International Journal of Pharmaceutics 388 (2010) 24–31

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

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

Vishal Sachdeva[a], Srujana Siddoju[a], Yi-Ying Yu[a], Hyun D. Kim[b], Phillip M. Friden[b], Ajay K. Banga[a,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Mercer University, Atlanta, GA 30341, United States
[b] Transport Pharmaceuticals, Framingham, MA 01701, United States

ARTICLE INFO

Article history:
Received 12 October 2009
Received in revised form 2 December 2009
Accepted 10 December 2009
Available online 16 December 2009

Keywords:
Iontophoresis
Terbinafine hydrochloride
Antifungal
Stratum corneum

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

* Corresponding author. Tel.: +1 678 547 6243; fax: +1 678 547 6423.
E-mail address: banga_ak@mercer.edu (A.K. Banga).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.12.029

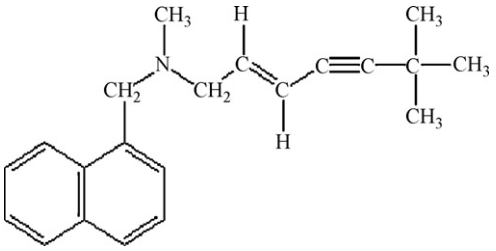

Fig. 1. Chemical structure of terbinafine base.

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

in all the receptor compartments was determined using a MI-410 combination pH electrode (Microelectrodes, Inc., NH, USA). Skin was mounted on the receptor compartments (effective area of diffusion was 0.64 cm^2) with the epidermal surface facing the donor chamber. The donor chambers were then placed on the mounted skin and secured in place using clamps. Terbinafine hydrochloride (4%, w/w) formulation (0.5 ml, pH 3.48) was then added to the donor chamber as donor solution. The anode (silver wire coil) was placed in the donor chamber and the cathode (custom made silver chloride electrode) was inserted into the receptor compartment through the sampling arm to perform anodal iontophoresis. For cathodal iontophoresis the arrangement of the electrodes was reversed with silver wire in the receptor compartment and the silver chloride electrode in the donor chamber. Using a constant current power supply (Keithley Instruments, Cleveland, OH, USA), anodal iontophoresis was performed for 1 h at 0.4 mA/cm^2 in most of the studies unless specified otherwise. Passive permeation studies in the absence of current ($n = 3$) served as the corresponding controls. Samples (0.5 ml) were withdrawn from the receptor compartment at predetermined time points and replenished with the same volume of fresh receptor buffer. Samples obtained were protected from light and air until analyzed using HPLC.

2.5. Tape stripped skin studies

In order to determine the primary barrier to the penetration of terbinafine into the skin, both passive permeation and anodal iontophoresis (0.4 mA/cm^2) were performed for 1 h across intact (with stratum corneum) and tape stripped (without stratum corneum) hairless rat skin. The latter was obtained by tape stripping the abdominal area of hairless rat skin. Trans-epidermal water loss (TEWL) measurements were recorded using a closed chamber evaporimeter (VapoMeter, Delfin Technologies Ltd., Kuopio, Finland) as an indirect measure to ensure complete removal of stratum corneum by the tape stripping process. Approximately 15 tape strips were able to remove the entire stratum corneum, as evidenced by an increase in TEWL values to 8–10 times the base value (obtained before tape stripping). Following 1 h permeation study, skin samples were cleaned of excess formulation, removed from the Franz cells and the drug was extracted. The amount of drug delivered into the underlying skin in the presence and absence of the stratum corneum was compared for both passive and iontophoretic groups.

2.6. Effect of current density and duration of current application

The influence of current density on the amount of drug delivered into the stratum corneum and the underlying skin was investigated by conducting anodal iontophoresis for 1 h at different current density values of 0.2, 0.3 and 0.4 mA/cm^2. For the duration of current application study, anodal iontophoresis was performed for 15, 30, 45 and 60 min at a current density of 0.3 mA/cm^2. Tape stripping and skin extraction was performed immediately after the study to determine the amount of drug delivered into the stratum corneum and underlying skin, respectively.

2.7. Post-iontophoretic delivery studies

To study drug permeation following iontophoretic delivery, 1 h anodal iontophoresis at 0.4 mA/cm^2 was followed by 23 h of passive permeation (post-iontophoretic period). Furthermore, in order to investigate whether the presence of the drug formulation has an impact on permeation during the post-treatment period, studies were performed with (formulation not removed, FNR or formulation partially removed; FPR) or without (formulation completely removed; FCR) the formulation in the donor compartment. Partial removal refers to removal of most of the formulation, leaving only a thin film on the treated skin area. Complete removal refers to the removal of all of the formulation followed by thorough cleaning with wet and dry Q-tips so to remove even trace amounts of drug formulation. At the end of 24 h (1 h treatment + 23 h post-treatment period), tape stripping and skin extraction studies were performed. Passive delivery, in the absence of current, using the same regimen as used for iontophoretic studies served as the corresponding controls for these studies.

2.8. Tape stripping

Following the permeation study, donor solution was removed and the skin mounted on the Franz cells was wiped three times with wet Q-tips soaked in receptor solution to remove excess formulation. Dry Q-tips were then rolled over the skin samples. In order to remove excess moisture, skin samples were gently dabbed with Kimwipes and placed on blotting paper. The treated area on each skin sample (0.64 cm^2) was tape stripped 15 times using 3 M Transpore tape (St. Paul, MN, USA) strips. The first two tape strips were discarded to avoid overestimation of the amount of terbinafine in the stratum corneum due to excess superficial formulation at the skin surface. It has been previously reported that the drug in the superficial stratum corneum layers, which is collected in the initial tape strips, is not absorbed into the system (Shah, 1998). The remaining tape strips were then placed in labeled six well plates for extraction with 0.01N HCl (0.5 ml). Extraction of drug from the strips was performed at room temperature for 3–4 h while shaking on a roller shaker (New Brunswick Scientific Co., Inc., NJ, USA). Samples were directly analyzed using HPLC.

