The plant defensin HXP124 has the potential to be a new safe and effective topical treatment for onychomycosis

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HXP124 has potent antifungal activity against pathogens that cause onychomycosis

Plant defensins with potent antifungal activity have the potential to be developed as effective and robust treatments for fungal infections in humans. The cysteine-stabilised structure of plant defensins makes them extremely stable and able to withstand extremes of pH and temperature. HXP124 is a novel plant defensin with potent antifungal activity against a range of clinically important fungal pathogens including *Candida* spp, *Cryptococcus* spp, dermatophytes and non-dermatophytic moulds. In particular, HXP124 has excellent activity against dermatophytes that infect skin and nails. In addition, HXP124 efficiently and rapidly penetrates human nails making it an ideal target for development of a novel onychomycosis treatment.

Onychomycosis, or fungal infection of the nail, affects about 14% of the population. These infections are long-term, hard to treat, can be painful and have a cosmetic impact the patient. Treatment options include systemic and topical therapies. Although effective, systemic therapies commonly have adverse effects, such as liver toxicity. Current topical treatments have low cure rates due to poor nail penetration, and thus typically require long treatment times.

Figure 1: NMR solution structure of HXP124 (blue) compared to the plant defensin NaD1 (pdb 1MR4).

Table 1: MIC of HXP124 against fungal pathogens

	HXP124 (µg/mL)		efinaconazole (µg/mL)	
	MIC	MFC	MIC	MFC
<i>Candida albicans</i>	30.00	30.00	<.03	30.00
<i>Trichophyton rubrum</i>	20.00	25.00	0.10	2.80
<i>Trichophyton interdigitale</i>	20.00	20.00	0.10	12.50
<i>Trichophyton mentagrophytes</i>	13.00	15.00	0.03	5.00

HXP124 adopts a typical plant defensin cystein-stabilised αβ-motif structure (Figure 1). **HXP124 inhibits the growth of a broad range of human fungal pathogens** (Table 1). The Minimal Inhibitory concentration (MIC, concentration required to inhibit all visible growth) and the Minimal Fungicidal Concentration (MFC, concentration required to reduce the number of viable cells by 99.9%) are similar indicating that **HXP124 has a fungicidal mode of action, even at low concentrations**. In contrast, the MFC for efinaconazole is 20-1000 times higher than the MIC indicating a largely fungistatic mode of action.

HXP124 kills fungi more efficiently than current antimycotic drugs

When *C. albicans* cells were treated with HXP124 (100 µg/mL), **more than 95% of the cells were killed within 30 mins** (Figure 2). In contrast, only ~10%, ~20% and ~30% of cells were killed by efinaconazole, tavaborole and terbinafine respectively in this time period.

Figure 2: (A) Flow cytometry of HXP124 treated *C. albicans* cells. *C. albicans* was cultured overnight at 30°C. The culture was diluted and HXP124 added at the concentrations indicated for 30 minutes. Propidium iodide was supplemented during HXP124 treatment to stain non-viable cells. (B) Cell death after treatment of *C. albicans* for 30 min with 100 µg/mL of HXP124 or various marketed antifungal drugs. Cells were stained with propidium iodide and dead and living cells were counted by flow cytometry.

HXP124 penetrates human nails and is active in an Infected Nail Model

For a topical onychomycosis treatment to be effective, the active ingredient must penetrate the nail plate to reach the nail bed, the main site of infection. We assessed the nail penetration of HXP124 using a method developed by Professor Howard Maibach, (UCSF Medical Centre). HXP124 penetrated human nails faster and more efficiently than the competitor molecules efinaconazole and terbinafine (Figure 3). **Within 7 days, more than 25% of the applied dose was collected from the underside of the nail each day.**

Figure 3: The amount of HXP124 (purple) terbinafine (grey) and efinaconazole (teal) collected on the underside of human nails each day. Molecules were quantified using RP-HPLC. Error bars represent standard error of the mean (HXP124 n=7, terbinafine n=3, efinaconazole n=3).

The ability of HXP124 to penetrate human nails and kill fungus growing on keratin as a sole nutrient source was assessed in the ChubTer® Infected Nail Model (Figure 4). HXP124 was applied onto nails that had been infected with the fungus *T. rubrum* (most common causative agent of onychomycosis), **After 7 days, HXP124 had reduced the amount of viable fungus by more than 95%.** HXP124 was as effective in this model as Jublia®.

Figure 4: Nails that had been infected with the fungus *Trichophyton rubrum* (most common causative agent of onychomycosis) were treated daily with HXP124, Jublia® (efinaconazole) and Penlac® (ciclopirox). Adenosine triphosphate (ATP) levels were used to indicate the level of viable fungus. This study was conducted by MedPharm (UK).

HXP124 has a favourable stability and toxicology profile

- ▶ HXP124 had a no observable adverse effect level (NOAEL) of 50 mg/kg in a GLP 42-day repeat-dose dermal irritation minipig model.
- ▶ HXP124 was classed as a non-sensitiser in the Buehler guinea pig sensitisation model.
- ▶ HXP124 has a classification of No Category for ocular irritation according to a bovine corneal opacity and permeability model.
- ▶ HXP124 does not perturbate hERG channels at > 5000-fold the anticipated clinical dose.
- ▶ HXP124 is stable in formulation at 40 degrees for almost 2 years.

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