In Vitro Effectiveness of Povidone-Iodine on *Acanthamoeba* Isolates from Human Cornea

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*Acanthamoeba* keratitis is a severe ocular infection secondary to accidental macro- or microscopic trauma of the cornea. Starting in 1985, a dramatic increase of this infection was recorded along with the spread of contact lens use. This protozoal disease is difficult to treat because of the scarcity of efficacious topical and systemic drugs. We evaluated the in vitro effectiveness of povidone-iodine (PVP-I [Betadine]), an agent with broad antibacterial and antiviral activity, compared to that of chlorhexidine (CXD), a cationic antiseptic, on *Acanthamoeba* isolates from patients with amebic keratitis. The results showed that PVP-I solution from 0.5 to 2.5% has a better antiamebic activity both on trophic and cystic stages of *Acanthamoeba* spp. than does CXD.

Small free-living amebae of the genus *Acanthamoeba* have been repeatedly recovered and isolated worldwide from a variety of environmental niches (12). 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 (9). 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 (13, 21).

The severity of the disease is due either to its misdiagnosis as herpes simplex keratitis or to a scarcity of effective topical and systemic drugs. However, clearance of this amebic ocular pathology has been reported when the disease is treated with systemic azolic compounds associated with topical drugs (neomycin plus polymyxin B plus bacitracin, propamidine isethionate, polyhexamethylene biguanide, chlorhexidine, hexamidine, etc.) (1, 3, 6, 11, 15). Nevertheless, the study of other chemotherapeutic agents with low corneal toxicity and high amebicidal activity is required. The aim of the present study was the comparative evaluation of the in vitro efficacy of povidone-iodine (PVP-I [Betadine]) and chlorhexidine (CXD) on trophic and cystic forms of *Acanthamoeba* spp. isolated from patients with amebic keratitis.

**MATERIALS AND METHODS**

**Amebic cultures.** (i) Trophozoites. Vegetative forms were from axenic cultures in 25-cm² Corning flasks with 10 ml of CGVS medium (22) and were kept at 37°C. Trophozoites in exponential growth (72 to 96 h) were concentrated by centrifugation at 500 × g for 10 min, counted in a hemacytometer, and adjusted to a final concentration of 10⁶ amebac/ml, and used immediately for testing.

(ii) Cysts. The cystic forms were obtained from cultures on 3% nonnutrient agar (NNA) plates seeded with *Escherichia coli* (optical density 5) incubated at 37°C for 7 to 12 days. The cysts were harvested, washed in phosphate buffered saline (PBS), and adjusted to a final concentration of 10⁶ cysts/ml.

**RESULTS**

Tables 1 and 2 report in vitro drug activities on the trophic and cystic forms of *Acanthamoeba* spp. The amebic strains...
were isolated from patients before treatment initiation, so the different sensitivities shown by the isolates were not related to previously acquired drug resistance.

Effects of drug on trophozoite growth. (i) PVP-I. The amebicidal activity on vegetative forms was more pronounced in the dilutions performed in distilled water than in those with CGVS axenic medium (Table 1). The TMACs of the reference strains, *A. mauritaniensis* VEPV-1 and *Acanthamoeba* sp. strain ANPV-1 isolates, were markedly lower (from 0.062 to 0.125%) than those observed for *A. mauritaniensis* VEPV-2 (from 2.5 to 5%) and *Acanthamoeba* sp. strain GEVP-1 (from 1 to 2.5%).

As for the dilutions of PVP-I in CGVS medium (Table 1), the TMACs of the various amebic strains were significantly higher than those of the distilled water dilutions, except for strain GEVP-1, for which antiamebic activity did not seem to be influenced by the medium used for testing.

(ii) CXD. The amebicidal activity of CXD on the trophozoites of the different tested strains was generally more homogeneous and did not seem to be related to the dilutions in distilled water or in CGVS medium (TMAC, 0.025 to 0.1%; Table 2).

Effects of drug on cystic forms. (i) PVP-I. The cysticidal action was observed at concentrations higher than those recorded for the trophozoites (Table 1). Also, the dilutions effective in CGVS medium were double those performed in water. A significant difference between the drug efficacy on the cysts maintained in microtiter wells (MCC, 0.25 to 1%) and that on NNA-*E. coli* subcultures (MCC, from 0.25 to 5%) was observed (Table 1).

PVP-I showed only a cystostatic effect on *Acanthamoeba* sp. strain GEVP-1, with growth of viable vegetative forms both in microtiter wells and on NNA-*E. coli* plates at the concentration of 10% (Table 1).

