Bilayered Nail Lacquer of Terbinafine Hydrochloride for Treatment of Onychomycosis

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ABSTRACT: The present study aimed to develop bilayered nail lacquer of terbinafine hydrochloride (TH) for treatment of onychomycosis. The composite nail lacquer formed an underlying drug-loaded hydrophilic layer and overlying hydrophobic vinyl layer. The hydrophilic lacquer made of hydroxylpropyl methylcellulose E-15 contained polyethylene glycol 400 (PEG 400) as a drug permeation enhancer. The vinyl lacquer was composed of poly (4-vinyl phenol) as a water-resistant film former. *In vitro* permeation studies in Franz diffusion cells indicated that the amount of TH permeated across the human cadaver nail in 6 days was 0.32 ± 0.14, 1.12 ± 0.42, and 1.42 ± 0.53 μg/cm^2 from control (hydrophilic lacquer devoid of PEG 400), monolayer (hydrophilic lacquer alone), and bilayered nail lacquers, respectively. A higher nail drug load was seen *in vitro* with the bilayered lacquer (0.59 ± 0.13 μg/mg) as compared to monolayer (0.36 ± 0.09 μg/mg) and control (0.28 ± 0.07 μg/mg) lacquers. The drug loss despite multiple washing was significantly low ($p < 0.001$) for the bilayered lacquer owing to the protective vinyl coating. Clinical studies demonstrated the efficacy of bilayered lacquer to achieve better drug load in the nail plate (1.27 ± 0.184 μg/mg) compared to monolayer (0.67 ± 0.18 μg/mg) and control (0.21 ± 0.04 μg/mg) lacquers. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:4267–4276, 2010

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INTRODUCTION

Onychomycosis is the fungal infection of the human nail affecting 19% of the global population.[1] It accounts for ~50% of the nail diseases in diabetic and elderly patients. Onychomycosis is commonly caused by dermatophytes though yeasts and candida may be the other causative organisms.[2] The disease is difficult to manage as it is chronic, hard to eradicate, and tends to relapse. The infected nails appear ugly, discolored, thickened, and dystrophic which makes a significant negative impact on the social life of the patient.

In the past, the treatment modality for onychomycosis was surgical extraction of the diseased nail which would be extremely traumatic and painful.[3] Currently, the disease is treated with oral or topical antifungal agents. Following oral administration the drug is absorbed into systemic circulation following which it eventually diffuses into the nail plate through the nail bed. Unfortunately about 20% of the patients fail to respond to the oral treatment in which the relapse of the disease is quite common.[4] Moreover, the oral therapy has been associated with systemic adverse effects and drug interactions. On the contrary, topical therapy based on nail lacquers circumvents most of the limitations of oral administration. But because of poor drug diffusion into the highly keratinized nail plate and the long duration of treatment, the topical monotherapy has been currently recommended only in the early stages of the disease or when the systemic therapy is contradicted.[5] However, the combination therapy has proved to achieve a higher success rate and demonstrated a better cost per cure rate compared to oral therapy alone.[6]

Considering these facts persistent attempts have been made to improve the efficacy of topical nail preparations.[7–9] Therefore, there is an urgent need to develop a topical formulation, which can be effectively used in topical monotherapy in the initial stages of onychomycosis or in combination with the oral therapy in the advanced stages.

Terbinafine hydrochloride (TH) is one of the most potent antifungal agent used to treat onychomycosis.

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The drug is known to have low minimum inhibitory concentrations (~0.001–0.01 μg/mL) and low minimum fungicidal concentrations (~0.003–0.006 μg/mL).[10] The oral therapy with TH has been associated with severe side effects, toxicity, drug interaction, and high rate of recurrence.[11] In this context there is a strong need for an effective, safe, and patient compliant topical formulation capable of delivering and maintaining therapeutic concentrations of terbinafine in the nail bed.

The key in the treatment of onychomycosis and other nail diseases is the delivery and maintenance of effective drug concentrations in deeper nail layers.[12] But most of the conventional nail lacquer preparations based on water-insoluble resins have limited potential to enhance the transungual drug delivery. Considering this, aqueous-based nail lacquers have been preferred for treatment of onychomycosis as they promote the nail hydration and the drug diffusion across the nail plate.[8] However, the water-soluble preparations suffer from the limitation of being easily wiped off or washed off the nail surface. Therefore, the present investigation aimed to develop a bilayered nail lacquer comprising of an underlying drug-loaded hydrophilic layer and overlying water-resistant film. The novel bilayered nail lacquer proposed for TH would be the first of its kind for the treatment of onychomycosis. The unique formulation is expected to overcome most of the limitations of the conventional lacquer formulations and ensure effective local therapy against fungi, dermatophytes, and molds.

