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Research Paper

A study on the effect of inorganic salts in transungual drug delivery of terbinafine

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Abstract

Objectives The poor success rate of topical therapy in nail disorders is mainly because of the low permeability of keratinized nail plates. This can be overcome by utilizing potent perungual drug penetration enhancers that facilitate the drug permeation across the nail plate. This study evaluated the efficacy of inorganic salts in enhancing the trans-nail permeation using a model potent antifungal agent, terbinafine hydrochloride.
Methods Permeation studies were carried out across human cadaver nail in a Franz diffusion cell using terbinafine solution (1 mg/ml; pH 3). Preliminary studies were carried out to assess the effect of salts (0.5 M) on the terbinafine permeation into and through the nail. Further, the influence of salt concentration (0.25–3 M) on permeation, the mechanism for the enhancement and the suitability of developing a formulation were also studied.
Key findings Terbinafine permeation (3–5 fold) through the nail and drug load (4–7 fold) in the nail were enhanced significantly when salts were used at 0.5 M concentration. Increase in salt concentration up to 1 M increased the permeation, which decreased with further increase in salt concentration (>1 M). Mechanistic studies revealed that the enhanced permeation by salts was mainly due to their ability to increase the nail hydration and also to increase the thermodynamic activity of the drug. The cumulative amount of terbinafine permeated at 24 h from the formulated gel ($9.70 \pm 0.93\ \mu g/cm^2$) was comparable with that of a solution ($11.45 \pm 1.62\ \mu g/cm^2$).
Conclusions Given the promising results from the permeation and drug load studies, it was concluded that inorganic salts could be used as potent transungual permeation enhancers.
Keywords hydration; nail; permeation enhancer; salts; terbinafine

Introduction

The most common causes of nail diseases are fungal infections and psoriasis. Onychomycosis, fungal infection of the nail, is the most prevalent (approximately 50%) of all nail diseases. Dermatophytes are the main causative organism for onychomycosis, while non-dermatophytic molds and yeasts are also involved.[1] Existing treatment modalities for onychomycosis include oral delivery and topical application. Topical drug delivery is the most appropriate therapy as it offers higher patient compliance and better proximity to the infected site and avoids potential systemic adverse effects.[2,3] Currently, topical formulations are available as nail lacquers, creams, ointments, gels, solutions and lotions.[4] However, the efficiency of these formulations is limited due to their inability to deliver a therapeutically effective amount of drug into and across the impermeable nail plate. Therefore, this therapy is limited for the treatment of superficial and minor subungual onychomycosis.[5,6]

Topical delivery of drugs by the usage of permeation enhancers is well known. A wide variety of proven permeation enhancers are available and are used in enhancing drug delivery across the skin. The skin contains about 15% of lipids arranged as lamellae in the intercellular space. Most skin penetration enhancers act by modulation of lipid structures. The nail plate has less lipid content (<1%) than the skin.[7] The major component of the nail plate is keratin. Enhancers of skin drug penetration that act predominantly on the keratinocyte pathway are relatively few, thus it is obvious that most skin permeation enhancers are unsuccessful in transungual drug delivery. Therefore, research is being carried out to screen novel entities for their potential transungual permeation enhancement ability. Many chemical agents, such as acetylcysteine, urea, salicylic acid, 2-n-nonyl-1,3-dioxalone,

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mercaptoethanol, thioglycolic acid and glycolic acid, have been screened for their ungual permeation enhancement capacity.[8–12] Reports on the capability of these agents to enhance transungual delivery are inconsistent.[2] Recently Malhotra and Zatz[10] reported the ineffectiveness of keratolytic agents, even at high concentration, when delivered from gel formulation. The authors also observed the failure of sodium metabisulfite to enhance penetration. Also, the efficiency of acetylcysteine was limited only on the dorsal nail layer and it was inactive in the intermediate and lower nail layers.[8]

It is well known that the cure rates with existing topical therapy remain much lower than those achieved by systemic antifungal therapy. Successful treatment of nail diseases by the topical route can be achieved by identifying potent perungual penetration enhancers to deliver drugs across the nail plate in effective amounts. It is also known that the efficiency of penetration enhancers vary with different formulation conditions and with different drugs. Therefore, one needs to identify the most appropriate enhancers for the given drug and formulation. In this direction, we developed a high-throughput technique for screening penetration enhancers, called TransScreen-N. It is a rapid microwell plate-based method that involves two different treatment procedures – simultaneous exposure treatment and sequential exposure treatment. The technique was developed to screen permeation enhancers for the delivery of terbinafine hydrochloride, a potent antifungal agent, which is the current treatment of choice in onychomycosis. During this process the efficiency of inorganic salts to act as perungual permeation enhancers was discovered. The drug load in nail pieces soaked in formulations containing salts as enhancers (sodium sulfite, sodium phosphate, potassium phosphate, calcium phosphate and ammonium carbonate) were found to be significantly higher than the control. TransScreen-N is a screening method only and the results are suggestive. The method does not reveal the drug permeation profile, flux, lag time or the possible mechanisms. Encouraged by results that suggested the potential permeation enhancement efficiency of salts in TransScreen-N, further research on this phenomenon was undertaken. The objective of this study was to evaluate the efficacy of inorganic salts in enhancing the transungual delivery of terbinafine hydrochloride.

