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Transungual Iontophoretic Transport of Polar Neutral and Positively Charged Model Permeants: Effects of Electrophoresis and Electroosmosis

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Abstract

Transungual iontophoretic transport of model neutral permeants mannitol (MA), urea (UR), and positively charged permeant tetraethylammonium ion (TEA) across fully hydrated human nail plates at pH 7.4 were investigated *in vitro*. Four protocols were involved in the transport experiments with each protocol divided into stages including passive and iontophoresis transport of 0.1 and 0.3 mA. Water and permeant uptake experiments of nail clippings were also conducted to characterize the hydration and binding effects of the permeants to the nails. Iontophoresis enhanced the transport of MA and UR from anode to cathode, but this effect (electroosmosis) was marginal. The transport of TEA was significantly enhanced by anodal iontophoresis and the experimental enhancement factors were consistent with the Nernst–Planck theory predictions. Hindered transport was also observed and believed to be critical in transungual delivery. The barrier of the nail plates was stable over the time course of the study, and no significant electric field-induced alteration of the barrier was observed. The present results with hydrated nail plates are consistent with electrophoresis-dominant (the direct field effect) transungual iontophoretic transport of small ionic permeants with small contribution from electroosmosis.

Keywords

transungual; iontophoresis; human nail plate; electroosmosis; electrophoresis

INTRODUCTION

The population of people having nail diseases is growing in recent years. Nail disease such as fungal infection can lead to severe health problems if left untreated in immuno-suppressed individuals.¹ Nail diseases result in changes in the physical and mechanical properties of the nail plates, such as pigmentation, thickening, hardening, or crumbling. Such conditions affect patients physically, socially, and psychologically and can seriously influence their quality of life. Many nail diseases are notoriously difficult to cure owing to the nail barrier and the deep-seated target site underneath the nail plate. Long treatments are usually needed and relapses are common.

Oral therapy has the inherent disadvantages of systemic adverse effects and drug interactions. Topical therapy is an attractive option due to its noninvasiveness, drug targeting to the site of action, avoidance of systemic side effects, increased patient compliance, and possibly reduced cost of treatment. However, the efficacy of topical therapy is limited by the low permeability

of the nail plates. Many pharmaceutical approaches have been utilized to optimize topical therapy. Scientists have tried to develop potent drugs of proper physicochemical properties in appropriate formulations to ensure effective drug concentrations at the site of action for transungual delivery.²⁻⁷ A number of chemical penetration enhancers have also been investigated to facilitate unguinal drug permeation.^{8,9} Physical enhancement methods such as thinning out the nail and laser therapy have generally been avoided due to their invasiveness.¹ Nail etching was explored to increase nail permeability and the findings are encouraging. Formulations and novel drug delivery systems are under development for the topical treatment of onychomycosis.¹⁰ Despite these efforts, thorough treatment of resistant nail diseases remains challenging.

Iontophoresis is a method to deliver a compound across a membrane with the assistance of an electric field.¹¹ Transdermal iontophoretic delivery of neutral and charged permeants has been extensively studied and reviewed.¹²⁻¹⁴ Three mechanisms are involved in iontophoretically enhanced transport: (a) direct interaction of the electric field with the charge of the ionic permeant (electrophoresis or electromigration), (b) convective solvent flow in the preexisting and newly created charged pathways (electroosmosis), and (c) electric field-induced pore induction (electroporation). For neutral permeants, transport enhancement is mainly due to electroosmosis. For ionic permeants, transport enhancement is a result of both electroosmosis and electrophoresis. Previous studies have shown that the Nernst–Planck theory and its modified form are good predicting tool of iontophoretic transport enhancement.^{15,16} Recent development in iontophoresis and the commercialization of iontophoresis products for topical therapy such as Numby Stuff[®] Phoresor[®] system (Iomed, Inc., UT) and LidoSite[®] Topical system (Vyteris, Inc., NJ) have encouraged researchers and scientists to extend the technology of transdermal iontophoresis to other tissues¹⁷⁻¹⁹ with diseases that encounter limited therapy efficacy using conventional treatment modalities.

The nail plate is a hard, yet slightly elastic, translucent, and convex structure made up of approximately 25 layers of dead, keratinized, and flattened cells. These cells are tightly bound to one another via numerous intercellular links, membrane-coating granules, and desmosomes. Although both the nail and the skin are derived from the epidermis, the nail plate is significantly different from skin. For example, the thickness of nail plate is approximately 0.25–0.6 mm, which is approximately 100-fold thicker than the stratum corneum. Unlike the stratum corneum, the nail plate behaves like a concentrated hydrogel rather than a lipophilic membrane.⁸ Hydration can affect the effective pore size of hydrogel and thus transungual transport. The nail is primarily enriched with highly disulfide-linked keratin.

