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In Vitro Profiling of Pramiconazole and *In Vivo* Evaluation in *Microsporum canis* Dermatitis and *Candida albicans* Vaginitis Laboratory Models[▽]

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The triazole antifungal pramiconazole (Stiefel, a GSK company) was compared with itraconazole, miconazole, and terbinafine *in vitro* and *in vivo*. Potent *in vitro* activities against *Candida* spp. (50% inhibitory concentration [IC_{50}], 0.04 to 1.83 μM) and *Microsporum* and *Trichophyton* spp. (IC_{50}, 0.15 to 1.34 μM) were obtained but not, however, against other filamentous molds and zygomycetes. In the *M. canis* guinea pig model and *C. albicans* vulvovaginitis rat model, pramiconazole was superior to the reference compounds after oral and topical administration.

Although considerable research is invested in finding novel strategies for the treatment of fatal invasive mycoses (6), nonfatal superficial mycoses believed to infect about 25% of the world population should not be overlooked (1). The most widespread dermatomycoses are caused by *Trichophyton*, *Epidermophyton*, and *Microsporum* species. Treatment is oral or topical with the allylamine terbinafine or any of the azoles (4, 13, 14). Yeasts also cause superficial infections of skin and mucous membranes, whereby vulvovaginal candidiasis (VVC) affects at least 75% of all women at least once in their lives (15, 21). Standard therapy involves intravaginal application of clotrimazole or miconazole or oral treatment with fluconazole or itraconazole (15). Although current treatment options may suffice, new antifungals would still be acceptable to improve treatment compliance or reduce adverse effects and drug interactions. The triazole pramiconazole shows good *in vitro* and clinical activity against dermatophytes and yeasts (12, 16, 17) and *Malassezia* infections (10, 11, 19). Laboratory data always refer to oral treatment of mice and guinea pigs (16, 17); however, no data on topical application are available. No data have yet been published on pramiconazole in VVC in comparison with reference drugs, although it is in clinical de-

TABLE 1. Cytotoxicity (CC_{50}) and activity (IC_{50}) against four dermatophyte and four *Candida* species

Human cell or fungal isolate	Activity[a] ± SD			
	TRB	MC	ITC	PRC
Cells				
MRC-5	63.00 ± 1.73	29.67 ± 13.32	49.33 ± 14.50	53.33 ± 18.48
Fungal isolates				
Microsporum canis B68128	0.10 ± 0.05	0.23 ± 0.16	2.02 ± 2.45	0.18 ± 0.06
Trichophyton mentagrophytes B70554	0.06 ± 0.04	0.40 ± 0.28	0.37 ± 0.39	0.15 ± 0.16
T. rubrum B68183	0.07 ± 0.05	0.33 ± 0.26	0.56 ± 0.48	0.35 ± 0.14
T. rubrum J941704	0.03 ± 0.02	0.14 ± 0.09	0.98 ± 1.31	0.19 ± 0.19
T. quinckeanum B68683	0.01 ± 0.01	0.79 ± 0.49	2.93 ± 2.91	1.34 ± 1.21
Candida albicans B59163	3.57 ± 1.39	0.30 ± 0.22	1.41 ± 1.20	0.04 ± 0.03
C. albicans B2630	64.00 ± 0.00	2.50 ± 2.08	1.39 ± 1.86	1.83 ± 2.34
C. glabrata B63155	30.66 ± 23.68	0.12 ± 0.06	3.74 ± 4.63	0.65 ± 0.15
C. kefyr B46120	6.33 ± 4.80	0.03 ± 0.03	0.40 ± 0.35	0.13 ± 0.06
C. krusei B68404	64.00 ± 0.00	1.40 ± 0.57	4.21 ± 4.90	0.87 ± 0.50

[a] For MRC-5 cells, the CC_{50} (the concentration at which 50% of the MRC-5 cells are killed) is given. For fungal isolates, the IC_{50} (the concentration in μM at which growth is inhibited for 50% compared to untreated controls) is given. Averages of five independent repeats are expressed, together with the standard deviation (SD). Conversion factors to be used for the IC_{50}s in μg/ml: TRB, × 0.33; MC, × 0.42; ITC, × 0.70; PRC, × 0.66.

