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Antifungal properties of selective serotonin reuptake inhibitors against *Aspergillus* species *in vitro*

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This study investigated the fungicidal activity of selective serotonin reuptake inhibitors (SSRIs) against clinical isolates of *Aspergillus fumigatus* (*n* = 11), *Aspergillus flavus* (*n* = 9), *Aspergillus terreus* (*n* = 10) and *Candida parapsilosis* (ATCC 22019). The common drugs fluoxetine, seroxate, sertraline, paroxetine and reboxetine were applied in a broth microdilution test. In addition, we examined whether a post-antibiotic effect occurs following short exposure to the drugs. The various SSRIs showed time- and dose-dependent effects and were fungicidal towards the organisms tested. Sertraline and fluoxetine were the most active drugs, yet there were differences in the susceptibility of the various isolates tested. A lag of regrowth was dependent on the various SSRIs tested and their concentration. Treatment for 4 h at concentrations of sertraline below and equipotent to the minimal fungicidal concentration resulted in a lag of regrowth of 8–24 h for isolates of *A. fumigatus* and *A. flavus*. In conclusion, our *in vitro* studies clearly demonstrate antifungal effects of SSRIs. Animal studies are needed to evaluate the potential role of these psychotropic drugs in the management of fungal infections.

Introduction

Years ago antimicrobial activity was described 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[2–4] and may reverse chloroquine resistance in *Plasmodium falciparum*.[5] These drugs have a significant antimicrobial activity, mainly against Gram-positive bacteria, yet are inactive against most enteric Gram-negative bacteria.[4]

SSRIs are increasingly being used as first-line therapy for severe premenstrual syndrome (PMS) and as antidepressants.[6] Recently, we found that sertraline (SSRI, Tresleen, Vienna, Austria) has *in vivo* and *in vitro* antifungal activity against *Candida* spp.[7] In this study we examined whether SSRIs also exert fungicidal effects against *Aspergillus* spp. conidia and hyphae. The common drugs fluoxetine, seroxate, paroxetine, sertraline and reboxetine were investigated. In addition, we studied whether a post-antibiotic effect results following short exposure to the various drugs tested.

Materials and methods

Strains

The *in vitro* tests were carried out on clinical isolates of *Aspergillus fumigatus* (*n* = 10), *Aspergillus flavus* (*n* = 9), *Aspergillus terreus* (*n* = 10) and *Candida parapsilosis* (ATCC 22019). Isolates were maintained as suspensions in water at room temperature, and subcultures were grown on Sabouraud glucose agar (Merck, Darmstadt, Germany) and incubated at 35°C for 5 days. One isolate of *A. fumigatus* (isolate AF 72) resistant to itraconazole *in vitro* and *in vivo*, kindly provided by D. W. Denning, was included in the study.

Drugs

Sertraline HCl (mol. wt 343 kDa), fluoxetine HCl (mol. wt 346 kDa), paroxetine HCl (mol. wt 365 kDa), citaloprame HCl (mol. wt 324 kDa) and reboxetine methansulphonate (mol. wt 410 kDa) were kindly provided by their respective manufacturers. The drugs were dissolved in sterile aqua ad injectionem (Fresenius, Linz, Austria) at room temperature for 30 min and centrifuged. Depending on the solu-

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bility of the drug substances (manufacturers' information) stock solutions were obtained and serial two-fold drug dilutions of the supernatant were prepared: sertraline HCl, 1900–7 mg/L; fluoxetine HCl and citaloprame HCl, 5000–19 mg/L; paroxetine HCl, 4000–15 mg/L; and reboxetine methansulphonate, 1000–3 mg/L.

Broth microdilution tests for conidia

Fungi were tested in a modified broth microdilution method as described previously.[8,9] The conidial suspension was harvested by flooding each colony with 2 mL of sterile 0.85% saline. Turbidity was measured with a spectrophotometer at 350 nm (DU-64 Spectrophotometer; Beckman, Toulerton, MN, USA); transmission was adjusted with sterile 0.85% saline to 78–82% for *A. flavus*, 87–82% for *A. fumigatus* and *A. terreus*, and 78–72% for *C. parapsilosis*. The suspension was further diluted in RPMI 1640 medium to obtain 1.2–8 $\times$ 10^5 cfu/mL for *Aspergillus* spp. and 3.2–4 $\times$ 10^4 cfu/mL for *C. parapsilosis*. A total of 100 mL of each of the drug dilutions was inoculated with 100 mL of the fungal suspensions, and the mixture was incubated at 35°C. Final concentrations used were 950–3 mg/L for sertraline, 2500–9 mg/L for fluoxetine and citaloprame, 2000–7 mg/L for paroxetine and 500–1.5 mg/L for reboxetine.

To determine the minimal fungicidal concentration (MFC), 100 μL volumes were taken from every well at 8, 24 and 48 h of incubation and spread on Sabouraud glucose agar. The number of cfu was counted after incubating the plates at 35°C for 72 h until growth of subcultures from the growth control well was apparent. The MFC was defined as the lowest drug concentration at which 99% of the inoculum was killed. Each experiment was done twice and carried out in duplicate.

