J. DRUG DEL. SCI. TECH., 24 (3) 301-310 2014

Transungual drug delivery: an update

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Topical therapy continues to be the treatment of choice for the patients and clinicians in treating certain infections of the nails. Topical treatment is widely accepted as an adjunct with oral therapy to improve the cure rates, reduce the treatment duration, cut down the treatment cost and enhance the therapeutic outcomes. However, effectiveness of topical therapy continues to pose a challenge owing to the poor permeability of the nail plate to many therapeutic agents and the prolonged treatment periods. Research over the past one decade has been focused to improve the transungual permeation using chemical penetration enhancers, mechanical methods and physical methods. Disrupting the dorsal surface of the nail by treating with penetration enhancers or etching agents or abrasion or filing of the nail plate has proved to drastically improve the efficacy of topical therapy. The present review is an effort to update the different chemical enhancers and etching agents used to enhance the transungual permeability.

Key words: Transungual – Onychomycosis – Penetration enhancers – Etching agents – Screening methods.

I. NAIL ANATOMY

The human nail apparatus is made of nail folds, nail matrix, nail plate and the nail bed. The nail folds are the wedge-shaped fold of the skins surrounding the sides of the nail plate. The nail fold present at the proximal end of the nail is termed as the proximal nail fold while those situated on either sides of the nail are called the lateral nail folds (*Figure 1*).

The dorsal surface of the proximal nail fold covers a part of the nail matrix and continues as the eponychium or the cuticle [1]. The nail folds that form soft keratinized flaps are made up of cornified epithelium which is similar to the normal skin. The nail matrix that is present just beneath the proximal nail plate basically consists of living, rapidly multiplying epidermal cells. The nail matrix is seen as a semilunar area totally recessed under the proximal nail fold or may extend as the lanula that may be more evident on the thumb and the toes rather than the fingers. The nail plate originates from the highly germinative nail matrix and is found to cover almost the entire nail bed. The nail plate is a hard, elastic, translucent and convex structure made of about 25 layers of flattened, dead, keratinized tightly bound cells and ranges in thickness of 0.25 to 0.6 mm. The nail plate is differentiated into the upper dorsal, the middle intermediate and the inner ventral layer that differ in thickness in a ratio of 3:5:2 respectively [2]. The dorsal layer is hard, whereas germinative epithelial intermediate layer is softer and more flexible. The ventral layer is soft and connects the nail plate to the underlying nail bed. The dorsal surface of the nail plate is considered to be the rate limiting barrier for the permeation of topically applied therapeutics. The human nail is uniquely designed as it is curved along the transverse as well as the longitudinal axes [3]. The unique design and composition of the nail plate contributes to its strength and physical characteristics.

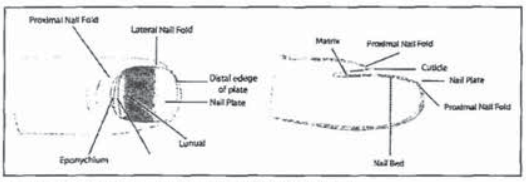

Figure 1 - Different parts of the nail apparatus.

The nail plate contains 7 to 12 % of water under normal ambient conditions that maintains the opacity, elasticity and flexibility of the nail while the content may increase to about 25 % at a relative humidity (%RH) of 100 % [4]. The nail plate also contains traces of lipids (0.1-1.0 %), composed of long chain fatty acids, free fats, cholesterol, squalene and phospholipids that are organized as bilayers and oriented parallel to the nail surface in the dorsal and ventral layers of the nail plate [5]. The dorsal and ventral layers of the nail plate contains relatively higher amounts of calcium, phospholipids and sulphydryl groups while the intermediate layer has more number of disulphide bonds but lower number of bound sulphydryl groups, phospholipids and calcium. The size, shape, thickness, surface ridging, curvature and the flexibility of the nail plate tends to vary within and among individuals depending on the site, age, disease states and seasons [1]. Nail bed is found to have a rich supply of nerves and lymphatic vessels and appears pink in color due to the underlying vascular network [6].

