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In vitro candidacidal activity of a synthetic killer decapeptide (KP) against *Candida albicans* cells adhered to resin acrylic discs

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BACKGROUND: Oral *Candida* spp., and *C. albicans* in particular, are considered as important aetiological agents in the pathogenesis of denture-induced stomatitis. Several studies have reported that *C. albicans* is able to easily adhere to different medical devices, such as vascular and urinary catheters or acrylic denture surfaces, and that adhesion is a fundamental step in the initial pathogenic process of colonization and further possible infection. Recently, a synthetic decapeptide (KP) derived from the sequence of a single-chain recombinant anti-idiotypic antibody, acting as a functional internal image of a microbicidal, broad spectrum yeast killer toxin, has been reported to kill *in vitro C. albicans* cells and to exert a therapeutic activity against experimental mucosal and systemic candidiasis.

METHODS: The aim of this study was to evaluate, through a CFU assay, the candidacidal activity of KP on sanded acrylic resin discs, previously colonized by *C. albicans* cells.

RESULTS AND CONCLUSIONS: At 100 μg/ml KP showed over 90% of killing activity on *C. albicans* cells adhered to resin discs, when compared with a scramble peptide used as control. The results of this study suggest a potential effect of KP on *C. albicans* cells adhered on the surface of resin materials, such as prosthetic dentures.

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Introduction

Candida spp. are frequently responsible for oral infections, including denture-related stomatitis, an inflammatory process affecting the oral mucosa of 30–60% of patients wearing removable dental prostheses (1, 2). Although several factors have been held responsible for the aetiology of denture stomatitis, such as bacterial infection, mechanical irritation or allergic reaction to denture base material, *Candida* spp., and *C. albicans* in particular, have been recognized as primary agents.

The adhesion of *Candida* cells is a critical step in the colonization of human mucosal surfaces, where yeast lives as a commensal causing disease when given the opportunity, and fungal adhesins are recognized virulence factors that contribute to pathogenesis (3). In individuals wearing dentures, *C. albicans* can adhere not only to host cells, but also to the surface of prosthetic devices or to adsorbed salivary macromolecules. Many different factors are involved in the adhesion of *C. albicans* to the acrylic surfaces. Among them, surface hydrophobicity, electrostatic forces, the presence of sucrose in the culture medium and germ tube formation seem to play a role in the process of adhesion. In addition, several studies on the adhesion of *C. albicans* to denture acrylic resins have shown that differences in surface topography affect the attachment of microorganisms to a surface, with a higher number of cells remaining on rougher surfaces after washing procedures (2, 4–7).

Recently, a synthetic decapeptide (KP) derived from the sequence of a single-chain recombinant anti-idiotypic antibody acting as a functional internal image of a microbicidal, broad spectrum yeast killer toxin, has been reported to kill *in vitro C. albicans* cells and to exert a therapeutic activity on experimental mucosal and systemic candidiasis (8, 9). Moreover, KP has shown a significant fungicidal activity on a large number of yeast strains belonging to different *Candida* species isolated from the oral cavity of diabetic and non-diabetic subjects, regardless of their pattern of resistance to conventional antifungal agents (10).

The aim of this study was to evaluate the fungicidal activity *in vitro* of the engineered peptide KP on *C. albicans* cells adhered to acrylic discs.

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Material and methods

Peptides

An engineered synthetic killer decapeptide (KP) was synthesized (NeoMPS, Strasbourg, France) for use in this study. KP synthesis and optimization through alanine scanning have been described in detail elsewhere (9). A scramble peptide containing the same amino acids of KP in a different sequence was also synthesized and included as a negative control.

Preparation of acrylic discs

Acrylic discs for the assay were prepared as described by Ellepola and Samaranayake with some modifications (11).

Transparent acrylic polymethyl metacrylate powder (1.5 g; Palapress, Kultzer, Italy) was spread on an aluminium foil-covered round glass plate (diameter 9 cm). One millilitre of monomer liquid (Palapress) was poured onto the surface of the plate and a second round glass plate similar to the first was immediately placed on top of the polymerizing mixture. The two plates were then fixed together and placed in water at 55°C under pressure (2 atm) for 15 min. The acrylic discs obtained were immersed in water for 1 day to remove the residual monomer, dried in air and were then sanded (100 μm) with a sandblaster (NYBRA-4800 S, Tecnogaz, Sala Baganza, Parma, Italy) on one side to increase the surface irregularities.

