In vitro acanthamoebicidal activity of a killer monoclonal antibody and a synthetic peptide

Pier Luigi Fiori1*, Antonella Mattana2, Daniele Dessì1, Stefania Conti3, Walter Magliani3 and Luciano Polonelli3

1Department of Biomedical Sciences, Division of Experimental and Clinical Microbiology, University of Sassari, Viale S. Pietro 43/B, 07100 Sassari, Italy; 2Department of Pharmaceutical Sciences, University of Sassari, Sassari, Italy; 3Dipartimento di Patologia e Medicina di Laboratorio, Sezione di Microbiologia, University of Parma, Parma, Italy

Received 28 July 2005; returned 1 November 2005; revised 12 December 2005; accepted 5 February 2006

Objectives: To evaluate the in vitro microbicidal activity against Acanthamoeba castellanii of a murine monoclonal anti-idiotypic antibody (KTmAb) and a synthetic killer mimotope (KP), which mimic a yeast killer toxin (KT) characterized by a wide spectrum of antimicrobial activity through interaction with specific cell wall receptors, mainly constituted by \( \beta \)-glucans.

Methods: Amoebicidal activity was investigated after incubation of trophozoites under different experimental conditions with laminarinase, KTmAb, KP and a scrambled decapeptide (SP). To confirm the specific interaction of KP with \( \beta \)-glucans, the experiments were also carried out in the presence of laminarin (\( \beta 1-3 \)-glucan) or pustulan (\( \beta 1-6 \)-glucan); both glucan molecules were co-incubated with KP or SP.

Results: KTmAb and KP exhibited a time-dependent killing activity, in comparison with SP or heat-inactivated KTmAb; this activity was completely abolished by pre-incubation with laminarin, but not by pustulan. Notably, in vitro amoebicidal activity was observed in the presence of laminarinase, an enzyme that specifically hydrolys \( \beta \)-glucans. Furthermore, KP specifically inhibited the growth of Acanthamoeba on infected contact lenses and the remaining adherent KP-treated trophozoites appeared strongly damaged.

Conclusions: The results indicate that the expression of \( \beta 1-3 \)-glucan receptors in the cell membrane is probably modulated during cell growth of A. castellanii and is critical for the killing activity of KT-like molecules. Our data confirm the broad antimicrobial spectra of KTmAb and KP, emphasize the crucial role of \( \beta 1-3 \)-glucan in microbial physiology and suggest the potential use of KTmAb and KP in the prevention and therapy of Acanthamoeba infections or in preventing Acanthamoeba contamination during storage of contact lenses.

Keywords: Acanthamoeba, antimicrobial peptides, anti-idiotypic antibodies, antibody-derived peptides, killer toxins

Introduction

Acanthamoeba are small, ubiquitous, free-living protozoa that can exist as motile trophozoites and double-walled cysts.1 Invasion in the human host can cause painful, sight-threatening as well as fatal infections.2 In particular, these protozoa have been associated with corneal infections (amoebic keratitis), especially in contact lens wearers, chronic but fatal granulomatous amoebic meningoencephalitis (GAE), and skin nodules and abscesses.3-7 More recently, Acanthamoeba have been recognized to be opportunistic pathogens of humans and other animals, and they are known to cause a spectrum of infections in immunocompromised individuals, including patients with AIDS.8-11 Eradication of these protozoa from the infection sites is difficult because, under adverse conditions, the amoebas encyst and the medical therapy is often less effective against cysts than against trophozoites.12 Acanthamoeba keratitis is usually treated with a combination of cationic antiseptics (polyhexamethylene...
bigenium), which inhibit cell membranes, and aromatic diamidines (propamidine isethionate), which inhibit DNA synthesis.\textsuperscript{13,14} Chlorhexidine, alone or in combination with propamidine isethionate, has also been applied successfully.\textsuperscript{15,16} The therapeutic treatment for GAE or disseminated acanthamoebiasis is more problematic. Although a few GAE cases, after surgical excision of the lesions, have been treated successfully using chemotherapy with ketoconazole or with fluconazole and sulfadiazine,\textsuperscript{17,18} at present, no clinical trial has yet been attempted for Acanthamoeba systemic infections.

