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Research Article

Antifungal Activity of Antidepressant Sertraline against *Candida* Species *In vitro*: A Potential Beneficial Association with Fluconazole

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Abstract

Among vaginal infections, vulvovaginal candidosis (VVC) is the second most frequent vaginal infection and is one of the most common clinical diseases caused by *Candida spp*. VVC is usually treated very effectively with azoles. However, complicated episodes require more intense and prolonged therapeutic approaches and even multidrug regimens.

Different studies reported an *in vitro* antimicrobial activity of selective serotonin reuptake inhibitors (SSRIs) antidepressants against several fungi including *Candida spp*. Thus, the main goal of this study was to determine the antimicrobial activity of the SSRI sertraline, alone and in combination with fluconazole, against twenty-nine *Candida spp*. strains.

Sertraline MIC and MLC values ranged from 4.9 to 78µg/ml and 9.8 to 78µg/ml, respectively. For the majority of the strains (23 from the 29 enrolled) the result corresponded to a fungicidal effect (MIC=MLC), while for the remaining strains the MLC value was twice the MIC value.

The combination of sertraline with fluconazole showed a synergic effect in yeast growth inhibition for the majority of the enrolled strains (FIX values between 0.07 and 0.28). In the resistant strains that showed a response to the synergistic effect, the fluconazole MIC value decreased up to 64-fold.

These results reinforce the potential of SSRIs to be used, alone or associated with conventional antifungal, as a new strategic therapeutic approach to treat resistant VVC.

INTRODUCTION

Vaginal infections represent one of the most frequent reasons for women to seek medical consultation. In fact more than 70% of adult women did have a vaginal problem and have used vaginal products to treat infections [1].

Among vaginal infections, vulvovaginal candidosis (VVC) is the second most frequent vaginal infection and is one of the most common clinical diseases caused by *Candida spp.* [2]. *Candida albicans* is the predominant cause of VVC, accounting for 50% to 70% of cases. However, the epidemiology of *Candida* infection has changed in recent years [3].

VVC is usually treated very effectively with azoles, including short-course topical treatments, in sporadic or uncomplicated cases, with no azoles resistance suspected [4].

Complicated episodes, reported in 10% of patients, require more intense and prolonged therapeutic approaches, often combining topical and oral therapies, or even multidrug regimens [5].

Since the first antimicrobial activity was described for a psychotropic drug, almost sixty years ago, a number of non antibiotic drugs have been shown to have some influence on the physiology and on the viability of microorganisms [6]. One group of these non antibiotic drugs is third generation antidepressants, the selective serotonin re-uptake inhibitors (SSRI). Specifically, sertraline has proven to have a significant antimicrobial activity, mainly against Gram positive bacteria [6].

The potential antifungal activity of this antidepressant was first observed in a clinical setting where three patients with premenstrual dysphoric disorder (PMDD) and VVC were treated with sertraline. Interestingly, VVC related clinical symptoms disappeared during the sertraline therapy but recurred after the treatment was stopped [7].

In accordance with these clinical findings, some studies reported the *in vitro* antimicrobial activity of this drug against *Saccharomyces cerevisiae, Aspergillus spp., Candida spp. and Cryptococcus neoformans*. However, the studies enrolled a small number of strains and the conclusions regarding the interest of this drug as a future antifungal agent were contradictory [8–11].

Thus, the main goal of this study was to determine the antimicrobial activity of the SSRI sertraline alone and in combination with fluconazole, against a significant number of *Candida spp*. strains in order to expand the knowledge regarding the possible usage of this drug as an antifungal, especially in the most difficult clinical cases.

MATERIAL AND METHODS

Drugs

Among antidepressant drugs we chose sertraline hydrochloride, which was generously provided by Bluepharma Pharmaceutical (Coimbra, Portugal). A stock solution with 2,500 µg/ ml in de mineralized water was prepared at room temperature, according to the manufacturer's instructions. From the stock solution, serial 1:2 dilutions were prepared using RPMI medium (Biochrom AG Germany) immediately at the beginning of the test.

Fluconazole (Sigma-Aldrich, Sintra, Portugal) stock solution was prepared in DMSO (Sigma-Aldrich, Sintra, Portugal) at a concentration of 512 µg/ ml. 1:2 dilutions were prepared using RPMI medium, as described [12].

Yeast strains

Twenty-nine *Candida* strains were included in this study corresponding to two American Type Culture Collection strains (*C. albicans* ATCC 10231 and *C. albicans* ATCC 90028) and twenty-seven clinical isolates distributed as follows: seven *C. albicans*, six *C. tropicalis*, four *C. glabrata*, two *C. krusei*, two *C. guillermondii*, one *C. sphaerica*, four *C. Parapsilosis* and one *C. lipolytica*. *Candida* isolates were characterized to species using molecular identification and API 32C testing (bio- Mérieux, Vercieux, France).

The strains were kept frozen in brain heart infusion broth (Difco Laboratories, Detroit, MI) with 5% glycerol (Sigma-Aldrich, Sintra, Portugal) at -70°C. Prior to testing, samples were thawed at room temperature and subcultured twice on SDA to assess their viability in culture.