The objectives of the present study were to understand the transport mechanisms of iontophoresis across fully hydrated nail plate and to explore the possibility of iontophoretically enhanced transungual transport to overcome the intrinsic barrier of the nail plate. Particularly, the following questions will be addressed: (1) Does iontophoresis enhance permeant transport across the nail plate? (2) What transport mechanisms contribute to the transport enhancement during iontophoresis? (3) Is the modified Nernst–Planck equation applicable to transungual iontophoresis? (4) Does convective solvent flow due to electroosmosis exist in transungual iontophoresis and what is the direction of electroosmosis? (5) What are the critical factors affecting transungual iontophoretic transport through the nail plate? (6) What is the effective pore size of the polar transport pathway in the nail plate? (7) Is the nail plate stable in long-term transport experiments and recovered to its original state after iontophoresis application? In the present study, positively charged permeant tetraethylammonium ion (TEA) and polar neutral permeants of different molecular sizes mannitol (MA) and urea (UR) were chosen as the model permeants. Transport experiments of MA and UR were conducted with constant direct current of 0.1 and 0.3 mA in anodal (anode in the donor) and cathodal (cathode in the donor) iontophoresis. Iontophoresis transport experiments of TEA and MA were conducted at

0.1 mA to examine the contributions of electrophoresis and electroosmosis and the applicability of the Nernst–Planck theory to predict flux enhancement. Hydration and permeant uptake studies were performed to assess water uptake and permeant partition into the nails, and the effect of permeant molecular size upon permeant uptake into the nail.

EXPERIMENTAL

Materials

Phosphate-buffered saline (PBS) tablets (0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride, pH 7.4, Sigma-Aldrich, St Louis, MO) were used to prepare the buffer solutions and 0.02% (w/v) sodium azide (99% purity, Acros, Morris Plains, NJ) was added as a bacteriostatic agent. ^3H -mannitol (10–30 Ci/mmol, 1 mCi/mL), ^{14}C -TEA bromide (1–5 mCi/mmol, 0.1 mCi/mL), and ^{14}C -salicylic acid (SA, 40–60 mCi/mmol, 0.1 mCi/mL) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). ^{14}C -urea (50–60 mCi/mmol, 0.1 mCi/mL) was purchased from Moravsek Biomaterials and Radiochemicals (Brea, CA). All these radiolabelled chemicals had purity of at least 97%. All materials were used as received. The physicochemical properties of UR, MA, TEA, and SA are summarized in Table 1.

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 solutions 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 solutions for 24 h to allow complete hydration before the transport experiments. The thickness of the hydrated nail plates ranged from 0.5 to 0.6 mm 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 solutions, wiped dry with Kimwipes tissue paper, and air dry before the hydration and uptake studies. The use of human tissues was approved by the Institutional Review Board at the University of Cincinnati, Cincinnati, OH.

Hydration and Uptake of Nail Clippings

In the hydration studies, the clean nail clippings were soaked in 10 mL of PBS solutions contained in a screw-capped vial at room temperature ($20\pm 2^\circ\text{C}$) for 24 h and then up to 7 days. After hydration, the nail clippings were removed, blotted dry with Kimwipes tissue paper, and quickly weighed (i.e., wet weight). The wet nail clippings were allowed to either air dry for 24 h or oven dry at 60°C to constant weights (i.e., dry weight). The percentage water content in nail clippings was expressed as the percentage of decrease in the weight of nail clippings after air dry or oven dry. The amounts of water in the nail clippings (g water/g dry nail) were calculated.

In the present uptake studies, the clean nail clippings were weighed and then equilibrated in 10 mL of PBS solutions spiked with 1 μL of ^3H -MA and/or 5 μL of ^{14}C -UR or ^{14}C -TEA at room temperature for 24 h. After equilibration, the nail clippings were removed and blotted dry with Kimwipes tissue paper to remove the solution on the nail surfaces. The nail clippings were then transferred into a separate vial containing 1 mL fresh PBS solutions to extract the permeant from the nail clippings for 24 h. After removal of nail clippings from the vial, the extraction solution was mixed with 10 mL of liquid scintillation cocktail (Ultima Gold™, PerkinElmer Life and Analytical Sciences, Shelton, CT) and assayed by a liquid scintillation counter (Beckman Counter LS6500, Fullerton, CA). Twenty microliters of equilibration solution were also taken and mixed with 1 mL of PBS solutions and 10 mL of liquid scintillation

cocktail for liquid scintillation counting. This method demonstrated extraction efficiency of greater than 90% for MA and TEA and 80% for UR and SA determined in a separate recovery experiment. The partition coefficient defined as the ratio of amount of permeant per gram dry nail clippings (air dried) to the amount of permeant per gram PBS solution was calculated.

Transport Studies

In our preliminary studies, it was found that the nail plates among individuals, even the fingernails from the same individual, showed different permeability results. Four protocols were randomly applied to the nail plates in the present transport studies. The treatment history of each nail plate is summarized in Table 2. Dual permeants were used in all experiments. The advantages of this experimental design were to minimize the influence of inter-sample variability on data interpretation and to maximize work productivity. Protocol 1 had five stages with MA and UR: passive transport experiment before iontophoresis (P1), anodal iontophoresis of 0.1 mA, second passive transport experiment (P2), cathodal iontophoresis of 0.1 mA, and third passive transport experiment (P3). Protocol 2 was the same as Protocol 1 except that anodal and cathodal iontophoresis of 0.3 mA were performed in the second and forth stages. Protocol 3 had two stages: passive transport (P1) and anodal iontophoresis of 0.1 mA with MA and TEA (9-h experiment). Protocol 4 had four stages including passive transport experiment of MA and UR (P1), passive transport experiment with MA and TEA (P2), anodal iontophoresis of 0.1 mA of MA and TEA (36-h experiment), and passive transport experiment with MA and UR or TEA (P3). A final passive transport experiment at the end of the study was used to check the stability of the nail plates.

