Getting Inside the Human Nail Plate: First Success

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Onychomycosis is a fungal infection of the nail plate – the most visible part of the nail apparatus (see sidebar). This infection is usually caused by species of *Epidermophyton, Microsporum* and *Trichophyton* resulting in nails that are opaque, white, thickened, friable and brittle.[1]

Onychomycosis is common, affecting 14% of the human population. Aging increases the incidence significantly, with the rate estimated to be 48% in persons 70 years of age. Discoloration, thickening, hardening or crumbling of the nail may result in pain while wearing shoes.

To cure the infection, the patient is obliged to take an oral systemic medication for months.[2] The currently approved oral medication has such side effects that the cure may be worse than the disease. Oral therapy has the inherent disadvantages of systemic adverse effects and drug interactions, while topical therapy is limited by the low permeability of the nail plates. Traditionally, the topical treatment of onychomycosis has been less than desirable due to the infection's deep-seated nature and to the ineffective penetration of the deep nail plate by topically applied drugs.

How can topical drugs be delivered into the nail? And, perhaps as importantly, how can one assess the drug content in the human nail to validate nail drug delivery?

The challenge was to develop a system to assay drug content within the inner nail bed where the infection resides. We developed a micrometer-controlled drilling instrument that removes and collects from the inner nail bed a powder sample from which by mass balance recovery we can determine the amount of penetrated radiolabeled drug. With such an assay procedure the effectiveness of topical nail drug delivery can be assessed.

Methodology

Nail anatomy consists of three structures. Starting from the outer structure, they are the nail plate, the nail bed and the nail matrix.

- The human nail plate consists of three layers. The dorsal layer is a dense, hard outer layer of cornified keratin only a few cells thick (approximately 200 µm). The intermediate layer of softer keratin constitutes roughly 75% of the plate's thickness. Below that is the ventral layer of soft keratin a few cells thick that connects to the nail bed below. In total, the human nail plate thickness is approximately 0.5 mm for finger nails and up to 1.3 mm for toe nails.
- The nail bed is non-cornified soft tissue under the nail plate. It is highly vascularized.
- Beneath the nail bed is the nail matrix, which is the heavily vascularized thick layer of highly proliferative epithelial tissue that forms the nail plate.

We devised two experiments to evaluate drug penetration through the human nail, using radiolabelled drugs. In the first experiment we sampled the ventral/intermediate

The Human Nail

The human nail, equivalent to claws and hooves in other mammals, evolved as our manual skills developed and protects the delicate tips of fingers and toes against trauma, enhances the sensation of fine touch and allows one to pick up and manipulate objects. The nail is also used for scratching and grooming, as a cosmetic organ and, sometimes, to communicate one's social status.

The nail plate, the most visible part of the nail apparatus, consists of tightly packed dead cells and is highly keratinized. It is also extremely variable among individuals. For example, the plates can be small, large, wide, narrow, hard, smooth, ridged, thin or thick.[3]

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layers to determine the penetration of each of four drugs in a test formulation with a penetration enhancer, and also, for comparison purposes, in a saline control. In a second experiment, we used only one drug in one test formulation with a penetration enhancer and also in a saline control, but we measured the amount of that drug recovered at various depths from the nail surface to the support bed.

Formulations: Nails have a high content of disulfide bonds (10.6% versus 1.2% for human skin), which makes the nails both strong and impenetrable. To deliver a therapeutically sufficient quantity of an antifungal drug to fungally-infected sites, such as nail plate, bed and matrix, a suitable carrier is needed to enhance drug penetration through the nail barrier.

In the case of the antifungal drugs urea, ketoconazole and salicylic acid, a lotion[a] containing the penetration enhancer dimethylsulfoxide (DMSO) had previously been shown to enhance skin penetration.[4-6] To test these three drugs, we prepared three formulations with [^{14}C]-urea, [^{3}H]-ketoconazole and [^{14}C]-salicylic acid at 0.002%, 0.1% and 0.07%, respectively, and corresponding saline controls with each drug at the same concentrations.

