Original Article

Comparison of the Effect of Non-Antifungal and Antifungal Agents on *Candida* Isolates from the Gastrointestinal Tract

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Abstract

Background: Non-antifungal drugs appear promising in treatment of opportunistic infections of *Candida* spp. that are often resistant to current antifungals.

Methods: The broth macrodilution method (NCCLS M27-P document) was used to compare the antifungal activity of trifluoperazine, propranolol, and lansoprazole with that of ketoconazole and amphotericin B, using 50 yeast isolates from the GI tract. The minimum fungicidal concentrations (MFCs), resistance rates and the time required for fungicidal activity of the drugs (2 – 48 hours) were determined.

Results: The most effective antifungal activity was exhibited by trifluoperazine. Its MFC was 32 µg/mL for *Candida albicans* (3.3% resistance) and *Candida* spp. (0% resistance) yeasts, and 64 µg/mL for *Candida tropicalis* with 10% resistance. The MFC for *C. albicans* and *Candida* spp. was comparable to that of ketoconazole. However, the time required for the inhibitory effect (6 hr) was shorter than that of ketoconazole (48 hr) or amphotericin B (24 hr). The time required for the inhibitory activity on *C. tropicalis* was 24 hr, which was shorter than that of ketoconazole and amphotericin B (48 hr). A considerable number (40%) of *Candida* spp. showed resistance to ketoconazole, and 20% of *C. tropicalis* showed resistance to amphotericin B. Trifluoperazine, an antipsychotic drug, exhibited effective antifungal activity with the MFC, comparable to ketoconazole (32 µg/mL). Among the three yeast groups, *C. tropicalis* showed resistance to trifluoperazine and amphotericin B, and *Candida* spp. was considerably resistant to ketoconazole.

Conclusion: Trifluoperazine could be considered as an alternative antifungal when encountering *Candida spp.* resistant to current antifungals.

Keywords: Antifungals, *Candida* spp., non-antifungals

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Introduction

Candida yeasts inhabit the mucosal surfaces of the human gastrointestinal (GI) tract and vagina as commensals or opportunistic pathogens. Among these, *C. albicans* is the most common species, followed by *C. tropicalis*, *C. parapsilosis*, and *C. glabrata*.[1] Reports demonstrate that fungal infections have increased significantly because of recent changes in medical practice, including the frequent use of antibacterial agents and the recruitment of indwelling medical prostheses. Furthermore, immunosuppressed patients suffering from AIDS and immunocompromised individuals who survive, chemotherapy or organ transplantation, comprise a population with a high susceptibility to invasive fungal infections.[2,3]

During the past two decades, the frequency of invasive fungal infections has increased dramatically in hospitalized patients throughout the world, and *Candida* has emerged as one of the leading causes of bloodstream infections,[2,4] with mortality rates of 38%–75%.[5,6] During the past 15 years, non-albicans *Candida* spp. have accounted for > 50% of episodes of fungemia.[7] Fluconazole and amphotericin B are two antifungals that have been extensively used against severe fungal infections. This has led to the emergence of fluconazole resistance in *C. albicans*[8] and *Candida* spp.[9] and amphotericin B resistance in the less common species of *Candida*.[10] A considerable increase in the rate of invasive fungal infections with high mortality warrants the need for more effective treatments. On the other hand, the use of current antifungal drugs has been restricted due to either the development of resistance[10] or their toxicity.[11] There are reports indicating that certain non-antifungal drugs used for conditions other than the infectious diseases could exhibit antifungal activity, because fungi and human cells share common pathways, both being eukaryotes.[12]

In this study, we compared the fungicidal activity of three non-antifungal drugs against 50 Candida yeast isolates from the GI tract with that of two antifungal agents, ketoconazole and amphotericin B. The non-antifungals included trifluoperazine (with antipsychotic activity), propranolol (the beta blocker with antiarrhythmic activity), and lansoprazole (proton pump inhibitor with antacid activity).

Materials and Methods

Yeast isolates

A total of 50 yeasts, previously isolated from the GI tracts of 50 patients with dyspepsia, were used in this study. They included 30 isolates of *C. albicans* (10 oral, 10 gastric, and 10 intestinal yeasts), 10 isolates of *C. tropicalis* (5 oral and 5 gastric yeasts), and 10 *Candida* spp. (5 oral and 5 gastric yeasts). All isolates were cultured from their frozen (-20°C) stocks on yeast extract glucose chloramphenicol (YGC) agar (Merck), and the single colonies were subcultured at least twice to ensure purity and viability of

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Table 1. The antifungal activity of three non-antifungal and two antifungal agents against 50 isolates of *Candida*, determined by the reference macrodilution method.

