Activity of the amidoamine myristamidopropyl dimethylamine against keratitis pathogens

Reanne Hughes¹, John Dart² and Simon Kilvington¹*

¹Department of Microbiology & Immunology, University of Leicester, Medical Sciences Building, P.O. Box 138, University Road, Leicester LE1 9HN; ²Department of Ophthalmology, Moorfields Eye Hospital, City Road, London EC1V 2PD, UK

Received 14 January 2003; returned 14 March 2003; revised 18 March 2003; accepted 18 March 2003

Objectives: Microbial keratitis accounts for up to 30% of blindness in some less developed societies. The development of a single broad-spectrum topical antimicrobial effective against bacteria, fungi and Acanthamoeba would have a major impact on reducing the morbidity and simplifying the treatment of microbial keratitis. To this end, the activity of the amidoamine myristamidopropyl dimethylamine (MAPD) was investigated against common causes of microbial keratitis.

Methods: Challenge test assays were used to study the efficacy of 50 mg/L MAPD against Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Fusarium solani and Acanthamoeba polyphaga.

Results: MAPD gave a 3.7 log kill of P. aeruginosa after 60 min, 5.4 log for S. aureus by 45 min and 5 log for C. albicans and F. solani within 15 min. A. polyphaga cysts were reduced by 4 log within 120 min.

Conclusions: The findings of this study confirm that MAPD is an effective Acanthamoeba cysticidal agent and extend the observation to demonstrate that it also possesses excellent antifungal and antibacterial activity. MAPD may represent a broad-spectrum therapeutic antimicrobial for keratitis and surgical prophylaxis and deserves further evaluation in these roles.

Keywords: Acanthamoeba, keratitis, amidoamine, antibacterial, antifungal

Introduction

Microbial keratitis is a serious condition, which is responsible for up to 30% of the prevalence of blindness in some less developed societies.¹ In tropical and subtropical climates, bacteria account for 50–60% and fungi for 35–50% of cases,¹² whereas in temperate climates, bacteria cause 90% of cases and fungi around 5%.¹ Most remaining cases are caused by the free-living amoeba Acanthamoeba.² Microbial keratitis is rare in the absence of predisposing factors, predominantly trauma, contact lens wear and ocular surface disease (i.e. herpetic keratitis, bullous keratopathy, dry eye). In developed societies, contact lens wear accounts for over half the cases with the remainder due to surface disease and, less commonly, trauma,³ whereas in tropical and subtropical communities, trauma in labourers accounts for half the cases of both bacterial as well as fungal keratitis.³⁵

Severe visual impairment following bacterial keratitis can usually be avoided by the early diagnosis and prompt therapy that is available in communities with high standards of health care. This is the situation in most temperate climates where blindness due to bacterial keratitis is now rare. However, where fungal keratitis is common, the management of corneal infection presents a much more difficult problem.²⁴⁵ In a recent series of keratitis cases, 63/391 (16%) required corneal graft surgery and 46 (12%) removal of the eye to treat the infection.⁴
Acanthamoeba keratitis, although less common than fungal keratitis, is similarly difficult to manage with a high morbidity.3 This difference in the outcome of therapy for bacterial keratitis, compared with fungal and amoebic keratitis, is multifactorial. However, this includes the difficulty and delay in diagnosis for the latter infections, and the lack of broad-spectrum agents effective against bacterial as well as fungal and amoebic infection. This is combined with the limited availability of effective antifungal and antiamoebic drugs compared with antibacterial drugs, the poor relationship of in vitro drug susceptibilities with the in vivo response in fungal and amoebic keratitis compared with bacterial keratitis, and the more complex host–organism interactions in amoebic and fungal disease.

