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Alteration of the diffusional barrier property of the nail leads to greater terbinafine drug loading and permeation

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ABSTRACT

The diffusional barrier property of biological systems varies with ultrastructural organization of the tissues and/or cells, and often plays an important role in drug delivery. The nail plate is a thick, hard and impermeable membrane which makes topical nail drug delivery challenging. The current study investigated the effect of physical and chemical alteration of the nail on the trans-ungual drug delivery of terbinafine hydrochloride (TH) under both passive and iontophoretic conditions. Physical alterations were carried out by dorsal or ventral nail layer abrasion, while chemical alterations were performed by defatting or keratolysis or ionto-keratolysis of the nails. Terbinafine permeation into and across the nail plate following various nail treatments showed similar trends in both passive and iontophoretic delivery, although the extent of drug delivery varied with treatment. Application of iontophoresis to the abraded nails significantly improved ($P < 0.05$) TH permeation and loading compared to abraded nails without iontophoresis or normal nails with iontophoresis. Drug permeation was not enhanced when the nail plate was defatted. Keratolysis moderately enhanced the permeation but not the drug load. Ionto-keratolysis enhanced TH permeation and drug load significantly ($P < 0.05$) during passive and iontophoretic delivery as compared to untreated nails. Ionto-keratolysis may be more efficient in permeabilization of nail plates than long term exposure to keratolysing agents.

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1. Introduction

Ungual and trans-ungual drug delivery continues to receive significant attention due to the need for efficacious topical therapies for onychomycosis given the potential risk of systemic adverse effects associated with the conventional oral therapy (Effendy, 1995). The major concern in topical therapy is the low trans-nail penetration into the deeper nail stratums because of the inherent limitation of low permeability of the keratinised nail plates (Baran and Kaoukhov, 2005). In one approach to address this issue, permeation enhancers were screened for their ability to enhance the trans-ungual permeation of antifungal agents (Van Hoogdalem et al., 1997; Kobayashi et al., 1998; Malhotra and Zatz, 2002; Hui et al., 2003; Hao et al., 2008). In another approach, drug discovery groups have synthesized and screened newer antifungal agents with low keratin binding and higher nail permeation properties (Tatsumi et al., 2002; Hui et al., 2007). Despite all these efforts, the success rates of topical therapies have so far been disappointing in comparison to systemic therapy (Murdan, 2008).

The barrier properties of a particular tissue are dictated by characteristics such as membrane composition and thickness, and the specific routes available for drug permeation through the tissue. In comparing nail and skin, for example, nail is a thick, hard and compact keratin membrane with low lipid content ($\leq$1%, w/w) whereas stratum corneum, which provides the primary barrier for skin, is thin (10–20 μm), highly flexible and has a high lipid content (10%) (Gupchup and Zatz, 1999). Additionally, the pathways available for drug permeation across the skin and nail differ; for example, the follicular route in skin is absent in nail (De Berker et al., 2007). Taken together, these characteristics of the nail make it a formidable barrier to drug permeation and the challenge to improve topical delivery of drugs into and through the nail remains formidable as well.

Recently the ability of iontophoresis to enhance the trans-ungual permeation of salicylic acid and terbinafine was demonstrated (Murthy et al., 2007a; Nair et al., 2009). In addition, the perm-selective nature of the human nail plate was found to be comparable to that of skin (Murthy et al., 2007b). Iontophoresis uses a low level electric current to actively facilitate drug transport across biological membranes (Batheja et al., 2006). The enhanced permeation of charged drug molecules by iontophoresis was principally

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due to electrorepulsion with a lesser contribution by electroosmosis (Hao and Li, 2008a). The influence of variables such as current level and density, vehicle pH, type of electrode, co-ions, drug concentration, and charge on the transport of drugs during iontophoresis in transdermal delivery is well-known (Kalia et al., 2004). A similar influence/effect of the above said variables in trans-ungual iontophoretic delivery was also assessed in earlier studies. It was observed that the increase in current density (0.1–1 mA/cm^2) enhanced the drug permeation linearly (Murthy et al., 2007a; Nair et al., in press). The role of drug concentration, ionic strength and vehicle pH on trans-ungual iontophoretic permeation was also investigated (Murthy et al., 2007a). Furthermore, Hao and Li (2008b) reported that pH and ionic strength had little effect on electroosmotic transport in trans-ungual iontophoresis.

Terbinafine is a potent antifungal agent which belongs to the allylamine class of antifungals, is highly effective in treating dermatophyte infections, and is the current treatment of choice in onychomycosis (Darkes et al., 2003). It possesses low minimum inhibitory concentrations (~0.001–0.01 μg/mL) and low minimal fungicidal concentrations (~0.003–0.006 μg/mL) against dermatophytes (Darkes et al., 2003). Terbinafine acts by blocking ergosterol biosynthesis by inhibiting the enzyme squalene epoxidase (fungistatic), which also leads to the toxic accumulation of intra-cellular squalene thereby exhibiting fungicidal activity (Ryder, 1992; Darkes et al., 2003). In the present study, the influence of barrier alteration on the passive and iontophoretic trans-ungual delivery (both permeation and drug load) was systematically investigated using terbinafine as a model drug.

