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Alteration of the diffusional barrier property of the nail leads to greater terbinafine drug loading and permeation

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ABSTRACT

The diffusional barrier property of biological systems varies with ultrastructural organization of the tissues and/or cells, and often plays an important role in drug delivery. The nail plate is a thick, hard and impermeable membrane which makes topical nail drug delivery challenging. The current study investigated the effect of physical and chemical alteration of the nail on the trans-ungual drug delivery of terbinafine hydrochloride (TH) under both passive and iontophoretic conditions. Physical alterations were carried out by dorsal or ventral nail layer abrasion, while chemical alterations were performed by defatting or keratolysis or ionto-keratolysis of the nails. Terbinafine permeation into and across the nail plate following various nail treatments showed similar trends in both passive and iontophoretic delivery, although the extent of drug delivery varied with treatment. Application of iontophoresis to the abraded nails significantly improved ($P < 0.05$) TH permeation and loading compared to abraded nails without iontophoresis or normal nails with iontophoresis. Drug permeation was not enhanced when the nail plate was defatted. Keratolysis moderately enhanced the permeation but not the drug load. Ionto-keratolysis enhanced TH permeation and drug load significantly ($P < 0.05$) during passive and iontophoretic delivery as compared to untreated nails. Ionto-keratolysis may be more efficient in permeabilization of nail plates than long term exposure to keratolysing agents.

1. Introduction

Ungual and trans-ungual drug delivery continues to receive significant attention due to the need for efficacious topical therapies for onychomycosis given the potential risk of systemic adverse effects associated with the conventional oral therapy (Effendy, 1995). The major concern in topical therapy is the low trans-nail penetration into the deeper nail stratums because of the inherent limitation of low permeability of the keratinised nail plates (Baran and Kaoukhov, 2005). In one approach to address this issue, permeation enhancers were screened for their ability to enhance the trans-ungual permeation of antifungal agents (Van Hoogdalem et al., 1997; Kobayashi et al., 1998; Malhotra and Zatz, 2002; Hui et al., 2003; Hao et al., 2008). In another approach, drug discovery groups have synthesized and screened newer antifungal agents with low keratin binding and higher nail permeation properties (Tatsumi et al., 2002; Hui et al., 2007). Despite all these efforts, the success rates of topical therapies have so far been disappointing in comparison to systemic therapy (Murdan, 2008).

The barrier properties of a particular tissue are dictated by characteristics such as membrane composition and thickness, and the specific routes available for drug permeation through the tissue. In comparing nail and skin, for example, nail is a thick, hard and compact keratin membrane with low lipid content (≤1%, w/w) whereas stratum corneum, which provides the primary barrier for skin, is thin (10–20 μm), highly flexible and has a high lipid content (10%) (Gupchup and Zatz, 1999). Additionally, the pathways available for drug permeation across the skin and nail differ; for example, the follicular route in skin is absent in nail (De Berker et al., 2007). Taken together, these characteristics of the nail make it a formidable barrier to drug permeation and the challenge to improve topical delivery of drugs into and through the nail remains formidable as well.

Recently the ability of iontophoresis to enhance the trans-ungual permeation of salicylic acid and terbinafine was demonstrated (Murthy et al., 2007a; Nair et al., 2009). In addition, the perm-selective nature of the human nail plate was found to be comparable to that of skin (Murthy et al., 2007b). Iontophoresis uses a low level electric current to actively facilitate drug transport across biological membranes (Batheja et al., 2006). The enhanced permeation of charged drug molecules by iontophoresis was principally

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due to electrorepulsion with a lesser contribution by electroosmosis (Hao and Li, 2008a). The influence of variables such as current level and density, vehicle pH, type of electrode, co-ions, drug concentration, and charge on the transport of drugs during iontophoresis in transdermal delivery is well-known (Kalia et al., 2004). A similar influence/effect of the above said variables in trans-ungual iontophoretic delivery was also assessed in earlier studies. It was observed that the increase in current density (0.1–1 mA/cm^2) enhanced the drug permeation linearly (Murthy et al., 2007a; Nair et al., in press). The role of drug concentration, ionic strength and vehicle pH on trans-ungual iontophoretic permeation was also investigated (Murthy et al., 2007a). Furthermore, Hao and Li (2008b) reported that pH and ionic strength had little effect on electroosmotic transport in trans-ungual iontophoresis.

