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# In Vitro Activities of Terbinafine in Combination with Fluconazole and Itraconazole against Isolates of *Candida albicans* with Reduced Susceptibility to Azoles

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**A checkerboard microdilution method was applied to study the in vitro interaction of terbinafine with either fluconazole and itraconazole against 30 strains of *Candida albicans*. Synergy was observed in 40% of the terbinafine-fluconazole interactions and in 43% of the terbinafine-itraconazole interactions, while antagonism was not observed. Even when only additivity was achieved, the combinations still showed beneficial effects since at least twofold reductions in the MICs of both drugs were found in 100% of the terbinafine-fluconazole interactions and in 76% of the terbinafine-itraconazole interactions.**

The increased incidence of fungal infections, particularly those caused by yeast fungi, has resulted in the development and introduction of a number of new antifungal agents including the broad-spectrum triazoles fluconazole and itraconazole. In the last few years, the development of fluconazole-resistant isolates of *Candida albicans* from patients with AIDS and oropharyngeal candidiasis has been often reported (5–7, 16, 18). An attractive therapeutic option in these circumstances might be a combination of antimicrobial agents with proven synergistic activity. The main aim of the study described here was to investigate the in vitro effects of terbinafine, the most active allylamine derivative, in combination with fluconazole and itraconazole. Terbinafine is an orally and topically active drug with primarily fungicidal activity against a broad spectrum of fungi, including dermatophytes, filamentous, dimorphic organisms, and some yeasts such as *Candida parapsilosis*, while it appears only fungistatic against *C. albicans* (2, 10, 13). In order to better assess the possible in vitro beneficial effect of terbinafine on the triazoles, we selected strains of *C. albicans* isolated from the oral cavities of human immunodeficiency virus (HIV)-infected patients.

**Sources of isolates.** Thirty recent clinical isolates of *C. albicans* were used throughout the study. All the strains were isolated from the oral cavities of HIV-infected patients suffering from oropharyngeal candidiasis while they were undergoing azole therapy. Each strain represented a unique isolate from a subject. Identification to the species level was performed by the morphological and biochemical method, and the strains were stored at −70°C until they were used in the study (19). *Candida krusei* ATCC 6258 was used as the control organism in all experiments (14, 17).

**Antifungal susceptibility testing.** Terbinafine (Sandoz Ltd., Basel, Switzerland), fluconazole (Pfizer Inc., New York, N.Y.), and itraconazole (Janssen Pharmaceutica, Beerse, Belgium) were obtained as reagent-grade powders from their respective manufacturers. Stock solutions were prepared in polyethylene glycol (terbinafine and itraconazole) or water (fluconazole). Serial twofold dilutions of each antifungal agent were prepared exactly as outlined in document M27-T of the National Committee for Clinical Laboratory Standards (NCCLS) (11). Final dilutions were made in RPMI 1640 medium (Gibco Laboratories, Milan, Italy) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma). Drug interactions were assessed by a checkerboard microdilution method. The final concentrations of the antifungal agents ranged from 0.125 to 8.0 μg/ml for terbinafine, 0.125 to 64 μg/ml for fluconazole, and 0.007 to 4.0 μg/ml for itraconazole. Yeast inocula, prepared spectrophotometrically and further diluted in order to obtain a concentration ranging from $1.0 \times 10^3$ to $5.0 \times 10^3$ CFU/ml (two times the inoculum), were added to each well of the microdilution trays (3, 11). The trays were incubated in air at 35°C, and the results were read at 48 h. Readings were performed spectrophotometrically with an automatic plate reader (model MR 700; Dynatech) set at 490 nm. MIC endpoints were determined as the first concentration of the antifungal agent tested alone and in combination at which the turbidity in the well was ≥50% less than that in the control well (14). Both on-scale and off-scale results were included in the analysis. The high off-scale MICs were converted to the next highest concentrations, while the low off-scale MICs were left unchanged. Drug interaction was classified as synergistic, additive, or antagonistic on the basis of the fractional inhibitory concentration (FIC) index (9). The FIC index is the sum of the FICs of each of the drugs, which in turn is defined as the MIC of each drug when used in combination divided by the MIC of the drug when used alone. The interaction was defined as synergistic if the FIC index was ≤0.50, additive if the FIC index was >0.50 to <2.0, and antagonistic if the FIC index was >2.0 (9).

