Drug Development and Industrial Pharmacy, 2011, 37(1): 72–79

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI:10.3109/03639045.2010.491832

informa
healthcare

ORIGINAL ARTICLE

A platform for predicting and enhancing model drug delivery across the human nail plate

Siva Ram Kiran Vaka[1], Satyanarayana Narasimha Murthy[1], John H. O'Haver[2] and Michael A. Repka[1,3]

[1]Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, MS, USA, [2]Department of Chemical Engineering, University of Mississippi, University, MS, USA and [3]National Center for Natural Products Research, University of Mississippi, University, MS, USA

Abstract

Purpose: The objective of the present study was to assess the effect of pretreatment using chemical etchants on the delivery of terbinafine hydrochloride (TH) and 5-fluorouracil (5-FU) into and across the human nail plate. Methods: The TranScreen-N method was used to screen five potential etchants. Based on these results, the dorsal surface of nails was pretreated with chemical etchants, 1% or 10% (w/w) phosphoric acid (PA) or 10% (w/w) lactic acid (LA) gels, for a period of 60 seconds. The nail pretreated with a plain gel formulation (no PA or LA incorporated) was used as the control. *Results*: Despite the differences in physicochemical properties between TH (log P = 3.3) and 5-FU (log P = –0.83), the in vitro permeation as well as drug load of these drugs in the nail plate was enhanced because of pretreatment with the PA gels, whereas LA pretreatment failed to enhance the drug load and permeation. Optical microscopic and atomic force microscopy studies revealed that the PA enhanced the trans-ungual drug delivery by decreasing the keratin density of the dorsal layer of the nail plate and by microstructural alterations. *Conclusions*: This study demonstrated that pretreatment of the nail plate with PA (1% or 10%, w/w) for a short duration could be a potential method of improving the efficiency of topical monotherapy treatment for nail diseases.

Key words: Chemical etchants, drug delivery, gels, microscopy, nail

Introduction

Onychomycosis is a fungal infection of the nail plate and/or nail bed affecting both finger and toenails. It has been reported that 10–40% of the world population is afflicted with onychomycosis and its incidence appears to be rising[1]. Causative agents of this disease are dermatophytes (90%), yeasts (7%), and nondermatophyte molds (3%)[2–6]. The primary fungi that cause onychomycosis are *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Elderly and diabetic patients are more vulnerable to onychomycosis because of poor blood circulation in extremities of fingers and/or toes. Nail psoriasis (also called psoriatic nail disease) is the second most prevalent (occurring in 80–90% of people with skin psoriasis) nail disease. This disease is caused due to psoriatic lesion within the nail matrix (pitting) or lesion within the nail bed (onycholysis) or subungual hyperkeratosis or as a result of trauma (splinter hemorrhages)[7].

Topical therapy of cutaneous tissues is a good choice of treatment over oral delivery as it offers better patient compliance, better drug targeting to the infected site, less systemic side effects and drug interactions. Although existing topical formulations such as creams, nail lacquers, ointments, lotions, solutions, and gels possess effective antifungal agents, they are less successful because of poor trans-ungual penetration of drugs. The poor permeation of drug molecules from topical formulations is known to be because of the impermeable nature of the highly keratinized dorsal nail plate. Moreover, drugs are known to depot in the dorsal layer of the nail plate thus failing to penetrate further into the ventral layers. Therefore, there is an unmet need to develop

Address for correspondence: Dr. Michael A. Repka, Department of Pharmaceutics, University of Mississippi, Faser 104B, PO Box 1848, University, MS 38655, USA. Tel: +1 662 915 1155, Fax: +1 662 915 1177. E-mail: marepka@olemiss.edu

(Received 4 Mar 2010; accepted 4 May 2010)

an efficient topical formulation that can enhance the permeation of antifungal drugs to the deeper target sites.

Etching was first introduced to the field of dentistry for tooth restoration using 30–40% phosphoric acid (PA)[8]. Repka et al.[9] carried out in vitro permeability studies by application of hot-melt extruded films containing ketoconazole on human nail samples treated with an etching agent (10% PA gel) and demonstrated that there was a significant increase in drug permeability compared to a control (unetched nail samples). The advantage of this technique compared to the existing methods (abrasion/ablation/microporation of nail plate, application of electric current/ultrasound, etc.) is its effectiveness even at very low concentrations, ease of application, and that it is a device-free treatment method.

Currently the success rate of topical therapy is only ~15% because of the lack of patient compliance and effective methods of drug delivery across the nail plate. The success rate of topical therapy could be improved by identifying potent etching agents, as a result of which effective amount of drugs can permeate across the nail plate. The efficiency of etching agents varies with different drugs. Therefore, one needs to identify the most appropriate etching agent for a given drug.