Terbinafine is a potent antifungal agent which belongs to the allylamine class of antifungals, is highly effective in treating dermatophyte infections, and is the current treatment of choice in onychomycosis (Darkes et al., 2003). It possesses low minimum inhibitory concentrations (~0.001–0.01 μg/mL) and low minimal fungicidal concentrations (~0.003–0.006 μg/mL) against dermatophytes (Darkes et al., 2003). Terbinafine acts by blocking ergosterol biosynthesis by inhibiting the enzyme squalene epoxidase (fungistatic), which also leads to the toxic accumulation of intra-cellular squalene thereby exhibiting fungicidal activity (Ryder, 1992; Darkes et al., 2003). In the present study, the influence of barrier alteration on the passive and iontophoretic trans-ungual delivery (both permeation and drug load) was systematically investigated using terbinafine as a model drug.

2. Materials and methods

2.1. Materials

Terbinafine hydrochloride (TH) [MW = 327.90 Da, aqueous solubility = 1 mg/mL, pKa = 7.1, log octanol/water partition coefficient of terbinafine (Alberti et al., 2001) = 3.3], was procured from Uquifa, Jiutepac, Mexico. Sodium sulfite and salicylic acid were purchased from Sigma–Aldrich, St. Louis, MO. Human cadaver nails, both male and female, aged between 49 and 86 years, with varying thickness of 0.4–0.7 mm were procured from Science care (Phoenix, AZ) and were stored at 4 °C until used. All other chemicals and reagents used were of analytical grade. All solutions were prepared in deionized water.

2.2. Analytical method

The amount of terbinafine in the samples was quantified by high performance liquid chromatography (HPLC) system (Waters, 1525) with an autosampler (Waters, 717 plus) consisting of a Phenomenex C18 (2) 100 R analytical column (4.6 mm × 150 mm, Luna, 5.0 μm) and a variable wavelength dual λ absorbance detector (Waters, 2487). Mobile phase consisted of aqueous solution (0.096 M triethyl amine, 0.183 M orthophosphoric acid) and acetonitrile (60:40) adjusted to pH 2 with orthophosphoric acid. Elution was performed isocratically at 32 °C at a flow rate of 1.0 mL/min. Injection volume was 20 μL and the column effluent was monitored at 224 nm. The method was validated by determination of linearity, precision, and accuracy. The range for the calibration curve was 2–1000 ng/mL (R^2 = 0.99). The coefficient of variation and the accuracy ranged 1.03–6.08% and −0.54 to −6.96%, respectively.

2.3. Nail treatments

2.3.1. Abrasion of dorsal or ventral nail layer

Nails were cleaned and adherent tissues were removed. The sandpaper (grade #180) until the top or bottom layer (3/10 of the total thickness) was removed. The complete removal of the dorsal/ventral layer was confirmed by microscopic examination of sections of the nail plate.

2.3.2. Defatting and keratolysing of nails

Cleaned nail pieces were defatted by placing them in a beaker containing chloroform:methanol (2:1) mixture (10 mL) and stirred for a period of 12 h. Similarly, nail pieces were stirred in salicylic acid solution (4 mg/mL) for 12 h to keratolyse the nails. Treatment period (12 h) was selected since the longer exposure (24 h) of nails with salicylic acid solution did not improve the permeation.

2.3.3. Pretreatment with keratolytic agents in conjunction with iontophoresis (ionto-keratolysis)

Each of the nail plates were soaked in 0.9% (w/v) saline for 1 h, cleaned and mounted on a nail adapter (PermeGear, Bethlehem, PA). The whole assembly was sandwiched between the two chambers in a Franz diffusion cell (Logan Instruments Ltd., Somerset, NJ). Each 500 μL solution of salicylic acid (4 mg/mL) or sodium sulfite (50 mg/mL) was placed in the donor compartment, and 5 mL of normal saline in the receiver. Cathodal iontophoresis was carried out by applying a constant current of 0.5 mA/cm^2 by placing the cathode (silver chloride electrode) in the donor compartment and the anode (silver electrode) in the receiver, for a period of 1 h. The nail plate was rinsed with pH 3 water before carrying out the permeation experiments.

