Journal of Pharmaceutical Sciences 105 (2016) 1201–1208

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Size and Charge Dependence of Ion Transport in Human Nail Plate

Sudhir M. Baswan, S. Kevin Li, Terri D. LaCount, Gerald B. Kasting*

James L. Winkle College of Pharmacy, The University of Cincinnati Academic Health Center, Cincinnati, Ohio 45267-0004

ARTICLE INFO

Article history:
Received 11 September 2015
Revised 10 December 2015
Accepted 11 December 2015
Available online 23 January 2016

Keywords:
diffusion
iontophoresis
membrane transport
mobility
nail
permeability
structure–transport relationship

ABSTRACT

The electrical properties of human nail plate are poorly characterized yet are a key determinate of the potential to treat nail diseases, such as onychomycosis, using iontophoresis. To address this deficiency, molar conductivities of 17 electrolytes comprising 12 ionic species were determined in hydrated human nail plate *in vitro*. Cation transport numbers across the nail for 11 of these electrolytes were determined by the electromotive force method. Effective ionic mobilities and diffusivities at infinite dilution for all ionic species were determined by regression analysis. The ratios of diffusivities in nail to those in solution were found to correlate inversely with the hydrodynamic radii of the ions according to a power law relationship having an exponent of -1.75 ± 0.27, a substantially steeper size dependence than observed for similar experiments in skin. Effective diffusivities of cations in nail were 3-fold higher than those of comparably sized anions. These results reflect the strong size and charge selectivity of the nail plate for ionic conduction and diffusion. The analysis implies that efficient transungual iontophoretic delivery of ionized drugs having radii upward of 5 Å (molecular weight, ca. ≥340 Da) will require chemical or mechanical alteration of the nail plate.

Introduction

Onychomycosis accounts for nearly 50% of all nail disorders and affects 2%-18% or more of the world's population.[1-6] Although the condition is treatable, the current therapeutic options are limited due to efficacy and safety issues. Oral therapy for the treatment of onychomycosis is recommended by only 35%-65% of the physicians due to the associated side effects, such as headache, risk of hepatotoxicity, and potential drug–drug interactions.[5] The clinical significance of formulations, such as medicated nail lacquers, patches, and creams for topical treatment of onychomycosis, is debatable. New techniques, such as use of chemical penetration enhancers,[7-10] micro-drilling,[11] nail abrasion,[12-14] acid etching,[15] and iontophoresis,[16-24] are also being evaluated as potential treatment methods for onychomycosis. The drug–device combination that uses iontophoresis to deliver high concentrations of medication directly to the site of action has considerable potential to improve the treatment of onychomycosis.[16-24] Although significant efforts have been made to evaluate and enhance transungual iontophoretic drug delivery, little has been performed to systematically characterize ion transport across the nail plate. It is fair to say that work to date on transungual ion transport has been largely phenomenological, with little guidance from electrotransport theory.

The main objective of this research was to evaluate the impact of ionic size and charge on transungual transport of both organic and inorganic ions. Transport parameters including ionic conductance at infinite dilution, ionic mobility, and diffusion coefficient of 12 ionic species in hydrated nail plate were obtained by performing conductivity and transport number studies using *in vitro* electrochemical and radiochemical methods. Also, gravimetric experiments were performed to determine the extent of hydration and keratin fiber volume fraction of the nail plate. Results are compared with similar information obtained recently for charged solutes in human stratum corneum[25] as amended[26] and to passive diffusion data for uncharged solutes in human nail plate.[27] A procedure for using the conductivity results to predict transungual iontophoretic drug delivery is proposed.

Materials and Methods

Materials

Sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), and potassium bromide (KBr) were obtained from

This article contains supplementary material available from the authors upon request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2015.12.011.

* *Correspondence to:* Gerald B. Kasting (Telephone: +1-513-558-1817; Fax: +1-513-558-0978).
E-mail address: gerald.kasting@uc.edu (G.B. Kasting).

http://dx.doi.org/10.1016/j.xphs.2015.12.011
0022-3549/

Fisher Scientific (Pittsburgh, PA); sodium bromide (NaBr), sodium sulfate (Na_2SO_4), tetraethylammonium bromide (TEABr), tetraethylammonium chloride (TEACl), sodium acetate (Na-Ac), tetraethylammonium acetate (TEA-Ac), tetraphenlyphosphonium bromide (TPPBr), tetraethylammonium benzoate (TEA-Bz), and olamine hydrochloride (Olam-Cl) from Sigma-Aldrich (St. Louis, MO); calcium bromide ($CaBr_2$) and potassium sulfate (K_2SO_4) from GFS Chemicals (Powell, OH); sodium benzoate (Na-Bz) from Alfa Aesar (Ward Hill, MA); and methyltriphenylphosphonium bromide (MTPPBr) from Strem Chemicals (Newburyport, MA). $CaCl_2$ and $CaBr_2$ had stated purity of ≥96%, TPPBr ≥97%, MTPPBr ≥98%, and the rest of the chemicals were ≥99% pure. KCl conductivity standard solutions (1000 and 10,000 μmho/cm at 25°C) were obtained from LabChem Inc. (Pittsburgh, PA). All aqueous solutions were prepared in deionized (DI) water (18.2 MΩ cm at 25°C, US Filter). Dialysis membranes (MWCO 6000 Da) were obtained from Bel-Art Products (Wayne, NJ). Optically clear silicone elastomer MED-6033 was obtained from NuSil Technology LLC (Carpinteria, CA). ^{22}NaCl (100-2000 Ci/g) and ^{14}C-TEABr (3.5 mCi/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Ag-AgCl electrodes, E215, and E252P were obtained from In-Vivo Metric (Healdsburg, CA).

