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Influence of pH on Transungual Passive and Iontophoretic Transport

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Abstract

The present study investigated the effects of pH on nail permeability and the transport of ions such as sodium (Na) and chloride (Cl) ions endogenous to nail and hydronium and hydroxide ions present at low and high pH, which might compete with drug transport across hydrated nail plate during iontophoresis. Nail hydration and passive transport of water across the nail at pH 1–13 were assessed. Subsequently, passive and iontophoretic transport experiments were conducted using ^{22}Na and ^{36}Cl ions under various pH conditions. Nail hydration was independent of pH under moderate pH conditions and increased significantly under extreme pH conditions ($\text{pH} > 11$). Likewise, nail permeability for water was pH independent at pH 1–10 and an order of magnitude higher at pH 13. The results of passive and iontophoretic transport of Na and Cl ions are consistent with the permselective property of nail. Interestingly, extremely acidic conditions (e.g., pH 1) altered nail permselectivity with the effect lasting several days at the higher pH conditions. Hydronium and hydroxide ion competition in iontophoretic transport was generally negligible at pH 3–11 was significant at the extreme pH conditions studied.

Keywords

pH; human nail plate; transungual transport; iontophoresis

INTRODUCTION

Drug transport into the nail is expected to be influenced by formulation factors, such as pH. pH can influence nail permeability by affecting the degree of drug ionization within the solution or by altering the keratin matrix within the nail itself. Walters et al.¹ found the penetration of miconazole to be independent of pH between pH 3.1 and 8.2. Conversely, Malhotra and Zatz² and Kobayashi et al.³ found pH to be an influential factor in determining nail permeability. Specifically, Malhotra and Zatz reported water permeation results that were significantly higher from a gel formulation at pH 12 as compared with gels at pH 2 or 7. They reasoned that the results were due to physical changes within the nail ultrastructure.² Kobayashi et al.³ found that changing their formulation a single pH unit reduced transungual permeation of 5-fluorouracil by half; these results were attributed to acid dissociation of the drug. The effects of pH upon nail permeability to ions have not been systematically studied, and little work has been done to understand the effects of pH on transungual iontophoresis.

Iontophoresis has gained substantial interest in recent years for drug delivery into and across nail for the treatment of nail diseases such as onychomycosis. Iontophoresis has certain

advantages over conventional approaches to the treatment of onychomycosis. Conventional approaches to the treatment of onychomycosis, which include reduction of the nail plate and oral administration of antifungal agents, are ineffective.⁴ Twenty-five percent of patients experience a relapse within 1 year of treatment.^{5,6} Moreover, reduction treatments that include mechanical reduction through surgery or abrasion and chemical reduction using concentrated urea solutions may temporarily eliminate dexterity and therefore a patient's ability to work while systemic medications may damage the liver and can be fatal.⁷ Topical monotherapies, which are desirable because they avoid systemic toxicity and first pass metabolism and have higher patient compliance, are similarly ineffective. They are associated with a low mycological cure rate—roughly 50%.⁴ The ineffectiveness of topical treatments results from the barrier resistivity of the nail plate to large and lipophilic molecules.^{1,8–13}

Iontophoresis has been demonstrated to be an effective physical enhancement method for topical drug delivery into and across nail.^{14–19} In previous studies, the fluxes of terbinafine hydrochloride¹⁴ and ciclopirox olamine¹⁸ across nail with iontophoresis were 16- and 10-fold higher than their passive transport counterparts, respectively. Prednisolone has also been iontophoretically delivered across nail.¹⁷ Characterization of the barrier properties of the nail under an electric field will help researchers identify the optimal conditions for transungual iontophoresis. The impact of hydration on the electrical resistance of the nail and on electrotransport *in vitro* and *in vivo*,²⁰ the effects of ionic strength and pH on transungual transport of neutral permeants,^{21,22} and the influence of ionic strength on the transport of a positively charged permeant across fully hydrated nail *in vitro* have been investigated.²³

To characterize the barrier properties of the nail, the present study sought to examine the impact of pH on the permeability of the nail, determine the transport of sodium (Na) and chloride (Cl) ions under different pH conditions, and identify potential interference of hydronium and hydroxide ions on iontophoretic transport of ions. Specifically, the objectives of this study were to (a) determine the effects of pH upon the nail barrier, (b) examine the effect of pH on nail permselectivity to small ions such as Na and Cl ions, (c) determine the transference number and effective electromobility of Na and Cl ions, which are present as endogenous ions in the nail matrix and subungual tissues, and (d) identify if there are any significant competitions from hydronium and hydroxide ions under low and high pH conditions. Hydration and passive water transport studies were first performed to examine the effects of pH on the barrier integrity of the nail plate. Then, transport studies were performed with Na and Cl ions to determine the impact of pH on passive and iontophoretic transport of charged permeants.

