International Journal of Pharmaceutics 384 (2010) 1–8

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Review

Transungual drug delivery: Current status

Rania Elkeeb[a,*,1], Ali AliKhan[b,1], Laila Elkeeb[c,1], Xiaoying Hui[a], Howard I. Maibach[a]

[a] Department of Dermatology, School of Medicine, University of California, San Francisco, CA 94143-0989, USA
[b] School of Medicine, University of California, Davis, CA, USA
[c] Department of Dermatology, School of Medicine, University of California, Irvine, CA, USA

ARTICLE INFO

Article history:
Received 9 June 2009
Received in revised form
22 September 2009
Accepted 1 October 2009
Available online 9 October 2009

Keywords:
Ungual drug delivery
Onychomycosis
Nail penetration
Antifungal
Onychopharmacokinetics

ABSTRACT

Topical therapy is highly desirable in treating nail disorders due to its localized effects, which results in minimal adverse systemic events and possibly improved adherence. However, the effectiveness of topical therapies is limited by minimal drug permeability through the nail plate. Current research on nail permeation that focuses on altering the nail plate barrier by means of chemical treatments, penetration enhancers as well as physical and mechanical methods is reviewed. A new method of nail sampling is examined. Finally limitations of current ungual drug permeability studies are briefly discussed.

Contents

* Corresponding author at: C/O Dr. H.I. Maibach, Department of Dermatology, School of Medicine, UCSF, 90 Medical Center Way, San Francisco, CA 9414-0989, USA. Tel.: +1 415 476 4997/738 8523; fax: +1 415 753 5304.
E-mail addresses: relkeeb@yahoo.com (R. Elkeeb), MaibachH@derm.ucsf.edu (H.I. Maibach).
[1] Rania Elkeeb, Ali Alikhan and Laila Elkeeb have contributed equally to the work.

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.10.002

1. Introduction

The importance of nail permeability to topical therapeutics has been realized, primarily in the treatment of onychomycosis, which affects approximately 19% of the population (Gupta and Scher, 1998). Topical therapy is highly desirable due to its localized effects, which results in minimal adverse systemic events and possibly improved adherence. Recent advances in topical transungual delivery have led to the development of antifungal nail lacquers. However, the effectiveness of topical therapies is limited by minimal drug permeability through the nail plate (Baran and Kaoukhov, 2005). Current research on nail permeation focuses on altering the nail plate barrier by means of chemical treatments (Kobayashi et al., 1998; Malhotra and Zatz, 2002) and penetration enhancers (Hui et al., 2003). Physical and mechanical methods are also under examination.

2. Topical drug delivery to the nail and available formulations

Mycotic nail infections infrequently resolve spontaneously, and may have a substantial impact on quality of life. Current treatment modalities include surgery, as well as oral and topical antifungal agents. However, a meta-analysis of randomized trials found little high quality evidence that any topical therapy is effective (Crawford and Hollis, 2007). Topical therapy is indicated when the nail matrix is not involved (in ≈74% of patients) (Effendy et al., 2005). It is preferred in elderly patients or patients receiving multiple medications, in order to minimize drug–drug interactions. Topical therapy is also preferred in patients with mild-to-moderate disease and for those unwilling to use systemic medications. Topical therapy minimizes adverse systemic drug reactions, like those associated with oral antifungal agents (Elewski and Hay, 1996).

Multiple classes of antifungal medications have been utilized; these include: polyenes (e.g. nystatin) which have both fungistatic and fungicidal properties in vitro; imidazoles (e.g. clotrimazole, tioconazole, econazole, ketoconazole, miconazole, sulconazole, and oxiconazole), which have fungistatic properties in vitro; and allylamines/benzylamines (e.g. naftifine, terbinafine, and butenafine), which have fungistatic and fungicidal properties in vitro (Tom and Kane, 1999).

Only one topical therapy has been FDA approved for onychomycosis: ciclopirox nail lacquer 8% solution. Ciclopirox inhibits the transport of essential elements into the fungal cell, thus disrupting DNA, RNA, and protein synthesis. It is a broad-spectrum antifungal with activity against dermatophytes and some non-dermatophyte molds.

Two randomized, controlled trials suggest that complete resolution occurs in approximately 7% of treated patients compared with 0.4% using placebo. Thus, only 1 of 15 patients using the lacquer will have a favorable outcome which involved reaching a clinically and mycologically cured target nail (treatment cure). Treatment cure comprised of a negative culture and negative potassium hydroxide (KOH) as well as global evaluation score = cleared (100% clearance of clinical signs of disease); furthermore, recurrence is common after discontinuing therapy (Gupta et al., 2000).

In Europe, amorolfine and ciclopirox (nail lacquer 8% solution) have been approved for onychomycosis treatment. Amorolfine, available as a nail lacquer, acts by inhibiting the biosynthesis of ergosterol, a component of the fungal cell membranes. Amorolfine is fungistatic and fungicidal and most effective against dermatophytes, but can be used for yeast and molds with lesser efficacy (Haria and Bryson, 1995).

The clinical efficacy of amorolfine therapy in 727 patients with toenail or fingernail onychomycosis was evaluated. A mycological and clinical cure was achieved in 45–50% of the patients treated with 5% amorolfine lacquer once or twice weekly for 6 months at 3 months post-treatment (Zaug and Bergstraesser, 1992).

3. Human nail

The chemical composition of the human nail differs significantly from other body membranes. The plate, composed of keratin molecules with many disulphide linkages and low associated lipid levels, does not resemble any other body membrane in its barrier properties – it behaves more like a hydrogel than a lipophilic membrane.

Drug transport into the nail plate is influenced by: physicochemical properties of a drug molecule (size, shape, charge, and hydrophobicity), formulation characteristics (nature of the vehicle and drug concentration), presence of permeation enhancers, nail properties (thickness and hydration), and interactions between the permeant and the keratin network of the nail plate. The chemical composition and some experimental evidence indicate that the aqueous pathway plays the dominant role in drug penetration through the nail. Furthermore, water is the principle nail plasticizer. Once hydrated, the nail becomes more elastic and possibly more permeable to topically applied substances. However, the effects of hydration on nail permeation requires elucidation (Gunt and Kasting, 2006).

