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Iontophoresis mediated in vivo intradermal delivery of terbinafine hydrochloride

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ABSTRACT

The objective of this study was to investigate the use of iontophoresis for the delivery of terbinafine hydrochloride (TH) into hairless rat skin in vivo. Drug formulation was applied to the abdominal skin and studies were performed using anodal iontophoresis. A current density of 250 μA/cm^2 was applied for 10, 15 and 20 min. Tape stripping and skin extraction were performed thereafter. For depot clearance studies, 20 min treatment was followed by tape stripping and skin extraction at 12, 24 and 48 h. Results indicated that iontophoresis delivered significantly more drug into the deeper skin as compared to controls ($p < 0.05$). Drug levels in the stratum corneum (SC) and underlying skin increased with increasing duration of current application. Depot clearance studies suggested drug depletion within 24 h from SC. A redistribution of terbinafine from the SC to the underlying skin over time was observed. Drug was detectable in the underlying skin for at least 48 h suggesting that formation of a drug depot persisted for at least 2 days following iontophoretic treatment. Thus, iontophoresis of TH may be useful in delivering higher drug levels more rapidly into the superficial and deep seated skin infection sites to form a depot providing sustained release.

1. Introduction

Nearly 20–25% of the world's population has fungal infections of skin and nail at some point, which are primarily caused by dermatophytes (Foster et al., 2004; Havlickova et al., 2008). It is an issue of concern especially in America and Europe, as the number of cases being reported is on the rise due to a variety of reasons, including changing lifestyles (tight fitting clothes and shoes), increasing geriatric populations, HIV infections and the use of immunosuppressant drugs. Infected patients suffer through pain and difficulties in their day to day activities, which affects them not just physically but also socially and psychologically (Murdan, 2002).

Fungal infections of the skin may be broadly classified as both superficial and deep seated, depending upon the site of infection. Current treatment for these fungal infections involves the use of drugs administered by oral and/or topical routes (Novartis, 1993). However, hepatotoxicity, hematological problems, drug interactions, and systemic side effects following oral delivery have been issues of concern especially for the potent antifungal drug, terbinafine (Amichai and Grunwald, 1998). The topical delivery route offers advantages over oral delivery due to the ease of the direct delivery of the drug to the infected site, decreased systemic exposure and associated adverse effects, and reduced cost for the treatment (Alberti et al., 2001c). However, poor drug penetration into the skin is the reason why delivery via this route alone is often unsuccessful. Furthermore, due to the relapsing nature of the infection, it is required that the drug be applied for long treatment durations (from weeks to months) (Novartis, 1993). Like human skin, the human nail is poorly permeable to antifungal drugs and a very small amount of drug is actually delivered into the nail plate and nail bed following a topical application. In order to understand this reduced drug delivery the structure of the nail plate, its composition and properties were extensively studied. It was found out that the poor permeability is due to the intact and highly keratinized nature of the nail plate (Murdan, 2002). Thus, despite of the common problem of poor permeability following topical application for both skin and nail, the delivery profile of the drugs through the two biological membranes of the skin and nails cannot be generalized and have to be investigated separately.

In order to meet the challenges mentioned above, several approaches have been investigated in the past to deliver higher drug levels into the skin layers following topical application. These include the use of chemical enhancers, as well as physical enhancement techniques, for different drug molecules (Wearley and Chien, 1990; Alberti et al., 2001b,c; Abla et al., 2006; Shukla et al., 2009). Among the various physical enhancement techniques available, such as sonophoresis, iontophoresis, electroporation, microporation and others, the use of iontophoresis appears to be the most promising for this study, as it is the only technique that applies a

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driving force to propel the drug into the deeper skin layers. All other techniques mentioned above involve surface modification in one or the other way and still rely on passive diffusion for drug migration into the skin.

The application of iontophoresis for the transdermal delivery of small and large molecules has been performed extensively and reported in literature (Kalia et al., 2004; Pillai et al., 2004; Batheja et al., 2006). However, its use for intradermal delivery of different drugs has not been explored fully. Dexamethasone and lidocaine are two examples of drugs known to be delivered intradermally with the use of iontophoresis as an enhancement technique (Nirschl et al., 2003; Dixit et al., 2007).

Iontophoresis can be briefly defined as the use of physiologically acceptable levels of electric current (in the μA range) to drive charged or neutral drug molecules across a biological membrane. Drugs can be transported via iontophoresis by two mechanisms: electro-repulsion and electro-osmosis. Electro-repulsion is the phenomenon in which charged drug molecules are repelled when placed under an electrode of the same polarity as the drug. Electro-osmosis, transports uncharged drugs along with bulk solvent flow that occurs in the presence of electric current (Banga, 1998; Guy et al., 2000; Pikal, 2001; Kalia et al., 2004).