*C. krusei* ATCC 6258 was tested 10 times versus all three antifungal agents alone and in combination. Terbinafine and fluconazole MICs for the control organism were >8.0 and 32 μg/ml, respectively, in all the experiments (14). Itraconazole MICs for the control organism ranged from 0.125 to 0.25 μg/ml (17). The combinations of terbinafine and fluconazole and of terbinafine and itraconazole had additive effects against *C. krusei* ATCC 6258, with FIC indices ranging from 0.56 to 1.00 and from 0.62 to 1.01, respectively. Terbinafine MICs for the 30 isolates of *C. albicans* ranged from 1.0 to >8.0 μg/ml, with an MIC at which 50% of the isolates are inhibited ($MIC_{50}$) and an $MIC_{90}$ of 8.0 and >8.0 μg/ml, respectively. Fluconazole MICs ranged from 1.0 to >64 μg/ml, with an $MIC_{50}$ and an $MIC_{90}$ of 8.0 and 64 μg/ml, respectively. Itraconazole MICs

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TABLE 1. Mode of interaction between terbinafine and fluconazole and between terbinafine and itraconazole against 30 isolates of *C. albicans*[a]

| Isolate no. | MIC (μg/ml) of the following drugs: | | | FIC index for T/F | MIC (μg/ml) of the following drugs: | | | FIC index for T/F |
|---|---|---|---|---|---|---|---|---|
| | T | F | T/F | | T | I | T/I | |
| 1 | >8.0 | 16 | 2.0/8.0 | 0.62 | >8.0 | 0.5 | 0.5/0.25 | 0.53 |
| 2 | 1.0 | 1.0 | 0.125/0.25 | 0.37 | 4.0 | 0.06 | 1.0/0.015 | 0.50 |
| 3 | >8.0 | 64 | 4.0/16 | 0.50 | >8.0 | 2.0 | 1.0/0.25 | 0.18 |
| 4 | >8.0 | 64 | 4.0/16 | 0.50 | >8.0 | 0.5 | 4.0/0.25 | 0.75 |
| 5 | >8.0 | 16 | 4.0/4.0 | 0.50 | >8.0 | 0.5 | 4.0/0.125 | 0.50 |
| 6 | 8.0 | 16 | 4.0/4.0 | 0.75 | 8.0 | 0.5 | 0.125/0.25 | 0.51 |
| 7 | 8.0 | 2.0 | 2.0/0.5 | 0.50 | 4.0 | 0.125 | 2.0/0.03 | 0.74 |
| 8 | >8.0 | 8.0 | 2.0/4.0 | 0.62 | >8.0 | 0.125 | 2.0/0.125 | 1.12 |
| 9 | 4.0 | 4.0 | 1.0/2.0 | 0.62 | 4.0 | 0.25 | 2.0/0.125 | 1.00 |
| 10 | >8.0 | >64 | 8.0/64 | 1.00 | >8.0 | 0.5 | 0.5/0.125 | 0.34 |
| 11 | 2.0 | 8.0 | 1.0/1.0 | 0.62 | 1.0 | 0.25 | 0.25/0.03 | 0.37 |
| 12 | >8.0 | 8.0 | 2.0/4.0 | 0.62 | >8.0 | 0.125 | 0.125/0.125 | 1.01 |
| 13 | >8.0 | 4.0 | 2.0/2.0 | 0.62 | >8.0 | 0.125 | 0.125/0.125 | 1.01 |
| 14 | >8.0 | 32 | 0.25/16 | 0.51 | >8.0 | 0.5 | 0.5/0.25 | 0.53 |
| 15 | >8.0 | 4.0 | 2.0/2.0 | 0.62 | >8.0 | 0.25 | 0.125/0.125 | 0.51 |
| 16 | 8.0 | 4.0 | 4.0/1.0 | 0.75 | 8.0 | 0.125 | 4.0/0.06 | 1.00 |
| 17 | 4.0 | 32 | 2.0/8.0 | 0.75 | 4.0 | 0.25 | 2.0/0.06 | 0.74 |
| 18 | 8.0 | 64 | 4.0/16 | 0.75 | >8.0 | 0.5 | 4.0/0.125 | 0.50 |
| 19 | >8.0 | 2.0 | 1.0/0.5 | 0.31 | 8.0 | 0.125 | 4.0/0.06 | 1.00 |
| 20 | 4.0 | 2.0 | 1.0/0.125 | 0.31 | 2.0 | 0.25 | 1.0/0.125 | 1.00 |
| 21 | 4.0 | 32 | 2.0/16 | 1.00 | 4.0 | 0.125 | 1.0/0.03 | 0.49 |
| 22 | >8.0 | 8.0 | 2.0/0.5 | 0.31 | 8.0 | 0.25 | 2.0/0.015 | 0.31 |
| 23 | >8.0 | 64 | 2.0/8.0 | 0.25 | >8.0 | 0.5 | 4.0/0.125 | 0.50 |
| 24 | 8.0 | 32 | 2.0/0.5 | 0.26 | 4.0 | 0.25 | 0.5/0.06 | 0.36 |
| 25 | 8.0 | 32 | 2.0/8.0 | 0.50 | 4.0 | 0.25 | 1.0/0.06 | 0.49 |
| 26 | 2.0 | 8.0 | 1.0/0.25 | 0.53 | 2.0 | 0.25 | 0.5/0.03 | 0.37 |
| 27 | 4.0 | 8.0 | 1.0/4.0 | 0.75 | 4.0 | 0.25 | 1.0/0.06 | 0.49 |
| 28 | 8.0 | 16 | 4.0/8.0 | 1.00 | 8.0 | 0.5 | 4.0/0.125 | 0.75 |
| 29 | >8.0 | 64 | 2.0/16 | 0.37 | 4.0 | 0.25 | 2.0/0.125 | 1.00 |
| 30 | 8.0 | 1.0 | 0.125/0.5 | 0.51 | 8.0 | 0.125 | 8.0/0.015 | 1.12 |

