Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 357 (2008) 61–69
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Chemical method to enhance transungual transport and iontophoresis efficiency

Jinsong Hao, Kelly A. Smith, S. Kevin Li *

Division of Pharmaceutical Sciences, College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267, USA

Received 3 October 2007; received in revised form 9 December 2007; accepted 16 January 2008
Available online 20 January 2008

Abstract

Transungual transport is hindered by the inherent small effective pore size of the nail even when it is fully hydrated. The objectives of this study were to determine the effects of chemical enhancers thioglycolic acid (TGA), glycolic acid (GA), and urea (UR) on transungual transport and iontophoresis efficiency. *In vitro* passive and iontophoretic transport experiments of model permeants mannitol (MA), UR, and tetraethylammonium (TEA) ion across the fully hydrated, enhancer-treated and untreated human nail plates were performed in phosphate-buffered saline. The transport experiments consisted of several stages, alternating between passive and anodal iontophoretic transport at 0.1 mA. Nail water uptake experiments were conducted to determine the water content of the enhancer-treated nails. The effects of the enhancers on transungual electroosmosis were also evaluated. Nails treated with GA and UR did not show any transport enhancement. Treatment with TGA at 0.5 M enhanced passive and iontophoretic transungual transport of MA, UR, and TEA. Increasing the TGA concentration to 1.8 M did not further increase TEA iontophoresis efficiency. The effect of TGA on the nail plates was irreversible. The present study shows the possibility of using a chemical enhancer to reduce transport hindrance in the nail plate and thus enhance passive and iontophoretic transungual transport.
© 2008 Elsevier B.V. All rights reserved.

Keywords: Transungual iontophoresis; Human nail plate; Chemical enhancer; Thioglycolic acid; Glycolic acid; Urea

1. Introduction

Nail diseases such as onychomycosis affect more than 30 million Americans. The current methods of treatment for onychomycosis achieve only limited success, require long treatment duration, and risk systemic side effects (Rodgers and Bassler, 2001; Repka et al., 2002; Ajit et al., 2003). Approximately one-third of the patients cannot be completely cured after 6 or more weeks of oral medication due to treatment failure and relapse (Gupta and Shear, 1999; Gupta et al., 2001; Finch and Warshaw, 2007). Recently, iontophoresis has been demonstrated as a promising method for enhancing drug delivery through the human nail plate (James et al., 1986; Murthy et al., 2007a,b). Iontophoresis is a noninvasive delivery method that employs an electric field to enhance the transport of a compound across a membrane (Kasting, 1992). In our previous study (Hao and Li, 2008), electrophoresis (direct-field effect) was shown to be the dominant driving force in transungual iontophoretic transport of small permeants across the fully hydrated nail plates. Contribution of electroosmosis to electrotransport was less than 10% of that due to electrophoresis for small permeants at pH 7.4 and ionic strength of 0.16 M. Changing formulation factors such as pH and ionic strength was found to affect transungual electroosmotic transport, but the contribution of electroosmosis in transungual iontophoresis remained small compared to the direct-field effect (Hao and Li, in press). Size exclusion effect of the nail plate was important in determining the permeability of the nail. No significant structure alteration of the nail was observed under the studied electric current conditions of 0.1 and 0.3 mA (Hao and Li, 2008).

In iontophoresis, the transport efficiency (transference number) for an ionic permeant is determined by the fraction of the electric current carried by the permeant to the total applied current. Accordingly, the maximum transference number is obtained under a 'single-ion' case in which competing ions are absent. This situation is merely theoretical. Endogenous counterions are always present. During iontophoretic delivery, an ionic permeant competes with both extraneous and endogenous co-ions and counter-ions, which greatly decreases the transport efficiency of the ion of interest (Phipps and Gyory, 1992). Dif-

* Corresponding author. Tel.: +1 513 558 0977; fax: +1 513 558 0978.
E-mail address: kevin.li@uc.edu (S.K. Li).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.01.027

ferent approaches have been explored to maximize transport efficiency in transdermal iontophoresis, including simplifying the charged additives in the system (Mudry et al., 2006), increasing the mole fraction of the charged drug (Marro et al., 2001), and utilizing polymeric electrolytes (Kochhar and Imanidis, 2004). The use of a chemical enhancer to improve transungual iontophoretic transport has not been studied.

The human nail plate is a highly ordered, epidermal appendage composed of sulfur-rich α-keratins (∼80%), water (10–30%), and lipids (0.1–1.0%). Primarily protein in nature, the nail has an isoelectric point (p*I*) between 4.9 and 5.4 (Murdan, 2002). As far as a biological membrane is concerned, the nail plate is extremely hard and impermeable; this is largely due to the physical and chemical stability of the chemical bonds (disulfide, peptide, hydrogen, and polar) found in keratins, the sandwich orientation of the keratin fibers, the presence of globular proteins that glue the keratin fibers together, the adhesiveness of nail cells to one another, and the design of the plate (curved in both the transverse and longitudinal axes). The water content of the nail imparts minimal flexibility and can influence permeability. The nail swells as water is absorbed such that the hydrated nail behaves like a hydrogel with a network of aqueous pores through which molecules can permeate. Aside from hydrating the nail, keratolytic agents or enhancers are believed to assist in increasing transungual permeability; specifically, urea and salicylic acid destabilize the hydrogen bonds and sulfhydryl compounds like cysteine cleave the disulfide linkages in the nail keratins (Murdan, 2002).

Glycolic acid (GA) has been widely used by the cosmetic industry to improve the appearance and texture of the skin. It is a skin penetrant, a humectant, and an exfoliating agent (Scholz et al., 1994; Campos et al., 1999). Urea (UR) is a well-known keratolytic agent that acts by solubilizing and/or denaturing keratins (Farber and South, 1978; Murdan, 2002). Thioglycolic acid (TGA) is a reducing agent that breaks disulfide bonds within hair keratins (Kuzuhara and Hori, 2003) and is thus used in permanent hair waving (Bolduc and Shapiro, 2001). TGA likely breaks down the disulfide bonds of nail keratins as well, leading to an increase in nail hydration and permeability. In addition, TGA treatment was reported to produce the greatest nail weight increase and enhancement of passive transport of caffeine among the enhancers studied (Khengar et al., 2007). Pretreatment of the nail with these chemicals is therefore expected to enhance transungual permeation and iontophoretic transport efficiency.