4. Nail sampling

Permeation studies with modified in vitro diffusion cells commonly utilized for flux determination. Drug is initially applied to the nail dorsal surface. Permeation is measured by sampling the solution on the ventral nail plate at successive time points, and calculating drug flux through the nail. This method bears similarities to skin penetration studies. However, skin penetration studies are not limited simply to determination of flux, but also include the separation of skin layers to quantify drug concentration in each layer.

A novel technique developed by Hui et al. enables the determination of drug concentration within the plate, where fungi reside. This method relies on a drilling system which samples the nail core without disturbing its surface (Fig. 1). This is achieved by the use of a micrometer-precision nail sampling instrument that enables finely controlled drilling into the nail with collection of the powder created by the drilling process. Drilling of the nail occurs through

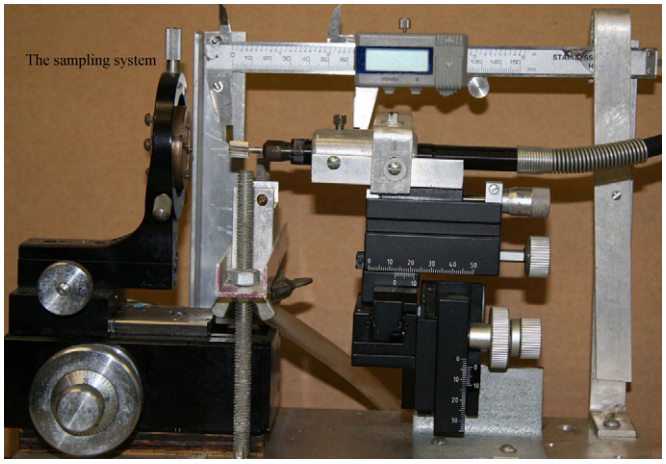

Fig. 1. The sampling device.

the ventral surface. The dorsal surface and ventrally-accessed nail core can be assayed separately. The dorsal surface sample contains residual drug, while the core from the ventral side provides drug measurement at the site of disease. This method permits drug measurement in the intermediate nail plate, which was previously impossible (Bronaugh and Maibach, 2005).

5. Enhancing nail penetration

Physical, chemical and mechanical methods have been used to decrease the nail barrier. Within each of these broad categories, many techniques exist to enhance penetration. Mechanical modes of penetration enhancement are typically straightforward, and have the most in vivo experience associated with them. In contrast, many of the chemical and physical methods discussed are still in the in vitro stages of development; laboratory studies are currently examining these techniques using human nail samples.

The goal of topical therapy for onychomycosis is drug penetration into deep nail stratums at amounts above the minimal inhibitory concentration (MIC). Effective penetration remains challenging as the nail is believed by some to be composed of approximately 25 layers of tightly bound keratinized cells, 100-fold thicker than the stratum corneum (SC) (Hao and Li, 2008b). Furthermore, De Berker et al. have observed increase in toe nail thickness along the nail. Mean nail plate thickness increased progressively along the entire length of the nail ranging between 590 μm and 1080 μm (De Berker et al., 1996). While there is disagreement on the exact thickness of the nail there is consensus that the nail structure is difficult to penetrate. In addition, poor permeability and prolonged transport lag time contribute to disappointing topical efficacy in nail diseases (Hao and Li, 2008b).

Chemical and physical modes of penetration enhancement may improve topical efficacy. There are two main factors to consider: physicochemical properties of the drug (polar compounds are more permeable) and binding of the drug to keratin within the nail. Binding to keratin reduces availability of the active (free) drug, weakens the concentration gradient, and limits deep penetration (Murthy et al., 2007b).

5.1. Mechanical methods to enhance nail penetration

Mechanical methods including nail abrasion and nail avulsion, have been used by dermatologists and podiatrists for many years – with varying results. Additionally, they are invasive and potentially painful. Thus, current research focuses on less invasive chemical and physical modes of nail penetration enhancement.

5.1.1. Nail abrasion

Simply stated, nail abrasion involves sanding of the nail plate to reduce thickness or destroy it completely. Sandpaper number 150 or 180 can be utilized, depending on required intensity. Sanding must be done on nail edges and should not cause discomfort (Di Chiacchio et al., 2003). An efficient instrument for this procedure is a high-speed (350,000 rpm) sanding hand piece (Baran et al., 2008). Additionally, dentist's drills have been used to make small holes in the nail plate, enhancing topical medication penetration (Di Chiacchio et al., 2003).

Nail abrasion thins the nail plate, decreasing the fungal mass of onychomycosis, and exposing the infected nail bed. In doing so, it may enhance the action of antifungal nail lacquer. The procedure may be repeated for optimal efficacy (Behl, 1973).

5.1.2. Nail avulsion

Total nail avulsion and partial nail avulsion involve surgical removal of the entire nail plate or partial removal of the affected nail plate, and under local anesthesia.

Keratolytic agents such as urea and salicylic acid soften the nail plate for avulsion. Urea or a combination of urea and salicylic acid have been used for nonsurgical avulsion (chemical avulsion) in clinical studies, prior to topical treatment of onychomycosis (Hettinger and Valinsky, 1991). Nail abrasion, using sandpaper nail files, prior to antifungal nail lacquer treatment may decrease the critical fungal mass and aid penetration (Di Chiacchio et al., 2003).

5.2. Chemical methods to enhance nail penetration

Studies examining the efficacy of chemical compounds with transungual penetration properties are currently underway. As would be expected, skin penetration enhancers do not usually have the same effect on nails (Walters et al., 1985). Thus far, only a few chemicals which enhance drug penetration into the nail plate have been described.

5.2.1. N-acetyl-L-cysteine and mercaptan compounds

Kobayashi et al. demonstrated that *N*-acetyl-L-cysteine and 2-mercaptoethanol, in combination, enhanced permeability of the antifungal drug tolnaftate into nail samples (Kobayashi et al., 1998). They suggested that these compounds may be generally useful in enhancing drug permeation across the nail plate.

Hoogdalem et al. evaluated the penetration-enhancing properties of *N*-acetyl-L-cysteine with the antifungal drug oxiconazole in vivo. *N*-acetyl-L-cysteine promoted oxiconazole retention in upper nail layers (Hoogdalem et al., 1997).