Materials and Methods

Materials

Terbinafine hydrochloride was procured from Uquifa (Jiutepec, Mexico). Sodium sulfite, sodium phosphate monobasic, potassium phosphate monobasic, calcium phosphate monobasic and ammonium carbonate were purchased from Sigma (MO, US). Human cadaver nails (second, third and fourth finger nails), both male and female with varying thickness (0.4–0.7 mm), were procured from Science Care (AZ, US) and were stored at 4°C and used within a week. All solutions were prepared in de-ionized water.

Analytical method

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

Preliminary in-vitro permeation studies

Nails were cleaned and adherent tissue was removed with a pair of scissors and scalpel and the nail was rinsed in water. Each of the nail plates was soaked in water for 1 h and mounted on a nail adapter (PermeGear, PA, US). The whole assembly was sandwiched between the two chambers of a Franz diffusion cell (Logan Instruments Ltd, NJ, US). Terbinafine hydrochloride solution (500 μl, 1 mg/ml with 0.5 M enhancer) was placed in the donor compartment and the receiver chamber was filled with 5 ml of acidified water (adjusted to pH 3 using 0.1 M hydrochloric acid or sodium hydroxide as the initial study data revealed that an ionic strength within 0.15 M does not affect the drug load and permeation). The enhancers used were ammonium carbonate, calcium phosphate, potassium phosphate, sodium phosphate and sodium sulfite. The active permeation area was 0.2 cm^2. The solution in the receiver compartment was stirred at 600 rev/min with a 3-mm magnetic stir bar. Samples were withdrawn at regular intervals for a period of 24 h from the receiver compartment and analysed. Similarly, control experiments (formulation with drug and no enhancer) were run in parallel for comparison.

Amount of drug in nail

The amount of terbinafine loaded in the nail was determined after the permeation study (24 h). Briefly, after in-vitro diffusion studies the nail plates were washed by a standardized procedure using water and 95% ethanol, until the surface was free from drug. The active diffusion area was cut into small pieces, weighed and placed in screw-cap pyrex vials. Sodium hydroxide (1.5 ml of 1 M) was added and the vials were incubated for 24 h in a shaker water bath at room temperature to allow the nail to dissolve. Extraction of drug was carried out by a slight modification of the method described by Dykes *et al.*[13] Briefly, after dissolving the nails in vials, 200 μl of 5 M hydrochloric acid was added to neutralize the mixture. Hexane (3 ml) was added to the vial to extract the drug and the vials were shaken manually for 30 min. The mixtures were transferred into centrifuge tubes and centrifuged at 4000 rev/min for 10 min. The hexane layer was collected, 1 ml of 0.5 M sulfuric acid–isopropyl alcohol (85 : 15) was added and the mixture was shaken vigorously for 30 min. The lower acidic aqueous layer, which holds the majority of terbinafine, was collected separately and the amount of drug in the nail was determined. This

extraction procedure was validated by spiking different drug concentrations (2–20 μg/ml) into sodium hydroxide solution in which the nail was previously dissolved. The recovery was found to be 84 ± 7%.

The amount of terbinafine diffused into the peripheral region (which was not in contact with the formulation or enhancer) was determined by dissecting the peripheral nail area (4–5 mm surrounding), which was washed, dried and weighed. The amount of drug was determined as described above.

Solubility measurements

A normal equilibrium solubility determination was carried out by the method of Okumara *et al.*[14] An excess amount of terbinafine hydrochloride was added and dissolved in a measured amount of solution containing different concentrations of sodium phosphate (0, 0.25, 0.5, 1.0, 2.0 and 3.0 M) in a glass vial to obtain a saturated solution. The pH of the solution was adjusted to pH 3 or 5 (using 0.1 M HCl or 0.1 M sodium hydroxide) and was monitored occasionally throughout the study period. The system was stirred for 24 h at room temperature and kept at rest for 1 h to assist the attainment of equilibrium. The solution was then filtered through a membrane filter (pore size 0.22 μm) and, after dilution, the solubility was determined.

Effect of salt concentration on permeation

Permeation of terbinafine in the presence of different concentrations of sodium phosphate (0.25, 0.5, 1, 2 and 3 M) was carried out as described previously in preliminary in-vitro permeation studies. The donor drug concentration was 1 mg/ml (pH 3). The amount of drug permeated after 24 h was measured by HPLC.