For the antifungal drug econazole, we used a nail lacquer formulation, which is a popular choice for topical antifungal treatment. Nail lacquer contains a film-forming agent and a solvent, in addition to the antifungal drug and, possibly, a penetration enhancer. Once the lacquer is applied, it forms a thin, water-insoluble film containing supersaturated antifungal drug. This provides a chemical gradient to drive drug flux as the drug is released. Thus, a lacquer formulation is suitable for topical treatment of nail diseases. We selected a commercial lacquer formulation[b]. The components of this lacquer formulation including econazole with the penetration enhancer 2-*n*-1,3-nonyl-dioxolane (18%)[7] were assembled into a test formulation in the lab prior to use. The control was the same formulation minus 2-*n*-1,3-nonyl-dioxolane.

To summarize, we prepared four test formulations and four corresponding saline controls – one pair for each drug. We tested nail penetration of urea, ketoconazole and salicylic acid from a commercial lotion. We tested nail penetration of econazole from a commercial nail lacquer.

Compounds: In each of the studies reported here a radiolabeled compound [^{3}H or ^{14}C] was used and chemical content was determined by radioactivity scintillation counting.

Human finger nail plates: Nail plates were collected from adult human cadavers and stored in a closed container at 0°C. Before each experiment, nail samples were gently washed with normal saline to remove any contamination, then rehydrated by placing them for 3 h on a cloth wetted with normal saline. Nail samples were randomly selected and allocated to test groups. Nail thickness was measured before testing in order to determine the drilling depth for each nail. Five nails were used for each formulation tested.

Dosing and surface washing procedures: A 5 mL dosing aliquot of each of the test formulations was applied to the surface of a nail plate with a microsyringe twice daily approximately 8 h apart for 14 days.

Starting on the second day, each morning before dosing, the surface of the nail was washed with cotton tips in a cycle, as follows: a dry tip, then a tip wetted with 50% skin cleansing liquid[c], then a tip wetted with distilled water, then another tip wetted with distilled water, then a final dry tip. This simulated a daily bathing. The nails treated with lacquer also received an alcohol wash (to remove residual lacquer that was insoluble to soap and water).

The washing samples from each cycle from each nail were pooled and collected by breaking off the cotton tip into scintillation glass vials. An aliquot of 5.0 ml methanol was added into each vial to extract the test material. The radioactivity of each sample was measured in a liquid scintillation counter.

[a] *Pennsaid lotion, Dimethaid Research Inc., Markham, Ontario, Canada. Pennsaid is a registered trademark of Dimethaid Research Inc.*

[b] *EcoNail. EcoNail is a trademark of MacroChem Corp., Lexington, Massachusetts.*

[c] *Ivory soap. Ivory is a registered trademark of Procter & Gamble, Cincinnati, Ohio.*

Table 1. Radiolabeled drug penetration into human nail from a test formulation containing a penetration enhancer versus a saline control without a penetration enhancer, measured by counting radioactivity scintillation (μgEq/g) at the ventral/intermediate layers of the nail plate

		Radioactivity Content		
Drug	Penetration enhancer	Test Formulation (μgEq/g)[a]	Saline Control (μgEq/g)	*p* Value
Ketoconazole	dimethylsulfoxide	53.9±10.6	34.0±15.9	0.048
Urea	dimethylsulfoxide	0.35±0.15	0.2±0.09	0.039
Salicylic acid	dimethylsulfoxide	10.2±0.6	7.0±1.1	0.008
Econazole	2-n-nonyl-1,3-dioxolane	11.2±2.6	1.8±0.3	0.008

[a] μgEq/g = microgram equivalents drug per gram of nail sample. Because radioactivity is used, the drug mass is referred to as "equivalents" because radioactivity was measured, not the drug itself. Mean ± SD (n=5) for nail powder drilled from ventral/intermediate layers of human nail plate.
Source: References 6,7

Nail incubation: To keep the nail at physiological levels of temperature and humidity, we incubated the nail in a Teflon[d] one-chamber diffusion cell[e]. The incubation period started 24 hrs prior to the first dose and ended 24 hrs after the final dose. A small cotton ball wetted with 0.1 ml normal saline was placed in the chamber beneath the nail plate to serve as a "nail bed" and provide moisture for the nail plate.[6,7] During the experiment, the holding tank temperature was 25±2°C and relative humidity was 44±8%. Hydration of the dorsal nail surface was 8.5±2.4 AU for saline formulation and 11.2±3.6 AU for the test formulations, where AU is Arbitrary Units, a digital expression of capacitance. The supporting cotton bed hydration was 115.9±9.9 AU for the saline control and 118.0±9.4 AU for the test formulations. Thus, there was no statistical difference between hydration conditions for nails treated with either the test formulation or the saline control.