Malhotra and Zatz screened nail penetration enhancers, including: mercaptan compounds, sulfites, bisulfites, keratolytic agents and surfactants in vitro. *N*-(2-mercaptopropionyl) glycine, demonstrated superior penetration enhancement to all other compounds, urea acted synergistically to increase nail permeation to the greatest extent (Malhotra and Zatz, 2002). However, post-treatment barrier integrity studies demonstrated that changes induced in the nail keratin matrix by these effective chemical modifiers were irreversible. It is believed that these enhancers act by breaking disulphide bonds, which are responsible for nail integrity thus producing structural changes in the nail plate (Malhotra and Zatz, 2002; Murdan, 2007).

5.2.2. 2-n-nonyl-1,3-dioxolane

Hui et al. have showed that 2-*n*-nonyl-1,3-dioxolane (SEPA®) enhances penetration of econazole (from a lacquer formulation) into the human nail (Hui et al., 2003). They demonstrated that econazole penetrates the nail six times more effectively in a lacquer containing 2-*n*-nonyl-1,3-dioxolane than in an identical lacquer without enhancer. Concentrations of econazole in the deep nail

layer and nail bed were significantly higher in the 'enhancer' group than in the control group. Furthermore, in the 'enhancer' group, econazole concentration in the deep nail layer was 14,000 times greater than the MIC necessary to inhibit fungal growth.

5.2.3. Keratolytic enhancers

Guerrero et al. described the effect of keratolytic agents (papain, urea, and salicylic acid) on the permeability of three imidazole antifungal drugs (miconazole, ketoconazole, and itraconazole) (Quintanar-Guerrero et al., 1998). In the absence of keratolytic agents, no transungual antifungal permeation was detected over a period of 60 days. Despite these findings, it is likely that the spectrophotometric method of analysis was insufficiently sensitive to accurately measure drug concentrations.

Permeation of these agents did not improve by pre-treatment with 20% salicylic acid (for 10 days) and the addition of 40% urea to the donor solution. However, pre-treatment with both 15% papain (for 1 day) followed by 20% salicylic acid (for 10 days), enhanced antimycotic permeation. Presence of ethanol (as a co-solvent) did not promote flux. Although ethanol is an effective skin permeation enhancer, it does not have a similar effect on the nail. Ethanol acts on the SC by altering intercellular lipids; however, the lipid content of the nail comprises just 0.15–0.76% of its total weight. The authors proposed that aggressive pre-treatment (with papain and salicylic acid) produced pore formation in the nail matrix, allowing for effective drug permeation which was supported by the SEM images they obtained.

Brown et al. investigated the effect of two novel penetration enhancers (PEs), thioglycolic acid (TA) a reducing agent and urea hydrogen peroxide (urea H_2O_2) an oxidizing agent on the in vitro nail permeability of penetrants of varying lipophilicity caffeine, methylparaben and terbinafine. TA increased the flux of CF and MP ~4- and ~2-fold, respectively, while urea H_2O_2 proved ineffective at enhancing permeability. Effects of the PEs were penetrant specific, but the use of a reducing agent (TA) followed by an oxidising agent (urea H_2O_2) dramatically improved human nail penetration while reversing the application order of the PEs was only mildly effective. Both nail PEs are likely to function via disruption of keratin disulphide bonds and the associated formation of pores that provide more 'open' drug transport channels (Brown et al., 2009).

5.2.4. Keratinolytic enzymes

Due to an abundance of keratin filaments, keratinic tissues like the SC, are effectively hydrolyzed by keratinase (Gradisar et al., 2005).

Mohorcic et al. hypothesized that keratinolytic enzymes may hydrolyze nail keratins, thereby weakening the nail barrier and enhancing transungual drug permeation. This group conducted permeation studies using modified franz diffusion cells and metformin hydrochloride as a model drug and found keratinase to markedly enhance drug permeation through bovine hoof membranes (Mohorcic et al., 2007).

In another study, human nail clippings were incubated in keratinase for 48 h, and subsequently examined with scanning electron microscopy. Keratinase clearly disrupted the nail plate, acting on both the intercellular matrix that holds the cells of the nail plate together and the dorsal nail corneocytes by corroding their surface (Mohorcic et al., 2007).

5.3. Physical methods to enhance nail penetration

Physical permeation enhancement may be superior to chemical methods in delivering hydrophilic and macromolecular agents (Murthy et al., 2007b). We discuss several physical enhancement methods, both established and experimental.

5.3.1. Iontophoresis

Iontophoresis involves delivery of a compound across a membrane using an electric field (electromotive force). The principle has been applied clinically for cutaneous anesthesia, hyperhidrosis management, antibiotic penetration, and herpes simplex treatment (Kassan et al., 1996). Currently both LidoSite® (lidocaine HCl/epinephrine topical iontophoretic patch) and GlucoWatch® (iontophoretic measurement of glucose in diabetics) are FDA approved. Iontophoresis has been used for various applications different from transdermal ophthalmic, dental, orthopaedic, etc. (Horwath-Winter et al., 2005; Nowicki et al., 2002; Chen et al., 2008). Drug diffusion through the hydrated keratin of a nail may be enhanced by iontophoresis.

Several factors contribute to this enhancement: electrorepulsion/electrophoresis, interaction between the electric field and the charge of the ionic permeant; electroosmosis, convective solvent flow in preexisting and newly created charged pathways; and permeabilization/electroporation, electric field-induced pore induction (Murthy et al., 2007b; Hao and Li, 2008b). While transport enhancement of neutral permeants relies on electroosmosis, transport enhancement of ionic permeants relies on electrophoresis and electroosmosis. The effects of electric current on nails are reversible in vitro; nail plates will return to normal after iontophoresis treatment (Hao and Li, 2008b).

Murthy et al. elegantly examined transport of salicylic acid (SA) across the human nail plate (Murthy et al., 2007b). In vitro transport studies were performed using specifically-designed diffusion cells. Compared to passive transport, iontophoresis significantly enhanced drug penetration through the nail. Iontophoretic trans-nail flux improved with higher SA concentrations (up to 2 mg/ml), higher current density (up to 0.5 mA/cm^2), higher buffer ionic strength (optimal strength at 50–100 mM), and higher pH. Murthy reported increased transungal glucose and griseofulvin flux with higher pH (pH > 5) in anodal iontophoresis (Murthy et al., 2007a). pH dependent transport due to cathodal iontophoresis followed the opposite trend (i.e. lower pH correlated with increased flux). Griseofulvin transport was enhanced ≈8-fold with iontophoresis.