2.9. Skin extraction

The area of the tape stripped skin exposed to formulation was punch biopsied and cut into small pieces using a scalpel blade. These skin pieces were transferred to tubes containing 1 ml of 5 M NaOH and incubated for 2 h at 60 °C. Following incubation, the mixture was cooled to room temperature and neutralized using 100 μl HCl (5N). Borate buffer (pH 10, 1.5 ml) was added and the tubes were vortexed (Fisher Scientific industries, Inc., Bohemia, NY, USA). Hexane (6 ml) was added to each tube and the drug was extracted by shaking on a roller mixer (New Brunswick Scientific Co., Inc., NJ, USA) for 60 min. The contents were then centrifuged for 10 min at 8000 × *g*. The upper organic layer containing extracted drug was separated and fresh hexane (6 ml) was added to the original tubes to extract the remaining drug from the aqueous layer using the above procedure. Meanwhile, the organic layer isolated previously was completely evaporated using nitrogen flushing to concentrate the drug. Following the second extraction, the tubes were centrifuged and placed in a −80 °C freezer to freeze the aqueous layer and obtain the entire organic layer. This organic layer was reconstituted into the tubes containing drug concentrate obtained earlier and vortexed. A solution (1.5 ml) of sulfuric acid (0.5 M) and isopropyl alcohol (85:15) was added to these tubes to back extract the drug from the organic layer by shaking for 30 min. The tubes were centrifuged at 8000 × *g* for 10 min and the upper organic layer was completely evaporated. 50 μl of the bottom layer was injected into the HPLC column for analysis, following filtration through a 0.22 μm filter.

2.10. Recovery studies

In order to determine the extraction efficiency, 50 μl of standard solutions having a known amount of drug were injected superficially into hairless rat skin samples (mean weight 250 mg) and allowed to equilibrate for 3 h. Four different amounts (5, 25, 125 and

250 μg) were injected into the skin pieces with three replicates for each amount. Following equilibration, the drug was extracted from the skin using the procedure described above. The actual amount extracted was determined using standard curve (0.5–500 μg). The extraction efficiency was found to be 75.0%. This value was taken into consideration while calculating the actual amount of drug present in the unknown skin samples.

2.11. Quantitative analysis

Terbinafine hydrochloride was quantified by high-performance liquid chromatography using an Alliance system (Waters Corp., MA, USA) with a photodiode array detector (waters, 2996) at 233 nm. Isocratic elution was performed using RP-18 Phenomenex column (Luna 5 μ C18 100 A, 250 mm × 4.6 mm, Phenomenex, Torrance, CA, USA). The mobile phase was acetonitrile, water and 100 mM ammonium formate, adjusted to pH 3.75 using formic acid (60:30:10, v/v). At a flow rate of 1.5 ml/min and 30 °C column temperature, the retention time was ~6 min. The run time employed was 13 min and the injection volume was 50 μl. The LOD was 0.25 μg and the lower limit of quantification was 0.5 μg. The range of standard curve prepared was 0.5–100 μg. The assay was sensitive for the range of interest.

2.12. Statistical analysis

Student's *t*-test and ANOVA (analysis of variance) was used to check the statistical significance. Post hoc analysis was performed using Tukey's analysis to determine the honestly significant difference (HSD). Mean of the replicate measurements ($n = 3$) with respective standard error (SE) was used to plot all graphs.

3. Results and discussion

3.1. Permeation studies

In this study, the influence of anodal iontophoresis on the delivery of terbinafine hydrochloride, both into and across the hairless rat abdominal skin, was investigated. The use of hairless rat skin in vitro; as an alternative to human skin, followed by in vivo studies in hairless rats for intradermal and transdermal drug delivery research has been reported in the past (Dickerson and John, 1964; Bronaugh et al., 1983; Morimoto et al., 1992; Lauer et al., 1997; Chen et al., 1998; Kikwai et al., 2005; Godin and Touitou, 2007; Paturi et al., 2009). Terbinafine is a small molecule and is categorized pharmacologically as an antifungal allylamine drug. It is highly lipophilic in the base form with a log *P* of 3.3. The hydrochloride salt of the drug (used here) on the other hand is relatively more polar. For the salt form of the drugs the appropriate terminology equivalent to octanol water partition coefficient is log *D* (logarithm of distribution coefficient; *D*). It is defined by Scherrer and Robert as "the ratio of the concentration of the compound of interest in the octanol (organic) layer to the concentration of all the species in the aqueous phase at a given pH, assuming that the organic layer contains only un-ionized species". The hydrochloride salt of terbinafine used in our study has a log *D* value of −0.32. This value was obtained using the values 3.3, 7.1 and 3.48 for log *P* (partition coefficient of the base form), pK_a (negative logarithm of salt's dissociation constant) and pH of the formulation, respectively in Eq. (1) (Scherrer and Howard, 1977; Kah and Brown, 2008). Furthermore, the salt form of the drug, upon ionization, will provide charged drug ions to enhance the electro-repulsive force between the ion and the active electrode. Thus, the high polarity of the charged drug molecule together with its ability to provide the chloride ions (essential for the completion of electrochemical reaction at anode when silver/silver chloride electrodes are used for iontophoresis) makes the hydrochloride salt more conducive for the iontophoresis mediated drug delivery (Behl et al., 1989; Kalia et al., 2004)

$$\log D_{\text{bases}} = \log P + \log\left[\frac{I}{I + 10^{\text{pK}_a - \text{pH}}}\right] \quad (1)$$

A glycerin-based formulation consisting of 4% (w/w) terbinafine hydrochloride was used as donor solution for the delivery studies. Besides the drug, the formulation consisted of 21% ethanol (95%), 5% tween 80, 40% glycerine, 0.3% hydroxyethylcellulose, 0.2% benzoic acid, 0.01% BHT and 0.01% di-sodium EDTA. This formulation was selected following optimization to maximize solubility, stability and iontophoretic delivery. The receptor solution consisted of propylene glycol (30%), ethanol (10%) and 10 mM

Table 1
Amount of drug delivered to different skin layers and receptor compartment during permeation studies.