2.4. Permeation studies with terbinafine

Nail plates were soaked in 0.9% (w/v) saline for 1 h immediately prior to use and mounted on a nail adapter (PermeGear, Bethlehem, PA). The whole assembly was sandwiched between the two chambers in a Franz diffusion cell (Logan Instruments Ltd., Somerset, NJ). TH solution (500 μL, 1 mg/mL adjusted to pH 3 using 0.01N HCl) was placed in the donor compartment. The active diffusion area exposed to both the donor and receiver compartments was 0.2 cm^2. The receptor compartment, which had a capacity of 5 mL and was filled with saline (adjusted to pH 3 using 0.01N HCl) which provides sink conditions due to increased drug solubility. The receiver compartment was stirred at 600 rpm with a 3-mm magnetic stir bar at room temperature. Samples were withdrawn from the receiver compartment after 24 h and analyzed for terbinafine concentration and the cumulative amount of terbinafine permeated into the receiver chamber normalized to the surface area exposed to the drug was expressed as μg/cm^2.

Anodal iontophoresis was carried out by fixing 0.5 mm diameter Ag/AgCl wire electrodes (Alfa Aesar, Wardhill, MA) at a distance of 2 mm from the nail surface in donor and receiver chambers. Iomed Phoresor II dose controller (Iomed Inc., Salt Lake City, UT) was used for application of a constant DC. The anode was connected to the donor and the cathode to the receiver chamber and a constant current (0.5 mA/cm^2) was applied for a period of 24 h.

2.5. Amount of drug in nail

After the *in vitro* diffusion studies, the nail plates were marked for active diffusion area (using permanent marker and metric punch), washed with water and alcohol five times each using a standardized protocol to avoid the washout of drug loaded in the nail while removing surface drug. In brief, washing was carried out by holding the nail with forceps and shaking twice by placing in 2 mL of water (pH 3). Five such washings were performed in fresh 2 mL of pH 3 water each time. The nail surface was cleaned using a cotton swab soaked in 95% ethanol and rinsed with 1 mL ethanol

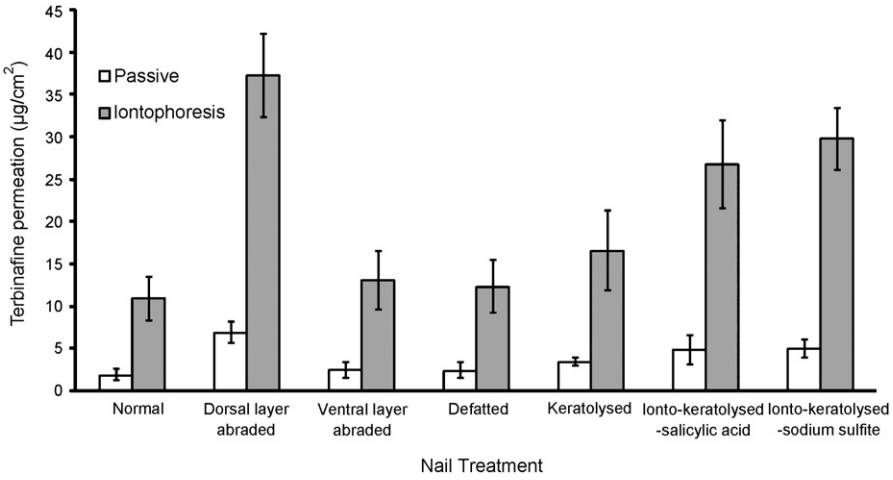

Fig. 1. Comparison of the amount of terbinafine permeated at the end of 24 h with various nail treatments. Ionto-keratolysis was carried out by cathodal iontophoresis (0.5 mA/cm^2) using salicylic acid/sodium sulfite for a period of 1 h. The diffusion area was 0.2 cm^2. Data expressed as means ± S.D. (n = 4).

nail surface was wiped with Kim wipe, active diffusion area was excised, weighed and dissolved in 1 M sodium hydroxide (1.5 mL) by constant overnight stirring. Extraction of drug was carried out by a slight modification of the method described by Dykes et al. (1990). Briefly, after dissolving the nails in the vials, 200 μL of 5 M hydrochloric acid was added to neutralize the mixture. Terbinafine was extracted by adding hexane (3 mL) to the vial and shaking manually for 30 min. The mixtures were transferred into centrifuge tubes and centrifuged at 4000 rpm for 10 min. The hexane layer was collected, 1 mL of 0.5 M sulfuric acid–isopropyl alcohol mixture (85:15) was added and the mixture was shaken vigorously for 30 min. The lower acidic aqueous layer, which holds the majority of terbinafine, was collected separately and the amount of drug in the nail was determined by HPLC. This extraction procedure was validated by spiking different drug concentrations (2–20 μg/mL) into sodium hydroxide solution in which the nail was previously dissolved. The recovery was found to be 84 ± 7%.