Hao and Li performed in vitro iontophoresis experiments on human nails with neutral and charged molecules. Anodal iontophoresis at 0.3 mA enhanced mannitol (MA) and urea (UR) transport compared to passive diffusion. Additionally, findings suggested only marginal contribution of electroosmosis in anodal iontophoretic transport of MA and UR using low electric current (≤0.3 mA). Electroosmosis contribution increased with permeant molecular size and current strength (Hao and Li, 2008b).

This group examined the effects of pH and ionic strength on electroosmotic transungual transport of neutral compounds (Hao and Li, 2008a). When pH was below the isoelectric point (p*I*) (pH < 5), the nail plates were positively charged, and electroosmotic flow occurred from cathode to anode. Conversely, when pH was above p*I* (pH > 5), the nail plates were negatively charged, and electroosmotic flow occurred from anode to cathode. Furthermore, electroosmosis improved significantly from pH 7.4 to 9 in anodal iontophoretic transport. As discussed previously, electroosmosis contribution was greater in MA than UR due to increased molecular size. Additionally, significant electroosmosis enhancement was seen only in MA, not UR, with pH changes (anodal transport at pH 7.4 and 9 and cathodal transport at pH 3). Electroosmosis correlated inversely with ionic strength, decreasing by four times when the solution ionic strength increased from 0.04 to 0.7 M. Nevertheless, the authors concluded that effects of electroosmosis were generally small, even at pH 9 and ionic strength of 0.04 M. They suggest more focus on the direct-field effect of iontophoresis, and less on electroosmosis.

Tetraethylammonium ion (TEA), a positively charged permeant, penetration was significantly enhanced with anodal iontophoresis at only 0.1 mA (i.e. permeability coefficients were 29-fold higher under iontophoretic transport than under passive transport) (Hao and Li, 2008b). Time-dependent instantaneous permeability coefficients for TEA did not begin to plateau until 15 h, while MA reached a plateau within 9 h. Additionally, contribution of electroosmosis was less than 10% of electrophoresis in TEA. Of interest, the Nernst–Planck theory was applied to successfully predict transungual iontophoretic transport for TEA. Hopefully, it will apply to antifungal drug penetration under iontophoresis.

5.3.2. Etching

"Etching" results from surface-modifying chemical (e.g. phosphoric acid) exposure, resulting in formation of profuse microporosities. These microporosities increase wettability and surface area, and decrease contact angle; they provide an ideal surface for bonding material. Presence of microporosities improves "interpenetration and bonding of a polymeric delivery system and facilitation of interdiffusion of a therapeutic agent" (Repka et al., 2004).

Once a nail plate has been "etched," a sustained-release, hydrophilic, polymer film drug delivery system may be applied. Bioadhesion, "a phenomenon related to the ability of biological or synthetic material to adhere to biological substrate," must be considered; improved bioadhesion results in superior application of a transungual bioadhesive drug delivery system (Repka et al., 2004).

Repka et al. used hot-melt extruded (HME) hydroxypropyl cellulose (HPC) films, in which active compound (e.g. 20% w/w ketoconazole) is embedded in a carrier formulation with meltable substances (i.e. polymeric materials or low melting point waxes) and other functional excipients. Over "etched" nails (secondary to phosphoric acid exposure), the authors hoped HME would further improve transungual ketoconazole delivery (Repka et al., 2004).

HME films were processed at 115–120 °C utilizing a Killion extruder. Extruded films demonstrated excellent content uniformity and post-processing drug content. HME films containing ketoconazole had 6-fold greater permeation in "etched" nail plates compared with normal nail plates. Ketoconazole 0.125% gel alone had a 60% higher permeability through "etched" nails than through normal nails. Bioadhesion of HME films was also significantly greater in "etched" (treated with 10% phosphoric acid gel for 60 s) vs. normal nails.

Repka et al., utilizing atomic force microscopy, scanning electron microscopy, and polarized light microscopy, demonstrated substantial dorsal nail surface disruption with phosphoric acid gel and tartaric acid (TTA); roughness scores increased 2-fold with phosphoric acid treatment vs. control (Repka et al., 2002). Roughness of the nail surface results in increased surface area, providing "greater opportunity for polymer chains to inter-diffuse and bond with the nail plate, improving bioadhesion and retention of a drug delivery system." Surface modifications influence polymer–substrate interactions – increasing adhesive force and toughness (Mididoddi et al., 2006).

Mididoddi et al. determined the influence of TTA on bioadhesion and mechanical properties in HME HPA films on human nails in vitro (Mididoddi et al., 2006). Though HPC alone has bioadhesive qualities, the TTA-incorporated film exhibited significantly greater bioadhesion than HPC film without TTA. This held true for various contact forces and bioadhesion parameters measured; TTA-containing film was 12-fold higher than film without TTA for one such parameter. Of note, film contact time affected bioadhesion more than contact force did.

5.3.3. Carbon dioxide laser

CO_2 laser may result in positive, but unpredictable, results. One method involves avulsion of the affected nail portion followed by laser treatment at 5000 W/cm^2 (power density). Thus, underlying tissue is exposed to direct laser therapy. Another method involves penetrating the nail plate with CO_2 laser beam. This method is followed with daily topical antifungal treatment, penetrating laser-induced puncture holes. The first method is preferred. In 9 onychomycosis patients treated with CO_2 laser, complete resolution and healing occurred in 6, with 7 reporting mild or no pain; average healing time was 21 days. Another trial of 50 patients demonstrated good or excellent results in 70% (35/50) (Rothermel and Apfelberg, 1987).

5.3.4. Hydration and occlusion

Hydration may increase the pore size of nail matrix, enhancing transungual penetration. Additionally, hydrated nails are more elastic and permeable. Iontophoresis studies have utilized this property to further enhance penetration (Hao and Li, 2008b). Solution pH and ionic strength have demonstrated no significant effect on nail hydration (Hao and Li, 2008a).