The availability of a broad-spectrum agent effective against the three major causes of keratitis, bacteria, fungi and amoeba, would bring substantial benefits to the treatment of this condition. We have previously reported the efficacy of the amidoxime myristamidopropyl dimethylamine (MAPD) against the cysts of Acanthamoeba.6 MAPD is a cationic amidoamine known also as stearamidopropyl dimethylamine, N-[3-(dimethylamino) propyl] octadecanamide or N,N-dimethyl-N′-tetradecanoyl-1,3-propylenediamine. It is present at 5 mg/L in the contact lens disinfecting system Opti-Free Express Multi-Purpose Disinfecting Solution (Alcon Laboratories, Inc., Fort Worth, TX, USA). The reported antimicrobial efficacy of the disinfection system was the rationale behind the selection of MAPD in this study for detailed investigation against fungal and bacterial causes of keratitis.

Materials and methods

Test solutions

MAPD was obtained from Alcon Laboratories, Inc. (Fort Worth, TX, USA). The powder was dissolved and diluted in 2 mM Tris–HCl, pH 7.2 for testing at 50 mg/L. Assays were carried out in 20 mL glass universal bottles with 0.1% Tween 80 used as neutralizer to the MAPD. In control experiments, one quarter strength Ringer’s solution was used (Oxoid, Basingstoke, UK).

Test organisms

Bacteria and fungi. Pseudomonas aeruginosa (ATCC 9027), Staphylococcus aureus (ATCC 6538), Candida albicans (ATCC 10231) and Fusarium solani (ATCC 36031) were chosen as representative of known ocular pathogens and were obtained from the ATCC, Rockville, MD, USA. All bacteria were cultured on tryptone soy agar (Oxoid CM129) and C. albicans on Sabouraud dextrose agar (Oxoid CM41) as described previously for disinfectant efficacy testing with these organisms.7 F. solani was cultured on potato dextrose agar (Oxoid CM139) in the dark at 25°C for 4 days, then exposed to daylight for a further 10–14 days to allow conidia formation. The cells were scraped off the plates into Dulbecco’s phosphate buffered saline with 0.05% (w/v) Tween 80. Cells were then vortexed for 10 s in the presence of a few 2 mm glass beads before being filtered through sterile glass wool to separate conidia from the hyphae. The conidia were pelleted at 2000g for 10 min and re-suspended in one quarter strength Ringer’s solution to give a final concentration of $1 \times 10^5$–$1 \times 10^9$/mL and stored at 4°C for testing within 7 days.

Acanthamoeba. The Acanthamoeba polyphaga strain was isolated from a keratitis case at Moorfields Eye Hospital, London in 1991. Trophozoites were maintained in a semi-defined axenic broth medium and cysts prepared from late log phase cultures using Neff’s chemically defined encystment medium as described previously.8 Mature cysts were sonicated for 5 s at 50% amplitude three times to break clumps before being adjusted to $5 \times 10^6$ cysts/mL and stored at 4°C for use within 14 days.

Assay methods

Bacteria and fungi. The bacterial and fungal strains were tested according to the internationally recognized, standard method used for the evaluation of contact lens solutions against ocular pathogens.7 Viable bacterial and fungal numbers were determined by making serial 10-fold dilutions of organism in triplicate across the rows of a microtitre plate. Fifty microlitres of each dilution was then inoculated as three drops, in triplicate, on appropriate agar culture media. After allowing the liquid to absorb, the plates were incubated in air at 32°C for 48 h.

Acanthamoeba. The method used to determine Acanthamoeba cyst killing was as described previously.6,8 Briefly, $1 \times 10^6$ cysts were inoculated into 10 mL of test solution. At 15 min intervals over 2 h, the solution was vortexed and, in quadruplet, 20 µL removed and added to 180 µL of 0.1 Tween 80 in a 96-well flat bottomed microtitre plate (Triple Red Laboratory Technology, Oxfordshire, UK) and left for 5 min to neutralize. Serial dilutions of 20 µL in 180 µL of one quarter strength Ringer’s solution (Oxoid) were then made across the rows of a microtitre plate in quadruplet. Then 25 µL of Escherichia coli (JM101: OD$_{600}$ 0.4) was added to each well and the plate sealed and incubated at 32°C for up to 7 days. The plates were inspected daily for 7 days by light microscopy with a ×10 lens objective for the presence of amoebal growth (encystment and trophozoite replication) in the wells.