Drugs	*C. albicans* (No. 30)				*C. tropicalis* (No. 10)				*Candida* spp. (No. 10)			
	MFC range (µg/mL)	MFC (µg/mL)	No. of susceptible isolates (%)	No. of resistant isolates (%)	MFC range (µg/mL)	MFC (µg/mL)	No. of susceptible isolates (%)	No. of resistant isolates (%)	MFC range (µg/mL)	MFC (µg/mL)	No. of susceptible isolates (%)	No Drugs. of resistant isolates (%)
TFP[a]	8 to > 64	32	29 (96.7)	1 (3.3)	32 to > 64	64	9 (90)	1 (10)	16–64	32	10 (100)	0 (0)
PRO[b]	32 to > 128	64	13 (43.3)	17 (56.6)	≥ 128	128	4 (40)	6 (60)	32 to >128	64	5 (50)	5 (50)
LAN[c]	32 to > 128	128	21 (70)	9 (30)	> 128	> 128	0 (0)	10 (100)	64 to >128	128	2 (20)	8 (80)
KET[d]	8–64	32	30 (100)	0 (0)	16–64	16	10 (100)	0 (0)	4 to >64	32	6 (60)	4 (40)
AMP[e]	0.25–2	0.5	30 (100)	0 (0)	1 to >4	2	8 (80)	2 (20)	0.5–2	1	10 (100)	0 (0)

trifluoperazine ; [b] propranolol; [c] lansoprazole; [d] ketoconazole; [e] amphotericin B

the yeasts. The yeasts were identified based on the color of their colonies on a Chromagar medium (bioMerioux).[13] Isolates of *C. albicans* produced green colonies, *C. tropicalis* produced blue colonies, and *Candida* spp. produced white to mauve colonies.

The Ethics Committee of the Digestive Diseases Research Center, Shariati Hospital, Tehran University of Medical Sciences approved this study based on the ethical principles of human research and experimentation expressed in the Declaration of Helsinki.

Drugs

The drugs included antifungals [ketoconazole (Arastoo, Tehran, Iran) and amphotericin B (Bristol-Myers Squibb, Rome, Italy)] and non-antifungal drugs [trifluoperazine (Industria Chimica Milanese S.p.A, Milan, Italy), propranolol (Daroopakhsh, Tehran, Iran) and lansoprazole (Sanovel, Turkey)]. Stock solutions of trifluoperazine (6 400 µg/mL), propranolol (12 800 µg/mL) and amphotericin B (400 µg/mL) were prepared in sterile distilled water. The stock solutions of lansoprazole (12 800 µg/mL) and ketoconazole (6 400 µg/mL) were prepared in 100% dimethyl sulfoxide (DMSO, Sigma). According to the proposed broth macrodilution reference method (M27-P document) recommended by the CLSI (formerly NCCLS),[14] each tube should have contained 0.1 ml of a 10× concentration of the drug. However, in this study DMSO at a concentration of 10% (vol/vol) had an inhibitory effect on *C. albicans*. Thus, we used 0.01 mL of 100× concentrations of lansoprazole and ketoconazole in DMSO. To observe consistency, stock solutions of the other drugs were also similarly prepared in water. Two-fold serial dilutions of stock solutions were prepared to obtain the concentrations in 0.01 ml of 4 to 64 µg/mL for trifluoperazine and ketoconazole, 16 to 128 µg/mL for propranolol and lansoprazole, and 0.125 to 4 µg/mL for amphoterecin B.

Susceptibility medium

RPMI 1640 medium (Biosera, England) with L-glutamine, without bicarbonate, buffered at pH 7.0 with hydroxyethyl piperazineethane sulfonic acid (an extracellular polar buffer similar to morpholinepropane sulfonic acid), was used for broth macrodilutions.