Diffusivity of water and other materials (i.e. drugs) increases as human skin becomes more hydrated (Kasting et al., 2003). Human stratum corneum retains up to ~300% of its weight in water (Kasting and Barai, 2003); when SC is saturated, diffusivity increases several-fold. In contrast, Gunt et al. demonstrated that nail hydration capacity occurs at ~25%, only twice its normal water content of 10–15%. Hence, unlike SC, transungual diffusivity does not dramatically increase with relative humidity (RH). Nonetheless, hydration still has a pronounced effect on drug penetration in the region of high water content (RH > 80%) (Gunt and Kasting, 2006).

Gunt and Kasting demonstrated that increasing ambient relative humidity (RH) from 15% to 100% enhanced permeation of [^{3}H]-ketoconazole by a factor of three in vitro (Gunt and Kasting, 2007). Flux increased from 0.175 μg/cm^2/h at 15% RH to 0.527 μg/cm^2/h at 100% RH. The most dramatic rise in [^{3}H]-ketoconazole flux occurred between 80% and 100% RH. Unbound [^{3}H]-ketoconazole concentrations across the nail were 10-fold higher than MIC values of ketoconazole.

Topical onychomycosis treatments typically require frequent application. Environmental exposure results in difficulty maintaining a constant drug level. A nail patch may be effective in multiple ways: constant drug exposure with less frequent application and increased hydration to the dystrophic nail. Susilo et al. conducted in vivo experiments examining nail patches containing a potent antifungal sertaconazole 3.63 mg; patches were replaced weekly. Penetration was remarkable, approaching 40–50%; mean sertaconazole concentrations were well above MIC at 2, 4, and 6 weeks (Susilo et al., 2006).

Decreases in transonychial water loss, ceramide concentration, and water binding capacity may result from onychomycosis. Occlusion may resolve these changes via reconstitution of water and lipid homeostatisis in dystrophic nails. Additionally, sertaconazole was able to amass in substantial subungual concentrations under occlusion (Susilo et al., 2006).

Grover et al. treated onychomycosis with avulsion and topical antifungal therapy (ketoconazole 2% cream vs. oxiconazole 1% cream), with and without occlusion. The overall efficacy rate was just 56% (15/27 patients cured); however, 71% of those in the occlusion group achieved cure vs. 38% in the non-occlusion group. Unfortunately, these results were not statistically significant, given small sample size (Grover et al., 2007).

Baden reported 5 patients who underwent avulsion and topical treatment (ketoconazole 2% cream vs. ciclopirox olamine 1% cream) under occlusion (with various methods). All patients were free of onchymycosis even 1.5 years later; both antifungals were equally effective (Baden, 1994).

5.4. New frontiers in physical penetration enhancement

5.4.1. Lasers

A patent has been filed for a microsurgical laser apparatus which makes holes in nails (Karrell, 1999); topical antifungals can be applied in these holes for onychomycosis treatment. Further work remains to characterize this new invention, termed the 'onycholaser.'

5.4.2. Phonophoresis

Phonophoresis describes the process by which ultrasound waves are transferred though a coupling medium onto a tissue surface. The induction of thermal, chemical, and/or mechanical alterations in this tissue may explain drug delivery enhancement. At a gross level, phonophoresis may result in improved penetration through the SC transcellularly or via increased pore size; at a cellular level, pores in the cell membrane (secondary to lipid bilayer alteration) may enhance drug diffusion (Kassan et al., 1996).

There exist no studies documenting phonopheresis on nail penetration. However, it has been used to enhance percutaneous penetration to joints, muscle, and nerves. Enhanced penetration of anesthetics, fluocinolone acetonide, and amphotericin B is recorded. It may serve as an effective monotherapy of keloid scars (Kassan et al., 1996).

Potential may exist for phonophoresis in onychomycosis therapy – further work remains. Advantages of phonophoresis (and iontophoresis) include: enhanced drug penetration, strict control of penetration rates, rapid termination of drug delivery, intact diseased surface, and lack of immune sensitization.

5.4.3. Ultraviolet light

A recently submitted patent discusses use of heat and/or ultraviolet (UV) light to treat onychomycosis (Maltezos and Scherer, 2005); several different instruments and methodologies are discussed which may effectively provide exposure. One method involves heating the nail, exposing it to UV light, and subsequently treating with topical antifungal therapy. Further studies examining heating and UV light in onychomycosis treatment will determine efficacy.

5.4.4. Photodynamic therapy of onychomycosis with aminolevulinic acid

Photodynamic therapy (PDT) is a medical treatment based on the combination of a sensitizing drug and a visible light used together for destruction of cells. PDT based on topical application of aminolevulinic acid (ALA) acid is used in oncological field. Topical PDT is being evaluated and modified to provide a once-off curative treatment for onychomycosis. This would negate the need for prolonged topical or systemic treatment regimens, with their associated poor success rates and potential for drug resistance, side effects, drug–drug interactions, and increased morbidity (Donnelly et al., 2005).

6. Formulations may improve penetration

Hui et al. demonstrated the importance of formulation in transungual drug delivery (Hui et al., 2004). The penetration of the antifungal ciclopirox was determined for a marketed gel (0.77% active drug), an experimental gel (2% active drug), and a marketed lacquer (8% active drug). Despite containing the lowest active drug concentration, the marketed gel produced the greatest transungual ciclopirox delivery. We hypothesize that this particular gel formulation may effectively improve nail hydration, thus enhancing nail permeability.

7. New drugs

Oxaboroles, a new class of antifungal agents, have been recently described. Oxaborole AN2690 penetrates the nail more effectively than ciclopirox, achieving impressive levels within and beneath the nail plate. Future studies will better characterize this agent, and likely support its use in onychomycosis (Hui et al., 2007).

In order to develop appropriate formulations for topical ungual application, there is a need for robust and validated in vitro techniques and models to enable the accurate prediction of the fate of the drug in vivo.

8. Limitations of current ungual drug permeability studies

8.1. Use of animal hooves as a model for nail penetration

Animal hooves provide an alternative to human nail in permeation studies. Myoung et al. utilized porcine hoof to investigate the effect of pressure sensitive adhesives on ciclopirox penetration (Myoung and Choi, 2003). Monti et al. used bovine hooves to investigate the permeation of compounds from an experimental nail lacquer (Monti et al., 2005). Care must be exercised when using the hoof as a model for the human nail plate in extrapolating data to predict ungual penetration in humans in vivo. Khengar et al. compared the swelling of human nail and horses' hooves as a result of solvent uptake in the presence of penetration enhancer systems. The increase in weight by the hooves was $40 \pm 9\%$ compared to a $27 \pm 3\%$ weight increase by human nails when immersed in control solutions (Khengar et al., 2007). This provides support to the findings Mertin et al. that the mammalian hoof is capable of taking up and retaining more water than human nail (36% vs. 27%) as animal hoof keratin is thought to be less dense than the human nail plate (Mertin and Lippold, 1997).

