

Interaction of sertraline with *Candida* species selectively attenuates fungal virulence in vitro

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Abstract

This study investigated whether the interaction between isolates of *Candida albicans* ($n=7$), *Candida parapsilosis* ($n=3$), *Candida krusei* ($n=2$), *Candida dubliniensis* ($n=1$) and sertraline, a typical selective serotonin reuptake inhibitor, alters candidal virulence. Sertraline treatment of *Candida* spp. significantly ($P<0.05$) affected hyphal elongation, phospholipase activity, production of secreted aspartyl proteinases and fungal viability. In addition, monocyte-derived macrophages (MDMs) treated with sertraline reduced inhibition of blastoconidia germination in comparison to MDMs alone. In conclusion, our findings suggest that the interaction between sertraline and *Candida* spp. may also diminish the virulence properties of this fungal pathogen in vivo.

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1. Introduction

Years ago antimicrobial activity was reported for psychotropic drugs of the Phenothiazine and Thioxanthene groups [1]. Since then, several non-antibacterial substances have been examined, and it was reported that selective serotonin reuptake inhibitors (SSRIs) influence the in vitro viability of bacteria [2,3] and may reverse chloroquine resistance in *Plasmodium falciparum* [4]. These drugs exert a significant antimicrobial activity, mainly against Gram-positive bacteria, yet are inactive against most enterobacteria [3].

SSRIs are increasingly being used as first-line therapy for severe premenstrual syndrome (PMS) and as antidepressants [5]. In humans, SSRIs modify the behavior of 5-hydroxytryptamine (5-HT) in the synapse space. These antidepressants act primarily on the 5-HT transporter protein (SERT) and block the reuptake process of 5-HT. SERTs, with molecular masses of 60–80 kDa and 12 trans-

membrane domains, are similar to other biogenic amine transporters and sodium- and chloride-dependent transporters [6].

Recently, we found that several SSRIs also exert fungicidal effects against *Aspergillus* spp., conidia and hyphae [7] and that sertraline (SSRI, Tresleen[®], Vienna, Austria) showed in vivo and in vitro antifungal activity against *Candida* spp. [8].

The present study investigated whether the interaction between sertraline and *Candida* spp. directly affects the virulence properties of this fungal pathogen in vitro.

2. Materials and methods

2.1. *Candida* spp. cultivation

Candida albicans CBS 5982 (Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands) and clinical isolates of *C. albicans* ($n=7$), *Candida parapsilosis* ($n=3$), *Candida krusei* ($n=2$) and *Candida dubliniensis* ($n=1$) were used in this study. The strains were initially grown on Sabouraud dextrose agar (Oxoid, Basingstoke, UK) at 37°C for 72 h. Cell culture flasks (Becton Dickinson, Lin-

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coln Park, NJ, USA) were inoculated at an initial concentration of 1×10^6 yeast cells ml^{-1} and incubated for 20 h at 30°C if not indicated otherwise.

2.2. Drug

Sertraline HCl (molecular weight 343 kDa) was kindly provided by Pfizer (Global Research and Development, Groton, CT, USA). The stock solution was prepared following the manufacturer's instructions: sertraline powder was dissolved in sterile aqua ad injectionem (Fresenius, Linz, Austria) at room temperature for 30 min and centrifuged. Serial two-fold drug dilutions from 950 $\mu\text{g ml}^{-1}$ to 0.37 $\mu\text{g ml}^{-1}$ were prepared and concentrations from 475 $\mu\text{g ml}^{-1}$ to 0.18 $\mu\text{g ml}^{-1}$ were used for in vitro tests.

2.3. Hyphal elongation

The morphology of isolates of *C. albicans* ($n=7$), *C. parapsilosis* ($n=3$), *C. krusei* ($n=2$) and *C. dubliniensis* ($n=1$) treated with or without sertraline was investigated by assessing hyphal elongation [9]. Therefore, 2×10^5 yeast cells ml^{-1} of RPMI 1640 were inoculated into microwell plates (Greiner, Kremsmünster, Austria) and incubated at 37°C. The morphology of the organisms was determined microscopically at the times indicated; a micrometer was used for length measurement. Each sample was assessed in triplicate, measuring 50 organisms per sample.

