

## Antifungal properties of 5-hydroxytryptamine (serotonin) against *Candida* species *in vitro*

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In this study the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of 5-hydroxytryptamine (5 HT, serotonin) against clinical isolates of *Candida albicans* ( $n = 11$ ), *Candida glabrata* ( $n = 9$ ), *Candida tropicalis* ( $n = 10$ ) and *Candida parapsilosis* (ATCC 22019) using a broth microdilution test were investigated. In addition, it was examined whether delayed regrowth as a post-antifungal effect results following short exposure to 5 HT. 5 HT showed antifungal activity towards all isolates of *Candida* spp. The isolates yielded comparable MIC and MFC values of 5 HT in the range 0.91–7.34 mM and 1.83–14.68 mM, respectively. A lag in regrowth was dependent on the concentration tested. Treatment for 3 h at concentrations of 5 HT below and equipotent to the MFC resulted in a delayed regrowth of 8–12 h for isolates of *Candida* spp. In conclusion, these *in vitro* studies clearly demonstrate antifungal effects of 5 HT. Identifying the mode of action could be of great help in developing and researching new antifungal drugs.

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## INTRODUCTION

Several years ago antimicrobial activity was described for psychotropic drugs of the phenothiazine and thioxanthene groups (Brown, 1975). Since then, several non-antibacterial substances have been examined and it has been reported that selective serotonin re-uptake inhibitors (SSRIs) influence the *in vitro* viability of bacteria (Cederlund & Mardh, 1993; Munoz-Bellido *et al.*, 1996, 2000) and may reverse chloroquine resistance in *Plasmodium falciparum* (Coutaux *et al.*, 1994). These drugs have significant antimicrobial activity, mainly against Gram-positive bacteria, yet they are inactive against most enteric Gram-negative bacteria (Munoz-Bellido *et al.*, 2000).

Recently, we found that sertraline, a typical SSRI has *in vivo* and *in vitro* antifungal activity (Lass-Flörl *et al.*, 2001a, b). Since fungicidal effects were observed at high concentrations, immunomodulatory effects or several modifications of fungal virulence by SSRIs were more likely to explain the *in vivo* outcome in our patients. In humans, SSRIs modify the behaviour of 5-hydroxytryptamine (5 HT) and act primarily on the 5 HT transporter protein (SERT) (Schloss & Williams, 1998). A block in the re-uptake process of 5 HT causes an increase in 5 HT during therapy with SSRIs (Dimmock *et al.*, 2000). This fact and the clinical phenomenon found in our

patients (Lass-Flörl *et al.*, 2001a) led us to examine the potential antifungal role of 5 HT. We determined the direct influence of 5 HT on the viability of clinical isolates of *Candida* spp. and studied whether delayed regrowth as a post-antifungal effect results following short exposure to 5 HT.

## METHODS

**Strains.** The *in vitro* tests were performed on clinical isolates of *Candida albicans* ( $n = 10$ ), *Candida glabrata* ( $n = 9$ ), *Candida tropicalis* ( $n = 10$ ) and *Candida parapsilosis* (ATCC 22019). Isolates were maintained as suspensions in sterile water at room temperature and subcultures were grown on Sabouraud glucose agar (Merck) incubated at 35 °C for 2 days.

**Drug.** According to the manufacturer's instructions, 5 HT ( $M_r$  212) (Sigma) was dissolved and further diluted in sterile water (Fresenius); final concentrations were 0.47 M–0.22 mM.

**Broth microdilution test.** Isolates were tested using the microbroth dilution method according to the National Committee for Clinical Laboratory Standards (1997) guidelines. A fungal inoculum size of  $1.6 \times 10^4$ – $6 \times 10^4$  c.f.u. ml<sup>-1</sup> was used. A total of 100 µl of each of the drug dilutions was inoculated with 100 µl of the fungal suspensions, and the mixture was incubated at 35 °C and evaluated after 24 h for growth. The minimal inhibitory concentration (MIC) end-point criterion was the lowest drug concentration showing no visible growth after 24 h of incubation. To obtain the minimal fungicidal concentration (MFC), 100 µl volumes were taken from every well and spread on Sabouraud Glucose Agar (Merck). The number of c.f.u. was counted after

**Abbreviations:** 5 HT, 5-hydroxytryptamine; MFC, minimal fungicidal concentration; MIC, minimal inhibitory concentration; SSRI, selective serotonin re-uptake inhibitor.

incubation of the plates at 35 °C for 48 h until growth of subcultures from the growth control well was apparent. MFC was defined as the lowest drug concentration at which 99% of the inoculum was killed.

**Lag in regrowth.** Lag in regrowth was assessed using a modification of the procedure of Nagl *et al.* (1999). 5 HT concentrations equipotent, one dilution above and one below to the MFC for each isolate were investigated. Fungal suspensions were prepared as described above and incubated with 5 HT for 1 and 3 h at 35 °C. Afterwards, these suspensions were centrifuged at 4000 g for 2 min and the supernatants were aspirated. Then, the fungi were washed twice with sterile water and refilled with RPMI 1640. Quantitative cultures of non-diluted samples and 1:100 and 1:1000 dilutions in water were spread on Sabouraud glucose agar and incubated at 35 °C. With a magnifier we visually examined the plates for growth of fungi and investigated colony size and colony counts every 12 h. We compared the time required for colony regrowth of untreated and treated isolates and examined the cultures for delayed growth. Each experiment was done twice and performed in duplicate.

## RESULTS

### Broth microdilution test

5 HT was effective towards the tested fungi, as shown in Table 1. The MIC and MFC ranges at 24 h for *Candida* spp. were 0.91–7.38 mM and 1.83–14.68 mM, respectively.