Antifungal activity

Sertraline and fluconazole anti-*Candida* activity was assessed according to the CLSI reference M27-A3 microdilution method [13]. Minimal inhibitory concentration (MIC) for sertraline was assessed after 48h of incubation under aerobic conditions at 37º C. MIC corresponded to the lowest concentration of the drug that prevented yeast growth, assessed by visual inspection of the microplates. Strains were grownin the absence of the drugs (positive control) and in pure non inoculated media(sterility control) under the same incubation conditions [14]. Minimal lethal concentration (MLC) was determined according to the previous described methods by Canton et al. [14]. In order to evaluate the effect of combining sertraline and fluconazole, the MIC value for fluconazole was also assessed. For this drug the MIC was defined as 50% reduction of the yeast growth by spectrophotometric evaluation at 530 nm, as described in the CLSI protocol [12]. The association effect of these two drugs in the yeast growth inhibition was assessed using the checkerboard technique [15]. Briefly six strains of C. *albicans* were placed in a two-dimensional microplate with 50 µl of each drug and incubated at 37º C for 48h under aerobic conditions. The interaction between both compounds was calculated using the fractional inhibitory concentration (FIC) and fractional inhibitory index (FIX). FIX interpretation defines a synergic effect as values ≤ 0.5, additive effect as values between 0.5 and 4.0, ; and antagonism when ≥ 4.0 [15].

All experiments were performed in duplicate and repeated in three independent experiments.

RESULTS

The antifungal effect of sertraline against the twenty-nine tested *Candida* strains is presented in Table (1), expressed in MIC and MLC values. Depending on the tested concentrations, the drug showed a fungistatic or fungicidal activity against all tested strains. In fact, the MIC and MLC values match for the majority of the strains (23 of the 29 strains tested), with exception of two *C. albicans*, one *C. glabrata,* one *C. tropicalis*, one *C. spaherica* and

Table 1: Antifungal activity of SSRI Sertraline against *Candida spp.*

Strain	MIC (µg/ml)	MFC (µg/ml)
Candidaalbicans ATCC 102	78	78
Candidaalbicans ATCC 9800	39	39
Candidaalbicans MP14	39	78
Candidaalbicans MP25	78	78
Candidaalbicans MP26	78	78
Candidaalbicans AP25A	39	39
Candidaalbicans AP26B	39	39
Candidaalbicans MP27	9.8	19
Candidaalbicans MP24	9.8	9.8
Candidaglabrata MP7	39	39
Candidaglabrata MP8	39	39
Candidaglabrata MP28	39	78
Candidaglabrata MP29	39	39
Candidaguilliermondi MP1	39	39
Candidaguilliermondi MP2	19	19
Candidakrusei MP16	39	39
Candidakrusei MP17	39	39
Candidasphaerica AP35B	4.9	9.8
Candidatropicalis MP4	78	78
Candidatropicalis MP5	78	78
Candidatropicalis MP37	78	78
Candidatropicalis MP38	78	78
Candidatropicalis MP39	78	78
*Candidatropicalis*MP36	78	78
Candidaparapsilosis MP34	39	39
Candidaparapsilosis MP32	39	39
Candidaparapsilosis MP9	9.8	19
Candidaparapsilosis MP12	78	78
Candidalipolytica MP40	19	19

one *C. parapsilosis* strain. For these six strains the MLC value was twice the MIC value.

Generally, MIC values ranged from 9.8 to 78µg/mL. Only for *C. sphaerica* a lower value was observed (MIC of 4.9µg/ml), being the most susceptible *Candida* specie. In contrast, the most resistant specie was *C. tropicalis* with all strains presenting the same MIC value of 78µg/ml.

Interestingly, and contrary to *C. tropicalis,* different MIC values were obtained for different strains from the same species.

Regarding the phenotypic classification of the six *Candida albicans* strains concerning their susceptibility to fluconazole, the obtained results were expressed in MIC values and are presented in Table (2). The results were interpret using CLSI M27-S4 criteria specific for *Candida albicans* specie[12]. Of the six strains enrolled in the test, five were resistant (R) and presented MICs between 16-128 µg/ml. Regarding the one sensible dose-dependent (S-DD) strain, the obtained MIC was 4 µg/ml.

The checkerboard technique was used to determine the effect of different concentrations of sertraline and fluconazole on the growth of six *Candida albicans* strains. Table (3) represents the MICs obtained for the two drugs, both alone and in combination. A synergistic effect was observed on five of the six tested strains with FIX values ranging from 0.07 to 0.28. All of the five strains were resistant to fluconazole. An additive effect was observed for the remaining strain, the only one with S-DD profile (FIX=0.50). When the drugs were used in combination, the fluconazole MIC for resistant strains decreased up to 64 fold. For all six strains, sertraline MIC decreased between 4 and 32 fold when tested in combination.

DISCUSSION

The need for novel antifungal regimens prompted this study regarding sertraline antifungal activity, which has already shown shown to exhibit antifungal activity towards *Candida spp*. [9,10,16]. In the current study the MICs assessed ranged from 4.9 to 78µg/ml, which are comparable with the previous reported values (MICs between 6.3 and 32µg/ml). The higher range of MIC concentrations here reported is probably related with the higher number of strains included.