Although drug therapy for Acanthamoeba keratitis has been revolutionized with the introduction of combination chemotherapy,\textsuperscript{19,20} failures can still occasionally occur; thus, further research is needed to exploit new therapeutic agents and strategies.\textsuperscript{21}

In recent years, a creative approach to new antimicrobial preventive and therapeutic strategies has been developed through the production and use of anti-idiotypic polyclonal (KTAbs), monoclonal (KTmAb) and recombinant single-chain (KTscFv) microbicidal antibodies (Abs), which mimic the internal structure of a killer toxin produced by the yeast Pichia anomala (PaKT), characterized by a wide spectrum of antimicrobial activity.\textsuperscript{22–26} In addition to their activity against Candida albicans,\textsuperscript{27} PaKT-immunological derivatives have shown in vitro microbicidal activity against other pathogenic eukaryotic and prokaryotic microorganisms, such as Pneumocystis carinii,\textsuperscript{27} multidrug-resistant Mycobacterium tuberculosis,\textsuperscript{28} antibiotic-resistant Gram-positive cocci\textsuperscript{29} and oral streptococci,\textsuperscript{30} Aspergillus fumigatus\textsuperscript{31} and Leishmania spp.\textsuperscript{32} More importantly, they have exhibited a significant therapeutic effect in experimental models of candidiasis, aspergillosis and pneumocystosis, suggesting new immunotherapeutic strategies to control mycoses.\textsuperscript{33–36} Even when cloned and expressed in the human commensal Streptococcus gordonii as a secreted or surface-displayed molecule, KTscFv retained its antimicrobial activity and had a strong therapeutic effect on Candida vaginal infections.\textsuperscript{37}

More recently, a killer decapetide (KP) was synthesized and engineered on the basis of the sequenced KTscFv gene. KP has been shown to exhibit a strong candidacidal activity in vitro. It was shown to cure rat vaginal infections caused by fluconazole-susceptible and fluconazole-resistant C. albicans strains and to protect immunocompetent as well as SCID mice against systemic candidiasis, thus acting as a functional mimotope of KTscFv.\textsuperscript{28} Furthermore, KP showed a significant antimicrobial activity in vitro against Cryptococcus neoformans and Paracoccidioides brasiliensis, as well as a relevant therapeutic effect in experimental murine systemic cryptococcosis\textsuperscript{39} and paracoccidioidomycosis.\textsuperscript{40} Although the mechanism of action of PaKT and its functionally equivalent derivatives is yet to be completely defined, it seems to rely on the interaction with a specific receptor (KTR), particularly expressed on neosynthesized cell walls, which has been recently identified as β-glucans.\textsuperscript{41} In vivo and in vitro studies reported previously also demonstrate that neither the anti-idiotypic monoclonal antibody nor the synthetic peptide KP are toxic against host cells.

The aim of this study was to evaluate the potential microbicidal activity of KTmAb and KP against the human pathogenic protocoz Acanthamoeba castellanii. The activity of laminarinase, an enzyme specific for β-glucan molecules, was also investigated. Results show that all these compounds exhibited an in vitro antiamoebic activity.

Material and methods

Chemicals

Laminarin (β1,3-glucanase) and laminarin (soluble β1,3-glucan) were obtained from Sigma-Aldrich (Milan, Italy); putulan (soluble β1,6-glucan) was obtained from Calbiochem (La Jolla, CA, USA).

Cultivation of amoebae

Trophozoites of A. castellanii isolated from the corneal ulcer of a soft contact lens wearer, grown axenically in sterile tissue culture flasks (25 cm\textsuperscript{2}) at 25°C in PYG medium,\textsuperscript{42} were used in this study. The species identification was based on cyst morphology and indirect immunofluorescence microscopy.\textsuperscript{43} Amoebas used as inocula were taken from logarithmic phase cultures.

Anti-idiotypic microbicidal monoclonal antibody

The production of the anti-idiotypic microbicidal monoclonal antibody (a rat IgM) used in this study (KTmAb–mAbK10) is described elsewhere.\textsuperscript{44} mAbK10 was precipitated from the supernatant obtained from the antibody secreting hybridoma with ammonium sulphate, dialysed against phosphate-buffered saline (PBS) and stored at 4°C. mAb concentrations were determined using capture ELISA by means of a pair of mouse mAbs against the heavy chain of rat Ig (LO-IMEX, Brussels, Belgium).

