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Research Paper

Effect of a novel penetration enhancer on the ungual permeation of two antifungal agents

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Abstract

Objectives The aim of this study was to demonstrate the effect of a novel permeation enhancer system using two existing marketed nail lacquers and the delivery of terbinafine through human nail samples *in vitro*.
Methods Initially a modified Franz cell was used, where sections of human nail serve as the barrier through which drug penetrates into an agar-filled chamber infected with dermatophytes. A second study was performed using a novel infected nail model where dermatophytes are incubated with and grow into human nail and ATP levels are used as biological marker for antimicrobial activity.
Key findings The novel permeation enhancing system increased the permeation of both existing drugs formulated in nail lacquers and terbinafine through human nail sections mounted in a modified Franz cell. Furthermore the ATP assay confirmed that the system also enhanced the permeation of terbinafine through infected cadaver nail resulting in a decrease in ATP levels equivalent to those of uninfected negative control samples.
Conclusions This study has clearly demonstrated that the use of a novel permeation enhancing system, which fundamentally alters the chemical structure of the nail, not only enhances the efficacy of the existing topical formulations but also enables the delivery and efficacy of terbinafine when applied ungually. Such a topically applied system has the possibility of overcoming the systemic side effects when terbinafine is delivered orally.
Keywords nail; onychomycosis; permeation enhancer; terbinafine; ungual

Introduction

Onychomycosis is a fungal infection of the nail that accounts for approximately 50% of all nail disorders and affects toenails substantially more than fingernails.[1] The prevalence of onychomycosis has been estimated to be around 5% in Western countries and has continued to increase in recent decades.[2–8] While onychomycosis may be caused by dermatophytes, yeasts or moulds it is accepted that the former are by far the predominant pathogens and probably account for more than 85% of all cases of fungal nail infections. Of the dermatophytes the most common cause of onychomycosis is *Trichophyton rubrum*.[5,9] Causative moulds include *Scopulariopsis brevicaulis* and *Scytaldium dimidiatum*.

Distal and lateral subungual onychomycosis (DLSO) is the commonest type of onychomycosis. Infection is initially a disease of the hyponichium, resulting in hyperkeratosis of the distal nail bed. It generally begins at the lateral edge of the nail rather than the central portion and spreads progressively proximally down the nail bed producing hyperkeratosis and thus onycholysis. Ultimately the underside of the nail is involved, which results in thickening of the nail. The nail may become friable and crumbles away. Sometimes the fungus proliferates in the space between the nail plate and nail bed (known as a dermatophytoma)[10] and is often the cause of treatment failure.[3]

It is important to treat onychomycosis, as it is an infection that does not resolve spontaneously. The infection may worsen, spread to other uninfected locations (other nails or to the surrounding skin) or infect other patients. Infections of the fingernails may be cosmetically unacceptable. Infections of the toenail can greatly affect the quality of life of patients and cause pain and morbidity.[9]

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Treatment for onychomycosis may be topical or oral. There have been four main oral therapies available for the treatment of onychomycosis: griseofulvin (Grisovin; Glaxo Wellcome); ketoconazole (Nizoral; Janssen-Cilag); itraconazole (Sporanox; Janssen-Cilag) and terbinafine (Lamisil; Novartis). Griseofulvin has been available since the 1950s and, due to its fungistatic activity against dermatophytes, requires long treatment periods (9–12 months for toenail infections) and has low cure rates and high relapse rates.[11,12] Ketoconazole was the first imidazole introduced for the treatment of onychomycosis in the 1980s. However, due to hepatotoxicity its use is now restricted to fingernail infections that have failed to respond to other therapies. The newer antifungals, itraconazole and terbinafine, are highly effective in the treatment of onychomycosis with mycological cure rates of 70–80% and treatment periods of 12–16 weeks.[12–14] The main disadvantages of systemic oral treatments is that they can cause systemic toxicity and may also cause significant drug interactions so it is important to review and document concomitant medications before commencing treatment.[13,15]

Topical therapies include amorolfine (Loceryl; Galderma) and ciclopirox (Penlac; Dermik).[16–20] Probably as a result of the poor drug penetration from these products through the nail, treatment times are long (12 months for toenail infections) and cure rates are low,[3,12,21] and alternative drugs and formulations to improve delivery are being sought.