II. DISEASES OF THE NAIL

The two most common infectious diseases that can affect the nails are onychomycosis and nail psorioisis. Onychomycosis is the fungal infection of the nail that contributes to 50 % of the total nail disorders [7]. The main pathogens in 90 % of these cases is usually *Trichophyton rubrum* while the other causative organisms include yeasts mainly *Candida albicans* and non-dermatophyte moulds. The infection is more prevalent in certain groups like the elderly, diabetics, miners and sports-active individuals. [8]. The other risk factors are immunosuppression owing to human immunodeficiency virus (HIV) infections, cancer and other atopic disorders. Based on the part of the nail affected and the pathophysiology, onychomycosis may be: (i) distal subungual which involves infection of the nail plate tip and the underlying nail bed; (ii) proximal subungual that affects the cuticle and the nail bed; (iii) superficial infection which is confined only to the nail plate; (iv) total dystrophic that infects the whole nail [9]. The infected nails appear ugly, discolored and thickened thereby posing serious cosmetic, medical social and emotional problems [10].

Onychomycosis is an infection that is difficult to treat since it is chronic, hard to eradicate and tends to commonly relapse. The only treatment option for onychomycosis in the past was surgical avulsion of the nail that would be extremely traumatic and painful [11]. However, currently the infection is treated with systemic and/or local antifungal

agents, considering the severity, patient population and choice, and cost effectiveness [12]. Systemic treatment involves prolonged oral dosing of powerful antifungal agents while the topical treatment is indicated only in cases where few nails are involved [13]. Moreover, the topical monotherapy, is generally recommended in the treatment of mild and distal infections, for superficial white onychomycosis and in cases where the nail matrix may not be involved [14]. Despite multiple therapeutic options, treatment failure has been common as about 20 % of the patients fail to respond to treatment due to which onychomycosis is considered as a "stubborn clinical problem" [15]. The therapeutic failures are due to the indiscriminate and extensive use of systemic antifungals which have increased the numbers of emerging resistant strains. Owing to the development of resistant strains, relapse of onychomycosis is common with a recurrence rates varying from 10 to 53 % [16].

Nail psoriasis is the other important disease of the nail that is found to be prevalent in 80-90 % of the patients with skin psoriasis which affects about 1 to 3 % of the total population [17]. The nail matrix, nail plate, and nail folds may get affected by psoriasis rendering the nails pitted, transversely ridged or thickened. Nail loss can also result in some cases from active shredding due to nail bed disease such as onycholysis or subungual hyperkeratosis [1]. Nail psoriasis warrants long term treatment durations and it is difficult to cure. The main treatment for psoriasis of nail plate is topical steroids vitamin D analogs, and 5-fluorouracil (5-FU), [18]. Systemic treatment for psoriatic nail has been recommended when the disease affects the skin or in case the function and quality of life has been drastically affected by the disease. In severe conditions, steroid injections are used while the other treatment options like superficial radiotherapy and electron beam therapy are found to be useful in some cases.

For many years the human nail plate was considered to be an impermeable barrier and the only treatment modalities adopted by clinicians were systemic therapy or surgical avulsion of the affected nail prior to topical application. Unfortunately systemic administration of antifungals would be hampered by the limited blood circulation to the affected nail bed leading to sub-therapeutic concentrations at the infected sites. The low drug concentration at the infected site invariably needs high oral doses of the drug for prolonged periods [19]. The high oral doses have been associated with severe adverse effects but most often the clearance of the infections has been temporary. In this context, the oral therapy in the treatment of nail disorders suffers from several limitations owing to severe side effects, contraindications, toxicities, drug interactions and long treatment periods that eventually incurs high treatment cost [20].

In contrast, the topical therapy to the nail would be an attractive therapeutic option as it obviates the systemic adverse effects and drug interactions commonly associated with oral therapy. The topical therapy has been the treatment of choice in children under 2 years due to its high efficacy owing to the low thickness of the nails. The topical treatment options remains inevitable when systemic treatment is strictly contradicted as in case of pregnant women [1]. Topical therapy is often recommended by clinicians in combination with oral therapy (Booster treatments) to improve the cure rates, reduce the treatment duration, cut down the treatment cost and thereby enhance the therapeutic outcomes [4].