Peptides

An engineered synthetic killer decapeptide (KP: AKVTMTCSAS) was used in this study. The synthesis of KP on the basis of the sequence of KTscFv and its optimization through alanine scanning have been described in detail elsewhere.\textsuperscript{38} A scrambled peptide (SP: MSTAVSKCAT), containing the same amino acids as KP in a different sequence, was included as a negative control.\textsuperscript{38}

Effect of mAb and peptides on Acanthamoeba growth

Experiments were performed in sterile tissue culture 96-well microtiter plates (Corning). Serial dilutions of mAbK10 (ranging from 12.5 to 50 μg/mL), laminarin (ranging from 0.5 to 1.5 μg/mL) and KP and SP decapeptides (ranging from 1 to 100 μg/mL) were made in 100 μL of PYG medium. Control cells were treated with (i) PYG medium alone, (ii) PYG medium containing the same concentrations of heat-inactivated (100°C for 10 min) mAb or laminarinase or (iii) SP. Trophozoites were washed twice in PBS buffer pH 7.2 then resuspended in PYG medium at a density of 10^5 cells/mL (100 μL/well); plates were then sealed and incubated at 37°C and 25°C. The antiamoebic activity of mAbK10, laminarinase and KP was evaluated by calculating the Acanthamoeba growth in comparison with controls. After incubation for 2, 4, 5 and 6 days, plates were observed using a Zeiss inverted microscope (Tilaval 31) to detect viable trophozoites, as described previously.\textsuperscript{44} The total well area was 32,1536 mm\textsuperscript{2}; to facilitate our task, the trophozoites included in the rectangle corresponding to the photographic field (area: 0.3215 mm\textsuperscript{2}) were counted. For each well we considered at least six of these rectangles. The total number of viable trophozoites per well was calculated according to the following formula: trophozoites/well = (Σ trophozoites counted in each rectangle/number of considered rectangles) × 100. Tests were performed in duplicate and experiments repeated at least three times.
Acanthamoebicidal activity of killer mimotopes

Evaluation of amoebicidal effect of KP

To better understand the kinetics of mortality during the first 72 h of incubation of protozoa exposed to KP and to find out whether the effect of KP could be related to amoebiostatic or amoebicidal activity, the viability of trophozoites exposed to peptides (KP and SP) was determined using a nigrosine or Trypan Blue exclusion test in a haemocytometer (Nageotte chamber). The cell viability of A. castellanii trophozoites was evaluated under different experimental conditions (as shown in the Results section). Experiments were performed in sterile test tubes at both 25°C and 37°C; briefly, 0.5 mL of a suspension containing 16 × 10⁴ trophozoites/mL in PYG medium was incubated for 3 days in the presence of killer and control peptides (doses ranging from 1 to 25 µg/mL); the number of killed cells was evaluated after 1, 2 and 3 days of incubation. In a group of different experiments, the killer activity of a higher concentration of KP (190 µg/mL) was evaluated after 6 h of incubation.

In order to confirm the specific interaction of KP with β-glucans, as described previously in C. albicans, the killing experiments were also carried out in the presence of laminarin (β1-3-glucan) or pustulan (β1-6-glucan). Both glucan molecules were co-incubated at a concentration of 4 µg/mL with 1 µg/mL KP or SP; the killing effect was evaluated after 6 h of incubation, as described above.

In order to find out whether viable trophozoites remaining after KP incubation were intrinsically or only occasionally resistant to the toxicity of the peptide, viable trophozoites remaining after 6 h of incubation with KP were washed with fresh medium, cultivated for 1 h in PYG and re-incubated in fresh PYG with 1 µg/mL KP (alone or co-incubated with 4 µg/mL laminarin or pustulan). Cell mortality was calculated after 6 and 24 h of incubation.

Immunofluorescence assay

Immunofluorescence assays were performed using mAbK10 conjugated with biotin, as described previously for C. albicans. Briefly, mAbK10 (1.5 mg/mL) was dialysed against 500 mL of buffer (0.1 M NaHCO₃/0.1 M NaCl adjusted to pH 7.4 with concentrated HCl) at 4°C with three changes over 2 days. After the dialysis, 30 µL of 10 mg/mL biotin (Sigma) in DMSO (Sigma) was added to each milligram of Ab. The mixture was incubated for 3 h at room temperature in the dark until used.

To better understand the kinetics of mortality during the first 72 h of incubation, performed counting the amoebas grown in each well of a haemocytometer (Nageotte chamber). Control cultures of A. castellanii after 6 days of incubation at 37°C produced ~7.4 × 10³ amoebas/well. Under the same experimental conditions, mAbK10 (at concentrations of 12.5, 25 and 50 µg/mL) inhibited the Acanthamoeba growth significantly, with the maximum inhibitory effect (75%) after 5 days of incubation at a concentration of 12.5 µg/mL (Figure 1a). The inhibitory effect described is not dose-dependent.