[a] T, terbinafine; F, fluconazole; T/F, terbinafine in combination with fluconazole; I, itraconazole; T/I, terbinafine in combination with itraconazole.

ranged from 0.06 to 2.0 μg/ml, with an $MIC_{50}$ and an $MIC_{90}$ of 0.25 and 0.5 μg/ml, respectively (Table 1). When terbinafine and fluconazole were given in combination, there were significant reductions in the geometric mean of the terbinafine MIC (from 8.4 to 1.7 μg/ml; $P = 0.0001$) and of the fluconazole MIC (from 11.8 to 2.9 μg/ml; $P = 0.002$). Forty percent (12 of 30) of the interactions were synergistic and 60% (18 of 30) were additive, while antagonism was not observed (Table 1). When synergy was documented, the median reductions in MIC were 4-fold (range, 4- to 16-fold) for terbinafine and 4-fold (range, 4- to 64-fold) for fluconazole. When additivity was documented, the median reductions in MICs were 2-fold (range, 2- to 64-fold) for terbinafine and 2-fold (range, 2- to 32-fold) for fluconazole. For 63% (14 of 22) of the isolates for which initial terbinafine MICs were ≥8.0 μg/ml, the MIC was reduced to ≤2.0 μg/ml upon combination with fluconazole. For 83% (five of six) of the isolates for which initial fluconazole MICs were ≥64 μg/ml, the MIC was reduced to ≤16 μg/ml upon combination with terbinafine (Table 1). When terbinafine and itraconazole were given in combination, there were significant reductions in the geometric mean of the terbinafine MIC (from 7.2 to 1.1 μg/ml; $P = 0.0001$) and of the itraconazole MIC (from 0.26 to 0.08 μg/ml; $P = 0.0001$). Forty-three percent (13 of 30) of the interactions were synergistic and 57% (17 of 30) were additive, while antagonism was not observed (Table 1). When synergism was documented, the median reductions in MIC were 4-fold (range, 4- to 32-fold) for terbinafine and 4-fold (range, 4- to 16-fold) for itraconazole. When additivity was documented, for 13 of 17 isolates the median reductions in MICs were 2-fold (range, 2- to 128-fold) for terbinafine and 2-fold (range, 2- to 4-fold) for itraconazole. For 55% (10 of 18) of the isolates for which the initial terbinafine MIC was ≥8.0 μg/ml, the MIC was reduced to ≤2.0 μg/ml upon combination with itraconazole. For 50% (5 of 10) of the isolates for which the initial itraconazole MIC was ≥0.5 μg/ml, the MIC was reduced to ≤0.125 μg/ml upon combination with terbinafine (Table 1).