TranScreen-N studies were performed to identify the trans-ungual etchants for two drugs, terbinafine hydrochloride (TH) and 5-fluorouracil (5-FU), which are widely used in treating nail diseases[10–12]. Of the five chemicals—lactic acid (LA), tartaric acid (TTA), glycolic acid (GA), citric acid (CA), and PA—evaluated, PA was identified as the most potent trans-ungual etchant. On the other hand, LA did not demonstrate any etching properties. TranScreen-N is a screening method only, the results of which are considered only suggestive. The method does not reveal the drug permeation profile, flux, lag time, or the possible mechanisms. Nevertheless, the method could be utilized to rapidly screen a large group of chemicals to identify potential trans-ungual etchants. The method has been briefly discussed in this manuscript, as the focus of the present manuscript is to demonstrate the efficiency of PA to enhance the trans-ungual delivery of drugs and to assess the mechanisms in further depth.

This current research provides a comprehensive investigation of utilization of pretreatment etching agents for enhancing drug delivery to the nail and nail bed. Systematic studies were performed to evaluate the validity of the TranScreen-N technique and also to assess the efficacy of the etchants, PA and LA, on the trans-ungual delivery of terbinafine and 5-FU. It should be noted that these two model drugs differ significantly with respect to their physicochemical properties, which is paramount to the investigation of this topical treatment technique. The importance of selection of an appropriate etching agent, as well as its concentration, was also studied and discussed.

Materials and methods

Materials

Terbinafine hydrochloride (TH; MW 327.90 Da) was procured from Uquifa (Jiutepac, Mexico), 5-FU from Alfa Aesar (Ward Hill, MA, USA). Human cadaver fingernails, both male and female with varying thicknesses of 0.4–0.7 mm were procured from Science Care (Phoenix, AZ, USA). LA, CA, GA, and TTA were purchased from Sigma-Aldrich (St. Louis, MO, USA), Zenith 40% PA gel by DMG America (Englewood, NJ, USA). All other chemicals and reagents used were of analytical grade. All solutions were prepared in deionized water.

Analytical method for terbinafine and 5-FU

The amount of terbinafine in the samples was quantified by high-performance liquid chromatography (HPLC) system (Waters, Milford, MA, USA, 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 an 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[13]. The range for the calibration curve was 2–1000 ng/mL ($R^2 = 0.99$).

Similarly, 5-FU was quantified by HPLC system (Waters, 1525) with an autosampler (Waters, 717 plus) consisting of a Symmetry C18 analytical column (4.6 mm × 150 mm, 5.0 μm). Mobile phase consisted of 50 mM monobasic potassium phosphate. Elution was performed isocratically at room temperature at a flow rate of 1.0 mL/min. Injection volume was 50 μL and the column effluent was monitored at 260 nm[14]. The range for the calibration curve was 10–1000 ng/mL ($R^2 = 0.99$).

Preparation of gel formulations for pretreatment of nail

Ten percent (w/w) LA, TTA, GA, CA, and PA gels were prepared with a polaxomer (Pluronic® F 127 Prill). A plain polaxomer gel without incorporation of these chemicals was used as the control.

TranScreen-N method of screening potential etchants for trans-ungual drug delivery

TranScreen-N, a high-throughput method of screening potential trans-ungual drug permeation enhancers, was recently reported with detailed development and validation of this method[15]. From the results of these studies, one can determine the potential enhancers from a variety of chemicals which can enhance the drug load in the nail as well as the drug uptake across the nail plate. In brief, nail pieces of dimensions 3 mm × 3 mm were weighed individually (~7 mg each) and pretreated for

60 seconds with the 10% (w/w) etchant gels (LA, CA, TTA, GA, and PA). The nail pieces pretreated for 60 seconds with plain gel (drug- and etchant-free formulation) served as the control. In one set of experiments, the treated nail plates were immersed in the drug solution for 24 hours. The nail samples were washed thoroughly and the drug load was determined after extraction. The loading factor (L) was calculated by the following equation:

$$L = \frac{\text{Drug loaded per milligram of the nail pretreated with potential etchants containing gel}}{\text{Drug loaded per milligram of the nail pretreated with plain gel}}$$

In a separate set of experiments, the pretreated nail plates were kept in contact with the drug solution for 2 hours. The 2-hour duration was considered in this case to avoid saturation of the nail plate. The nail plates were then washed using a standard protocol, dissolved in sodium hydroxide, extracted and analyzed by HPLC[15]. The drug uptake rate enhancement factor (R) was calculated by the following equation,

$$R = \frac{\text{Amount of drug penetrated per unit weight of the nail pretreated with potential etchants-containing gel}}{\text{Amount of drug penetrated per unit weight of the nail pretreated with plain gel}}$$

