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Transdermal iontophoretic delivery of terbinafine hydrochloride: Quantitation of drug levels in stratum corneum and underlying skin

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ABSTRACT

The objective of this study was to determine the effect of iontophoresis on the delivery of terbinafine hydrochloride (4%, w/w) into and across hairless rat skin. *In vitro* skin uptake and permeation studies were performed using Franz diffusion cells. Anodal iontophoresis was applied for 1 h at current densities of 0.2, 0.3 and 0.4 mA/cm^2. In addition, iontophoresis was applied for 15, 30, 45 and 60 min. Studies were conducted in which the formulation was either removed or left in contact with the skin following iontophoresis and then passive delivery was assessed 23 h later. Tape stripping and skin extraction were performed to quantify drug levels in the stratum corneum and the underlying skin, respectively. The samples were analyzed using HPLC. The amount of drug delivered into the stratum corneum following iontophoresis was not significantly different from the amount delivered passively ($p > 0.05$). However, drug levels in the underlying skin were significantly higher for the iontophoretic group. The amount of terbinafine delivered into the skin layers was influenced by current density and duration of current application. Leaving the drug formulation in contact with the skin during the post-iontophoretic period had a significant effect on drug levels delivered into skin layers. Iontophoresis enhanced the delivery of terbinafine hydrochloride into the skin layers and, therefore, may be used to improve the treatment of skin fungal infections.

1. Introduction

Terbinafine ($C_{21}H_{25}N$, TBF) is a synthetic allylamine antifungal agent used for the treatment of superficial fungal infections of the skin and nail (Novartis, 1993). It has a molecular weight of 291 Da and a pK_a of 7.1 (Alberti et al., 2001a). The drug is lipophilic and keratinophilic in nature, with an octanol/water partition coefficient (log *P* value) of 3.3 (Gupta and Shear, 1997; Alberti et al., 2001a,b). Fig. 1 shows the chemical structure of the molecule. Terbinafine is commercially available as oral (125 or 250 mg tablets) and topical (1% topical cream and spray solution) formulations (under the trademark Lamisil® as well as several other generic versions). The oral tablet is indicated for the treatment of onychomycosis (fungal infection of nail caused by dermatophytes such as *Trichophyton rubrum*, *T. mentagrophytes*) and several tineal skin infections (*Tinea corporis*, *T. cruris*, *T. pedis*), whereas the topical formulation is indicated for the treatment of fungal skin infections caused by dermatophytes (trichophytons), such as athlete's foot (*T. pedis*), jock itch (*T. cruris*), ringworm (*T. corporis*), and pityriasis (*T. versicolor*) (Novartis, 1993).

The stratum corneum is the primary site of action for terbinafine in superficial cutaneous fungal infections (Alberti et al., 2001c). The drug acts by blocking ergosterol biosynthesis in the fungal cell wall through the noncompetitive and irreversible inhibition of squalene epoxidase (a complex membrane-bound enzyme distinct from the CYP 450 enzyme system). This results in decreased ergosterol synthesis and increased intracellular accumulation of the toxic precursor; squalene, causing cell membrane disruption and cell death (Ryder, 1992).

In order to treat fungal infections effectively, the drug must be present at the site of action at a concentration above the minimum inhibitory concentrations (MIC) during the entire treatment period (Pershing et al., 1994). This necessitates oral administration of large doses for prolonged periods (2–6 weeks for skin infections and 6–12 weeks for nail infections) (Novartis, 1993). In addition, oral administration has been shown to be associated with drug-drug interactions (inhibition of CYP2D6, an important phase I metabolizing enzyme), hepatotoxicity, gastrointestinal and systemic side effects, lactose intolerance and other adverse effects (Amichai and Grunwald, 1998). An improved topical drug delivery approach could overcome these limitations as it provides immediate access to the site of infection and reduces unwanted systemic drug exposure (Alberti et al., 2001c). However, a major limitation of topical delivery for skin infections is poor bioavailability. Results

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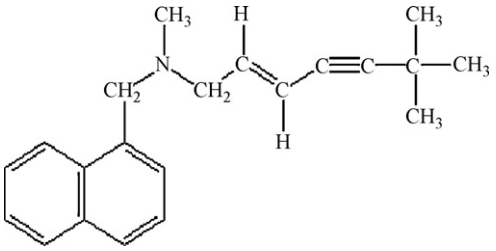

Fig. 1. Chemical structure of terbinafine base.

have shown a bioavailability of less than 5% in humans even after a week's treatment period (Novartis, 1993).

In the past, several enhancement techniques, such as chemical enhancers and iontophoresis, have been used to improve the transdermal and trans-ungual delivery of small and large drug molecules (Alberti et al., 2001b,c; Pikal, 2001; Murdan, 2002; Hui et al., 2003; Kalia et al., 2004; Pillai et al., 2004; Alba et al., 2006; Barry, 2006; Batheja et al., 2006; Kanikkannan et al., 2006; Hao and Li, 2008; Elkeeb et al., 2009). One of the more powerful enhancement techniques is iontophoresis, as it is the only one that provides a physical driving force to move drugs through biological membranes. This technique involves the use of small amounts of physiologically acceptable electric current (in the μA range) to drive charged or neutral drug molecules across the skin or nail. Electrostatic repulsion and electro-osmosis are the two processes which propel the drug molecules during iontophoresis. The former is the process in which ionized drug is driven across the barrier (skin/nail) when placed under the electrode of the same polarity, while the latter accounts for the movement of neutral molecules along with the bulk fluid flow (Kalia et al., 2004).