The fate of the drug following topical application to the surface of the nail plate has been pictorially portrayed in *Figure 2*. A significant pre-absorptive loss is prone to occur following topical application of the formulation due to routine day-to-day activities. In addition, considerable amount of the drug may get bound to the keratin of the nail plate, eventually reducing the amount of drug delivered to the nail bed. Therefore, in order to maintain therapeutic drug concentrations at the target site, the rate at which the drug is delivered to the nail bed must suffice for the loss owing to tissue binding, metabolism and systemic clearance from the nail bed [21].

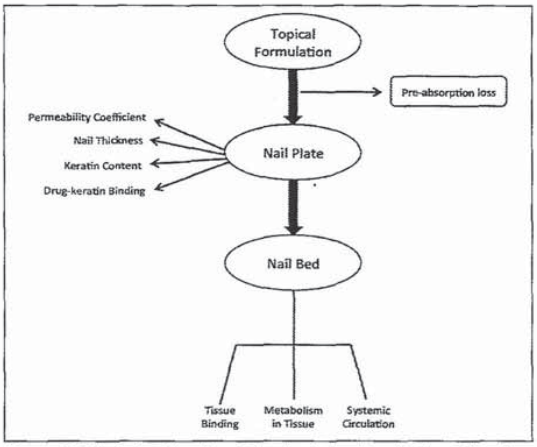

Figure 2 - The fate of the drug following topical application of the drug to the nail plate.

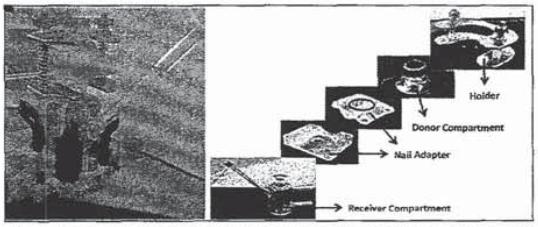

Figure 3 - The Franz diffusion cell with the nail adapter used for ungual permeation studies. The right side picture shows the individual parts.

III. *IN VITRO* TRANSPORT STUDIES

In order to predict the permeation of the therapeutic agents into and across the human nail plate a number of *in vitro* models have been developed and assessed. The *in vitro* data generated is a valid predictor of the *in vivo* performance of the topical nail products. The data also serves as a useful index to compare the newly developed topical products and helps to optimize the composition of the topical nail products. The vertical Franz Diffusion Cells (FDC) set up used at present to determine the permeability of the nail plate is as shown in portrayed in *Figure 3*.

The barrier across which permeability has to be assessed is mounted on a custom made nail adapter usually made of Teflon. The nail adapter with the nail plate is sandwiched between the donor and the receptor compartments of the vertical FDC. Hoof membranes sourced from bovine [22], porcine [23], Horse [24], or sheep [25], are used as barriers to predict the permeation across the nail plate. In addition to these, keratin films [26], nail clippings from healthy human volunteers [27] and human cadaver nail plates [28] are also used as barriers for the *in vitro* studies. The solution of the permeant is charged into the donor compartment while the receptor compartment is composed of a suitable buffer measuring about 5 mL. The contents of the receptor compartment are maintained at a temperature of 37 °C and a stirring speed of 600 rpm with a magnetic bead. The drug permeated across the barrier is determined at predetermined time points during the study. The drug loaded following the *in vitro* permeation studies into the barrier is determined by estimating the drug content of the barrier.

IV. FACTORS INFLUENCING THE DRUG PERMEATION ACROSS THE NAIL PLATE

By virtue of its thickness, unique chemical composition and rela-

tively compact and dense nature, human nail is known to considerably hinder the penetration of topically applied drugs. The clinical evidence documented so far has suggested that the success in treatment of fungal nail infections by topical antifungals lies in effectively overcoming the barrier of the nail plate [4]. The diffusion of the topically applied therapeutic agents is determined by the physicochemical properties of the permeant, formulation characteristics, presence of permeation enhancers, nail properties and interaction between the permeant and the nail keratin [29]. Some of the important factors are discussed below