In the present study, the effects of chemical enhancers on passive and iontophoretic transport of model permeants across the fully hydrated nail plates and the barrier properties of the nail plates were investigated. Polar, neutral permeants of different molecular sizes, mannitol (MA) and UR, as well as positively charged tetraethylammonium (TEA) ion were selected as the model permeants. Passive and iontophoretic transport experiments of MA and UR or TEA across the fully hydrated nail plates with/without the pretreatment of the enhancers were conducted. The concentrations of TGA examined were 0.5 and 1.8 M. The concentrations of GA and UR used were 0.5 and 2.5 M, respectively. The effects of the enhancers on nail water uptake were also assessed in nail hydration studies to provide insights into the mechanisms of the enhancers.

2. Materials and methods

2.1. Materials

Phosphate-buffered saline (PBS) of pH 7.4 (0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride) was prepared by dissolving PBS tablets (Sigma–Aldrich, St Louis, MO) in distilled, deionized water. Tetraethylammonium chloride (TEACl) solution of pH 7.4 (0.15 M) was prepared by reacting tetraethylammonium hydroxide (20% w/w, Acros, Morris Plains, NJ) with hydrochloric acid and subsequently adjusting the pH. Enhancer solutions of different molarities were prepared by dissolving appropriate amounts of the enhancer powders in PBS. The enhancers were thioglycolic acid sodium salt (Bacteriological grade, MP Biomedicals, Solon, OH), glycolic acid (99% purity, Acros, Morris Plains, NJ), and urea (99.9% purity, Amresco, Solon, OH). Sodium azide (99% purity, Acros, Morris Plains, NJ) of 0.02% (w/v) was added to all solutions as a bacteriostatic agent. ^{3}H-mannitol (1-^{3}H(N)–, 10–30 Ci/mmol) and ^{14}C-tetraethylammonium bromide (1-^{14}C, 1–5 mCi/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). ^{14}C-urea (50–60 mCi/mmol) was purchased from Moravek Biomaterials and Radiochemicals (Brea, CA). All radiolabeled chemicals had purity of at least 97%. All materials were used as received.

2.2. Preparation of nail samples

Human fingernail plates (male, age 55–83) were obtained from Science Care Anatomical (Phoenix, AZ). The frozen nail plates were thawed in PBS at room temperature. Adhering tissues on the nail plates were removed with a pair of forceps. The nails were then rinsed with and soaked in PBS for at least 24 h to allow complete hydration before the transport experiments. The thickness of the hydrated nail plates, ranging from 0.5 to 0.8 mm, was measured using a micrometer (Mitutoyo, Kawasaki, Kanagawa, Japan) at the end of the experiments. Nail clippings were obtained from healthy volunteers (male and female, age 30–50) using nail clippers. The nail clippings were cleaned with a pair of forceps, rinsed with PBS, and dried both with Kimwipes® and by leaving them in open containers overnight before the hydration studies. The use of human tissues was approved by the Institutional Review Board at the University of Cincinnati, Cincinnati, OH.

2.3. Hydration of nail clippings

The nail hydration studies were divided into three stages. In Stage I, clean nail clippings were weighed and soaked in 1 ml of PBS containing different amounts of enhancers in a screw-capped vial at room temperature ($20 \pm 2\,^{\circ}$C) for 24–48 h until a constant nail weight was obtained. After hydration, the nail clippings were removed, blotted dry with Kimwipes®, and quickly weighed (i.e., wet weight). The wet nail clippings were

then allowed to oven dry at 60 °C for 24 h to a constant weight (i.e., dry weight). The percentage water content in the nail clippings was determined by dividing the difference between the wet and dry weights by the dry weight, i.e., (wet weight − dry weight)/dry weight × 100%. In Stage II, the reversibility of nail hydration was checked by re-performing the hydration study in fresh PBS using the same enhancer-treated nail samples immediately after Stage I, and the percentage water content was determined. In Stage III, the same nail samples were re-hydrated again approximately 2 weeks after the completion of Stage II. As a control, a hydration study was also conducted in PBS in the absence of enhancers using the same method. Two other controls were nail hydration in deionized water and in 0.15 M TEACl, both without the enhancers.

2.4. Transport study strategy

As described previously (Hao and Li, 2008), a multi-stage protocol with the same nail plates and a dual permeant strategy (i.e., concurrent MA and UR delivery in passive transport experiments and concurrent MA and TEA transport in iontophoresis experiments) were employed in the present study to minimize the influence of intersample variability on data interpretation. Although UR is a keratolytic agent (Farber and South, 1978), the trace amount of UR (approximately 20 nmol) used in the present study as a model permeant would not affect the nail structure. The transport experiments to examine the effects of TGA included seven stages (Fig. 1) at each TGA concentration: passive transport of MA/UR (Passive 1, day 1–3), iontophoresis of MA/TEA (Anodal 1, day 4–5), passive transport of MA/UR (Passive 2, day 6–8), TGA treatment (day 8–9), passive transport of MA/UR (Passive 3, day 9–11), iontophoresis of MA/TEA (Anodal 2, day 12–13), and passive transport of MA/UR (Passive 4, day 14–16). After Passive 2, the nail plates were treated with TGA solutions for 36 h. To examine the effects of UR and GA, Anodal 1 and Passive 2 were not performed because this control step appeared unnecessary due to the reproducible data found in Anodal 1 and Passive 2 in the present TGA studies. In this case, the nail plates were treated with UR or GA solutions for 36 h after Passive 1, which was followed by Passive 3, Anodal 2, and Passive 4. The experiments of Passive 1 were used solely to check the integrity of the nail plates. The results obtained from Passive 1 were not included in the analysis as the nail plates in the first passive transport experiment sometimes had lower permeability coefficients than those in the later stages, possibly due to incomplete hydration of the nail plates (Hao and Li, 2008). Enhancer pretreatment was chosen in this protocol to prevent the interference of ionized TGA and GA with iontophoretic transport of the permeants under the conditions in the present study. The long enhancer pretreatment allowed the examination of the maximum enhancer effect in this mechanistic and feasibility study.