Permeation of drugs through the skin has been extensively documented in the last 60 years,[22–29] while permeation of drugs through the nail remains a relatively undocumented area. Compared with the thin stratum corneum, the much thicker nail plate means a much longer diffusional pathway for drug delivery. In addition, in contrast to the elastic and pliable stratum corneum, the nail plate is dense and hard. However, when the thickness difference is taken into consideration, the water permeation rate of the nail is about 10 times higher than that of the stratum corneum. As such it would be expected that the permeation characteristics of the nail are very different from the stratum corneum, and this is supported by the current literature.[3,30]

The physical and chemical differences between the nail and stratum corneum are probably the reasons for the lack of efficacy of the topical nail antifungal formulations presently on the market as they have been developed based on knowledge of the barrier properties of the skin. Thus, when designing topical formulations for perungual drug absorption it is essential to consider the physicochemical properties of the drug molecule (e.g. size, shape, charge, log P, etc), the formulation characteristics (e.g. vehicle, pH, drug concentration) and possible penetration enhancers, as well as any possible interactions between the drug and keratin. Monti *et al.*[31] showed that incorporation of the water-soluble film-forming agent hydroxypropyl chitosan (HPCH) in a nail lacquer resulted in a decreased lag time in the uptake of ciclopirox compared with Penlac. The authors suggested that this was likely to be due to the adhesive properties of HPCH when in contact with biological membranes, and the formation of hydrogen bonds between the hydroxypropyl groups on the HPCH and the keratin, resulting in intimate contact between the vehicle and the keratin matrix thus enhancing transfer of ciclopirox from the vehicle in to the nail matrix. Malhotra and Zatz[32] investigated a wide range of possible nail permeation enhancers using tritiated water as a probe. They postulated that any potential permeation enhancer would induce changes in the primary or higher-order structure of keratin, thus making the nail more permeable. Malhotra and Zatz tested a wide range of compounds including mercaptan compounds, sulfites and bisulfites, keratolytic agents and surfactants. They concluded that the most effective enhancing agents produced irreversible changes to the keratin matrix compromising its barrier function, whereas those compounds that were not as effective produced minor reversible changes to the barrier function, which was quickly restored.[32] A range of non-chemical physical enhancement techniques, including abrasion or etching of the nail surface, ultrasound and micropration, have also been investigated by a range of authors and are comprehensively reviewed elsewhere.[33]

Described in this paper is a novel penetration enhancer consisting of sequential application of a reducing agent and an oxidising agent used as a pre-treatment before application of a pharmaceutically active agent. Khengar *et al.*[34] previously tested a range of reducing and oxidising agents using a nail swelling model to predict the enhancing effect of such agents. The authors identified thioglycolic acid and urea hydrogen peroxide as being the reducing and oxidising agents that produce the greatest level of enhancement of those tested. Brown *et al.*[35] subsequently tested the enhancing potential of these agents alone and in combination with each other and discovered that treatment of the nail with thioglycolic acid followed by urea hydrogen peroxide had the greatest penetration-enhancing effect on a range of model permeants. This pre-treatment (thioglycolic acid followed by hydrogen peroxide) is not a product on its own, it has no therapeutic effect and contains no active ingredients; it is a penetration enhancer that is capable of altering the barrier properties of the nail such that it becomes more permeable to antifungal agents such as ciclopirox, amorolfine and terbinafine.

This study aims to demonstrate the effect of a novel penetration-enhancing system on the permeation of two drugs, amorolfine and ciclopirox, presented in two marketed nail lacquers, and terbinafine applied in a spray system developed in-house.