1. Molecular size

Smaller molecules are known to penetrate well through the nail plate compared to the larger molecules. The dense keratin network is known to increase the path length of the permeant owing to its greater pore tortuosity. In addition, penetration rate would drop owing to the increased friction between the between the diffusing molecules and the keratin network as the molecular size of the permeant increases. Attempt was made to establish a meaningful correlation between the molecular weight of a series of therapeutic agents and the permeability coefficient. Further, the correlation established was used to predict the therapeutic efficacy of a series of antimycotics considering the aqueous solubility and minimum inhibitory concentration (MIC) [30]. An inverse relationship is known to prevail between permeability of nail plate for several model permeants and the molecular size of diffusing molecules [31]. Generally, molecules that exceed 300 Daltons in size may face hindrance while permeating the nail plate and therefore are likely to demonstrate a poor clinical efficacy [32].

2. Polarity of the permeant

The human nail is known to behave like a hydrogel with a high ionic strength to the diffusing molecules. However, owing to the traces of lipids in the nail plate (~1 %), researchers speculate the possibility of existence of a minute lipid pathway that could assist the transport of lipophilic molecule across the nail plate. By and large, the permeation of the molecules through the nail plate is dictated by the partition coefficient of the diffusing molecule. The permeation of homologous alcohols (C2 to C5) across the nail plate was found to decrease with increase in the hydrophobicity or the alkyl chain length [33]. The reduction in the permeation of the long chain alcohols was ascribed to the hydrophilic nature of the nail plate. However, the better transport of extremely long chain alcohols like decanol and dodecanol was attributed to the utilization of the lipid pathway prevalent in the nail plate by these molecules. In this context, lipid formulations which have the potential to exploit the lipid pathway have been proposed off late for lipophilic therapeutic agents [34, 35].

3. Nature and pH of the vehicle

The human nail is found to be 1000 times more permeable to water than the skin [36]. Permeability studies across cadaver nail plates indicated that the permeability coefficient of water was found to be approximately three times higher for water compared to ethanol suggesting that hydrated nail is more permeable to water than to ethanol [33]. The nail is known to swell and soften on contact with water or a hydroalcoholic solution. As a result, the keratin network is likely to expand leading to the formation of larger pores that would ease the transport of the permeant across the nail plate.

The pH of the aqueous vehicle along with the pKa value of the permeant determines the extent of ionization and therefore its aqueous solubility. Generally, acidic compounds are in the ionized or soluble state at higher pH values while the basic compounds are more soluble at low pH values. The saturation solubility and hence the thermodynamic activity of the drug is determined by the pH of the aqueous vehicle in such cases. The amount of drug permeated across the nail plate is eventually a function of its thermodynamic activity in the vehicle. Based on this hypothesis, aqueous solvents are considered to be ideal for lipophilic drugs whereas hydrophilic drugs require lipophilic solvents in order to ensure a high thermodynamic activity [37]. However, considering hydrophobic nature of most antimycotics agents, lipophilic vehicles have been investigated in the past for topical applications to the nail [38]. Though the lipophilic vehicles fail to neither hydrate or soften the nail plate nor expand the keratin network, they have been successful in enhancing the transport of certain drugs across the nail plate.

4. Surface charge of the permeant

The charge the permeant carries is known to determine its diffusion through the nail plate [39]. The nail keratin having an isoelectric point (pI ~ 5.0), is known to carry a net positive charge at a pH values below 5.0 while it bears a negative charge at a pH values higher than 5.0. It is likely that a negatively charged molecule is repelled from the nail surface at pH values of above 5.0 while a positively charged molecule is repelled at pH values lower than 5.0. The electrostatic interaction between the charged nail surface and the surface charge of the diffusing ions is termed as "Donnan effect" [21].