Two different concentric circles (diameters 5.5 and 4.8 cm) were drawn on the sanded surface of each disc. To avoid the yeast suspension leaking during the experiment, an incision was also made on the circumference of the large circle. The area of the smaller circle was evaluated in this study for the colony-forming unit (CFU) assay.

The discs were put under UVA light for 3 h for each side to promote the surface sterilization before the use.

Candidacidal activity of KP

The fungicidal activity of KP on *C. albicans* cells adhered to acrylic discs was evaluated *in vitro* by a CFU assay. A reference *C. albicans* strain (UP10S), recognized to be sensitive to KP, was grown on Sabouraud dextrose agar plates at 30°C for 24 h. The yeast cells from a single colony were suspended in 199 Medium (Sigma, Sigma-Aldrich, Dorset, UK) and incubated at 37°C for 1 h in shaking conditions (180 rpm). *Candida* cells were counted in a Burker haemocytometer (Emergo, Landsmeer, the Netherlands) and the yeast cell suspension was properly diluted in sterile distilled water to achieve a final concentration of $5–6 \times 10^2$ viable cells⁄ml. Five millilitre of this suspension were inoculated on the sanded surface of each acrylic disc and put inside a Petri dish to cover the smaller circle area. Each disc was then incubated at 37°C for 2 h to promote the adhesion of yeast to the acrylic surface. Discs were then washed with sterile water and put inside new Petri dishes. Five millilitre of sterile water containing 100 μg⁄ml of KP were added to the same smaller circle area, while the scramble peptide at the same concentration was used as a control, and the plates were incubated at 37°C. Each assay was carried out in triplicate for statistical purposes.

Following incubation for 5 h at 37°C, the acrylic discs were re-washed, put into new Petri dishes, coated with 15 ml of melted Sabouraud dextrose agar, allowed to solidify and incubated at 30°C for 48 h before CFU enumeration. Only the colonies comprised in the smaller circle area were counted.

In order to test the fungicidal activity of KP on a higher concentration of *Candida* cell, a second experiment was performed with a yeast cell suspension of viable *C. albicans* cells 25 times greater than the first one. The methodology used in this second experiment was identical to the one previously described. However, in this case, to facilitate the count of *Candida* colonies, the discs were observed at 250× by a stereomicroscope (Wild M3Z, Heerbrugg, Switzerland). The colonies in three different fields were counted for the discs treated with the scramble peptide and KP.

The candidacidal activity of KP was evaluated as percentage reduction in the average number of CFU compared to the scramble peptide control. Statistical significance was assessed by the two-tailed Student's *t*-test.

Results

In this study, the synthetic decapeptide KP has shown a significant fungicidal activity upon the *C. albicans* cells that adhered to the disc acrylic surface.

With a *Candida* inoculum of 675 ± 70 cells⁄ml, KP, at a concentration of 100 μg⁄ml, showed 93.54% of killing in comparison to the scramble peptide used as a control. The average number of yeast colonies observed was 286.5 ± 26.16 in the discs treated with scramble peptide, and 18.5 ± 21.9 in discs treated with KP ($P = 0.007$; Fig. 1).

The experiment was repeated at the same concentration of KP (100 μg⁄ml) using a yeast suspension approximately 25 times greater than the first one (17 900 ± 1131 cells⁄ml). The fungicidal activity of KP in comparison with the scramble peptide was 91.44% (*Candida* colonies average number in three different microscopic fields 20 ± 7 vs. 233.66 ± 39.27; $P = 0.01$).

Discussion

The aim of this study was to evaluate the activity of a synthetic killer peptide (KP), derived from the sequence of a single-chain, recombinant, anti-idiotypic antibody acting as a functional internal image of a yeast killer toxin, on *C. albicans* cells previously adhered to acrylic discs (9).