After 24 h of hydration in PBS, the nail plate was mounted between side-by-side diffusion cells (Dana Enterprise, West Chester, OH) with the aid of a custom-made silicone nail adapter. The adapter was constructed with silicone elastomer (MED-6033, NuSil Silicone Technology, Carpinteria, CA) to fit the nail plate curvature, and was tested to show no noticeable permeant-to-adapter binding. The dorsal side of the nail plates faced the donor chamber and the ventral side faced the receptor chamber. The diffusion cells have effective diffusion area of approximately 0.64 cm^2 and cell volume of 2 mL. The donor solutions were PBS solutions containing trace amounts of radiolabelled permeants (2 μL of ^3H -MA and 10 μL of ^{14}C -UR or ^{14}C -TEA) added immediately before transport studies, and the receptor solutions were fresh PBS. The transport studies were performed at room temperature ($20 \pm 28^\circ\text{C}$) for 9–36 h in iontophoretic transport and 46 h in passive diffusion experiments. Ten microliters of donor solution and 1 mL of receptor solution were withdrawn at predetermined time intervals 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 and assayed by the liquid scintillation counter.

Generally, iontophoretic transport experiments were immediately conducted at the end of passive permeation experiments without replacement of the donor solutions unless otherwise stated. Passive permeation experiments after iontophoresis were performed at least 12 h after the end of the iontophoresis experiments with fresh donor solutions. Between passive permeation experiments and for passive experiments after iontophoresis, the diffusion chambers on both sides of the nail sample were rinsed with PBS at least three times and at least over 12 h by replacing the PBS. In iontophoretic transport studies, either 0.1 or 0.3 mA constant current DC was applied with a constant current iontophoretic device (Phoresor II Auto, Model PM 850, Iomed, Inc., Salt Lake City, UT) using Ag and Ag/Cl as the driving electrodes. The voltage drop across the nail plates was monitored using a multimeter (Fluke 73III, Everett, WA) before and during iontophoresis. The electrical resistance of the nail plates before the iontophoresis experiments was measured by applying 0.1 mA current across the nail for 30 s and using Ohm's law.

The cumulative amount of permeant transported through the nail plate was plotted against time, and the steady-state flux of permeant was calculated from the slope of the linear portion of the plot. Transport lag time was estimated by extrapolating the linear region (usually, the last four data points) in the receptor permeant amount versus time plot to the abscissa (i.e., the x-intercept). Passive lag time was determined only in the first transport experiment of each nail and in experiments of more than 2-day washing between the transport experiments. In the passive transport experiments, steady-state flux was generally observed within 20 h from the start of the experiments. In the iontophoretic transport experiments, “true” steady-state transport was never observed for the charged permeant TEA, and the last four data points in the cumulative amount versus time plot were used to estimate the flux in data analysis. These results would be discussed in the Results and Discussion section. The steady-state permeability coefficient or apparent instantaneous permeability coefficient, P (cm/s), is defined as the flux divided by the concentration of permeant in the donor chamber. The instantaneous flux of permeant between two time points (J) was determined as described previously:²⁰

$$J = \frac{\Delta Q}{A \Delta t} \quad (1)$$

where A is the diffusional surface area, and $\Delta Q/\Delta t$ is the slope of the cumulative amount of the permeant transported across the nail into the receptor chamber versus time plot. Enhancement factor (E) is defined as the ratio of the permeability coefficient of iontophoretic transport to that of passive transport.

THEORY AND EQUATIONS

The steady-state iontophoretic flux ($J_{\Delta\psi}$) of a permeant through a homogeneous porous membrane can be described by the modified Nernst–Planck model as described previously.^{15,16}

$$J_{\Delta\psi} = -\varepsilon \left\{ HD \left[\frac{dC}{dx} + \frac{CzF}{R_{\text{gas}}T} \frac{d\psi}{dx} \right] \pm WvC \right\} \quad (2)$$

where ψ is the electric potential in the membrane, F is the Faraday constant, R_{gas} is the gas constant, T is the temperature, v is the average velocity of the convective solvent flow, ε is the combined porosity and tortuosity factor for the membrane, and C , x , z , and D are the concentration, the position in the membrane, the charge number, and the diffusion coefficient of the permeant, respectively. H and W are hindrance factors for Brownian diffusion and for pressure induced parabolic convective solvent flow, respectively. Assuming a single pore size and cylindrical pore geometry in the porous membrane, the hindrance factor for Brownian diffusion can be expressed by:

$$H(\lambda) = \frac{6\pi(1-\lambda)^2}{K_t} \quad (3)$$

where λ is the ratio of permeant radius (r_s) to pore radius (R_p).

$$K_t = \frac{9}{4}\pi^2 \sqrt{2}(1-\lambda)^{-5/2} \left[1 + \sum_{n=1}^2 a_n(1-\lambda)^n \right] + \sum_{n=0}^4 a_{n+3}\lambda^n \quad (4)$$

where $a_1 = -1.217$, $a_2 = 1.534$, $a_3 = -22.51$, $a_4 = -5.612$, $a_5 = -0.3363$, $a_6 = -1.216$, and $a_7 = 1.647$.

