Antifungal activity of Histatin-5 against non-albicans Candida species.

Abstract
Fungicidal effects of Histatin-5 against 26 oral isolates belonging to 5 non-albicans Candida species were examined. Fifty μM of Histatin-5 killed more than 95% of C. tropicalis and C. guilliermondii isolates, and more than 90% of C. parapsilosis and C. krusei. However, and C. glabrata were less sensitive to the peptide (mean 62.9%). Our results, taken together, demonstrated that Histatin-5 possessed the fungicidal activity against Candida species, other than C. glabrata.

The AIDS epidemic, improved life-sustaining therapy, and aggressive anticancer therapy have contributed to the rise in the number of severly immunocompromised patients (3). This has led to an increase in oral and systemic fungal infection, and the concomitant increased usage of antifungal agents for prophylaxis is most likely the main cause of the development of antifungal drug resistance (20).

Recently, the importance of non-albicans Candida species in the candidal infection and candidaemia has been reported (5,10,21). Chakrabarti et al. (1996) has suggested that non-albicans Candida spp. with fluconazole resistance have become more prominent in recent years (1). In addition, unfortunately, most of currently available antifungal drugs, such as polyene and azole antimycotics, have undesirable toxic and other side effects (16). Therefore, the search for more effective but less toxic anticandidal therapeutic agents cannot be overemphasized. Peptide antibiotics, which are believed to interact with the microbial membrane leading to the disruption of cellular integrity and cell death, may be a promising new class of antifungal agents (3).

Histatins are a group of small, cationic antifungal peptides present in human saliva (11,12) They were also detected in human serum (7). In saliva, secretory IgA, lactoferrin (8,9), lysozyme (19), and histatins (12) have all been demonstrated to exert anticandidal activities. Among these molecules, histatins are most likely to be the protein responsible for the in vitro salivary candidicidal activity (4). In fact, among several protective proteins in saliva, only the histatin concentration decreased (statistically significant decrease) in the saliva of AIDS patients who developed candidiasis (6). The decrease in histatin concentration in the saliva of AIDS patients (4) may partially explain why more than 70% of AIDS patients develop oral candidiasis over the course of the disease (17). Since histatins are naturally occuring molecules they are considered to be nontoxic to mammalian cells (18). This property and the
fungicidal potencies make histatins promising natural therapeutic or preventive agents against fungal infection. Among the three major histatins (Hsn-1, -3 and -5), Hsn-5 is the most potent peptide in killing Candida albicans isolates (22).

However, little is known about the antifungal activity of this peptide against non-albicans Candida species from oral cavity. Thus, in the present study, we analysed the fungicidal potential of Hsn-5 against 26 isolates of Candida spp. representing 5 species.

Four isolates of C. guilliermondii (GU1, GU2, GU3, GU4), 8 of C. glabrata (GL1, GL2, GL3, GL4, GL5, GDH 1306, GDH 1407, GDH 2269), 4 of C. parapsilosis (P1, P2, P3, P5), 4 of C. krusei (BOX1, CK2, CK14, CK 16) and 6 of C. tropicalis (T1, T2, T3, T5, GDH 1362, ATCC13803) were used in the present study. All the isolates, other than ATCC strain were oral isolates gifted from Prof. Samaranayake, and identified by sugar assimilation test using the API 20C system (API Products, Biomeroux, Lyon, France) and "germ tube" test (15) in Oral Biology Unit of Hong Kong University.

A loopful of the yeast was inoculated in yeast nitrogen base medium (Difco, Detroit, USA) containing 250 mM glucose and grown aerobically at 37 °C. After 18 h incubation, the yeast was harvested in the mid exponential growth phase, washed twice with PBS containing 1 mM phosphate (pH 6.8) and resuspended to a final concentration using a haemocytometer. All yeast cells suspended, remained in the blastospore phase.

Hsn-5 (DSHEKRHHGYKRKFHEKHHSHRGY) was purchased from Sigma Chem. Co. (St Louis, MO, USA).

Antifungal activity of Hsn-5 was examined according to the method of Edgerton et al. (1998) with some modifications (2). Fungicidal assays were performed on exponential Candida cells in the presence or absence of 50 μM of Hsn-5. Briefly, Candida cells were washed twice with 1 mM sodium phosphate buffer (Na2HPO4/NaH2PO4; pH6.8), and resuspended at 1.8 x 10^5 cells/ml. Twenty microliter of cell suspensions were mixed with 20 μl of 1 mM phosphate buffer containing the indicated protein and incubated for 90 min at 37 °C with shaking. Control tubes were incubated with 20 μl of 1 mM phosphate buffer alone.

The reaction was stopped by addition of 360 μl of yeast nitrogen base (YNB); 40 μl of the suspension (containing 360 cells) were spread onto Sabouraud dextrose agar plates and incubated for 48 h at 37 °C.

The assays were carried out on two independent occasions with quadruplicated samples on each occasion. Loss of viability was calculated as [1 - (colonies from suspension with peptides/colonies from suspension with no protein)] x 100 (2).
All of the numerical data obtained here were analysed by an one-way analysis of variance (ANOVA) and subjected to Tukey's multiple range test.

Hsns are present in saliva of healthy adults at concentrations of 50 - 425 $\mu$g/ml (2). Hsn-5 is the most potent candididal member of the family that kills pathogenic *Candida* species from 90 to 100% at physiological concentrations (15 - 30 $\mu$M)(13). Indeed, Edgerton et al. (1998) has examined the antifungal activities of histatins, including Hsn-5, and shown that concentration of Hsn-5 (31.25 $\mu$M) was required to kill more than 95% of *C. albicans* isolate derived from palate of denture stomatitis patients (2). Thai and Bobek (1997), and Helmerhorst et al. (1999), employed the range of concentration of Hsn-5 from 0 to 100 $\mu$M (3,18). In the preliminary study, loss of viability of isolates of *Candida* caused by Hsn-5 gradually increased at the concentration of 25-50 $\mu$M, and plateaued at 50-100 $\mu$M of peptide (data not shown). Thus, we employed 50 $\mu$M Hsn-5, to assess the fungicidal effects. At the concentration, *C. tropicalis* (ranged 91.4± 4.7 % to 100± 0 %; mean 97.7%) and *C. guilliermondii* (92.2± 1.7% to 99.6± 0.8%; mean 97.1%) was most sensitive to Hsn-5, followed by *C. parapsilosis* (74.1± 7.4% to100± 0%; mean 93.0 %) and *C. krusei* (82.0± 4.1 % to 98.2± 1.1%; mean 90.8%), and *C. glabrata* (44.4± 10.6% to 87.5± 2.37%; mean 62.9%) was significantly resistant to Hsn-5 (ANOVA; p<0.01; Fig. 1). The latter finding is consistent with the results of several studies (3,4,18). While several possible mechanisms of Hsn-induced killing of *C. albicans* has been proposed, the detail of the mechanisms is still unknown. Thus further studies on the mechanisms of the peptide should solve the problem of the peptide which is less effective against *C. glabrata*.

In conclusion, our results, taken together, demonstrated the therapeuic potentials of Hsn-5 against the infection by *Candida* species, though the antifungal effects against isolates of *C. glabrata* were relatively low. Further trials to improve the fungicidal potential of basic peptides should be required.

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References


Legend to Figure

Fig. 1 Histatin-5 (50μM) induced loss of viability of isolates of Candida species.