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# Chemical method to enhance transungual transport and iontophoresis efficiency

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**Abstract**

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


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

## 1. Introduction

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

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

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

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

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

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

## 2. Materials and methods

### 2.1. Materials

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

### 2.2. Preparation of nail samples

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

### 2.3. Hydration of nail clippings

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