The effective pore size of the membrane can be calculated from the ratio of the permeability coefficients of the permeants obtained from the passive permeation experiments using Eqs. 3-5.

$$\frac{P_i}{P_j} = \frac{H_i D_i}{H_j D_j} \quad (5)$$

For the iontophoretic transport of neutral permeants, the Peclet number (Pe)²¹ is determined from the enhancement factor (E_v) by:

$$E_v = \frac{Pe}{1 - \exp(-Pe)} \quad (6)$$

For electrophoresis dominant iontophoretic transport, the enhancement factor ($E_{\Delta\psi}$) is proportional to the average electrical potential across the membrane based on the Nernst–Planck model:

$$E_{\Delta\psi} = \frac{zF}{R_{\text{gas}} T} \Delta\psi \quad (7)$$

and the transport lag time of iontophoresis ($t_{\Delta\psi}$) can be expressed by:²²

$$t_{\Delta\psi} = \frac{6t_{\text{passive}}}{E_{\Delta\psi}^2} \left(E_{\Delta\psi} \coth\left(\frac{E_{\Delta\psi}}{2}\right) - 2 \right) \quad (8)$$

where t_{passive} is the transport lag time for passive permeation.

RESULTS AND DISCUSSION

Hydration and Uptake Studies

The rate of water absorption in the nail clippings demonstrated a fast and a slow phase (data not shown). Upon soaking the nails in PBS, the nails approached 90% of complete hydration within half an hour in the fast phase. This was followed by a slow phase in which equilibrium was observed in 1 day. After that, no further change in nail hydration was observed up to 7-day monitoring. These results imply that at least 24 h of hydration is required to fully hydrate the nail plates prior to subsequent transport experiments. The kinetics of water evaporation from the nail clippings showed similar biphasic profiles as those in water uptake. Figure 1 presents the water and permeant uptake results. When the fully hydrated nail clippings were air dried at room temperature, water uptake of the nail clippings was $24 \pm 3\%$. More water was removed from the nail upon oven drying of the wet nail clippings ($36 \pm 5\%$). Drying the nail clippings in a desiccator at room temperature gave essentially the same result as oven drying (data not shown). These results suggest that water absorbed into the nails existed in two forms, that is, unbound and bound water. The air drying method only removed unbound water while the oven drying method could remove loosely bound water along with unbound water. Previous near infrared (NIR)-Fourier transform (FT)-Raman spectroscopy study of human nails indicated that water was bound to protein both in dry and in wet nails.²³ The difference in water content of nail clippings determined from the different drying methods could be explained by the interaction of water and the protein of the nail. The water contents in the nail clippings determined in the present study were also in agreement with those reported previously.²³ Gniadecka et al.²⁴ reported that over 80% of water in the nails under normal conditions without pretreatment was in bound form and less than 20% of water was free water. The water content of the fingernail *in vivo* determined by portable NIR spectroscopy was in the range of 7–25% and varied according to the conditions of the nails.²⁵

To study the interactions between the nail and the permeants, the partition coefficients of MA, TEA, SA, and UR were compared. In Figure 1, UR showed a higher partition coefficient than those of MA and TEA. This can be attributed to size exclusion of the larger molecular size MA and TEA compared to UR. The higher UR uptake than that of MA implies that the nail matrix

has pore size within the same order of magnitude as the molecular sizes of the permeants. Because TEA, MA, and SA have similar molecular sizes, comparison of their partition coefficients provides insights into possible charge–charge interactions and binding of the permeants to the nail. For MA and TEA, the partition coefficients are essentially the same, indicating the absence of significant charge–charge interactions between the nail and TEA. SA showed a significantly higher partition coefficient than those of MA and TEA, implying high SA affinity to the nail chippings. However, the high SA uptake is not likely a result of charge–charge interactions between SA and the nail matrix. This is because the keratin in the nail plate has an isoelectric point (pI) of 4.9–5.4 and is net negatively charged at physiological pH of 7.4.⁸ SA (pK_a 2.9) is also negatively charged at pH 7.4 in PBS. Accordingly, the uptake of SA into the nail clippings should be lower not higher than MA and TEA due to electrostatic repulsion between the negatively charged membrane and the like-charge molecule. Binding due to other SA interactions with the nail may account for the large SA uptake. Hui et al.²⁶ found that SA in saline formulation interacted and bound with the nail surface and was unavailable for absorption further into the nail. In their study, a tested SA carrier formulation freed SA from the interactions and made it available for nail absorption. SA was also reported to bind to proteins,²⁷ consistent with the results in the present uptake study. Because of SA binding, SA was not chosen as a permeant in subsequent transport experiments in the present study. The effects of SA binding to the nail plate upon its permeation will be investigated in a future study.

