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 antimycotic activity both on trophic and cystic stages of Acanthamoeba sp., 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 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, polyhexamethyleneguanidine, chlorhexidine, hexamidine, etc.) (1, 3, 6, 11, 15). Nevertheless, the study of other chemotherapeutic agents with low corneal toxicity and high activity (2, 6) has been repeatedly recovered and isolated worldwide from a variety of environmental niches (12).

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 mauritaniensis VEPV-1, A. mauritaniensis 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 Specialty Chemicals, Kortenberg, 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).

Determination of the minimum amebicidal concentration (MAC). 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 gently removed by aspiration and replaced with 100 μl of distilled water or CGVS medium. The drug dilutions were performed in sterile distilled water or CGVS medium.

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The minimum cysticidal concentration (MCC) was the lowest concentration of the drug that prevented excystment and trophozoite replication after a week of incubation. 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 excystment and the multiplication of viable vegetative forms. The microplates were prepared and incubated as for the trophozoite assay. After 48 h, the wells 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 reseeded, 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.

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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 distilled water. A significant difference between the drug efficacy on the reference 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 effect was observed at concentrations higher than those recorded for the trophozoites (Table 1). Also, the dilutions effective in CGVS medium were doubled those performed in distilled water. A significant difference between the drug efficacy on the cysts maintained in microtiter wells (MCC, 0.25 to 1%) and on NNA-E. coli plates is needed to determine the cidal or static effects 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, 0.1% to 0.25%)

### 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). 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).

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### TABLE 1. In vitro sensitivities of Acanthamoeba sp. trophozoites and cysts to PVP-I

<table>
<thead>
<tr>
<th>Amebic strain</th>
<th>PVP-I + H₂O</th>
<th>PVP-I + CGVS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMAC (%)</td>
<td>MCC (%)</td>
</tr>
<tr>
<td></td>
<td>Microtiter plate</td>
<td>NNA</td>
</tr>
<tr>
<td>A. polyphaga (ATCC 30461)</td>
<td>0.125–0.25</td>
<td>0.25–0.5</td>
</tr>
<tr>
<td>A. castellani (ATCC 50370)</td>
<td>0.025–0.025</td>
<td>0.05–0.1</td>
</tr>
<tr>
<td>A. mauritaniensis VEPV-1</td>
<td>0.025–0.025</td>
<td>0.05–0.1</td>
</tr>
<tr>
<td>A. mauritaniensis VEPV-2</td>
<td>0.025–0.025</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Acanthamoeba sp. strain ANPV-1</td>
<td>0.025–0.025</td>
<td>0.025–0.025</td>
</tr>
<tr>
<td>Acanthamoeba sp. strain GEPV-1</td>
<td>0.025–0.025</td>
<td>0.0125–0.025</td>
</tr>
</tbody>
</table>

### TABLE 2. In vitro sensitivities of Acanthamoeba sp. trophozoites and cysts to CXD

<table>
<thead>
<tr>
<th>Amebic strain</th>
<th>CXD + H₂O</th>
<th>CXD + CGVS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMAC (%)</td>
<td>MCC (%)</td>
</tr>
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<td></td>
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<td>0.1</td>
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<td>A. mauritaniensis VEPV-1</td>
<td>0.025–0.025</td>
<td>0.05–0.1</td>
</tr>
<tr>
<td>A. mauritaniensis VEPV-2</td>
<td>0.05–0.1</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Acanthamoeba sp. strain ANPV-1</td>
<td>0.025–0.025</td>
<td>0.025–0.025</td>
</tr>
<tr>
<td>Acanthamoeba sp. strain GEPV-1</td>
<td>0.025–0.025</td>
<td>0.0125–0.025</td>
</tr>
</tbody>
</table>
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-Ncidal values were obtained both in subcultures in microtiter wells (MCC, 0.25 to 1%) and on NNA-Ncidal values were obtained both in subcultures in microtiter wells (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