Figure 1. (a) Inhibitory effect demonstrated by different concentrations (µg/mL) of the murine anti-idiotypic monoclonal antibody mAbK10 on the growth of Acanthamoeba castellanii cultured in PYG medium at 37°C. Control, open squares; mAbK10 50 µg/mL, open triangles; mAbK10 25 µg/mL, open upside-down triangles; mAbK10 12.5 µg/mL, filled diamonds. (b) Effect of laminarinase enzyme. Control, open squares; laminarinase 1.5 µg/mL, open triangles; laminarinase 1.0 µg/mL, open upside-down triangles; laminarinase 0.5 µg/mL, filled diamonds. (c) Inhibitory effect abolition after heat-inactivation of mAbK10 and laminarinase. Control, open squares; heat-inactivated mAbK10 25 µg/mL, open triangles; heat-inactivated laminarinase 1.0 µg/mL, open upside-down triangles. Values are the means ± the standard errors of at least three different experiments.

Effect of KP on colonization of contact lenses

In order to study the effect of KP on colonization of soft contact lenses (Acti-Toric, Hydron, Hamble, UK), a total of 10⁴ Acanthamoeba trophozoites resuspended in 1 mL of PYG medium were incubated in a 24-well plate with a soft contact lens in the presence of KP or SP (10 µg/mL). The colonization by protozoa was then observed microscopically after 1–5 days of incubation at 37°C.

Results

In vitro antiamoebic activity

The method used to evaluate the growth of Acanthamoeba in microtitre plates was very sensitive and reproducible. The results were not significantly different from those obtained in preliminary experiments, performed counting the amoebas grown in each well with a Nageotte chamber. Control cultures of A. castellanii after 6 days of incubation at 37°C produced ~7.4 × 10³ amoebas/well.
of at least three different experiments.

SP 100 µg/mL, open squares; SP 10 µg/mL, open upside-down triangles; KP 100 µg/mL, filled diamonds. Values are the means ± the standard errors of at least three different experiments. The results obtained at 37°C was completely ineffective. As an example, Figure 2 shows the results obtained at 37°C and 25°C. Under both conditions KP caused a significant dose-dependent inhibition of *Acanthamoeba* growth, whereas SP was completely ineffective. As an example, Figure 2 shows the results obtained at 37°C.

**Figure 2.** (a) Culture in PYG medium at 37°C of the engineered killer KP. Control, open squares; KP 1 µg/mL, open triangles; KP 10 µg/mL, open upside-down triangles; KP 100 µg/mL, filled diamonds. (b) Inhibitory effect of SP control decapeptides on *Acanthamoeba castellanii* growth. Control, open squares; SP 1 µg/mL, open triangles; SP 10 µg/mL, open upside-down triangles; SP 100 µg/mL, filled diamonds. Values are the means ± the standard errors of at least three different experiments.

Similar results were obtained for *A. castellanii* trophozoites cultured at 37°C in the presence of laminarinase at concentrations of 0.5, 1.0 and 1.5 µg/mL (Figure 1b). Significantly, the heat-inactivation of mAbK10 and laminarinase completely abolished their inhibitory effect on amoeba growth (Figure 1c). Experiments performed at 25°C showed the same results.

The effect of KP or SP on *Acanthamoeba* growth (at concentrations of 1, 10 and 100 µg/mL) was evaluated at 37°C and 25°C. Under both conditions KP caused a significant dose-dependent inhibition of *Acanthamoeba* growth, whereas SP was completely ineffective. As an example, Figure 2 shows the results obtained at 37°C.

**Amoebicidal effect of KP**

To further investigate whether *Acanthamoeba* growth inhibition by KP was correlated to amoebistatic or amoebicidal activity, cell mortality of *A. castellanii* trophozoites exposed to the peptide for a period ranging from 1 to 3 days (at concentrations of 1, 10 and 25 µg/mL) was evaluated. Results showed a dose- and time-dependent killing activity of the peptide; interestingly the peak activity was at 25 µg/mL after 24 h of incubation: the mortality decreased from 57% after 1 day of incubation to 20% after 3 days of incubation (Figure 3a). This apparently non-logical result could be explained by the active multiplication of KP-resistant protozoa during days 2 and 3 of incubation with KP. In order to better understand the acute effect of KP on trophozoite viability, other experiments were performed to evaluate mortality of amoebas after a short incubation period with low and high concentrations of KP. After 6 h of incubation, KP at concentrations of 1 and 190 µg/mL caused a dose-dependent increase in cell mortality of 15% and 29%, respectively (Figure 3b), which remained stable after the removal of the medium containing KP, followed by 18 h of re-incubation in fresh PYG medium (data not shown). All experiments were performed at 37°C and 25°C and showed the same results.

