Enhancing the Skin Flux of Tolnaftate Utilizing the Novel Excipient, Dodecyl-2-N,N-Dimethylaminopropionate (DDAIP)

Susan R. Meier-Davis*, Salma Debar1, Richard Martin1, Mohamed Hachicha1 and Bassam Damaj1

Abstract
Penetration of drug through the stratum corneum to reach the underlying epidermal and dermal layers is a critical step for effective therapy by the topical route. To enhance permeation, various penetration enhancers have been utilized in combination with active drugs. The novel excipient, dodecyl-2-N,N-dimethylaminopropionate (DDAIP), was utilized to assess the epidermal penetration of tolnaftate. Tolnaftate, an anti-fungal agent, is approved as an over-the-counter (OTC) product for the treatment of superficial fungal infections including, athlete’s foot, ringworm and jock itch. Utilizing human cadaver skin mounted on Franz cells, tolnaftate skin flux was evaluated with and without the presence of DDAIP. Over a 24-hour period, DDAIP at a concentration of 0.5% enhanced tolnaftate permeation through human cadaver skin relative to the marketed tolnaftate formulation (1%). Increased tolnaftate penetration may decrease the number of applications and treatment duration required for dermatophyte therapy.

Keywords: Tolnaftate; Permeation; Excipient; Dermatophyte; DDAIP

Introduction
Causative organisms responsible for fungal infections of the skin, classified as dermatophytes include Microsporum, Trichophyton, and Epidermophyton spp [1,2]. Dermatophytosis is generally limited to keratin-producing cells of the skin but may become more invasive due to trauma, moisture, secondary infection or patient status [2-4]. The superficial nature of the infection allows the fungus to be easily transmissible on contact. Therefore, early diagnosis and treatment initiation not only limit the primary infection but also transmission [1,2]. The treatment regimen frequently employed to treat these superficial epidermal fungal infections is topical application of tolnaftate [3]. Tolnaftate, a thio-carbamate, is fungicidal at its marketed concentration of 1%. The customary treatment regimen is initiation not only limit the primary infection but also transmission [3]. Tolnaftate is poorly absorbed and, hence, a good candidate for topical treatment of superficial infections. However, increased penetration of the anti-fungal may reduce the number of applications and the overall treatment duration necessary to clear the infection [6]. Resistance to anti-fungal therapy increases with duration of therapy [7]. Consequently, development of a tolnaftate formulation with increased epidermal permeation could not only decrease the treatment duration but also decrease the potential for organism resistance.

Efficient permeation of pharmaceutical agents through the stratum corneum is dependent upon permeation enhancers. Permeation enhancers are thought to enhance delivery of topically applied drugs by increasing the thermodynamic activity of the stratum corneum or traversing between the intercalated lipid layers [8,9]. A number of permeation enhancers have been used to enhance topical drug delivery including isopropl myristate, capric acid, lauric acid, myristic acid, oleic acid, Tween 20, Tween 80, sodium lauryl sulfate and Span 80 [10,11]. DDAIP is a novel excipient that also may act as a permeation enhancer in combination with pharmaceutical agents. The putative mechanism for enhanced permeation by DDAIP is due to the temporary changes of the lipid bi-layer permeation dynamics including loosening of the tight junctions between skin cells [12]. Additionally, at the pH of skin, DDAIP remains stable for >100 hours, potentially allowing prolonged permeation (unpublished data). The objective of this study was to determine whether DDAIP enhances the permeation of the marketed formulation of tolnaftate. Increased permeation of tolnaftate may contribute to enhanced cure rates of superficial dermatophyte infections.

Materials and Methods
All reagents used in this study were analytical reagent grade or better. Tolnaftate was commercially available (Lot 14007) and contains 1% tolnaftate, butylated hydroxytoluene, cetanol, liquid paraffin, methylparaben, polyoxyethylene cetyl ether, propylene glycol, propylparaben, purified water, sorbitan monostearate and stearyl alcohol. A tolnaftate formulation was developed with the novel excipient, dodecyl-2-N,N-dimethylaminopropionate (DDAIP, lot number R1050) and evaluated for skin penetration against the OTC product, both consisting of a final tolnaftate concentration of 1%. Human cadaver skin was obtained from the University of California, San Diego Skin Bank and stored at -80°C. The skin was thawed by immersing in 0.9% sodium chloride for 30 minutes prior to the experiment. In vitro skin flux was conducted by mounting the human cadaver skin sections (US Tissue & Cells-lots 118122127 and 118235129) onto Franz diffusion cells (Permegear, Model # VC9).