Materials and Methods

Thioglycolic acid and urea hydrogen peroxide addition compound were purchased from Sigma-Aldrich (Dorset, UK). Human nail clippings were donated from healthy volunteers, and cadaver nails obtained from a human tissue bank (Science Care Anatomical, Phoenix, USA) following approval by the King's College Research Ethics Committee (study ref. No. 04/05-126). Validated ChubTur and TurChub permeation cells were kindly donated by MedPharm Ltd (Surrey, UK). Ethanol (EtOH) was purchased from BDH Chemicals Ltd (Dorset, UK). Acetonitrile (ACN), triethylamine, orthophosphoric acid, potassium dihydrogen orthophosphate (KH_2PO_4) and Ringers solution were supplied by Fisher (Loughborough, UK). MedPharm's lysing agent was supplied by MedPharm Ltd. *T. rubrum* was originally isolated from a patient suffering from onychomycosis and was a gift from Cardiff University. Sterile gauze filter (Propax, 7.5 cm × 7.5 cm 8-ply gauze swab, BP Type 13) was obtained from Smith & Nephew (Hull, UK). Adenosine 5-triphosphate standard disodium salt trihydrate (substantially vanadium free) was obtained from Sigma (Poole, UK). BacTiter-Glo kit and BacTiter-Glo substrate were obtained from Promega (Southampton, UK).

TurChub assay

The TurChub assay uses a modified Franz cell, where sections of human nail serve as the barrier through which the drug initially penetrates into an agar-filled receptor chamber where the dermatophytes (*T. rubrum*) grow. The cells are dosed with the test formulation and then incubated for a set period of time, at a set temperature. After incubation the presence of any zone of inhibition is measured (examples of which are shown in Figure 1).

Preparation of human nail clippings (distal)

Initially, distal nail clippings were obtained from volunteers' toe nails, which had been grown to a minimum length of 3 mm. All volunteers were required to not have used nail varnish or polish on their toe nails within 6 months and have not shown any signs of disease to their nails within six months. All volunteers were asked to remove the distal nail sections using either scissors or standard nail clippers. The nail clippings were then placed into an appropriate container e.g. plastic bag, vial, envelope etc for transfer to our laboratory. The nail clippings were then placed into an 8 ml bijou bottle per donor/donation and labelled with any details supplied.

Preparation of 3 mm × 3 mm distal nail segments

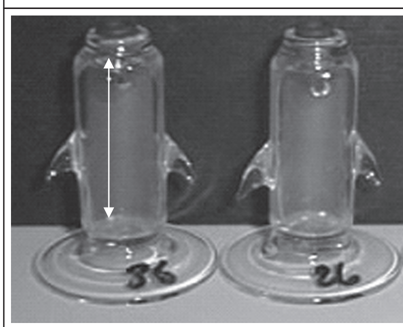
Using scissors, the nails clippings were cut into pieces, which were a minimum of 3 mm by 3 mm. The nail clippings were immersed in a 70% ethanol in water solution and vortex mixed for 1 min. The ethanol solution was then decanted and replaced with a fresh 70% ethanol solution and vortex mixed for a further minute. The ethanol solution was then decanted and replaced with Ringer's solution, vortex mixed for 1 min and decanted and replaced with fresh Ringer's. This process of washing with Ringer's was carried out a total of three times, replacing the wash solution at each phase. Once the washing process was complete, the nail clippings were placed into a sterile Petri dish without a lid and air dried under a laminar flow cabinet for 30 min at room temperature. Once the nail clippings were dry, they were placed into new 8 ml bijou bottles (separate bottle per donor, per batch). Nails were measured for thickness using a sterilised pair of callipers.