2.4. Determination of extracellular phospholipase activity

The extracellular phospholipase activity of *C. albicans* ($n=7$) was measured using the egg yolk agar method [9]. Briefly, the egg yolk agar consisted of Sabouraud Dextrose Agar (SDA) (in 184 ml of distilled water), 13.0 g; NaCl, 11.7 g; CaCl_2 , 0.111 g; and 10% sterile egg yolk and was poured into 60 mm diameter Petri dishes (Becton Dickinson). *C. albicans* strains were plated at a concentration of 1×10^7 yeast cells ml^{-1} in aliquots of 10 μl onto egg yolk agar. After incubation of the agar at 37°C for 72 h, the diameter of the precipitation zone around the colonies was determined.

2.5. Assay for Candida-secreted aspartyl proteinases (Saps)

C. albicans and *C. dubliniensis* (2×10^6 colony-forming units (CFU) ml^{-1}) were incubated in 1 ml of Sap induction medium (pH 4.0) consisting of 1% bovine serum albumin (BSA, fraction V, Sigma, St. Louis, MO, USA), 2% glucose, 0.1% KH_2PO_4 , 0.05% MgSO_4 and 1% 100 \times minimum essential medium vitamins (Sigma). Every other day various amounts of sertraline (237 to 0.25 $\mu\text{g ml}^{-1}$) or phosphate-buffered saline (PBS) were added. After growth in this medium at 20°C for 7 days under continuous rotation, colony-forming units were counted and Sap

activity was assessed using a modified version of the original protocol by Ollert et al. [10]. Briefly, the suspension was centrifuged at $3500 \times g$ for 10 min. The amount of proteinase antigen in the supernatant was determined by absorbing aliquots of the supernatant to microwell plates (Greiner, Kremsmünster, Austria). Antigen detection was accomplished using the monoclonal mouse IgG antibody FX 7⁻¹⁰ (2 $\mu\text{g ml}^{-1}$) (kindly provided by Dr. Borg-von Zeppelin, Göttingen, Germany), which reacts with an epitope of Sap2.

2.6. Germination assay

The ability of macrophages to inhibit germination of blastoconidia of *C. albicans* ($n=7$), *C. parapsilosis* ($n=3$) and *C. krusei* ($n=2$) was determined using a modification of Waldorf et al. [11]. After differentiation of human monocytes to monocyte-derived macrophages (MDMs) in 24-well plates according to a method of Kacani et al. [12], non-adherent cells were discarded by washing with RPMI 1640. Sertraline was added to each well at different concentrations. The macrophages were challenged with blastoconidia at a ratio of 1:1 and centrifuged for 5 min at $100 \times g$ to facilitate contact between blastoconidia and effector cells. Incubation was continued for a total of 4 h at 37°C in a CO_2 incubator, after which the plates were centrifuged, the supernatants discarded and 0.5 ml of 2.5% deoxycholate (Sigma, Vienna, Austria) was added to each well to lyse macrophages. The plates were stained with lactophenol cotton blue (Merck, Vienna, Austria), and an inverted microscope was used to visualize germination of blastoconidia. The inhibition rate was calculated from the percentage of blastoconidia which did not germinate out of a total of 100 examined per well.

2.7. Statistics

Statistical significance was determined using the Student's *t*-test analysis. All comparisons were two-sided, and $P < 0.05$ was considered significant.

3. Results

3.1. *C. albicans* and non-*C. albicans* morphology after treatment with sertraline

Significant differences in hyphal elongation were observed for *Candida* spp. treated with sertraline as compared to untreated yeasts (Table 1).

3.2. Effect of sertraline on the activity of extracellular phospholipases of several *C. albicans*

Treatment with sertraline significantly decreased phospholipase activity as compared to control (Table 2).

Table 1
Effect of sertraline on growth of *C. albicans* ($n=7$) and non-*C. albicans* ($n=5$) isolates