Experimental conditions	Stratum corneum (μg)		Underlying skin (μg)		Receptor compartment (μg)	
	ITP	Passive	ITP	Passive	ITP	Passive
1 h studies						
Anodal ITP	16.42 ± 0.84	NA	38.70 ± 6.38	NA	0	NA
Cathodal ITP	3.32 ± 1.35	NA	1.21 ± 0.25	NA	0	NA
Passive	NA	23.51 ± 3.42	NA	13.82 ± 1.64	NA	0
Tape stripped skin	NA	NA	357.28 ± 12.53	371.78 ± 59.52	0	0
Effect of current density during 1 h anodal ITP						
0.2 mA/cm^2	9.42 ± 1.51	NA	11.64 ± 0.98	NA	0	NA
0.3 mA/cm^2	11.00 ± 2.06	NA	14.40 ±1.06	NA	0	NA
0.4 mA/cm^2	16.42 ± 0.84	NA	38.70 ± 6.38	NA	0	NA
Effect of duration of current application during anodal ITP at 0.3 mA/cm^2						
15 min	9.46 ± 0.72	NA	9.26 ± 0.62	NA	0	NA
30 min	7.01 ± 1.43	NA	12.20 ±1.27	NA	0	NA
45 min	12.20 ± 0.94	NA	18.22 ± 3.06	NA	0	NA
60 min	11.01 ± 2.06	NA	14.45 ± 1.06	NA	0	NA
Post-treatment studies (1 h anodal ITP + 23 h post-treatment period)						
ET	16.42 ± 0.84	23.51 ± 3.42	38.70 ± 6.38	13.82 ± 1.64	0	0
FNR	27.20 ± 8.67	40.80 ± 8.33	250.77 ± 53.83	80.40 ±17.03	0	0
FPR	23.33 ± 2.65	8.66 ± 1.00	123.29 ± 10.18	41.61 ± 4.36	0	0
FCR	10.73 ± 0.88	5.74 ± 1.15	52.83 ± 3.23	16.10 ± 0.72	0	0

ITP, iontophoresis; NA, not applicable; ET, end of treatment; FNR, formulation not removed; FPR, formulation partially removed; FRC, formulation removed completely. Current density and duration of study were 0.4 mA/cm^2 and 1 h (unless specified).

NaCl in de-ionized water. This composition was selected based on the high solubility of terbinafine hydrochloride in this solvent system. NaCl was added because the presence of anions in the receptor solution is required to complete the electrical circuit and allow rapid ramp up to the desired current levels, as the release of chloride ions from the cathode (silver chloride electrode in this study) is not instantaneous. The amount of salt added was kept low, so as to minimize competition with the drug ions for transport and maximize the delivery efficiency (Behl et al., 1989). At the end of the permeation experiment, tape stripping and skin extraction studies were performed to quantify drug levels in the stratum corneum and the underlying skin, respectively. These methods of quantifying drug levels in different skin layers have been employed previously and have gained considerable acceptance (Denouel et al., 1995; Escobar-Chavez et al., 2008; Herkenne et al., 2008).

3.2. Mechanism of iontophoretic drug transport

In order to identify the underlying mechanism of drug transport during iontophoresis, both anodal and cathodal iontophoresis was performed for 1 h at 0.4 mA/cm^2 using hairless rat skin. The amount of drug delivered into the stratum corneum and underlying skin was determined (Table 1). A significantly larger amount of drug was delivered into the stratum corneum and the underlying skin by anodal iontophoretic delivery as compared to cathodal delivery ($p < 0.05$, Fig. 2). This is due to the fact that, at the pH of the formulation used (3.48), nearly 99% of the drug was ionized (pK_a ~7.1) and positively charged. These charged drug molecules were repelled to move into the skin by the electro-repulsive force when placed under a like charged electrode (anode). Electro-osmosis did not seem to contribute towards the drug transport under the given conditions. This is because, as the pH of the formulation is reduced to 4 (the iso-electric point of the skin), the contribution of electro-osmosis towards electro-transport of the drug molecule decreases and its direction eventually reverses with further reduction in pH (Behl et al., 1989; Pikal and Shah, 1990; Banga, 1998). This is further evidenced by the significantly lower delivery under cathodal iontophoresis when compared to anodal iontophoresis or passive delivery (Fig. 2). Higher delivery under anodal iontophoresis was probably due to drug transport occurring by electro-repulsion as the predominating transport mechanism despite the presence of the opposing effect of electro-osmosis (from cathode to anode) at the pH of the formulation used. The lower delivery observed under cathodal iontophoresis when compared to passive delivery could be explained by electro-attraction,

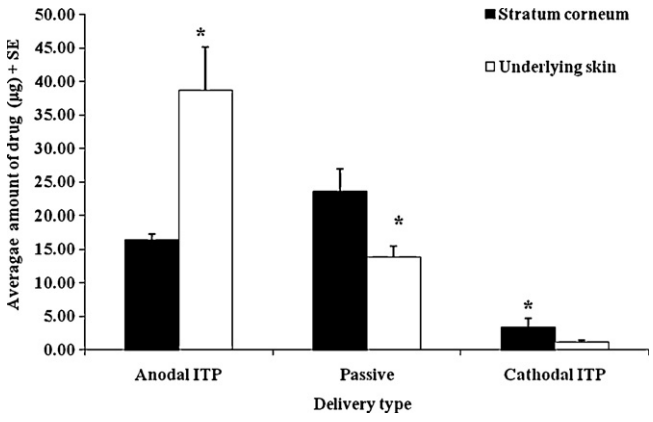

Fig. 2. Average amount of drug in stratum corneum (black bars) and underlying skin (white bars) following anodal iontophoretic, passive and cathodal iontophoretic delivery for 1 h. Iontophoresis was performed at 0.4 mA/cm^2. Asterisk represents significant difference ($p < 0.05$) from other delivery methods. The error bars shown are mean standard error.