In vitro drug permeation studies

Each of the nail plates were soaked in 0.9% (w/v) saline for 1 hour immediately prior to use and mounted on a nail adapter (PermeGear, Bethlehem, PA, USA). The dorsal surface of the nails were pretreated with gels (1%, w/w PA gel; 10%, w/w PA gel; and 10%, w/w LA gels) for a period of 60 seconds. The nails pretreated with plain gel formulations (no PA or LA incorporation) were used as the controls. After 60 seconds, the nails were washed with water for 2 minutes to remove the excess gel. The whole assembly was sandwiched between the two chambers of Franz diffusion cells (Logan Instruments Ltd., Somerset, NJ, USA). Drug solutions (TH/5-FU; 500 µL, 1 mg/mL) were placed in the donor compartment. The receiver chamber was filled with 5 mL of saline (pH 3) in the case of TH studies and 5 mL of saline (pH 7) in that of the 5-FU studies to maintain sink conditions. The active permeation area was 0.2 cm^2. The solution in the receiver compartment was stirred at 600 rpm with a 3 mm magnetic stir bar. The temperature of the whole assembly was maintained at 37 ± 0.5°C by circulating water. Samples were withdrawn at regular intervals of time for a period of 48 hours from the receiver compartment and analyzed by HPLC.

Drug load

After the in vitro drug delivery studies using TH, the nail plates were marked for active diffusion area, excised, and then washed using a standardized protocol to avoid the washout of drug loaded in the nail while removing surface drug[13]. After washing, the nail surfaces were wiped with Kimwipes®, weighed, and then dissolved in 1 M sodium hydroxide (1.5 mL) by constant overnight stirring. Extraction of drug was carried out by the method described by Nair et al.[13] This extraction procedure was validated by spiking different drug concentrations (2–20 µg/mL) into a sodium hydroxide solution in which the nail was previously dissolved. The recovery was found to be 84.36 ± 7.46% of that applied.

Similarly after drug delivery studies using 5-FU, the active diffusion area of the nail plates was excised and then washed with 10 mL of water to remove the drug adhering to the nail surface. After washing, the nails were wiped with Kimwipes®, weighed, and dissolved in 1 M sodium hydroxide (1.5 mL) by constant overnight stirring. This solution was neutralized by adding 5 N HCl. To this, 3 mL of *n*-proponal–diethyl ether (16:84, v/v) was added and shaken manually for 30 minutes. The mixture was centrifuged for 5 minutes and the organic layer was separated. One-milliliter water was added to this organic layer and shaken vigorously for 30 minutes. The lower aqueous layer was collected separately and the amount of drug in the nail was determined. The extraction procedure was validated by spiking different drug concentrations (2–20 µg/mL) in nail solutions prepared by dissolving the nails in sodium hydroxide and the recovery was found to be 73.84 ± 8.21% (theoretical).

Atomic force microscopy studies

All human nail plates were obtained for atomic force microscopy (AFM) studies. The dorsal surface of nails was pretreated with gels. The nails treated with plain gel were used as controls. AFM studies were performed in ambient air (ca. 25°C and 50% relative humidity) using a Multimode AFM (Digital Instruments, Inc., Santa Barbara, CA, USA). A J-type scanner was used in tapping mode with a maximum scan size of 125 µm and a scan rate of 1 Hz. The cantilever was 125 µm in length with a 4 µm silicon tip. The scanning frequency was close to or at the resonant frequency of approximately 348.5 kHz. Image analysis was performed by the NanoScope IIIA v4.23 software. Images were flattened using third order, least square fit before analyzing. Images were scanned utilizing a scanning force set point ratio between 0.7 and 0.9. The root mean square roughness (R_q) was calculated as follows:

$$R_q = \sqrt{\frac{\sum (Z_i - Z_{\text{ave}})^2}{N}}$$

where Z_i is the height of a local point (average height of a pixel); Z_{ave} the average height in a given area (given amount of pixels); and N the number of pixels in a given area[16].

Optical microscopy studies

The dorsal surface of the human nail plates, subjected to pretreatment using 1% PA, 10% PA, and 10% LA for a period of 60 seconds were used for these studies. The nails treated with plain gel were used as controls. A Cryostat (Leica Cryostat CM 3050S) was used to take 50 µm thick microscopic sections. The nail sections were fixed in Tissue-Tek® (Electron Microscopy Sciences, Hatfield, PA, USA) embedding media for sectioning and stained with methylene blue. The sections were examined under an optical microscope (Zeiss MI, Thornwood, NY, USA) with an Axio Cam (Thornwood, NY, USA).

Data analysis

Statistical analysis was carried out using GraphPad Instat 3 software. The *t*-test was selected as the test for significance and $P < 0.05$ was considered statistically significant. The data points provided in the graphs are an average of three trials. The error bars represent the standard deviation.

Results and discussion

Etchants have been used in dentistry for tooth restoration for decades. Buonocore et al. studied the pattern on enamel caused by 30–40% PA. These researchers reported that the etched enamel was characterized by the formation of profuse microporosities which allowed the penetration of monomers to form resin tags that provided micro-mechanical retention of composite restorations[8]. Repka et al.[9] reported that nail samples treated with PA gel for 60 seconds were significantly more permeable to ketoconazole (log $P = 3.3$) as compared to untreated nail plates.

