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Phase I Study of the Novel Antifungal Agent Dapaconazole (Zilt®) in Healthy Volunteers

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Abstract: The study aims to evaluate the tolerability of multiple-dose topical dapaconazole tosylate, a new imidazole antifungal drug, in healthy volunteers. Twenty-four healthy volunteers (12 men) with skin pigmentation classified as I-III in the Fitzpatrick scale enrolled in this open-label, two-treatment study with daily application of 40 mg of dapaconazole for 14 consecutive days. Drug application was monitored by a physician and photographs were taken before and 1 h after application to evaluate possible dermatological reactions. Medical evaluations including physical examination, laboratory tests and electrocardiograms were performed to evaluate possible systemic adverse events. To evaluate systemic dapaconazole absorption blood samples were collected before and 2, 4 and 6 h after products application on the first day of treatment. The same occurred in days 7 and 13, but an extra 24 h sample was collected after application of the products. Dapaconazole plasma levels were measured by high-performance liquid chromatography coupled to tandem mass spectrometry. No volunteers had dermatological reactions to the formulations. Only one blood sample had detectable levels of dapaconazole (0.23 ng mL^{-1}). One volunteer presented hypertriglyceridemia (424 mg dL^{-1}) after the 14 days of treatment. Three months after the last dose triglycerides were back to normal range (151 mg dL^{-1}). Dapaconazole 2% (Zilt®) showed a safe adverse event profile for topical application in daily doses of 40 mg for up to 14 days in healthy individuals.

Key words: Pharmacokinetics, skin, bioavailability, topical imidazole

INTRODUCTION

From the beginning of the 20th century until shortly after the Second World War, potassium iodide was the standard treatment for cutaneous fungal infections like tinea and sporotrichosis (Dostrovsky *et al*., 1960; Urabe and Nagashima, 1969). In 1946, the first polyene antifungals were obtained from the fermentation of *Streptomuces griseus*, which later led to the development of nystatin and amphotericin B (Whiffen *et al*., 1946). Progress of antifungal drug development has since then lagged behind that of the antibacterial antibiotics. The main reason for this is that, before emergence of HIV/AIDS as a global disease, the incidence of fungal infections was believed to be very low, which prevented extensive investments in this research area (Ghannoum and Rice, 1999).

Until the discovery of the azole antifungal drugs, amphotericin B was the only treatment for disseminated fungal infections. Therefore, the antifungal azole compounds constituted a considerable advance in the treatment of superficial and systemic infections of fungal etiology. The first generation of azoles was developed in the late 1960s and included several imidazole compounds, such as miconazole, clotrimazole and ecoconazole (Botter, 1971; Buchel *et al*., 1972; Thienpont *et al*., 1975).

Nowadays, resistance to commonly used antifungals is a significant problem, especially in the nosocomial setting, either in invasive or superficial mycosis (Sternberg, 1994; Rodloff *et al*., 2011; Faller *et al*., 2011; Canton *et al*., 2011; Lockhart *et al*., 2012; Verweij and Warris, 2013). Thus, the emergence of drug-resistant fungal infections has generated the need for new antifungal drugs.

The compound dapaconazole, 1-(2-(2,4-dichlorophenyl)-2-(4-(trifluoromethyl)benzyloxy)ethyl)-1H-imidazole, CAS 1269726-67-1, is a novel imidazole derivative and exhibits interesting anti-fungal properties (Table 1) (Kepler *et al*., 2012). A dose finding study has already been performed in 33 healthy male volunteers. Doses evaluated ranged from 0.5-250 mg (5 g of creams

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Table 1: Minimum Inhibitory Cconcentration (MIC) of miconazole nitrate and dapaconazole tosylate against various fungal species

			MIC (μg mL^{-1})	
Fungal species	Source	CFU mL^{-1}	Miconazole-N	Dapaconazole-T
Aspergillus niger	Standard sp.	-	>16.0	8.000
Microsporum gypseum	Clinical isolate	-	0.5	0.250
Microsporum canis	Clinical isolate	100	0.5	0.125
Trichophyton verrucosum	Clinical isolate	4700	>16.0	4.000
Trichophyton rubrum	Clinical isolate	22500	>16.0	4.000
Trichophyton mentagrophytes	Clinical isolate	2100	0.5	0.250

CFU: Colony formation units, Miconazole-N: Miconazole nitrate, Dapaconazole-T: Dapaconazole tosylate

with concentrations ranging from 0.01-5%) applied in single doses to healthy skin. Only 8% of blood samples collected for bioavailability evaluation had detectable levels of dapaconazole. Detections ranged from 0.24-8.0 ng mL^{-1} (De Moraes *et al*., 2014). No volunteers had dermatological reactions to any cream concentration evaluated. The present study was designed to evaluate the safety and tolerability of chronic treatment (14 consecutive days) of 2 g of a 2% topical formulation (daily dose of 40 mg) of dapaconazole (Zilt®). The study was performed in healthy volunteers of both sexes.

