TRANSUNGUAL DRUG DELIVERY

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ABSTRACT

Topical therapy is desirable in treatment of nail diseases like onychomycosis (fungal infection of nail) and psoriasis. The topical treatment avoids the adverse effects associated with systemic therapy, thereby enhancing the patient compliance and reducing the treatment cost. However the effectiveness of the topical therapies has been limited due to the poor permeability of the nail plate to topically applied therapeutic agents. Research over the past one decade has been focused on improving the transungual permeability by means of chemical treatment, penetration enhancers, mechanical and physical methods. The present review is an attempt to discuss the different physical and chemical methods employed to increase the permeability of the nail plate. Minimally invasive electrically mediated techniques such as iontophoresis have gained success in facilitating the transungual delivery of actives. In addition drug transport across the nail plate has been improved by filing the dorsal surface of the nail plate prior to application of topical formulation. But attempts to improve the trans-nail permeation using transdermal chemical enhancers have failed so far. Attempts are on to search suitable physical enhancement techniques and chemical transungual enhancers in view to maximize the drug delivery across the nail plate.

Keywords: *Transungual, drug delivery, nail, permeation*

Nail anatomy

The human nail apparatus is made up of nail folds, nail matrix, nail plate and nail bed. The nail folds are the invaginating wedge-shaped skin folds on sides of the nail plate. The nail fold located at the proximal end of the nail is called proximal nail fold while those present on the lateral side are termed as lateral nail folds. Beneath the proximal nail plate is located a small area of living tissue termed as nail matrix which consists of highly proliferative epidermal tissue.

The section of the nail has been shown in Fig. 1. The nail plate originates from the nail matrix and covers the entire nail bed. The nail plate is about 0.25 to 0.6 mm thick, hard, elastic, translucent and convex in shape. The plate is composed of approximately 25 layers of flattened, dead, keratinized tightly bound cells. Nail plate can be differentiated into three layers the upper dorsal, followed by the intermediate and the inner ventral layer varying in thickness in a ratio of 3:5:2 respectively (1). The dorsal layer is few layers thick and forms the hardest layer of the nail plate. The intermediate layer is the softer, more flexible and the thickest layer of the nail plate. The ventral layer is 1-2 cells thick and made up of soft keratin and connects the nail plate to the underlying nail bed.

The nail plate is mainly made up of the

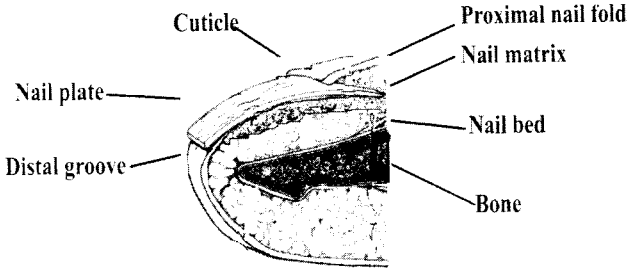

Fig. 1*:* Cross section of the human nail apparatus

fibrous protein keratin, about 80% of which would be the hard hair-type keratin while the remainder is the soft skin-type keratin (2). The hair-type keratin is concentrated in the intermediate nail layers whereas the skin-type keratin is present in the dorsal and ventral layers (3). The nail plate contains 7 to 12% of water under normal conditions while the content is about 25% at a relative humidity of 100%. The water content is found to be responsible in maintaining the opacity, elasticity and flexibility of the nail. The nail plate also contains traces of lipids (0.1–1.0 %) organized as bilayers and oriented parallel to the nail surface in the dorsal and ventral layers (4).

The nail bed is made of thin, soft, epithelium that extends the whole length of the nail. The nail plate closely adheres and overlays the nail

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bed. Nail bed acts as a holder for the nail plate. The nail bed is pink in color due to the underlying vascular network. In addition, the nail bed has a rich supply of lymphatic vessels.

Diseases affecting the nail

The human nails can be affected by a number of diseases which can range from relatively harmless conditions like pigmentation in case of heavy smokers to more painful, and devastating conditions where the nail may be dystrophied, hypertrophied, inflamed or infected (3). The diseases of the nail are known to make a serious emotional and psychological impact on the social life of the affected.