In the present study, the two drugs selected differ significantly in their physicochemical properties. TH is a potential antifungal drug, less soluble in water (solubility is maximum at pH 3), cationic (at pH 3), and also is relatively more lipophilic (log $P = 3.3$)[17]. On the other hand, 5-FU is highly water soluble, anionic (at the formulation pH 7), and is highly hydrophilic (log $P = -0.83$)[18]. Therefore, one of the goals of the current project was to assess the impact of pretreatment on the delivery of these two different categories of drugs with significantly different physicochemical properties.

Screening of different etching agents by TranScreen-N method, suggested that PA could be a potential permeation enhancer. In the case of TH, the R and L values were 2.65 ± 0.24 and 1.67 ± 0.19, respectively. In the case of 5-FU the R value was 2.14 ± 0.13 and L was 1.71 ± 0.27. However, other chemicals that were used as pretreatment agents resulted in significantly smaller R and L values than PA. The R and L values of the chemicals used for pretreatment were in the order PA > CA > TTA > GA > LA. Therefore, further studies were performed using PA and LA as a representative potential etchant and nonetchant, respectively, as well as to validate the TranScreen-N technique.

When the nail plate was pretreated with 1% and 10% (w/w) PA gel for 60 seconds, the permeation of TH was enhanced by twofold and fivefold, respectively, over the control (2.79 ± 0.40 µg/cm^2). Upon pretreatment with 10% (w/w) LA for 60 seconds, the amount of drug permeated was not significantly different ($P = 0.142$) from that of control (Figure 1). In case of 5-FU studies also, pretreatment with 1% and 10% PA gel enhanced the permeation of 5-FU by twofold and threefold, respectively, over the control (1.54 ± 0.06 µg/cm^2). Again 10% (w/w) LA pretreatment for 60 seconds resulted in a cumulative permeation of 1.83 ± 0.13 µg/cm^2, which was not significantly different ($P = 0.127$) from that of the control (Figure 2). Despite the differences in physicochemical properties between TH and 5-FU, permeation of both the drugs was enhanced due to pretreatment with the PA gel. It is noteworthy that the interpretations from TranScreen-N were exactly translated in the case of in vitro Franz diffusion cell experimental results. The studies clearly demonstrated the ability of PA to act as an etchant for trans-ungual drug delivery and the lack of any etching properties of LA. These results provide validity to the TranScreen-N technique.

The drug delivered into the nail bed is generally cleared rapidly by the dermal microcirculation, in vivo. Therefore, there is need for providing a depot to replenish the drug level in the nail bed. Forming a drug depot in the nail plate is one of the potential ways of achieving prolonged therapeutic levels of drug within the nail bed. It is likely that the drug loaded in the nail plate will eventually release into the nail bed to compensate for the drug lost because of systemic clearance. Therefore, it

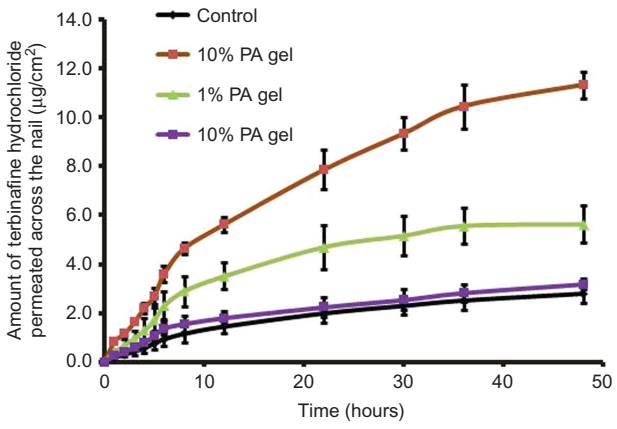

Figure 1. In vitro permeation of TH across the human nail plate pretreated with plain gel (control), 1% PA gel, 10% PA gel, and 10% LA gel for 60 seconds. Data expressed as mean ± SD ($n = 3$).

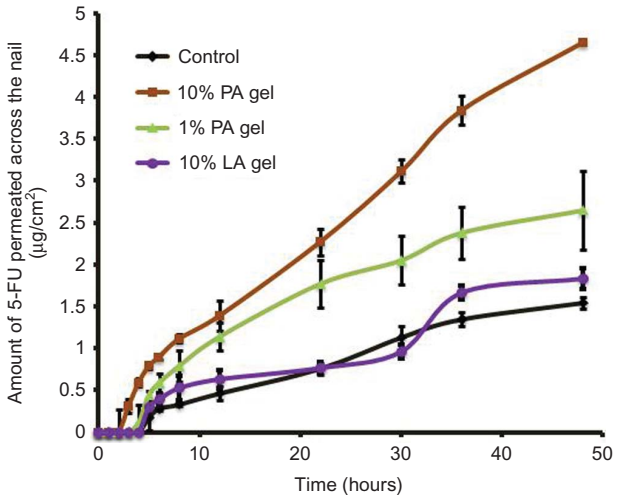

Figure 2. In vitro permeation of 5-FU across the human nail plate pretreated with plain gel (control), 1% PA gel, 10% PA gel, and 10% LA gel for 60 seconds. Data expressed as mean ± SD ($n = 3$).

would be advantageous if the etchants not only increased permeation, but also enhanced the drug loading within the nail plate.

