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

Cornelia Lass-Flörl, Dietmar Fuchs, Maximilian Ledochowski, Cornelia Speth, Manfred P. Dierich and Reinhard Würzner

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 fungical 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 fungical 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

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.

Correspondence:
Cornelia Lass-Flörl
Cornelia.Lass-Florl@uibk.ac.at

Received: 25 June 2002
Accepted: 24 September 2002

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 *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-Florl et al., 2002). Even so, it is known that several antimycotic drugs interfere with platelets (Helmeš 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

<table>
<thead>
<tr>
<th>Species</th>
<th>5 HT concn (mM)</th>
<th>MIC range</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>MFC range</th>
<th>MFC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MFC&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> (n = 11)</td>
<td>1.83–7.34</td>
<td>3.67</td>
<td>7.34</td>
<td>1.83–14.68</td>
<td>7.34</td>
<td>14.68</td>
<td></td>
</tr>
<tr>
<td><em>C. glabrata</em> (n = 9)</td>
<td>0.91–3.67</td>
<td>1.83</td>
<td>3.67</td>
<td>1.83–7.34</td>
<td>3.67</td>
<td>7.34</td>
<td></td>
</tr>
<tr>
<td><em>C. tropicalis</em> (n = 10)</td>
<td>1.83–3.67</td>
<td>3.67</td>
<td>7.34</td>
<td>1.83–7.34</td>
<td>3.67</td>
<td>7.34</td>
<td></td>
</tr>
<tr>
<td><em>C. parapsilosis</em> (ATCC 22019)</td>
<td>3.67–7.34</td>
<td></td>
<td></td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Exposure time to 5 HT (h)</th>
<th>Species</th>
<th>No. of isolates with certain effects (% in parentheses)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NE</td>
</tr>
<tr>
<td>1</td>
<td><em>C. albicans</em> (n = 11)</td>
<td>5 (45), 8 (73), 11 (100)</td>
</tr>
<tr>
<td></td>
<td><em>C. glabrata</em> (n = 9)</td>
<td>5 (55), 9 (100), 9 (100)</td>
</tr>
<tr>
<td></td>
<td><em>C. tropicalis</em> (n = 10)</td>
<td>4 (40), 8 (80), 10 (100)</td>
</tr>
<tr>
<td>3</td>
<td><em>C. albicans</em> (n = 11)</td>
<td>0 (0), 0 (0), 8 (73)</td>
</tr>
<tr>
<td></td>
<td><em>C. glabrata</em> (n = 9)</td>
<td>0 (0), 0 (0), 7 (78)</td>
</tr>
<tr>
<td></td>
<td><em>C. tropicalis</em> (n = 10)</td>
<td>0 (0), 0 (0), 8 (90)</td>
</tr>
</tbody>
</table>

*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 & Spletstoesser, 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 physiologic and pathophysiologic 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 by 5 HT assessed in 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 could contribute synergistic effects when SSRIs are administered. Identifications of selective serotonin reuptake inhibitors against Aspergillus species in vitro. J Antimicrob Chemother 47, 775–779.

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.

REFERENCES