MATERIALS AND METHODS

Materials

Phosphate-buffered saline (PBS) of pH 7.4 was prepared by dissolving PBS tablets (0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride) purchased from Sigma-Aldrich (St. Louis, MO) in distilled, deionized water. Hydrochloric acid (12.1 M, certified ACS plus) and sodium hydroxide (0.1 M) were obtained from Fisher Scientific (Fair Lawn, NJ). pH 2–12 solutions were prepared by pH modulation of PBS using hydrochloric acid (12.1 M) or sodium hydroxide (0.1 M). pH 1 solution of 0.1 M hydrochloric acid and 0.05 M NaCl was prepared by hydrochloric acid and sodium chloride (99% purity; Acros, Morris Plains, NJ). pH 13 solution of 0.1 M sodium hydroxide and 0.05 M NaCl was prepared by sodium hydroxide and sodium chloride. Sodium azide (99% purity; Acros) was added at 0.02% (w/v) to all solutions, except in the solutions of pH 1 and 13, as a bacteriostatic agent. Sodium azide was not added to the pH 1 and 13 solutions because their innate pH should be enough to inhibit microbial growth. The conductivities of the solutions were checked with a pH/CON 510 benchtop meter (Oakton Instruments, Vernon Hills, IL) and found to be consistent within the conductivity range expected. ³H-water (1.0 mCi/mL) and ²²NaCl (100–2000 Ci/g) were

purchased from Perkin-Elmer Life and Analytical Sciences (Boston, MA). Na^{36}Cl was acquired from Amersham Biosciences (Piscataway, NJ). The radioactive materials had a purity of at least 97%. All materials were used as received.

Nail Sample Preparation

Frozen, human cadaver fingernails (male, aged 49–91) were acquired from ScienceCare Anatomical (Phoenix, AZ). Nail plates were inspected visually for abnormalities, such as cracking and stored in a -20°C freezer until use. Prior to use, the nails were thawed at room temperature in PBS. The nails were subsequently cleaned, rinsed with PBS, and wiped dry with Kimwipes®. The cleansing process consisted of forceps removal of residual tissues and trimming of the nail for smoothness and fit. The thickness of the nail plates was measured using a micrometer (Mitutoyo, Kawasaki, Kanagawa, Japan); the thickness range was 0.51–0.89 mm. Nail clippings were acquired from healthy volunteers using nail clippers (male and female, aged 23–45) or from cadaver nail plates too small to use in the diffusion cell experiments (male and female, aged 24–82). The clippings were cleaned in a similar manner to the nail plates. The use of human tissues was approved by the Institutional Review Board at the University of Cincinnati (Cincinnati, OH).

Hydration Studies

Nail hydration studies were divided into three stages, all performed at room temperature ($20 \pm 2^{\circ}\text{C}$). In Stage I, clean nail clippings with known weights were immersed in 1 mL PBS solutions of pH 2, 5, 9, 11, and 12 in sealed scintillation vials. Nail clippings were also immersed in pH 1 and pH 13 solutions. After hydration for 3 days (Stage IA), the nail clippings were removed from the solution, dried with Kimwipes®, and quickly weighed (i.e., wet weight). The same nail clippings were then reimmersed in the same solution for 2 weeks (Stage IB) and the wet weight was determined following the same procedure as in Stage IA. Subsequent to Stage IB, the nail clippings were dried in an oven set at 60°C until a constant weight was achieved (i.e., dry weight). The percentage water content in the nail clippings (%WC) is equivalent to the difference between wet and dry weights ($\text{weight}_{\text{wet}}$ and $\text{weight}_{\text{dry}}$, respectively) divided by the dry weight and multiplied by 100%:

$$\% \text{WC} = \frac{\text{weight}_{\text{wet}} - \text{weight}_{\text{dry}}}{\text{weight}_{\text{dry}}} \times 100\% \quad (1)$$

Stages II and III were conducted immediately after Stage I hydration and drying. In Stage II, the nail clippings were soaked in 1 mL aliquots of PBS (pH 7.4) for 2 days. After the removal of the nails from this solution, the nails were dried with Kimwipes®, weighed, and oven dried at 60°C overnight. Stage III was performed similarly to Stage II 2 weeks later. The percentages for water content were calculated using Eq. (1) with the wet and dry weights observed during each individual stage.

Transport Study Strategies

The influence of pH on transungual transport was tested using side-by-side diffusion cell experiments in which nails were treated with multistage transport protocols. Testing the same nails in a multistage experimental design allowed the nails to serve as their own control and simultaneously reduced intersample variability. Four main multistage transport protocols were employed in which the nail plates were secured between the diffusion half cells such that the dorsal plate faced the left chamber and the ventral plate faced the right chamber as depicted in Figure 1A.

Within all four protocols, two main stage types were used: control and test. During the control stages, near physiological conditions (pH 7) were maintained on the dorsal side of the nail plate. Test stages were defined as any stage in which a pH condition other than pH 7 was used to treat the dorsal plate; test conditions included pH 1, 2, 3, 5, 9, 10, 11, and 13. Test conditions were performed in ascending or descending pH order to exert the least possible stress on the nail plate. PBS of pH 7.4 always served as the solution in contact with the ventral plate to mimic *in vivo* conditions. Control stages were placed sporadically throughout the protocols between the test stages to ensure that the integrity of the nail was maintained. Nails were excluded from the analysis if the control stage data were not within 50% of each other. Before the first transport experiment on each nail, a hydration stage was conducted. Data from this hydration stage in all protocols were not analyzed. This stage was precautionary—checking the barrier integrity and ensuring complete hydration of the nail plate.