In this study we investigated the in vitro interaction between terbinafine and two triazoles, fluconazole and itraconazole, with a large number of *C. albicans* isolates. Recently, Fothergill et al. (8) found a synergistic interaction between terbinafine and triazoles not only against isolates of *C. albicans* but even against isolates of *Candida glabrata* and *Cryptococcus neoformans* (8). Although both classes of antifungal agents inhibit ergosterol biosynthesis, they do so by blocking the pathway at different steps. Azoles inhibit lanosterol 14-demethylase, an enzyme belonging to the superfamily of cytochrome P-450, while terbinafine inhibits the non-cytochrome P-450 enzyme squalene epoxidase (2). A classic example of a proven synergistic interaction between two antibacterial compounds acting at different steps of the same pathway is trimethoprim and the sulfonamides, which exert their individual antibacterial activities at sequential points in the metabolism of folate (9). Aside from producing a deficiency in ergosterol, terbinafine has additional antifungal activity due to the intracellular accumulation of squalene, which disrupts fungal cell membranes (2).

To better assess the possible in vitro beneficial effect of terbinafine on the triazoles, we selected strains of *C. albicans*

with reduced susceptibility to azoles. In order to avoid subjective interpretation of MIC endpoints, we used a spectrophotometric reading. It has been shown that spectrophotometric MICs reflecting either a $\geq$50% inhibition or a $\geq$70% inhibition of growth relative to control growth provided a good agreement with the NCCLS reference MICs for several antifungal drugs (1, 4, 15). Our in vitro results confirmed a limited activity of terbinafine when it was used alone against isolates of *C. albicans*, having found an $MIC_{50}$ and an $MIC_{90}$ of 8.0 and >8.0 μg/ml, respectively (2, 13). These MICs are severalfold higher than the achievable terbinafine levels in serum (10). It has been shown that maximum concentrations in plasma of approximately 0.9 and 1.7 to 2.0 μg/ml are achieved within 2 h of oral administration of terbinafine at 250 and 500 mg, respectively (2, 10). On the other hand, our data indicate a clear enhancement of the in vitro activity of the allylamine compound when tested in combination with the triazoles. The findings that 40% of the terbinafine-fluconazole interactions and 43% of the terbinafine-itraconazole interactions were synergistic and that antagonism was not observed are encouraging. It should be pointed out that the results of any in vitro testing of antibiotic combinations are definition dependent. Although in recent reports the synergy between antibacterial or antifungal drugs has been defined by a FIC index of $\leq$1.0, in this study we selected a more stringent criterion for the definition of synergy (12, 20). However, our data indicated that even when only additivity was achieved, the combination of terbinafine and triazoles still showed beneficial effects, having found at least a twofold reduction in the MICs of both drugs in 100% (18 isolates) of the terbinafine-fluconazole interactions and in 76% (13 of 17 isolates) of the terbinafine-itraconazole interactions.

In conclusion, our in vitro results demonstrated an effective interaction between terbinafine and triazoles against isolates of *C. albicans*. Clinical studies are warranted to further elucidate the potential utility of this combination therapy.

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