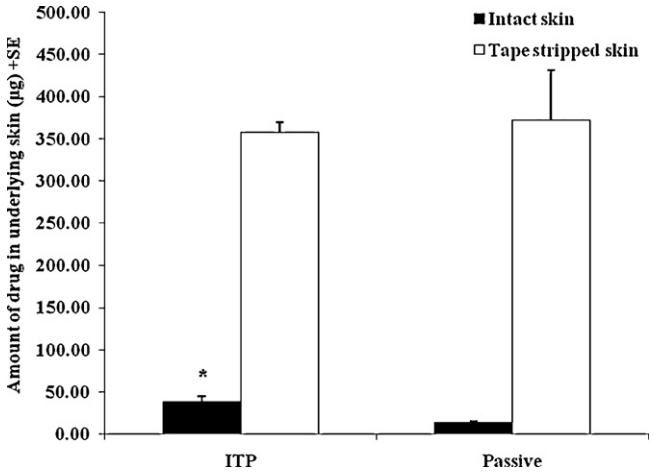

Fig. 3. Average amount of drug in the underlying skin following anodal iontophoretic and passive delivery through the intact (black bar) and tape stripped (white bar) hairless rat skin. Anodal iontophoresis was performed at 0.4 mA/cm^2 for 1 h. Asterisk represents significant difference ($p < 0.05$) from corresponding control group. The error bars shown are mean standard error.

which would have occurred between the positively charged drug molecules and negatively charged electrode (cathode). This process would likely predominate over electro-osmosis (occurring from cathode to anode direction), and thereby actively draw the drug away from the skin. Based on these findings, it seems that electro-repulsion was the major mechanism of transport for terbinafine hydrochloride in this iontophoretic system. This methodology to confirm the mechanism of drug transport under iontophoresis has been used earlier (Murthy et al., 2007). Also, Guy et al. (2000) previously reported that electro-repulsion is the primary mechanism of transport for low molecular weight compounds and that the contribution of electro-repulsion decreases with an increase in molecular weight. Since anodal iontophoresis was able to deliver significantly higher drug levels, it was employed for all the permeation studies.

3.3. Tape stripped skin studies

To determine the primary barrier to the permeation of terbinafine through the skin, both passive permeation and anodal iontophoresis (0.4 mA/cm^2) were performed for 1 h across intact (with stratum corneum) and tape stripped (without stratum corneum) hairless rat skin. The stratum corneum drug levels were not significantly different (Fig. 3, Table 1, $p > 0.5$) following either passive or iontophoretic delivery across the intact skin suggesting that the drug binding sites in the stratum corneum were probably saturated with the drug in both passive and iontophoretic delivery. The drug's lipophilic characteristics and its high affinity for binding to the keratinocytes most likely explains these observations (Uchida and Yamaguchi, 1993). The drug levels delivered into the underlying skin following iontophoresis (38.70 ± 12.53 μg) was however significantly (3-fold) higher than passive (13.82 ± 1.64 μg) delivery, in the presence of stratum corneum ($p < 0.5$). These results suggest the presence of a rate limiting step (barrier) which was restricting the movement of drug into the underlying skin. This rate limiting step was, however, overcome with the application of current as it was able to propel more drug into the deeper skin layers as compared to passive delivery. In order to identify the actual rate limiting step, delivery studies were then performed across tape stripped skin. The results obtained did not show a significant difference in the amount of drug delivered into the underlying skin following either delivery method (357.28 ± 12.53 and 371.78 ± 59.52 μg for iontophoretic and passive delivery, respectively, Fig. 3). Moreover, the levels delivered were 10–20 times

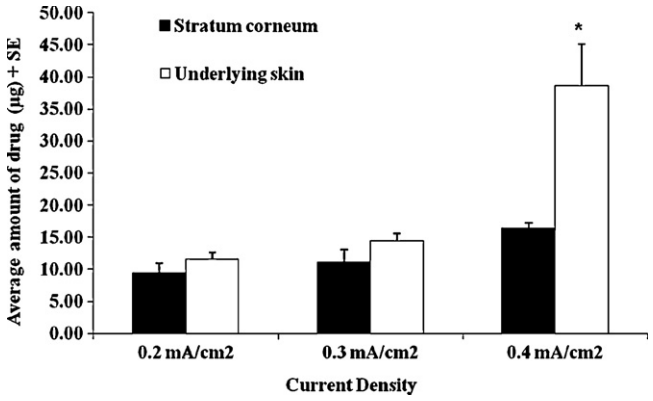

Fig. 4. Average amount of drug in stratum corneum (black bars) and underlying skin (white bars) following anodal iontophoresis at 0.2, 0.3 and 0.4 mA/cm^2 for 1 h. Asterisk represents significant difference ($p < 0.05$) from other groups. The error bars shown are mean standard error.