5. Nail plate effect

The nail plate is known to be about 100 times thicker than the *stratum corneum* of the skin though both the membranes are rich in keratin [4]. Due to its thickness, the nail plate is known to pose considerable obstacle to the transport of permeants to the infected nail bed. Further, nails infected with onychomycosis are found to be thicker than the healthy human nail plates due to the presence of the fungi and owing to the damage caused. An inverse relationship is known to exist between the thickness of the nail plate and the penetration of the topically applied therapeutic agents. Wetting of the nail plate or filing of the nail plate surface was found to cause a significant increase in the TOWL, which is usually considered as a measure of permeability through the nail plate [40]. Abrasion of the dorsal surface of the nail plate was found to increase the permeation of terbinafine hydrochloride (THC) by ~ 4 fold, which proved that the dorsal layer is the rate limiting barrier for the transport of permeants [41]. Filing or vigorous debridement of the dorsal surface of the nail prior is likely to enhance the success of the topical therapy [42].

V. METHODS TO ENHANCE TRANSUNGUAL DRUG DELIVERY

A better understanding of the barrier properties on the nail plate has been helpful to rationally design topical formulations that can improve the ungual and trans-ungual delivery of therapeutic agents. Topical therapy is the most preferred mode of transungual drug delivery as it is noninvasive and helps in regional delivery of actives to the infected sites. It has to be noted that most of the transdermal permeation enhancers have proved to be ineffective in enhancing the transungual drug delivery owing to the low lipid content in the nail plate (0.1-1 %) when compared to that in the skin (~10 %). Owing to its thickness, compactness and unique composition, the nail acts as a formidable barrier to the penetration of topically applied drugs. Further, binding of the drug to the nail plate keratin further decreases the free (active) drug and eventually the concentration gradient thereby limiting the drug penetration into deeper tissues [39]. Despite these constraints, the drug penetration into the nail plate can be improved using agents that break the physical and chemical bonds that maintain the integrity of the nail plate keratin. The disulfide, peptide, hydrogen and polar bonds in the nail plate keratin appear to be potential soft targets which could be breached by transungual penetration enhancers [43]. Exploiting this attempts have been made to enhance the efficacy of topical therapy using chemical penetration enhancers and etching agents.

The transungual chemical permeation enhancers identified till date can be classified into:

1) solvents:
   a) water,
   b) other solvents: dimethyl sulfoxide (DMSO), methanol;
2) keratolytic agents: urea, salicylic acid, papain, etc.;
3) thiolytic agents:
   a) thiols: N-acetyl cysteine, thioglycolic acid, 2-mercaptoethanol, etc.,
   b) sulfites: sodium sulfite, sodium metabisulfite etc.,
   c) hydrogen peroxide;
4) enzymes: keratinase;
5) etchanting agents:
   a) phosphoric acid,
   b) tartaric acid;
6) miscellaneous penetration enhancers:
   a) inorganic salts,
   b) hydrophobins,
   c) dioxalane,
   d) polyethylene glycols,
   e) lipid vehicles.

1. Solvents as permeation enhancers

1.1. Water

The trace amount of lipids in the nail plate and good number of experimental evidences has collectively indicated that the aqueous pathway plays a predominant in the penetration of drug through the nail plate [29]. Water is known to be the principle plasticizer present in the nail plate that imparts a certain degree of opacity, elasticity and flexibility to the nail. The degree of hydration of the nail is known to govern the permeability of the nail plate as demonstrated in number of studies. Nail plate has a tendency to hydrate, soften and swell similar to hydrogels on coming in contact with aqueous solutions. The permeability coefficients of the homologous alcohol diluted with saline was found to be five-fold higher when compared to the neat alcohols suggesting the facilitating role of water in increasing the permeation of water soluble permeants through the human nail plate [44]. Further, the permeation of methanol a hydrophilic alcohol and n-hexanol a hydrophobic alcohol was reduced when the proportion of water in the donor solution was decreased. A five-fold drop in the permeability coefficient of n-hexanol was noted as the concentration of dimethyl suphoxide in the binary mixture with water was increased to 86 % [45]. A similar reduction in the permeability coefficient of n-hexanol was observed when the donor contained traces or no water in isopropanol-water binary mixture. The decrease in the permeation of the two solutes on depletion of the water in the donor clearly confirmed the role of water in promoting the permeation of compounds of varied polarity through the nail plate.