MATERIALS AND METHODS

Reagents: Cream containing dapaconazole tosylate 2.0% (Zilt®) and a formulation containing only vehicle were provided by Biolab Sanus Farmacêutica Ltda. (Brazil). The composition of the vehicle was as follows (w/w): Glycerol 5%, dimethicone 1%, methylparaben 0.18%, propylparaben 0.02%, edetate disodium 0.1%, octyl palmitate 10%, stearic acid 4%, glyceryl stearate/ PEG-100 stearate 14%, purified water 65%.

Human volunteers: Twenty-four volunteers (twelve men) participated in the study. Their median age was 32 years (19-46 years); mean body weight was 66.5 kg (44.0-93.0 kg), mean height was 165 cm (145-183 cm) and mean Body Mass Index (BMI) was 24.00 kg m^{-2} (19.63-27.60 kg m^{-2}).

No volunteers had any significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal or hematological diseases, as determined by their medical history, physical examination and routine laboratory tests (hematology, blood biochemistry and urine analysis) and had negative serology for HIV, hepatitis C and B (except for serologic scars). Women were negative for serum β-HCG. Because dermatological reactions are more easily evaluated in subjects with lighter skin pigmentation (Fitzpatrick scale I to III (Fitzpatrick, 1988)) and little body hair, volunteers with these characteristics were selected for the studies. Subjects were instructed to abstain from taking any drug including Over-The-Counter (OTC) medicines for 2 weeks prior to and during the study period. The study was performed according to the revised declaration of Helsinki for biomedical research involving human subjects (WMA., 2008) and the volunteers gave full informed consent. The protocol was approved by the Committee of Research Ethics of the State University of Campinas (UNICAMP), Campinas, Brazil (approval number 474.845).

Study design: The study had an open-label, two-treatments (vehicle and Zilt®) design. At first, twelve men were treated with Zilt® daily for 14 consecutive days. Treatments were performed by topical administration of 2 g of the cream on a fixed area (4×4 cm^2) on the right superior region of the back. Concomitantly with treatments on the right side, volunteers received 2 g of the vehicle on an equivalent area on the left superior region of the back, as a control for possible dermatological reactions to the vehicle. The products were removed 1 h after application. After treatment in men, twelve women were treated with the same administration scheme.

Before enrolment volunteers were tested with the vehicle in order to evaluate any reactions to its components.

Dermatological evaluation: Application of the product was performed by a physician and photographic documentation was made of the sites where Zilt® (right upper back) and vehicle (left upper back) were applied. Photographs were taken daily before and immediately after the application of the products, as well as before and immediately after product removal (1 h after products administration).

Dapaconazole bioavailability: To evaluate systemic bioavailability of dapaconazole following topical application, venous blood samples (7 mL) were collected before and 2, 4 and 6 h after products application on the first day of the study. The same occurred in days 7 and 13, but an extra 24 h sample was collected after application of the products (days 8 and 14, before product administration). Collected blood samples were centrifuged at 2000 g for 10 min, plasma was separated and stored at -20°C until assayed, for pharmacokinetic studies.

Dapaconazole quantification: Plasma dapaconazole was quantified by high-performance liquid chromatography coupled to tandem mass spectrometry as previously described (De Moraes *et al*., 2014).

RESULTS

Twenty-four volunteers received treatment. There were no dermatological reactions, such as dry skin, itching, altered pigmentation or erythema after 14 days of daily exposure to 40 mg of dapaconazole (Fig. 1).

Only one sample of the 294 had detectable levels of dapaconazole (volunteer 14, sample of 6 h of day 13, 0.23 ng mL^{-1}).