The amount of terbinafine diffused into the lateral region was determined by dissecting the lateral nail area (4–5 mm surrounding the active diffusion area), washed, dried and weighed. The amount of drug was determined as described before.

2.6. Data analysis

Enhancement was calculated as the ratio of the amount of drug permeated (μg/cm^2) in 24 h with various treatments to its corresponding control. Statistical analysis was performed by one-way analysis of variance (ANOVA) and t-test using Graphpad prism 5, graphpad software, Inc., CA, USA, to test the effects of various treatments. P value less than 0.05 was considered statistically significant. The data points provided in the graphs are an average of four trials. The error bars represent the standard deviation.

3. Results and discussion

3.1. Permeation of TH across the nail plate

3.1.1. Passive permeation

The reports in the literature and our previous study demonstrated that the maximum amount of drug is retained in the dorsal layer of the nail during permeation across the nail plate (Hui et al., 2002; Nair et al., in press) when compared to the intermediate and ventral layers. This suggests that the upper most layer of the nail plate, which is relatively more dense and harder than the permeation of drugs into the deeper layers of the nail plate and the nail bed. Therefore, removal of the upper layer may improve drug delivery across the nail plate. To test this hypothesis, a passive permeation study using terbinafine was initially carried out for a period of 24 h after the complete removal of the dorsal nail layer by abrasion with sandpaper. This process eliminated almost 3/10th of the total nail thickness. The donor vehicle pH was adjusted to 3, as the drug possesses a higher solubility at acidic pH levels. The amount of drug permeated passively within 24 h across the nail plate devoid of the upper layer (abraded nails) during delivery was found to be 6.87 ± 1.26 μg/cm^2, which was ~4-fold higher than the drug permeation through the intact nail (control: 1.89 ± 0.66 μg/cm^2) (P = 0.0004). In another set of experiments, the ventral layer of nail was abraded (3/10th of thickness) and the TH permeation was assessed. The results indicated that TH permeation was not altered significantly (2.48 ± 0.91 μg/cm^2 after passive permeation, P = 0.3343) compared to intact nails without a decrease in the nail thickness, suggesting that reduction in nail thickness alone did not result in the observed permeation enhancement with the dorsal layer abraded nails. These results further confirm that the dorsal layer is a significant barrier to drug permeation in topical nail drug delivery, which is in agreement with the earlier report (Kobayashi et al., 1999). Further, this suggests that abrasion of the dorsal nail layer could be used to enhance drug delivery of antifungal agents during topical therapy. This technique is generally reported to be an effective and inexpensive method for treating hyperkeratosis of the nail plate or partial removal of the plate to resolve haematomas (Di Chiacchio et al., 2003). Although this technique possesses many advantages, the application of this in practice could be limited to severely diseased nails (Baran et al., 2008).

Chemical permeation enhancers that are presumed to act on the nail plate composition were next examined for their ability to increase the permeation of terbinafine across the human nail plate. Similar to skin, the nail plate also could have two potential pathways of drug permeation, one being permeation across the keratin matrix and the second being permeation through the lipid domain which constitutes approximately 1% (w/w) of the nail. TH permeation studies were carried out across deplipidized or keratolysed nail plates. The nail was defatted by soaking the nail plates in a chloroform–methanol mixture (2:1) overnight. Similarly, the nail plates were kept immersed in salicylic acid solution (4 mg/mL) for effective keratolysis. Inconsistent reports exist on the ability of salicylic acid to enhance the permeation of antifungal agents in topical nail delivery (Kobayashi et al., 1998; Murdan, 2002; Malhotra and