The permeability of the nail plate at different states of % RH, has shown that diffusivity of water increased logarithmically by nearly 400 folds as the % RH increased from 15 to 100 % [46]. Scanning electron microscopy (SEM) analysis undertaken recently has revealed that hydration of the finger nails was found to increase the pores size and promote the interconnection of the pores that in turn could enhance the drug transport. Mercury intrusion porosimetric (MIP) studies further confirmed the modification in the porous microstructure of the nail plate [47].

The hydration of the nail was found to play a key role in transungual delivery of topically applied water insoluble actives as well. The effect of hydration of the nail plate on the *in vitro* permeation of ketoconazole a poorly water-soluble drug through excised human nails was assessed [48]. The steady state flux of radiolabeled ketoconazole which was solvent casted on the nail plate increased by nearly three-fold as the % RH to which the nails were exposed increased from 15 to 100 % with a drastic 2-fold enhancement as the % RH increased from 80 to 100 %. Considering the poor aqueous solubility of ketoconazole, the increased flux can be explained by increased flexibility and structural expansion of the keratin matrix on hydration with water, that would have allowed the high molecular weight (Mol. wt: 531.44) to diffuse with ease. The results conclusively suggested that the formulations or treatment modalities that improve the nail hydration have the potential to improve the penetration of topically applied therapeutic agents. Considering the ability of water to enhance the transport of topically applied actives across the nail plate, water soluble nail lacquers composed of hydrogels were developed for molecules of vivid polarity [22, 49-51].

1.2. Other solvents

Dimethyl sulfoxide (DMSO) is known to interact with the lipid domains of the *stratum corneum* thereby increasing their fluidity and promoting the partitioning of drug into the skin. Though, the solvent is not expected to demonstrate the same efficacy as a transungual penetration enhancer, considering the traces of lipids in the nail plate, there are few papers that report increase in the transungual penetration with DMSO. DMSO was found to increase the penetration of topically applied antimycotics [52]. Further, pretreatment of the nail with DMSO was found to increase the penetration of amorolfine [53]. A maximum penetration depth of one fourth the depth of the total nail plate was observed compared to other lipophilic solvents in a human subject study when DMSO was used as an enhancer [54].

The depth of penetration of urea, salicylic acid and ketoconazole into the human nail plate from test formulations containing DMSO was 2-fold higher compared to control formulations containing saline [55]. With salicylic acid in particular, greater amount of drug was bound to the dorsal surface of the nail plate for the control that limited the drug availability to the deeper areas. On the contrary, higher amount of salicylic acid was delivered to deeper areas of the nail plate with the formulation composed of DMSO.

An increase in permeation of the caffeine across cadaver human nail plate was noted when DMSO or methanol was used as an enhancer in formulations [56]. The test formulations of caffeine (2 %w/v) with DMSO (5 %) or methanol (5 %) in either water or 20 % v/v ethanol while the reference formulations had similar compositions but were devoid of any enhancers. When DMSO was used as an enhancer, the permeability coefficients of caffeine increased by ~3.3 and 2-fold in ethanolic and aqueous systems respectively, when compared to the corresponding reference formulations. Likewise, the caffeine loaded into the nail plates following the permeation studies were 1.55 and 1.18-fold higher for ethanolic and aqueous systems respectively, in presence of DMSO compared to the corresponding reference formulations.

Similarly, in presence of methanol, the permeability coefficients of caffeine through the human cadaver nail plate increased by a factor of 4.8 and 3.2 fold for ethanolic and aqueous systems respectively when compared to the corresponding reference formulations. Correspondingly, the caffeine content in the nail plate after the permeation studies increased by ~1.7 and 1.61-fold from ethanolic and aqueous systems respectively for the test formulations compared to the corresponding references. The surface topography revealed an increase in the roughness of the dorsal nail surface treated with the test formulations compared to that treated with the reference formulations. The mechanism of action of DMSO and methanol as a transungual penetration enhancer continues to remain unclear though the authors attribute the penetration enhancement to the depletion of the lipids present in the dorsal surface of the nail plate.