Preparation of growth media

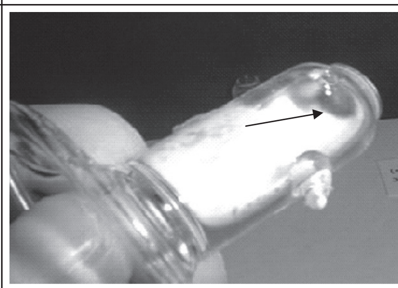
Briefly, to prepare standard Sabouraud dextrose agar (SDA), 65 g of the powdered agar was suspended in 1 l of distilled water. The mixture was then heated to boiling point while stirring to dissolve the powdered agar completely. The agar solution was then sterilised in an autoclave for 15 min at 121°C.

Approximately 2 cm zone of inhibition (indicated by white arrow)

No zone of inhibition



Total kill



Very small zone of inhibition e.g. 3 mm

Figure 1 Four examples of typical zone of inhibition results observed in the TurChub cell test system

Briefly, to prepare potato dextrose agar (PDA), 39 g of the powdered agar was suspended in 1 l of purified water. The mixture was then heated to boiling point while stirring to dissolve the powdered agar completely. The agar solution was then sterilised in an autoclave for 15 min at 121°C.

Preparation of *Trichophyton rubrum* suspension

T. rubrum, originally isolated from a patient suffering from onychomycosis, was sub-cultured onto fresh SDA slopes and reference samples were placed into a glycerol solution and cryogenically frozen. Isolates of the dermatophytes were also transferred into Ringer's solution and onto PDA and incubated at 28°C for seven days using a previously tested procedure to produce conidia. The fungal colonies were then covered with 5 ml of Ringer's solution and suspensions made by gently probing the surface with the tip of a Pasteur pipette, generating a mixture of conidial and hyphal fragments. The spore suspension was then filtered through sterile gauze (Propax, 7.5 cm × 7.5 cm 8-ply gauze swab, BP Type 13; Smith & Nephew) to remove mycelium. The density of the suspension was assessed using a UV spectrophotometer at 600 nm, and the spore suspension adjusted until a spore count of approximately 1×10^7 cfu/ml was achieved by diluting with Ringer's solution. A serial dilution of the final spore suspension and plate count was also carried out for confirmation. The identity of the isolated strain was verified by microscopy and culture on agar.

Preparation of TurChub cells

The receiver compartment of the TurChub was filled with a pre-determined calibrated volume of SDA. The agar was then inoculated with 50 μl of previously prepared *T. rubrum* suspension. Nail clippings (approximate size: 3 mm × 3 mm) were mounted into the validated TurChub gasket system, to ensure no leakage of formulation around the nail. The gasket system was then mounted into the TurChub cell ensuring complete contact with the agar in the receiver compartment and clamped in place between the donor and receiver compartments. An initial feasibility study was performed to determine the relative efficacy of Penlac and Loceryl when applied to human nail in the TurChub cells. The nail sections used in this study were cut to a uniform thickness of 5 μm using a microtome. The nail lacquers (0.5 ml) were applied to the apical surface of the nail, the cells occluded with Parafilm and incubated at 25°C for seven days.

The main study was performed using full thickness human nails in order to closely mimic the in-vivo situation in a patient suffering from onychomycosis. In this study the nails were dosed with the novel dual system penetration enhancers before application of the same nail lacquers used in the feasibility study. Penetration enhancer 1 (PE1) was 5% thioglycolic acid (w/w) prepared in 20% EtOH–water (v/v). Penetration enhancer 2 (PE2) was urea hydrogen peroxide solution prepared in water (Millipore) such that the concentration of the hydrogen peroxide was 15% (w/v). Cells were dosed with PE1 by applying 0.5 ml to the donor compartment of the ChubTur cell so that the apical surface of the nail was submerged. Following a period of 20 h, the PE1 solution was removed and the donor compartment flooded three times with water to wash away any residual penetration enhancer. Any remaining moisture was removed using a dry tissue. PE2 was then applied in the same way and after 20 h, PE2 was again washed out of the donor chamber and any excess solution was removed using a dry tissue. The relevant nail lacquer was applied (0.5 ml) to the dry clean donor compartment after the application of the penetration enhancer. The diffusion cells were then sealed with Parafilm to prevent evaporation and incubated for seven days. A set of control cells in which no penetration enhancer was applied before the nail lacquers were also prepared in the same way.