MATERIALS AND METHODS

Materials

TH (MW 327.90 Da) was procured from Uquifa (Jiutepac, Mexico). Human cadaver fingernails, both male and female with varying thicknesses of 0.4–0.7 mm were procured from Science Care (Phoenix, AZ). Methocel E 15 Premium LV-Hydroxypropyl methylcellulose (HPMC E-15 LV) was the gift sample from Dow Chemical Company (Midland, MI). Poly (4-vinyl phenol) [average molecular weight 25,000], polyethylene glycol 400 (PEG 400), and dibutyl phthalate were purchased from Sigma–Aldrich (St. Louis, MO). All other chemicals and reagents used were of analytical grade.

Methods

Analytical Method

The amount of TH in the samples was quantified by high-performance liquid chromatography (HPLC) system (Waters, Hunlingdon Vly, PA, 1525) equipped with an autosampler (Waters 717 Plus) and a variable wavelength dual λ absorbance detector (Waters 2487). Elution was performed isocratically using Phenomenex C18 (2) 100 R analytical column (4.6 mm × 150 mm, Luna, Torrance, CA 5.0 μm) at a temperature of 32°C. The mobile phase consisting of aqueous solution (0.096 M triethyl amine, 0.183 M orthophosphoric acid) and acetonitrile (60:40) was set to pH 2.0 and a flow rate of 1.0 mL/min. The sample injection volume was 20 μL and the column effluent was monitored at 224 nm. The method developed was validated for the linearity, precision, and accuracy. The range of calibration curve was found to be 2–1000 ng/mL ($R^2 = 0.99$) with the coefficient of variance and accuracy ranging from 1.03% to 6.08% and −0.54% to −6.96%, respectively.

Preparation of Nail Lacquers

The nail lacquers formulated in the present study were the drug-loaded hydrophilic lacquer (HPMC lacquer containing PEG 400) and hydrophobic vinyl lacquer. The hydrophilic nail lacquer was composed of TH (5%, w/v), HPMC E-15 (6%, w/v), PEG 400 (10%, v/v), ethanol (60%, v/v), and purified water (q.s.). TH was dissolved in a mixture of water and ethanol (pH 3.0) on a bath sonicator. HPMC E-15 was soaked overnight in the hydroalcoholic mixture (pH 3.0) and sonicated to ensure complete polymer dissolution. The two hydroalcoholic solutions were mixed thoroughly on a magnetic stirrer to obtain a clear homogeneous solution to which PEG 400 was added and the stirring continued. The pH, viscosity, drying time, and TH content of the hydrophilic nail lacquers was found to be ~4.0, ~500 cps, and 300 ± 75 s, respectively. A "control" hydrophilic nail lacquer of HPMC E-15 containing TH but devoid of PEG 400 and a drug free "placebo" hydrophilic lacquer of HPMC E-15 containing PEG 400 were similarly prepared for comparison.

The hydrophobic nail lacquer was prepared by dissolving poly (4-vinyl phenol) in ethyl acetate at concentration of 10% (w/v). Dibutyl phthalate was used as a plasticizer in the lacquer at a concentration of 4% (v/v).

The different lacquer formulations repeatedly referred throughout the article are "control lacquer" (TH-loaded HPMC lacquer devoid of PEG 400), "monolayer lacquer" (TH-loaded HPMC lacquer containing PEG 400), "bilayered lacquer" (monolayer lacquer overlaid with the hydrophobic lacquer), and "placebo lacquer" (drug-free HPMC lacquer containing PEG 400).

Microscopy Studies

Sulforhodamine B was dissolved in the hydrophilic nail lacquer at a concentration of 1 mg/mL while methylene blue was solubilized in the hydrophobic vinyl lacquer at similar concentration. The hydrophilic lacquer was applied to a human cadaver nail

plate and allowed to dry (for about 5 min). The vinyl lacquer was applied on top of the drug-loaded hydrophilic layer and dried to form a bilayer. Cross-sections of the nail with the adhering lacquer films were taken perpendicular to the surface of the nail using a microtome (Rotary Microtome, Reichert-820, Buffalo, NY). The sections were mounted on a gelatinous slide, viewed, and photographed under an optical microscope (Zeiss, MicroImaging, with Axiocam, Thornwood, NY).

