

## NON-AZOLE ANTIFUNGALS AND CALCINEURIN INHIBITORS EXHIBIT SYNERGISTIC ACTIVITY AGAINST *C. ALBICANS*

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**Purpose:** Azole drugs target the ergosterol biosynthetic enzyme lanosterol 14 $\alpha$ -demethylase and are a widely applied class of antifungal agents due to their broad therapeutic window, wide spectrum of activity, and low toxicity. Unfortunately, azoles are generally fungistatic, and resistance to fluconazole is emerging in several fungal pathogens. It was recently established that the protein phosphatase calcineurin allows survival of *Candida albicans* during the membrane stress exerted by azoles. The calcineurin inhibitors cyclosporin A (CsA) and tacrolimus (FK506) are dramatically synergistic with azoles, resulting in potent fungicidal activity. The purpose of this research was to determine whether terbinafine and fenpropimorph—two non-azole antifungal drugs that target other enzymes in the ergosterol biosynthesis pathway—also exhibit dramatic synergistic antifungal activity against *C. albicans* when combined with CsA or FK506.

**Summary:** The *in vitro* antifungal susceptibilities of various *C. albicans* strains were tested using disk diffusion halo assays. Strains were grown in YPD media overnight, resuspended in top agar, and poured onto YPD solid media. Sterile drug disks containing combinations of terbinafine, fenpropimorph, FK506, CsA, and L-685,818 or solvent controls were placed over the solidified top agar. Cells were incubated for 24 to 48 hours at 37°C. The minimum inhibitory concentration (MIC) of the different drug combinations was determined according to standard NCCLS criteria for wild-type and mutant *C. albicans* strains. To determine the presence of drug synergy, fractional inhibitory concentration (FIC) and FIC index values were calculated for each drug combination.

**Results:** Both terbinafine and fenpropimorph exhibited potent fungicidal synergism with FK506 and CsA in *C. albicans*. Similarly, *C. albicans* mutant strains lacking the calcineurin B subunit were markedly hypersensitive to terbinafine and fenpropimorph. The FK506 binding protein FKBP12 was required for FK506 synergism with ergosterol biosynthesis inhibitors and mutations that conferred FK506-resistance abolished this synergism. In wild-type *C. albicans*, there was evidence of drug synergy between the non-immunosuppressive FK506 analog L-685,818 and both ergosterol biosynthesis inhibitors. Additionally, an *erg24/erg24* mutant which lacks the enzyme target of fenpropimorph was hypersensitive to both FK506 and CsA.

**Conclusions:** In order to combat the growing problem of drug-resistant microorganisms, we must be innovative in our approaches to drug design and vigilant in monitoring current therapies whose properties can be exploited for novel therapeutic purposes. These studies demonstrate that the activity of non-azole antifungal agents that target ergosterol biosynthesis can be enhanced by inhibition of the calcineurin signaling pathway, extending their spectrum of activity and providing an alternative approach to overcoming antifungal drug resistance.