Infected nail model

A second study was performed using a novel infected nail model developed by MedPharm Ltd. In this infected nail model, ATP levels were used as biological marker for antimicrobial activity across human cadaver nails and distal nail clippings. Each nail was stored in an individual bag in a freezer before use. Before cutting the full cadaver nails into 3 mm × 3 mm segments, the nails were removed from the freezer (leaving them in the individual sealed bags) and placed in a laminar flow cabinet for 30 min to defrost. The cadaver nail was then placed into a sterile universal tube before cutting into segments. As the thickness, rigidity and permeability of cadaver nails varies across the entire nail, only the distal section of the cadaver nails was used. The underside of the nail was also irregular, and all loose material was removed where possible by gently scraping with a scalpel blade. The nails were then sterilised by brief immersion in 70% ethanol, washing in Ringer's solution and gently heating to 60°C for 15 min. A pair of sterile callipers were used to measure the length (in mm) and width (in mm) of the sterile cadaver nail, to determine how many 3 × 3 mm sections could be obtained and these were cut using a scalpel. Each nail segment was designated a number to maintain traceability. The thickness of the nail segments were then individually measured using callipers. Distal nail clippings were prepared as detailed above. The underside of the nails were initially inoculated with *T. rubrum* (5 μl of 1×10^7 cfu/ml) and incubated under controlled temperature and humidity to stimulate growth of the organism on and into the nails. Sacrificial samples were set up to monitor the growth of the organism on the nail samples over the incubation period, and control nails without any organisms were also set up and incubated to ensure no contamination. After establishing the growth of the organisms on the nails, the nails were dosed with one of the test formulations as detailed below. The performance of the formulation was determined after removing the nail sample from the cell and recovering viable microorganisms using a previously validated bioluminescence ATP method.

Dosing regime

As a result of the data obtained in the TurChub study it was decided that the infected nail model would be used to compare the efficacy of both the commercial products (Loceryl and Penlac) with that of a spray product containing terbinafine developed in-house and a range of controls. The terbinafine spray was formulated by adding terbinafine (1%) to the urea hydrogen peroxide (0.5%) component of the pre-treatment enhancing system, in a co-solvent (15% ethanol) to which a film-forming agent (copovidone) was also added before addition of hydrofluorolkane as the propellant. After the 14-day incubation period one group of infected nails were removed from the incubator and dosed with one actuation of thioglycolic acid spray formulation and returned to the incubator for 8 h. After this period, the thioglycolic acid spray formulation was washed off using sterilised water and wiped using a sterile cotton swab. Then one actuation of the urea hydrogen peroxide spray formulation also containing the active (terbinafine), or a placebo formulation containing only urea hydrogen peroxide, was applied to the nail surface.

A second group of infected nails were dosed with either Penlac or Loceryl only (1 μl; this volume was calculated proportionally for the size of the nail clipping based on the directions in the patient information leaflet for application to an infected nail *in vivo*), while a third set were used as an inoculated only control and received no further treatment. Following dosing, all cells were placed back into the incubator for a further seven days. Three weeks after initial inoculation with *T. rubrum*, all cells were removed from the incubator, dismantled and analysed for the presence of ATP. The nails were aseptically removed from the ChubTur cells and placed into a 96-well Nunc micro-titre plate. The micro-titre plate was then placed in the fluorescence plate reader, whereby BacTiter-Glo, containing the lysing agent was added by automated injection at set intervals and the fluorescence measured after a pre-determined period of time to ensure consistency and complete lysis of all viable organisms.