The two most common diseases that affect the nails are onychomycosis and nail psoriasis. Onychomycosis is the fungal infection of the nails that has been responsible for 50% of the nail disorders (5). The pathogens in 90% of the cases are dermatophytes usually *Trichophyton rubrum* and *T. mentagrophytes*. The other causative organisms include yeasts mainly *Candida albicans* and nondermatophyte moulds. Onychomycosis is found to be more prevalent in the elderly and diabetic patients (6). The other risk factors are immunosuppression (as in Human Immunodeficiency Virus infections and Cancer) and atopic disorders. Therefore the incidence of the disease seems to increase due to growing elderly population and extensive use of immunosuppressants in infections with HIV. Life style factors like wearing of tight fit clothing and shoes and routine visits to communal recreational facilities and health clubs have influenced the prevalence of onychomycosis. The disease is known to involve the toe nail in majority of the cases with the infection spreading over the nail plate, nail bed and surrounding skin folds. Based on the site of involvement and the pathophysiology, onychomycosis has been classified into four types. They include (i) Distal subungual: infection involving the tip of the nail plate and underlying nail bed; (ii) Proximal subungual: infection in the cuticle and nail bed; (iii) Superficial: infection only involving the nail plate and (iv) Total dystrophic onychomycosis where the whole nail is involved. The infected nails look ugly, discolored and thickened, adversely affecting the quality of life of the patients, reducing the physical and social functioning.

Nail psoriasis is reported to be prevalent in 80–90% of the patients with skin psoriasis that affects about 1 to 3% of the total population. The disease can affect the nail plate including the surrounding soft tissue. The affected nails may be pitted, transversely ridged, thickened or lost. Nail loss may be the result of active shredding due to nail bed disease such as onycholysis or subungual hyperkeratosis.

Current treatment options

Onychomycosis is difficult to treat since it is chronic, hard to eradicate and tends to relapse. The only treatment option for onychomycosis in the past was surgical avulsion that would be extremely traumatic and painful (7). The disease is currently treated with systemic or local antimycotics either alone or in combination, depending on the severity of the condition. Systemic therapy involves oral administration of antifungals such as terbinafine, itraconazole, griseofulvin etc.

The main treatment for psoriasis of nail plate is topical steroids, vitamin D analogs and 5-Fluorouracil. Systemic treatment for psoriatic nail has been recommended when the disease is present in tandem with severe skin psoriasis or in case the function and quality of life has been significantly hampered by the disease. In severe condition, steroid injections may be used. The other treatment options like superficial radiotherapy and electron beam therapy are found to be useful in some cases. However, the disease is difficult to cure but may respond to combination therapy.

Limitations of the current treatment methods

The orally administered drugs for most of the nail diseases have to be systemically distributed and subsequently reach the infected site located in the nail bed. Unfortunately, systemic administration of antifungals has been hampered by the limited blood circulation into the affected nail bed leading to poor drug transport. This needs oral administration of high doses of the drug for prolonged periods (8). A large number of patients fail to respond to the oral therapy owing to the sub-therapeutic concentrations attained at the infected site. In addition, the high oral doses have been associated with severe adverse effects but most often the clearance of the infection has been

temporary. Moreover, it has been thought that indiscriminate and extensive systemic antimicrobial therapy has led to an increase in the number of emerging resistant strains of the microorganisms (9). In this context, the oral therapy has limited success rate in the treatment of nail diseases due to severe side effects, contraindications, toxicities, drug interactions and high treatment cost.

On the contrary, topical therapy would be an attractive alternative in treatment of nail diseases as it can overcome most of the limitations associated with systemic therapy. Topical treatment is known for its non invasiveness and regional delivery to the infected site. Topical therapies obviate the side effects and drug interactions associated with systemic therapy and enhance the patient compliance and the treatment cost in many cases. Topical therapy has been able to deliver and maintain therapeutic concentrations of the drug to the nail bed using different physical enhancement techniques and chemical transungual penetration enhancers.

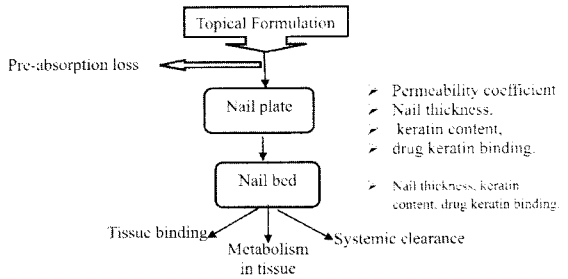

Fig. 2: The fate of the drug following topical application to the nail plate.

The fate of the drug on topical application to the nail plate has been pictorially represented in Fig. 2. A significant pre-absorptive loss tends to occur due to routine day to day activities following topical application. The drug absorbed through the nail plate may bind to the keratin reducing the amount of the bioavailable drug. The drug has to partition into the nail bed and maintain concentrations exceeding the minimum inhibitory concentration to be effective. The rate at which the drug is delivered into the nail bed must compensate for the losses due to binding, metabolism and clearance of the drug from the nail bed. Loading high amounts of drug into the nail and the nail bed will most likely increase the success of topical monotherapy.