Each experiment comprised either 6 or 9 samples derived from two cadaver skin donors. The receptor compartment contained a solution of phosphate-buffered isotonic saline (PBS), pH 7.4 ± 0.1 with 20% ethanol, 0.1% gentamicin sulfate and 0.05% thio-urea, maintained at 32.0 ± 0.5°C. The test formulations (100 µL) were applied directly onto the cadaver epidermis (0.64 cm²). Samples were collected at specified times from the receptor compartment and the skin was homogenized for determination of tolnaftate levels. The skin
and receptor samples were analyzed for tolnaftate concentrations using liquid chromatography tandem mass spectrometry (LC-MS/MS).

**Analytical method**

The quantification of tolnaftate was conducted using LC-MS/MS by High Standard Products, Inc. The Shimadzu 20AD Integrated System was used with a Phenomenex Luna C18(2) Column (50 x 2 mm). For the mass spectrometry, the Applied Biosystems API 5000 was used with a TurboIonSpray (electrospray) source and samples were prepared using an acetonitrile precipitation.

**Data evaluation**

If the result for any sample was less than the limit of quantitation (LLOQ), then that sample was treated as a non-data value. Values <LLOQ were assigned a value of zero for the purpose of calculating key parameters. A suspected outlier result was confirmed if it was outside the range of the mean ± 2 standard deviations of the replicate values. The amount of permeated drug, Q, was calculated from the following equation:

\[ Q = C_t V + \sum_0^t 0.2 (C_t - x) \]

Where \( C_t \) is the concentration (µg/mL) of the sample at timepoint \( t \) and \( V \) is the volume of the cell (5.2 mL) and \( t-x \) represent all previous sampling timepoints. \( 0.2 (C_t - x) \) is the correction for all of the sampling amounts at previous timepoints. The flux is calculated by dividing \( Q \) by the area of the Franz Cell, which is 0.64 cm² at each timepoint. Skin content was reported as amount tolnaftate/g tissue. Statistics were calculated using the Student’s unpaired t test.

**Results**

Topical application of a 1% tolnaftate formulation with 0.5% DDAIP resulted in a significant increase (~57%) of tolnaftate concentrations in the skin homogenates after 24 hours, relative to the tolnaftate OTC formulation (Figure 1). Similarly, the cumulative flux, as determined by tolnaftate concentrations in the receptor compartment, was significantly increased with the tolnaftate/DDAIP formulation over a 24-hour period. The mean cumulative flux of 1% tolnaftate in 0.5% DDAIP over this duration was increased more than three times in comparison to the 1% tolnaftate without 0.5% DDAIP (Figure 2).

**Discussion**

Tolnaftate has been used extensively for the treatment of superficial mycoses since its approval more than forty years ago. Now available as an over-the-counter (OTC) product, novel formulations have not been developed, although cyclodextrin and combination products with glucocorticoids have been proposed [13,14]. The significantly increased tolnaftate concentrations achieved in the skin and receptor compartments with the tolnaftate/DDAIP formulation provide evidence that DDAIP enhances skin penetration of the drug. The putative mechanisms of action for DDAIP are creating reversible tight junction gaps and interacting with the polar region of the phospholipid bilayer allowing intercalation of skin ceramides [unpublished,15,16]. DDAIP (Figure 3) is a novel excipient present in the drug, Vitaros®, approved for the treatment of erectile dysfunction in Canada. The safety of DDAIP has been evaluated consistent with procedures outlined in the ICH HARMONISED TRIPARTITE GUIDELINE, “Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals” and FDA Guidance for Industry, “Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients” [17,18]. Based upon the nonclinical safety studies, DDAIP is not mutagenic or clastogenic. Further, in a two-year carcinogenicity assessment in mice, no tumors were found at dose levels up to 200 mg/kg/day. Topical administration of DDAIP in the Tg.AC transgenic mouse model was not tumorigenic at dose levels of 25 mg/kg/day. Topical administration to the mouse does not induce local or systemic toxicity at dose levels of 100 or 200 mg/kg/day, respectively. Consequently, nonclinical safety data support DDAIP administration by the topical route at dose levels of ≥25 mg/kg/day. Clinical safety evaluation of topical DDAIP revealed no sensitization or phototoxicity potential. In clinical trials applying DDAIP topically to >5000 patients, the excipient was generally well-tolerated. This study evaluated a novel tolnaftate formulation containing 0.5% DDAIP. Utilizing human cadaver skin, the in vitro skin flux and permeation of tolnaftate were significantly increased with the tolnaftate/DDAIP formulation. Enhanced penetration may...
decrease the number of applications and treatment duration required for dermatophyte therapy [6,7]. Although further formulation optimization is warranted, these results suggest a beneficial role for DDAIP in topical dermatophyte therapy.

The authors declare no competing interests.

References

Author Affiliation
NexMed Inc. 11975 El Camino Real, Suite 300, SanDiego, CA 9213, USA