2.5. Transport studies

Transport experiments were conducted at room temperature (20 ± 2 °C) with constant stirring similar to those described previously (Hao and Li, 2008) except that the donor solution used in the iontophoresis experiments was TEACl. After 24 h of hydration in PBS, the nail plates were mounted between side-by-side diffusion half-cells (Dana Enterprise, West Chester, OH) with custom-made nail adapters such that the dorsal side of nail plates faced the donor chamber and the ventral side faced the receptor chamber. The diffusion cells had an effective diffusion area of approximately 0.64 cm^2 and a cell volume of 2 ml. The adapters, which had a similar circular opening of 0.64 cm^2 in the center, were constructed from silicone elastomer (MED-6033, NuSil Silicone Technology, Carpinteria, CA) to fit the curvature of the nail plate. Preliminary studies showed no noticeable permeant-to-adapter binding. The receptor solution was PBS. In the passive transport experiments, the donor solution was PBS containing trace amounts of radiolabeled permeants (2 μCi of ^{3}H-MA and 1 μCi of ^{14}C-UR) added immediately before the transport experiments. In the iontophoretic transport experiments, the donor solution was 0.15 M TEACl (pH 7.4) with trace amounts of radiolabeled permeants (2 μCi of ^{3}H-MA and 1 μCi of ^{14}C-TEA), and was replaced with fresh solution every 12 h to maintain a constant donor solution composition during iontophoresis. A constant direct current of 0.1 mA was applied with a constant current iontophoretic device (Phoresor II Auto, Model PM 850, Iomed, Inc., Salt Lake City, UT) using Ag and Ag/AgCl as the driving electrodes. The anode was in the donor and the cathode was in the receptor. The voltage drop across the nail plates was monitored using a multimeter

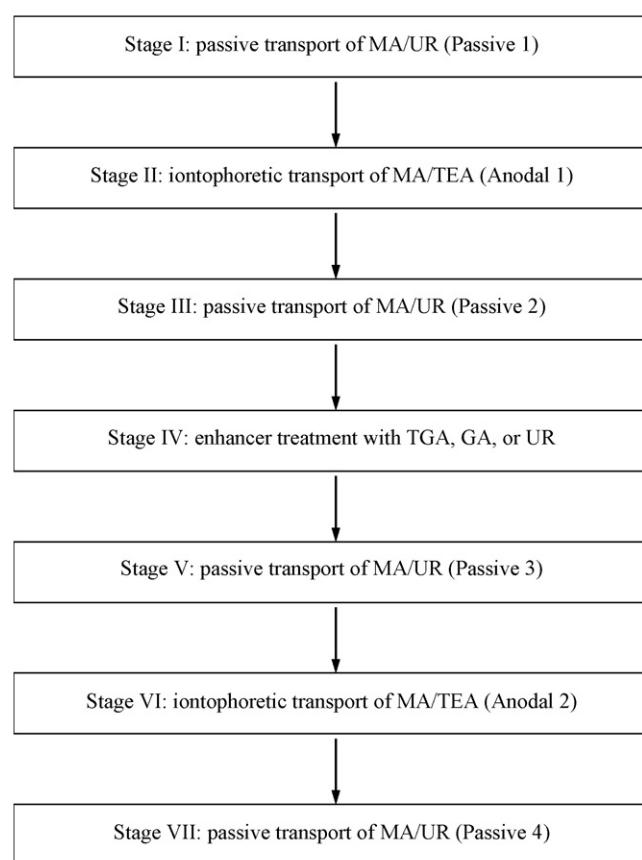

Fig. 1. A schematic diagram of transport experimental procedure.

(Fluke 73III, Everett, WA) during iontophoresis. The electrical resistance of the nail plates was calculated using Ohm's law. The electrical resistance of the nail plates before the iontophoresis experiments was also measured by applying 0.1 mA current across the nail for 30 s and using Ohm's law. At predetermined time intervals, 10 μl of donor solution and 1 ml of receptor solution were withdrawn and 1 ml fresh PBS was added to the receptor to maintain a constant volume in the receptor. The samples were mixed with 10 ml of liquid scintillation cocktail (Ultima Gold^{TM}, PerkinElmer Life and Analytical Sciences, Shelton, CT) and assayed by a liquid scintillation counter (Beckman Counter LS6500, Fullerton, CA). The cumulative amount of permeant transported through the nail plate (Q) was plotted against time (t), and the steady-state flux of permeant (J) was calculated from the slope of the linear portion of the plot:

$$J = \frac{\Delta Q}{A\,\Delta t} \tag{1}$$

where A is the diffusion area and $\Delta Q/\Delta t$ is the slope of the cumulative amount against time. The steady-state permeability coefficient (P) is defined as the flux divided by the concentration of the permeant in the donor. The durations of the iontophoresis and passive transport experiments were 36 and 48 h, respectively. Both chambers of the diffusion cells were rinsed with fresh PBS at least three times by replacing the donor and receptor solutions over 12 h between the transport experiments of each stage, unless otherwise stated. In the enhancer-pretreated nail experiments, both sides of the nail plates were treated with the enhancer solution. Immediately after the passive transport experiment and the PBS rinsing, the enhancer solution was placed in the donor and receptor. After treating the nail plates with the enhancer solution for 36 h, the donor and receptor compartments were rinsed with fresh PBS three times. Passive and iontophoretic transport experiments were then performed immediately as described above.

2.6. Theory and equations

The steady-state iontophoretic flux ($J_{\Delta\psi,i}$) of permeant i through a homogeneous porous membrane can be described by the modified Nernst–Planck model (Li et al., 1997; Zhu et al., 2001):

$$J_{\Delta\psi,i} = -\varepsilon_{\rm p}\left\{H_i\,D_i\left[\frac{{\rm d}C_i}{{\rm d}x} + \frac{C_i\,z_i\,F}{R_{\rm gas}\,T}\frac{{\rm d}\psi}{{\rm d}x}\right] \pm W_i\,v\,C_i\right\} \tag{2}$$

where ψ is the electric potential in the membrane, F the Faraday constant, $R_{\rm gas}$ the gas constant, T the temperature, v the average velocity of the convective solvent flow, and $\varepsilon_{\rm p}$ the combined porosity and tortuosity factor for the membrane. C_i, x, z_i, and D_i are the concentration, membrane position, charge number, and diffusion coefficient of the permeant, respectively. H_i is the hindrance factor for diffusion and W_i is the hindrance factor for pressure induced parabolic convective solvent flow. The diffusion coefficient of the permeant is related to its mobility (u_i) and charge by the Einstein relation:

$$D_i = \frac{u_i\,R_{\rm gas}\,T}{z_i\,F} \tag{3}$$

The enhancement factor is defined as the ratio of the iontophoretic flux over the passive flux at the same donor concentration. For the iontophoretic transport of a neutral permeant, the Peclet number (${\rm Pe}_i$) is determined from the enhancement factor ($E_{v,i}$) by (Peck et al., 1996):

$$E_{v,i} = \frac{{\rm Pe}_i}{1-\exp(-{\rm Pe}_i)} \tag{4}$$

For iontophoretic transport of a charged permeant, the transference number (t_i) is defined as the fraction of the total current carried by the permeant i and is a measure of iontophoretic delivery efficiency (Phipps and Gyory, 1992):

$$t_i = \frac{C_i\,u_i\,z_i}{\sum_j C_j\,u_j\,z_j} \tag{5}$$

where the subscript j represents all ions in the membrane including the permeant i. The transference number was determined experimentally in the present transport experiments by (Phipps and Gyory, 1992):

$$t_i = \frac{z_i\,F\,J_{\Delta\psi,i}}{I} \tag{6}$$

where I is the current density applied.

2.7. Statistical analysis

The Student's t-test for two-tailed distribution was used to evaluate the significance of transport and hydration parameters among untreated and enhancer-treated groups. Differences were considered to be significant at a level of $p < 0.05$. The means ± standard deviations (S.D.) of the data are presented.

3. Results

3.1. Hydration studies

A previous study showed that the nail approached 90% of complete hydration in an hour after immersion in PBS. This was followed by a slow equilibration process to complete hydration over 1 day (Hao and Li, 2008). In the presence of the enhancers, the nail took 1–2 days to achieve its maximum water uptake. Fig. 2 shows the results in the nail hydration experiments. In Stage I, the average water content of control nail clippings in PBS was 39 ± 6%. There was no significant difference between the water contents of nail clippings in PBS, TEACl, and deionized water (data not shown). At 0.5 M, TGA increased the water content of the nails to 47 ± 6%. TGA at higher concentrations did not further increase the water content of the nails. UR had no effect on the nail water contents at concentrations of 0.3 and 1.0 M but increased the water content to 51 ± 6% at 2.5 M ($p < 0.05$). The water content of 0.5 M GA-treated nails (43 ± 3%) was not

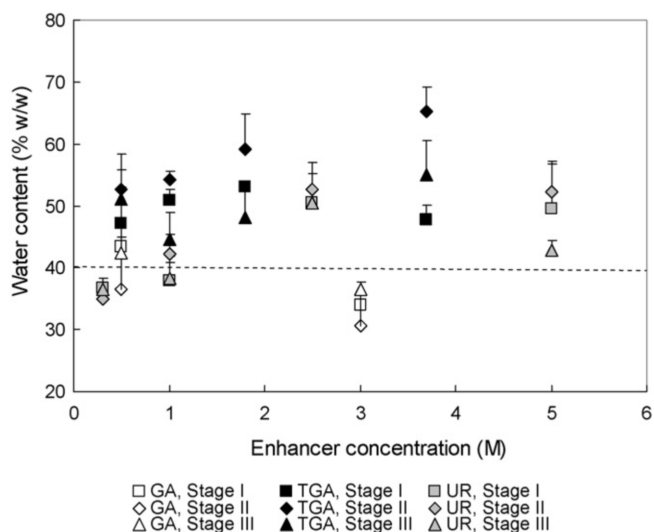

Fig. 2. The percentage water content in nail clippings treated with the enhancer solutions. Different enhancers (GA, TGA, and UR) and various concentrations of these enhancers were used. Percentage water content = (nail wet weight − nail dry weight)/nail dry weight × 100%. Data represent the average of 3–6 nail samples. Dashed line shows the average percentage water content in control nail clippings of Stages I, II, and III (40 ± 6%).

significantly different from that of the control ($p > 0.05$). Increasing the concentration of GA to 3.0 M slightly decreased the nail water content compared to the control. These results indicate that both TGA and UR increased the hydration capability of the nails. At the concentration examined in the present study, GA did not affect nail hydration.

It should be noted that the water content data presented in Fig. 2 were not corrected for possible deposit of the enhancers in the dry nail samples. This may introduce uncertainties to the nail water content results, especially with the enhancer of high molecular weight at high concentrations. For example, the corrected water contents of nails treated with 1.8 and 3.7 M TGA were increased from 53% and 48% to 59% and 60%, respectively. However, these calculations are believed to overestimate the amount of the enhancer deposited in the dry nail samples because the actual concentration of the enhancer in the nail is expected to be lower than that in the bulk solution due to the size exclusion property of the nail. Also, the data in Stage I in the presence of the enhancers were generally not significantly different from those in Stage II in the absence of the enhancers, further suggesting that the correction for the amount of the enhancers deposited in the nails was not significant.

3.2. *Enhancer effects on passive transport*

Fig. 3 presents the passive permeability coefficients of MA and UR across the nail plates treated with different enhancers at various concentrations. Treatment with TGA at 0.5 and 1.8 M increased passive permeation of both MA and UR across the nail plates. The permeability coefficients for MA and UR across the nail plates treated with 0.5 M TGA were increased by approximately four and two times, respectively. At the higher TGA concentration of 1.8 M, flux enhancement increased to approximately eight- and fourfold compared to the controls for MA

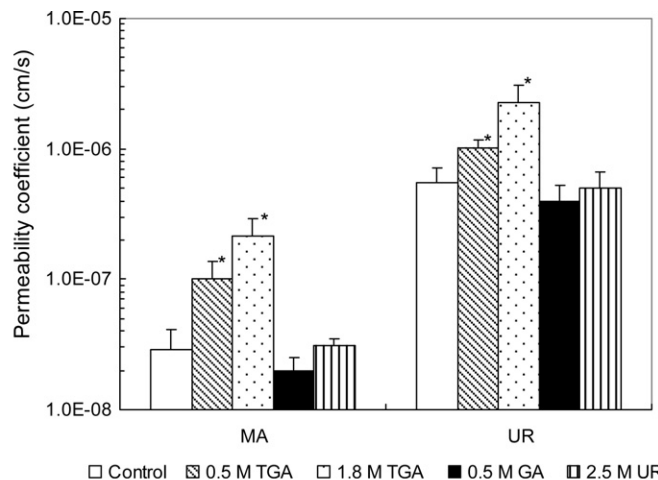

Fig. 3. Permeability coefficients of MA and UR obtained from Passive 3 with the enhancer-treated nails. Data represent the mean ± S.D., $n = 3$–9. The average permeability coefficients obtained with untreated nails serve as the control. The asterisks indicate significant differences between the enhancer-treated groups and the control groups.

and UR, respectively. These results indicate that TGA treatment enhanced passive permeation across the nail plates and larger enhancement was observed at the higher TGA concentration. No passive transport enhancement of MA and UR was observed with the nails treated with 0.5 M GA or 2.5 M UR.