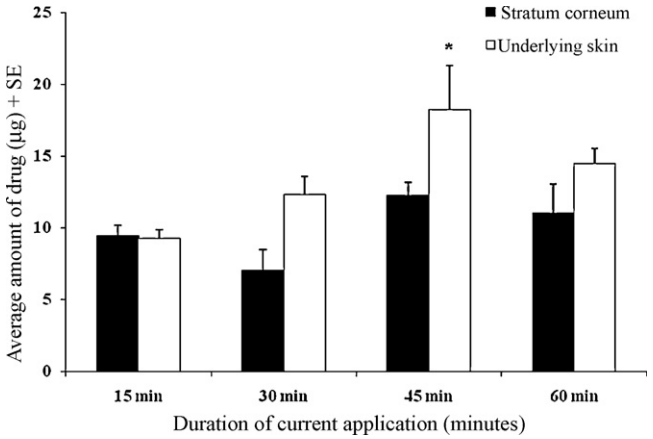

Fig. 5. Average amount of drug in stratum corneum (black bars) and underlying skin (white bars) following anodal iontophoresis at 0.3 mA/cm^2 for 15, 30, 45 and 60 min. Asterisk represents significant difference ($p < 0.05$) from other groups. The error bars shown are mean standard error.

higher than those delivered in the presence of stratum corneum, suggesting that the stratum corneum is the primary barrier to drug penetration into the skin. Also, in the absence of stratum corneum, the drug does not have to partition out into the more hydrophilic environment of the underlying skin. Instead, the hydrochloride salt directly diffuses into the tape stripped skin to give high drug levels, with no advantage seen with iontophoresis. Based on all these observations it can be concluded that the inability of the drug to partition "out" from the stratum corneum into the relatively more hydrophilic layers (epidermis and underlying skin) of the skin is the major rate limiting step for the permeation of this antifungal drug. These findings are in accordance with the observations reported earlier (Herkenne et al., 2008).

3.4. Effect of current density and duration of current application

The influence of iontophoretic parameters (current density and duration of current application) on the amount of drug delivered into the stratum corneum and the underlying skin was also investigated. For this, anodal iontophoresis was conducted for 1 h at current density values of 0.2, 0.3 and 0.4 mA/cm^2. Increasing the current density from 0.2 to 0.4 mA/cm^2 significantly increased the amount of drug delivered into the stratum corneum and the underlying skin ($p < 0.05$, Table 1, Fig. 4). However, a linear relationship between the two variables was not observed, as evidenced by the lack of significance in the difference between the amount of drug delivered using current densities of 0.2 and 0.3 mA/cm^2 (determined by Tukey's Honestly Significant Difference (HSD) test). In order to investigate the influence of duration of current application, anodal iontophoresis was performed for 15, 30, 45 and 60 min at a current density of 0.3 mA/cm^2. While there was a trend toward the amount of drug delivered increasing as a function of the duration of current application, a clear linear relationship was not observed. A significant difference was not observed in the amount of drug delivered into the stratum corneum at any of the durations tested ($p > 0.05$, Table 1, Fig. 5), suggesting that the stratum corneum was saturated fairly rapidly. For the drug levels in the underlying skin, a significant difference was observed for the 45 min time point (determined by Tukey's HSD test). This suggests that increasing the duration of current application to 45 min was able to increase the drug levels delivered into the underlying skin, but a further increase in time (to 60 min) did not do so. This is evidenced by the lack of statistical significance in the difference in the levels delivered into the underlying skin during 45 and 60 min of current application (determined by Tukey's Honestly Significant Difference (HSD) test). This could be due to the relatively rapid saturation of the skin binding sites with the drug. The difference in the levels might be more prominent at lower treatment durations when the skin would not be saturated with the drug.

3.5. Post-iontophoretic delivery studies

The influence of the presence or absence of drug formulation on further drug penetration into the skin during the post-treatment period was investigated in vitro. The amount of drug delivered into the stratum corneum and underlying skin layers (epidermis–dermis) for all the four groups ET (end of the treatment group where formulation was removed at the end of 1 h treatment), FNR (formulation not removed group where formulation was left in the donor cell for the entire study), FPR (formulation partially removed group where only a thin film of formulation was left in contact with skin after 1 h treatment) or FCR (formulation completely removed group where the formulation was completely removed from the donor compartment immediately after 1 h treatment) were compared.

Stratum corneum drug levels: For the drug levels delivered into the stratum corneum, significant difference was observed between the iontophoretic and passive delivery for the FPR and FCR groups ($p < 0.05$, Table 1 and Fig. 6). In addition, a statistical comparison

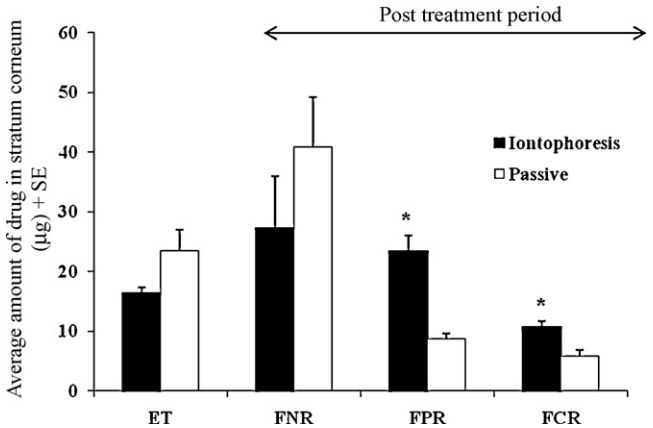

Fig. 6. Average amount of drug in stratum corneum for iontophoretic (black bars) and passive (white bars) groups following 24 h study. Terms ET, FNR FPR and FCR stands for end of treatment, formulation not removed, formulation partially removed and formulation completely removed, respectively. Asterisk represents significant difference ($p < 0.05$) from their corresponding controls. The error bars shown are mean standard error.