ATP assay: calibration

ATP calibration standards of known concentrations (0, 1, 5, 10, 25, 50, 100 and 200 ng/ml) were prepared by diluting the stock ATP standard (1 mg/ml), sequentially, in water. These standards (100 μl) were then placed into a sterile Nunc 96-well white micro-titre plate followed by the addition of 100 μl of the MedPharm lysing agent and Promega BacTiter-Glo assay reagent. The solution was mixed for a period of 30 s and the total amount of light that was emitted from the well measured every 10 s over a total period of 10 min using a Biotek FLx800 micro-titre fluorimeter/luminometer. The average of the relative light units measured over the 10 min was then calculated.

Statistical analysis

Statistical analysis of the effect of pre-treatment with the enhancing system on the efficacy (demonstrated by level of ATP activity) of Penlac, Loceryl and the terbinafine spray formulation compared with positive and negative controls was performed using the Kruskal–Wallis test. A significance level of $P < 0.05$ denoted significance in all cases. In all cases the number of replicates was five.

Results and Discussion

TurChub assay

The initial study was performed using nail samples cut to a uniform thickness of 5 μm using a microtome. In this study a small zone of inhibition was detected following a single application of Penlac (3.7 ± 5.7%, mean ± SD) and a larger zone of inhibition following a single application of Loceryl (93.7 ± 4.6%). A secondary study was performed using full-thickness human nail sections; in this study no zone of inhibition was detected for either Penlac (0.00 ± 0.00%) or Loceryl (0.00 ± 0.00%). Penlac and Loceryl were also applied to the full-thickness nail sections following an initial pre-treatment with the permeation enhancing system. Following this pre-treatment a significantly increased zone of inhibition was detected for both commercial formulations (pre-treatment + Penlac 100 ± 0.00%, pre-treatment + Loceryl 46.5 ± 6.3%) through full-thickness nail samples. Indeed the pre-treatment followed by application of Penlac resulted in complete kill in all replicates of the sample such that there was no detectable presence of *T. rubrum* in the cell and the samples were identical to the negative control samples that were uninfected (100 ± 0.00%) (Figure 2).

Data from the initial study using 5-μm nail sections indicates that one application of Loceryl alone appears to be a more potent treatment for onychomycosis than Penlac using this model. However, in the second part of the study using full-thickness nail sections, neither Loceryl nor Penlac demonstrated any efficacy against *T. rubrum*. This implies that in an in-vivo situation, where penetration of the full thickness of the nail would be required to guarantee complete cure, both Penlac and Loceryl would perform poorly. However, this may be an unfair comparison as each lacquer was only applied once in this study as opposed to the repeated applications (Loceryl once or twice per week until the nail has grown out and Penlac daily until the nail has grown out) indicated in their use for the treatment of onychomycosis. Also patient instructions for the use of both Loceryl and Penlac involve debridement and cleaning of the nail before application of the lacquer, a process that was not followed in this study. Nevertheless, historical data supports these findings with incidences of successful treatment very low, and relapse rates high, despite the extremely long treatment times involved. In addition, the relative effectiveness of the two marketed formulations *in vivo* seems to be depicted in the TurChub in-vitro model.

The purpose for deviating from the standard directions for use, given in the patient information leaflet for Penlac and Loceryl, was to demonstrate the ability of the penetration-enhancing system to alter the barrier properties of the nail after only a single application. Data from this study confirm the ability of a thioglycolic acid/hydrogen peroxide pre-treatment to significantly enhance the effectiveness of both Penlac and Loceryl across full-thickness nail *in vitro*, even after only one application. A greater improvement in the efficacy of Penlac compared with Loceryl was seen in the presence of the penetration enhancer and there are at least two possible explanations for this. The most likely is due to the fact that ciclopirox (Penlac) has a lower molecular weight than amorolfine (Loceryl) and as such has a greater inherent permeability, making the effect of the enhancement more apparent. An alternative possibility is that the pre-treatment helps to maintain ciclopirox in solution on the surface of the nail and thus sustains drug absorption, resulting in greater efficacy. Such results suggest that any potential formulation should be developed and optimised for the drug selected. The increased rate of ungual permeation observed for both ciclopirox and