Transport of Neutral Permeants and Electroosmosis

Electroosmosis can enhance the transport of neutral permeants during iontophoresis. The direction of electroosmosis is related to both the polarity of the electric fields and the net charge of the transport pathway in a membrane. Table 3 shows the permeability coefficients of the nail plates for MA and UR in the passive and iontophoretic transport experiments under Protocols 1 and 2. Passive transport lag times for MA and UR were also estimated and their average values were approximately 12 and 4 h, respectively. In order to assess the contribution of electroosmosis, the Peclet numbers were determined using the enhancement factors of the permeants and Eq. 6, provided that there was no electric field-induced alteration of the nail barrier. The enhancement factors and Peclet numbers are summarized in Table 4. From the results in Tables 3 and 4, anodal iontophoresis enhanced the transport of MA and UR over passive diffusion. The Peclet numbers of both permeants were relatively small under the conditions in this study. Because Peclet number describes electroosmosis contribution to iontophoretic transport,²¹ these results suggest marginal contribution of electroosmosis in anodal iontophoretic transport of MA and UR when low electric current (up to 0.3 mA) was applied across the nail plate. The positive Peclet numbers during anodal iontophoresis suggest that the convective solvent flow was from the anode to cathode. When the electric current was increased from 0.1 to 0.3 mA, the contribution of electroosmosis increased proportionally (Pe increased from 0.3 to 0.9 for UR and 0.7 to 2.6 for MA, Tab. 4). Different from anodal iontophoresis, cathodal iontophoresis showed no significant transport enhancement for MA and UR at both current levels. The Peclet numbers of MA and UR were not significantly different from zero and did not increase when the electric current was increased during cathodal iontophoresis. The Peclet numbers of MA and UR also show that the contribution of electroosmosis increased with the molecular size of the permeant. The Peclet numbers of MA were larger than those of UR in anodal iontophoresis (2.4 and 2.9 times larger at 0.1 and 0.3 mA, respectively). This trend of increasing Peclet number with increasing molecular size is consistent with the theory of electroosmotic transport and is in agreement with the findings that flux enhancement of neutral permeants due to electroosmosis were molecular size dependent.²¹

As noted in the previous section, the keratin of the nail plate is believed to be negatively charged at physiological pH. It is therefore expected that the convective solvent flow due to electroosmosis would be from the anode to cathode to enhance the transport of neutral permeants in anodal transungual iontophoresis. In the present study with fully hydrated nail plates, the convective solvent flow of electroosmosis was found to be from the anode to the cathode, consistent with the negatively charged keratin structure of the nails. It should be pointed out that electroosmosis is a function of the pore charge, pore size, and voltage applied. As a result, electroosmosis can be affected by nail hydration and the present findings can be limited to fully hydrated nails. Future studies will investigate the effects of hydration upon electroosmotic enhanced transport during transungual iontophoresis.

Hindered Transport in Transungual Permeation

A previous study has demonstrated a relationship between the permeability of nail and permeant molecular weight,²⁸ suggesting size selectivity in transungual transport. Similar molecular weight-permeability dependence was observed in the present nail permeability and nail uptake studies. As can be seen in Tables 1 and 3, the ratios of the passive permeability coefficients of UR to those of MA are significantly larger than the free diffusion coefficient ratios of the permeants. This hindered transport effect can be responsible for the low diffusion coefficients of the permeants in the fully hydrated nail plates and their long transport lag time observed in the present study. This effect would also significantly reduce the transference efficiency of the permeants during iontophoresis due to the larger hindrance effects on the electro-mobilities of the permeants than of the endogenous ions in the body (e.g., NaCl); note that the equivalent solute hydration radius of UR is similar to that of Na ion and MA is similar to that of small compounds such as TEA. From Eqs. 3 to 5 and the UR and MA passive permeability data, the average effective pore radius of the nails was estimated to be 0.7 nm.

Transport of Positively Charged Permeant and Electrophoresis

Anodal iontophoretic transport of TEA and MA was carried out at 0.1 mA with the nail plates in Protocols 3 and 4. TEA and MA have similar molecular sizes, and thus the concurrent experiments of TEA and MA would allow the delineation of the contributions of electroosmosis and electrophoresis to transungual iontophoretic delivery. Table 5 shows the permeability coefficients of each permeant in each stage in the protocols. The enhancement factors of TEA and MA and the Peclet numbers of MA are summarized in Table 6. In the 9-h iontophoresis experiments, the permeability coefficient of TEA was significantly enhanced during anodal iontophoresis. As expected, the enhancement factors of MA were less than 2 with Peclet numbers of approximately 1 in this experiment, consistent with the results in Protocol 1. Figure 2 is a plot of the instantaneous permeability coefficients of TEA and MA against time during iontophoresis in Protocol 3. Whereas the instantaneous permeability coefficients of MA reached a plateau within 9 h, the permeability coefficients of TEA continued to increase and had not reached steady-state at the end of the 9-h iontophoresis study. These results suggest that longer duration iontophoresis experiments were required to achieve steady-state for TEA. To this end, 36-h anodal iontophoretic transport experiments were performed for TEA and MA in Protocol 4. Figure 3 shows that the instantaneous permeability coefficients of TEA drastically increased in the first 9 h of the 36-h experiment. At 15 h, the permeability coefficients of TEA began to plateau. For MA, the instantaneous permeability coefficients were relatively constant from 9 to 36 h and were not statistically different from the results in the 9-h study ($p=0.62$).