Broth microdilution test for hyphae

The conidial stock solutions were prepared as described above in RPMI 1640 containing 10 mM HEPES (Sigma, St Louis, MO, USA). A modified culture technique was used as described earlier.[9] Briefly, 100 μL of conidial suspensions were added to 96-well plates (Costar, Vienna, Austria), and the plates were incubated at 30°C for 16–22 h for the formation of hyphae. At this time, drug concentrations were added similarly to conidial tests and the MFC was determined as described above. Each experiment was done twice and carried out in duplicate.

Post-antibiotic effect

Conidial suspensions were prepared as described above and incubated with the various antidepressants for 1 and 4 h at 35°C. Lag of regrowth was assessed using a modification of the procedure of Nagl *et al.*[10] Concentrations equipotent to, one dilution above and one below the MFC for each isolate were investigated. Afterwards, fungi were washed twice with sterile water, centrifuged at 4000**g** for 2 min and refilled with RPMI 1640. Quantitative cultures of non-diluted samples and 1:100 and 1:1000 dilutions in aqua were spread on Sabouraud glucose agar, incubated at 35°C and examined visually for growth every 12 h. We compared the time required for colony count of untreated and treated isolates and examined the cultures for a lag of regrowth. Each experiment was done twice and carried out in duplicate.

Results

Broth microdilution tests for conidia and hyphae

The SSRIs showed time- and dose-dependent effects and were fungicidal towards the tested fungi, as shown in Table 1. Sertraline, followed by fluoxetine, were the most active drugs with isolate-dependent *in vitro* susceptibility. For sertraline the MFC ranges at 8 h (48 h) for *Aspergillus* conidia and hyphae were 14–475 mg/L (3–237 mg/L) and 7–118 mg/L (3–59 mg/L), respectively. For sertraline the MFC for *C. parapsilosis* was 29–59 mg/L during 48 h of incubation. Paroxetine, citaloprame and reboxetine showed comparable MFCs, yet with higher values. The MFC ranges of the various SSRIs at 24 h were similar to the MFCs at 48 h for *Aspergillus* conidia and hyphae (data not shown).

Post-antibiotic effect

Lag of regrowth depended on the individual SSRIs and the concentration tested, as shown in Table 2. Treatment for 4 h with sertraline at concentrations below and equipotent to the MFCs (48 h) for conidia showed a lag of regrowth of 8–24 h for some isolates. No effects were seen after an exposure time of 1 h. Concentrations higher than the MFC resulted in a lag of regrowth and/or a decrease in cfu count for sertraline. A lag of regrowth for fluoxetine, reboxetine, paroxetine and citaloprame was shown for some isolates after an exposure time of 1 and 4 h.

Discussion

The five SSRIs tested in this study displayed different potencies with respect to both antifungal killing and lag of fungal regrowth. Sertraline and fluoxetine showed the highest activity against *Aspergillus* spp. and *C. parapsilosis* with differences in susceptibility of the various isolates tested.

A number of non-antibiotic drugs such as anti-inflammatory drugs,[11] mucolytic agents[12] and proton pump inhibitors[13] exert an influence on the physiology and viability of bacteria. The precise mechanism by which these drugs affect bacteria is not yet known,[14] and activity against fungi has not been reported before to our knowledge.

Table 1. *In vitro* activity of the selective SSRIs against 30 isolates of *Aspergillus* spp.

(a) MFC ranges

			MFC[a] range (mg/L)									
			sertraline		fluoxetine		paroxetine		citaloprame		reboxetine	
Fungi	cfu/mL	Inoculum	8 h	48 h	8 h	48 h	8 h	48 h	8 h	48 h	8 h	48 h
A. fumigatus	$1.2–8 \times 10^5$	C	29–237	7–118	78–1250	39–625	250–2500	250–500	312–1250	312–1250	500	500
($n = 10$)		H	7–118	7–59	156–312	39–156	62–2000	62–125	625–500	312–2500	500	500
A. fumigatus	$3.6–4.2 \times 10^5$	C	14–29	7–14	78–156	39–78	500–1000	250–500	1250	625–1250	1000	1000
AF 72		H	7–14	3–7	312	156–312	2000	1000–2000	2500	1250	1000	500
A. flavus	$1.5–4.4 \times 10^5$	C	14–475	3–237	156–2500	39–1250	62–1000	62–500	312–1250	156–625	1000	500–1000
($n = 9$)		H	29–118	7–59	78–156	39–78	125–500	62–500	625–2500	312–1250	500	500
A. terreus	$2.4–5.2 \times 10^5$	C	29–237	14–118	59–2500	39–1250	500–1000	500	625–1250	312–1250	500	500–1000
($n = 10$)		H	29–118	14–29	625	312–625	2000	500–1000	312–625	156–625	500–1000	500
C. parapsilosis ATCC 22019	$3.2–4 \times 10^4$	B	59	29–59	78–156	156	500	500	1250	1250	1000	1000

[a]MFC, minimal fungicidal concentration (99% killing); C, conidia; H, hyphae; B, blastoconidia.