Results indicate that the peak of killer activity is obtained from 6 to 24 h, and suggest the same ligand–receptor relation between KP and β-glucan molecules described previously in a number of prokaryotic and eukaryotic microbial systems.23,25–30,34–36,38,40,41 Data obtained can be explained by the presence of specific receptors expressed by a sub-population of susceptible target cells. Moreover, the low difference in the ability to kill amoeba cells by increasing KP dose from 1 to 190 µg/mL might be explained by saturation of specific receptors expressed in the sub-population of protozoa.

**Characterization of KP binding site: effect of laminarin and pustulan on KP activity**

Assays were carried out to test whether different β-glucans, used at doses four times the concentration of KP, could modify KP activity on amoeba viability. Our findings showed that, after 6 h of incubation, the presence of laminarin (β1-3-D-glucan)
abolished the killing effect of 1 µg/mL KP completely (15% mortality; confirming the results obtained in the experiments described above), whereas pustulan (β1-6-α-glucan) was completely ineffective (Figure 4). These data confirm that the binding site of KP is a β1-3-α-glucan molecule, as demonstrated previously in different microorganisms.27–29,31,39–41

The killing effect of KP and the inhibition by laminarin were also studied after re-incubation of protozoa that survived the killer activity of the peptide with KP (alone or co-incubated with laminarin or pustulan). After washing cultures with fresh medium and another 24 h of incubation with KP at a concentration of 1 µg/mL, a total of 25% trophozoites were still killed; also in this case, the effect was completely inhibited by co-incubating the peptide with 4 µg/mL laminarin but not with 4 µg/mL pustulan (data not shown). The persistence of the same sensitivity to the killing effect in the population of KP-resistant cells after re-incubation with fresh peptide demonstrates that Acanthamoeba trophozoites that survived KP cannot merely be considered to be mutants or intrinsically resistant to killer activity; data also suggest that β1-3-α-glucan receptor molecules are only expressed in a sub-group of trophozoites (25–30%), as demonstrated in different microbial pathogens. The killing of the same percentage of KP-surviving cells also indicates that protozoa are able to re-express KP receptors when they are re-incubated in KP-free medium.

**Detection of KP binding by immunofluorescence**

By using biotinylated mAbK10 in immunofluorescence assays, it has been possible to demonstrate a specific binding of the mAb to the cell surface of several trophozoites. Amoebas not reactive were also present, demonstrating different levels of expression of mAb receptors, confirming the modulation of KP-receptor expression in a population of protozoa (Figure 5).

**Effect of KP on contact lens colonization**

The growth of Acanthamoeba on contact lenses was specifically inhibited by KP; thus, an infected lens incubated in the presence of this peptide appeared less colonized by protozoa than one incubated with SP. Moreover, contact-lens-adherent KP-treated trophozoites appeared to be strongly damaged from the first days of culture. The same effect was observed in protozoan cultures exposed to KP. Figure 6 shows trophozoite morphology after incubation of the contact lens with KP for 2 days (Figure 6a); in contrast, typical well-spread amoeboid forms were present after incubation with SP (Figure 6b).

**Discussion**

Medical therapy for Acanthamoeba infections is still difficult because antiprotozoal drugs are not completely effective in some anatomical sites. In fact, while there are numerous reports on drugs used for local therapy of Acanthamoeba keratitis, in general therapy based on the use of cationic disinfectants (biguanides–chlorhexidine and polyhexamethylene biguanide, used alone or in combination), systemic therapeutic treatment for GAE or disseminated acanthamoebiasis is less amenable to antimicrobial treatment than ophthalmological infections. Moreover, some anti-amoebic drugs show only an amoebistatic effect, molecules used for keratitis treatment are toxic for the host cells and some eye drop preparations are not well tolerated when used for prolonged periods. Finally, drug resistance is an emerging problem, and new compounds with amoebicidal activity are needed for treatment when resistance is encountered, especially when drugs are used over long periods of time.