In the 36-h study, the permeability coefficients of the nail plate for TEA under iontophoretic transport were approximately 29-fold higher than those under passive transport. This suggests that iontophoretically enhanced transungual delivery of charged permeants is practical although the transport lag time can be long. The long transport lag time for TEA can be partly

explained by the low permeant diffusivity in the nail and the thickness of the nail plate, but a longer than 4-h iontophoresis transport lag time for TEA is not expected according to the TEA passive lag time (ranging from approximately 10 to 20 h), the iontophoresis transport enhancement factor, and the theory (Eq. 8) assuming a homogenous transport pathway with no electric field-induced membrane alteration during iontophoresis. The shorter apparent lag time for MA than TEA during iontophoresis may suggest different transport pathways for electrophoresis and electroosmosis in transungual iontophoretic transport or may be due to the experimental design with the small enhancement effect of electroosmosis in the present study. Future studies will be conducted to investigate this phenomenon.

To determine the relative contributions of electrophoresis and electroosmosis in transungual iontophoretic transport, the enhancement factors of TEA and MA were compared. The ratios of anodal iontophoretic permeability coefficients of MA to those of TEA (P_{MA}/P_{TEA}) and the ratios of the Peclet numbers to electrophoresis enhancement factors ($Pe/E_{\Delta\psi}$) are presented in Table 6. The $Pe/E_{\Delta\psi}$ ratios reflect the relative velocity of the convective solvent flow of electroosmosis to that due to electrophoresis. The results indicate that the contribution of electroosmosis was relatively small and generally less than 10% of electrophoresis. Electrophoresis is the dominant transport mechanism of positively charged permeants across the fully hydrated nail during iontophoresis.

As transport enhancement of TEA under anodal iontophoresis was governed by electrophoresis, the applicability of the Nernst–Planck theory to predict iontophoretic transport across the nail plates was also examined. The applied voltage across the nail plates in the 36-h iontophoresis experiments was from 0.4 to 1.0 V among the samples. Using the applied voltage and the Nernst–Planck theory assuming negligible electroosmosis contribution (Eq. 7), the average enhancement factor of electrotransport of the charged permeant across the nail plates was calculated to be around 26. This value is in close agreement with the experimental values obtained in the present study (Tab. 6). Therefore, transungual iontophoretic transport was predicted by the Nernst–Planck theory under the experimental conditions used in the present study. The electrical resistance of the nail plates during iontophoresis was also measured using the applied electric current, the voltage data, and Ohm's law. The electrical resistance of the nail plates during 0.1 mA iontophoresis was around 4 to 10 k Ω , and was not significantly different from the values determined before iontophoresis.

Reversibility and Stability of Nail Plates

In the present study, the nail plates were mounted in the diffusion cells with PBS for up to 3 months to allow the use of the same nail samples in Protocols 1–4. These nail samples were treated with electric current repeatedly. Nail stability and reversibility were therefore important in this experimental design and were monitored by comparing the passive permeability coefficients of MA and UR throughout Protocols 1–4. Once the nail samples were mounted in the diffusion cells, they were in the same cells without disassembling the setup until the end of the study except for Nail A in Protocol 1. Nail A in P1 of Protocol 1 was in a different diffusion cell assembly (the diffusion cells were disassembled and reassembled at the end of the protocol) and should not be included in this analysis. This is because the alignment of the nail adapter openings of the diffusion cells might be different and not cover the same nail surface before and after cell reassembling. In addition, the effective diffusion area of the nail plate in the diffusion cells could be slightly different after the reassembling of the nail in the diffusion cells. The first passive transport experiments for the nail samples were P1 of Protocol 2 for Nail A, P1 of Protocol 3 for Nails B and C, and P1 of Protocol 4 for Nails E and F, which generally showed lower permeability coefficients and higher electrical resistance than those obtained in the later transport studies, possibly due to incomplete nail hydration. A final passive diffusion experiment of MA and UR was conducted for the nail plates at the end of Protocol

4. Table 7 presents the data showing that the permeability coefficients of both MA and UR and the nail electrical resistance were essentially the same after the first passive transport experiments. These results suggest that, once the nails were fully hydrated, the passive permeability coefficients of the nails were stable for up to 2 months and were not influenced by the applied electric current or possible degradation over the time course in the present study. Any effects due to the electric current upon the nail samples were reversible, and the nail plates returned to their normal state after iontophoresis application.

Transungual Drug Delivery

The low permeability of hydrated nail plates and long transport lag time are two of the main reasons of the poor treatment efficacy of topical therapy of nail diseases, particularly for nail diseases related to the nail bed underneath the nail plate. To achieve therapeutic concentration of the drug at the target site under the nail plate is critical for effective treatment. It is possible to increase the flux of a drug compound across the nail plate by applying an electric current across the nail. The present study shows that iontophoresis enhances the transport of a charged compound across the nail plate according to the Nernst–Planck theory with relatively little contribution from electroosmosis. This information will be helpful in predicting transungual iontophoretic transport and in choosing the right drug candidates for transungual iontophoretic delivery in the future. In addition to enhanced drug delivery across the nail plate, enhanced drug delivery into the nail plate is also desired if the target site is within the nail matrix. The drug-loaded nail plate can also serve as a drug depot releasing the drug to the nail bed and surrounding tissues for prolonged nail treatment after iontophoresis. However, this aspect is beyond the scope of the present investigation.