Susceptibility testing procedure

The broth macrodilution method was performed according to the NCCLS M27-P document.[14] Subcultures on YGC agar incubated at 35°C for 24 – 48 hr were used for inoculation. Two to three colonies, each at least 1 mm in diameter, were suspended in 5 mL of sterile 0.85% saline. The suspension was vortexed for 10 sec, and the cell density was adjusted to the turbidity of 0.5 McFarland's standard. The final cell density of 0.5×10^3 to 2.5×10^3 CFU/mL was obtained by 1:100, and then 1:20 dilutions of the yeast suspension with RPMI 1640 broth medium. The cell density was confirmed by performing a colony count on potato dextrose agar (PDA) (Merck). A volume of 0.9 mL of each yeast suspension was dispensed into tubes containing 0.01 mL of the different drug concentrations, and 0.09 mL of RPMI 1640 was added to each tube to obtain a final volume of 1 mL. Tubes including drug diluents only were used as growth controls. All tubes were incubated at 35°C for 48 hr. Tubes showing no visible growth were used for determination of the minimum fungicidal concentrations (MFCs). A volume of 0.1 mL from each tube was cultured on a PDA. The plates were incubated at 35°C for 48 hr. The MFC was determined as the lowest drug concentration that yielded no colonies. Resistant strains were determined as those inhibited by concentrations of > 128 µg/mL of propranolol and lansoprazole, > 64 µg/mL of trifluoperazine and ketoconazole, and > 4 µg/mL of amphotericin B. The MFC of the drugs for each group of yeasts was selected out of a range of effective concentrations that inhibited the majority of yeasts. The selected MFCs and the frequency of resistance were compared within the three yeast groups. The antifungal activity of trifluoperazine on yeast isolates from the oral cavity (20), stomach (20), and intestine (10) was also compared.

Medium acidification for lansoprazole activation

Proton pump inhibitors such as lansoprazole are acid-activated reagents that undergo acid-catalyzed conversion to the active sulfenic acid and sulfonamide derivatives.[15] It has been determined that the best condition for lansoprazole to exhibit its fungicidal activity is in acidified medium (pH 4). For this purpose, the pH of the final suspension was adjusted to 4 using 1M HCl prior to dispensing into the tubes. The growth control tube was similarly acidified.

Determination of the time required for fungicidal activity

One isolate of each yeast species and the MFCs were recruited for determining the time required for the fungicidal activity of the drugs. The broth macrodilution method was used and a colony count was performed after 8, 24, and 48 hr for propranolol, lansoprazole, ketoconazole, and amphotericin B; and after 2, 4, 6, 8, 24, and 48 hr for trifluoperazine.

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Table 2. The antifungal activity of trifluoperazine, ketoconazole, and amphotericin B against three groups of yeasts, determined by the reference broth macrodilution method.

Organisms (No.)	Trifluoperazine			Ketoconazole			Amphotericin B		
	MFC (µg/mL)	No. of susceptible isolates (%)	No. of resistant isolates (%)	MFC (µg/mL)	No. of susceptible isolates (%)	No. of resistant isolates (%)	MFC (µg/mL)	No. of susceptible isolates (%)	No. of resistant isolates (%)
Oral yeasts (20)	32	19 (95)	1 (5)	32	19 (95)	1 (5)	0.5	18 (90)	2 (10)
Gastric yeasts (20)	32	20 (100)	0 (0)	32	17 (85)	3 (15)	1	20 (100)	0 (0)
Intestinal yeasts (10)	64	9 (90)	1 (10)	16	10 (100)	0 (0)	1	10 (100)	0 (0)

Statistical analysis

SPSS software (version 18) was used for statistical analysis. The statistical tests applied were chi-square and one-way ANOVA (Duncan). $P < 0.05$ was considered as significant.

Results

The macrodilution method was used to compare the fungicidal activity of trifluoperazine, propranolol, and lansoprazole with that of ketoconazole and amphotericin B on 50 yeast isolates from the GI tract. Trifluoperazine exhibited a considerable fungicidal activity on *C. albicans* and *Candida* spp. (MFC 32 µg/mL), and on *C. tropicalis* (MFC 64 µg/mL). One out of 30 yeasts (3.3%) in the *C. albicans* group, 1/10 (10%) in *C. tropicalis* group, and none in the *Candida* spp. group were resistant to trifluoperazine. The time required for the fungicidal activity of trifluoperazine against *C. albicans* and *Candida* spp. was 6 hr. However, *C. tropicalis* was inhibited after 24 hour.

Propranolol showed a lower fungicidal activity with an MFC of 64 µg/mL for *Candida* spp. and *C. albicans*, and 128 µg/mL for *C. tropicalis*. Seventeen out of 30 yeasts (56.6%) among *C. albicans* strains, 6/10 (60%) of *C. tropicalis* strains, and 5/10 (50%) of *Candida* spp. strains showed resistance. The antifungal activity of propranolol was observed after a 48 hr incubation period for *C. albicans* and *C. tropicalis* and 24 hr for *Candida* spp.

Lansoprazole showed a weak fungicidal activity even when its concentration was increased to 128 µg/mL. Nine out of 30 (30%) of *C. albicans* isolates, 10/10 (100%) of *C. tropicalis* strains, and 8/10 (80%) of the *Candida* spp. group exhibited resistance. The time required for the antifungal activity of this drug was determined to be 24 hr for *C. albicans* and *Candida* spp. (Table 1).