Protocol 1 investigated the effect of pH on nail permeability using water. Protocol 1 included seven passive stages in which nail plates were treated dorsally with solutions of varying pH (1, 4, 7, 10, and 13) dosed with 1.0 μCi ^3H -water. The seven stages comprised three control stages and four test stages. The stages were performed in both ascending and descending pH orders to investigate the effects of directionality. The data for the descending pH experiments are not presented in the present study because the pH 13 condition was found to irreversibly alter the structure of the nail such that all data obtained during stages performed after treatment with pH 13 solution were not representative of the other pH conditions.

In Protocols 2–4, the nail plates were studied using 1.2 μCi $^{22}\text{NaCl}$ or Na^{36}Cl . Protocol 2 tested the reversibility of the nail barrier when the nail was treated with extremely acidic solutions during passive and iontophoretic delivery. Protocol 2 included four stages: three control stages and a test stage. The test stage was performed third, and the test condition was pH 1. Protocols 3 and 4 were performed to determine the permeability and transference of the endogenous Na and Cl ions and conditional hydronium and hydroxide ions. Protocol 3 was the passive transport study and had four stages (two control and two test stages) during which nail plates were treated dorsally with solutions of varying pH values (3, 5, 7, 9, and 11). The test stages were performed in ascending order (pH 9 followed by pH 11 conditions) and descending order (pH 5 followed by pH 3 conditions) to ensure that the moderate pH conditions being studied were performed first for the best chance of reversibility. Protocol 4 was the iontophoretic transport study and included a passive control stage followed by four to five iontophoretic stages during which the nail plates were treated with solutions of varying pH values (2, 3, 5, 7, 9, and 11).

The transport experiments included both dorsal and ventral transport (transport from the dorsal to the ventral plate and from the ventral to the dorsal plate, respectively). For the water transport experiments, (Protocol 1) ^3H -water was always delivered dorsally. For the Na and Cl ion transport experiments (Protocols 2–4), the directionality of delivery depended upon the pH of the test solution as illustrated in Figure 1B. Specifically, chamber spiking was determined according to the test solution pH and the reported isoelectric point (pI) of the nail which is between 4.9 and 5.4.²⁴ For test solutions with pH values at or below pI , ^{22}Na was placed into the chamber facing the dorsal side of the nail in the ^{22}Na transport study and ^{36}Cl was placed on the ventral side in the ^{36}Cl study. For test solutions with pH 9 or 11, the reverse was true. For the hydration and control stages (pH 7), the location of ^{22}Na and ^{36}Cl ions was the same as that of the first test stage. During iontophoretic stages, the anode and cathode were matched to ^{22}Na and ^{36}Cl , respectively. Each transport testing protocol was performed in at least triplicate with nail plates from different human donors, and the same nail donors each providing up to eight separate nail plates were used in each protocol.

Transport Experiments: General Procedure

In the transport experiments, fully hydrated nail plates were sandwiched between custom-made silicone adaptors (MED-6033, NuSil Silicone Technology, Carpinteria, CA) and mounted in side-by-side diffusion cells with a compartmental volume of 2 mL (Dana Enterprise, West Chester, OH). Preliminary studies showed no noticeable permeant-to-adaptor binding. Both the half cells and the adaptors had an effective diffusion area of 0.64 cm². Fully hydrated nail plates have been used in transungual iontophoretic studies. As hydration concurrently increases nail conductance^{20,25} and nail permeability via nail swelling, the transport data of fully hydrated nail demonstrate the potential of transungual iontophoretic delivery. Additionally, the use of fully hydrated nail mimics occluded conditions present during patch treatments which may be encountered in the clinical application of transungual iontophoretic drug delivery. Although fully hydrated nail data could overestimate the initial transport across the nail (prior to complete hydration) during iontophoresis patch applications or transport across the nail under physiologic conditions without occlusion such as from nail lacquers, the hydrated nail model was believed to be appropriate for the purposes of the present study.

At the start of each experiment, the appropriate chamber was spiked with the radiolabeled compound. The duration of the passive transport experiments was 12 h, and that of iontophoretic delivery was 8 h. In all iontophoretic stages, a constant current device (Phoresor II Auto, Model PM 850; Iomed, Inc., Salt Lake City, UT) and Ag and Ag/AgCl electrodes were used to apply a direct electric current of 0.1 mA across the nail plates. Although Cl ion was generated at the anode and consumed at the cathode during iontophoresis, the changes in Cl ion concentration in the donor and receiver chambers were insignificant, in the range of a few micromoles under the current conditions, and should not interfere with the outcomes of the study. For both passive and iontophoretic transport, the resistance of the nail was determined before and after each stage using the voltage drop across the nail measured with a multimeter (Fluke 73III, Everett, WA) at 0.1 mA and Ohm's law. Between stages, the cells were rinsed three to five times and the nails were allowed to equilibrate with the solutions of the upcoming stage for at least 12 h. Constant stirring and room temperature (20 ± 2°C) were maintained during all stages and equilibration periods.

