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ORIGINAL ARTICLE

Possible mechanisms of the antifungal activity of fluconazole in combination with terbinafine against *Candida albicans*

Alireza Khodavandi[1], Fahimeh Alizadeh[2], Nasim Aghai Vanda[3], Golgis Karimi[4], and Pei Pei Chong[5]

[1]*Department of Paramedical Sciences, Gachsaran Branch, Islamic Azad University, Gachsaran, Iran,* [2]*Department of Microbiology, Yasouj Branch, Islamic Azad University, Yasouj, Iran,* [3]*Department of Microbiology, Science and Research Branch, Islamic Azad University, Fars, Iran,* [4]*Department of Nutrition, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Malaysia, and* [5]*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Malaysia*

Abstract

Context: Candidiasis is a term describing infections by yeasts from the genus *Candida*, the majority *Candida albicans*. Treatment of such infections often requires antifungals such as the azoles, but increased use of these drugs has led to selection of yeasts with increased resistance to these drugs.
Objective: Combination therapy would be one of the best strategies for the treatment of candidiasis due to increased resistance to azoles.
Materials and methods: The antifungal activities of fluconazole and terbinafine were evaluated *in vitro* alone and in combination using broth microdilution test and time kill study. Eventually the expression level of selected genes involved in ergosterol biosynthesis of *Candida* was evaluated using semi-quantitative RT-PCR.
Results: The obtained results showed the significant MICs ranging from 0.25 to 8 μg/mL followed by FICs ranged from 0.37 to 1 in combination with fluconazole/terbinafine. Our findings have demonstrated that the combination of fluconazole and terbinafine could also significantly reduce the expression of *ERG1*, *3*, and *11* in the cell membrane of *Candida* in all concentrations tested ranging from 1.73- to 6.99-fold.
Discussion and conclusion: This study was undertaken with the ultimate goal of finding the probable targets of fluconazole/terbinafine in *C. albicans* by looking at its effects on cell membrane synthesis.

Keywords

Candidiasis, gene expression, synergistic activity

History

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Introduction

Candidiasis is a common term that usually results from overgrowth of *Candida albicans* in the human body treated by antifungal agents such as azoles (like fluconazole) and allylamines (like terbinafine) as the common antifungal drugs (Ferahbas et al., 2006; Rossie & Guggenheimer, 1997). The primary target of azoles may be the heme protein, which cocatalyzes cytochrome-P450-dependent 14α-demethylation of lanosterol in the last stage of ergosterol biosynthesis, while allylamines act by inhibiting the early stages. Indeed, the inhibition of squalene epoxidase by terbinafine (early step) or 14α-demethylase by fluconazole (last step) of ergosteroln's biosynthesis has principal role in a play of depletion of ergosterol and agglomeration of sterol precursors, resulting some alteration in the structure and function of cell membrane in *Candida* cells (Borecká-Melkusová et al., 2009; Espinel-Ingroff, 2008; Ghannoum & Rice, 1999; Spampinato & Leonardi, 2013).

Correspondence: A. Khodavandi, Department of Paramedical Sciences, Gachsaran Branch, Islamic Azad University, Gachsaran, Iran. Tel: +987423332036. Fax: +987423333533. E-mail: cpp@upm.edu.my, khodavandi@iaug.ac.ir, alireza_khodavandi@yahoo.com

Sometimes, *Candida* seen in immunocompromised or hospitalized individuals is resistant to main types of antifungal agents (Park & Perlin, 2005). Ergosterol biosynthesis genes including *ERG*1, *ERG3*, and *ERG11* are the most significant genes involved in the resistance to azoles and the other antifungals such as allylamines. In contrast, the up-regulation of these genes resulting in alteration of enzyme targeted by fluconazole (encoded by *ERG11*) or terbinafine (encoded by *ERG1*) which may result in the resistance to drugs. Moreover, mutation of these genes could lead to resistance to these antifungals (Borecká-Melkusová et al., 2009; Espinel-Ingroff, 2008).

Nowadays increasing incidence of resistance to antifungal therapy has required that novel therapies be used. Some reports have shown that combination of fluconazole/terbinafine was effective to inhibiting *Candida* growth *in vitro* (Ghannoum & Elewski, 1999; Perea et al., 2002). In the present study, *in vitro* antifungal activities of fluconazole and terbinafine alone and in combination against *C. albicans* were examined. Subsequently, the expression of significant genes involved in ergosterol biosynthesis of *Candida* such as *ERG1*, *ERG3*, and *ERG11* were analyzed with fluconazole/terbinafine combination therapy.