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was used to characterize the physical state of TH in the casted hydrophilic lacquer film. DSC thermograms of TH, casted HPMC lacquer film, and physical mixture of TH and HPMC E-15 (in ratio of 5:6) were recorded in differential scanning calorimeter (Perkin Elmer Pyris 1 DSC, Waltham, MA). Samples weighing about 2 mg were heated from 25 to 225°C in flat-bottomed hermetically sealed aluminum pans at a linear heating rate of 10°C/min. Ultra pure nitrogen was purged at a flow rate of 30 mL/min and the data recorded was interpreted using Pyris Manager™ software.

Bioadhesivity Studies

Bioadhesion is one of the key factors to be considered in development of a successful transnail drug delivery system in onychomycosis.[13] The adhesion of HPMC lacquer films to the cadaver nails was determined using Texture Analyzer® equipped with a 50-kg load cell (TA.XT2*i*, Technologies Corp., Scarsdale, NY/ Stable Micro Systems, Godalming, Surrey, UK) (Fig. 1). The TH loaded or the placebo HPMC lacquer was spread on a glass slide that was secured on the base of Texture analyzer®. The cadaver nail plate of 6 mm diameter was mounted on TA-96 probe. The probe was lowered from a height of 25 mm at a speed of 1 mm/s with a force of 3.5 N till the nail plate touched the surface of the lacquer film. When the nail plate detected the surface of the lacquer film, a trigger force of 0.5 N was applied for 30, 60, or 120 s. After the contact time was lapsed the probe was withdrawn from the surface film at a preset speed of 0.5 mm/s and the force required to withdraw the nail from the lacquer film was recorded as peak adhesive force (PAF). The area under the curve (AUC) that represents the work of adhesion was determined from the force deflection profiles. The two parameters for the placebo and drug-loaded lacquer films were recorded in triplicate and interpreted using Texture Expert™ software.

Figure 1. TA.XT2*i* Texture Analyzer (from Texture Analyzer Corp., Scarsdale, NY) used for bioadhesivity testing of the nail lacquer formulations.

In Vitro Drug Permeation Studies

Cadaver nails were cleaned and the adherent tissue was removed with a pair of scissors and a scalpel. Each nail plate having an average thickness of 0.5 mm was washed with water, soaked in normal saline for 1 h prior to use and mounted on a nail adapter having a active diffusion area of 0.2 cm^2 (Permegear, Hellertown, PA). The whole assembly was sandwiched between the two chambers of a Franz diffusion cell (Logan Instruments Ltd, Somerset, NJ). About 20 μL of the TH-loaded hydrophilic nail lacquer was applied onto the nail plate in the donor compartment and allowed to dry for 5 min. On drying, 20 μL of the vinyl nail lacquer was applied on top of the drug-loaded hydrophilic film to form a bilayered lacquer film on the surface of the nail plate. The receiver chamber was filled with 5 mL of saline set to pH 3.0, maintained at a temperature of 37°C and stirred at 600 rpm with a 3-mm magnetic bead. Samples were withdrawn from the receiver compartment at regular time interval for a period of 6 days and analyzed for TH by HPLC. The second set of experiment was performed by application of 20 μL of the drug-loaded hydrophilic nail lacquer alone on the nail plate that formed monolayer lacquer film. The third set of trails performed by application of 20 μL of control nail lacquer alone was run in parallel for comparison.

Drug Load in the Nail Plate

The amount of TH loaded in the nail plate following the permeation studies was determined following the validated extraction procedure reported earlier.[14] The active diffusion area on the nail plates measuring 0.2 cm^2 were marked (using permanent marker) and cut using metric punch, washed by standardized procedure with water and ethanol (95%) until the surface was free from drug. The active diffusion area was cut into small pieces, weighed, and taken in screw cap pyrex vials. The vials were incubated with 1.5 mL

of sodium hydroxide (1 M) on a shaker water bath at room temperature for 24 h to allow the nail to completely dissolve. The solutions obtained were neutralized with 200 μL of 5 M hydrochloric acid and extracted with hexane (3 mL) by vigorous agitation for 30 min. The resulting mixture was transferred into tubes and centrifuged at 4000 rpm for 10 min to separate the organic phase. The hexane layer was separated and treated with 1 mL of 0.5 M sulphuric acid/isopropyl alcohol (85:15) and shaken vigorously for 30 min. The lower acidic aqueous layer containing the drug was collected and assayed for the drug content. The extraction procedure was validated by spiking different drug concentrations (2–20 μg/mL) in sodium hydroxide in which the nail was previously dissolved.