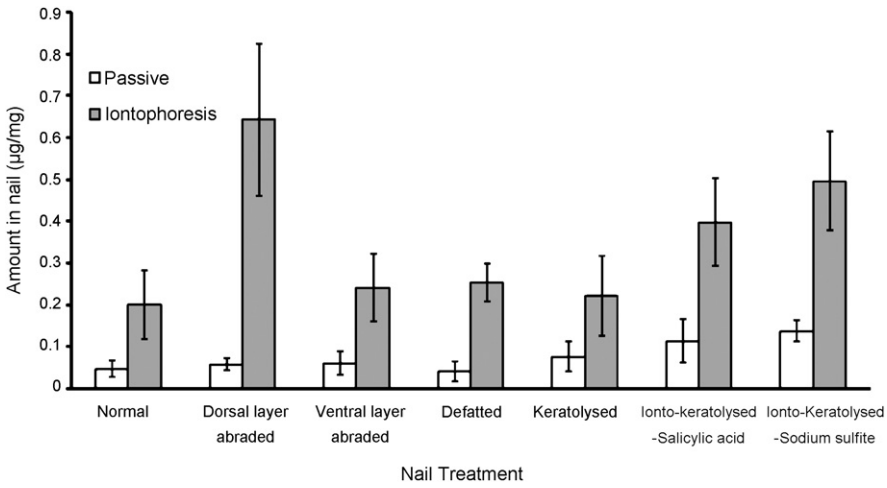

Fig. 2. The amount of TH loaded (µg/mg) at 24 h in the active diffusion area of nail during permeation studies with various nail treatments. Ionto-keratolysis was carried out by cathodal iontophoresis (0.5 mA/cm^2) using salicylic acid/sodium sulfite for a period of 1 h. The diffusion area was 0.2 cm^2. Data expressed as mean ± S.D. (n = 4).

permeated over 24 h of passive delivery following keratolysis was found to be 3.47 ± 0.52 µg/cm^2, which is a moderate enhancement (~2-fold, P = 0.0094) over passive delivery in normal nails (Fig. 1). It has been proposed that the enhancement in drug permeation by salicylic acid pretreatment is likely due to an increase in the nail hydration level and the disruption of the keratin tertiary structure (Kobayashi et al., 1998). In contrast, the passive drug permeation in defatted nails (2.43 ± 0.94 µg/cm^2) was not statistically significant when compared with untreated nails (P = 0.3834). These results suggest that permeation enhancers which disrupt keratin structure are likely to improve the delivery of terbinafine. However, the agents that interact with the lipids in the nail plate may not be effective in enhancing the trans-ungual delivery of TH. It appears that the nail plate may not have a continuous lipid pathway running through the nail plate which could again be due to the relatively small fraction of lipid constituting the nail plate.

Iontophoresis is a technique in which low level electric current is used to enhance drug delivery across biological membranes. Application of a driving force with the same polarity as that of the compound to be delivered should enhance its delivery across the hydrated nail plate. Recently our group demonstrated the ability of short term iontophoresis to enhance the trans-ungual delivery of terbinafine (Nair et al., 2009; Nair et al., in press). We later hypothesized that the combination of iontophoresis with chemical permeation enhancers (ionto-keratolysis) could improve the effect of the enhancers by driving more of the enhancer into the nail matrix. Salicylic acid and sodium sulfite (keratolytic agents) were selected and combined with iontophoresis as a pretreatment in the permeation studies. These enhancers are anionic in nature and can be driven into the nails by the application of cathodal iontophoresis (0.5 mA/cm^2). Salicylic acid solution (4 mg/mL: pH 5 and pKa 3.1) or sodium sulfite solution (50 mg/mL; pH 8.6) was placed (500 µL) in the donor chamber and cathodal iontophoresis was carried out for 1 h as a pretreatment of the nails prior to permeation studies with TH. After the ionto-keratolysis, the donor compartment enhancer solution was replaced with TH solution (1 mg/mL) and the drug was allowed to permeate passively. Ionto-keratolysis for a short duration (1 h) with salicylic acid or sodium sulfite enhanced the amount of drug permeated within 24 h [5.01 ± 1.09 µg/cm^2 (P = 0.0157) and 4.87 ± 1.75 µg/cm^2 (P = 0.0034), respectively] versus the normal untreated control nails (1.89 ± 0.66 µg/cm^2) (Fig. 1). Sodium sulfite is postulated to act by reducing the disulfide linkages in keratin (Malhotra and Zatz, 2002). However, the amount of terbinafine permeated in this set of studies was less than the remove the dorsal layer. Interestingly, the amount of passive TH permeation after ionto-keratolysis (0.5 mA/cm^2 for 1 h) with salicylic acid was ~1.5-fold higher (P = 0.0435) than the nails soaked in a solution of salicylic acid for 12 h. This shows that ionto-keratolysis results in a higher structural alteration as compared to long exposure of nails to keratolytic agents, which may not be a clinically feasible technique.