To assess hindered transport across the nails and the effects of the enhancers upon transport hindrance, the ratios of the permeability coefficients of UR to MA were calculated and presented in Fig. 4. A decrease in the permeability coefficient ratio is indicative of a reduction in transport hindrance and an increase in the effective pore size in the transport barrier. The data in the figure show that the ratio after treatment with 0.5 M TGA was 11, half of the ratio of the control. This decrease implies an increase in the effective pore size of the TGA-treated nail plates. Increasing the TGA concentration to 1.8 M did not further decrease this

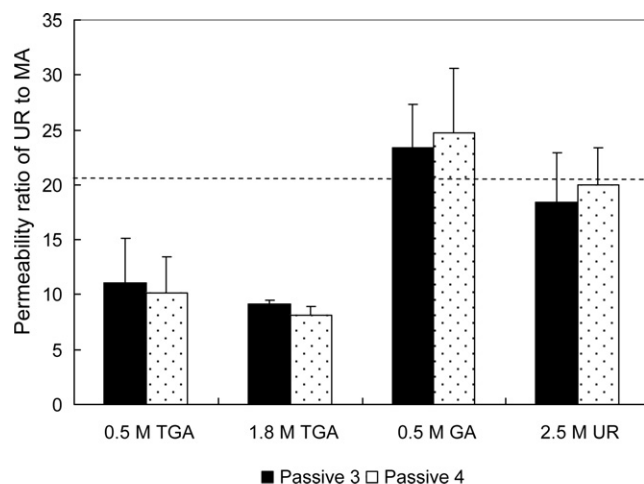

Fig. 4. The permeability ratios of UR to MA obtained from the passive transport experiments across the fully hydrated nail plates treated with the enhancers. Data represent mean ± S.D., $n = 3$–9. The average permeability coefficient ratio with untreated nail plates serves as the control. The dashed line shows the average permeability coefficient ratio of the control (21 ± 7, $n = 9$).

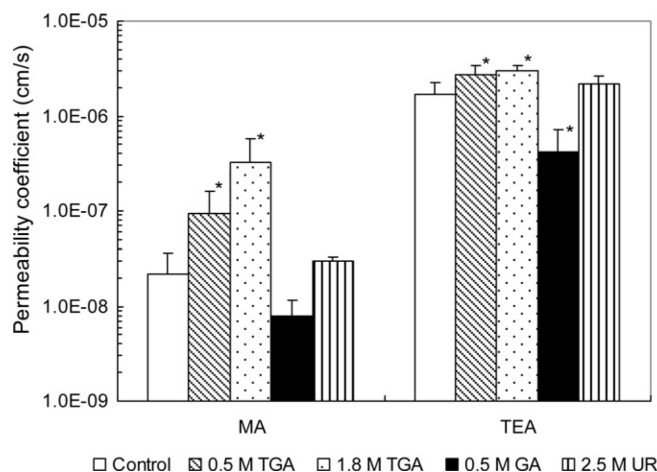

Fig. 5. Permeability coefficients of MA and TEA obtained in Anodal 2 with the nail plates treated with the enhancers. Data represent mean ± S.D., $n = 3$–9. The data for the control bars are the average permeability coefficients in Anodal 1 with the nail plates before the enhancer treatment. The asterisks indicate significant differences between the enhancer-treated groups and the control groups.

ratio, suggesting essentially the same hindrance transport in the nail plates treated with TGA at both concentrations. Fig. 4 also substantiates the assertion that GA and UR were not effective enhancers under the conditions in the present study. The data show that the ratios of the passive permeability coefficients of UR to MA were essentially the same for the nail plates treated with 0.5 M GA and 2.5 M UR.

3.3. Enhancer effects on anodal iontophoretic transport

Fig. 5 shows the permeability coefficients of MA and TEA obtained in the anodal iontophoresis transport experiments. The permeability coefficients of both MA and TEA across the nail plates treated with TGA at concentrations of 0.5 and 1.8 M were increased compared to the anodal iontophoresis control. The permeability coefficients of MA and TEA with UR-treated nail plates were not statistically different from those of the control nails ($p > 0.05$). These results indicate that the treatment of UR did not enhance anodal iontophoretic transport of MA and TEA. GA did not affect anodal iontophoretic transport of MA ($p > 0.05$) and interestingly decreased the transport of TEA ($p < 0.05$).

The efficiency of iontophoretic transport of a permeant is generally assessed by the transference number in constant current iontophoresis. The transference numbers of TEA calculated from Eq. (6) are summarized in Table 1. For the control nails, the transference number of TEA in anodal iontophoresis was 0.15 ± 0.05. The transference numbers increased to 0.26 ± 0.07 and 0.27 ± 0.04 after the nail plates were treated with 0.5 and 1.8 M TGA, respectively. Although TGA treatment increased the efficiency of TEA iontophoresis, the higher TGA concentration in the treatment did not further increase the efficiency. Treatment with UR at 2.5 M did not significantly increase the transference number of TEA ($p > 0.05$) in anodal iontophoresis. GA treatment decreased the anodal iontophoretic transport efficiency of TEA ($p < 0.05$).

Table 1
Enhancement factor ($E_{v,i}$), apparent Peclet number (Pe_i), transference number (t_i), and nail plate voltage determined in the 0.1 mA anodal iontophoretic transport experiments[a]

Enhancer	MA		TEA	Voltage (V)
	$E_{v,i}$[b]	Pe_i	t_i	
Control[c]	0.78 ± 0.42	−0.59 ± 0.88	0.15 ± 0.05	0.74 ± 0.27
0.5 M TGA	0.88 ± 0.32	−0.32 ± 0.71	0.26 ± 0.07	0.41 ± 0.10
1.8 M TGA	0.83 ± 0.33	−0.45 ± 0.81	0.27 ± 0.04	0.44 ± 0.21
0.5 M GA	0.45 ± 0.05	−1.42 ± 0.16	0.04 ± 0.03	0.56 ± 0.10
2.5 M UR	0.95 ± 0.02	−0.11 ± 0.06	0.20 ± 0.04	0.76 ± 0.16

[a] Mean ± S.D., $n = 3$–9.
[b] The enhancement factor was calculated from the ratio of the iontophoretic permeability coefficient to corresponding passive permeability coefficient before and after the enhancer treatment.
[c] The control represents the data obtained from transport experiments with nail plates before the enhancer treatment.