Two adverse events were detected:

- Volunteer 16 presented hypertrigly ceridemia (424 mg dL^{-1}) on the week following the end of the 14 days of treatment. Three months after the last dose triglycerides were back to normal range (151 mg dL^{-1})
- Volunteer 20 presented low hematocrit (35%) after treatment. This adverse event was considered unrelated to therapy

Volunteers 8, 12 and 24 dropped out due to personal reasons.

DISCUSSION

The present study results demonstrated that chronic treatment with 40 mg day^{-1} of dapaconazole tosylate for 14 days applied to healthy skin can be considered safe in healthy volunteers. This conclusion was based on the absence of local dermatological reactions at the site of the application and the low systemic bioavailability after topical application.

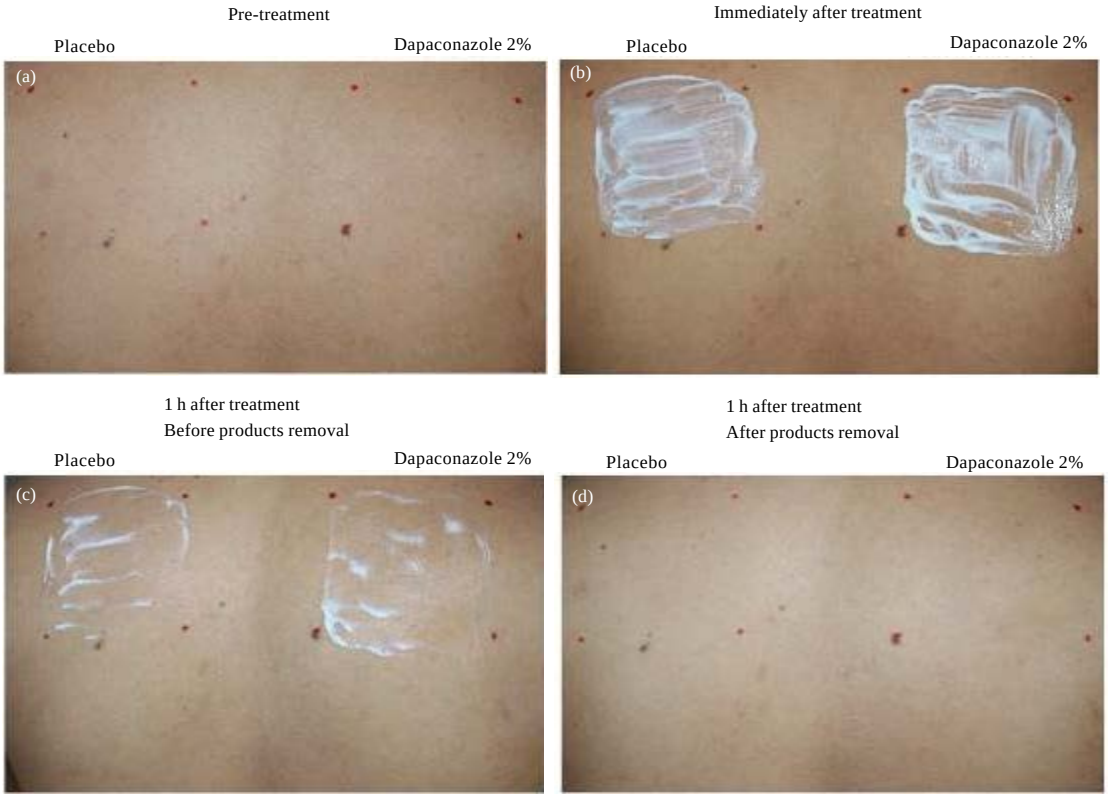

Fig. 1(a-d): Photographic documentation of possible dermatological reactions in volunteer 3 on day 14 of treatment. Figure show the back of the volunteer, (a) Before and (b) Immediately after the application of the 40 mg daily dose of Zilt® on the right side and placebo on the left side, 1 h after products administration, (c) Before and (d) After products removal

The common dermatological reactions to imidazole antifungals include erythema, dry skin, burning sensation, scaling and pruritus (Leiste *et al*., 1989; Jegasothy and Pakes, 1991; Suschka *et al*., 2002; Watanabe *et al*., 2007; Sharma *et al*., 2011). These adverse reactions are important factors in securing patient compliance with therapy, a major determinant of the success of the treatment of dermatomycosis (Ali *et al*., 2007; Weinberg, 2009).