In this study, we investigated the use of anodal iontophoresis for the intradermal delivery of TH in vivo using a hairless rat model. Terbinafine is a potent synthetic allylamine antifungal drug which is approved by FDA for use in superficial skin and nail fungal infections. It has a MW of 291 Da, p*K*a of 7.1 and a log *P* value of 3.3 and is keratophilic and lipophilic in nature (Gupta and Shear, 1997; Alberti et al., 2001c). The drug is known to interfere with ergosterol biosynthesis in fungal cell wall synthesis by non-competitive and irreversible inhibition of the enzyme squalene epoxidase. This results in the depletion of ergosterol (required for fungal growth and survival) and accumulation of the toxic metabolite squalene. The later is responsible for the fungicidal activity of terbinafine (Ryder, 1992).

The effect of different durations of anodal iontophoresis on the delivery of TH into hairless rat abdominal skin was assessed in vivo using a custommade electrode system (described in Section 2). Tape stripping and skin extraction studies were performed to determine drug levels into the SC and underlying skin (epidermis-dermis) layers, respectively (Alberti et al., 2001a; Herkenne et al., 2008). Drug depot clearance studies were also performed to determine the drug's residence time in the SC and underlying skin.

2. Materials and methods

2.1. Materials

The TH formulation (MW 327.90, 4% w/w) was provided by Transport Pharmaceuticals Inc. (TPI, Framingham, MA). Methanol, propylene glycol, hydrochloric acid (HCl), sodium chloride, isopropyl alcohol, sodium hydroxide (NaOH), sulfuric acid, sodium borate for the preparation of borate buffer, formic acid, hexane, acetonitrile (HPLC grade), ammonium acetate and extraction tubes were purchased from Fisher Scientific (Pittsburgh, PA). Silver backing carbon electrode integrated with poly urethane foam and sponge electrode used as anode and cathode, respectively, were provided by TPI. De-ionized water was used for preparing all the solutions and samples.

2.2. Animals for in vivo studies

Male hairless rats (8–10 weeks old and weighing 350–400 g) were obtained from Charles River Laboratories (Wilmington, MA) and housed in the vivarium at Mercer University. The animals were quarantined and allowed to acclimatize for at least a 7-day time period before being used in any study. All the animal studies were conducted according to the protocol approved by the Institutional Animal Care and Use Committee (IACUC) at Mercer University.

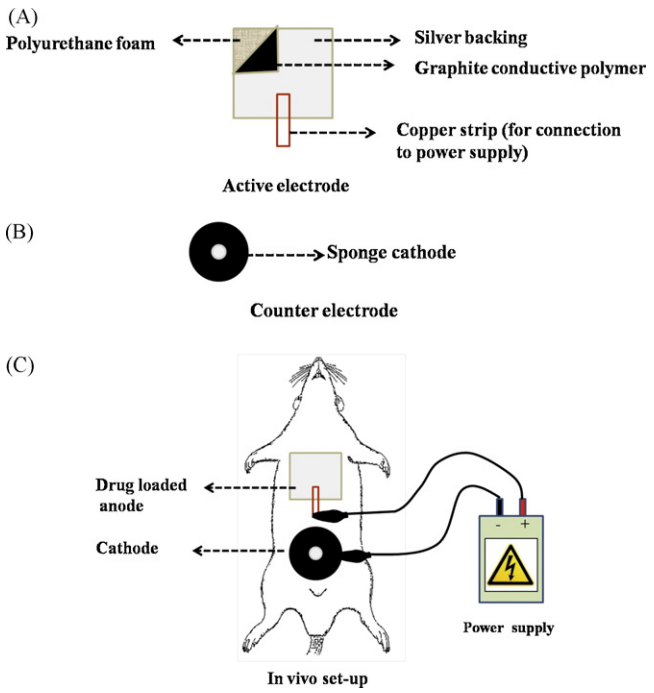

Fig. 1. (A) The anode (active electrode) used in the study. It consisted of graphite conductive polymer with silver backing (1 ml) integrated with a medical grade hydrophilic open cell polyurethane foam. A small conductive copper strip, approximately 3 mm × 12 mm, adhered on the silver side of the anode such that it protruded outwards; as shown in the figure. (B) Custom made Vermed sponge electrode, which was used as cathode. (C) The in vivo set up used for performing anodal iontophoresis in hairless rat model. Drug loaded anode and cathode was placed on abdominal surface and connected to constant current power source.