On a closer examination of electroosmosis, the Peclet numbers of MA calculated with Eq. (4) are summarized in Table 1. Except for the study using GA, the enhancement factors of MA were not significantly different from unity, and the apparent Peclet numbers were not different from zero. For all nail plates, the enhancers showed no significant effect on the iontophoretic transport of MA compared with those of the control ($p > 0.05$). This suggests that the contribution of electroosmosis to iontophoretic transport of the permeant was negligible in the presence of the enhancers.

Table 1 also lists the voltage across the nail plates measured in the iontophoretic transport experiments with and without the enhancer treatments. Under the constant electric current conditions in the present study, a decrease in the voltage indicates a drop in the electrical resistance of the nail. The average voltage across the nails dropped from 0.74 ± 0.27 V for untreated nails to 0.41 ± 0.10 V for 0.5 M TGA-treated nail during 0.1 mA iontophoresis. Increasing the concentration of TGA did not further decrease the nail electrical resistance. The lower voltage across the TGA-treated nails implied an enlarged effective pore size and/or increased porosity in the nail plates. There was a tendency of a slight decrease in the nail resistance after 0.5 M GA treatment, but the effect was not statistically significant. The treatment of 2.5 M UR did not reduce the nail electrical resistance. The lower nail electrical resistance after the enhancer treatment has practical significance in the development of a miniature transungual iontophoretic device. When a nail has lower electrical resistance, lower voltage and battery power consumption would be needed in a constant current iontophoresis system.

3.4. Reversibility of enhancer effects on nail hydration and passive transport

Reversibility of the enhancer effects on both water uptake and passive transungual transport were assessed. In the hydration experiments, the enhancer-treated nail clippings (Stage I) were re-equilibrated in PBS in the absence of the enhancers in Stages II and III hydration to determine nail hydration reversibil-

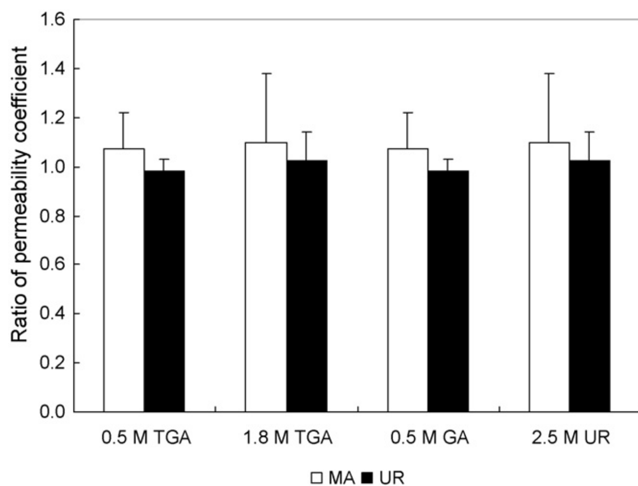

Fig. 6. Test of long-term reversibility of the enhancer effects on passive permeation of MA and UR across the nail plates treated with the enhancers. The ratios of the permeability coefficients were calculated by dividing the permeability coefficients obtained 6 days after enhancer treatment in Passive 4 by that obtained immediately after enhancer treatment in Passive 3. Data represent the mean ± S.D. of 3–9 experiments.

ity after the initial enhancer treatment in Stage I. The reversibility study results are shown in Fig. 2. The control nail clippings showed essentially the same water contents of 40 ± 6%, irrespective of the hydration stage. The results of the nail clippings treated with 0.5 and 1.8 M TGA indicate that the effect of TGA on water uptake in the nail was irreversible; the water contents of the three stages of 0.5 and 1.8 M TGA were not statistically different ($p > 0.05$). The differences in water contents among different stages of 2.5 M UR-treated nails were also insignificant ($p > 0.05$), suggesting that the effect of UR on nail water uptake was essentially irreversible. GA was not an effective enhancer for nail hydration and the data were not analyzed.

Fig. 6 shows the permeability coefficient ratios comparing the passive transport permeability coefficients of MA and UR 6 days after the enhancer treatment in Passive 4 transport experiments to those immediately after the treatment in Passive 3. As expected, due to the ineffectiveness of GA and UR as chemical enhancers in transungual transport, both MA and UR had ratios close to unity for GA and UR treatments. For TGA, the permeability coefficient ratios were also close to unity. These results suggest that the effect of the enhancer on hindered transport across the nail plates was irreversible. In addition, the results also imply that iontophoresis did not alter the nail barrier after the enhancer treatments as Passive 4 was carried out after Anodal 2.

4. Discussion

4.1. Transungual transport hindrance

The relative size of a molecule to a pore plays an important role in molecule transport in liquid-filled pores (Deen, 1987). If the pore size is in the same order of magnitude as the molecular size of a permeant, hindered transport will be encountered. In this case, the effective diffusion coefficient and mobility of the permeant are smaller than those in bulk solution, and convective transport is slower than that of the bulk water flow. The fully hydrated nail plate behaves like a hydrogel with a network of aqueous solution filled pores. Although the water content (or the porosity) of the fully hydrated nail was approximately 40% (Fig. 2), the permeability of the nail plates for polar permeants was relatively low with such high porosity (Fig. 3). This suggests significant hindered transport in transungual permeation with an effective pore size in the same order of magnitude as the permeant molecular sizes. Our previous study (Hao and Li, 2008) used the permeability coefficient ratio of two permeants of different molecular sizes to assess transungual hindered transport and found that the effective pore radius of the fully hydrated nail plate was less than 1 nm. The present study examined the hypothesis of chemically enhanced transungual transport by the mechanisms of enlarging the nail effective pore size and/or increasing its porosity. The decrease in the permeability coefficient ratio caused by TGA (Fig. 4) suggests an increase in the effective pore size and a reduction in the size exclusion effect of the nail plate. The following sections will discuss the effects of the enhancers examined in the present study.