To promote *in vitro Candida* adhesion and then test KP in 'adverse' conditions, the discs were sanded on one side at 100 μm to increase the surface roughness, mimicking the anatomical features of old dental prostheses and causing possible alterations of the dentures due to the prolonged usage. Furthermore, the germination phase of the *C. albicans* cells is also recognized as an

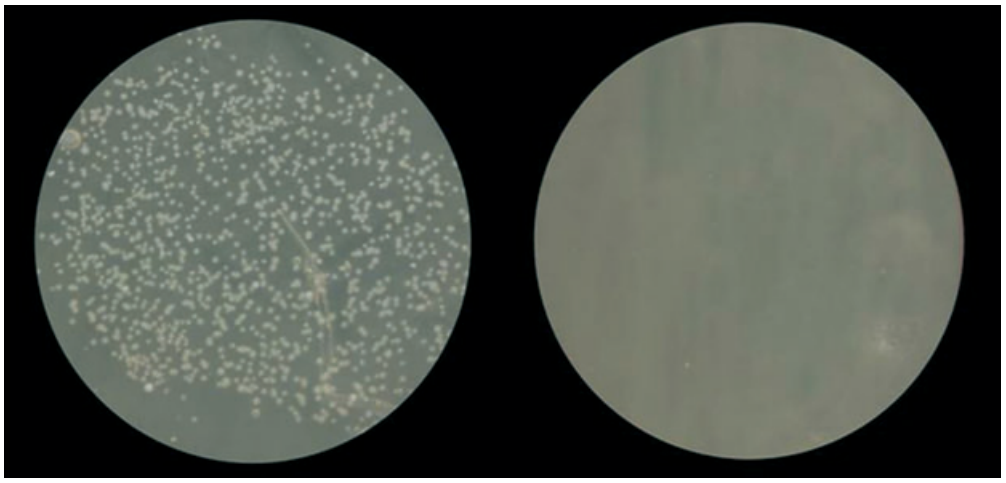

Figure 1 *In vitro* activity of KP (100 μg⁄ml in sterile water) on *Candida albicans* cells previously adhered to acrylic disc surface. Left: yeast cells treated with the scramble peptide; right: yeast cells treated with KP.

important factor in the adhesion of yeast to acrylic surfaces (12, 13). Several studies have shown how the adhesion of *C. albicans* to plastic materials is correlated with germ-tube induction, with a molecular re-organization of the cell wall that may facilitate the adhesion of the yeast to the biomaterials by expressing a fibrillar layer associated with the outer part of the cell wall (12, 14).

Germ-tube induction was then promoted prior to the use of yeast cell suspension, in order to increase the adherence ability of *C. albicans* to acrylic surfaces. In addition, a strong KP activity presumably mediated by the interaction with cell wall β-glucans an mainly expressed during the germination phase of *C. albicans* (9), was reported.

In this study, KP, used at a concentration of 100 μg⁄ml, has shown a significant fungicidal activity on *Candida* cells adhered to the discs. This killer activity was observed both in the first series of experiments where a lower yeast suspension ($5–6 \times 10^2$ viable cells⁄ml) was used as well as in the second series with a 25-fold suspension of *C. albicans* cells, a condition more predictive of denture-wearing subjects less careful in maintaining oral hygiene.

This study has tested for the first time the candidacidal activity of KP on *C. albicans* cells adhered to acrylic surfaces, such as oral prostheses, in order to verify its potential action in different conditions compared to those evaluated to date. The effect of KP has already been shown *in vitro* on several *Candida* spp. (10), on different KTR-possessing pathogenic micro-organisms and on *in vivo* mucosal and⁄or systemic models of animal candidosis, cryptococcosis and paracoccidioidomycosis showing an important therapeutic activity (9, 15–18). Moreover, KP is shown to inhibit *ex vivo* HIV-1 replication (19).

Results from this study show a potential candidacidal effect of KP on *C. albicans* cells even when adhered to acrylic surfaces. It is well known that bacteria and yeast, such as *Candida* spp., are able to easily develop complex structures in a self-producing organic polymeric matrix (biofilms) on different surfaces (i.e. dental prostheses), that determine higher resistance to conventional antifungal drugs and to usual hygiene procedures (20–22). In particular, it has been observed that *Candida* biofilms directly contribute to the development of denture-induced stomatitis (23). It could be of interest, therefore, to study KP activity on a standardized and specific model of denture-*Candida* biofilm, in order to recreate the conditions favourable for the development of oral *Candida* infections.

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