2.3. Electrode assembly for iontophoresis

The anode consisted of a custom-made graphite conductive polymer (1 ml) integrated with a medical grade hydrophilic open cell polyurethane foam (Fig. 1A). The conductive polymer consisted of a graphite loaded vinyl coating on one side and a thin silver layer on the other side. The surface area of this electrode was 2 cm^2. A small conductive copper strip, approximately 3 mm × 12 mm, was adhered on the silver side of the anode such that it protruded outwards (Fig. 1A). This protruding area allowed connection between the anode and power source using alligator clips (Fig. 1C). A Vermed (Bellows Falls, VT) sponge electrode was used as the cathode (Fig. 1B).

2.4. Trans-epidermal water loss (TEWL)

A non-invasive, portable, closed-chamber evaporimeter (VapoMeter®, Delfin Technologies Ltd., Kuopio, Finland) was used as an indirect measure to ensure complete removal of the SC by tape stripping. To determine TEWL values, the device was placed over clean and dry abdominal skin and held in place for a set amount of time. The TEWL measurements were recorded in triplicates before (for baseline values) and after (indicator of SC removal) every 5 tape strips during the tape stripping procedure. The values thus obtained were plotted as a function of the number of tape strips with the TEWL values on the *y*-axis and the tape strip number on the *x*-axis.

2.5. Tape stripping

For tape stripping, the treated and marked abdominal surface area was cleaned thoroughly using wet (with water, two times) and dry Kim wipes (two times). TEWL measurements were recorded prior to tape stripping using a VapoMeter®, as described above. 3 M transpore tape (St. Paul, MN) strips of equal size were cut and weighed. Each pre-weighed tape strip was placed over the treated abdominal surface area (2 cm^2) and pressurized by rolling the glass rod 40 times (to maximize the adhesion between the tape strip and skin). The strip was then removed using forceps with force at once. To ensure complete removal of the SC from the entire surface, the direction of strip removal was altered and tape stripping was performed 30 times. TEWL measurements were made in triplicates after every 5 tape strips. These tape strips were then weighed and placed in labeled 6-well plates for extraction with 0.01 N HCl. The first five tape strips were extracted individually and the remaining strips were extracted in groups of five. Drug extraction consisted of shaking on a roller shaker (New Brunswick Scientific Co., Inc., Edison, NJ) at room temperature for 3–4 h. Samples were directly analyzed using HPLC. The drug amounts in the first two strips were not included in the analysis to eliminate any possible surface contamination.

2.6. Skin isolation and extraction

Following tape stripping, the rats were euthanized by CO_2 asphyxiation. The tape stripped abdominal skin area was isolated using scissors and forceps. The drug was then extracted from the skin samples using a modified version of the method reported by Yeganeh and McLachlan (2000). Briefly, the skin sample was cut into small pieces and hydrolyzed by incubating for 2 h at 60 °C with 1 ml 5 M NaOH in extraction tubes. Following incubation, the mixture was cooled down to room temperature and neutralized using 100 μl HCl (5 N). Borate buffer (pH 10, 1.5 ml) was added and tubes were vortexed (Fisher Scientific industries, Inc., Bohemia, NY). Thereafter, the drug was extracted into hexane (6 ml) by shaking the tubes for 60 min on a roller mixer (New Brunswick Scientific Co., Inc., Edison, NJ). The organic layer was separated following centrifugation for 10 min at 8000 × *g* and evaporated in order to concentrate the drug by purging with nitrogen. The remaining drug in the borate buffer layer was extracted again with fresh hexane (6 ml). Following this extraction, the contents were centrifuged and the aqueous layer was frozen at −80 °C (so that entire organic layer could be separated easily). The organic layer was reconstituted with the drug concentrate obtained earlier and from the latter the drug was extracted into 1.5 ml of a mixture of sulfuric acid (0.5 M) and isopropyl alcohol (85:15) by shaking for 30 min. The tubes were centrifuged at 8000 × *g* for 10 min following extraction and the organic layer was completely evaporated as explained earlier. The aqueous layer containing the extracted drug was injected (50 μl) into the HPLC column for analysis, following filtration through a 0.22 μm filter.

2.7. Recovery studies

These studies were performed to determine the drug extraction efficiency. For this, 50 μl of drug solutions having known drug amounts were injected superficially into hairless rat skin samples (mean weight 250 mg) in vitro. The skin samples were left 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. These skin samples were then extracted for drug using the procedure described above. The actual amount of drug extracted was determined using standard curve having a range of 0.5–500 μg. The extraction efficiency was found to be 75% and was used in all calculations to determine the actual amount of drug in the skin samples.