In vitro permeability of several antifungal agents was examined in porcine hoof membranes using diffusion cells. Drugs were suspended in ethanol 42% (v/v) in the donor chamber, and ethanol 42% (v/v) also served as the receptor fluid (Mertin and Lippold, 1997). The maximum flux of the 10 antimycotics through the nail plate was predicted on the basis of their penetration rates through the hoof membranes and their water solubilities using the following equation:

$$\log\ P_N = 3.723 + 1.751\ \log\ P_H$$

where P_N is the nail plate permeability coefficient and P_H is the permeability through hoof membrane derived experimentally. An efficacy coefficient against onychomycosis was calculated from the maximum flux and the minimum inhibitory concentration. Using this experimental data from this study, mathematical models were developed and utilized to predict human nail permeability and maximum flux.

These results may indicate that animal hooves do not necessarily provide a representative model in which diffusion through the nail can be evaluated. The hoof is more permeable than the human nail plate. Hoof proteins have a significantly lower disulfide linkages compared to the human nail plate (Baden et al., 1973). As a result, the hoof may be less susceptible to compounds, which break the disulfide linkages currently being investigated as potential perungual penetration enhancers. In such cases, enhancement of perungual absorption in the hoof may be less than the enhancement that could be achieved in human nail plates. Taken together careful validation studies with a variety of molecular weights, and solubilities should clarify functional similarities and difference between the two. The current data is insufficient to establish a correlation between animal hooves and human nail plate to be able to use it as a model for human nail plate in permeation studies.

8.2. Use of nail clippings as a model of nail penetration

Nail clippings have been previously used as a model for the human nail plate. It is easier to obtain nail clippings from healthy volunteers and use them for in vitro testing; this model, however, is short of the nail bed so it might not be the best model for nail studies. This model needs to be validated and compared to the use of avulsed human cadaver nail plates model so that we would be able to predict permeability through human nail with the use of data from animal hooves.

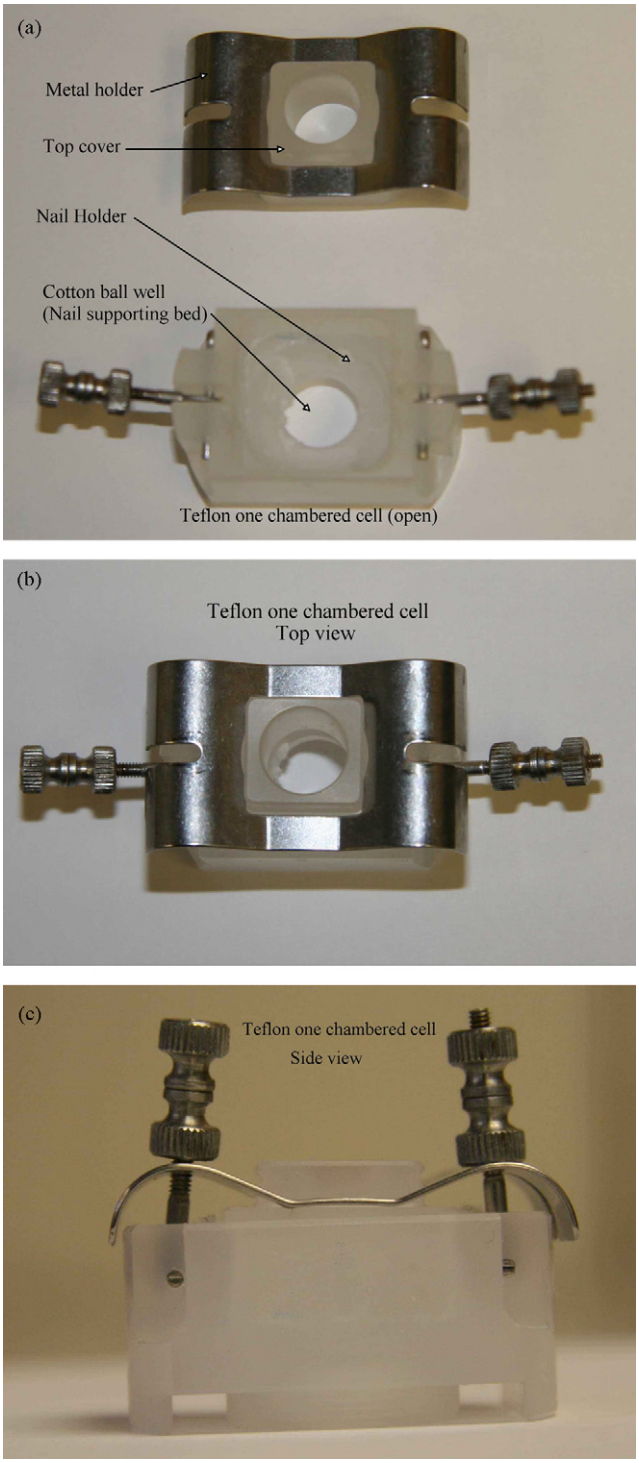

Fig. 2. Nail support and incubation system: (a) Teflon one-chamber diffusion cell components, (b) Teflon one-chamber diffusion cell top view, and (c) Teflon one-chamber diffusion cell side view.

8.3. Super hydration method

The most commonly used in vitro method in studying drug permeation through the nail is performed using modified diffusion cells. This method is similar to skin penetration studies where permeation is measured by sampling the solution on the ventral nail plate at successive time points, and calculating drug flux through the nail. Hui et al. slightly modified this approach by using a cotton ball soaked in saline, to provide moisture (but not saturation) and hydrating the nail throughout the experiment. The setup is as follows: a Teflon one-chamber diffusion cell is used to hold each nail in order to mimic physiological conditions (Fig. 2a–c), a small cotton ball wetted with normal saline is placed in the chamber to serve as a "nail bed" and provide moisture for the nail plate. As previously mentioned the hydration may increase the pore size of nail matrix thus promoting transungual penetration. Additionally, from everyday life experience upon soaking of nails we can easily notice that the nails become more soft, flexible and elastic. Data obtained from previous studies need to be interpreted differently as it is lacking the effect of nail hydration thus affecting nail permeation.