3.1.2. Iontophoretic permeation

To investigate the effect of iontophoresis on the permeation of terbinafine through the barrier modified nails, anodal iontophoresis (0.5 mA/cm^2 for 24 h) was carried out using abraded, defatted, keratolysed and ionto-keratolysed (using salicylic acid and sodium sulfite for 1 h) nails. The drug solution was delivered at pH 3, which also ensured that the drug was fully ionized for optimal delivery via electrorepulsion. The amount of terbinafine permeated in 24 h through nails subjected to different treatment processes and control are depicted in Fig. 1. The significant increase in permeation due to iontophoresis was obvious in all the cases when compared to passive delivery. Surprisingly, the fold enhancement due to iontophoresis was comparable (~5–6-fold) in all the cases, irrespective of the nail treatment. The plausible explanation for this constant enhancement in drug permeation by iontophoresis could be due to the following reasons. In the present study, the experimental conditions of iontophoresis (duration, electrical dose and drug concentration) were the same in all cases, irrespective of the nail treatments. There were no other counterions present in the donor drug solution other than H^+ ions. Due to the identical experimental condition, the ratio of terbinafine ion to H^+ ions remains the same in all cases. Hence the percentage of total charge transported by terbinafine ions remains approximately constant.

Iontophoretic drug permeation through treated nails showed a pattern similar to that observed in passive permeation studies (Figs. 1 and 2). As expected, TH permeation was enhanced (~3-fold) in nails without the dorsal layer (37.21 ± 4.93 µg/cm^2) when compared to the normal untreated nails during iontophoresis (P < 0.0001). However, the permeation was not enhanced when the ventral nail was abraded (13.06 ± 3.47 µg/cm^2, P = 0.3668). Defatted and keratolysed nails did not significantly enhance terbinafine permeation even in conjunction with iontophoretic delivery when compared to normal nails. The amount of terbinafine permeated across the defatted and keratolysed nail was 12.32 ± 3.12 (P = 0.5238) and 16.59 ± 4.73 µg/cm^2 (P = 0.0812), respectively, and was comparable to that obtained with untreated nails

pretreatment methods could not sufficiently alter the nail barrier properties to enhance iontophoretic drug permeation. In contrast, ionto-keratolysis for 1 h with salicylic acid or sodium sulfite followed by anodal iontophoresis (24 h) of TH significantly enhanced the terbinafine permeation to a similar extent [26.72 ± 5.16 ($P = 0.0016$) and 29.74 ± 3.61 μg/cm^2 ($P = 0.0001$) using salicylic acid and sodium sulfite, respectively], when compared to normal nails (iontophoresis). Interestingly, the amount permeated using ionto-keratolysis followed by iontophoretic delivery of TH was ~4-fold higher than the passive permeation in nail without the dorsal layer (abraded nail).

3.2. Drug load in nail

3.2.1. Passive delivery

It is well-known that during the process of permeation across a tissue barrier, some amount of drug is retained in the barrier, and the amount of drug retained is likely to be influenced by the thickness of the barrier. Thus, it was hypothesized that the amount of drug loaded into the nail would be significant given the thickness of the nail. Further, it was hypothesized that the drug loaded in the nail would release over time such that the nail bed and surrounding tissues would be bathed in drug for an extended period of time, which would also prolong the therapeutic effect. Fig. 2 compares the drug load in the active diffusion area of the nail using both passive and iontophoretic delivery with nails subjected to various pretreatments. The drug loaded in the dorsal/ventral layer abraded, defatted and keratolysed nails was comparable to the normal nail on a per-weight basis (mg/g). However, nails subjected to ionto-keratolysis (1 h) with salicylic acid or sodium sulfite followed by passive delivery (24 h) showed a significantly higher drug load (~2.5-fold) ($P = 0.0014$) in comparison to the control (passive untreated nails).