The use of iontophoresis for the transdermal and intradermal delivery of antifungal drugs into the various skin layers has not been previously explored. Currently, oral and topical terbinafine formulations are indicated only for superficial cutaneous fungal infections (dermatomycosis). Its use in the treatment of deep seated skin fungal infections (subcutaneous/cutaneous mycoses) is considered ineffective, most likely due to its inability to deliver adequate drug levels into the deeper epidermis or dermis layers of the skin where the infection exists. Hence, it would be beneficial to investigate whether higher drug levels in deeper skin layers could be attained with the use of iontophoresis.

Iontophoresis has recently gained heightened attention for use in trans-ungual delivery. Topical formulations are generally ineffective for the complete treatment of onychomycosis due to poor permeation across the human nail. Current treatment involves the use of oral and/or topical formulations for prolonged periods of time (6 weeks–3 months), creating patient compliance issues. Nair et al. (2008, 2009a,b) recently reported the use of iontophoresis as a promising technique to rapidly deliver significant levels of drug, both into and across the nail. This research group also investigated the use of iontophoresis for the *in vitro* delivery of terbinafine across the human nail plate. They reported a 16-fold enhancement in trans-ungual permeation and a 6-fold enhancement in drug load as compared to control. The drug levels delivered into the nail plate in their experiments were found to be significantly higher than those reported by Faergemann et al. following oral administration of 250 mg of drug for 28 days (Nair et al., 2008). More literature demonstrating successful use of iontophoresis for trans-ungual delivery has been reported recently (James et al., 1986; Murthy et al., 2007; Hao and Li, 2008; Amichai et al., 2009). However, it is anticipated that delivery of drug to both the nail and skin would be advantageous for the treatment of onychomycosis so that drug is delivered not only to the nail and nail bed, but also to the soft tissues surrounding the nail (e.g. lateral and proximal folds). Hence, determining drug levels in the skin following iontophoresis was investigated.

In this study, iontophoresis was used to deliver terbinafine hydrochloride into and across hairless rat skin *in vitro*. Subsequently, drug levels were determined in the stratum corneum (using tape stripping method), the underlying skin (using skin extraction method) and the receptor compartment. Studies to identify the rate limiting barrier for the penetration of terbinafine into the skin and predominant process driving the drug during iontophoresis were performed. The effect of current density, duration of current application and the presence or absence of formulation during the post-iontophoretic period were also investigated.

2. Materials and methods

2.1. Materials

The terbinafine hydrochloride (MW 327.90; 4%, w/w) formulation used in these studies was provided by Transport Pharmaceuticals, Inc., Framingham, MA. Methanol, propylene glycol, sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH), isopropyl alcohol, sulfuric acid, sodium borate for the preparation of borate buffer, hexane, formic acid, acetonitrile (HPLC grade), ammonium acetate and extraction tubes were purchased from Fisher Scientific (NJ, USA). Silver wire (0.5 mm diameter) and silver chloride used for the preparation of electrodes were purchased from Sigma Aldrich (St. Louis, MO, USA). De-ionized water was used for preparing all the solutions and during analysis by HPLC.

Male hairless rats, 8–10 weeks old weighing 350–400 g, were obtained from Charles River Laboratories (Wilmington, MA, USA) and housed in the Mercer University animal facility. All animals were quarantined and acclimated to the animal facility before their use in any study. The animal studies were conducted as per the protocol approved by Institutional Animal Care and Use Committee (IACUC) at Mercer University.

2.2. Skin isolation and preparation

Abdominal skin was freshly excised and prepared for use in each in vitro study. Rats were euthanized by CO_2 asphyxiation and gently laid in the area prepared for surgery. The abdominal skin was isolated using scissors and forceps. Following skin isolation, subcutaneous fat (if present) was carefully removed. The skin was then cleaned using de-ionized water and cut into appropriate size. These skin pieces were then mounted on the receptor compartment of the vertical Franz diffusion cells.

2.3. Preparation of electrodes

A planar coil of silver wire was prepared manually and used as anode. The cathode was custom made by coating a melt of silver chloride on a fine silver wire. The coating procedure was continued until a uniform and sufficient coat of silver chloride was obtained. The electrodes were freshly prepared on the day of the experiment.

2.4. Permeation experiments

In vitro permeation studies ($n \geq 3$) were performed using vertical Franz diffusion cells (Permegear, Inc., Hellertown, PA, USA). The receptor compartment was thoroughly washed prior to use and then filled with receptor buffer (5 ml, consisting of 10% ethanol, 30% propylene glycol, and 10 mM sodium chloride in de-ionized water, pH 5.8). The temperature of the cells was maintained at 37 °C throughout the experiment using a water circulation jacket built around the receptor chambers. The pH of the receptor buffer