The amount of TH laterally diffused into the peripheral regions was determined by dissecting the peripheral nail area (4–5 mm surrounding the active diffusional area) which is further washed, dried, and weighed. The drug content in the peripheral region was determined following the extraction procedure described above.

Resistance to Multiple Washing

The ability of the nail lacquer to withstand multiple washings was assessed by employing a washing procedure developed and validated in-house. Nails were mounted on a nail adapter with an active diffusion area of 0.2 cm^2 (Permegear). The whole assembly was sandwiched between the two chambers of a Franz diffusion cell (Logan Instruments Ltd). About 20 μL of the drug-loaded hydrophilic nail lacquer was applied onto the nail plate in the donor compartment. After complete drying of the hydrophilic layer (about 5 min.), about 20 μL of the vinyl lacquer was applied on top of the hydrophilic drug-loaded film to form a bilayered film. On drying of the vinyl film, about 500 μL of distilled water (pH 3.0) was loaded into the donor compartment. The water was allowed to stand for 1 min after which it was collected to estimate the amount of TH dissolved. Each washing step with distilled water is repeated 50 times and the drug content in each washing was determined from which the cumulative amount of TH lost from the bilayer lacquer after 50 washings was computed. The second set of experiments was carried out by application of 20 μL of the drug-loaded hydrophilic nail lacquer alone. The resulting hydrophilic monolayer was subjected to multiple washings following the same protocol. The third set of trial was performed similarly on application of the control nail lacquer alone and the cumulative amount of TH lost after multiple washings was calculated. After subjecting the lacquers to multiple washings, the residual amount of TH present in the lacquers retained on the nail plate in each case was also determined by extracting the nail plate following the procedure described previously.

Human Subject Study

A clinical study was conducted in healthy human subjects to determine the efficacy of the developed nail lacquer formulations. The total study period spanned 6 weeks comprising of 2 weeks of treatment period continued by 4 weeks of follow-up. Written consent was obtained from each participating subject before the performance of the screening procedure. The study plan was considered adequate by the human ethical committee of Dehradun Institute of Technology, Faculty of Pharmacy, Dehradun, India (Ref. No. DITP #2009/01A). The inclusion criteria were healthy human subjects in the age group of 20–50 with a mean weight of 65 kg. Subjects with nail disease or those allergic to terbinafine were excluded from participation.

The subjects were divided into three groups with six volunteers in each group: group I was applied with control nail lacquer. Group II was applied with hydrophilic nail lacquer alone that resulted in formation of a monolayer film. Group III was applied with hydrophilic nail lacquer and allowed to dry. On drying, the vinyl lacquer was applied on top of the hydrophilic lacquer film to obtain a bilayered lacquer film. The drying time of the hydrophilic lacquers in all cases was around 5 min. About 20 μL of lacquers were applied to the big toe of the either feet on daily basis for a period of 2 weeks. Volunteers were instructed to avoid exposure to water within 1 h of application and avoid activities like swimming and dish washing during the study period. The nail clippings were sampled at the beginning of the study (0 weeks), after the treatment period of 2 weeks and on follow-up (at the end of 2 and 4 weeks after stopping the application of the lacquer). The content of the TH in the clippings was estimated by HPLC following the validated extraction procedure outlined earlier. The volunteers are monitored for the local and systemic side effects throughout the study period.

Data Analysis

Statistical analysis was performed by one-way analysis of variance and *t*-test in Graph pad Instat 5 software (GraphPad Software, Inc., La Jolla, CA). p-Value <0.05 was considered statistically significant. The data points presented in the graphs are an average of three/six trials. The error bars represent the standard deviation (SD).

RESULTS AND DISCUSSION

An ideal transungual formulation should require less frequent applications, must be easy and convenient to

use and possess good adhesivity to the nail.[15] The specific objectives for developing the bilayered nail lacquer were (i) to maximize the adhesion of the drug-loaded lacquer film to the nail surface employing a hydrophilic bioadhesive polymer, (ii) to retain the supersaturated hydrophilic lacquer film on the nail plate for prolonged period of time using a durable water resistant hydrophobic layer, and thereby (iii) to ensure delivery of effective amounts of TH across human nail by exploiting the combined effects of occlusion and transungual penetration enhancement.