In this paper we describe the amoebicidal effect of an anti-idiotypic murine monoclonal antibody (KTmAb) that mimics a KT from the yeast P. anomala. The wide spectrum of antimicrobial activity of KTmAb and the recombinant single-chain KTscFv has been reported previously against a number of pathogens, including different bacterial and fungal species, both in vitro
and/or in animal models.\textsuperscript{23,25–30,34–36,38,40,41} Regarding the activity of these Abs in protozoan systems, their cytotoxic effect has been demonstrated only \textit{in vitro} for two Leishmania species (\textit{Leishmania major} and \textit{Leishmania infantum}).\textsuperscript{32} In particular, KTmAb showed a dose-dependent microbicidal activity against the promastigote stage and was able to bind to the cell surface. In this model the major limitation for the use of KTmAb in the therapy of human and animal leishmaniasis is the intracellular location of the protozoon, since extracellular promastigote forms are specific for vector insects, but during human infection only the amastigote forms reside in macrophage-derived cells.

Interestingly, in this paper we also demonstrate that a decapeptide (KP) derived from the sequence of KTscFv is able to exert a similar cytotoxic effect to KTmAb on protozoa. These results confirm previous data on the wide-spectrum microbicidal activity of KP both \textit{in vitro} and in animal models.\textsuperscript{25,38–40} Since \textit{Acanthamoeba} is a typical extracellular microorganism, the use of this amoebicidal small peptide could represent an alternative and safe pharmacological approach in the treatment of local and systemic \textit{Acanthamoeba} infections in humans.

Previous studies have demonstrated that the microbicidal effect of both KTmAb and KP is due to the specific recognition of β-glucan components expressed at the surface of the different microorganisms. The importance of β-glucans in \textit{Acanthamoeba} physiology has been well described.\textsuperscript{45,46} In particular, β-glucan might be an intermediate stage in cellulose synthesis during \textit{Acanthamoeba} growth. Results reported in our paper confirm that the β-glucans are the target molecules expressed in \textit{Acanthamoeba} trophozoites. In fact, the same amoebicidal effect of antibody or decapetide is observed using laminarinase, an enzyme that specifically hydrolyses β-glucans, suggesting that the killing effect is strictly related to the cellular expression of these molecules. Interestingly, the growth of the flagellate protozoon \textit{Trichomonas vaginalis} is not affected either by KTmAb or by enzymatic activity of laminarinase (data not shown). Results also demonstrate that the interaction of both KTmAb and KP with β1-3-glucans is highly specific; in fact their microbicidal effect is abolished in the presence of laminarin (β1-3-glucan), but not of pustulan (β1-6-glucan). These data indicate that \textit{Acanthamoeba} expresses β1-3-glucans at the cellular surface, and that these components, widely represented in different microorganisms, play a critical role in microbial physiology. This observation has been confirmed by immunofluorescence assays using biotinylated KtmAb, also demonstrating the modulation of receptor expression in \textit{Acanthamoeba} (Figure 5).

The recognition of different cells by the antibody and the presence of a number of non-reactive cells suggest that the β1-3-glucan receptor is not constantly expressed during \textit{Acanthamoeba} growth. This could explain results obtained in the analysis of the inhibitory effect of KTmAb and KP. In particular, killing by KP is dose-dependent, decreases from 24 to 72 h exposure and can also be observed after 6 h of incubation (15–30% cell mortality, after exposure to 1 and 190 μg/mL KP). Moreover, when remaining viable cells (after 6 h of incubation with 1 μg/mL KP) are re-exposed for an additional 6 or 24 h to the same concentration of decapetide, the same rate of mortality (25%) is observed. These results might indicate that the expression of the β1-3-glucan receptor is modulated during cell growth and that microbial cells could be killed by KP only if receptors are expressed at the cell surface. The same modulation of β1-3-glucan receptors has been demonstrated in all KP-sensitive prokaryotic and eukaryotic microbial systems tested previously.

Results reported in this paper confirm the broad antimicrobial spectrum of mAbK10 and KP, and also demonstrate that β1-3-glucans are molecules that play a crucial role in the physiology of a large number of microorganisms, including protozoa. Moreover, our data also suggest a potential use of the antimicrobial peptide KP in the prevention and therapy of \textit{Acanthamoeba} infections or in preventing \textit{Acanthamoeba} contamination during storage of contact lenses.

Acknowledgements

This work was supported by Ministero dell’Istruzione, dell’Università e della Ricerca di Italy (PRIN 2005) and by the University of Sassari (Progetto di ricerca sul 60%, Centro di Eccellenza sulla Biodiversità).

Transparency declarations

None to declare.
Acanthamoebic activity of killer mimotopes

References


44. Mattana A, Biancu G, Alberti L et al. In vitro evaluation of the effectiveness of the macrolide rokitamycin and chlorpromazine against
Fiori et al.