Ketoconazole showed fungicidal activity at the MFC of 16 µg/mL for *C. tropicalis* and 32 µg/mL for *C. albicans* and *Candida* spp. None of *C. albicans*, or *C. tropicalis*, and 6/10 (60%) of *Candida* spp. group were resistant to ketoconazole. This antifungal agent was effective on the three species of *Candida* yeasts after 48 hr.

Finally, amphotericin B showed the highest fungicidal activity at an MFC of 0.5 µg/mL for *C. albicans*, 1 µg/mL for *Candida* spp., and 2 µg/mL for *C. tropicalis*. Only 2/10 (20%) of the *C. tropicalis* isolates showed resistance. The fungicidal activity of amphotericin B was observed after 24 hour for *C. albicans* and *Candida* spp.; this time was 48 hr for *C. tropicalis* (Table 1).

Comparison of susceptibility rates of recruited yeasts to the three non-antifungals showed that trifluoperazine was significantly more effective than propranolol and lansoprazole ($P = 0.001$). Furthermore, susceptibility rates of these yeasts to recruited antifungals were significantly higher than non-antifungals ($P = 0.000$).

A comparison of antifungal activity of trifluoperazine on yeast isolates from the oral cavity, stomach, and intestine showed that out of 20 oral yeasts, 19 were susceptible (MFC 32 µg/mL) and only 1 (5%) was resistant. All 20 gastric yeasts were susceptible to trifluoperazine (MFC 32 µg/mL). Nine out of 10 intestinal yeasts (90%) were susceptible to trifluoperazine, although its concentration was increased to 64 µg/mL. The difference between resistance rates of oral, gastric, and intestinal yeasts to trifluoperazine was not significant ($P = 0.418$). No significant difference in resistance rates to ketoconazole ($P = 0.308$) and amphotericin B ($P = 0.219$) was observed among the three groups of yeasts (Table 2).

Discussion

GI tract mucosa appear to be a common place for yeast colonization,[16] through which the *Candida* species could penetrate and cause disseminated candidiasis in immunocompromised patients.[17] *C. albicans* has been recognized as the predominant causal agent of candidiasis.[18] However, the extensive use of antifungals for prophylaxis or for the treatment of patients with impaired immunity has led to the emergence of non-albicans *Candida* species and an increase in resistance to current antifungals.[19,20] Accordingly, non-antifungal drugs with antifungal activity could be regarded as promising compounds to use when current antifungals become limited due to their toxicity or the emergence of resistance.[12] The results of this study showed that the three recruited non-antifungal drugs, trifluoperazine (an antipsychotic drug), propranolol (a beta blocker), and lansoprazole (a proton pump inhibitor) have antifungal activity against the three recruited groups of GI yeasts, *C. albicans*, *C. tropicalis*, and *Candida spp*.

Trifluoperazine exhibited the most effective fungicidal activity on all the three yeast groups ($P = 0.001$). The MFC was determined as 32 µg/mL, which was similar to the MFC of ketoconazole. Among the three *Candida* groups, 96.7% of *C. albicans* and 100% of *Candida* spp. strains were inhibited by the MFC of 32 µg/mL, whereas 90% of *C. tropicalis* strains were inhibited at 64 µg/mL. The time required for the antifungal activity of trifluoperazine was 6 h for *C. albicans* and *Candida spp*., while amphotericin B was effective after 24 hr and ketoconazole after 48 hr. The antifungal activity of trifluoperazine on *C. tropicalis* was determined after 24 hr, and for ketoconazole and amphotericin B after 48 hr. The antifungal activity of trifluoperazine and ketoconazole on 101 *C. albicans* clinical strains has been studied and the minimum inhibitory concentrations (MICs) were determined as 65.8 µg/mL for trifluoperazine and 27.9 µg/mL for ketoconazole.[21] Studies on the antifungal activity of trifluoperazine on *Saccharomyces cerevisiae* revealed that within 30 min of addition, the drug caused membrane damage and interference with the cell cycle. Thus, it was proposed that trifluo-

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perazine might be used against the pathogenic yeasts in systemic infections. Since trifluoperazine accumulates in the central nervous system, it could also be applied against fungal meningitis and encephalitis.[22] The combination of trifluoperazine and ketoconazole in various ratios was found to exhibit a synergistic effect that was considerably higher when compared with ketoconazole alone.[21]

