P1401

Isolation and characterization of a putative multiple drug resistance gene from *Trichophyton mentagrophytes*

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Onychomycosis is the thickening and discoloration of the nail plate most commonly caused by dermatophytes, yeasts, or nondermatophyte moulds. It is a significant disorder that is estimated to affect more than 2% to 13% and 6.5% of the US and Canadian populations, respectively. The incidence of onychomycosis has increased because of the large invasion of dermatophytes, especially *Trichophyton rubrum*, and *T mentagrophytes* from West Africa and Southeast Asia to North America and Europe, and may now account for 50% of all nail disorders. It is the most common nail disease in adults, with the toenails much more likely to be infected than the fingernails. The incidence of onychomycosis has been increasing, owing to such factors as diabetes, immunosuppression, and increasing age and lifespan. Despite the potency of preexisting and new agents, a major obstacle to effective clinical treatment remains the large minority of patients that are unresponsive to these antifungals. Griseofulvin shows a mycological cure rate of only 24% with Trichophyton species, whereas terbinafine, the most potent agent, shows a rate of 60% to 80%. Furthermore, there is an appreciable relapse rate for all these drugs, with griseofulvin, itraconazole, and terbinafine exhibiting rates of 40%, 22%, and 9%, respectively. It is not clear why some patients are unresponsive to treatment or why relapse occurs. While many factors, such as ongoing trauma to the nail unit, poor peripheral perfusion, and decreased immunity because of the presence of comorbid conditions may contribute to the failure, one important possibility may be the presence of *T rubrum* or *T mentagrophytes* drug-resistant strains in these cases. Patients who are initially unresponsive may mostly carry drug-resistant strains, whereas patients experiencing a relapse may originally have carried both sensitive and resistant colonies before treatment. In the latter case, recolonization by more resistant strains may lead to relapse. In this poster, we describe the isolation of a *T mentagrophytes* gene, named Tmmdr1, which has significant homology to the ATP-binding cassette genes that confer multiple drug resistance in a wide variety of prokaryotic and eukaryotic organisms. We present the sequence of the entire transcriptional unit and evidence of its expression in strains exhibiting increased ketoconazole resistance.

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P1407

Phase IV study of the efficacy and safety of a pulse terbinafine/ciclopirox lacquer regimen with a continuous terbinafine regimen

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Onychomycosis is the most common nail disorder, affecting 2% to 13% of the general population and increasing in prevalence with age. The treatment of toenail onychomycosis with terbinafine is an established practice. By reducing the total dose of terbinafine and combining it with a topical therapy, it was hypothesized that similar or greater efficacy of treatment may be obtained compared to terbinafine monotherapy, without increasing the risks of adverse events. The objective of this study was to determine the comparability of a pulse terbinafine/ciclopirox nail lacquer regimen with a standard continuous terbinafine regimen. Eight hundred and sixty-four subjects (550 evaluable at week 84) with dermatophyte toenail onychomycosis were enrolled in this randomized, parallel-group, evaluator blinded trial. Four hundred and fourteen subjects were treated with daily ciclopirox nail lacquer for 48 weeks and three 2-week pulses of terbinafine from weeks 12 to 24. Some of these subjects also received terbinafine booster therapy at week 36 and/or week 60 if they met certain criteria. This pulse treatment group showed an effective cure of 48.4% at week 84. Effective cure was defined as mycological cure (negative KOH and culture) with <10% visually affected area of nail remaining. This was significantly higher than the effective cure (32.8%) of patients treated with a placebo nail lacquer (48 weeks) and once daily terbinafine from weeks 12 to 24 ($n = 450$; $P < .001$). Efficacy between the two regimens was similar for mild, moderate, and severe involvements of onychomycosis. Terbinafine-related adverse events were similar in number between groups, and typical in type and frequency to those reported in the terbinafine prescribing information. The reported lacquer-related adverse events were similar to those reported in the ciclopirox prescribing information. Most events were mild to moderate and transient. The distribution of serious adverse events was similar between groups, and no serious adverse events were related to study treatments. The effective cure rates suggest that a combination of pulse terbinafine and ciclopirox nail lacquer may provide increased clinical efficacy compared to terbinafine monotherapy for moderate to severe dermatophyte onychomycosis.