3.2.2. Iontophoretic delivery

The utility of iontophoresis to enhance the drug load in nail by driving molecules into the deeper nail layers, as opposed to passive delivery, was reported in our earlier study (Nair et al., 2009; Nair et al., in press). A similar trend was observed in the current study as well. Iontophoretic drug delivery resulted in a large amount of drug being loaded into the nail plate (3–10-fold) when compared to passive delivery, irrespective of the nail treatments (Fig. 2). A significant enhancement in drug load (~3-fold) was found in the dorsal layer abraded nails (0.64 ± 0.18 μg/mg, $P = 0.0045$), when compared to normal nails loaded using iontophoresis (0.20 ± 0.08 μg/mg). In contrast, no significant difference in drug load was observed when the ventral nail layer was abraded (0.24 ± 0.03 μg/mg) or the nail which was subjected to defatting (0.25 ± 0.05 μg/mg) or keratolysis (0.22 ± 0.09 μg/mg) processes (Fig. 2). However, ionto-keratolysis for a short duration (1 h) with salicylic acid or sodium sulfite followed by anodal iontophoresis (24 h) of TH enhanced the drug load in the nail plate by ~2-fold ($P = 0.0254$) and ~3-fold ($P = 0.0063$) respectively, when compared to iontophoresis with the untreated nail. The results obtained further validate the capability of cathodal iontophoresis to drive the keratolytic agent into the nail. The enhancement in drug load could be due to an increase in nail keratin binding sites (due to greater surface area) and/or creation of pockets for drug retention by the keratolytic agents when they are driven into the nail matrix using iontophoresis.

Generally the whole nail apparatus, including the nail that is not accessible to topical treatment due to the proximal and lateral nail folds which overlap the nail, will be affected in nail diseases. The drug loaded in the active diffusion area represents the area of nail that is in direct contact with the formulation. In vivo, this would be the exposed part of the nail available for application of the drug formulation. Therefore, for optimal efficacy the drug loaded in the active diffusion area must diffuse laterally into the area that is not exposed to the formulation. In the present experiments, the amount of drug loaded into the nail surrounding the active diffusion area was also assessed. The amount of drug reaching the peripheral nail area depends on the concentration gradient between the active diffusion area and peripheral nail area. In the case of iontophoresis, it also depends on the current flow into the peripheral tissue regions. Table 1 summarizes the amount of terbinafine loaded in the peripheral region of the nail during various treatments. The peripheral drug load was higher with iontophoresis when compared to passive delivery. The amount of drug present in the peripheral nail is still well above the MIC (~0.001–0.01 μg/mL) (Darkes et al., 2003) of terbinafine for dermatophytes. The drug load in the peripheral area also followed a similar trend that was observed in the active diffusion area with the different treatments.

Table 1
Amount of terbinafine loaded in the peripheral nail areas during passive and iontophoresis processes with different nail treatments.

Nail treatments	Amount in peripheral area (μg/mg)	
	Control	Iontophoresis
Normal nails	0.012 ± 0.006	0.031 ± 0.018
Dorsal layer abraded	0.009 ± 0.004	0.097 ± 0.032
Ventral layer abraded	0.011 ± 0.006	0.054 ± 0.019
Defatted nails	0.013 ± 0.008	0.042 ± 0.025
Keratolysed nail	0.014 ± 0.005	0.046 ± 0.009
Ionto-keratolysis with salicylic acid	0.017 ± 0.005	0.069 ± 0.011
Ionto-keratolysis with sodium sulfite	0.021 ± 0.006	0.080 ± 0.027

4. Conclusion

Permeation studies using dorsal/ventral layer abraded nails indicated that the dorsal (upper) layer is the primary barrier in topical nail delivery. Permeation of TH through and drug load in the nail subjected to various treatments showed similar trends in both passive and iontophoretic delivery, although the extent of drug delivery varied with treatment. Iontophoresis, being an active delivery process, enhanced TH delivery in all the cases. Permeation was not altered in defatted nails, suggesting that the lipid component of the nail is not a significant barrier to terbinafine penetration. However, enhanced drug delivery was observed when the keratolytic agents were driven deep into the nail matrix using iontophoresis (ionto-keratolysis) or the dorsal layer was abraded. This study demonstrates that chemical alteration (ionto-keratolysis using salicylic acid or sodium sulfite) or physical alteration (abrasion of dorsal nail layer-debridement) could be effective pretreatment methods for enhancing topical trans-ungual delivery.

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