Donor and receiver samples were collected regularly at predetermined time points. Ten microliters were removed from the donor compartment and one milliliter was withdrawn from the receiver compartment. A corresponding volume of fresh buffer was always added to the receiver chamber following sampling to maintain the receiver volume. Ten milliliters of liquid scintillation cocktail (Ultima Gold TM; PerkinElmer Life and Analytical Sciences, Shelton, CT) were added to each sample and the samples were analyzed by a liquid scintillation counter (Beckman Counter LS6500, Fullerton, CA). To avoid interference from quenching in data analyses, ²²NaCl and Na³⁶Cl donor and receiver sample volumes were matched by adding PBS (0.15 M, pH 7.4) into the donor samples. The cumulative amount of permeant delivered across the nail (*Q*) was plotted against time (*t*). The steady-state flux (*J*) was calculated using the slope of the linear portion of this curve, correcting for the diffusion area (*A*):

$$J = \frac{\Delta Q}{A \Delta t} \quad (2)$$

and the transport lag time was determined by the *x*-intercept of this line.

Statistical Analysis

Data were analyzed using SAS[®] 9.1 Software. Specifically, one-way analysis of variance was performed using the proc glm function. GLM or the general linear model calculated the *p*-value for the *F*-test, which confirms or refutes differences between treatments. When differences

were confirmed, the lsmeans function with proc glm was used to render all pairwise comparisons to isolate the statistically significant conditions. Statistically significant differences were established when $p < 0.05$.

THEORY AND EQUATIONS

Nernst–Planck theory describes the flux of ions in electrolyte solutions through homogeneous pore pathways within a membrane exposed to an electric field.^{26,27} Accordingly, it extends Fickian diffusion to include the movement of ions in response to electrostatic forces established by concentration gradients as well as an applied electrical potential. The effective diffusion coefficient of such ions (D_i) is related to the effective electromobility according to the Einstein relation:²⁸

$$D_i = \frac{u_i R_{\text{gas}} T}{z_i F} \quad (3)$$

where F is the Faraday constant, R_{gas} the gas constant, T the temperature, z_i and u_i the valence of the permeant and its effective electromobility, respectively. The effective diffusion coefficient can be calculated using the passive permeability coefficient (P) and the concentration in the donor chamber (C):

$$P = \frac{J}{C} = \frac{D_i}{h} \quad (4)$$

where h is the thickness of the nail plate. The effective diffusion coefficient and effective electromobility are related to the free aqueous diffusivity and electromobility taking into the account of factors such as the porosity and tortuosity of the membrane barrier and hindered transport.

As the ions interact with the electric field, their transport across a membrane may be enhanced or retarded. The enhancement factor ($E_{\Delta\psi,i}$) for electromigration-dominated iontophoresis is proportional to the average electrical potential across the membrane ($\Delta\psi$). Experimentally, the enhancement factor $E_{\Delta\psi,i}$ is defined as the ratio of the iontophoretic flux ($J_{\Delta\psi,i}$) to the passive flux ($J_{p,i}$) when the donor concentrations are equal and can be calculated from the iontophoretic and passive permeability coefficients ($P_{\Delta\psi,i}$ and $P_{p,i}$, respectively)

$$E_{\Delta\psi,i} = \frac{z_i F}{R_{\text{gas}} T} \Delta\psi = \frac{J_{\Delta\psi,i}}{J_{p,i}} = \frac{P_{\Delta\psi,i}}{P_{p,i}} \quad (5)$$

Iontophoretic permeability coefficient is defined as iontophoretic flux divided by donor concentration. For electrically assisted delivery of charged species, the transference number of an ion is an important indicator of iontophoretic efficiency or the extent of electric current carried by the ion. Empirically, the transference number (t_i) is defined as the fraction of the current carried by the ionic species I_i to the total applied current I_T and is expressed in terms of the effective mobilities of the ionic species of interest, i , and all other ions in the system, j , (μ_i and μ_j) and their concentrations within the system (C_i and C_j):²⁹

$$t_i = \frac{I_i}{I_T} = \frac{C_i \mu_i |z_i|}{\sum_j C_j \mu_j |z_j|} \quad (6)$$

Experimentally, the transference number is a function of the iontophoretic flux, valence z_i , and the current density applied I_c .²⁹

$$t_i = \frac{|z_i| F J_{\Delta\psi, i}}{I_c} \quad (7)$$

RESULTS

Effect of pH on Hydration

Figure 2 presents the water uptake into the nail as a function of pH. In the pH 2–11 range, nail hydration was not affected by pH; water uptake values were not statistically different from the control ($40 \pm 6\%$) for all three stages ($p > 0.05$). Under extreme pH conditions of pH 12 and 13, water uptake was greatly impacted by pH, and the nail hydration results differed significantly from the control. At pH 12, nail hydration was approximately 110% in Stage IA and decreased slightly to 87% and 83% during PBS treatments in Stages II and III. The nail clippings dissolved completely in pH 13 solution before the completion of Stage IB (during the 2 weeks of treatment in Stage IB). The dissolution of the nail clippings involved the nail fragmenting and becoming an ivory paste. Using an estimate of the oven dry weight, the pH 13 hydration results were 91 % after 24 h and 123 % after 1 week. pH 1 hydration data show water uptake of approximately 52% in Stages IA and IB (which is not statistically different from the control, $p > 0.05$) and the hydration returned to the control level during PBS treatments in Stages II and III.