8.4. Correlation of in vitro to in vivo studies

The in vitro data obtained with the use of modified diffusion cells together with nail clippings, avulsed human cadaver nail plates and animal hooves as model for human nail plates required comparison to in vivo data with the use of radioisotopes and atomic mass spectroscopy. In vitro human or animal studies assume that penetration is a passive process and that there is no viable component to it. We strongly believe that in vitro studies must be correlated to what happens in vivo. Until these correlations are defined biological interpretation remains tenuous. Additional references are found in a recent overview (Murdan, 2008).

References

Baden, H.P., 1994. Treatment of distal onychomycosis with avulsion and topical antifungal agents under occlusion. Arch. Dermatol. 130, 558–559.
Baden, H.P., Goldsmith, L.A., Fleming, B., 1973. A comparative study of the physicochemical properties of human keratinized tissues. Biochim. Biophys. Acta 322, 269–278.
Baran, R., Hay, R.J., Garduno, J.I., 2008. Review of antifungal therapy and the severity index for assessing onychomycosis: part I. J. Dermatolog. Treat. 19, 72–81.
Baran, R., Kaoukhov, A., 2005. Topical antifungal drugs for the treatment of onychomycosis: an overview of current strategies for monotherapy and combination therapy. J. Eur. Acad. Dermatol. Venereol. 19, 21–29.
Behl, P.N., 1973. Abrasion in the treatment of nail disorders. Indian J. Dermatol. 18, 77–79.
Bronaugh, R.L., Maibach, H.I., 2005. Percutaneous Absorption: Drugs, Cosmetics, Mechanisms, Methodology. Taylor & Francis, Boca Raton.
Brown, M.B, Khengar, R.H., Turner, R.B., Forbes, B., Traynor, M.J., Evans, C.R., Jones, S.A., 2009. Overcoming the nail barrier: a systematic investigation of ungual chemical penetration enhancement. Int. J. Pharm. 370, 61–67.
Chen, L.J., Meng, Q.F., Chen, Y.M., Smales, R.J., Yip, K.H., 2008. Effect of fluoride iontophoresis on the microtensile bond strength between dentin and two adhesive systems. J. Dent. 36, 697–702.
Crawford, F., Hollis, S., 2007. Topical treatments for fungal infections of the skin and nails of the foot. Cochrane Database Syst. Rev. (3): CD001434. doi:10.1002/14651858.CD001434.pub2.
De Berker, D., Mawhinney, B., Sviland, L., 1996. Quantification of regional matrix nail production. Br. J. Dermatol. 134, 1083–1086.
Di Chiacchio, N., Kadunc, B.V., De Almeida, A.R., Madeira, C.L., 2003. Nail abrasion. J. Cosmet. Dermatol. 2, 150–152.
Donnelly, R.F., Mccarron, P.A., Lightowler, J.M., Woolfson, A.D., 2005. Bioadhesive patch-based delivery of 5-aminolevulinic acid to the nail for photodynamic therapy of onychomycosis. J. Control. Release 103, 381–392.
Effendy, I., Lecha, M., Feuilhade De Chauvin, M., Di Chiacchio, N., Baran, R., 2005. Epidemiology and clinical classification of onychomycosis. J. Eur. Acad. Dermatol. Venereol. 19, 8–12.
Elewski, B.E., Hay, R.J., 1996. Update on the management of onychomycosis: highlights of the Third Annual International Summit on Cutaneous Antifungal Therapy. Clin. Infect. Dis. 23, 305–313.