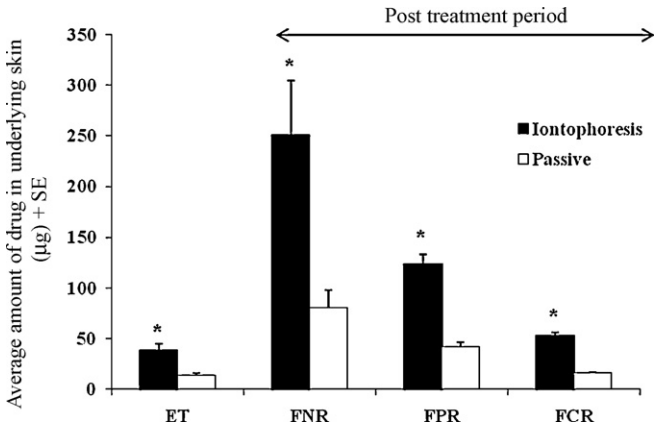

Fig. 7. Average amount of drug in underlying skin for iontophoretic (black bars) and passive (white bars) groups following 24 h study. Terms ET, FNR FPR and FCR stands for end of treatment, formulation not removed, formulation partially removed and formulation completely removed, respectively. Asterisk represents significant difference ($p < 0.05$) from their corresponding controls. The error bars shown are mean standard error.

across the four groups using ANOVA did not reveal any statistically significant differences, suggesting that the presence or absence of drug following the initial 1 h treatment period had no impact on the levels of drug detected after 24 h. These results suggest a saturation of the stratum corneum fairly rapidly upon exposure of the drug to the skin, most likely through binding of the drug to the keratin in this skin layer. There was, however, a trend suggesting that when the drug formulation was removed following the 1 h delivery period, the amount of drug in the stratum corneum decreases, reaching levels below that after the 1 h delivery period (ET group).

Underlying skin drug levels: For the skin drug levels during the post-treatment period we found that the levels were significantly higher for the iontophoretic than the passive delivery in all the groups ($p < 0.05$, Table 1 and Fig. 7). Nearly three times more drug was delivered into the underlying skin following iontophoresis when compared to passive delivery in the ET group. This increase is generally referred as the enhancement ratio. This initial enhancement, produced due to the driving force provided by iontophoresis, was observed to be maintained during the entire study (even though the current application was discontinued after 1 h). This is evidenced by the fact that a similar enhancement ratio is observed for all the groups (FNR, FCR and FPR) at the end of the post-treatment period of 23 h. The influence of maintaining the formulation in contact with the skin following treatment in the four iontophoretic groups was also determined using ANOVA. A significant difference was observed in the drug levels delivered ($p < 0.05$). Using Tukey's it was shown that the drug levels delivered in the FNR group were significantly higher as compared to all other groups (ET, FPR and FCR). This was probably due to maintaining the formulation in contact with the skin throughout the entire study duration, allowing higher drug delivery into the underlying skin layers. The ET group's drug levels were also significantly lower than FPR group, suggesting that leaving a thin film of formulation on the skin also increased drug levels in the underlying skin during the post-iontophoretic period. However, no significant difference was observed between the ET and FCR groups ($p > 0.05$) suggesting that drug levels delivered at the end of the 1 h treatment was maintained throughout the post-treatment period and no significant increase or decrease was observed when the formulation was completely removed. Similar observations were recorded upon comparison of the drug levels delivered into the underlying skin layers passively for the four groups. Thus, the drug levels in the underlying skin can be significantly enhanced by maintaining the formulation in contact with the skin beyond the period of iontophoretic delivery. Similar observations were made in a study by Volpato et al. (1998). These authors investigated the distribution of acyclovir in human skin layers following transdermal iontophoresis. They observed that when the formulation was left in contact with the skin (for 5 h) following 30 min of iontophoresis there was a significant increase in skin drug levels. Based on these findings the authors also hypothesized that repeated application of current might not be required to deliver high drug levels into the skin. Instead, utilizing a dosing regimen in which an iontophoretic pulse is followed by maintenance of a drug reservoir on the treated skin area may be more effective (Volpato et al., 1998).

3.6. Receptor analysis

No drug was detected in the receptor solution in any of the delivery studies, indicating that drug was not delivered across the skin. The presence of drug in the skin and the absence of detectable amounts in the receptor compartment suggest the formation of a drug depot/reservoir in the skin. This tendency of terbinafine to form a depot in the skin following topical application has been reported previously (Schafer-Korting et al., 2008). The accumulation of the drug in the underlying skin layers (epidermis–dermis) may be due to the association of the drug with the positively charged sites present in the skin, rendering it unable to partition further into the receptor compartment. Such associations have been previously reported for other lipophilic, positively charged drugs (Guy et al., 2000; Upasani and Banga, 2004). An important implication of this observation is that the drug tends to stay close to the site of delivery in the skin and thus should not cause unwanted systemic exposure when delivered using iontophoresis through skin.

3.7. Implications for drug levels in underlying skin

The presence of drug in the skin and the absence of detectable amounts in the receptor compartment suggest the formation of a drug depot/reservoir in the skin. The results confirm that the applied current was able to propel more drug into the deeper skin layers. The implications of the terbinafine depot that is formed in the underlying skin following iontophoretic delivery are that it could be used in the treatment of deep seated (cutaneous and subcutaneous) skin fungal infections, including infection of the soft tissues adjacent to the nail in onychomycosis.

Faergemann et al. suggested the use of fluconazole (triazole antifungal agent) for fungal infections localized deep in the epidermis–dermis based on the drug's ability to accumulate in the skin. The authors found that fluconazole was delivered in high concentrations to the stratum corneum, ecrine sweat glands and epidermis–dermis following oral administration to human volunteers (Faergemann and Laufen, 1993). They also found that terbinafine was mostly concentrated in the sebum, stratum corneum and peripheral nail clippings in patients following oral administration (Faergemann et al., 1994). However, in the results presented here, high concentrations of terbinafine were delivered into the epidermis–dermis of skin during the iontophoretic treatment period. This suggests that iontophoresis has the potential to deliver significant drug levels deep into the skin, thus allowing its possible use for the treatment of deep seated cutaneous fungal infections. Delivery to the soft tissues surrounding the nail may also help ensure complete eradication of the fungal infection associated with onychomycosis. Given the levels of drug delivered in the studies reported here, lower current levels and/or a shorter duration of current application may be sufficient for efficacious drug delivery. This mode of drug delivery has several potential advantages. Primarily, sustained release of drug from the drug depot into the surrounding infected areas will eliminate the need for frequent

topical application or oral administration and minimize the chance of recurrence.