Effect of pretreatment on permeation

Pretreatment of nails was carried out by soaking the nail plates in 0.5 M sodium phosphate solution (pH 3) for 24 h. The nail plates were then washed with 10 ml of water (pH 3) five times and mounted on a nail adapter. Drug solution (500 μl; 1 mg/ml; pH 3) was placed in the donor compartment. For control trials, the nail was soaked in water (pH 3). Permeation studies were carried out as described in preliminary in-vitro permeation studies.

Effect of pH

The influence of pH on the permeation of drug was assessed using 1 mg/ml of terbinafine hydrochloride solution (pH 5) in the absence of salt. Permeation was carried out as described previously in preliminary in-vitro permeation studies and the amount of terbinafine permeated after 24 h was determined.

Permeation studies using dialysis membrane

Permeation studies were carried out across the dialysis membrane (MWCO 1000, 7 Spectra/Por; Spectrum Laboratories, Inc., CA, US) with varying concentration of sodium phosphate (0.25, 0.5, 1, 2 and 3 M). The membrane was mounted between the donor and receiver chamber of a Franz diffusion cell (Logan Instruments Ltd., NJ, US). Drug–enhancer solution (500 μl; 1 mg/ml; pH 3) was placed in the donor compartment while the receiver chamber was filled with 5 ml of the respective sodium phosphate solutions without drug (pH 3), to maintain uniform osmotic pressure. The permeation area was 0.64 cm^2. The solution in the receiver compartment was stirred at 600 rev/min with a 3-mm magnetic stir bar. Samples were withdrawn at regular intervals from the receiver compartment and analysed. Control experiments were run in parallel for comparison using terbinafine solution (1 mg/ml; pH 3).

Water uptake

Nail pieces (4 × 4 mm) were incubated at 37 ± 0.5°C for 24 h, weighed (W1) and soaked for 24 h in water (pH 3) or 0.5 M sodium phosphate solution (pH 3). Then the nail plates were removed and wiped with Kimwipe to remove the surface water. Nails were placed in a screw-cap Pyrex vial containing pre-weighed anhydrous sodium sulfate (W2) and kept at room temperature until the weight of sodium sulfate (W3) remained constant (48 h). The increase in the weight of sodium sulfate was noted and the percentage water uptake of the nail was calculated by the following equation.

$$\%\ \text{Water uptake} = [(W3 - W2)/W1] \times 100 \qquad (1)$$

Permeation using poloxamer gel

The poloxamer gel was prepared by a 'cold process'. Drug solution (1 mg/ml; pH 3) was slowly added to poloxamer (21% w/v) with constant stirring at low temperature (4–5°C) until the polymers were uniformly dispersed. The required amount of permeation enhancer (0.5 M sodium phosphate) was incorporated into the drug dispersion while mixing, and the pH was readjusted to 3 using 0.1 M HCl. The dispersion was mixed and then kept in a refrigerator (4–5°C) overnight (12 h).

The drug content was determined by accurately weighing 1 g of each gel, which was diluted with ethanol (95%), filtered and assayed by HPLC. The drug content in the gel was found to be 0.84–0.89 mg/g.

Nail plates were soaked in water for 1 h and mounted on a nail adaptor. Five-hundred milligrams of the viscous gel (with or without enhancer) was placed in the donor compartment and the permeation studies were carried out for a period of 24 h, as previously described in preliminary in-vitro permeation studies.

Statistical analysis

Statistical analysis on the effect of different salts on the permeation of terbinafine through the nail, drug load in the nail and the influence of concentration of sodium phosphate on the drug permeation across the nail and dialysis membrane was performed using two tailed Kruskal–Wallis test (Graphpad Prism 5; Graphpad software, Inc., CA, US). In all cases, post-hoc comparisons of the means of individual groups were performed using Dunn's test. The effect on enhancement of terbinafine permeation across nail and dialysis membrane with different concentrations of sodium phosphate was analysed using Mann–Whitney *U*-test. The data points provided in the graphs are an average of four trials. The error bars represents the standard deviation. $P < 0.05$ denoted significance in all cases.

Results

Figure 1 represents the effect of different inorganic salts at 0.5 M concentration on the permeation of terbinafine hydrochloride across the nail plate. It is evident that the permeation was significantly enhanced (3–5 fold, Kruskal–Wallis test, Dunn's test, $P = 0.008$) in the presence of salts when compared with the control. The cumulative amount of drug permeated after 24 h with different salts (ammonium carbonate (8.09 ± 1.85 μg/cm^2), sodium phosphate (11.44 ± 1.62 μg/cm^2), calcium phosphate (7.75 ± 0.59 μg/cm^2), potassium phosphate (10.47 ± 1.92 μg/cm^2), sodium sulfite (6.93 ± 0.72 μg/cm^2)) was found to be higher than the control (2.24 ± 0.72 μg/cm^2). The profiles also indicated that the drug permeation was higher in the initial period (up to 12 h) and followed slow release afterwards, with all the enhancers studied. In contrast, control experiments exhibited much less permeation and the lag time to attain steady-state diffusion was ~8 h.