Interestingly and very importantly, the etching procedure with the PA gel increased the drug load in the nail plate as well. In the case of TH, the drug load in the control after 48 hours of permeation studies was 272.04 ± 15.90 ng/mg. Pretreatment with 1% and 10% PA increased the drug load significantly to 333.86 ± 14.13 ng/mg and 445.27 ± 25.14 ng/mg, respectively (Figure 3). The load of 5-FU was increased significantly from 136.84 ± 32.25 ng/mg (control) to 277.50 ± 40.24 ng/mg and 392.65 ± 42.43 ng/mg upon pretreatment with 1% and 10% PA (Figure 4). LA failed to increase the drug load significantly in the nail plate (279.06 ± 39.16 ng/mg in the case of TH and 152.57 ± 31.63 ng/mg in case of 5-FU).

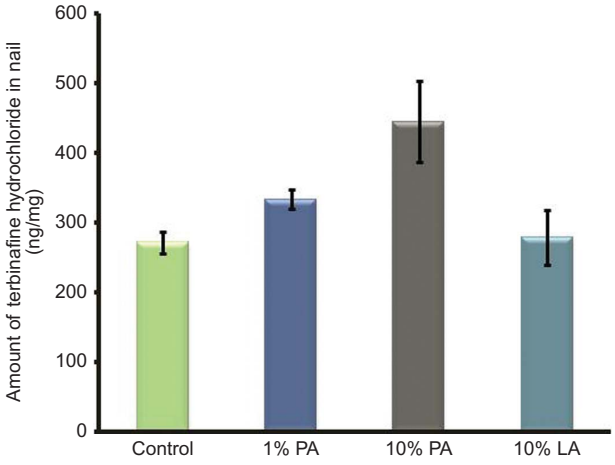

Figure 3. Terbinafine hydrochloride load in the nail plate subjected to in vitro permeation studies for 48 hours after pretreatment of nail plate with plain gel (control), 1% PA gel, 10% PA gel, and 10% LA gel for 60 seconds. Data expressed as mean ± SD ($n = 3$).

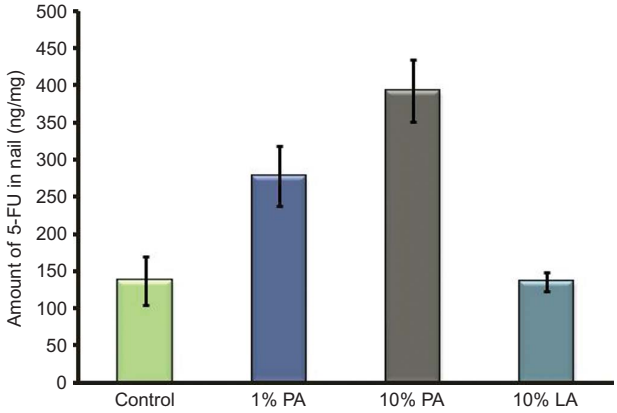

Figure 4. 5-FU when subjected to in vitro permeation studies for 48 hours after pretreatment of nail plate with plain gel (control), 1% PA gel, 10% PA gel, and 10% LA gel for 60 seconds. Data expressed as mean ± SD ($n = 3$).

To further assess the effect of PA and LA on the nail plate, two types of microscopic studies were performed. Morphological changes were investigated by optical microscopy and microstructural changes were investigated by utilizing AFM. Anatomically the nail plate is divided into dorsal and ventral layers. The dorsal layer consists of densely packed cells that primarily act as a barrier for drug permeation across the nail plate. The methylene-blue-stained sections of the nail plates were observed under the microscope to investigate the morphological changes brought about because of pretreatment with PA and LA. The dorsal layer in the case of the nail plates treated with the plain gel appeared to be denser than the nail plates which were pretreated with PA suggesting that PA is capable of penetrating the dorsal layer of the nail plate and it is likely that it effectively etched the dense keratin network (Figure 5). No such morphological changes were observed in case of nail plates treated with LA. In addition, the pretreatment with PA gel was found to decrease the thickness of the dorsal layer. The percentage decrease in thickness {(difference in thickness, before and after etching/initial thickness) × 100} of the dorsal nail plate when it was pretreated with 1% and 10% PA gel was 27 ± 3% and 61 ± 5%, respectively, whereas the percentage decrease in thickness of the dorsal layer of the nail plate was <3 ± 2% when pretreated with 10% LA.