Nail Sample Preparation

Frozen, human cadaver fingernails (Caucasian men, age 26-86) were obtained from Science Care Anatomical (Phoenix, AZ). A total of 75 index, middle, and ring fingernails from both left and right hands of 13 donors were selected for the experiments. The frozen nail plates were thawed at room temperature (25 ± 2°C) in DI water and cleaned by removing adhering tissues with forceps and cotton swabs. The nails were then rinsed with DI water and inspected for any visual deformities, such as cracks or hairline fractures. The thickness of nail plates, ranging from 0.21 to 0.85 mm, was measured using a point micrometer (Mitutoyo, Kawasaki, Kanagawa, Japan). The use of human cadaver nails was approved by the Institutional Review Board at the University of Cincinnati (Cincinnati, OH).

Nail Adapter Preparation

The nail adapters were fabricated from a thermally curable silicone elastomer MED-6033, which comprised 2 parts. These materials were mixed together in equal proportions by volume and allowed to stand for 2-3 h to remove air bubbles formed during the mixing process, before pouring into the molds. The silicone mix within the molds was then cured for 30 minutes at 60 ± 2°C to form sturdy but flexible nail adapter halves. A circular hole, 9 mm in diameter (0.64 cm^2), was punched in the center of both halves of the silicone nail adapter. The shape and design of these custom-made silicone nail adapters were similar to Teflon nail adapters made by PermeGear Inc. (Bethlehem, PA); however, they had an additional advantage of not requiring adhesive glue, which is used to seal the gaps between the nail and Teflon nail adapters. These custom-made silicone nail adapters accommodated the nail curvature better than Teflon adapters and demonstrated no inter-compartmental leakage in preliminary experiments.

Nail Hydration Studies

Hydration experiments were performed on 6 fingernails selected randomly from different donor sets. Nail plates were immersed in DI water in a glass vial and placed in a water bath (32.0 ± 0.1°C) for 24 h. The nail samples were removed after 24 h, excess water was wiped off with Kimwipes® and cotton swabs, and then wet mass (m_{wet}) of the nail plate was measured on an analytical balance (Mettler Toledo AB-135SP®) reading to ±0.01 mg. The nail samples were then dried at 60°C overnight or until constant weight was achieved (m_{dry}). The water content w (wt%) in hydrated nail plate was determined by Equation 1. The water uptake capacity of dry nail plate (v') was determined using Equation 2.

$$w(\text{wt }\%) = \frac{m_{wet} - m_{dry}}{m_{wet}} \times 100\% \quad (1)$$

$$v'(\text{g } H_2O/\text{g dry nail}) = \frac{m_{wet} - m_{dry}}{m_{dry}} \quad (2)$$

Assuming nail plate to comprise only keratin and water, the fiber volume fraction (φ_f) is given by[28]:

$$\varphi_f = 1 - \frac{\rho_m v'}{\rho_1 + \rho_m v'} \quad (3)$$

where ρ_m is the density of dry nail (keratin density ~1.3 g/cm^3)[29] and ρ_1 is the density of water (~1.0 g/cm^3).

Conductivity Measurements

The molar conductivities of all the electrolyte solutions and of human nail plate immersed there were determined by a 4-terminal resistance method[25,30,31] in side-by-side diffusion cells (PermeGear Inc.). The method was that used in Franz diffusion cells by LaCount and Kasting[25] except for variations as described subsequently. Commercially available cylindrical Ag-AgCl electrodes, E215 and E252P (In-Vivo Metric), were used for all electrolytes except for those containing bromide. For the latter, homemade Ag-AgBr electrodes were used as Ag-AgCl electrodes are fouled by bromide ions.[32] Ag-AgBr electrodes were constructed from a 1.0-mm diameter silver wire (99.999%; Alfa Aesar) electrolyzed in 0.1 M KBr solution and were stored in the dark before use due to its sensitivity to light. Electrodes were placed as shown in Figure 1. The 98.28 kΩ standard resistor described previously[25] was replaced by a 10.01 kΩ (±0.02%) precision standard resistor (Ohm-labs Inc., Pittsburgh, PA) to better match the 3-10 KΩ resistance of the hydrated nail plate immersed in physiological saline solution.[33] A 10 V peak-to-peak, 20 Hz sinusoidal signal was applied across a 1 MΩ current-limiting resistor using a waveform generator (Agilent 33220A) to yield a 6.5-6.9 μA root mean square AC current across the nail plate. The AC potential generated across the nail plate (maximum of 0.5 V peak to peak) was measured by the inner pair of electrodes using a sensitive multimeter (Agilent 34410A).

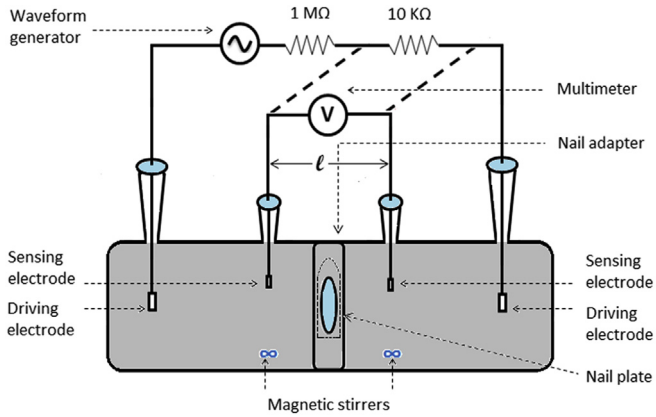

Figure 1. Diffusion cell configuration for 4-terminal resistance measurements.