2.8. Drug delivery studies

In vivo studies ($n \geq 4$) were performed in hairless rats using anodal iontophoresis. The rats were anesthetized using an intraperitoneal injection of ketamine and xylazine. The abdominal surface was cleaned thoroughly twice using a wet paper towel and then dried with a Kim wipe. The baseline TEWL value was then recorded in triplicate using the VapoMeter®. The anode was loaded with a measured volume (200 μl) of 4% (w/w) TH formulation (pH 3.48) and allowed to soak into the pad for 60 s. The drug-loaded anode and cathode were placed over the abdominal surface of the anesthetized rats at least 1 cm apart, ensuring no contact between the two electrodes (Fig. 1C). The electrodes were secured in place using surgical tape ensuring complete contact with the skin surface. The electrodes were then connected to a constant current device (Keithley Instruments, Cleveland, OH) and a current density of 250 μA/cm^2 was applied for 10, 15 and 20 min. Following treatment, the drug applicator was removed and the skin was cleaned and tape stripped. The underlying skin was isolated and extracted for drug. Studies performed without current application served as corresponding passive controls.

2.9. Depot clearance studies

For depot clearance studies (Fig. 2), drug delivery was performed for 20 min using the procedure described above. Following treatment, the anode and cathode were removed and the treated area was marked using permanent marker and thoroughly cleaned twice using wet and dry cotton Q-tips ensuring complete removal of adhering formulation. The treated area was visually inspected for burns or redness and TEWL measurements were recorded. Thereafter, the treated area was covered using a crape bandage and the rats were placed in the cages with complete access to food and water during the post-treatment period. The rats were then anesthetized 12, 24 or 48 h following treatment and tape stripping was performed using the procedure described earlier. TEWL measurements were recorded before, during and after tape stripping. Tape stripped skin was then isolated and extracted for drug. Passive delivery studies without current application were also performed at all time points as controls.

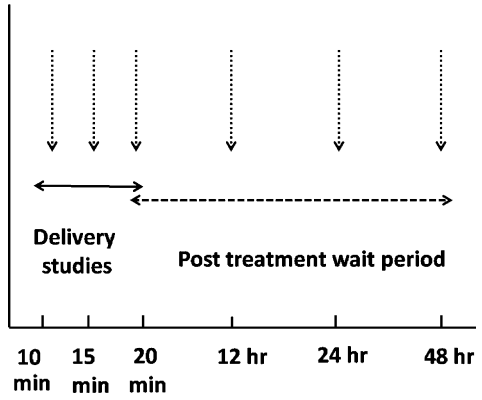

Fig. 2. Graphical representation of the studies performed during delivery and depot clearance studies. Double sided solid arrow indicates the delivery studies performed for either 10, 15 or 20 min. Double sided dashed arrow indicates the post-treatment wait period from 0 h after 20 min treatment to 48 h. Downward dotted arrows indicate the time points at which tape stripping and skin extraction was performed during delivery and depot clearance studies.

2.10. Quantitative analysis

TH was quantified using an Alliance high performance liquid chromatography system (Waters Corp., MA, USA), equipped with a photodiode array detector (Waters 2996). Isocratic elution was performed using RP-18 Phenomenex column (Luna 5μ C18 100 A, 250 mm × 4.6 mm, Phenomenex, Torrance, CA). The mobile phase consisted of 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 run time employed was 11 min and the injection volume was 50 μl. Detection was performed at 233 nm to give a peak with retention time of ~6 min. The LOD was 0.25 μg and the lower limit of quantification was 0.5 μg. The standard curve was prepared over the range of 0.5–100 μg.

2.11. Statistical analysis

Analysis of variance (ANOVA) and Student's *t*-test were employed in the study to determine statistical significance. All graphs were plotted using mean of replicate measurements ($n \geq 4$) with respective standard error (SE).

3. Results

3.1. Intradermal drug delivery studies

The amount of drug delivered into the SC (Fig. 3A) and deeper skin layers (Fig. 3B) using iontophoresis was found to be greater than the passive control group and increased with the time of current application. The amount of drug delivered into the SC following the 10, 15 and 20 min treatment periods was 20.60 ± 2.77 μg, 40.96 ± 11.67 μg and 48.21 ± 8.80 μg, respectively, for iontophoretic delivery and 6.60 ± 0.61 μg, 20.75 ± 5.33 μg and 25.78 ± 3.75 μg, respectively, for the passive delivery group (Fig. 3A). The amount of drug delivered into the underlying skin (Fig. 3B) using iontophoretic delivery (1.45 ± 0.13 μg, 4.71 ± 0.68 μg and 10.51 ± 1.28 μg for the 10, 15 and 20 min treatments, respectively) was significantly higher ($p < 0.05$) than passive delivery (0.61 ± 0.06 μg, 1.67 ± 0.11 μg and 4.36 ± 0.74 μg, respectively).