(ii) CXD. As observed for the trophic growth, in the case of cystic forms there were no significant differences for the medium used (water or CGVS), whereas MCCs for NNA plates were higher (0.1%) than those in microcysts (from 0.0125 to 0.1%). The only exception was *A. mauritaniensis* VEPV-2, on which a 0.1% CXD concentration showed only a cystostatic activity.

**DISCUSSION**

*Acanthamoeba* keratitis is a severe disease related to the use of soft contact lenses (13, 21). Over the years various therapeutic regimens have been proposed, but none has shown constant effectiveness in achieving a clinical and parasitological cure. An important factor that might influence treatment is the identification and culture of the *Acanthamoeba* strains and a subsequently in vitro assay for known antiparasitic agents (6, 20). This method would aid the clinician in planning the therapeutic regimen in order to obtain the best possible outcome (9). In our study we used a modified protocol first suggested by Kilvington et al. (8). In agreement with other authors (4, 14), we believe that the effectiveness of an antimicrobial agent on *Acanthamoeba* spp. is of clinical value when it causes the complete destruction of both trophozoite and cystic stages. It is impossible to evaluate the effects of pharmaceutical agents on cyst sensitivity by microscopic observation alone (16). 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 (17).

We evaluated the efficacy of two disinfectants, PVP-I and CXD, on various amebic isolates from patients with proven cases of *Acanthamoeba* keratitis.

A pharmacological test in distilled water was performed to partially simulate the environmental conditions which lead to the survival of these protozoa. In all cases, the trophic forms were more sensitive than the cysts, as previously reported (4, 16). Trophozoites of strains VEPV-1 and ANPV-1 were more sensitive to PVP-I (TMAC,

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<th>Amebic strain</th>
<th>PVP-I + H₂O</th>
<th>PVP-I + CGVS</th>
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<tr>
<td></td>
<td>TMAC (%)</td>
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<td>Microtiter plate</td>
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<td><em>A. polyphaga</em> (ATCC 30461)</td>
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<td><em>A. castellanii</em> (ATCC 50370)</td>
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<td>0.25–0.5</td>
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<tr>
<td><em>A. mauritaniensis</em> VEPV-2</td>
<td>2.5–5</td>
<td>0.25–0.5</td>
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<td><em>Acanthamoeba</em> sp. strain ANPV-1</td>
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<td>&lt;0.25</td>
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<tr>
<td><em>Acanthamoeba</em> sp. strain GEVP-1</td>
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<td>&gt;10</td>
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<tr>
<td></td>
<td>TMAC (%)</td>
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0.031 to 0.125%) than those of the other two isolates (TMAC, 2.5 to 5%). The cysticidal action of PVP-I was higher in dilutions with CGVS medium than in distilled water; however, cidal values were obtained both in subcultures in microtiter wells (MCC, 0.25 to 1%) and on NNA-cidal values were obtained both in subcultures in microtiter wells (MCC, 0.25 to 1%) and on NNA-
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PVP-I showed only a cystostatic effect on Acanthamoeba sp.
strain GEPV-I. Nevertheless, the concentrations of PVP-I for
three other Acanthamoeba spp. isolates were lower than those regarded as toxic for the corneal stroma (≥2.5%) (5, 7).

The amebicidal activity can vary among the species consid-
ered and also among amebic isolates belonging to the same species as reported by other authors (6, 16). This finding is
most likely related to different degrees of pathogenicity and virulence among species or strains. CXD showed a more
homogeneous activity against trophic forms of all the isolates, with TMAC values between 0.025 and 0.1%. Both PVP-I and
CXD showed only a static effect on the cystic stages of the four strains tested, with the presence of viable forms also at dilu-
tions higher than 0.1%.

The results obtained showed that the concentrations of CXD needed to achieve complete destruction of cystic stages were greater than those previously reported (6, 18, 19). Ho-
ever, it should be noted that CXD activity in these studies was
evaluated in association with other drugs or disinfectants.

In conclusion, our study emphasizes the importance of cul-
tivating Acanthamoeba strains and species causing keratitis in
vitro and the importance of performing a drug sensitivity assay on the isolate at the beginning of therapy or at a later stage if
resistance develops and change to another drug is indicated.

In particular, our results demonstrated that PVP-I shows
better antiamebic activity on both the trophic and the cystic stages of the protozoan than CXD. Although our results need
to be confirmed with other amebic strains and species, in
association with other drugs in vitro, and in experimental animal models, the topical use of PVP-I has been proven to be effec-
tive in the treatment of Acanthamoeba keratitis.

REFERENCES
18. Seal, D. V., J. Hay, and C. M. Kirkness. 1995. Chlorhexidine or polyhexa-
methylene biguanide for Acanthamoeba keratitis. Lancet 345:136–137.