Effect of pH on Transungual Transport of H₂O

Figure 3 shows the passive permeability coefficients of water across nail under the influence of pH. The transport lag time of water was around 3 h. Under most pH conditions studied, pH had minimal to no effect on water permeability of the nail. Between pH 1 and 10, the average permeability coefficient for water was approximately $3.1 \times 10^{-6} \pm 0.9 \times 10^{-6}$ cm/s. This corresponds to transungual water flux of approximately 1×10^2 g/m²/h. The flux values observed in the present study are several fold larger than the transonychia water loss (TOWL) values reported previously.^{31,32} The discrepancy is likely due to the differences in the hydration states of the nails in the present and previous studies.³³ The water permeability coefficients in the present study are consistent with those presented previously for fully hydrated nail under physiological conditions determined using desorption data.³⁴ Under the extremely alkaline condition of pH 13, there was a marked increase in nail permeability. The permeability coefficient for water increased an order of magnitude to $2.4 \times 10^{-5} \pm 0.7 \times 10^{-5}$ cm/s and this effect was not reversible over 1–4 days at neutral or acidic pH. Permeation of hydronium and hydroxide ions under pH 1 and 13 conditions, respectively, was not expected to be significant compared to that of water due to the relatively low concentration of these ions (0.1 M) to that of water (~55 M) under these conditions.

Effect of pH on Passive Transungual Transport of Na and Cl Ions

Figure 4 presents the passive permeability coefficients of the nail for Na and Cl ions under the conditions of pH 1–11. Between pH 3 and 11, nail permeability coefficients for both ions were in the order of 10^{-7} cm/s. The permeation of small ions across the nail is moderately affected

by pH with a trend of increasing passive permeability coefficients of Na ion and decreasing passive permeability of Cl ion when the pH increases, albeit not statistically significant due to nail-to-nail and experimental variability. At pH 1 a drastic change to nail permeability was observed and the permeability coefficient for Cl ion was 25 times that for Na ion. It should be noted that the permeability coefficients presented at pH 7 in Figure 4 are anomalous because they are representative of an average of both ventral and dorsal transport. The difference between dorsal and ventral delivery is not statistically significant ($p > 0.05$) but there is a trend toward higher permeability coefficient of ion transport from the ventral to dorsal side of the nail compared to that from the dorsal to ventral side.

Per comparison of the permeability coefficients from the control stages, the effects of pH on nail permeability were reversible between pH conditions 3 and 11 and only partially reversible for the pH 1 condition as shown in Figure 5. Specifically, the figure shows that, initially under the pH 7 condition, the passive permeability coefficient for Na ion was higher than that for Cl ion with the ratio of permeability coefficient of Cl ion to that of Na ion (P_{Cl}/P_{Na}) = 0.4. In the second pH 7 passive stage after the treatment of pH 1 solution in the pH 1 transport experiment the permeability coefficient of Cl ion was still significantly higher than that of Na ion (P_{Cl}/P_{Na} = 5.7). This pH effect that favored the transport of Cl ion over Na ion was partially reversible and persisted over roughly 4 days when the nail was treated with neutral and alkaline pH solutions (P_{Cl}/P_{Na} = 1.5 in the third pH 7 passive stage).

Cumulative amounts of ions transported across the nail plate at 3 and 12 h were in the range of 0.3–1.4 and 2.1–3.9 μmol for Na ion and 0.2–0.4 and 1.5–2.9 μmol for Cl ion, respectively, in the PBS systems of pH 3–11, in which the concentration of Na ion in the donor chamber was approximately 0.15 M (Na ion from NaCl, phosphate buffer, and sodium azide) and Cl approximately 0.14 M (from NaCl and KCl). Although Na and Cl ions are small, the apparent passive transport lag times for Na and Cl ions across the nails were observed to be in the order of 2–3 h under the conditions studied; this is similar to the lag times for water. The apparent effective electromobilities of Na ion estimated using Eq. (3), the passive permeability data, and an average nail thickness of 0.6 mm were 0.7, 1.0, 1.0, 1.0, and $1.6 \times 10^{-6} \text{ cm}^2/\text{s/V}$ and those of Cl ion were 1.1, 0.9, 0.5, 0.8, and $0.7 \times 10^{-6} \text{ cm}^2/\text{s/V}$ at pH 3, 5, 7, 9, and 11, respectively.

Effect of pH on Iontophoretic Transungual Transport of Na and Cl Ions

Figure 6 presents the iontophoretic permeability coefficients and transference numbers for Na and Cl transport across the nail under pH conditions from 1 to 11. At pH 1, iontophoretic transport of Na and Cl ions was significantly lower than those in the other conditions (pH 3–11). At pH 2, the transference number of Na ion remained to be low, but that of Cl ion was significantly higher and comparable to those in the other pH conditions. The pH 3–11 range shows a trend of increasing transference numbers and permeability coefficients for Na ion and a decrease in the transference number and permeability for Cl as the pH increases similar to those observed in Figure 4. Cumulative amounts of Na ion transported across the nail plate were between 1.4 and 2.5 μmol at 2 h and between 10 and 23 μmol at 8 h, in the PBS systems of pH 2–11 during iontophoresis at 0.1 mA. Cumulative amounts of Cl transport across the nail plate were 0.8–2.2 μmol at 2 h and 8–20 μmol at 8 h under similar conditions. Apparent transport lag times of Na and Cl ions for the nails were in the order of 2 h under the iontophoretic conditions. At pH 1, the iontophoretic fluxes of Na and Cl ions were relatively low compared to their passive counterparts and the results under other pH conditions. It is interesting to point out that the iontophoretic enhancement factor for the Cl ion at pH 1 was less than unity, contrast to the significant flux enhancement for the Cl ion under other conditions (the enhancement factor was 8.7 ± 1.4 for Cl ion at pH 3–11). The voltage applied across the nail was $0.10 \pm$

0.03, 3.1 ± 2.6 , 2.5 ± 1.6 , 0.7 ± 0.2 , 0.6 ± 0.2 , 0.8 ± 0.3 , and 1.1 ± 0.5 V at pH of 1, 2, 3, 5, 7, 9, and 11, respectively.