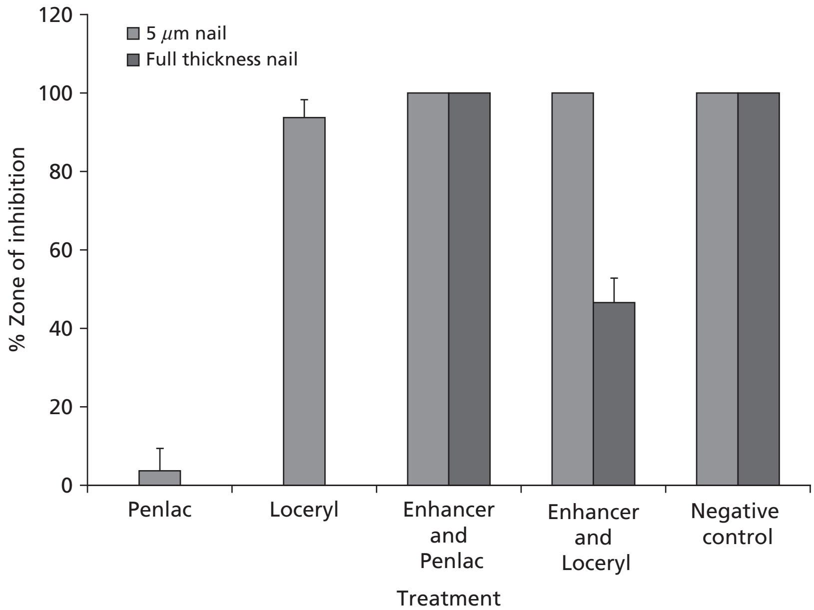


Figure 2 Comparison of zones of inhibition observed in the TurChub cell test system following application of the test systems to the nail surface. Figures are calculated as a percentage of the maximum possible zone of inhibition, mean ± SEM, $n = 6$

amorolfine in the presence of the permeation enhancing system could lead to a significant decrease in required treatment time and, combined with an increase in patient compliance, result in an increase in overall successful treatment outcomes.

Infected nail model

The infected nail model was used to compare the effectiveness of Penlac and Loceryl with a spray product containing terbinafine developed in-house and a range of controls. The terbinafine spray formulation was significantly better than Penlac and Loceryl at a 95% confidence level ($P < 0.05$) using infected cadaver nails. The terbinafine spray formulation (incorporating the enhancers) gave complete kill in all repeats of the sample, and levels of ATP recovered from the nails (5.4 ± 3.2% of infected control levels) were equivalent to negative control whereby no organisms were added to the nail (5.3 ± 1.6% of infected control levels). In comparison, the spray vehicle (i.e. a placebo containing no terbinafine), Penlac and Loceryl all showed no statistically significant decrease ($P > 0.05$) in ATP levels recovered from the nails, compared with infected controls that received no treatment (Figure 3) (78.9 ± 32.8%, 87.4 ± 23.1% and 79.0 ± 32.8% of infected control levels, respectively). When the distal nail sections from healthy volunteers were treated with Penlac (31.9 ± 1.7%) and Loceryl (24.0 ± 0.7%) both formulations showed a statistically significant decrease ($P < 0.05$) in percentage ATP levels compared with the infected control (100%) but the levels did not decrease to those seen in the negative untreated control (1.93 ± 1.37%).