Several investigators have studied the human nail structure over the past three decades via polarized light microscopy and scanning electron microscopy[19–24]. Recently, Repka et al.[16] demonstrated that AFM is a valuable technique for analyzing the human nail plate by providing both qualitative and semiquantitative information for the evaluation of nail morphology pertaining to drug delivery. The AFM is a type of scanning probe microscope that can be used to image micron- to nanometer-sized topography. The AFM utilized in this study can obtain topographic information of surfaces

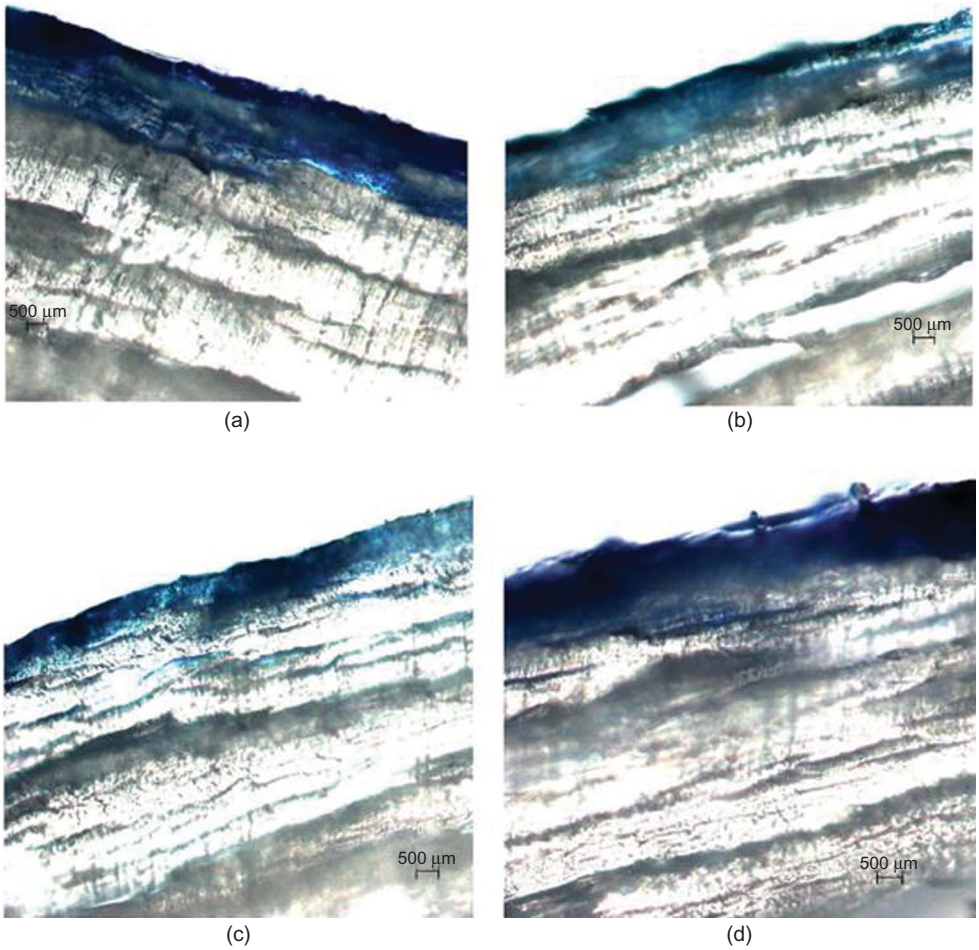

Figure 5. Methylene-blue-stained microscopic sections of the human nail plates pretreated with (a) plain gel (control), (b) 1% PA gel, (c) 10% PA gel, and (d) 10% LA gel for 60 seconds.

with minimal sample preparation[25]. Figure 6 represents the atomic force micrographs of the human nail plates treated with plain gel, 10% PA, and 10% (w/w) LA gels. Quantitative topographic analysis via NanoScope IIIA v4.23 software demonstrated significant differences in mean roughness scores of nail plates treated with the PA gel and the plain gel. When the dorsal surface of the nails was treated with 10% LA there was no change in topography and it almost resembled that of the nails treated with plain gel.

Figure 7 is a graphic representation of mean roughness scores of the dorsal nail plate pretreated with the plain gel, PA gels, and 10% LA gel. As can be visualized, the peak to valley roughness was decreased by pretreatment with 10% (w/w) LA (less than the nails treated with plain gel). For example, the mean roughness score for the nail treated with the plain gel was 85 ± 5.6 nm versus that of the 10% (w/w) LA-treated nails (72.9 ± 3.9 nm). Conversely, the mean roughness scores of 1% (w/w) PA (118.4 ± 7.8 nm) and 10% (w/w) PA (147.8 ± 14.3 nm) were significantly higher than the nails treated with the plain gel. The mean roughness scores reported by Repka et al. when 10% (w/w) TTA (112.2 ± 11.7 nm, included in Figure 7) was used as surface modifier, was almost equal to that observed for 1% (w/w) PA (118.4 ± 7.8 nm) in this study, indicating that PA is a relatively more potent etchant.