<i>Candida</i> spp.	Time	Range of hyphal elongation at various sertraline concentrations ($\mu\text{g ml}^{-1}$)						
		29	7	3	1.5	0.75	0.39	Control
<i>C. albicans</i> ($n=7$)	3 h	0.0 \pm 0.0*	0.6 \pm 0.4*	1.3 \pm 0.3*	3.7 \pm 2.1*	8.5 \pm 1.6*	15.5 \pm 2.4	17.3 \pm 1.6
<i>C. parapsilosis</i> ($n=3$)		0.0 \pm 0.0*	0.7 \pm 0.6*	1.9 \pm 0.7*	3.9 \pm 1.1*	7.5 \pm 0.7*	17.5 \pm 3.4	19.6 \pm 2.6
<i>C. krusei</i> ($n=3$)		0.0 \pm 0.0*	0.5 \pm 0.3*	1.2 \pm 0.4*	2.8 \pm 0.5*	6.5 \pm 1.9*	15.9 \pm 2.5	16.4 \pm 1.7
<i>C. albicans</i> ($n=7$)	6 h	0.0 \pm 0.0*	3.2 \pm 2.1*	6.3 \pm 0.9*	10.1 \pm 0.3*	16.3 \pm 2.4*	25.4 \pm 3.6	26.5 \pm 6.2
<i>C. parapsilosis</i> ($n=3$)		0.0 \pm 0.0*	3.7 \pm 1.1*	7.2 \pm 1.1*	12.3 \pm 3.3*	14.1 \pm 3.4*	33.1 \pm 2.1	39.4 \pm 5.8
<i>C. krusei</i> ($n=2$)		0.0 \pm 0.0*	4.2 \pm 3.1*	6.9 \pm 1.4*	11.1 \pm 3.3*	15.3 \pm 3.4*	29.6 \pm 2.3	32.2 \pm 5.3
<i>C. albicans</i> ($n=7$)	9 h	0.0 \pm 0.0*	12.4 \pm 3.5*	14.6 \pm 0.8*	18.3 \pm 1.3*	20.4 \pm 6.4*	29.6 \pm 1.5	34.3 \pm 5.3
<i>C. parapsilosis</i> ($n=3$)		0.0 \pm 0.0*	11.4 \pm 2.4*	13.6 \pm 1.6*	19.3 \pm 2.2*	21.4 \pm 2.8*	39.6 \pm 3.5	43.8 \pm 4.1
<i>C. krusei</i> ($n=3$)		0.0 \pm 0.0*	13.4 \pm 3.5*	15.6 \pm 5.8*	17.3 \pm 3.3*	22.4 \pm 2.3*	36.6 \pm 2.5	39.8 \pm 5.3

In all samples with $>1.5 \mu\text{g ml}^{-1}$ sertraline blastoconidia were also detected.

* $P < 0.05$ in comparison to control.

Values are means \pm S.D. of three experiments, done in duplicate.

3.3. Effect of sertraline on production of *Candida*-secreted aspartyl proteinases (Saps)

Growth of *Candida* was significantly impaired at $237 \mu\text{g ml}^{-1}$ sertraline (Fig. 1A, black columns) and Sap production was clearly and significantly impaired at 7 and $118 \mu\text{g ml}^{-1}$ (Fig. 1A, white columns), concentrations which do not have an effect on fungal growth. The maximum Sap reduction at low sertraline concentrations which did not affect the growth of *Candida* was $>80\%$ Sap reduction at $118 \mu\text{g ml}^{-1}$ sertraline. Similar data were observed for *C. dubliniensis* as shown in Fig. 1B.

In order to investigate whether the decrease in Sap concentration is due to a direct impact on Saps or an inhibition of production, sertraline was added at different concentrations to the supernatants of the positive control, which had been grown in induction medium for 7 days. After further 16 h at 20°C , Sap activity was not found to be decreased at $7 \mu\text{g ml}^{-1}$ (Fig. 2), a concentration at which a significantly lower Sap production was recorded after 7 days coculture (Fig. 1). At 118 and $237 \mu\text{g ml}^{-1}$ the decrease in Sap production over 7 days was >80 and $>90\%$, respectively, but the direct impact assessed by overnight culture was only approximately 20 or 50%,

respectively (Fig. 2). Thus, the sertraline concentrations required to inhibit Saps are about one magnitude lower than those needed to inhibit fungal growth, and most of the inhibition is due to an inhibition of production and only partly to a direct impact.

3.4. Inhibition assay

MDMs cultured in medium alone (control) inhibited germination of blastoconidia of *C. albicans* at (mean \pm S.D.) $39 \pm 7\%$, of *C. parapsilosis* at $37 \pm 3\%$ and of *C. krusei* at $41 \pm 5\%$. Addition of $7 \mu\text{g ml}^{-1}$ sertraline to *C. albicans* isolates led to a statistically significant increase in inhibition in comparison to the control ($P < 0.05$, Table 3). Similar data were found for non-*C. albicans* strains: significant inhibition of blastoconidia for *C. parapsilosis* and *C. krusei* was at 7 and $14 \mu\text{g ml}^{-1}$ sertraline, respectively.

4. Discussion

The present study shows that the direct interaction between sertraline and *C. albicans* and non-*C. albicans* isolates significantly affects fungal virulence properties.