4. Conclusion

Anodal iontophoresis enhanced the delivery of terbinafine hydrochloride, a water soluble form of a hydrophobic drug, into the skin. Rapid delivery to the target site was achieved with the use of this technique. The drug formed a depot in the deeper layers of the skin and did not permeate across the skin into the receptor solution over the duration of the experiment. Significantly more drug was recovered from the underlying skin following iontophoretic delivery as compared to passive delivery. The tendency of lipophilic drugs to form a depot in skin can be utilized for sustained drug delivery by appropriately characterizing and evaluating the formed depot. Furthermore, iontophoretic delivery of terbinafine into the soft skin tissues, such as those surrounding the nails, could be advantageous in ensuring continuous drug levels at the site of infection, which could translate into less frequent administrations and/or reduced chance for recurrence.

Acknowledgements

We want to thank Transport Pharmaceuticals, Inc. for funding this research project. We would also like to acknowledge Dr. Sameer Late for his helpful discussions throughout the project. Special thanks to Anushree Herwadkar for proofreading this manuscript.

References

Alba, N., Naik, A., Guy, R.H., Kalia, Y.N., 2006. Iontophoresis: clinical applications and future challenges. In: Smith, E.W., Maibach, H.I. (Eds.), Percutaneous Penetration Enhancers, 2nd ed. Taylor & Francis Group, pp. 177–219.

Alberti, I., Kalia, Y.N., Naik, A., Guy, R.H., 2001a. Assessment and prediction of the cutaneous bioavailability of topical terbinafine, in vivo, in man. Pharm. Res. 18, 1472–1475.

Alberti, I., Kalia, Y.N., Naik, A., Bonny, J., Guy, R.H., 2001b. Effect of ethanol and isopropyl myristate on the availability of topical terbinafine in human stratum corneum, in vivo. Int. J. Pharm. 219, 11–19.

Alberti, I., Kalia, Y.N., Naik, A., Bonny, J.D., Guy, R.H., 2001c. In vivo assessment of enhanced topical delivery of terbinafine to human stratum corneum. J. Control. Release 71, 319–327.

Amichai, B., Nitzan, B., Mosckovitz, R., Shemer, A., 2009. Iontophoretic delivery of terbinafine in onychomycosis: a preliminary study. Br. J. Dermatol..

Amichai, B.A., Grunwald, M.H., 1998. Adverse drug reactions of the new oral antifungal agents—terbinafine, fluconazole, and itraconazole. Int. J. Dermatol. 37, 410–415.

Banga, A.K., 1998. Electrically Assisted Transdermal and Topical Drug Delivery. Taylor & Francis, Bristol, PA.

Barry, B.W., 2006. Penetration enhancer classification. In: Smith, E.W., Maibach, H.I. (Eds.), Percutaneous Penetration Enhancers, 2nd ed. Taylor & Francis group, pp. 3–16.

Batheja, P., Thakur, R., Michniak, B., 2006. Transdermal iontophoresis. Exp. Opin. Drug Deliv. 3, 127–138.

Behl, C.R.,K.S., Kumar, S., Malick, A.W., Terzo, S. Del., Higuchi, W.I., Nash, R.A., 1989. Iontophoretic drug delivery: effects of physiochemical factors on the skin uptake of nonpeptide drugs. J. Pharm. Sci. 78, 355–360.

Bronaugh, R.L., Stewart, R.F., Congdon, E.R., 1983. Differences in permeability of rat skin related to sex and body site. J. Soc. Cosmet. Chem. 34, 127–135.

Chen, T., Langer, R., Weaver, J.C., 1998. Skin electroporation causes molecular transport across the stratum corneum through localized transport regions. J. Invest. Dermatol. Symposium proceedings/the Society for Investigative Dermatology, Inc 3, 159–165.

Denouel, J., Keller, H.P., Schaub, P., Delaborde, C., Humbert, H., 1995. Determination of terbinafine and its desmethyl metabolite in human plasma by high-performance liquid chromatography. J. Chromatogr. B: Biomed. Appl. 663, 353–359.

Dickerson, J.W., John, P.M., 1964. The effect of sex and site on the composition of skin in the rat and mouse. Biochem. J. 92, 364–368.

Elkeeb, R., Alikhan, A., Elkeeb, L., Hui, X., Maibach, H.I., 2009. Transungual drug delivery: current status. Int. J. Pharm..

Escobar-Chavez, J.J., Merino-Sanjuan, V., Lopez-Cervantes, M., Urban-Morlan, Z., Pinon-Segundo, E., Quintanar-Guerrero, D., Ganem-Quintanar, A., 2008. The tape-stripping technique as a method for drug quantification in skin. J. Pharm. Pharm. Sci. 11, 104–130.

Faergemann, J., Zehender, H., Millerioux, L., 1994. Levels of terbinafine in plasma, stratum corneum, dermis–epidermis (without stratum corneum), sebum, hair and nails during and after 250 mg terbinafine orally once daily for 7 and 14 days. Clin. Exp. Dermatol. 19, 121–126.

Faergemann, J.A., Laufen, H., 1993. Levels of fluconazole in serum, stratum corneum, epidermis–dermis (without stratum corneum) and eccrine sweat. Clin. Exp. Dermatol. 18, 102–106.

Godin, B., Touitou, E., 2007. Transdermal skin delivery: predictions for humans from in vivo, ex vivo and animal models. Adv. Drug Deliv. Rev. 59, 1152–1161.

Gupta, A.K., Shear, N.H., 1997. Terbinafine: an update. J. Am. Acad. Dermatol. 37, 979–988.

Guy, R.H., Kalia, Y.N., Delgado-Charro, M.B., Merino, V., Lopez, A., Marro, D., 2000. Iontophoresis: electrorepulsion and electroosmosis. J. Control. Release 64, 129–132.