3.2. Depot clearance studies

The results obtained from the depot clearance studies are plotted in Fig. 4A and B. The drug levels in the SC (Fig. 4A) at the 0 and 12 h time points following treatment were found to be 48.21 ± 8.8 μg and 4.71 ± 0.94 μg (for iontophoretic delivery) and 25.78 ± 3.75 and 5.05 ± 0.64 μg (for passive delivery), respectively. At the 24 and 48 h time points, no drug was found in the SC for either treatment group. The amount of drug delivered using iontophoresis was significantly higher only for the 0 h time point ($p < 0.05$). Similarly, the amount of drug delivered into the underlying skin showed a characteristic decreasing trend as the post-treatment period increased (Fig. 4B). The amount of drug delivered into the skin at the 0, 12, 24 and 48 h time points was found to be 10.51 ± 1.28 μg, 14.27 ± 2.26 μg, 5.11 ± 0.65 μg and 0.72 ± 0.06 μg for iontophoretic delivery and 4.36 ± 0.74 μg, 6.86 ± 1.12 μg, 2.94 ± 0.61 μg and 0 μg for passive delivery, respectively. The amount of drug delivered using iontophoresis was significantly higher than that for passive delivery at all time points ($p < 0.05$).

3.3. TEWL measurements

The TEWL values were recorded in all of the studies prior to and at the end of treatment. The former value served as the base value for comparison with the value obtained at the end of treatment. The baseline TEWL values did not change significantly after iontophoretic or passive treatment in any of the studies, suggesting there was no effect on barrier function as a result of treatment. TEWL values recorded during the tape stripping procedure for the 48-h time point depot clearance study is shown in Fig. 5A (for iontophoretic delivery) and Fig. 5B (for passive delivery). The values essentially leveled off at approximately 10 times the base value after 20–25 tape strips in most of the studies, suggesting complete removal of the SC by tape stripping. Similar observations were recorded for all trials in all studies.

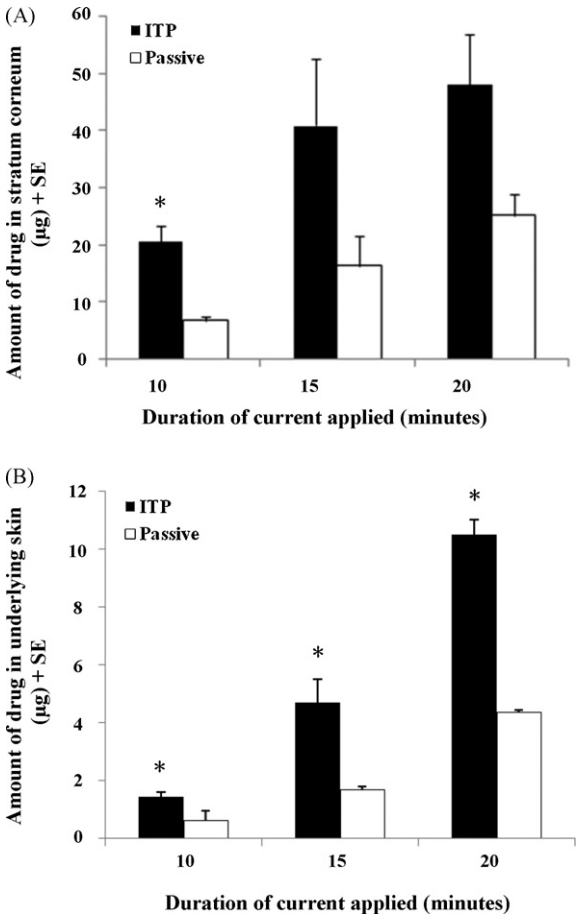

Fig. 3. (A) The average drug levels delivered into the SC following 10, 15 and 20 min of iontophoretic (ITP, black bars) and passive (white bars) delivery. (B) The average drug levels delivered into the underlying skin following 10, 15 and 20 min of iontophoretic (ITP, black bars) and passive (white bars) delivery. The error bars indicate the mean standard error. *Statistically significant difference when compared with results for passive trials ($p < 0.05$).

4. Discussion

The use of anodal iontophoresis for the intradermal delivery of a potent antifungal allylamine drug was investigated in this study. The antifungal drug terbinafine, which is indicated for the treatment of skin and nail fungal infections, is a small lipophilic molecule with a log *P* value of 3.3. In order to maximize iontophoretic delivery, the hydrochloride salt form of the drug (TH, MW 327 Da) with a log *D* (log distribution coefficient; equivalent term for log *P* for ionizable salts) value of −0.32 was used in this study. This log *D* value was calculated by inserting the log *P* value of the base (3.3), the pH of the formulation used in the study (3.48), and the p*K*a of the base (7.1) in the equation described in literature (Scherrer and Howard, 1977; Kah and Brown, 2008). At the pH of the formula-