Conclusions

From the in vitro drug delivery and microscopic studies, it can be concluded that PA (1% or 10%, w/w) is an effective etching agent providing significant surface modification of the human nail. However, 10% (w/w) LA had no effect as an etchant as it did not enhance significantly the in vitro drug delivery compared to the control and demonstrated a roughness score less than that of the nails treated with the plain gel. These data provide validity to TranScreen-N, the high-throughput method of screening potential trans-ungual drug delivery enhancers.

From the microscopic studies it may be concluded that by application of 10% (w/w) PA as an etchant, the dorsal surface integrity of the nail plate is diminished as a result of which the permeation of antifungals, as well as other drugs to the deeper target sites is enhanced.

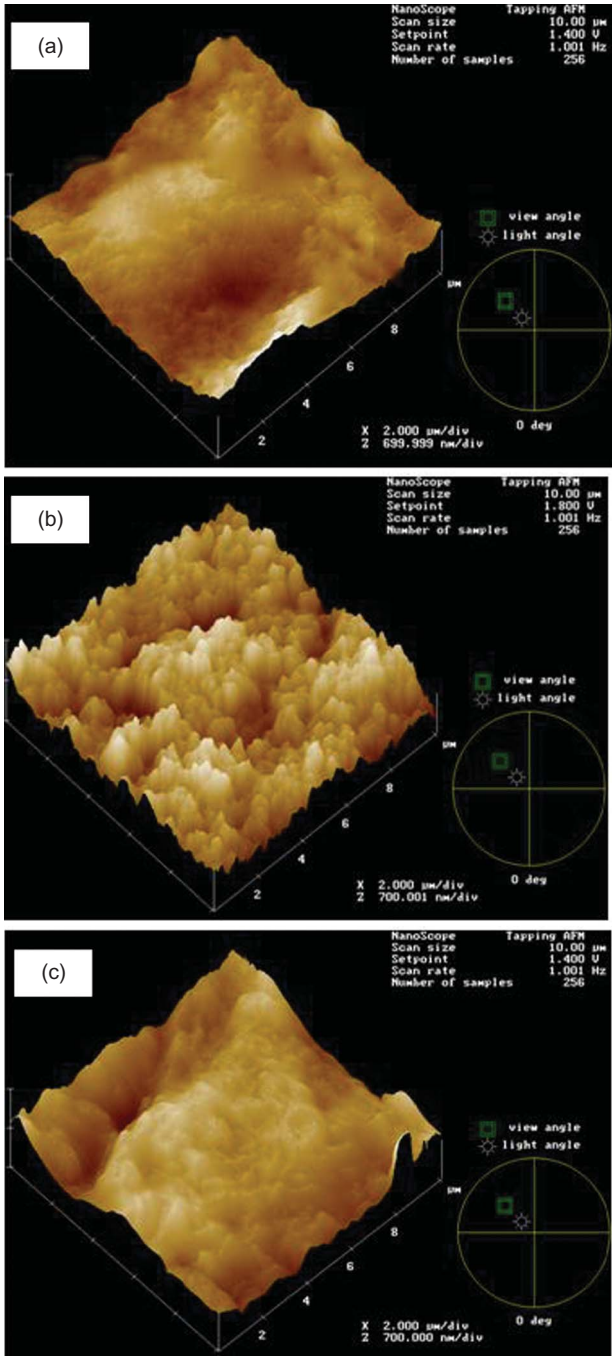

Figure 6. Atomic force micrographs of the human dorsal nail plate pretreated with (a) plain gel (control), (b) 10% PA gel, and (c) 10% LA gel for 60 seconds.

Thus, these morphological changes are likely to benefit the topical treatment of nail infections as well as other nail conditions. However, selection of an appropriate etching agent (and concentration) is paramount to providing optimal results for nail maladies.

Etching is a noninvasive technique of structural alteration and does not compromise the cosmetic appearance of the nail plate. Moreover, etching is a short pretreatment method which does not require a clinician's supervision.