4.2. Chemical enhancers

UR interacts with the hydrogen bonds in the nail keratins, causing nail hydration and softening (Kobayashi et al., 1998; Murdan, 2002). With the hydration and swelling, the nail plate has a less dense structure with larger pores for the diffusion of permeants. Consequently, permeant transport is expected to be enhanced. In the present study, UR at 2.5 M increased water uptake into the nail clippings (Fig. 2) but had no effect on passive and iontophoretic transungual transport of the permeants (Figs. 3 and 5). A possible explanation is that the pores created in the UR-treated nails are so small that no permeants but water can penetrate into the pores. Therefore, nail water uptake was increased but no enhancement on transungual permeant transport was observed. Both the transport data (Figs. 3 and 5) and the electrical resistance data (Table 1) support this explanation.

GA weakens the binding properties of the epidermal lipids responsible for adhering dead skin cells to one another (Fartasch et al., 1997). Unlike skin which has a considerable lipid composition, the nail is almost entirely keratin (Gniadecka et al., 1998; Murdan, 2002). The present results suggest that GA is unable to increase the uptake of water into the nail clippings (Fig. 2) and enhance permeant transport through the nail plates (Figs. 3 and 5), and therefore, does not work in the nail as it does on skin.

Compounds containing sulfhydryl groups such as acetylcysteine, cysteine, and mercaptoethanol can reduce disulfide bonds. These sulfhydryl compounds were shown to be effective transungual enhancers when formulated in aqueous as well as in lipophilic vehicles (Kobayashi et al., 1998, 1999). TGA, a sulfhydryl compound, is used in the industry to break the disulfide bonds in the hair cortex (Kuzuhara and Hori, 2003). Similar to hair, nail keratins are rich in disulfide bonds. TGA is therefore expected to destabilize the nail keratin network, increase nail water uptake (Fig. 2), and enhance transungual transport (Fig. 4) by two different mechanisms. TGA can increase the size

of the pre-existing pores or create larger pores, thereby reducing transport hindrance and increasing nail permeability. Permeability could also be increased without altering the hindrance factor if TGA only creates more pores of the same size as the pre-existing pores. The transport data obtained from the TGA-treated nails suggest that both mechanisms may be involved. TGA at 0.5 M increased the effective pore size in the nail plate and/or created new pores as evidenced by the decrease in the permeability ratio of UR to MA after the treatment (Fig. 4). In addition, TGA treatment increased the passive permeability coefficients of MA by more than twofold (Fig. 3); this was significantly greater than the enhancement in nail hydration (Fig. 2). This supports the explanation of a decrease in transport hindrance. At the higher TGA concentration of 1.8 M, the treatment seems to create more pores of similar size, causing higher permeability (Fig. 3) with similar transport hindrance (Fig. 4). All these effects were shown to be irreversible in the present study.

4.3. Transungual iontophoresis efficiency

Although only model permeants were employed in the present study, the data provide insights into the mechanisms of the chemical enhancers and transungual drug delivery. Membrane transport is mainly related to the physicochemical properties of the permeant such as molecular size, charge, and/or electromobility. Permeants of similar molecular sizes, charge, electromobilities, and lipophilicities would show similar nail uptake and permeation behavior when there is no specific interaction between the permeants and the nail. The transference number, defined as the ratio of the current carried by the permeant to the total current carried by all ionic species in the system, measures the efficiency of iontophoretic transport of a permeant. As can be seen in Eq. (5), a constant percentage increase of the mobilities of all ions (the permeant, co-ions, and counter-ions) will result in the same transference number. A method to enhance iontophoresis efficiency in constant current iontophoresis is to increase the flux of the permeant to a greater extent than those of the small co-ions and/or counterions such as sodium and chloride. This can be achieved by increasing the effective pore size of the nail with a chemical enhancer. From the data in the present study, the transference number of TEA was increased by approximately two times after 0.5 M TGA treatment (Table 1). This is consistent with the two times decrease in the ratio of the permeability coefficients of UR and MA after 0.5 M TGA treatment (Fig. 4) as the molecular size of UR is similar to those of the small ions and MA close to that of TEA (Zhu et al., 2001). The essentially constant transference numbers of TEA after the treatments of 0.5 and 1.8 M TGA (Table 1) can be explained by the relatively constant effective pore size induced by the treatments of TGA at 0.5 and 1.8 M as discussed in Section 4.2.

4.4. Transungual electroosmosis

In the present study, iontophoretic transport of MA across the untreated and treated nails (Anodal 1 and Anodal 2) was not significantly different compared to their corresponding passive transport (Passive 2 and Passive 3). This observation is different from that of anodal iontophoresis in PBS at pH 7.4 in a previous study (Hao and Li, 2008), where the transport of MA was enhanced by electroosmosis from the anode to cathode during iontophoresis. This discrepancy is believed to be related to the 0.15 M TEACl used in the donor in the present study. A separate study was conducted under the same experimental setting to examine the transport of MA from the PBS cathode to the 0.15 M TEACl anode. The data suggest the reduction or reversal of electroosmotic solvent flow (unpublished data). A possible explanation is that under the 0.15 M TEACl experimental condition, TEA moved from the anode to the cathode, instead of the sodium ion in the previous study (Hao and Li, 2008), at a significantly slower rate compared to the chloride ion due to transport hindrance in the nail. The TEA ions might alter the charge density of the pore microenvironment in the nail. However, the data are not conclusive and the discrepancy may not be entirely due to the phenomenon of electroosmosis. In summary, the results in the present study continue to indicate that the effect of electroosmosis is secondary to electrophoresis in transungual iontophoretic transport with and without the enhancer treatments. Electroosmosis is not an important factor in transungual delivery as concluded previously (Hao and Li, 2008).

5. Conclusions

The present study has demonstrated the feasibility of using a chemical enhancer as a means to improve transungual passive permeation and iontophoretic transport efficiency. To our knowledge, this is the first mechanistic study on the effects of chemical enhancers on transungual iontophoretic transport. Among the three enhancers studied, TGA was an effective transungual penetration enhancer. It increased both passive and iontophoretic transungual transport. The effect of TGA on the nail plates was also found to be irreversible. The mechanisms of TGA are related to the increase in nail hydration accompanying the reduction of transport hindrance and an increase in nail porosity. TGA-treated nails had lower electrical resistance than the control during iontophoresis, and this would allow longer iontophoresis applications by reducing battery power consumption in a transungual iontophoresis device. GA and UR were not effective enhancers under the experimental conditions in the present study.