In vitro models for study of trans-ungual drug permeation

The permeability of nail plate has been systematically studied over the last three decades using different *in vitro* models. Bovine hoof membrane, nail clippings from healthy human volunteers and avulsed human cadaver nail plates have been used as models for the nail plate. In one of the recent studies, the whole toe excised from cadavers was used as a model for the study. The researchers delivered drug topically and isolated individual parts of the nail apparatus and analyzed the amount of drug delivered.

Permeation studies were initially performed in 1970's using brass cup filled with saline. The open end of the cup was covered with a nail plate and weight loss of the cup was considered as a measure of permeability. Later on in the next decade a two compartment horizontal diffusion cell in which the nail plate, separating the two compartments was totally immersed in the media was used to determine the water flux. A modified vertical Franz diffusion cell made of stainless steel was used in 1990's for the first time to determine the permeability through hoof membrane as well as human nail plate.

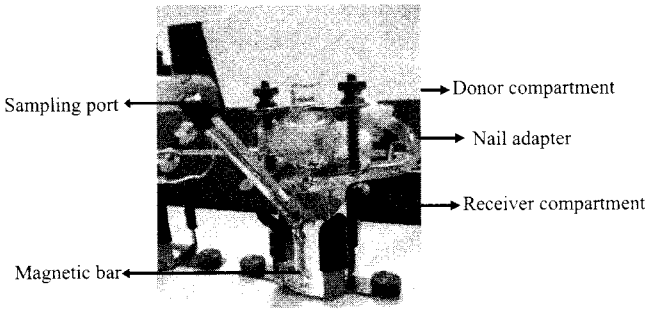

Fig. 3: Vertical Franz diffusion cell set up used to determine the nail permeability

The vertical Franz diffusion cell set up used at present to assess the permeability through the nail plate is shown in Fig. 3 (10). The nail is mounted on a custom made nail adapter made of teflon. The nail adapter is sandwiched between the donor and receptor compartments of the vertical Franz diffusion cell. The receptor compartment having a capacity of 5 ml is thermostatically controlled and stirred using a magnetic stir bar. The receptor compartment has a sampling port for

timely sample withdrawals.

Factors affecting transungual drug permeation

The diffusion of the permeants across the nail plate is said to be determined by the following factors

i. Molecular size of the permeant
ii. Polarity of the permeant
iii. Nature and pH of the vehicle
iv. Surface charge

The permeability coefficient of the diffusing molecules through the human nail plate is found to decrease with the increase in the molecular weight of drugs (11). The decrease in the permeability coefficient with increase in the lipophilicity of permeant is due to the hydrophilic nature of the nail plate (12). The permeation in most cases decreases with the increase in the carbon chain length or the lipophilicity of the permeant. The interrelationship between the permeability coefficient and the lipophilicity of the permeant has been well demonstrated in a series of homologous alcohols varying in the carbon chain length. In addition, a minute lipid pathway is known to exist in the nail plate owing to the low lipid content of the nail (1%). Highly lipophilic molecules were found to permeate across the nail utilizing this lipid pathway.

Water plays a facilitating role in promoting the diffusion of the permeants through the nail plate. The nails swell on coming in contact with the aqueous or hydroalcoholic solution. As a result the keratin network expands leading to formation of larger pores thereby promoting the permeation of the diffusing molecules (13).

The pH of the aqueous vehicle determines the extent of ionization, aqueous solubility of the permeant and its interaction with the nail plate. Many compounds are known to exhibit pH dependent solubility profiles. Thermodynamic activity that is represented as a fraction of the saturation solubility is therefore determined by the pH of the vehicle in such cases. The amount of drug permeated across the nail plate is directly related to the thermodynamic activity of the permeant (10).

The nail keratin has an isoelectric point of 5.0 and thus carries a net positive charge at pH below 5.0 while a net negative charge at pH above 5.0 (14). Nail plate shows selective absorption of cationic and anionic drugs at pH more than and less than 5, respectively.

Methods to enhance transungual drug permeation

Many of the nail disorders can successfully be treated only when the applied drug is able to permeate the dense keratinized nail plate. This has been made possible utilizing different chemical enhancers or physical techniques.