Hao, J., Li, S.K., 2008. Transungual iontophoretic transport of polar neutral and positively charged model permeants: effects of electrophoresis and electroosmosis. J. Pharm. Sci. 97, 893–905.

Herkenne, C., Alberti, I., Naik, A., Kalia, Y.N., Mathy, F.X., Preat, V., Guy, R.H., 2008. In vivo methods for the assessment of topical drug bioavailability. Pharm. Res. 25, 87–103.

Hui, X., Chan, T.C., Barbadillo, S., Lee, C., Maibach, H.I., Wester, R.C., 2003. Enhanced econazole penetration into human nail by 2-n-nonyl-1,3-dioxolane. J. Pharm. Sci. 92, 142–148.

James, M.P., Graham, R.M., English, J., 1986. Percutaneous iontophoresis of prednisolone—a pharmacokinetic study. Clin. Exp. Dermatol. 11, 54–61.

Kah, M., Brown, C.D., 2008. Logd: lipophilicity for ionisable compounds. Chemosphere 72, 1401–1408.

Kalia, Y.N., Naik, A., Garrison, J., Guy, R.H., 2004. Iontophoretic drug delivery. Adv. Drug Deliv. Rev. 56, 619–658.

Kanikkannan, N., Babu, R.J., Singh, M., 2006. Structure–activity relationship of chemical penetration enhancers. In: Smith, E.W., Maibach, H.I. (Eds.), Percutaneous Penetration Enhancers, 2nd ed. Taylor & Francis group, pp. 17–34.

Kikwai, L., Babu, R.J., Prado, R., Kolot, A., Armstrong, C.A., Ansel, J.C., Singh, M., 2005. In vitro and in vivo evaluation of topical formulations of spantide ii. AAPS PharmSciTech. 6, E565–E572.

Lauer, A.C., Elder, J.T., Weiner, N.D., 1997. Evaluation of the hairless rat as a model for in vivo percutaneous absorption. J. Pharm. Sci. 86, 13–18.

Morimoto, Y., Hatanaka, T., Sugibayashi, K., Omiya, H., 1992. Prediction of skin permeability of drugs: comparison of human and hairless rat skin. J. Pharm. Pharmacol. 44, 634–639.

Murdan, S., 2002. Drug delivery to the nail following topical application. Int. J. Pharm. 236, 1–26.

Murthy, S.N., Wiskirchen, D.E., Bowers, C.P., 2007. Iontophoretic drug delivery across human nail. J. Pharm. Sci. 96, 305–311.

Nair, A.B., Kim, H.D., Chakraborty, B., Singh, J., Zaman, M., Gupta, A., Friden, P.M., Murthy, S.N., 2009a. Ungual and trans-ungual iontophoretic delivery of terbinafine for the treatment of onychomycosis. J. Pharm. Sci..

Nair, A.B., Kim, H.D., Davis, S.P., Etheredge, R., Barsness, M., Friden, P.M., Murthy, S.N., 2009b. An ex vivo toe model used to assess applicators for the iontophoretic ungual delivery of terbinafine. Pharm. Res. 26, 2194–2201.

Nair, A.B., Vaka, S.R., Sammeta, S.M., Kim, H.D., Friden, P.M., Chakraborty, B., Murthy, S.N., 2008. Trans-ungual iontophoretic delivery of terbinafine. J. Pharm. Sci..

Novartis, 1993. Prescribing information for lamisil (terbinafine hydrochloride), antifungal agent Dorval, control no. 107515.

Paturi, J., Kim, H.D., Chakraborty, B., Friden, P.M., Banga, A.K., 2009. Transdermal and intradermal iontophoretic delivery of dexamethasone sodium phosphate: quantification of the drug localized in skin. J. Drug Target..

Pershing, L.K., Corlett, J., Jorgensen, C., 1994. In vivo pharmacokinetics and pharmacodynamics of topical ketoconazole and miconazole in human stratum corneum. Antimicrob. Agents Chemother. 38, 90–95.

Pikal, M.J., 2001. The role of electroosmotic flow in transdermal iontophoresis. Adv. Drug Deliv. Rev. 46, 281–305.

Pikal, M.J., Shah, S., 1990. Transport mechanisms in iontophoresis. Ii. Electroosmotic flow and transference number measurements for hairless mouse skin. Pharm. Res. 7, 213–221.

Pillai, O., Kumar, N., Dey, C.S., Borkute, Sivaprasad, N., Panchagnula, R., 2004. Transdermal iontophoresis of insulin: Iii. Influence of electronic parameters. Methods Find. Exp. Clin. Pharmacol. 26, 399–408.

Ryder, N.S., 1992. Terbinafine: mode of action and properties of the squalene epoxidase inhibition. Br. J. Dermatol. 126 (Suppl. 39), 2–7.

Schafer-Korting, M., Schoellmann, C., Korting, H.C., 2008. Fungicidal activity plus reservoir effect allow short treatment courses with terbinafine in tinea pedis. Skin Pharmacol. Physiol. 21, 203–210.

Scherrer, R.A., Howard, S.M., 1977. Use of distribution coefficients in quantitative structure–activity relationships. J. Med. Chem. 20, 53–58.

Shah, V.P., 1998. Topical Dermatological Drug Product NDAS and ANDAS—In Vivo Bioavailability, Bioequivalence, In Vitro Release and Associated Studies. US Department of Health and Human Services, pp. 1–19.

Uchida, K., Yamaguchi, H., 1993. Studies on the affinity of terbinafine with keratin. Jpn. J. Med. Mycol. 34, 207–212.

Upasani, R.S., Banga, A.K., 2004. Response surface methodology to investigate the iontophoretic delivery of tacrine hydrochloride. Pharm. Res. 21, 2293–2299.

Volpato, N.M., Nicoli, S., Laureri, C., Colombo, P., Santi, P., 1998. In vitro acyclovir distribution in human skin layers after transdermal iontophoresis. J. Control. Release 50, 291–296.