2. Materials and methods

2.1. Materials

Terbinafine hydrochloride (TH) [MW = 327.90 Da, aqueous solubility = 1 mg/mL, pKa = 7.1, log octanol/water partition coefficient of terbinafine (Alberti et al., 2001) = 3.3], was procured from Uquifa, Jiutepac, Mexico. Sodium sulfite and salicylic acid were purchased from Sigma–Aldrich, St. Louis, MO. Human cadaver nails, both male and female, aged between 49 and 86 years, with varying thickness of 0.4–0.7 mm were procured from Science care (Phoenix, AZ) and were stored at 4 °C until used. All other chemicals and reagents used were of analytical grade. All solutions were prepared in deionized water.

2.2. Analytical method

The amount of terbinafine in the samples was quantified by high performance liquid chromatography (HPLC) system (Waters, 1525) with an autosampler (Waters, 717 plus) consisting of a Phenomenex C18 (2) 100 R analytical column (4.6 mm × 150 mm, Luna, 5.0 μm) and a variable wavelength dual λ absorbance detector (Waters, 2487). Mobile phase consisted of aqueous solution (0.096 M triethyl amine, 0.183 M orthophosphoric acid) and acetonitrile (60:40) adjusted to pH 2 with orthophosphoric acid. Elution was performed isocratically at 32 °C at a flow rate of 1.0 mL/min. Injection volume was 20 μL and the column effluent was monitored at 224 nm. The method was validated by determination of linearity, precision, and accuracy. The range for the calibration curve was 2–1000 ng/mL (R^2 = 0.99). The coefficient of variation and the accuracy ranged 1.03–6.08% and −0.54 to −6.96%, respectively.

2.3. Nail treatments

2.3.1. Abrasion of dorsal or ventral nail layer

Nails were cleaned and adherent tissues were removed. The dorsal or ventral surface of the nail was physically abraded using sandpaper (grade #180) until the top or bottom layer (3/10 of the total thickness) was removed. The complete removal of the dorsal/ventral layer was confirmed by microscopic examination of sections of the nail plate.

2.3.2. Defatting and keratolysing of nails

Cleaned nail pieces were defatted by placing them in a beaker containing chloroform:methanol (2:1) mixture (10 mL) and stirred for a period of 12 h. Similarly, nail pieces were stirred in salicylic acid solution (4 mg/mL) for 12 h to keratolyse the nails. Treatment period (12 h) was selected since the longer exposure (24 h) of nails with salicylic acid solution did not improve the permeation.

2.3.3. Pretreatment with keratolytic agents in conjunction with iontophoresis (ionto-keratolysis)

Each of the nail plates were soaked in 0.9% (w/v) saline for 1 h, cleaned and mounted on a nail adapter (PermeGear, Bethlehem, PA). The whole assembly was sandwiched between the two chambers in a Franz diffusion cell (Logan Instruments Ltd., Somerset, NJ). Each 500 μL solution of salicylic acid (4 mg/mL) or sodium sulfite (50 mg/mL) was placed in the donor compartment, and 5 mL of normal saline in the receiver. Cathodal iontophoresis was carried out by applying a constant current of 0.5 mA/cm^2 by placing the cathode (silver chloride electrode) in the donor compartment and the anode (silver electrode) in the receiver, for a period of 1 h. The nail plate was rinsed with pH 3 water before carrying out the permeation experiments.

2.4. Permeation studies with terbinafine

Nail plates were soaked in 0.9% (w/v) saline for 1 h immediately prior to use and mounted on a nail adapter (PermeGear, Bethlehem, PA). The whole assembly was sandwiched between the two chambers in a Franz diffusion cell (Logan Instruments Ltd., Somerset, NJ). TH solution (500 μL, 1 mg/mL adjusted to pH 3 using 0.01N HCl) was placed in the donor compartment. The active diffusion area exposed to both the donor and receiver compartments was 0.2 cm^2. The receptor compartment, which had a capacity of 5 mL and was filled with saline (adjusted to pH 3 using 0.01N HCl) which provides sink conditions due to increased drug solubility. The receiver compartment was stirred at 600 rpm with a 3-mm magnetic stir bar at room temperature. Samples were withdrawn from the receiver compartment after 24 h and analyzed for terbinafine concentration and the cumulative amount of terbinafine permeated into the receiver chamber normalized to the surface area exposed to the drug was expressed as μg/cm^2.

Anodal iontophoresis was carried out by fixing 0.5 mm diameter Ag/AgCl wire electrodes (Alfa Aesar, Wardhill, MA) at a distance of 2 mm from the nail surface in donor and receiver chambers. Iomed Phoresor II dose controller (Iomed Inc., Salt Lake City, UT) was used for application of a constant DC. The anode was connected to the donor and the cathode to the receiver chamber and a constant current (0.5 mA/cm^2) was applied for a period of 24 h.

2.5. Amount of drug in nail

After the *in vitro* diffusion studies, the nail plates were marked for active diffusion area (using permanent marker and metric punch), washed with water and alcohol five times each using a standardized protocol to avoid the washout of drug loaded in the nail while removing surface drug. In brief, washing was carried out by holding the nail with forceps and shaking twice by placing in 2 mL of water (pH 3). Five such washings were performed in fresh 2 mL of pH 3 water each time. The nail surface was cleaned using a cotton swab soaked in 95% ethanol and rinsed with 1 mL ethanol (95%)—this alcohol washing procedure was repeated 5 times. The

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