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

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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.

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 ergosterol’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 & Leonard, 2013).

Sometimes, *Candida* seen in immunocompromised or hospitalized individuals is resistant to main types of antifungal agents (Park & Perlin, 2005). Ergosterol biosynthesis genes including *ERG1*, *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 enzymes 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; Espinell-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* (Ghanoun & 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.