CONCLUSIONS

Enhanced transport of neutral permeants such as mannitol and urea across fully hydrated human nail plates was observed only at the higher electric current of 0.3 mA during anodal iontophoresis. Unlike mannitol and urea, transport of positively charged TEA was drastically enhanced by 0.1 mA iontophoresis. Electrophoresis was the dominant mechanism governing the iontophoretic transport of charged permeants and the contribution of electroosmosis was minimal. Once fully hydrated, the barrier properties of the nails were stable. No irreversible electroporation was observed in the iontophoresis experiments. The effective pore size of the fully hydrated nail plates was about 0.7 nm. Thus, hindered transport played a critical role in the low permeability of the nail plate for the permeants. Although overcoming the inherent barrier of the nail plate is challenging, transungual iontophoresis provides a promising alternative to conventional physical and chemical enhancement methods.

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Figure 1. Permeant and water partition coefficient (amount/g dry nail divided by amount/g PBS) of nail clippings. Except for the oven-dry water data, dry nail weight was determined by the air-dry method. Data represent the average and standard deviation of 3–9 measurements.

Figure 2. Time-dependent apparent instantaneous permeability coefficients of TEA (open symbols) and mannitol (closed symbols) through Nails A (diamonds), B (tri-angles), and C (squares) during 9-h anodal iontophoresis.

Figure 3. Time-dependent apparent instantaneous permeability coefficients of TEA (open symbols and dashes) and mannitol (closed symbols and crosses) across Nails A (diamonds), B (triangles), C (squares), E (circles), and F (dashes and crosses) during 36-h anodal iontophoresis.

Table 1
Physicochemical Properties of the Permeants at 37°C

Permeant	Diffusion Coefficient ($\times 10^5$, cm ² /s)	Effective Hydration Radius (nm)
UR	1.8	0.27 ^a
MA	0.90	0.41 ^a
TEA ⁺	1.1	0.36 ^a
SA ⁻	1.2	0.33 ^b

^aData from Li et al.²⁰

^bData from Li et al.¹⁵

Table 2

Treatment History of the Nail Plates^a

Nail A (Day)	Nail B (Day)	Nail C (Day)	Nail D (Day)	Nail E (Day)	Nail F (Day)
P (1-3)	P (1-3)	P (1-3)	P (1-3)	P (1-3)	P (1-3)
Asl (3)	Asl (3)	Asl (3)	Ash (3)	P (4-6)	P (4-6)
P (4-6)	P (11-13)	P (11-13)	P (4-6)	All (7-8)	All (7-8)
Csl (6)	Asl (13)	Asl (13)	Csh (6)	P (12-14)	P (12-14)
P (7-11)	P (14-16)	P (14-16)	P (7-11)	P (22-24)	P (22-24)
P (22-24)	Csl (16)	Csl (16)			
Asl (24)	P (17-19)	P (17-19)			
P (28-30)	Ash (19)	Ash (19)			
Ash (30)	P (20-22)	P (20-22)			
P (31-33)	Csh (22)	Csh (22)			
Csh (33)	P (23-25)	P (23-25)			
P (34-36)	All (33-34)	All (33-34)			
All (44-45)	P (37-39)	P (37-39)			
P (48-50)	P (40-42)	P (40-42)			
P (51-53)	P (59-61)	P (59-61)			
P (70-72)					

^aPassive=P; 0.3 mA, 9-h anodal iontophoresis=Asl; 0.3 mA, 9-h cathodal iontophoresis=Csh; 0.1 mA, 9-h anodal iontophoresis=Asl; 0.1 mA, 9-h cathodal iontophoresis=Csl; and 0.1 mA, 36-h anodal iontophoresis=All. The time when the experiments were performed (day from the start of the study) is shown in parentheses. In the symbols Ash, Csh, Asl, Csl, and All, the first, second, and third letters designate the direction (anodal and cathodal), the duration (short 9-h and long 36-h), and the electric current (high 0.3 mA and low 0.1 mA) of iontophoresis, respectively.

Table 3

Permeability Coefficients ($\times 10^8$, cm/s) of Mannitol (MA) and Urea (UR) across the Nail Plates in the Transport Experiments of Protocols 1 and 2

Nail	P1		Anodal		P2		Cathodal		P3	
	MA	UR	MA	UR	MA	UR	MA	UR	MA	UR
Protocol 1, 0.1 mA iontophoresis										
A	1.7	30	2.3	36	1.6	28	2.2	25	1.5	28
B	0.85	30	1.1	34	0.88	26	1.3	29	0.84	28
C	2.8	53	4.5	60	2.4	46	2.2	42	2.6	47
Protocol 2, 0.3 mA iontophoresis										
A	4.4	51	7.8	72	3.8	46	4.9	43	3.9	50
B	0.84	28	2.9	45	0.99	26	2.0	22	0.86	28
C	2.6	47	9.0	71	2.6	46	2.0	32	2.7	50
D	3.3	47	9.6	73	4.3	53	5.6	37	4.5	58

Table 4
Enhancement Factor (E_v) and Peclet Number (Pe) Determined in the Iontophoretic Transport Experiments in Protocols 1 and 2