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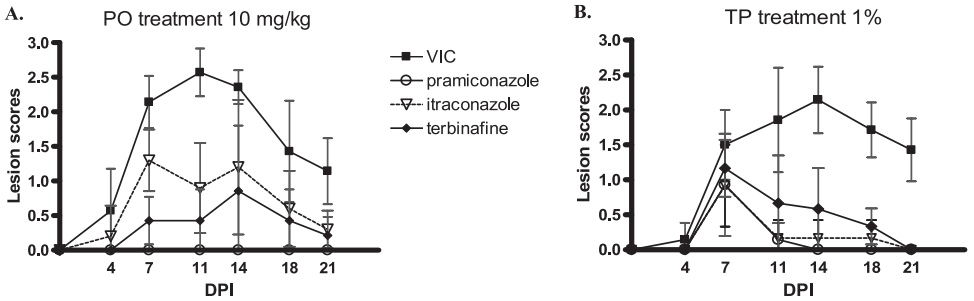

FIG. 1. Comparative efficacy of pramiconazole, terbinafine, and itraconazole after oral (PO) dosing (once daily [s.i.d.]) at 10 mg/kg (A) and after topical (TP) treatment (twice daily [b.i.d.]) with 1% (wt/wt) cream (B) against *M. canis* in guinea pigs. The scores assigned to the animals are shown on the *y* axis; the *x* axis represents the days after infection. VIC, vehicle-treated control group; DPI, days postinfection.

velopment for these indications (8, 9). The specific aims of this laboratory study were (i) to perform an *in vitro* profiling of pramiconazole and (ii) to evaluate oral and topical treatment schemes against *Microsporum canis* in guinea pigs and *Candida albicans* VVC in rats.

Miconazole (MC), itraconazole (ITC), and terbinafine (TRB) were purchased from Sigma, while pramiconazole (PRC) was provided by Stiefel-GSK. The fungal isolates were obtained from the Scientific Institute of Public Health (IHEM, Brussels, Belgium) and cultivated on Sabouraud dextrose agar (SDA) (Oxoid). For all species, a stock of 5×10^6 CFU/ml was prepared in RPMI-MOPS medium with 10% glycerol and stored in liquid nitrogen for later use in all *in vitro* tests. Fresh inocula were used for animal infections. The *in vitro* susceptibility screens were performed as previously described (7). Briefly, 10 μl of prediluted compound solution was spotted onto 96-well plates (U-bottom; Greiner Bio-One) with 64 μM as the highest concentration; 10^3 CFU in 200 μl RPMI-MOPS was added to each well. After incubation, growth inhibition was measured after adding 10 μl/well 0.005% (wt/vol) resazurin (Sigma), allowing fluorimetric reading (λ_{ex}, 550 nm; λ_{em}, 590 nm) (23). Activity is expressed as IC_{50}, i.e., the concentration that inhibits growth for 50% compared to nontreated controls. Cytotoxicity was simultaneously tested on human lung fibroblasts ($MRC\text{-}5_{SV40}$) (Invitrogen). Five independent replicates were performed for each observation.

The *in vitro* IC_{50}s for reference drugs were comparable to the ranges in literature (2, 5, 20), and available data on pramiconazole were also confirmed (16, 17) (Table 1). TRB performs marginally better against dermatophytes. Except for *Trichophyton quinckeanum*, PRC activity remained below 0.5 μM. Against *Candida* spp., activities remained below 1 μM, except for *C. albicans* B2630. PRC failed to show activity (IC_{50}, >64 μM) against the other filamentous molds and zygomycetes (data not shown).

All animal experiments were approved by the Ethical Commission of the University of Antwerp (2008/015). Compounds were formulated in polyethylene glycol 200 (PEG_{200}) for oral (PO) dosing and in $PEG_{400–1,500}$ (3:2, wt/wt) for topical (TP) administration. Each treatment was evaluated for six animals grouped into two independent experiments. Group averages of lesion scores (LS) or intravaginal burdens were used to plot graphs, and the area under the infection curve (AUC) was calculated for each animal as a measure for infection burden. An unpaired *t* test (two tailed, $P \leq 0.05$) was used to determine levels of significance between the different experimental groups.

The dorsum of each female guinea pig was shaved and scarified with a steel brush. An inoculum (*M. canis* B68128) of 10^6 CFU in 100 μl was applied to the scarified skin. Oral dosing at 10 mg/kg started about 2 h before infection and was continued for 5 days. Topical treatment using a 1% formulation was applied twice daily for 4 days starting on the morning after infection. Skin lesions were evaluated every 3 to 4 days (Fig. 1). Lesion scoring systems as found in the literature (18, 24) were slightly modified to include both lesion size and severity. Upon oral administration at 10 mg/kg (Fig. 1A), PRC performed much better than ITC and TRB, with complete suppression of lesion development, which contrasts with the *in vitro* data in which TRB was better than PRC ($P = 0.004$) and ITC ($P = 0.005$). The latter can be explained by the better pharmacokinetic properties of PRC (Table 2), the lower protein binding (17) and higher metabolic stability (3). After TP application, PRC was better than TRB ($P = 0.041$), but no difference was observed between PRC and ITC (Fig. 1B).