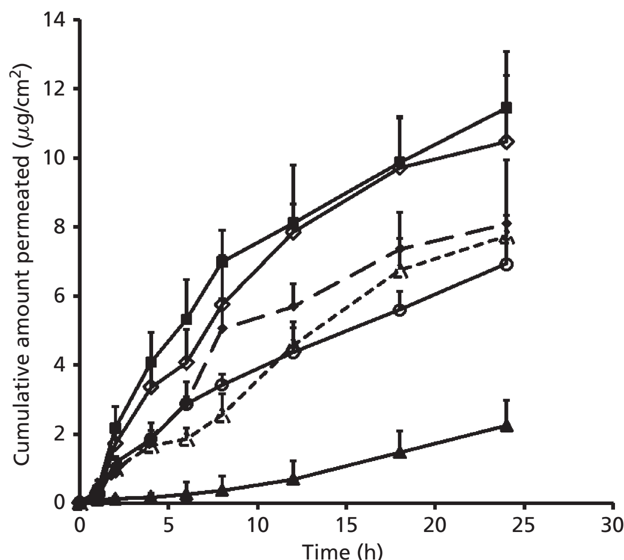

Figure 1 Permeation profile of terbinafine hydrochloride in the presence of different salts. Permeation was carried out using terbinafine hydrochloride solution (1 mg/ml; pH 3) and the salt concentration was 0.5 M. ▲, control; ◆, ammonium carbonate; ◇, potassium phosphate; ■, sodium phosphate; △, calcium phosphate; ○, sodium sulfite. Data are expressed as mean ± SD, $n = 4$.

The drug load in the active diffusion area and peripheral area of the nail plate after 24 h of drug permeation studies with different enhancers and the control are represented in Figure 2. The inorganic salts enhanced the drug load in both the active diffusion area (4–7 fold, Kruskal–Wallis test, Dunn's test, $P = 0.011$) and peripheral area (5–10 fold, Kruskal–Wallis test, $P = 0.0275$).

Table 1 summarizes the solubility of terbinafine with different concentration of sodium phosphate at pH 3 and pH 5. The solubility decreased with increasing salt concentration at both pH 3 and pH 5.

In the next step, permeation studies were carried out to evaluate the effect of the concentration of sodium phosphate (0, 0.25, 0.5, 1.0, 2.0 and 3.0 M) on drug permeation across the nail plate using terbinafine solution (1 mg/ml pH 3), and the data obtained are presented in Figure 3. The drug permeation increased with increase in salt concentration up to 1 M and decreased with further increase in salt concentration (2–3 M) (Kruskal–Wallis test, $P = 0.001$). A similar effect was also observed when the permeation was carried out at different salt concentrations across the dialysis membrane (Figure 4).