Chemical enhancement of transungual drug delivery

The barrier of the nail plate can be altered using agents that break the physical and chemical bonds that maintain the integrity of the nail keratin. To select and identify the right transungual penetration enhancer for the given drug a more rapid and simple methods have been put forward in view to overcome the tedious and expensive diffusion studies. Preformulation screening of putative transungual enhancers was undertaken employing nail swelling as a surrogate marker of penetration enhancement. To confirm the relationship between human nail swelling and altered nail barrier a permeation study across human nail was performed. But nail swelling cannot always be considered as an indication for the increased transungual permeation. In this context, Transcreen-N™ has been proposed as a high throughput screening procedure to identify the right enhancer from a large pool of penetration enhancers for the given compound. About 47 different enhancers were screened during the study and the outcome of the technique was validated against the *in vitro* permeation data. The procedure also identifies whether the enhancer can be used in pretreatment or as a coapplication agent.

The transungual chemical permeation enhancers identified till date fall under the following categories.

1. Agents that cleave the nail disulphide bonds
2. Nail softeners and Hydrating agents
3. Keratolytic enzymes
4. Miscellaneous penetration enhancers

Agents that cleave the nail disulphide bonds

Some of the thiol compounds which have been used as transungual penetration enhancers include pyrithone, N-(2-mercaptopropionyl) glycine, N-acetyl cysteine, and thioglycolic acid. These compounds were found to irreversibly alter

the structure of the keratin matrix. The mechanism involved in the enhancement of the permeation across the nail, is the reduction of the disulphide linkage in the keratin matrix. Considerable amount of nail swelling and drug uptake are observed when human nails were soaked in formulations containing thiol compounds. Sulphites are also known to increase the transungual flux by reacting with the nail keratin and reducing the disulfide links.

Nail softeners and Hydrating agents

The facilitating role of water in transungual permeation of alkanols is well demonstrated in the past. Nail hydration on contact with water is thought as the possible mechanism for the higher flux from certain vehicles. Urea and salicylic acid are known to soften and hydrate the nail plate. The swelling and hydration of the nail plate would enhance the drug permeation as a consequence of formation of a less dense structure with large pores. Urea in combination with N-(2-mercaptopropionyl) glycine or hydrogen peroxide was found to increase permeability of nail plate.

Keratolytics

Keratolytic enzymes are known to hydrolyze the keratin matrix of the nail plate altering its barrier property and enhancing the transungual permeation. Nail clippings on incubation with the enzymes has been found to result in separation of the corneocytes on the dorsal surface of the nail. The enzyme treated hoof membranes demonstrated increased flux of drug. Papain is an endopeptidase containing sulfhydryl group has been reported as a transungual penetration enhancer.

Miscellaneous enhancers

There are many other solvents and chemicals reported in the literature as good drug permeation enhancers across the nail plate. However, the mechanisms have been resolved only in some cases. N-methyl-2-pyrrolidone, dimethylsulfoxide, Labrasol, Transcutol and sodium dodecyl sulfate are found to increase the permeability of the nail plate. The infrared rays were found to cause conformational changes in the keratin structure of the hooves membrane. Transcreen-N™ the high throughput screening procedure indicated that inorganic salts such as sodium metabisulphite, sodium citrate, potassium phosphate, and ammonium carbonate were found to increase the drug load as well as drug uptake rate of terbinafine hydrochloride. In addition, polyethylene glycols were identified as potential transungual enhancers of the drug.

Physical methods used to enhance the transungual permeation

1. Abrasion of the nail plate surface
2. Pulsed lasers
3. Microporation
4. Low frequency ultrasound
5. Iontophoresis

Abrasion of the nail plate surface

The dorsal layer of the nail is found to be the major barrier for the permeation of drug into the nail plate. One of the methods employed to physically enhance the transungual delivery is filing the surface of the nail plate using an abrasive. Therefore, removal of the dorsal layer by filing has been helpful to overcome the barrier and facilitate permeation of the topically applied drug into the deeper nail bed. Vigorous debridement of the surface of the nail plate has increased the success rate of topical therapy for different nail disorders (15). The abrasion of dorsal surface of the nail using electrical equipment or dental drills has proved to be beneficial in clinics to improve the efficacy of the topical treatment.

Tartaric acid and phosphoric acid have been used as etchants or nail surface modifiers to modify the surface of nail and thus facilitate the drug delivery across the nail plate (8). The surface modifiers are found to disrupt the dorsal surface of the nail plate and enhance the permeability across the nail, thereby, improving the topical treatment in nail infections. In addition, the pretreatment of the human nails with the etching agents increases the bioadhesion of the topical transungual films to the surface of the nail. The enhanced bioadhesion and permeability is due to the increased surface area resulting from interaction of the etchant with the nail surface.