The number of surviving Acanthamoeba in the microtitre plates at each time point was determined using Reed and Muench computation as previously described for Acantha-
**Activity of myristamidopropyl dimethylamine against keratitis pathogens**

moeba cyst viability. The reduction in viable organisms was plotted as change in log viability for each time point compared with viability at time zero.

**Results and discussion**

The activity of MAPD against the test organisms is shown in Figure 1. MAPD at 50 mg/L gave a 3.7 log kill of *P. aeruginosa* after 60 min or 4.4 log after 120 min. With *S. aureus*, a 5.4 log reduction occurred by 45 min. For *C. albicans* and *F. solani*, a 5.1–5.45 log kill resulted by the first time point of 15 min. *A. polyphaga* cysts showed 1.54 log kill at 60 min and 4.18 by 120 min. No decline in viability was found in the control experiments using one quarter strength Ringer’s solution (results not shown). When testing the antimicrobial efficacy of MAPD, care should be taken in the choice of materials as the compound has been shown to bind to certain plasticware resulting in reduced activity; therefore, glassware was used in this study.

Empirical therapy for keratitis is now widely used, where bacterial keratitis predominates, because of the effectiveness of broad-spectrum topical antibacterials. However, in tropical and subtropical environments, where both fungal and bacterial keratitis are equally common and facilities for diagnosis are often limited, there is no effective broad-spectrum empirical therapy that can be used for both classes of pathogen. In this situation, the choice of antibacterial, antifungal (or antiamoebic) therapy often has to be made on the basis of imprecise clinical criteria with a correspondingly high failure rate. Other disinfectants are being evaluated as therapeutic agents in keratitis, as well as for surgical prophylaxis. Provodine-iodine has a similarly broad range of antimicrobial activity and is in widespread use as an antiseptic in the preparation of the eye before cataract surgery but has been disappointing as topical therapy for fungal keratitis. However chlorhexidine shows promise as an antifungal agent in addition to its use in *Acanthamoeba* keratitis.

Here, we have confirmed the efficacy of the compound against *Acanthamoeba* cysts and extended the observation to include fungi and bacteria associated with keratitis. The antimicrobial mode of action of MAPD is not known, but probably results in cytoplasmic membrane damage leading to an irreversible loss of essential cellular components following binding to the cell wall, as has been described in bacteria for the disinfectants polyhexamethylene biguanide (PHMB) and chlorhexidine.

The development of a single broad-spectrum topical antimicrobial effective against bacteria, fungi and *Acanthamoeba* would be expected to have a major impact on reducing the morbidity and simplifying the treatment of microbial keratitis. MAPD is effective in vitro against all three major classes of corneal pathogen and the alternative chemical structure and low molecular weight of MAPD (mol. wt 300) compared with PHMB (average mol. wt 2340) and chlorhexidine digluconate (mol. wt 898) might permit better penetration into the cornea to achieve adequate therapeutic levels. MAPD, along with other amidoamines currently being studied, is a potential candidate for investigation as a broad-spectrum therapeutic antimicrobial for keratitis and for surgical prophylaxis and deserves further evaluation in these roles.

**Acknowledgements**

This study was supported by research grants from Alcon Laboratories, Inc., Fort Worth, TX, USA and the British Society for Antimicrobial Chemotherapy (GA324). Part of these findings were presented at the 102nd General Meeting of the American Society for Microbiology, Salt Lake City, UT, 2002 (poster A-149).

**References**

and laboratory results of fungal keratitis: a 10 year review at a referral eye care center in South India. *Cornea* 21, 555–9.