Nail	Anodal				Cathodal			
	MA		UR		MA		UR	
	E_v	Pe	E_v	Pe	E_v	Pe	E_v	Pe
Protocol 1, 0.1 mA iontophoresis								
A	1.4	0.6	1.2	0.4	1.4	0.8	0.9	-0.3
B	1.3	0.5	1.1	0.3	1.5	0.9	1.1	0.2
C	1.6	1.1	1.1	0.2	0.9	-0.1	0.9	-0.2
Mean $\pm$ SD	1.4 $\pm$ 0.2	0.7 $\pm$ 0.3	1.2 $\pm$ 0.1	0.3 $\pm$ 0.1	1.3 $\pm$ 0.3	0.5 $\pm$ 0.5	1.0 $\pm$ 0.1	-0.1 $\pm$ 0.2
Protocol 2, 0.3 mA iontophoresis								
A	1.8	1.3	1.4	0.7	1.3	0.5	0.9	-0.1
B	3.4	3.3	1.6	1.0	2.0	1.6	0.8	-0.4
C	3.4	3.3	1.5	0.9	0.8	-0.5	0.7	-0.7
D	2.6	2.4	1.5	0.9	1.3	0.6	0.7	-0.7
Mean $\pm$ SD	2.8 $\pm$ 0.8	2.6 $\pm$ 0.9	1.5 $\pm$ 0.1	0.9 $\pm$ 0.1	1.4 $\pm$ 0.5	0.5 $\pm$ 0.9	0.8 $\pm$ 0.1	-0.5 $\pm$ 0.3

Table 5

Apparent Permeability Coefficients ($\times 10^8$, cm/s) of Tetraethylammonium (TEA) and Mannitol (MA) Obtained from the Passive and 0.1 mA Anodal Iontophoresis Transport Experiments in Protocols 3 and 4

Nail	Protocol 3				Protocol 4			
	P1		Anodal, 9-h		P2 and P3 ^a		Anodal, 36-h	
	MA	TEA	MA	TEA	MA	TEA	MA	TEA
A	5.1	2.9	7.5	34	4.8	4.4	6.0	103
B	0.53	0.10	0.82	10	0.89	1.9	1.6	60
C	1.5	1.6	2.6	43	2.7	5.0	5.4	133
E	—	—	—	—	1.7	1.8	3.0	69
F	—	—	—	—	8.1	4.3	8.5	116

^aThe permeability coefficient in P2 or the average permeability coefficient in P2 and P3.

Enhancement Factors (E_v and $E_{\Delta\psi}$) and Peclet Number (Pe) Determined in the 0.1 mA Anodal Iontophoresis Experiments in Protocols 3 and 4

Nail	MA		TEA		P_{MA}/P_{TEA}	$Pe/E_{\Delta\psi}$
	E_v	Pe	$E_{\Delta\psi}$			
Protocol 3, 9-h iontophoresis						
A	1.5	0.81	a		a	a
B	1.6	0.96	a		a	a
C	1.9	1.3	a		a	a
Protocol 4, 36-h iontophoresis						
A	1.3	0.48	23		0.06	0.02
B	1.8	1.3	32		0.03	0.04
C	1.9	1.5	27		0.04	0.06
E	1.8	1.4	38		0.04	0.04
F	1.0	0.1	27		0.07	0.004
Mean±SD	1.6±0.4	1.0±0.6	29±6		0.05±0.02	0.03±0.02

^a $E_{\Delta\psi}$, P_{MA}/P_{TEA} , and $Pe/E_{\Delta\psi}$ were not calculated in the 9-h iontophoresis study because TEA transport was not in steady-state.

Table 7

Stability of the Nail Plates as Indicated by the Permeability Coefficients ($\times 10^8$, cm/s) of Mannitol (MA) and Urea (UR) in Passive Transport Experiments and Electrical Resistance (R, k Ω) Measured before or after the Transport Experiments

Nail	Day ^a	MA	UR	R ^b
A	1–3	1.7	30	6.4 ^e
	4–6 ^c	1.6	28	7.9 ^e
	22–24 ^c	5.1	— ^d	5.8
	28–30	4.4	51	4.7
	34–36	3.9	50	5.1
	48–50	4.6	56	4.0
	70–72	6.2	56	4.3
B	1–3	0.53	— ^d	13 ^e
	11–13	0.85	30	9.4
	17–19	0.84	28	9.6
	23–25	0.86	28	9.5
	37–39	0.95	34	8.2
	59–61	0.97	34	8.2
	1–3	1.5	— ^d	6.5 ^e
C	11–13	2.8	53	4.5
	17–19	2.6	47	5.0
	23–25	2.7	50	4.7
	37–39	3.0	54	4.1
	59–61	3.1	54	4.3
E	1–3	0.83	24	— ^f
	4–6	1.6	— ^d	10 ^e
	12–14	1.7	— ^d	— ^f
	22–24	2.0	36	— ^f
F	1–3	10	46	— ^f
	4–6	9.8	— ^d	6.5 ^e
	12–14	6.4	— ^d	— ^f
	22–24	6.6	57	— ^f

^aThe time when the experiments were performed (from the start of the studies).

^bUnless otherwise specified, electrical resistance was measured before the passive transport experiments.

^cDiffusion cells were disassembled and reassembled at the end of Protocol 1.

^dExperiments were conducted with MA and TEA, UR data are not available.

^eElectrical resistance measured after the passive transport experiment.

^fElectrical resistance data are not available.