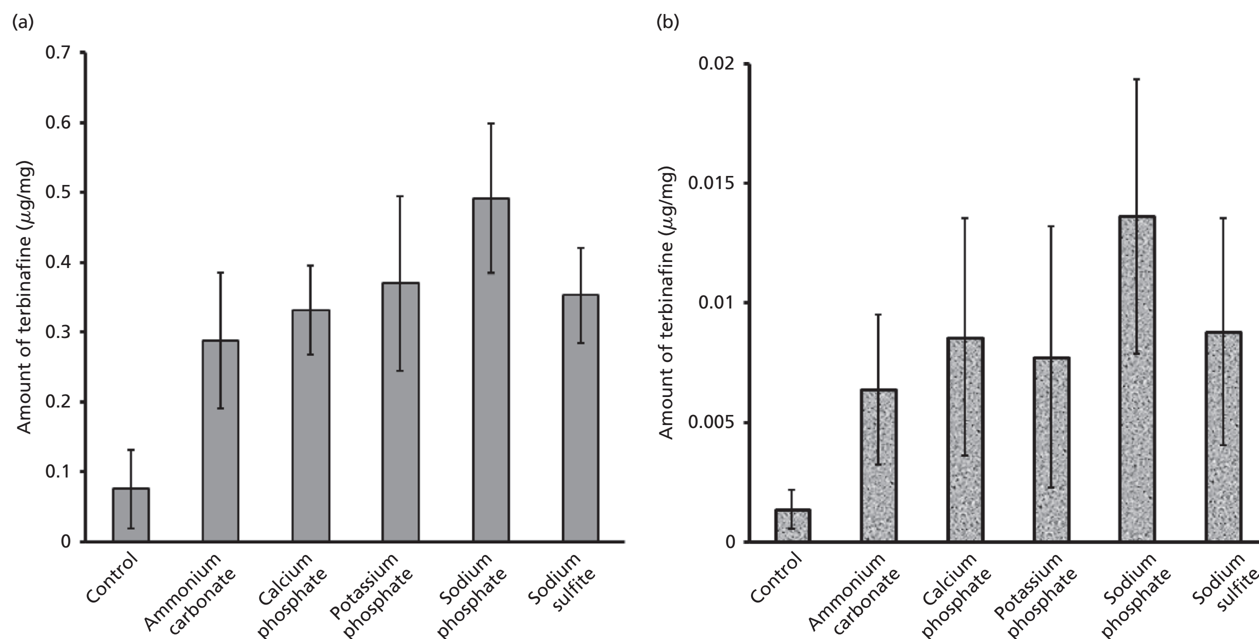

Figure 2 The amount of terbinafine hydrochloride loaded (μg/mg) in the nail diffusion area (a) and peripheral nail (b) during permeation studies in the presence of different salts (0.5 M) in 24 h. The diffusion area was 0.2 cm^2. Data are expressed as mean ± SD, $n = 4$.

Table 1 Solubility of terbinafine hydrochloride in water (pH 3 and 5) with different concentration of sodium phosphate

Concentration of sodium phosphate (M)	Terbinafine hydrochloride solubility (mg/ml)	
	pH 3	pH 5
0	1.57 ± 0.23	1.12 ± 0.16
0.25	1.41 ± 0.18	0.86 ± 0.10
0.5	1.19 ± 0.25	0.74 ± 0.14
1.0	0.98 ± 0.20	0.63 ± 0.08
2.0	0.79 ± 0.16	0.54 ± 0.12
3.0	0.69 ± 0.11	0.42 ± 0.11

Solubility studies were carried out at room temperature (25 ± 1°C). Each value represents the mean ± SD, n = 5.

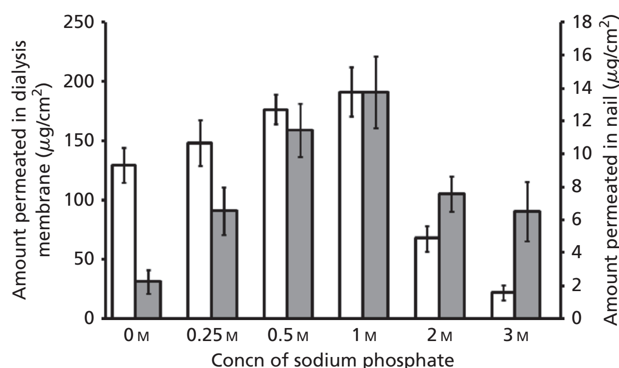

Figure 3 Comparison of the cumulative amount of terbinafine permeated across the dialysis membrane and nail at the end of 24 h with different concentrations (0, 0.25, 0.5, 1.0, 2.0 and 3 M) of sodium phosphate. The diffusion area of the dialysis membrane and nail were 0.64 cm^2 and 0.2 cm^2, respectively. Open columns, dialysis membrane; shaded columns, nail. Data are expressed as mean ± SD, n = 4.

Mechanistic aspects on the enhancement of terbinafine permeation by inorganic salts were studied by pretreatment of nails with sodium phosphate solution (0.5 M for 24 h) and then followed by the permeation studies. The amount of terbinafine permeated after 24 h across the treated and untreated nail plate was 3.03 ± 0.52 and 2.39 ± 0.44 $\mu g/cm^2$, respectively. Water uptake study indicated a higher percentage water uptake by the nail in the presence of inorganic salt (36.34 ± 4.68%) when compared with control (26.27 ± 4.55%). The cumulative amount of terbinafine permeated after 24 h from the formulated gel containing 0.5 M sodium phosphate (9.70 ± 0.93 $\mu g/cm^2$) was found to be comparable with the amount of drug permeated from solution (11.44 ± 1.62 $\mu g/cm^2$) (Figure 5).

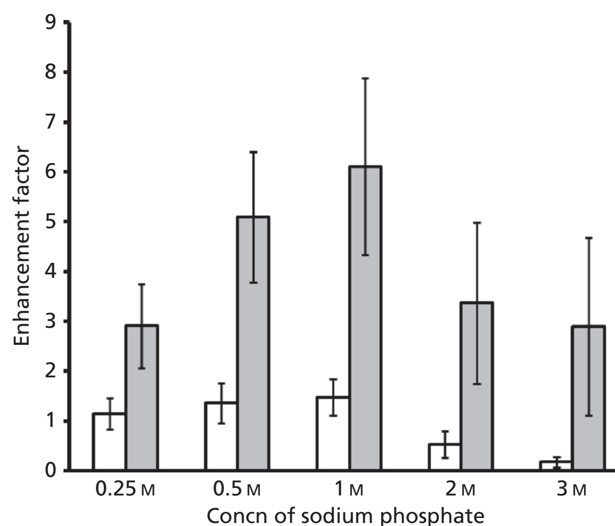

Figure 4 Comparison of enhancement in terbinafine hydrochloride permeation across nail and dialysis membrane in the presence of different concentration of sodium phosphate. Enhancement was calculated with respect to control (1 mg/ml, pH 3 water). The donor constituted 1 mg/ml of terbinafine hydrochloride. Open columns, dialysis membrane; shaded columns, nail. Data are expressed as means ± SD, n = 4.