Table 2
Phospholipase activity of *C. albicans* ($n=7$) treated with sertraline

	Phospholipase activity (precipitation zone)
Control	0.83 \pm 0.1
$237 \mu\text{g ml}^{-1}$	NZ*
$118 \mu\text{g ml}^{-1}$	NZ*
$59 \mu\text{g ml}^{-1}$	NZ*
$29 \mu\text{g ml}^{-1}$	0.62 \pm 0.5*
$14 \mu\text{g ml}^{-1}$	0.79 \pm 0.8*
$7 \mu\text{g ml}^{-1}$	0.81 \pm 0.9

NZ, no zone of precipitation.

* $P < 0.05$ in comparison to control.

Precipitation zone represents the ratio of the diameter of colony to cloudy zone plus colony diameter.

Table 3
Inhibition of blastoconidia germination by MDMs incubated with various concentrations of sertraline

	Germination of <i>C. albicans</i> blastoconidia ($n=7$) (mean \pm S.D.)
Control	$39 \pm 7\%$
$59 \mu\text{g ml}^{-1}$	NG*
$29 \mu\text{g ml}^{-1}$	NG*
$14 \mu\text{g ml}^{-1}$	$5 \pm 4\%*$
$7 \mu\text{g ml}^{-1}$	$12 \pm 4\%*$
$3 \mu\text{g ml}^{-1}$	$27 \pm 9\%$
$1.5 \mu\text{g ml}^{-1}$	$36 \pm 7\%$

NG, no germination of *C. albicans* blastoconidia.

* $P < 0.05$ in comparison to control.

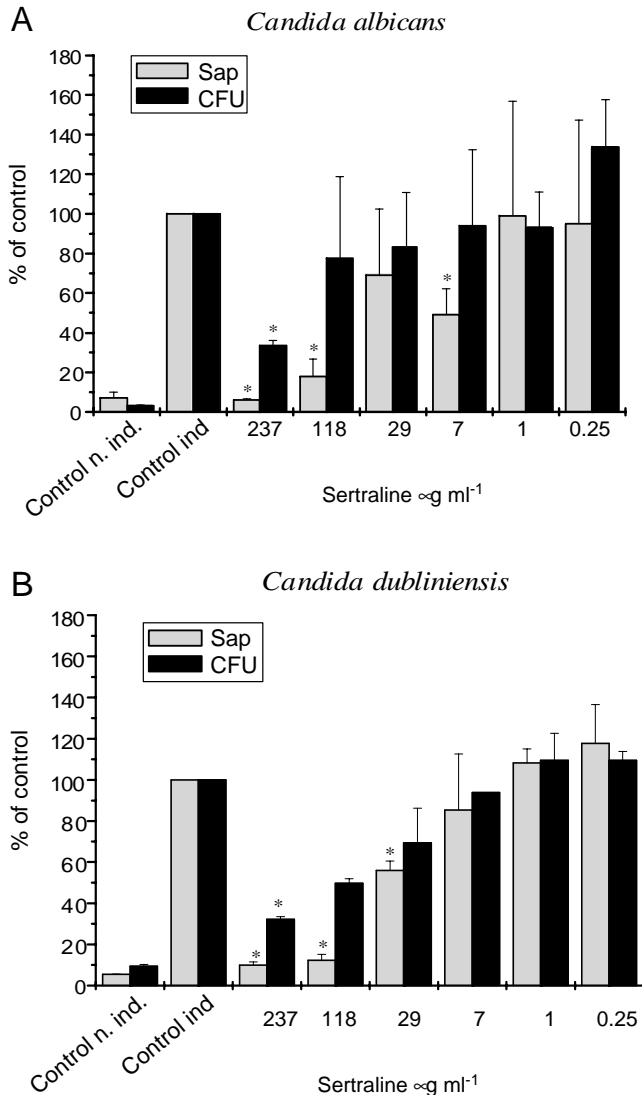


Fig. 1. Decrease in the production of Sap and the growth of *C. albicans* (A) and *C. dubliniensis* (B) (CFU, colony-forming units) incubated in induction medium for 7 days at room temperature in the presence of sertraline. The amounts of sertraline detailed were added every other day. Results are presented as the percentages of the controls treated with saline (control-induced) and are means \pm S.D. of four separate experiments determined at the end of the 7-day incubation period. 'Control not ind.' is a control where *C. albicans* Sap production was not induced, i.e. the yeast was incubated in medium without BSA or vitamins. * $P < 0.05$ in comparison to control.