(b) MFC_{90}s

		MFC_{90} (mg/L)	
Compound		8 h	48 h
Sertraline	C	237	118
	H	118	59
Fluoxetine	C	1250	625
	H	625	312
Paroxetine	C	1000	500
	H	2000	1000
Citaloprine	C	1250	625
	H	2500	1250
Reboxetine	C	1000	500
	H	500	500

C, conidia; H, hyphae.

Table 2. Post-antibiotic effects of *Aspergillus* spp. ($n = 30$) treated with different concentrations of the various SSRIs for 1 and 4 h

		Lag of regrowth of *Aspergillus* spp.									
	SSRI	sertraline		fluoxetine		paroxetine		citaloprame		reboxetine	
Fungi	concentration	1 h	4 h	1 h	4 h	1 h	4 h	1 h	4 h	1 h	4 h
A. fumigatus	>MFC[a]	NE–LAG ↓	LAG ↓ ↓–CFU ↓	LAG ↓	LAG ↓ ↓	NE	LAG ↓	NE–LAG ↓	LAG ↓	NE–LAG ↓	NE–LAG ↓
($n = 11$)	MFC[b]	NE	LAG ↓–LAG ↓ ↓	NE	NE–LAG ↓	NE	NE–LAG ↓	NE	NE–LAG ↓	NE	NE
	<MFC[c]	NE	LAG ↓	NE	NE	NE	NE	NE	NE	NE	NE
A. flavus	>MFC[a]	LAG ↓ –CFU ↓	CFU ↓	LAG ↓	LAG ↓	NE–LAG ↓	LAG ↓	NE–LAG ↓	LAG ↓	NE–LAG ↓	LAG ↓
($n = 9$)	MFC[b]	NE	LAG ↓–LAG ↓ ↓	NE	LAG ↓	NE	NE–LAG ↓	NE	NE	NE	NE
	<MFC[c]	NE	LAG ↓	NE	NE	NE	NE	NE	NE	NE	NE
A. terreus	>MFC[a]	NE–LAG ↓	CFU ↓	NE	LAG ↓–LAG ↓ ↓	NE–LAG ↓	LAG ↓	NE–LAG ↓	LAG ↓	NE–LAG ↓	LAG ↓
($n = 10$)	MFC[b]	NE	LAG ↓–LAG ↓ ↓	NE	NE	NE	NE–LAG ↓	NE	NE	NE	NE
	<MFC[c]	NE	NE–LAG ↓	NE	NE	NE	NE	NE	NE	NE	NE

CFU ↓, 80–90% killing of inoculum; LAG ↓, lag of regrowth for 8–12 h; LAG ↓ ↓, lag of regrowth for 12–24 h; NE, no effects.
[a]Concentration one dilution above the MFC (48 h) for each isolate.
[b]Concentration equipotent to the MFC (48 h) for each isolate.
[c]Concentration one dilution below the MFC (48 h) for each isolate.

In humans, SSRIs modify the behaviour of 5-hydroxytryptamine (5HT) in the synapse space.[15] These antidepressants act primarily at the 5HT transporter protein (SERT) and block the reuptake process of 5HT.[14,15] SERTs, with mol. wts of 60–80 kDa and 12 transmembrane domains are similar to other biogenic amine transporters and part of sodium- and chloride-dependent transporters.[15] It is probable that antifungal activity results from an interaction of SSRIs and fungal transporter systems, as has been noted for *Staphylococcus aureus* and chlorpromazine.[14]

We observed, however, that the various neuronal monoamine reuptake inhibitors were active against the two types of inocula tested. Recently, we found that in order to kill hyphae *in vitro*, antifungals had to be applied at significantly higher concentrations.[9] Surprisingly, similar phenomena were not seen for the various SSRIs tested. In contrast, MFCs for conidial inocula were higher as compared with hyphal inocula in some isolates, as shown in Table 1. Importantly, our data show that the broad antifungal effect is also exerted on the itraconazole-resistant *A. fumigatus* strain.

Considering the bioavailability of these antidepressant drugs, it remains unclear whether our *in vitro* findings are of relevance *in vivo*. The maximal achievable concentrations range between 0.1 mg/L for fluoxetine and 0.05 mg/L for sertraline,[15] and were clearly lower than the MFCs for the strains tested. Nevertheless, concentrations of sertraline in the cerebrospinal fluid and brain are >40-fold higher (2 mg/L) as compared with plasma levels.[16] It is possible that these serum levels may be sufficient to modify fungal virulence. A lag of regrowth was observed after exposure to the agents, and the extent of this effect depended on the concentration and incubation time of the various psychotropic substances. The maximum duration of lag of regrowth was observed at incubation times shorter than those required for killing, but at concentrations similar to the MFC.

In conclusion, SSRIs act against *Aspergillus* spp. in at least two steps: reversible attenuation and, if incubation is prolonged, irreversible changes resulting in loss of viability. Our *in vitro* findings probably provide a rationale for the local treatment of fungal infections with formulae containing SSRIs. Animal models and clinical trials are highly warranted to evaluate the potential role of SSRIs in the management of fungal infections. Identification of the mode of action could be of great help in the development and research of new antifungal drugs.

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