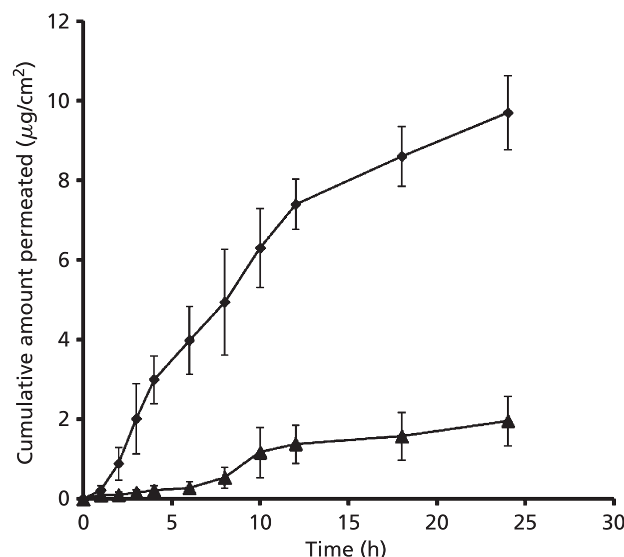

Figure 5 Effect of sodium phosphate on the permeation of terbinafine hydrochloride from poloxamer gel across the nail plate. The concentration of sodium phosphate used was 0.5 M. ♦, sodium phosphate; ▲, control. Data are expressed as mean ± SD, n = 4.

Discussion

Preliminary in-vitro permeation studies were carried out across the nail plate with different inorganic salts using terbinafine hydrochloride solution (1 mg/ml; pH 3). The concentration of all salts was kept at 0.5 M for the purpose of comparison. The donor vehicle was adjusted to pH 3 as the drug possesses high solubility at this pH as compared with higher pH. From Figure 1 the ability of salts to enhance the permeation of terbinafine across the nail plate and reduce the lag time to attain steady-state diffusion is evident. All the salts were comparable in their enhancement efficiency (3–5 fold), although with sodium phosphate the difference to the control was significant (~5-fold enhancement). This is the first report to our knowledge on the enhancement effect of inorganic salts in transungual drug delivery.

During permeation across biological membranes, a certain amount of drug is retained in the barrier. The drug retention is a factor of its solubility in the membrane, pH–pKa conditions of the experiment and concentration of the drug. Retention due to irreversible binding generally hampers the permeation of a drug into deeper tissues in topical drug delivery. However, in the case of nail, we observed that the terbinafine loaded in the nail during the permeation process formed a depot and released subsequently to provide prolonged therapeutic effect in the affected target tissue underneath the nail plate.[15] It is evident from Figure 2a in our study that the presence of salts significantly enhanced the drug load (4–7 fold) compared with the control. Like permeation, the highest drug load was observed with sodium phosphate (0.47 ± 0.16 μg/mg). Further, the amount of drug permeated across the nail plate was found to be proportional to the drug load in the nail.

The amount of drug loaded in the active diffusion area represents the area of nail that is in direct contact with the formulation. *In vivo*, this would be the exposed part of the nail available for application of drug formulation. Onychomycosis affects the whole nail apparatus, which includes the nail that is not accessible due to overlap by the nail fold. Therefore, the drug loaded in the active diffusion area is required to diffuse peripherally into the area that is unexposed to formulation. This peripheral diffusion, in turn, plays a crucial role in the success of the onychomycosis therapy. In our permeation experiments, the amount of drug loaded into the surroundings of the active diffusion nail area was also assessed. The amount of drug reaching the peripheral nail area depends on the concentration gradient between the active diffusion area and peripheral nail area. It is obvious that the salts enhanced the drug load in the peripheral nail as they could enhance the drug load in the active diffusion area (Figure 2b).

Given the promising results in the preliminary permeation studies, further studies on the effect of salt on transungual drug delivery was investigated using sodium phosphate as a representative salt enhancer. An increase in salt concentration did lead to increased drug permeation. However, the drug permeation decreased at higher salt concentrations (2–3 M) (Figure 3). To obtain further insight into the mechanisms responsible for the enhancement of drug permeation by inorganic salts, further studies were carried out. Generally, drug penetration enhancers act by modifying the structure of the barrier or by driving the drug into the barrier. If the permeation enhancers are capable of bringing about irreversible changes in the barrier property of the tissue, then the permeability of the nail plate would be enhanced upon pretreatment of the nail plate with the penetration enhancer. Hence to assess the effect of salts on the barrier property of nail, permeation studies were carried out across nails that had been pretreated with sodium phosphate solution (0.5 M for 24 h). The permeation of terbinafine in 24 h across the treated (3.03 ± 0.52 μg/cm^2) and control (2.39 ± 0.44 μg/cm^2) nail plate suggests that the terbinafine permeation enhancement observed (in preliminary studies and the effect of salt concentration in the subsequent studies) was not due to any irreversible structural alteration of the nail plate.