DISCUSSION

pH Effects on the Integrity of Human Nail

Penetration enhancers have been investigated to aid in the delivery of antifungal agents across human nail in the treatment of onychomycosis.^{30,35,36} Penetration enhancers are believed to alter the physical and chemical structures of the nail which is usually accompanied by nail swelling. Nail swelling is believed to increase the porosity and effective size of the permeation pathway across the nail and enhance the diffusion of molecules such as drugs in the nail. In the present study, nail hydration was shown to be independent of pH under moderate pH conditions but was increased under extreme pH conditions such as pH 12 and 13. Under highly alkaline pH condition of pH 13, nail disintegration was observed. These hydration results are supported by the passive permeation data of water. The passive permeability coefficients of water were relatively constant at pH values between 1 and 11, but increased approximately 10 times at pH 13. The relatively constant nail hydration and passive water permeability at pH conditions of 1–11 provided the basis for the systematic study of the effects of pH upon nail permselectivity and ion competition during iontophoresis under these conditions; these results imply that the nail barrier structure was not significantly altered and was not responsible for the observed changes in the passive or iontophoretic transport of Na and Cl ions under these pH conditions in the transport experiments.

The present nail hydration study also examined nail reversibility after the pH treatments. The nail degradation observed at pH 13 was irreversible. The degradation at pH 12 could be partially reversible as indicated by a 20% drop in hydration values between Stage I and a month later at the end of Stage III. Malhotra and Zatz² found similar water permeation results and suspected that alkaline solutions may catalyze the β -elimination of disulfide linkages or degrade the cysteine linkages as seen in hair keratins though the latter is seen mainly at higher temperatures. Alkaline solutions at pH ≥ 12 may therefore be useful in enhancing transungual drug uptake and penetration. At pH 1, a slight elevation in hydration was observed and as such the acidic solutions may be useful in enhancing drug transport to a lesser extent compared to those at pH ≥ 12 without the destructive effects of alkaline solutions.

pH Effects on Nail Permselectivity and Reversibility

Nail permselectivity has been investigated in previous studies using electrokinetic theory in electroosmosis experiments, charge–charge interactions in uptake and transport experiments with charged molecules, and electrical measurements with membrane potentials.^{15,21–23,37} The results in the present study serve to verify the previous findings with a different experimental approach—using passive and iontophoretic transport of small ions, particularly, Na and Cl ions. However, it is not easy to delineate the effect of nail permselectivity on electrotransport from other factors such as hindered transport and competition for electric current by other coions and counterions in the present system.

The effect of pH on transungual transport of neutral permeants has been previously examined.²² Under symmetric conditions in which the pH of the donor and receiver solutions was identical, passive transport was shown to be independent of pH, suggesting that the effect of hindered transport upon nail permeation remained relatively constant between pH 3 and 9. Assuming constant transport hindrance across the nail at pH 3–9 and negligible effects of diffusion potential, the observed iontophoretic transport pattern in Figure 6 is consistent with nail permselectivity with the pI at approximately pH 5. The data for the small ions in the present study continue to support the previous permselectivity findings^{15,21–23} that the nail favors the

permeation of positively charged permeants at pH above the pI and that of negatively charged permeants at pH below pI . The electrical resistance of the nail was lowest for the pH 1 condition at $\sim 1\text{ k}\Omega$, highest under the pH 2 condition at $30\text{ k}\Omega$, and around $10\text{ k}\Omega$ at pH between 5 and 11. The resistance data suggest that nail permselectivity did not significantly affect the electrical resistance of the nail under moderate pH conditions.

The reversibility of the treatment effects of pH on nail permeability was examined. Between pH 3 and 11, the effects of pH were generally reversible. For pH 1 and 2 conditions, however, the treatment effects persisted several days to a week at higher pH (neutral and alkaline pH) posttreatment. The partial reversibility of these treatments suggests that these highly acidic solutions likely affect the chemical environment of the transport pathways in the nail, not the physical structure of the nail as is with the case of the highly alkaline conditions of $\text{pH} \geq 12$. Clinically, these observations can be significant. Acidic solutions of low pH may be useful in selectively altering the permselectivity of the nail toward negatively charged drugs when the nail is treated with these solutions in a pretreatment step to enhance transungual delivery of these drugs at neutral pH up to 1 week after the pretreatment. This finding is also important in transungual iontophoretic delivery as nail permselectivity would not recover to its initial state over an extended period of time if the pH of the system drops to approximately $\text{pH} \leq 2$.