Pulsed Lasers

To overcome the natural barriers that limit the penetration of topically applied drugs, pulsed laser systems have been employed (16). On topical application, laser energy would be absorbed by the keratin in the nail plate and the scattered heat would lead to vaporization and thus removal of the nail

layers. The effect of different laser systems on nail plate ablation rates, ablation efficiencies, and subsequent craters morphology has been studied. The *in vitro* application of laser on the nail plate resulted in the formation of craters. The shape, size, wall smoothness of the craters formed and presence of cracks and melted and resolidified tissue are determined by the type of laser employed. Ultrashort pulsed laser are found to display best ablation efficiencies without producing any cracks or thermal damage to the nail plate. However, the efficacy of laser *in vivo* on transungual permeation has to be established.

Microporation

PathFormer (Path Scientific, Carlisle, USA) is an FDA approved microcutting device used to create a hole of specific depth in the nail plate without affecting the nail bed in order to drain subungual hematomas (17). The advantage of the device is that it uses the electrical resistance of the nail bed as a feedback to stop and retract the drill when it has penetrated through the nail plate. The other advantage of the device is that it eliminates the need for anesthesia. The nail plate is drilled using a 400 μm diameter tissue cutter in a hand held device containing two small electric motors. The electrical resistance of the highly keratinized nail plate is found to be 5 M while it may drop to few k depending on the depth of the hole (18). Holes of varying depths corresponding to the electrical resistances ranging from 90 to 25 k were drilled to assess the tolerability of the technique in healthy adult subjects. The procedure is found to cause minimal pain and so well tolerated all the subjects. The same device was employed in some clinical trials to drill holes prior to application of terbinafine cream and placebo cream. Though transungual permeation of terbinafine was promoted, the number of holes drilled and the extent of enhancement obtained is not revealed (19).

Low frequency Ultra sound

The potential of low frequency ultrasound as a physical transungual enhancement technique has been assessed through bovine hoof membranes (20). Low frequency ultrasound (20 kHz) was applied to the membranes using ultrasound probe held at a distance of 13 mm from the surface through a liquid coupling medium. A 50% intensity level was employed as a pretreatment procedure for 1 minute time in a pulsatile manner (on and off). The enhanced drug permeation may be enhanced due to the ultrasound induced disruption of the hoof membrane. Though the mechanism of membrane disruption is yet to be elucidated, it was proposed that inertial cavitation or formation of pits was mechanism involved.

Iontophoresis

The application of electric current has proved to enhance the delivery of charged molecules across the nail plate. The enhanced transungual drug transport by iontophoresis has been attributed to electrorepulsion and membrane permeabilization (10). The pH of the vehicle, buffer ionic strength and current density are found to influence the transungual permeation across the nail plate. The pH of the vehicle determines the extent of ionization which in turn influences the iontophoretic transport through the nail plate. The nail plate with an isoelectric point (pI) of 5.0 carries a net negative charge at pH above 5.0 and a net positive charge below pH 5.0. Anodal iontophoretic transport is favored at pH above 5.0 while cathodal iontophoretic transport was promoted at pH below 5.0 (14). An optimum buffer ionic strength need to be maintained during iontophoresis to facilitate the drug transport. The iontophoretic transport flux can be enhanced by increasing the current density but the maximum current density applied in most of the cases would be 0.5 mA/sq. cm.

Iontophoresis has been employed to accomplish rapid transungual delivery of the antifungals in treatment of onychomycosis (21). The drug loaded into the nail plate by iontophoresis is found to form a depot in the nail. The drug loaded was subsequently released at concentrations exceeding the minimum inhibitory concentration for prolonged period of time in a progressive manner.

Conclusion:

The permeability of the drug molecules through the compact and highly keratinized nail plate is found to be poor. This results in poor delivery of the actives into the deeper nail layers and nail bed which are believed to be the site of many nail disorders. The drug molecules should be of low molecular weight and relatively polar in

nature in order to permeate the nail plate. In this context, many chemical and physical approaches have been investigated to breach the impermeable nail barrier and promote the transungual permeation of topically applied drugs. Electrically mediated techniques such as iontophoresis have shown good promise in facilitating the transungual delivery of actives. In addition, drug transport across the nail plate has been improved by filing the dorsal surface of the nail plate prior to application of topical formulation. But attempts to improve the trans-nail permeation using transdermal chemical enhancers have not been convincing so far. More attempts to choose suitable physical enhancement techniques and chemical transungual enhancers to maximize the drug delivery across the nail plate should be made in near future.

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