Propranolol inhibited 43.3% of *C. albicans* and 50% of *Candida* spp. strains at the MFC of 64 μg/mL. It also inhibited 40% of *C. tropicalis* isolates when its concentration was increased to 128 μg/mL. Reports indicate that the antifungal activity of propranolol involves the inhibition of hyphal growth by interference with the cAMP-EFG1 pathway in *C. albicans.*[23] It appears that the formation of hyphae plays an important role in fungal virulence and tissue invasion.[24] Lansoprazole exhibited a low antifungal activity, however a considerable number (70%) of *C. albicans* strains were inhibited at the MFC of 128 μg/mL. None of the *C. tropicalis* strains were inhibited, even at the MFC of > 128 μg/mL, and only 20% of *Candida* spp. strains were inhibited at 128 μg/mL. It has been reported that the activated form of lansoprazole (AG 2000) at concentrations of > 200 μM inhibited the hyphal growth of *C. albicans*. Since the membrane proton pump H^+-ATPase activity increases during the hyphal growth of *C. albicans*, it has been suggested that ATPase might be the target molecule of lansoprazole.[25]

Among the three non-antifungal drugs (trifluoperazine, propranolol, and lansoprazole), the most effective antifungal activity against the three groups of yeast isolates from the GI tract was exhibited by trifluoperazine (P = 0.001). The MFC of trifluoperazine was determined as 32 μg/ml for *C. albicans* yeasts with a 3.3% resistance rate. The MFC for *C. tropicalis* was 64 μg/mL with 10% resistance, and for *Candida spp*. it was 32 μg/mL with no resistance. The MFC of trifluoperazine (32 – 64 μg/mL) determined for *C. albicans* and *Candida spp*. was comparable to that of ketoconazole. However, the time required for the inhibitory effect of trifluoperazine on *C. albicans* and *Candida* spp. (6 hour) was shorter than that required for ketoconazole (48 hour) or amphotericin B (24 hr) activity. On the other hand, the MFC of trifluoperazine was 64 μg/mL for *C. tropicalis* and the time required for inhibitory activity was 24 hr compared with that of ketoconazole and amphotericin B, which was 48 hr.

Among the three yeast groups, *C. albicans* isolates showed a resistance rate of 3.3% to trifluoperazine (MFC 32 μg/mL) with no resistance to ketoconazole (MFC 32 μg/mL) or amphotericin B (MFC 0.5 μg/mL). These yeasts showed a considerable resistance to propranolol (56.6%) and lansoprazole (30%) even when the MFCs were increased to 128 μg/mL. In *C. tropicalis* group, 10% were resistant to trifluoperazine (MFC 64 μg/mL), 60% showed resistance to propranolol (MFC 128 μg/mL), and 100% to lansoprazole (MFC > 128 μg/mL); they were all inhibited by ketoconazole (MFC 16 μg/mL). However, 20% were resistant to amphotericin B (MFC 2 μg/mL). In the *Candida* spp. group, trifluoperazine inhibited all (MFC 32 μg/mL), 50% were resistant to propranolol (MFC 64 μg/mL), 80% to lansoprazole (128 μg/mL), and 40% to ketoconazole (MFC 32 μg/mL). However, there was no resistance to amphotericin B (1 μg/mL). These results indicated that among the three yeast groups, *C. tropicalis* strains showed resistance to trifluoperazine and amphotericin B, and *Candida spp*. strains were considerably resistant to ketoconazole.

Yeasts from the oral cavity, stomach, and intestine showed a considerable susceptibility to trifluoperazine (P = 0.418). However, intestinal isolates were inhibited at a higher MFC (64 μg/mL) of trifluoperazine compared with the oral and gastric yeasts, which were inhibited by a lower MFC (32 μg/mL).

The increase in opportunistic fungal infections due to *Candida* spp. overgrowing *C. albicans* has been reported by several investigators.[19,20,26] These infections are often associated with high mortality rates among hospitalized patients[3,27,28] mainly due to resistance of *Candida* spp. to current antifungals,[19,20,28] such as azole compounds.[29,30] This could be important, particularly in high risk groups, such as patients with candidemia, after failure in treatments with azole compounds.[31] Performance of a susceptibility test and recruitment of effective drugs with lower toxicities have been recommended in order to control the emergence of drug-resistant strains of *Candida* species.[31] Accordingly, trifluoperazine an antipsychotic drug that exhibited an effective antifungal activity with the MFC comparable to ketoconazole, could be considered as an alternative antifungal when encountering yeasts with high resistance to current antifungals.

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Autumnal view of the foggy Jungle(photo by M.H.Azizi MD, November 2011)

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