### Lag in regrowth

The delay in colony regrowth depended on the concentration tested, as shown in Table 2. Treatment for 3 h with 5 HT at concentrations below and equipotent to the MFCs showed a lag in regrowth of 8–12 h for several isolates. Minor effects were seen after an exposure time of 1 h. Concentrations higher than the MFC resulted in a decrease in the c.f.u. count.

## DISCUSSION

Our study revealed that 5 HT can inhibit and kill isolates of *C. albicans*, *C. glabrata* and *C. tropicalis*. In addition, a delay in fungal regrowth depended on the concentration tested.

There is a surprising coincidence of an increased rate of infection and low 5 HT levels in certain diseases, e.g. AIDS (Larsson *et al.*, 1989), Down's syndrome (Tu & Zellweger, 1965) and Chediak–Higashi syndrome (Rendu *et al.*, 1983). A possible role of 5 HT in antifungal host defence has been suggested by a few other studies. Christin *et al.* (1998) reported that platelets, which contain 5 HT attach to the cell wall of hyphae of *Aspergillus fumigatus* and damage this organism. Furthermore, we observed that 5 HT is able to kill conidia and hyphae of *Aspergillus* spp. *in vitro* (Lass-Flörl *et al.*, 2002). Even so, it is known that several antimycotic drugs interfere with platelets (Helmeste *et al.*, 1998): miconazole and econazole inhibit platelet uptake of 5 HT and could therefore

**Table 1.** Antifungal concentrations of 5 HT against *Candida* species

Species	5 HT concn (mM)					
	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>	MFC range	MFC <sub>50</sub>	MFC <sub>90</sub>
<i>C. albicans</i> (n = 11)	1.83–7.34	3.67	7.34	1.83–14.68	7.34	14.68
<i>C. glabrata</i> (n = 9)	0.91–3.67	1.83	3.67	1.83–7.34	3.67	7.34
<i>C. tropicalis</i> (n = 10)	1.83–3.67	3.67	3.67	1.83–7.34	3.67	7.34
<i>C. parapsilosis</i> (ATCC 22019)	3.67–7.34			7.34–14.68		

**Table 2.** *In vitro* effects of *Candida* spp. (n = 30) treated with different concentrations of 5 HT

Exposure time to 5 HT (h)	Species	No. of isolates with certain effects (% in parentheses)*		
		NE	LAG	c.f.u.↓
1	<i>C. albicans</i> (n = 11)	5 (45), 8 (73), 11 (100)	6 (55), 3 (27), 0 (0)	0 (0), 0 (0), 0 (0)
	<i>C. glabrata</i> (n = 9)	5 (55), 9 (100), 9 (100)	4 (45), 0 (0), 0 (0)	0 (0), 0 (0), 0 (0)
	<i>C. tropicalis</i> (n = 10)	4 (40), 8 (80), 10 (100)	6 (60), 2 (20), 0 (0)	0 (0), 0 (0), 0 (0)
3	<i>C. albicans</i> (n = 11)	0 (0), 0 (0), 8 (73)	6 (55), 8 (73), 3 (27)	5 (45), 3 (27), 0 (0)
	<i>C. glabrata</i> (n = 9)	0 (0), 0 (0), 7 (78)	5 (55), 7 (78), 2 (22)	4 (45), 2 (22), 0 (0)
	<i>C. tropicalis</i> (n = 10)	0 (0), 0 (0), 9 (90)	8 (80), 10 (100), 1 (10)	2 (20), 0 (0), 0 (0)

\*Data are presented in the order (i) 5 HT concentration one dilution above the MFC for each isolate, (ii) 5 HT concentration equipotent to the MFC for each isolate and (iii) 5 HT concentration one dilution below the MFC for each isolate. NE, No effects; LAG, lag in regrowth of 8–12 h; c.f.u.↓, 80–90% killing of inoculum.

contribute synergistic effects in the defence against fungal infections. Recently, it was reported that incubation of neutrophils with 5 HT results in a modulation of their bactericidal efficacy (Schuff-Werner & Spletstoeser, 1999).

However, since our *in vitro* effects were observed at high 5 HT concentrations the relevance for direct antifungal host defence remains unclear. *In vivo*, 5 HT levels occur under physiological and pathophysiological conditions (Harenbe *et al.*, 2000). Base levels in several tissues is probably determined by platelets present in those tissues rather than by 5 HT localized in parenchymal cells. Similar considerations apply to 5 HT levels during inflammation. 5 HT content in platelets is quite variable (range about  $748.3 \pm 448.3$  ng per  $10^9$  cells; Kumar *et al.*, 1990) whereas the serum 5 HT levels can exceed  $7987$  ng ml<sup>-1</sup> in several circumstances (Harenbe *et al.*, 2000). It is possible that these levels may be sufficient to modify fungal virulence. A lag in regrowth was observed after exposure to 5 HT and the extent of this effect depended on the concentration of 5 HT and the incubation time. The maximum duration of lag in regrowth was observed at incubation times lower than required for killing, yet at concentrations similar to the MFC.

In conclusion, 5 HT acts against *Candida* spp. in at least two steps: reversible attenuation and, if incubation is prolonged, irreversible changes, resulting in loss of viability. The data encourage us to focus on the relationship between *Candida* spp. and 5 HT and to define the role of 5 HT in antifungal host defence. It can be imagined that 5 HT acts in several ways, both directly on the fungi and indirectly on the defence system. At any rate, 5 HT could contribute synergistic antifungal effects when SSRIs are administered. Identification of the mode of action of 5 HT would be of great help in the research and development of new antifungal drugs.

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