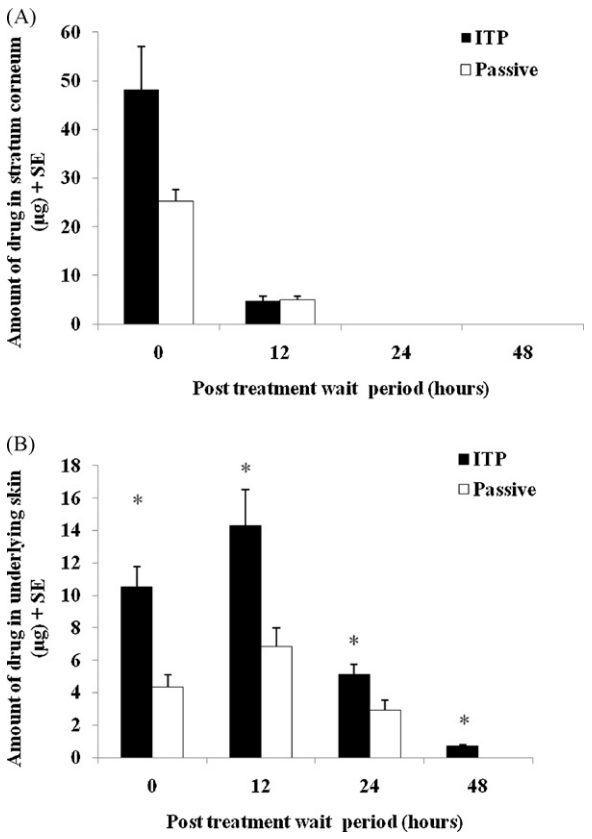

Fig. 4. (A) The average drug levels remaining in the SC during post-treatment wait period following 20 min of iontophoretic (ITP, black bars) and passive (white bars) delivery. (B) The average drug levels remaining in the underlying skin during post-treatment wait period following 20 min of iontophoretic (ITP, black bars) and passive (white bars) delivery. The error bars indicate the mean standard error. *Statistically significant difference when compared with results for passive trials ($p < 0.05$).

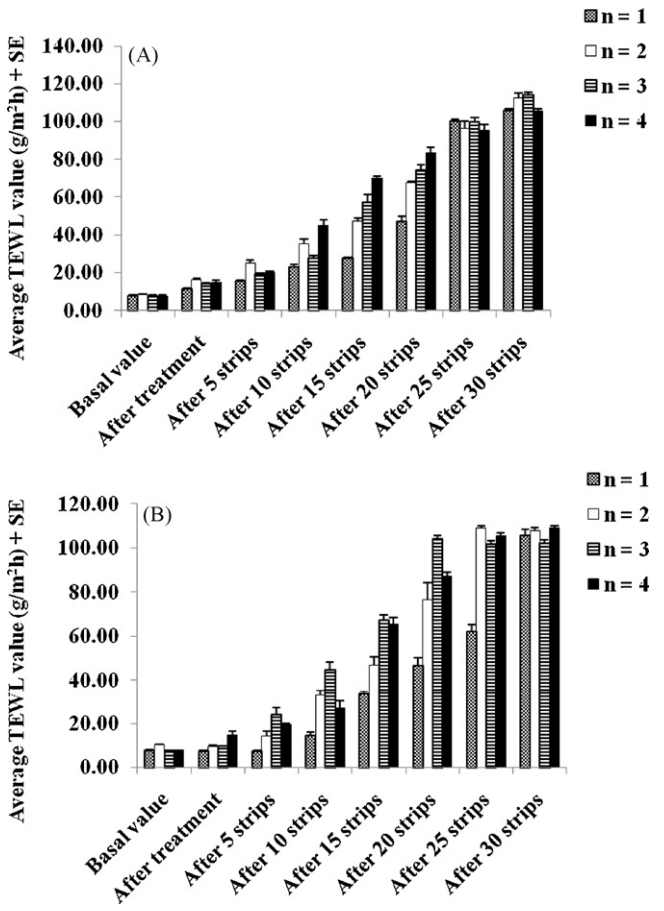

Fig. 5. (A) The average TEWL values recorded during the tape stripping procedure for all four trials following 20 min iontophoretic treatment and 48 h of post-treatment wait period. (B) The average TEWL values recorded during the tape stripping procedure for all four trials following 20 min passive treatment and 48 h of post-treatment wait period. The error bars indicate the mean standard error.

tion used (3.48) the acid salt of the drug will ionize to nearly 99% to furnish positively charged drug ions. Furthermore, it is known that the primary mechanism of drug transport for terbinafine ions under current application is electro-repulsion (Nair et al., 2009a,c). Knowing the degree of ionization and the primary drug transport mechanism at the selected pH, it can be hypothesized that using the hydrochloride salt form of this drug will enhance the iontophoretic efficiency by increasing the electro-repulsive force between the anode and positively charged drug ion (Behl et al., 1989; Kalia et al., 2004).

