectiveness of an antimicrobial agent on *Acanthamoeba* is of clinical value when it causes the complete destruction of the cystic form. It is impossible to evaluate the effects of pharmaceutical agents on cyst sensitivity by microscope observation alone.[15] Subcultivation of cysts treated with antimicrobial agents on NNA – *E. coli* plates is needed to determine the cidal or static effects of an antimicrobial agent.

Evaluation of the efficacy of two disinfectants, PVP-I and Virkon® was studied on various amoebic isolates from patients with proven cases of *Acanthamoeba* keratitis.

The results obtained showed that the concentrations of Virkon® needed to achieve complete destruction of the cystic stages was 0.25%, and PVP-I was 0.04%. The amoebicidal activity can also vary among species. In vitro study of the sensitivity assay is helpful for the beginning of therapy or at a later stage if resistance develops and change to another disinfectant is indicated. The PVP-I and Virkon® showed very good antiamoebic activity on the cystic stages of *Acanthamoeba*. However, these results need to be confirmed with other amoebic strains and species, in association with other disinfectants *in vitro*.

CONCLUSION

In conclusion, our study emphasizes the importance of cultivating *Acanthamoeba* strains and species causing keratitis *in vitro* and the importance of performing disinfectant sensitivity assay on the isolate at the beginning of therapy. At a later stage if resistance develops a change to another disinfectant is indicated. Our results demonstrated that PVP-I and Virkon® show very good antiamoebic activity on the cyst form of *Acanthamoeba in vitro*. This results need to be confirmed with other amoebic strains and species, in association with other disinfectants *in vitro*.

REFERENCES

1. Whitcher JP, Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. Bull World Health Organ 2001;79:214-21.
2. Jones DB. Dicision-making in the management of microbial keratitis. Ophthalmology 1981;88:814-20.
3. Allan BD, Dart JK. Strategies for the management of microbial keratitis. Br J Ophthalmol 1995;79:777-86.
4. Sridhar MS, Gopinathan U, Garg P, Rao GN. *Aspergillus fumigatus* keratitis with wreath pattern infiltrates. Cornea 2001;20:534-5.
5. Ostler HB. Disease of the external eye and adnexa. Baltimore: Williams & Wilkins, 1993: 173-91.
6. Florakis GJ, Moazami G, Schubert H, Koester CJ, Auran JD. Scanning slit confocal microscopy of fungal keratitis. Arch Ophthalmol 1997;115: 1461-3.
7. Ma P, Visvesvara GS, Martinez AJ, Theodore FH, Daggett PM, Sawyer TK. *Naegleria* and Acanthamoeba infections: review. Rev Infect Dis 1990;12:490-513.
8. Kilvington S, White DG. *Acanthamoeba* : biology, ecology and human disease. Rev Med Microbiol 1990;51:12-20.
9. Kilvington S, Larkin DFP, White DG, Beeching JR. Laboratory investigation of *Acanthamoeba* keratitis. J Clin Microbiol 1990;28:2722-5.
10. Martinez AJ, Visvesvara GS. Free-living amphizoic oppotunistic amebas. Brain Pathol 1997; 7:583-98.
11. Gatti S, Cevini C, Bruno A, Penso G, Rama P, Scazlia M. *In vitro* effectiveness of povidone-iodine on *Acanthamoeba* isolates from human cornea. Antimicrob Agents Chemother 1998; 42:2232-4.
12. Isenberg SJ, Apt L, Wood M. A control trial of povidone-iodine as prophylaxis against ophthalmia neonatorum. N Engl J Med 1995;332:562-6.
13. Visvesvara GS, Stehr-Green JK. Epidemiology of free-living ameba infection. J Protozool 1990;37:S25-S33.
14. Varga JH, Wolf TC, Jensen HG, Parmley VC, Rowsey JJ. Combine treatment of *Acanthamoeba* keratitis with propamidine, neomycin and polyhexamethylene biguanide. Am J Ophthalmol 1993;115:466-70.
15. Schuster FL. Comparative effects of selected azole compounds on trophic and cystic stages of *Acanthamoeba polyphaga*. J Eukargot Microbiol 1993;40:563-9.

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