The other possible mechanism responsible for the penetration enhancement is thermodynamic driving due to the concentration gradient. To explain the enhancement of permeation based on thermodynamic activity, one should know the solubility of drug in different concentrations of sodium phosphate solution. From Table 1, it can be noted that the solubility of terbinafine hydrochloride decreased with an increase in salt concentration. It is well known that permeation flux is maximal from saturated solutions of drug due to the maximum thermodynamic activity. In this study the donor concentration used was 1 mg/ml and from Table 1 it is apparent that the thermodynamic activity of terbinafine increased in the salt solution in the order of $0.25 < 0.5 < 1$ M. At 1 M concentration, the solubility was 0.98 ± 0.20 mg/ml, which has the maximum thermodynamic activity of unity. Thus, it appears that increase in thermodynamic activity is one of the mechanisms for the enhanced terbinafine permeation (cumulative amount permeated in 24 h) across the nail plate. At concentrations >1 M the thermodynamic activity still remained one, but the absolute solubility of drug decreased with increasing salt concentration. This is the likely reason for the decrease in drug permeation from salt solution concentrations above 1 M. Although there was a meaningful relationship interpreted between the permeation data and thermodynamic activity, this experiment does not rule out the possible real time effect of salts on the nail plate.

To understand the relative contribution of thermodynamic activity and the potential real time effect of salts on the nail plate, two sets of permeation studies were carried out. In the first set, permeation studies were carried out across the nail using terbinafine hydrochloride solution (1 mg/ml) at pH 5 (in the absence of salt) in which the thermodynamic activity of the solution would be higher than at pH 3 due to low drug solubility. The drug solubility at pH 3 and 5 was 1.57 ± 0.23 and 1.12 ± 0.16 mg/ml, respectively. The cumulative amount of drug permeated across the nail at pH 5 was 1.5-fold (3.38 ± 0.39 μg/cm^2) that observed at pH 3. This study further confirms that the thermodynamic activity has a significant role to play in the transungual drug permeation enhancement by salts. Here the important point to note is that the solubility of terbinafine at pH 5 in the absence of any salt (1.12 ± 0.16 mg/ml) was comparable with its solubility at pH 3 in the presence of 0.5 M sodium phosphate (1.19 ± 0.25 mg/ml). However, the enhancement in drug permeation observed in the latter case was significantly higher (5 fold) than in the former.

In the second set of studies, the permeation experiments were carried out at different salt concentrations across a dialysis membrane. The salt concentration on both sides of the membrane was the same so as to avoid a concentration gradient. In this case, the cumulative amount permeated showed a similar salt-concentration-dependent trend as observed with the nail permeation studies (Figure 3). However, it is noteworthy that the permeation enhancement factor (in dialysis membrane) over the control was lower (Mann–Whitney *U*-test, $P = 0.0286$) than that observed across the nail plate (Figure 4). The above two sets of studies suggests that there could be additional mechanisms contributing to the permeation enhancement by salts, besides thermodynamic activity.

Water is known to be a plasticizer for nail as it hydrates up to ~25% of the nail weight.[16] It is well known that most of the permeation enhancers generally increase the hydration level of nail keratin molecules. Increased hydration of keratin molecules is believed to provide a continuous pathway to the diffusion of molecules across the nail plate and thereby

enhances the permeation and drug load.[17] Moreover, it is also reported that the pH of the solution does not influence the nail hydration level.[18] Hence it was thought rational to determine the water uptake of nail in the presence and absence of salt at pH 3. The calculated percentage of water uptake by nail in the presence of salt enhancer was 36.34 ± 4.68%, and for the control it was 26.27 ± 4.55%. The apparent difference in hydration level due to the presence of sodium phosphate is one of the key mechanisms responsible for the transungual drug permeation enhancement effect of salts. However, quantifying the water content in the nail plate at different salt concentrations would be a challenging task due to the difference between the tissues and due to the lack of any simple and sensitive method of measuring water in a way applicable to the present case. Neverthless, from this study we can speculate that the increase in salt concentration increases the hydration of keratin in the nail plate. When the drug concentration in the salt solution is constant (as in the case of salt concentrations <1 M), the increased hydration would in turn increase the drug loading in the nail. At salt concentrations above 1 M, the absolute drug concentration is less, which is likely the reason for the relative decrease in the drug load, despite the increased hydration of nail plate at higher salt concentration.