The drug formulation used in this study was glycerine-based and consisted of 4% (w/w) TH, 21% ethanol (95%), 5% Tween 80, 40% glycerine, 0.3% hydroxyethylcellulose, 0.2% benzoic acid, 0.01% BHT and 0.01% di-sodium EDTA (Sachdeva et al., 2010). The formulation was selected to ensure maximum solubility, stability and delivery under iontophoretic delivery. The electrode system (Fig. 1A and B) used for in vivo iontophoresis was designed to ensure sufficient flexibility in order to maintain contact with both hard (nail) and soft tissues (skin) simultaneously (Nair et al., 2009b).

The selection of an in vivo animal model, as an alternative to human subjects, for dermatological studies poses several challenges. The use of primates is restricted and not all animal facilities house the large pig animal model. Thus, rodents are often used as alternative in vivo models, but they have some limitations as their skin differs from human skin with respect to its thickness and composition of skin layers, follicle density, sebaceous gland number and permeability profiles. Despite these limitations, rodent models are valued for their easy availability, small size and convenient housing/handling, making them one of the most popular models for dermatological research. Hairless rat models were used in this study. They were selected over other hairy skin rodent models (such as Sprague–Dawley rats), as hairless rats are known to mimic human skin better in the absence of a hairy coat (Bronaugh et al., 1982; Morimoto et al., 1992; Lauer et al., 1997; Chen et al., 1998; van Ravenzwaay and Leibold, 2004; Godin and Touitou, 2007). Several transdermal studies using hairless rat models have been performed and reported in the past (Yanagimoto et al., 1999; Yeganeh and McLachlan, 2000; Kikwai et al., 2005; Paturi et al., 2010).

Tape stripping and skin extraction techniques to quantify drug amounts in the SC and the underlying skin, respectively, have gained wide acceptability to determine skin bioavailability following topical application and results have been reported in the literature (Escobar-Chavez et al., 2008; Herkenne et al., 2008).

During the delivery studies, significantly more drug was delivered into the SC following 10 min of iontophoresis as compared to passive delivery for the same duration. This was due to the driving force of current, which propelled the drug into and across the SC. However, the drug levels in the SC appeared to level off after 10 min of current application and the difference between the iontophoretic and passive group was no longer significantly different. ANOVA performed for the drug levels delivered into the SC following 10, 15 and 20 min iontophoresis showed that there was no significant difference between the groups ($p > 0.05$). This difference was, however, significant for the passive delivery and the levels continued to increase with increase in duration of treatment. This was probably due to the rapid saturation of the SC with

iontophoretic delivery as compared to passive delivery. This explanation is confirmed by the observation that significantly higher drug levels were delivered into the underlying skin for the iontophoretic group at all three time points (10, 15 and 20 min) when compared to passive delivery ($p < 0.05$). Furthermore, ANOVA performed between the iontophoretic groups showed that increased drug levels were delivered into the underlying skin as the duration of current application was increased, with the highest levels delivered following 20 min of iontophoresis ($p < 0.05$). Similar results were obtained when ANOVA was performed for passive delivery, though the amount of drug delivered was significantly lower than that for iontophoretic delivery.

In the drug depot clearance studies, immediately after iontophoretic or passive delivery ($T = 0$), drug levels in the SC were relatively high. However, by 12 h post-delivery this had reversed (Fig. 4A). The drug levels decreased from 48.21 ± 8.8 μg to 4.71 ± 0.94 μg (for the iontophoretic group) and 25.78 ± 3.75 μg to 5.05 ± 0.64 μg (for the passive group), during the first 12 h of the post-treatment period. This decrease in drug levels was statistically significant and suggests that following termination of the treatment the drug was cleared from the SC and/or migrating into the underlying skin layers. The absence of a significant difference in the SC drug levels in either group at the 12-h time point suggests that this drug clearance and/or migration process was similar for both the experimental and the control group. By 24 h, the drug was undetectable in the SC suggesting that the drug's residence time in the SC was somewhere between 12 and 24 h following either delivery method.