For VVC, female rats were ovariectomized 3 weeks before infection and estrus was induced with 1 mg estradiol-benzoate plus 200 μg progesterone on days −3, 2, and 7. Rats were infected intravaginally with 10^7 CFU *C. albicans* B2630. Treatment schedules were identical to those of the guinea pig model. At days 4, 9, and 14 after the infection, vaginal swabs were taken to estimate *Candida* burdens. Oral PRC and ITC at 10 mg/kg were both highly effective but not significantly different. At 5 mg/kg, PRC outperformed ITC ($P = 0.021$) (Fig. 2A). After intravaginal application, superiority of PRC over ITC and MC was significant (Fig. 2B).

In conclusion, pramiconazole has potent *in vitro* anti-der-

TABLE 2. Pharmacokinetics (PK) after oral administration of pramiconazole, itraconazole, and terbinafine to guinea pigs

PK parameter	Result with:		
	PRC[b]	ITC[c]	TRB[d]
C_{max} (μg/ml)[a]	0.18	0.35	0.06
T_{max} (min)	240	120	42
$t_{1/2}$ (h)	23	13.9	6.6

[a] Plasma concentrations were normalized to a dose of 10 mg/kg.
[b] 40 mg/kg; source, Janssen Pharmaceutica, unpublished data.
[c] 20 mg/kg; source, Sobue et al. (22).
[d] 10 mg/kg; source, Janssen Pharmaceutica, unpublished data.

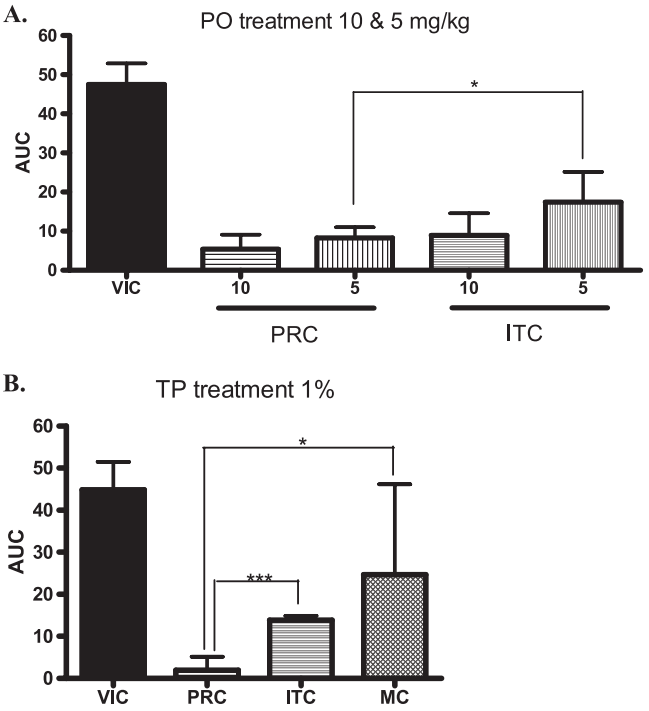

FIG. 2. Comparative efficacy of pramiconazole (PRC) and itraconazole (ITC) after oral (PO) treatment (once daily [s.i.d.]) at 10 and 5 mg/kg (A) and comparison with itraconazole (ITC) and miconazole (MC) results after topical (TP) treatment (twice daily [b.i.d.]) with 1% (wt/wt) cream (B) in the *C. albicans* vaginitis model in rats. The AUC representing the entire infection burden over the 3 days of sampling is shown on the *y* axis. The different groups are shown on the *x* axis. VIC, vehicle-treated control group. *, $P = 0.01$ to 0.05 (two-tailed *t* test). ***, $P < 0.001$ (two-tailed *t* test).

matophyte and anti-yeast activities comparable to those of current reference drugs. In dermatomycosis and VVC animal models, oral pramiconazole performs better than itraconazole and terbinafine and shows a higher intrinsic *in vivo* efficacy, as also demonstrated after topical application. Our findings support the potential of pramiconazole as a promising candidate for treatment of topical mycoses.

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