2.Keratolytic agents

Keratolytic agents are known to disrupt the tertiary structure and the hydrogen bonds present in the keratin thereby enhancing the permeation of therapeutic agents through the nail plate. These agents are known to act by softening and swelling the nail plate especially in presence of water [37]. The swelling and softening of the nail plate is likely to enhance the drug permeation as a consequence of formation of a less dense keratin structure with large pores.

The effect of keratolytic agents like papain, urea and salicylic acid on the *in vitro* permeation of miconazole nitrate, ketoconazole and itraconazole through human nail were studied [57]. The permeation studies across nail plates carried out in side by side diffusion cells with 60 % ethanol as donor and receiver fluid indicated no permeation of the three antimycotics in 60 days in the absence of keratolytic agents. Moreover, a 'single-step pretreatment' with salicylic acid (20 %) alone for 10 days nor addition of urea (40 %) to the donor solution failed to induce any permeation of the antimycotics. However, a "2-step pretreatment" with papain (15 %) for one day followed by salicylic acid (20 %) for 10 days resulted in a steady state flux of 6.66×10^5, 1.15×10^5 and 0.13×10^5 mg/cm^2/s for miconazole nitrate, ketoconazole and itraconazole respectively with an effective diffusion constants of 6.29×10^8, 3.60×10^8 and 3×10^8 cm^2 sec^{-1}, respectively. Further, the lag times for miconazole, ketoconazole and itraconazole were found to be 32.15, 56.22 and 67.5 min, respectively. SEM revealed that the "2-step pretreatment" procedure was found to damage and fracture the dorsal nail surface, which in turn would have created pathways for drug penetration.

Concentrated solutions of urea and salicylic acid have been used as hydrating and softening the nail in topical treatment of onychomycosis. The benefits of using urea (40 %) for non-surgical nail avulsion are low risk of infection, hemorrhage, a quick improvement after avulsion and absence of pain during and after treatment. In clinical trials, urea in combination with salicylic acid was found to effective in increasing the penetration of bifonazole penetration into the nail plate [58].

Urea and salicylic acid are known to increase the permeation of tritiated water through human nail in combination with N-(2-mercaptopropionyl) glycine from aqueous gel formulations [59]. Urea in combination with other cysteine derivatives is reported to improve the penetration of permeants from aqueous formulations through human nail [43]. Cysteines are thiols that act on disulfide bonds in the nail keratin whereas urea acts on the hydrogen bonds to facilitate the cleavage of disulfide linkages. Urea (20 %) in combination with N-acetyl cysteine (NAC) (5 or 10 %) was found to enhance the *in vitro* permeation of miconazole nitrate through the nail plate by 2 to 2.5 fold. Further, the concentration of miconazole in the nail following the studies exceeded the MIC [60].

3. Compounds that cleave the disulfide bonds

3.1. Thiols

Thiols are a group of compounds containing sufhydryl groups (-SH) that have shown promise as transungual penetration enhancers. The mechanism involved in the enhancement of the transungual permeation is the reduction of the disulfide linkage in the nail keratin matrix as shown below [60]. Thiols are known to get oxidized while reducing the disulfide linkage of the nail keratin as shown in *Equation 1*:

$$\text{Nail-S-S-Nail} + \text{R-SH} \leftrightarrow 2\ \text{Nail-SH} + \text{R-S-S-R} \qquad \textbf{Eq. 1}$$

where Nail-S-S-Nail represents for the disulfide linkage of the nail plate keratin while R-SH stands for a thiol. The reduction of the keratin is known to destabilize the disulphide bonds, compromise the barrier integrity of the nail plate and thereby promote the transungual permeation [61]. Thiols are found to be more effective in aqueous or hydroalcoholic vehicles which tend to hydrate, swell and soften the nail plate. Once the disulfide bonds are broken, they are less likely to be reformed in the dead nail plate and hence the action of the thiols as transungual penetration enhancers is irreversible. Thiol compounds which have been investigated as transungual penetration enhancers are pyrithione (PTO), N-(2-mercaptopropionyl) glycine (MPG), N-acetyl cysteine (NAC), cysteine, 2-mercaptoethanol (MPE) and thioglycolic acid (TGA).