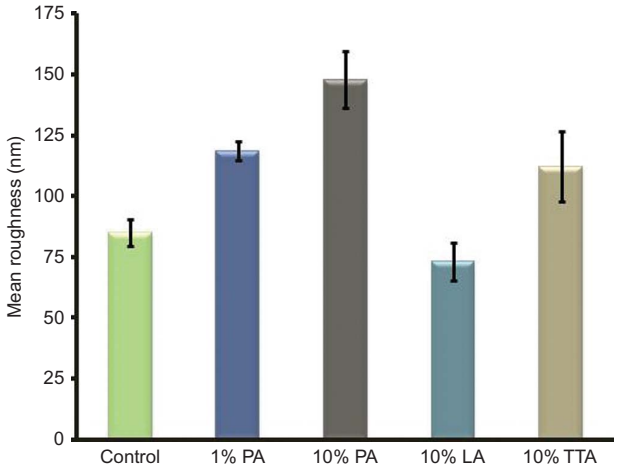

Figure 7. Mean roughness scores of the dorsal surface of the human nail plates upon pretreatment (as determined by NanoScope IIIA software) with different gels for 60 seconds.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References

1. Rich P, Scher RK. (2002). An atlas of diseases of the nail. London: Parthenon.
2. Debruyne D, Coquerel A. (2001). Pharmacokinetics of antifungal agents in onychomycosis. Clin Pharmacokinet, 40:441–72.
3. Niewerth M, Korting HC. (1999). Management of onychomycosis. Drugs, 58:283–96.
4. Piraccini BM, Tosti A. (1999). Drug induced nail disorders: Incidence, management and prognosis. Drug Saf, 21:187–201.
5. Tom CM, Kane MP. (1999). Management of toenail onychomycosis. Am J Health Syst Pharm, 56:865–71.
6. Tosti A, Baran R, Piraccini BM. (1999). "Endonyx" onychomycosis: A new modality of nail invasion by dermatophytes. Acta Derm Venereol, 79:52–3.
7. Murdan S. (2008). Enhancing the nail permeability of topically applied drugs. Expert Opin Drug Deliv, 5:1267–82.
8. Buonocore MG. (1995). A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res, 34:849–53.
9. Repka MA, Mididoddi PK, Stodghill SP. (2004). Influence of human nail etching for the assessment of topical onychomycosis therapies. Int J Pharm, 282:95–106.
10. de Jong EM, Menke HE, van Praag MC, van De Kerkhof PC. (1999). Dystrophic psoriatic fingernails treated with 1% 5-fluorouracil in a nail penetration-enhancing vehicle: A double-blind study. Dermatology, 199:313–8.
11. Drakes MJM, Scott LJ, Goa KL. (2003). Terbinafine: A review of its use in onychomycosis in adults. Am J Clin Dermatol, 4:39–65.
12. Gupta AK, Ryder JE, Lynch LE, Tavakkol A. (2005). The use of terbinafine in the treatment of onychomycosis in adults and special populations: A review of the evidence. J Drugs Dermatol, 4:302–8.
13. Nair AB, Vaka SRK, Sammeta SM, Kim HD, Friden PM, Chakraborthy B, et al. (2009). Trans-ungual iontophoretic delivery of terbinafine hydrochloride. J Pharm Sci, 98:1788–96.
14. Nassim MA, Shirazi FH, Cripps CM, Veerasinghan S, Molepo MJ, Obrocea M, et al. (2002). An HPLC method for the measurement of 5-fluorouracil in human plasma with a low detection limit and a high extraction yield. Int J Mol Med, 10:513–6.

15. Murthy SN, Vaka SRK, Sammeta SM, Nair AB. (2009). Tran-Screen-N™: Method for rapid screening of trans-ungual drug delivery enhancers. J Pharm Sci, 98:4264–71.
16. Repka MA, O'Haver J, See CH, Gutta K, Munjal M. (2002). Nail morphology studies as assessments for onychomycosis treatment modalities. Int J Pharm, 245:25–36.
17. Alberti I, Kalia YN, Naik A, Bonny J, Guy RH. (2001). Effect of ethanol and isopropyl myristate on the availability of topical terbinafine in human stratum corneum, in vivo. Int J Pharm, 219:11–9.
18. Quigley JM, Lloyd DG. (2002). A topological study of prodrugs of 5-flurouracil. Int J Pharm, 231:241–51.
19. Baden HP (1987). The nail, diseases of the hair and nail. Chicago, IL: Year Book Medical Publishers.
20. Baden HP, Kibilus J. (1984). A comparative study of the immunologic properties of hoof and nail fibrous proteins. J Invest Dermatol, 83:327–31.
21. Germann H, Barran W, Plewig G. (1980). Morphology of corneocytes from human nail plate. J Invest Dermatol, 74:115–8.
22. Mauro J, Lumpkin LR, Dantzig PI. (1975). Scanning electron microscopy of psoriatic nail pits. N Y State J Med, 75:339–42.
23. Montagna W, Parakkal PF. (1974). Nails in the structure and function of skin. New York: Academic Press.
24. Thorndike EE. (1968). A microscopic study of the marmoset claw and nail. Am J Phys Anthropol, 28:247–61.
25. Binning G, Quate CF, Gerber C. (1986). Atomic force microscope. Phys Rev Lett, 56:930–3.