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P1700

Resist microbial resistance: Overcoming *Cryptococcus neoformans* biofilms with chitosan

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Introduction: The formation of microbial biofilms, a mechanism for enhancing resistance to host defense and antibiotics, marks a dangerous advance in the evolution of pathogen virulence. The efficacy of host defense and antibiotic therapy for acute and chronic wound infections is undermined by this modern defense mechanism, making treatment a challenge. *Cryptococcus neoformans* not only forms such protective biofilms, but is often fortified by fungal cell melanization, quenching host immune oxidative stress such as released nitric oxide (NO). We hypothesized that if these biofilms could be overcome, the efficiency of this protective mechanism against host defense and antimicrobial agents would be significantly reduced. Chitosan, a cationic biostructural polymer, has recently received attention for its antimicrobial and food preservation properties. We investigated the susceptibility of *C neoformans* cells in biofilm and planktonic states to chitosan and subsequent introduction of oxidative stress in the form of NO.

Methods: Biofilm formation in vitro on polystyrene plates demonstrated adequate colony formation and subsequent polymer based matrix formation. The effect of various dilutions of chitosan solutions at both pH 4 and pH 5.5 on fungal mass and metabolic activity of *C neoformans* biofilms were measured by colony counts and 2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]- 2H-tetrazolium hydroxide (XTT) reduction, respectively.

Results: Cryptococcal cells encapsulated in biofilms and melanized biofilms were shown to be refractory to concentrations of gaseous NO ranging from 1 to 80 μM, while planktonic cells were quite sensitive to these concentrations. However, both *C neoformans* cells and preformed biofilms were susceptible to both the chitosan solutions alone and subsequent addition of NO, as compared to controls of equivalent acidity. Both significant killing impact and decrease in metabolic activity were noted with all three cell lines.

Conclusions: These data suggest that although the biofilm phenotype increases resistance against host immune mechanisms, *C neoformans* vulnerability to chitosan may offer a novel approach to overcome this pathogenic system and aid in the treatment of resistant cutaneous infections.

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P1701

Novel anti-inflammatory activity of sertaconazole nitrate is mediated via activation of a p38/COX-2/PGE2 pathway

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Sertaconazole nitrate is a broad-spectrum antifungal compound with efficacy against dermatophytes, yeasts, and some Gram-positive bacteria. In addition to its antifungal efficacy, recent studies have shown that sertaconazole possesses intrinsic antiinflammatory activity, thus having the potential of providing clinical benefit beyond fungus eradication. We sought to determine the cellular mechanisms by which sertaconazole exerts its antiinflammatory activity in keratinocytes and human peripheral blood mononuclear cells (PBMCs). Paradoxically, sertaconazole was found to activate the proinflammatory p38 mitogen activated protein kinase. Treatment of keratinocytes with other antifungal agents, such as butoconazole, fluconazole, terconazole, tioconazole, or ketoconazole, indicated that only sertaconazole nitrate induced a direct stimulation of P38 MAP kinase; thus, sertaconazole activates a signaling pathway distinct from other antifungal agents. Sertaconazole treatment also resulted in the induction of cyclooxygenase-2 and the subsequent release of prostaglandin E2. Knocking down p38 in keratinocytes using siRNA resulted in an inhibition of sertaconazole-induced PGE2 release, confirming that activation of p38 was required for PGE2 production. Additionally, in stimulated keratinocytes and human PBMCs, sertaconazole was found to suppress the release of proinflammatory cytokines. Treatment with anti-PGE2 antiserum or the COX-2 inhibitor NS398 reversed the inhibitory effects of sertaconazole on the release of proinflammatory cytokines, linking endogenous PGE2 with the antiinflammatory effects. Finally, in an in vivo mouse model of TPA induced—dermatitis, the sertaconazole-mediated inhibition of TPA-induced ear edema was reversed by NS398. Biochemical analysis of tissue biopsies revealed increase in PGE2 levels in sertaconazole-treated mice. Because other antifungal agents did not activate this signaling pathway, it seems unlikely that activation of the p38-COX 2-PGE2 pathway contributes to the antifungal activity of the compound. Thus activation of the p38-COX 2-PGE2 pathway by sertaconazole is a novel pathway for providing antiinflammatory therapeutic benefits. Furthermore, the antiinflammatory properties of sertaconazole may contribute to the efficacy of the drug in the treatment of cutaneous fungal conditions and provide greater antiinflammatory activity compared with other antifungal agents.

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