Thiol compounds like PTO and MPG were found to act as transungual penetration enhancers in aqueous as well as lipophilic gels [59]. *In vitro* permeation studies in FDC indicated that aqueous gels of hydroxyethyl cellulose (HEC) containing MPG (10 %) enhanced the permeation of tritiated water across human finger nails by 2.49-fold compared to control gels. Owing to its small size, MPG was thought to get well incorporated in the nail matrix thereby enhancing the flux of tritiated water. Moreover, when urea (20 %) was incorporated to the aqueous gel containing MPG the transport of tritiated water was further increased (3.54-fold compared to the control). It was also noted that the MPG levels were more critical than the urea levels in increasing the permeation of tritiated water. Similarly, lipophilic gel of hydroxypropyl cellulose (HPC) containing PTO (10 %) in DMSO increased the permeation of the tritiated water by 2.59-fold compared to control.

NAC and MPE were found to increase the transungual permeation of drugs with varied polarity and molecular size from aqueous as well as lipophilic vehicles [37]. *In vitro* permeation performed in a side-by-side diffusion cells maintained at 37 °C using human nail plate as barrier indicated that the flux of 5-FU (Mol. wt: 130, aqueous solubility: 17.1 mg/mL) was increased by NAC (3 %) and MPE (3 %) by 13 and 16-fold, respectively, from aqueous vehicles (40 % ethanol) and by 6.7 and 8.4 fold, respectively, from lipophilic vehicles (10 % ethanol in isopropyl myristate) compared to corresponding controls. Though it appeared that the two enhancers affected the nail barrier integrity in both vehicles, the enhancers too seem to penetrate well and get better incorporated into the nail from aqueous vehicle, increasing the drug diffusion due to the swelling and softening of the nail plate that resulted in the cleavage of the disulphide bonds. Further, it was also observed that the permeation flux of 5-FU from the aqueous vehicle was found to be proportional to the concentrations of NAC (0.1, 0.5, 1, 3, 5, 10 %).

Though no detectable amounts of tolnaftate (Mol. wt: 307, aq. Sol.: 0.39 mg/mL) permeated across the nail plate from any solvent systems devoid of the enhancers, the drug flux containing NAC and MPE were found to be 0.137 and 0.058 μg/cm^2/h, respectively, from aqueous vehicle and 0.053 and 0.223 μg/cm^2/h, respectively, from lipophilic vehicle. The poor permeation of tolnaftate in the nail plate can most likely be attributed its high molecular weight and poor aqueous solubility of the molecule. SEM imaging and MIP has been used off late to study the NAC induced surface and microstructural changes in the human finger nail plate [47]. SEM revealed that NAC (10 %) pretreatment induced the formation of pores on the nail surface that significantly increased the nail roughness. MIP used to model the porous structure indicated that NAC (10 %) pretreatment was found to alter the internal microstructure of the nail and render the nail more porous. Infrared spectroscopy and impedance spectroscopy have indicated spectral changes in the amide linkages of keratin following NAC treatment that were attributed to the disruption of the nail plate protein owing to the action of NAC [62].

TGA is known to reduce the –S-S– bonds in the nail keratin to –SH where the addition of hydrogen results in the cleavage of disulphide linkage and compromise the barrier integrity of the nail plate. Pretreatment of the nail surface with aqueous solution (20 % ethanol) of TGA (5 %) increased the permeation of topically applied radiolabelled mannitol and caffeine by 2.8-fold and 3.8-fold compared to enhancer free control respectively [24]. Pretreatment with TGA was found to increase the water content of the nail plate and enhance the passive permeation of model permeants like mannitol (Mol. wt: 182) and urea (Mol. wt: 60) through the nail plate [63]. Pretreatment with 0.5, 1.8 and 3.7 M TGA increased the nail plate weight to 47, 59 and 60 %, respectively, in phosphate buffer saline (PBS), which indicated that TGA had the potential to hydrate the human nail clippings in a concentration dependent manner. The permeability coefficient of mannitol and urea across nail plates pretreated with 0.5M TGA was found to increase by 4-fold and 2-fold, respectively, compared to the control. Likewise, pretreatment with 1.8 M TGA was found to further increase

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