A brief review of topical imidazoles available commercially today shows that these antifungal drugs have dermatological reactions as a common adverse event. In a study involving 250 patients with *Tinea corporis* comparing topical treatment with sertaconazole nitrate 2% cream or miconazole nitrate 2% cream twice a day for 2 weeks, the main dermatological reactions observed were: Burning in 3 (2.7%) patients receiving sertaconazole and in 2 (1.8%) receiving miconazole; pruritus in 2 (1.8%) patients of the sertaconazole group and in 3 (2.7%) of the miconazole group and dry skin in 2 (1.8%) patients receiving sertaconazole and in 4 (3.6%) individuals receiving miconazole (Sharma *et al*., 2011). In a similar study evaluating the safety of clotrimazole 1% cream once a day and ketoconazole 2% cream twice a day for four weeks in 106 patients (53 in each group) with *Tinea pedis*, dermatological reactions included burning after application of the product in one patient of each group (1.9%) and redness and scaling of one patient (1.9%) in the group receiving ketoconazole (Suschka *et al*., 2002). Another study evaluated the safety of the recently developed topical imidazole luliconazole in 224 patients with *Tinea pedis*. Patients received one of the 3 cream formulations evaluated (0.1, 0.5 and 1% luliconazole). The overall safety evaluation showed 2 cases of eczema (0.1 and 1%), 1 case of dermatitis (1%), 1 case of pruritus (0.5%) and 1 case of erythema (0.1%), all considered mild by the authors (Watanabe *et al*., 2007). In 955 patients treated with 1% oxiconazole nitrate in trials in the United States, the following adverse events were reported: itching (1.6%), burning (1.4%), irritation (0.4%), erythema (0.2%), maceration (0.1%) and fissuring (0.1%) (Jegasothy and Pakes, 1991). A study comparing 2% fenticonazole spray once daily versus 1% naftidine spray, once daily for 2-4 weeks, for dermatomycosis had 6% (3/50) of fenticonazole-treated patients reporting burning sensations after product application (Leiste *et al*., 1989). Daily occlusive patch applications of efinaconazole in concentrations up to 10% in healthy volunteers over a three-week period did not significantly change cumulative irritancy indices, when compared with vehicle and control-treated areas (Del Rosso *et al*., 2013).

Topical azole drugs have the therapeutic advantage of treating superficial fungal infections without generating high plasma levels of the drug, compared with orally administered azoles and thus show fewer drug interactions (Yu *et al*., 2005; Galatti *et al*., 2007; Alexandra *et al*., 2008; Wey *et al*., 2008; Broos and van Puijenbroek, 2010). A study with 2% miconazole nitrate cream in infants with diaper dermatitis showed that 80% had detectable levels of miconazole ranging from 5.2-7.4 ng mL^{-1} after 7 days of treatment with at least 5 applications per day. This study, however, did not specify the amount of cream (dose) applied at each intervention. No adverse events were noted in the infants with these plasma levels of miconazole (Eichenfield and Bogen, 2007). When administered intravaginally, a single-dose of an ovule containing 1200 mg of miconazole nitrate generated a mean maximum concentration (Cmax) of 10.7 ng mL^{-1} (Stevens *et al*., 2002). A single oral dose of 400 mg of posaconazole generated a mean Cmax of 611 ng mL^{-1} (Courtney *et al*., 2003). Oral fluconazole generates plasma levels of 2000 ng mL^{-1} after a 100 mg dose (Debruyne, 1997). Thus, dapaconazole plasma levels were much lower than those reported by several studies with topical miconazole nitrate, oral posaconazole or fluconazole.

There is no report in the literature of hypertriglyceridemia induced by treatment with topical antifungal azoles. However, orally administered azoles such as ketoconazole and itraconazole are both known to cause hypertriglyceridemia as a commonly reported but low incidence adverse event (Rollman *et al*., 1985; Tucker *et al*., 1990; De Lima Barros *et al*., 2011). Since the volunteer that had elevated triglycerides had no blood samples with a detectable level of dapaconazole, this systemic adverse event is unlikely to be related to the treatment.

CONCLUSION

Because of the absence of dermatological reactions to the formulation and the low systemic bioavailability, Zilt® showed a safe adverse event profile for topical application in daily 40 mg doses for up to 2 weeks in healthy individuals of both sexes. The lack of dermatological reactions also should improve patient compliance during the chronic treatment necessary for many fungal infections of the skin. This study gives support to efficacy evaluations of Zilt®.

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