It is thought that the thioglycolic acid component of the pre-treatment system causes reduction of S–S bonds in the keratin to –SH, where the addition of hydrogen results in the cleavage of the disulfide bridge, which in turn leads to the formation of cysteine amino acid molecules, as is known to happen in hair-care products.[36] In hair-care products it is well known that application of urea hydrogen peroxide results in the re-formation of the broken disulfide bridges,[37] although in the nail previously reported data has shown that application of urea hydrogen peroxide following initial administration of thioglycolic acid actually causes a further increase in the permeability of the nail,[35] thus suggesting that in the case of the nail the S–S bonds are not re-formed in the same way as in the hair. One possible explanation for this phenomenon is that as a result of the fairly high concentration and prolonged application (20 h) of the thioglycolic acid, disulfide bonds do not re-form because the initial cleavage of the bridges is damaging to the nail keratin structure resulting in a

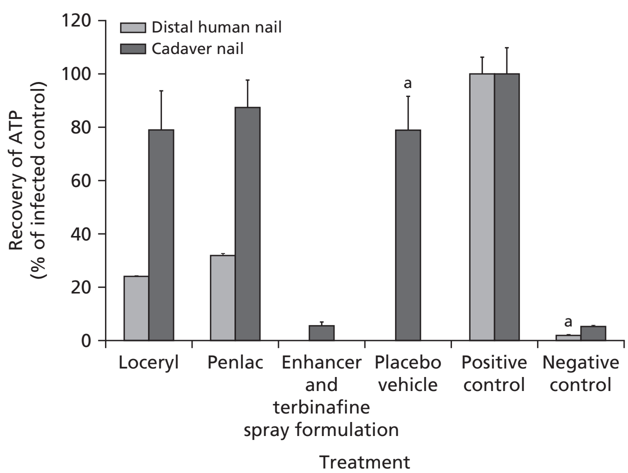


Figure 3 Comparison of % ATP recovered compared with infected control following a single 1 μl dose at t = 0 and incubated for 7 days treatment. Figures are mean ± SEM, $n = 5$ with exception of 'a' where $n = 4$, cell rejected after failing interQC

semi-permanent (longer than 20 h) reduction in the integrity of the nail barrier and therefore increased drug permeation.

Although combination of the permeation enhancing system with all three active agents investigated resulted in increased permeation of all three agents and increased apparent efficacy of each, as demonstrated by the infected nail model, the best results were observed when using terbinafine as the active agent. As such the increased effectiveness of terbinafine may not only be as a result of its enhanced ungual absorption but also because of its increased potency (minimum inhibitory concentration (MIC), 0.016–0.125 μg/ml)) compared with ciclopirox and amorolfine (MIC, 0.25–2 μg/ml and 0.04–0.063 μg/ml, respectively).

Conclusions

The nail is a very difficult barrier to overcome when attempting to deliver drugs to the underside of the nail or the nail bed.[3,21,38] This is reflected in the low success rates and high relapse rates of the products currently available for the topical treatment of onychomycosis.[11,12] This study has clearly demonstrated that the use of a novel permeation-enhancing system fundamentally alters the chemical structure so that the rate of permeation of a range of antifungal agents is significantly increased. This proof of concept study involved the sacrificial termination of all samples at the end of the study and further work is required to understand the nature of the changes occurring in the nail structure and the length of duration of the effect. As demonstrated in this study, this penetration-enhancing system could potentially be combined with the current topical treatments for onychomycosis, Penlac and Loceryl, to create a single treatment with enhanced permeation of their antifungal agents (ciclopirox and amorolofine, respectively). As shown in this study, a permeation-enhancing system could also potentially be formulated into a nail lacquer or spray formulation containing terbinafine. Terbinafine is the current gold standard oral treatment for onychomycosis but has significant systemic side effects that limit its use in this indication. If terbinafine could be delivered topically in this way, the chances of systemic toxicity would be significantly reduced.

Declarations

Conflict of interest

This work was funded by MedPharm Ltd who have submitted a patent application for the work described within. Three of the authors are direct employees of MedPharm Ltd, the remaining authors have collaborated with MedPharm on this and other previous and ongoing research projects.

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