Gradisar, H., Friedrich, J., Krizaj, I., Jerala, R., 2005. Similarities and specificities of fungal keratinolytic proteases: comparison of keratinases of Paecilomyces marquandii and Doratomyces microsporus to some known proteases. Appl. Environ. Microbiol. 71, 3420–3426.
Grover, C., Bansal, S., Nanda, S., Reddy, B.S., Kumar, V., 2007. Combination of surgical avulsion and topical therapy for single nail onychomycosis: a randomized controlled trial. Br. J. Dermatol. 157, 364–368.
Gunt, H., Kasting, G.B., 2006. Hydration effect on human nail permeability. J. Cosmet. Sci. 57, 183–184.
Gunt, H.B., Kasting, G.B., 2007. Effect of hydration on the permeation of ketoconazole through human nail plate in vitro. Eur. J. Pharm. Sci. 32, 254–260.
Gupta, A.K., Fleckman, P., Baran, R., 2000. Ciclopirox nail lacquer topical solution 8% in the treatment of toenail onychomycosis. J. Am. Acad. Dermatol. 43, S70–80.
Gupta, A.K., Scher, R.K., 1998. Oral antifungal agents for onychomycosis. Lancet 351, 541–542.
Hao, J., Li, S.K., 2008a. Mechanistic study of electroosmotic transport across hydrated nail plates: effects of pH and ionic strength. J. Pharm. Sci. 97, 5186–5197.
Hao, J., Li, S.K., 2008b. Transungual iontophoretic transport of polar neutral and positively charged model permeants: effects of electrophoresis and electroosmosis. J. Pharm. Sci. 97, 893–905.
Haria, M., Bryson, H.M., 1995. Amorolfine. A review of its pharmacological properties and therapeutic potential in the treatment of onychomycosis and other superficial fungal infections. Drugs 49, 103–120.
Hettinger, D.F., Valinsky, M.S., 1991. Treatment of onychomycosis with nail avulsion and topical ketoconazole. J. Am. Podiatr. Med. Assoc. 81, 28–32.
Hoogdalem, E.J., Hoven, W.E., Terpstra, I.J., Zijtveld, J., Verschoor, J.S.C., Visser, J.N., 1997. Nail penetration of the antifungal agent oxiconazole after repeated topical application in healthy volunteers, and the effect of acetylcysteine. Eur. J. Pharm. Sci. 5, 119–127.
Horwath-Winter, J., Schmut, O., Haller-Schober, E.M., Gruber, A., Rieger, G., 2005. Iodide iontophoresis as a treatment for dry eye syndrome. Br. J. Ophthalmol. 89, 40–44.
Hui, X., Baker, S.J., Wester, R.C., Barbadillo, S., Cashmore, A.K., Sanders, V., Hold, K.M., Akama, T., Zhang, Y.K., Plattner, J.J., Maibach, H.I., 2007. In vitro penetration of a novel oxaborole antifungal (AN2690) into the human nail plate. J. Pharm. Sci. 96, 2622–2631.
Hui, X., Chan, T.C., Barbadillo, S., Lee, C., Maibach, H.I., Wester, R.C., 2003. Enhanced econazole penetration into human nail by 2-*n*-nonyl-1,3-dioxolane. J. Pharm. Sci. 92, 142–148.
Hui, X., Wester, R.C., Barbadillo, S., Lee, C., Patel, B., Wortzmman, M., Gans, E.H., Maibach, H.I., 2004. Ciclopirox delivery into the human nail plate. J. Pharm. Sci. 93, 2545–2548.
Karrell, M.L., 1999. Laser Apparatus for Making Holes and Etchings. USPTO, USA.
Kassan, D.G., Lynch, A.M., Stiller, M.J., 1996. Physical enhancement of dermatologic drug delivery: iontophoresis and phonophoresis. J. Am. Acad. Dermatol. 34 (4), 657–666.
Kasting, G.B., Barai, N.D., 2003. Equilibrium water sorption in human stratum corneum. J. Pharm. Sci. 92, 1624–1631.
Kasting, G.B., Barai, N.D., Wang, T.F., Nitsche, J.M., 2003. Mobility of water in human stratum corneum. J. Pharm. Sci. 92, 2326–2340.
Khengar, R.H., Jones, S.A., Turner, R.B., Forbes, B., Brown, M.B., 2007. Nail swelling as a pre-formulation screen for the selection and optimisation of ungual penetration enhancers. Pharm. Res. 24, 2207–2212.
Kobayashi, Y., Miyamoto, M., Sugibayashi, K., Morimoto, Y., 1998. Enhancing effect of *N*-acetyl-L-cysteine or 2-mercaptoethanol on the in vitro permeation of 5-fluorouracil or tolnaftate through the human nail plate. Chem. Pharm. Bull. (Tokyo) 46, 1797–1802.
Malhotra, G.G., Zatz, J.L., 2002. Investigation of nail permeation enhancement by chemical modification using water as a probe. J. Pharm. Sci. 91, 312–323.
Maltezos, G., Scherer, A., 2005. Treatment of Toenail Fungus. USPTO, USA.
Mertin, D., Lippold, B.C., 1997. In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: prediction of the penetration rate of antimycotics through the nail plate and their efficacy. J. Pharm. Pharmacol. 49, 866–872.
Mididoddi, P.K., Prodduturi, S., Repka, M.A., 2006. Influence of tartaric acid on the bioadhesion and mechanical properties of hot-melt extruded hydroxypropyl cellulose films for the human nail. Drug Dev. Ind. Pharm. 32, 1059–1066.
Mohorcic, M., Torkar, A., Friedrich, J., Kristl, J., Murdan, S., 2007. An investigation into keratinolytic enzymes to enhance ungual drug delivery. Int. J. Pharm. 332, 196–201.
Monti, D., Saccomani, L., Chetoni, P., Burgalassi, S., Saettone, M.F., Mailland, F., 2005. In vitro transungual permeation of ciclopirox from a hydroxypropyl chitosan-based, water-soluble nail lacquer. Drug Dev. Ind. Pharm. 31, 11–17.
Murdan, S., 2007. 1st meeting on topical drug delivery to the nail. Expert. Opin. Drug Deliv. 4, 453–455.
Murdan, S., 2008. Enhancing the nail permeability of topically applied drugs. Expert. Opin. Drug Deliv. 5, 1267–1282.
Murthy, S.N., Waddell, D.C., Shivakumar, H.N., Balaji, A., Bowers, C.P., 2007a. Iontophoretic permselective property of human nail. J. Dermatol. Sci. 46, 150–152.
Murthy, S.N., Wiskirchen, D.E., Bowers, C.P., 2007b. Iontophoretic drug delivery across human nail. J. Pharm. Sci. 96, 305–311.
Myoung, Y., Choi, H.K., 2003. Permeation of ciclopirox across porcine hoof membrane: effect of pressure sensitive adhesives and vehicles. Eur. J. Pharm. Sci. 20, 319–325.
Nowicki, K.D., Hummer 3rd, C.D., Heidt Jr., R.S., Colosimo, A.J., 2002. Effects of iontophoretic versus injection administration of dexamethasone. Med. Sci. Sports Exerc. 34, 1294–1301.
Quintanar-Guerrero, D., Ganem-Quintanar, A., Tapia-Olguin, P., Kalia, Y.N., Buri, P., 1998. The effect of keratolytic agents on the permeability of three imidazole antimycotic drugs through the human nail. Drug Dev. Ind. Pharm. 24, 685–690.
Repka, M.A., Mididoddi, P.K., Stodghill, S.P., 2004. Influence of human nail etching for the assessment of topical onychomycosis therapies. Int. J. Pharm. 282, 95–106.
Repka, M.A., O'haver, J., See, C.H., Gutta, K., Munjal, M., 2002. Nail morphology studies as assessments for onychomycosis treatment modalities. Int. J. Pharm. 245, 25–36.
Rothermel, E., Apfelberg, D.B., 1987. Carbon dioxide laser use for certain diseases of the toenails. Clin. Podiatr. Med. Surg. 4, 809–821.
Susilo, R., Korting, H.C., Greb, W., Strauss, U.P., 2006. Nail penetration of sertaconazole with a sertaconazole-containing nail patch formulation. Am. J. Clin. Dermatol. 7, 259–262.
Tom, C.M., Kane, M.P., 1999. Management of toenail onychomycosis. Am. J. Health Syst. Pharm. 56, 865–871.
Walters, K.A., Flynn, G.L., Marvel, J.R., 1985. Physicochemical characterization of the human nail: solvent effects on the permeation of homologous alcohols. J. Pharm. Pharmacol. 37, 771–775.
Zaug, M., Bergstraesser, M., 1992. Amorolfine in the treatment of onychomycoses and dermatomycoses (an overview). Clin. Exp. Dermatol. 17, 61–70.