Drug combination has been one consistent strategy to overcome antimicrobials limitations and the potential association of sertraline and fluconazole was been previously reported, though with different results [9,11].

In the present study, a synergistic effect resulted from the combination of these drugs upon all five fluconazole resistant strains. This synergism was more evident when the combined concentrations were far below the MICs for each compound (Table 3). It was also found that, for all tested strains, increasing one of the compounds concentrations (reaching MIC values) while maintaining the concentration of the second compound at a low value, resulted in a shift from a synergistic to an additive effect (data not shown).

With regard to the S-DD strain tested, an additive relationship was evident.

Contrarily, Zhai et al., reportedan an antagonism between fluconazole and sertraline in six *Candida* strains. These apparently contradictory results are due to a different analysis methodology used on the two studies. In fact, when the same analysis method is

Table 2: Phenotypic classification of six Candida strains based on their susceptibility to fluconazole.

Strain	Fluconazole MIC-2[a]	Phenotype[b]
Candidaalbicans ATCC 10231	16	R
Candidaalbicans MP14	64	R
Candidaalbicans MP25	64	R
Candidaalbicans MP26	128	R
Candidaalbicans AP25A	4	SDD
Candidaalbicans AP26B	32	R

[a]MIC-2: Drug concentration that caused a 50% reduction in turbidity compared to the growth control.
[b]S-DD: Susceptible Dose Dependent; R, resistant.

Table 3: Effect of sertraline and fluconazole alone and in combination against *Candida albicans* strains[a].

Strain	MIC STR (µg/ml)	MIC STR comb (µg/ml)	MIC FLC (µg/ml)	MIC FLC comb (µg/ml)	FIC STR	FIC FLC[b]	FIX[c]	Effect
Candidaalbicans ATCC 10231	78	2.4	16	4	0.03	0.25	0.28	Synergistic
Candidaalbicans MP14	39	4.9	64	2	0.13	0.03	0.16	Synergistic
Candidaalbicans MP25	78	4.9	64	2	0.06	0.03	0.09	Synergistic
Candidaalbicans MP26	78	4.9	128	2	0.06	0.01	0.07	Synergistic
Candidaalbicans AP25A	39	9.8	4	1	0.25	0.25	0.50	Additive
Candidaalbicans AP26B	39	2.4	32	2	0.06	0.06	0.12	Synergistic

[a]SRT: Sertraline; FLC: Fluconazole; Comb: Combination Of The Two Drugs.
[b]FIC: Fractional Inhibitory Concentration
[c]FIX: The Sum of the FIC for SRT and that for FLC.

applied and the FIX indexes are recalculated for Zhai et al. results, an additive effect is present for some concentration ranges.

Also, Heller et al., reported an antagonist effect of sertraline when combined with itraconazole [11]. These different results are consequence of a different FIX scale. When the results are reanalyzed using the scale defined by Ernst EJ, the relation between sertraline and itraconazole is, in fact, an additive one [15].

Furthermore, these results are in accordance with other previous studies using sertraline and other SSRIs [17,18] and highlight the importance of including these drugs to inhibit the growth of fluconazole resistant strains.

The evident advantage of combining these two drugs leads us to focus our attention on the underlying possible mechanism of action.

The first mechanism suggested for psychotropic drugs was that they may act as efflux pumps Inhibitors, since they also act on human cells as pump inhibitors [6]. However, the fact that SSRIs not only show antifungal activity against fungi, but also impair a number of processes present in bacteria (slime synthesis, swarming) may indicate that they act on fundamental metabolic processes as protein translation [6,9,19,20].

In addition to sertraline, the anti-*candida* activity of SSRIs has already been reported for other molecule, fluoxetine [17]. When comparing the results of both studies, that are basically identical in methodology and strains, all species with exception of *C. glabrata* showed lower MIC and MLC values for sertraline, which indicated a more potent antifungal activity of this drug when compared with fluoxetine. Moreover, and as previously concluded by T. Young et al., the more lipophilic of the two compounds (sertraline logP=5.06; fluoxetine logP=4.05) was also the most efficient against all *candida* strains [10]. This fact may suggest that a non-specific cytotoxic effect may be involved in the antifungal activity of SSRIs.

Although sertraline's mechanism of action was not elucidated, the fact that the drug exhibits fungicidal or fungistatic effect, depending on the tested Concentrations, points us to a multi factorial approach. Studies regarding the drug's mechanism of action are mandatory to confirm this hypothesis.

Concluding, our results do support the use of combined therapies that allow for a lower required dosage of individual drugs, a decrease in host toxicity and enhanced antimicrobial activity, among others benefits [21,22].

Despite that further studies are needed to elucidate the specific mechanism of action of sertraline and other promising SSRIs like fluoxetine, these results do encourage the potential of SSRIs to be used, alone or associated with classic antifungal drugs, as new strategical therapeutic approaches to resistant candidosis.

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