Effect of caspofungin on trophozoites and cysts of three species of *Acanthamoeba*

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Objectives: Amoebic keratitis is difficult to treat, without total efficacy in some patients because of cysts that are less susceptible than trophozoites to the usual treatments. We investigated here the *in vitro* effectiveness of caspofungin, a new antifungal, against three species of *Acanthamoeba*.

Methods: Trophozoites and cysts of *Acanthamoeba castellanii, Acanthamoeba culbertsoni* and *Acanthamoeba polyphaga* were incubated with caspofungin at concentrations varying from 16 to 500 mg/L.

Results: The trophozoites of the three tested species were susceptible *in vitro* to caspofungin at a concentration of 250 mg/L (206 μM). Furthermore, this drug was cysticidal at a concentration of 500 mg/L (412 μM) against *A. castellanii* and *A. culbertsoni*.

Conclusions: Caspofungin could represent, if *in vivo* studies confirm its efficacy, a new anti-*Acanthamoeba* compound.

Keywords: Caspofungin, *Acanthamoeba castellanii*, *Acanthamoeba culbertsoni*, *Acanthamoeba polyphaga*, keratitis

Introduction

*Acanthamoeba* spp. are causative agents of granulomatous amoebic encephalitis, and more frequently of keratitis in the case of minor corneal surface injury caused by contact lenses or other agents.1 At least eight species of *Acanthamoeba* have been implicated in corneal infections: *Acanthamoeba castellanii, Acanthamoeba culbertsoni, Acanthamoeba polyphaga, Acanthamoeba hatchetti, Acanthamoeba rhyzodes, Acanthamoeba lundunensis, Acanthamoeba quina* and *Acanthamoeba griffini.*2

The effective therapy for pathologies attributable to these free-living amoebae has to be improved and new drugs are needed, particularly when a resistance to classical therapeutics is encountered. Some azoles such as miconazole, ketoconazole, fluconazole and itraconazole and other antifungals such as amphotericin B or 5-flucytosine have already been used in antimicrobial combinations in different clinical situations.3–6 We describe in this study the effect of caspofungin, the first licensed compound of a new class of agents belonging to the echinocandins, on trophozoites and cysts of three *Acanthamoeba* species: *A. castellanii, A. polyphaga* and *A. culbertsoni*.

Materials and methods

*A. castellanii* (ATCC 30234), *A. polyphaga* (ATCC 30461) and *A. culbertsoni* (ATCC 30171) were used in this study. The trophozoites of *Acanthamoeba* were first grown in 150 cm2 tissue culture flasks in PYG medium at 30°C.7 For experiments carried out in 96-well microtitre plates, amoebae were used at a final cell concentration of 105/mL in PYG medium. An aliquot (100 μL) of this suspension was transferred into wells with caspofungin diluted in sterile normal saline (0.9% NaCl) from 16 to 500 mg/L (Cancidas®; obtained from MSD, Paris, France).8 Caspofungin is an echinocandin, a synthetically modified lipopeptide with a molecular weight of 1213.42 g/mol, which inhibits the synthesis of β-1-glucan in fungal cell walls.9 Trophozoites were incubated in PYG without caspofungin to serve as a control. The number of viable amoebae was determined by examination of the wells with an inverted microscope after 1, 4 and 24 h of incubation with the antifungal. The evaluation of cell death was determined with Toluidine Blue staining of amoebae.

The cysticidal properties of the same concentrations of caspofungin were also investigated on the three species of *Acanthamoeba*. Cysts were first prepared from the trophozoite cultures by using a chemically defined encystment medium.7 Mature cysts were stored...
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at 4°C for 1 week before use. The cysts were then suspended in the same encystment medium at a final concentration of 10^7/mL and antifungal at the defined concentrations was added. The number of viable cysts was estimated after 12, 24 and 48 h of incubation with the antifungal. For that, the content of each tested well was washed two times then resuspended in PYG medium where the viable microorganisms excysted. The subsequent growth and replication of the trophozoites were observed microscopically after 5 days using Toluidine Blue staining. These cytotoxicity assays of caspofungin on the amoebal forms (trophozoites and cysts) were performed in two independent experiments, each time in triplicate.

Results

The effect of caspofungin on the trophozoites of *Acanthamoeba* was dependent on the tested concentrations (Figure 1). The concentrations of 250 and 500 mg/L totally inhibited the growth, whereas lower concentrations had limited activity on the viability of trophozoites. After 24 h of exposure at a concentration of 16 mg/L, 12% of the trophozoites of *A. castellanii* and 21% of the trophozoites of *A. culbertsoni* were affected versus 71% of the trophozoites of *A. polyphaga*.

Caspofungin was also tested for its ability to kill the encysted stages of the three *Acanthamoeba* strains. For each strain, the cysts were less susceptible than the trophozoites. A 100% eradication of the cysts was achieved for *A. castellanii* and *A. polyphaga* with a concentration of 500 mg/L. This concentration did not prevent the excystation of a low percentage of the *A. culbertsoni* treated cysts. In addition, the percentage of viable forms increased when the concentration of caspofungin decreased (Figure 2).

Discussion

The association between keratitis produced by *Acanthamoeba* and the use of contact lenses is now firmly established. Although this infective keratitis is a relatively rare corneal disease, the emergence of drug-resistant strains and the recurrence of dormant infectious forms underscore the need for more effective treatments. A number of drugs (cationic antiseptics, azole antifungals, and antibiotics such as macrolides) have been tested *in vitro* and *in vivo* with varying degrees of efficacy, leading to some recovery, but also to some failures. Present therapeutic regimens rely on topical applications of antimicrobials, including a combination of propamidine isothiocyanate and neomycin or chlorhexidine. These treatments are poorly effective against the cystic stages of the parasite. The encysted stage of *Acanthamoeba* species appears to cause most of the problems, so an important consideration is to use drugs that are not only amoebicidal, but also cysticidal. Our study indicates that caspofungin has *in vitro* activity against trophozoite and cyst stages of the tested *Acanthamoeba* species. Although the cysts were less susceptible than the trophozoites, a 100% eradication of the cysts was achieved for two of the *Acanthamoeba* strains at 500 mg/L (412 μM). Furthermore, in a recent study, no drug-related adverse effects were noticed when caspofungin was used in the topical administration of the drug.
treatment of fungal keratitis in a rabbit model, even at a concentration of 5 mg/mL (4.12 mM). According to our results, this new antimicrobial agent could be promising to treat, alone or in combination (with another anti-amoebic drug such as an antibiotic or an antiseptic), Acanthamoeba keratitis. Further studies have to be conducted in an animal model to verify the absence of toxicity of caspofungin at these high concentrations and to confirm the efficacy of this compound on Acanthamoeba keratitis in vivo.

Transparency declarations

None to declare.

References