In contrast, the amount of drug in the underlying skin was significantly higher for the iontophoretic group as compared to the passive group at all time points ($p < 0.05$). A characteristically decreasing trend with an increase in post-treatment period was also observed in both the iontophoresis and passive groups (Fig. 4B). However, an interesting observation was that at the 12-h time point, the drug levels increased from 10.51 ± 1.28 μg to 14.27 ± 2.26 μg (for iontophoretic group) and 4.36 ± 0.74 μg to 6.86 ± 0.98 μg (for passive group), respectively, contrary to the decrease in drug levels observed for the SC at this time point. This suggests that the drug migrated from the SC into the deeper layers of the skin over time by passive diffusion. However, the decrease in SC drug levels and increase in deeper skin layer's drug levels were disproportionate, suggesting simultaneous clearance of drug from both of these sites. ANOVA performed on the treatment groups at different points (0, 12, 24 and 48 h) showed that there was a significant difference in the decreasing drug levels with an increase in the post-treatment wait period. A significant amount of drug remained in the deeper skin layers for the iontophoretic group, which persisted for at least 48-h post-delivery. The drug level in the passive group, however, was undetectable at the 48 h time point. This depot formed in the deeper skin layers may be beneficial to maintain the therapeutic levels at the site of infection for a longer duration.

TEWL was used in these studies to monitor changes in skin permeability as a result of the iontophoretic treatments as well as to ensure complete removal of the SC during the tape stripping procedure. TEWL measurements were recorded using a portable and closed chamber evaporimeter. TEWL signifies water escape from inside the body into the surrounding atmosphere, through the epidermal surface, predominantly by diffusion or evaporation. This loss of water occurs continuously but is kept to minimal levels due to the excellent barrier properties of the skin, more specifically by the SC. It has been reported that when the SC is disrupted, the TEWL values (g/m^2 h) increase to approximately 8–10 times of their base values. The percentage change in relative humidity with time is the underlying principal for the TEWL measurements in this study. The device has been reported to be used in dermatology, academic transdermal research and other relevant research areas in the past (Shah et al., 2005; Fluhr et al., 2006; Zhai et al., 2007). The TEWL values shown in Fig. 5A and B increased with additional tape strips and reached a constant value (10 times the base value) with no further increases seen with more skin stripping. Thus, 20–25 tape strips were able to remove the SC completely. In this study, we found insignificant change in the TEWL values relative to the base value at the end of treatment. This suggests that iontophoresis performed at the selected current density did not cause any irritation or redness of skin. The same was found to be true for passive delivery with the formulation alone. This was further confirmed by visual inspection. This use of TEWL as an indirect measure of skin irritation and redness has been reported before (Murahata et al., 1986).

Higher drug levels were delivered into the underlying skin layers using anodal iontophoresis. The therapeutic implication of this finding could be to broaden the spectrum of potential drug candidates that could be used for the treatment of deep-seated fungal infections. Currently, only fluconazole (Faergemann and Laufen, 1993) and itraconazole are approved for these infections. Other available antifungal drugs, which may be beneficial for these infections, cannot be used because of their inability to effectively penetrate the skin barrier following topical application. However, with the use of an enhancement technique such as iontophoresis, this challenge can potentially be overcome. The hypothesis, however, needs to be validated by selecting suitable drug candidates (effective against the causative organism for the infection being treated) and determining whether iontophoretic delivery can achieve therapeutic drug levels in the deeper skin layers at the target site. Furthermore, the relevance of this study could be extended for the treatment of onychomycosis (nail fungal infection). It is suggested by the dermatologists that during onychomycosis the fungus might be present in the surrounding soft skin tissues beside the nail unit. Thus, for complete treatment it may be beneficial for the fungus to be removed not only from the nail plate and nail bed, but also from the skin surrounding the nail unit to prevent rebound or reinfection. Iontophoretic delivery of TH into the nail unit was studied and the technique's potential for effective drug delivery was reported recently (Amichai et al., 2009; Nair et al., 2009a,b,c). Our study showed the potential for the faster and deeper delivery of drug into the skin tissue. From the results obtained in these studies together with the results obtained from iontophoretic nail drug delivery research, it can be anticipated that iontophoresis may be useful to deliver required drug levels into the skin and the nail simultaneously for the effective treatment of nail fungal infection. The iontophoretic application of a terbinafine gel to human toe nail has been evaluated and further human studies are now needed.

5. Conclusion

The use of iontophoresis for the intradermal delivery of TH into hairless rat skin was investigated in vivo in this study. The amount of drug delivered increased with an increase in duration of current application, with high levels initially in the SC. Migration of drug from the SC into the deeper skin layers was observed, such that by 24 h no drug remained in the SC. Concurrent with this was an increase in drug in the deeper skin layers, suggesting a redistribution of drug from the surface to the underlying skin. Drug was still detectable in the underlying skin layers 48 h following iontophoretic delivery. These results suggest the formation of a terbinafine depot in the deeper layers of the skin that persists for at least 2 days. Thus, iontophoresis could be used to deliver high drug levels rapidly into the SC (target site for superficial fungal infections) and the underlying skin (target site for cutaneous/subcutaneous infections).

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