The last phase of study was intended to develop a suitable formulation and assess the permeation across the nail plate. As mentioned earlier there are different formulations available for topical application to the nails. Most of these are nail lacquers in which a film-forming polymer is dissolved in a rapidly evaporating solvent.[19] Often penetration enhancers are incorporated into the nail lacquers even though most of the nail penetration enhancers would be effective in the presence of water (or when the nail is hydrated). It is certain that the nail hydration level achieved on application of a nail lacquer would be much lower than the hydration level that could be attained by the application of a hydrogel. The gel formulations hold a significant amount of water and possess an ability to overcome the contour of the nail. The ability of gel to permeate a greater amount of drug than from a nail lacquer across the nail plate was demonstrated by Hui *et al*.[20] Therefore, in our study, a gel was formulated using poloxamer gel (21% w/v) by cold process. Permeation studies were conducted (24 h) under similar experimental conditions to those used in the preliminary permeation study: enhancer concentration, 0.5 M sodium phosphate; drug content, ~1 mg/g; and pH 3. The amount of drug permeated across the nail plate was assessed, as depicted in Figure 5. It is obvious that the permeation profile and enhancement results observed with the drug solution (Figure 1) were translated to the gel formulation also. This study shows that the salt permeation enhancers could be employed in gel formulations for topical application.

Conclusions

Results from this study indicate that inorganic salts are promising transungual permeation enhancers. Greater benefit can be derived with respect to the drug permeation when the salts are used at optimum concentrations in the formulation. Mechanisms contributing to the enhancement of drug permeation across the nail plate by salts include their ability to increase the nail hydration and also to increase the thermodynamic activity of the drug. These enhancers are inexpensive, safe and readily available, which makes them more attractive when compared with other enhancers.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Elewski BE. Onychomycosis, pathogenesis, diagnosis, and management. *Clin Microbiol Rev* 1998; 11: 415–429.
2. Murdan S. Drug delivery to the nail following topical application. *Int J Pharm* 2002; 236: 1–26.
3. Bodman MA *et al.* Topical treatments for onychomycosis. *J Am Podiatr Med Assoc* 2003; 93: 136–141.
4. El Mahrab Robert M, Kalia YN. New developments in topical antifungal therapy. *Am J Drug Deliv* 2006; 4: 231–247.
5. Effendy I. Therapeutic strategies in onychomycosis. *J Eur Acad Dermatol Venereol* 1995; 4(Suppl. 1): S3–S10.
6. Afsaneh A *et al*. Common nail disorders and fungal infections. *Adv Skin Wound Care* 2007; 20: 346–357.
7. Gupchup GU, Zatz JL. Structural characteristics and permeability properties of the human nail, a review. *J Cosmet Sci* 1999; 50: 363–385.
8. Van Hoogdalem EJ *et al.* Nail penetration of the antifungal agent oxiconazole after repeated topical application in healthy volunteers, and the effect of acetylcysteine. *Eur J Pharm Sci* 1997; 5: 119–127.
9. Kobayashi Y *et al.* 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* 1998; 46: 1797–1802.
10. Malhotra GG, Zatz JL. Investigation of nail permeation enhancement by chemical modification using water as a probe. *J Pharm Sci* 2002; 91: 312–323.
11. Hui X *et al.* Enhanced econazole penetration into human nail by 2-n-nonyl-1,3-dioxolane. *J Pharm Sci* 2003; 92: 142–148.
12. Hao J *et al.* Chemical method to enhance transungual transport and iontophoresis efficiency. *Int J Pharm* 2008; 357: 61–69.
13. Dykes PJ *et al.* Determination of terbinafine in nail samples during systemic treatment for onychomycoses. *Br J Dermatol* 1990; 123: 481–486.
14. Okumara M *et al.* Skin permeability of water soluble drugs. *Chem Pharm Bull* 1989; 37: 1404–1409.
15. Nair AB *et al.* Trans-ungual iontophoretic delivery of terbinafine. *J. Pharm. Sci.* 2008 (epub ahead of print).
16. Gunt HB, Kasting GB. Equilibrium water sorption characteristics of the human nail. *J Cosmet Chem* 2007; 58: 1–9.
17. Hui X *et al.* Enhanced human nail drug delivery, nail inner drug content assayed by new unique method. *J Pharm Sci* 2002; 91: 189–195
18. Hao J, Li SK. Mechanistic study of electroosmotic transport across hydrated nail plates: effects of pH and ionic strength. *J Pharm Sci* 2008; 97: 5186–5197.
19. Marty J-PL. Amorolfine nail lacquer, a novel formulation. *J Eur Acad Dermatol Venerol* 1995; 4: S17–S21.
20. Hui X *et al.* Ciclopirox delivery into the human nail plate. *J Pharm Sci* 2004; 93: 2545–2548.