Transference Numbers of Competing Endogenous Ions in Transungual Iontophoresis

Transungual iontophoretic transport depends on the charge of the barrier in the nail. According to the literature, the free aqueous diffusivities of Na and Cl ions are 1.334×10^{-5} and $2.032 \times 10^{-5}\text{ cm}^2/\text{s}$, respectively,³⁸ and the transference numbers for Na and Cl ions in bulk solution are 0.4 and 0.6, respectively. The transference numbers calculated in the present study at pH 3–5 followed a similar trend as those in the bulk solution but were quantitatively different possibly due to the presence of other ions such as potassium and phosphate ions in the system. At lower and higher pH (pH 1–2 and 7–11), the transference numbers of Na and Cl ions changed drastically. At pH 7–11, the transference numbers of the Na ion were higher than those of the Cl ion due to nail permselectivity as discussed in the preceding section. At pH 1–2, the transference numbers of Na and Cl ions were likely affected by the hydronium ion. When the concentration of hydronium ion was 0.1 and 0.01 M at pH 1 and 2, significant ion competition from hydronium for the electric current in iontophoretic transport was observed. The low applied voltages to maintain the constant electric current in the pH 1 iontophoresis experiments (relative to those at pH 3–11) also support this conclusion. Within experimental variability, the estimated transference number for the hydronium ion across nail at pH 1 could be as high as 0.8. This corresponds to hydronium effective electromobility of approximately 80 and 14 times of those of Na and Cl ions, respectively, across the nail under this condition where the electromobility ratios were calculated by the relationship ($t_{\text{Na}} + t_{\text{Cl}} + t_{\text{H}} = 1$) and Eq. (6). At pH 3–11, ion competition from the hydronium and hydroxide ions was found to be minimal. These results suggest that hydronium and hydroxide ion competition during iontophoresis can generally be ignored at pH 3–11 under the conditions studied.

Ion Transport across the Nail Plate

The transport of Na and Cl ions is crucial to understanding transungual iontophoretic delivery. Particularly, a comparison of the transport of small Na and Cl ions in present study and that of the larger permeants in previous studies could provide valuable information of the transport barrier of the nail. The effective electromobility data of these endogenous ions determined in the present study also allow the prediction of iontophoretic transport of drugs using the electrotransport theory (e.g., Eqs. 5 and 6). In addition, the transport data of the small ions can represent the maximum passive and iontophoretic transungual delivery under the pH conditions studied. Under the moderate pH conditions (pH 3–11) in the present study, the amounts of Na and Cl ion transported across the nail at 2–3 h after passive and 0.1 mA iontophoretic delivery

(~0.16 mA/cm²) were in the order of 10⁻¹ and 1 μmol, respectively. The cumulative amount delivered increased to the order of 1 and 10 μmol at 8–12 h of passive and iontophoretic delivery, respectively, and 10² μmol after 48 h of iontophoresis. The transport lag time of the ions was in the order of a few hours. The Na and Cl ion transport data in the present study were more than an order of magnitude larger than those of the drug molecules in previous studies, and this suggests significant transport hindrance across the nail plate. For example, the deliverable amounts of salicylate, which is approximately two times larger in size than the Cl ion, transported across a 0.25 cm² area of nail were reported to be between 0.04 and 0.09 μmol after 12 h of 0.5 mA/cm² iontophoresis in a previous study conducted in a similar pH range (pH 3–7).¹⁶ For the larger drugs terbinafine hydrochloride and ciclopirox olamine, relatively low transungual transport was also observed.^{14,18} In summary, the present results suggest significant transport hindrance in the hydrated nail plate to the small ions Na and Cl (present as endogenous ions in the body) and large ionic permeants in transungual iontophoretic delivery. Hindered transport and the resulting low effective diffusivity of the ions in the thick nail plate also lead to the long transport lag time observed for the small ions in the present study.

A caveat for the analysis of the present experimental results was the experimental variability even with the multiple stage experimental design employed in the present study. For example, the transference numbers for Na and Cl ions did not sum to unity. The transference numbers at pH 5 close to the suggested nail *pI* are also different from those expected from the free aqueous electromobilities for Na and Cl ions of 0.4 and 0.6, respectively. In addition to experimental variability, a possible explanation is the existence of other ions such as the potassium, mono- and dibasic phosphate, and azide ions in the system and their competition for transungual electric current during iontophoresis. However, a separate study conducted with 0.15 M NaCl at pH ~7 in both the donor and receiver chambers without these competing ions showed that the transference numbers for Na and Cl ions (0.5 ± 0.2 and 0.2 ± 0.1 for Na and Cl, respectively) were similar to those in the present study. This suggests that the contribution of these ions to electric current was likely to be small. In addition, the present transference number results were consistent with those given for Na ion in two separate reports, which utilize different methodologies.^{37,39} Another possible explanation is that the ions existing in the nail plate could interfere with the transference number measurements. Extending the duration of the pH 7 iontophoresis experiments from 12 to 48 h in a follow-up study showed a slightly higher transference number of Na ion (0.7 ± 0.1) and longer transport lag time, but those of Cl ion were unchanged. The observed longer apparent transport lag time for Na ion transport is believed to be related to the positively charged ions initially within the nails. The release of these preexisting ions in the nail to the receiver chamber would occur and the exchange of radioactive Na ion (permeating from the donor to the receiver) with these ions in the nail was necessary before steady-state transport of the radioactive Na ion could be established. One other possible explanation particularly for the deviation between the experimental result in the pH 5 iontophoresis experiments and that anticipated at the nail *pI* is the pH gradient in the nail. Due to the neutral pH on the dorsal side of the nail in the pH 5 experiments, the resultant pH in the nail could be higher than the *pI* and this could lead to higher transference of Na ion than that of Cl ion. Nevertheless, all these variabilities and factors are believed to be insignificant and should not affect the main findings and conclusions in the present study.