Aqueous-based nail lacquers are known to promote the nail hydration and the drug diffusion across the nail plate and hence play a major role in treatment of onychomycosis.[8] Since the nail plate is known to behave like a hydrogel upon hydration, one can expect a better adhesion of the hydrophilic lacquer film with the contours of the nail plate. In addition, the water-based nail lacquers are free from the burning sensation when applied to the highly sensitive nail bed. Hydroxypropyl methylcellulose was selected as the film former as it forms a nonsticky, nonglossy bioadhesive film with good plasticity.[16] The HPMC film formed has a matte and natural look that would be preferred by majority of the mycosis patients.

PEG 400 was selected as a penetration enhancer based on the outcome of TranScreen-N™, a high-throughput method developed in our laboratory to screen compounds for their ability to enhance the transungual delivery of TH across nail.[17] The upper vinyl film was employed to shield the underlying hydrophilic film from being washed off during the routine day-to-day activities.

The cross-section of the full thickness cadaver nail along with the applied bilayered nail lacquer has been shown in Figure 2. It is clearly evident from the photograph that the underlying hydrophilic lacquer film was able to establish a good contact with the contours of the nail plate. An intimate contact of the drug-loaded film with the nail plate is a key for successful topical transungual delivery.[8] The proximity of the occlusive hydrophobic vinyl film and the underlying hydrophilic drug-loaded film is distinctively visible from the cross-section. The close contact of the occlusive vinyl film would be crucial in maintaining the nail in a hydrated state.

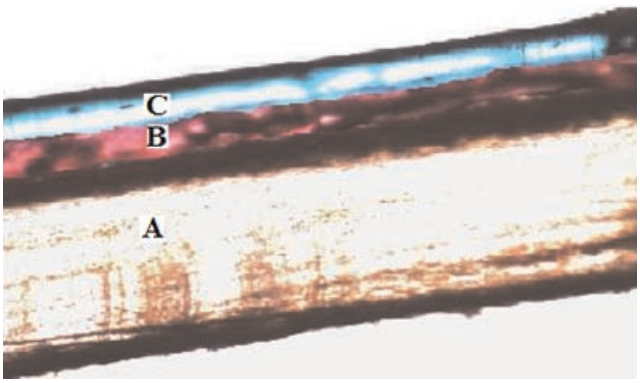

Figure 2. Cross-section of the nail (A) with applied with the bilayered nail lacquer showing the drug-loaded hydrophilic lacquer (B) over laid with the hydrophobic vinyl lacquer (C).

DSC thermogram of TH, casted drug-loaded lacquer film and physical mixture of TH and HPMC E-15 are represented in Figure 3. The DSC scan of TH presented a sharp endothermic peak at 215.88°C with the peak onset at 211.45°C that corresponds to the melting point of the drug.[18] The enthalpy of fusion (ΔH_f) for TH in pure state was found to be 54.93 J/g. A broader endothermic peak at 209.33°C with the peak onset at 203.05°C was observed in the thermogram of the physical mixture composed of TH and HPMC E-15 in a ratio of 5:6. The appearance of endothermic peak ruled out the physical incompatibility between TH and the polymer and indicated the crystalline nature of TH in the physical mixture as well. The enthalpy of fusion of the physical mixture was found to be 25.62 J/g that corresponded to the amount of TH in the physical mixture (~45%, w/w). The complete absence of the endothermic peak in the DSC of the casted drug-loaded lacquer film indicated the chances of existence of TH in amorphous state as a solid solution in HPMC E-15 lacquer film. In addition, the absence of the endothermic peak ruled out the possibility of crystallization of TH in the casted lacquer films.

HPMC E-15 is a well-known bioadhesive polymer containing a high proportion (7–12%) of hydroxylpropyl groups that would render a high surface charge density to the lacquer film. The good bioadhesion of the casted lacquer films with the nail has been attributed to the hydrogen bonds formed between the carboxylic and hydroxyl group with the biological substrate.[19] The PAF and AUC values for the drug loaded and placebo films were found to increase with increase in the contact time intervals as represented in Figure 4. The contact time is reported to affect the extent of hydration and swelling of polymeric films that in turn would influence the adhesion to the nail plate.[20] Though the PAF and AUC at any contact time period were higher for the placebo lacquer films compared to TH-loaded films they were not significantly different from each other ($p > 0.05$, t-test). The values of PAF and AUC for the drug-loaded films were comparable to those obtained by Mididoddi and Repka[19] with bioadhesive cellulose-based films for transungual applications. The results of the studies indicated that the drug-loaded hydrophilic lacquer film have sufficient adhesiveness to be retained on the surface of nail plate for prolonged time periods.

The cumulative amount of TH permeated across the nail plate at the end of 144 h (6 days) was found to

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