The morphology of several *Candida* spp. was strongly altered since hyphal elongation was significantly decreased by sertraline. The lack of hyphal transformation for the various isolates of *Candida* spp. was observed at sertraline concentrations between 0.35 and 0.75 $\mu\text{g ml}^{-1}$, which are close to tissue concentrations: the maximal achievable sertraline concentrations in vivo range between 0.05 and 2 $\mu\text{g ml}^{-1}$ [13]. The ability of *C. albicans* to undergo morphologic changes is an important virulence factor [14]. Therefore, it is likely that the inhibition of hyphal elongation is responsible for the favorable in vivo outcome during ser-

traline intervention in patients with recurrent vulvovaginal candidiasis [8]. It is widely recognized that formation of hyphae enhances adherence and tissue invasion, with more efficient release of proteolytic enzymes. Isoforms of Saps, which are encoded by at least 10 related Sap genes, are involved in adherence and thus represent major virulence factors of the opportunistic yeast *C. albicans* [15]. The majority of Sap proteins is secreted by those *C. albicans* cells that directly adhere to the epithelial surface [16], and a dominant Sap isoenzyme in vitro and possibly also in vivo is Sap2 [10,15], which is the predominant isoenzyme investigated here.

In addition to being fungicidal at higher concentrations, sertraline also exerts an inhibitory effect on yeasts by inhibiting Sap (at least Sap2) release, even at concentrations which are a magnitude lower. The mechanism by which sertraline acts on the biology of fungi is not yet known. It is probable that antifungal activity results from an interaction of sertraline with fungal membrane transporter systems [7].

Furthermore, extracellular phospholipase activity, which has been shown to be predictive for mortality in a murine mouse model of disseminated candidiasis, was also affected by treatment of *Candida* with sertraline, at yet higher concentrations.

So far, innate and humoral immunity and cell-mediated immunity are all involved in host defense against *C. albicans* infection [17]. Although the contribution of each appears to be site-specific, innate immunity by polymorphonuclear neutrophils and macrophages dominates protection against systemic candidiasis. In the presence of sertraline, the inhibition of blastoconidia germination was superior by MDMs to that of MDMs alone. One explanation could be a drug-induced enhancement of oxi-

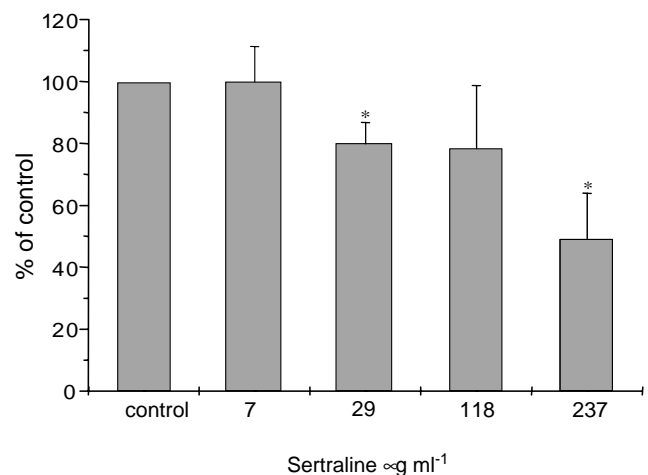


Fig. 2. Amounts of Sap in the supernatants of the positive control, which was grown in induction medium for 7 days at room temperature and was afterwards supplemented with different amounts of sertraline for further 16 h at 20°C to investigate a direct effect on the Saps produced. Results are presented as the percentages of the unsupplemented positive control (0 μg) and are means \pm S.D. of four separate experiments. * $P < 0.05$ in comparison to control.

ductive burst [18], another may lie in the intracellular activity of sertraline. This drug is probably internalized with inoculum and exerts an effect similar to that seen in the extracellular fluid.

In conclusion, our findings show that sertraline treatment of *Candida* spp. significantly ($P < 0.05$) affected hyphal elongation, phospholipase activity, production of secreted aspartyl proteinases, viability of the fungus and the alteration of host cells in vitro. These data probably reflect a direct impact of sertraline on release or expression of virulence factors since fungicidal effects were observed at higher concentrations. So far, the effects observed and the presence of an increased concentration of 5-hydroxytryptamine, which also affects fungal viability [19], during sertraline treatment may finally diminish the virulence of *Candida* spp. in vivo.

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