CONCLUSION

The effects of pH on nail water uptake and water permeation across nail plates were determined. Nail hydration was independent of pH under the conditions of pH 3–11 and was significantly elevated above pH 11. These findings are consistent with the permeability coefficients of water across nail, which were similar between pH 1 and 10 and an order of magnitude higher at pH

13. The effects of pH on the transport of Na and Cl ions, under passive and iontophoretic conditions, were also investigated. These results were consistent with the nail being a permselective membrane. Under moderate pH conditions, the passive permeability coefficients for Na and Cl ions were in the order of 10^{-7} cm/s and those of 0.1 mA iontophoresis (~ 0.16 mA/cm²) were 10^{-6} cm/s, which correspond to apparent Na and Cl effective electromobilities $\sim 10^{-6}$ cm²/s/V. Hydronium and hydroxide ion transport was minimal during iontophoresis at pH 3–11 under the conditions in the present study. At pH 1, iontophoretic transport of hydronium ion becomes significant and the ion competes with Na and Cl ion transport. These results have four main practical implications. First, the pretreatment or cotreatment of nail plates with solutions of extremely alkaline pH (pH >11) may be useful for enhancing transungual drug delivery. Second, the pretreatment of nail with solutions of extremely acidic pH (pH <3) may be useful for enhancing passive and iontophoretic delivery of negatively charged drugs across nail under neutral pH conditions. Third the effects of pH upon iontophoretic drug delivery under the acidic conditions may not mirror their respective passive drug delivery counterparts given the inherent competition for current between hydronium ion and drug under these conditions. Fourth and finally, the results suggest that pH modulations—between pH 3 and 11—in formulation development do not affect the intrinsic barrier of the nail for drug delivery.

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Figure 1.

(A) Diffusion cell set-up for transport experimental Protocols 1–4. Fully hydrated nail plates were secured between diffusion cells using custom-made silicone nail adaptors such that the dorsal plate faced the left diffusion chamber in the diagram and the ventral plate faced the right diffusion chamber. (B) Transport Protocols 2–4 for Na and Cl ion delivery. The dorsal plates were treated with PBS solutions of various pH conditions (second column), and the ventral plate was always treated with PBS of physiologic pH (fifth column). $^{22}\text{NaCl}$ and Na^{36}Cl dosing was based on the pH condition and *pI* of nail (third and sixth columns). At or below *pI*, $^{22}\text{NaCl}$ was delivered dorsally and Na^{36}Cl was delivered ventrally. At or above pH 9, the reverse was true. At pH 7, dosing was matched to the initial testing stage. The “+” and “-” signs in the first and seventh columns indicate the polarity of the electrode in the diffusion chamber.

Figure 2.

Percentage water content in nail clippings treated with solutions of various pHs. The pH 13 hydration data are incomplete and are only estimates. Data represent the mean and standard deviation of four nail samples. Symbols: Stage IA (closed diamonds), Stage IB (open diamonds), Stage II (closed squares), and Stage III (closed triangles). The dotted line represents the average percentage water content in control nail clippings (fully hydrated at physiologic ionic strength and pH), which was previously established by Hao et al.³⁰ to be $40 \pm 6\%$ ($n = 6$). The asterisk indicates statistical significance from the control ($p < 0.05$).

Figure 3. pH effects on the passive permeability of water across fully hydrated human nails. Nails were treated per Protocol 1. Data represent the mean and standard deviation of three to six nail samples. The asterisk indicates statistical significance from the other pH conditions ($p < 0.05$).

Figure 4. pH effects on the passive permeability of sodium (open diamonds) and chloride (closed diamonds) ions across fully hydrated human nails treated with Protocol 3. pH 7 data are averages of both the ventral and dorsal permeabilities. Data represent the mean and standard deviation of three to four nail samples.

Figure 5. pH effects on the permselectivity and reversibility of passive transport of sodium (white bars) and chloride (black bars) ions across fully hydrated human nails. Reversibility of the effects of the pH 1 solution on the nail was checked 2 and 4 days posttreatment at pH 7 (2nd 7 and 3rd 7, respectively). The results in the first pH 7 stage (1st 7) were different from those posttreatment (2nd 7 and 3rd 7). Data represent the mean and standard deviation of three to four nail samples.

Figure 6.

Effects of pH on iontophoretic transport and transference number of sodium and chloride ions across fully hydrated human nails treated with Protocol 4. The iontophoretic transport experiments of sodium (open symbols) and chloride (closed symbols) ions were performed from dorsal to ventral (diamonds) and from ventral to dorsal (squares) of nails, depending on the pH and ions tested. Permeability coefficients are indicated in black and transference numbers are indicated in gray. In this study, iontophoretic permeability coefficient of 1×10^{-7} cm/s is equivalent to transference number of 0.01 at 0.1 mA except for sodium ion at pH 1. For sodium ion at pH 1, a permeability coefficient of 1.5×10^{-7} cm/s is equivalent to a transference number of 0.005 at 0.1 mA. Data represent the mean and standard deviation of three to four samples.