Acknowledgements

This research was supported in part by NIH Grant GM063559. The thesis work of Kelly A. Smith was sponsored by a grant from Boehringer Ingelheim Cares Foundation. The authors thank Dr. Gerald B. Kasting for his assistance and insights. The authors also thank Iomed, Inc. for kindly supplying some of the iontophoretic devices used in this study.

References

Ajit, C., Suvannasankha, A., Zaeri, N., Munoz, S.J., 2003. Terbinafine-associated hepatotoxicity. Am. J. Med. Sci. 325, 292–295.

Bolduc, C., Shapiro, J., 2001. Hair care products: waving, straightening, conditioning, and coloring. Clin. Dermatol. 19, 431–436.

Campos, P.M.B.G.M., Ricci, G., Semprini, M., Lopes, R.A., 1999. Histopathological, morphometric, and stereologic studies of dermocosmetic skin formulations containing vitamin A and/or glycolic acid. J. Cosmet. Sci. 50, 159–170.

Deen, W.M., 1987. Hindered transport of large molecules in liquid-filled pores. AIChE J. 33, 1409–1425.

Farber, E.M., South, D.A., 1978. Urea ointment in the non-surgical avulsion of nail dystrophies. Cutis 22, 689–692.

Fartasch, M., Teal, J., Menon, G.K., 1997. Mode of action of glycolic acid on human stratum corneum: ultrastructural and functional evaluation of the epidermal barrier. Arch. Dermatol. Res. 289, 404–409.

Finch, J.J., Warshaw, E.M., 2007. Toenail onychomycosis: current and future treatment options. Dermatol. Ther. 20, 31–46.

Gniadecka, M., Nielsen, O.F., Christensen, D.H., Wulf, H.C., 1998. Structure of water, proteins, and lipids in intact human skin, hair, and nail. J. Invest. Dermatol. 110, 393–398.

Gupta, A.K., Konnikov, N., Lynde, C.W., 2001. Single-blind, randomized, prospective study on terbinafine and itraconazole for treatment of dermatophyte toenail onychomycosis in the elderly. J. Am. Acad. Dermatol. 44, 479–484.

Gupta, A.K., Shear, N.H., 1999. The new oral antifungal agents for onychomycosis of the toenails. J. Eur. Acad. Dermatol. Venereol. 13, 1–13.

Hao, J., Li, S.K., 2008. Transungual iontophoretic transport of polar neutral and positively charged model permeants: effects of electrophoresis and electroosmosis. J. Pharm. Sci. 97, 881–893.

Hao, J., Li, S.K., in press. Mechanistic study of electroosmotic transport across hydrated nail plates: effects of pH and ionic strength. J. Pharm. Sci.

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

Kasting, G.B., 1992. Theoretical models for iontophoretic delivery. Adv. Drug Deliv. Rev. 9, 177–199.

Khengar, R.H., Jones, S.A., Turner, R.B., Forbes, B., Brown, M.B., 2007. Nail swelling as a pre-formulation screen for the selection and optimisation of ungual penetraton enhancers. Pharm. Res. 24, 2207–2212.

Kobayashi, Y., Miyamoto, M., Sugibayashi, K., Morimoto, Y., 1998. Enhancing effect of *N*-acetyl-L-cysteine or 2-mercaptoethanol on the in vitro permeation of 5-fluorouracil or tolnaftate through the human nail plate. Chem. Pharm. Bull. (Tokyo) 46, 1797–1802.

Kobayashi, Y., Miyamoto, M., Sugibayashi, K., Morimoto, Y., 1999. Drug permeation through the three layers of the human nail plate. J. Pharm. Pharmacol. 51, 271–278.

Kochhar, C., Imanidis, G., 2004. In vitro transdermal iontophoretic delivery of leuprolide under constant current application. J. Control. Release 98, 25–35.

Kuzuhara, A., Hori, T., 2003. Reduction mechanism of thioglycolic acid on keratin fibers using microspectrophotometry and FT-Raman spectroscopy. Polymer 44, 7963–7970.

Li, S.K., Ghanem, A.H., Peck, K.D., Higuchi, W.I., 1997. Iontophoretic transport across a synthetic membrane and human epidermal membrane: a study of the effects of permeant charge. J. Pharm. Sci. 86, 680–689.

Marro, D., Kalia, Y.N., Delgado-Charro, M.B., Guy, R.H., 2001. Optimizing iontophoretic drug delivery: identification and distribution of the charge-carrying species. Pharm. Res. 18, 1709–1713.

Mudry, B., Guy, R.H., Delgado-Charro, M.B., 2006. Transport numbers in transdermal iontophoresis. Biophys. J. 90, 2822–2830.

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

Murthy, S.N., Waddell, D.C., Shivakumar, H.N., Balaji, A., Bowers, C.P., 2007a. Iontophoretic permselective property of human nail. J. Derm. Sci. 46, 150–152.

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

Peck, K.D., Srinivasan, V., Li, S.K., Higuchi, W.I., Ghanem, A.H., 1996. Quantitative description of the effect of molecular size upon electroosmotic flux enhancement during iontophoresis for a synthetic membrane and human epidermal membrane. J. Pharm. Sci. 85, 781–788.

Phipps, J.B., Gyory, J.R., 1992. Transdermal ion migration. Adv. Drug Deliv. Rev. 9, 137–176.

Repka, M.A., O'Haver, J., See, C.H., Gutta, K., Munjal, M., 2002. Nail morphology studies as assessments for onychomycosis treatment modalities. Int. J. Pharm. 245, 25–36.

Rodgers, P., Bassler, M., 2001. Treating onychomycosis. Am. Fam. Physician 63, 663–672.

Scholz, D., Brooks, G.J., Parish, D.F., Burmeister, F., 1994. Fruit acid extracts, a fresh approach to skin renewal. Int. J. Cosmet. Sci. 16, 265–272.

Zhu, H., Peck, K.D., Li, S.K., Ghanem, A.H., Higuchi, W.I., 2001. Quantification of pore induction in human epidermal membrane during iontophoresis: the importance of background electrolyte selection. J. Pharm. Sci. 90, 932–942.