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 hundred 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.

MATERIALS AND METHODS

Amebic isolates tested. The following amebic isolates were tested: Acanthamoeba polyphaga (reference strain ATCC 30461; from human corneal scraping), Acanthamoeba castellani (reference strain ATCC 50370; from human eye infection), Acanthamoeba maunatensis VEPV-1, A. maunatensis VEPV-2, Acanthamoeba sp. strain ANPV-1, and Acanthamoeba sp. strain GEPV-1.

Pharmaceutical agents. The sensitivities of the amebic isolates to the following two pharmaceutical agents were determined. PVP-I (ASTA Medica Laboratories, Milan, Italy) is a solution of polyvinylpyrrolidone 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 (5). 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 (7).

CXD (ICI Speciality Chemicals, Kontenberg, Belgium) is a cationic antiseptic that inhibits membrane function (10) and has been previously shown to have a good in vitro and, anecdotally also in humans, anti-Acanthamoeba activity (2, 6).

The drug dilutions were performed in either sterile distilled water or CGVS medium. Each dilution was tested in quadruplicate.

Experimental design. Experiments were performed according to the method of Kilvington et al. (8) with modifications. For the trophozoite assay, serial twofold dilutions of 50 µl of the drugs were made with either sterile distilled water or CGVS axenic medium in the wells of a Corning tissue-culture microtiter plate. Control wells of the two different tests received, respectively, 50 µl of distilled water or CGVS medium.

Fifty microliters of the calibrated trophozoite suspension was added to each well, and then the plates were sealed and incubated at 37°C. After 48 h, the plates were observed with a Leitz inverted microscope (>10 and ×25 objectives); then the well solutions were gently removed by aspiration and replaced with 100 µl of CGVS culture medium. The plate was resealed, reincubated at 37°C for 24 h, and examined once again.

The trophozoite minimum amebicidal concentration (TMAC) was defined as the lowest concentration of the drug that caused complete destruction of the vegetative forms in the wells.

In the cyst assay the cystic forms adhere to the wells before and after drug exposure. The addition of a living E. coli suspension (optical density = 0.2) to the wells of the microplate favors the encystment and the multiplication of viable vegetative forms. The microplates were prepared and incubated as for the trophozoite assay. After 48 h, the wells were checked microscopically with the inverted microscope to detect viable cysts or trophozoites. The well solution was aspirated and, after two washes with 200 µl of PBS, 100 µl of PBS with living E. coli suspension was added to each well. The test was performed in quadruplicate. Two wells of each dilution were sealed, reincubated, and checked after 7 days. After gentle aspiration, the cysts were transferred from the other two wells of each dilution to NNA-E. coli plates and incubated at 37°C for 7 days in order to test their viability.

The minimum cysticidal concentration (MCC) was the lowest concentration that prevented encystment and trophozoite replication after a week of incubation.

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 bicidal activity on vegetative forms was more pronounced in different sensitivities shown by the isolates were not related to were isolated from patients before treatment initiation, so the (from 2.5 to 5%) and *Acanthamoeba* sp. strain GEPV-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 GEPV-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 cidal 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 GEPV-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 microwells (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,
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- E. coli plates (MCC, 0.25 to 1%).

PVP-I showed only a cystostatic effect on Acanthamoeba sp. strain GEPV-I. Nevertheless, the concentrations of PVP-I for A. polyphaga and A. castellanii reference strains and for the 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 considered 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 dilutions 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). However, 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 cultivating 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 effective in the treatment of Acanthamoeba keratitis.

REFERENCES