Nail sampling procedure: The objective was to determine drug concentration within the nail where the disease resides. Treatment dosing is done to the surface of the nail. The drilling system samples the inner core of the nail without disturbing the nail surface. The two parts (surface and inner core) can be assayed separately. The surface contains only residual drug after washing. The drilled out core (from the ventral side) is thus a true drug measurement at the target site where the disease resides.

Drug penetration into the nail was sampled by a unique micrometer-controlled nail sampling instrument that enabled finely controlled drilling into the nail and collection of the powder created by the drilling process. The nail is fastened in a cutting holder below the cutter and surrounded by a funnel containing a filter paper. The funnel is attached to a vacuum pump. During drilling, the vacuum draws the powder debris onto the filter paper so it can be collected and measured.

After completion of the dosing and incubation phase, the nail plate was transferred from the diffusion cell to a clean cutting holder for sampling. The nail plate was secured in position so that the ventral surface faced the cutter and the dorsal-dosed surface faced the holder. The cutting holder was moved to bring the plate surface just barely in contact with the cutter tip. The drill was then turned on and a fine adjustment moved the stage toward the cutter tip, removing a powder sample from the nail. In this way, a hole approximately 0.3-0.4 mm in depth and 7.9 mm in diameter was drilled in each nail, enabling the harvest of powder sample from the center of each nail's ventral surface. We'll refer to these samples as having been taken from the "ventral/intermediate nail plate."

After the nail had delivered its ventral/intermediate nail plate powder sample, it was removed from the sampling instrument. The nail outside the dosing area was cut away and discarded. The nail within the dosing area but outside the sampling area was trimmed away and saved; we refer to this as the "remainder nail plate." It surrounds the dorsal layer above the sampling area where the powder samples were taken; we refer to this as the "sampling area dorsal nail plate." The ventral/intermediate nail plate powdered samples, the sampling area dorsal nail plate and the remainder nail plate were individually collected into a glass scintillation vial and

[d] *Teflon is a registered trademark of E.I. DuPont de Nemours and Company, Wilmington, Delaware.*
[e] *Permegear, Inc., Hellertown, Pennsylvania.*

Table 2. Mass balance recovery of econazole following 14-day human nail treatment with a test formulation containing a penetration enhancer* or a saline control without a penetration enhancer, measured as a percent of the radioactive dose recovered

Site	Test Formulation	Saline Control
Sampling area dorsal nail plate	11.4±3.6%	20.1±3.0%
Ventral/intermediate nail plate (powdered samples)	1.4±1.1	0.2±0.08
Remainder nail plate 5.6±3.9	3.2±2.3	
Supporting bed (cotton ball)	0.7±0.3	0.0±0.0
Surface washes	71.8±12.5	72.8±5.1
Total	90.8±16.4	96.4±7.3

* 18% 2-*n*-nonyl-1,3-dioxolane
Source: Reference 7

weighed. The nail samples were then dissolved by adding 5.0 ml of a solvent[f]. The total mass of nail collected was measured by the difference in weight of the plate before and after drilling.[6,7]

Results

Table 1 summarizes the penetration of ketoconazole, urea, salicylic acid and econazole into the human inner nail plate. Each test formulation contained a drug delivery enhancer[6,7] and was compared to a saline control formulation without any penetration enhancer. In each case the test formulation enhanced drug delivery ($p < 0.05$).

Table 2 summarizes the econazole mass balance recovery following the 14-day nail treatment. Overall recovery of applied dose was 90.8±16.4% for the test formulation and 96.4±7.3% for the saline control, indicating that essentially all the dose was accounted for.

Table 2 also indicates what happens to chemicals put on the nail in this experiment. Approximately 72% was washed off the surface. Dose absorbed from the surface of the nail ended up in the sampling area dorsal nail plate (11.4%), the ventral/intermediate nail plate (1.4%) and the supporting bed (0.7%), which is the cotton ball that the nail rested on. Notice that econazole recovery in the test formulation is greater for both the ventral/intermediate nail plate and the supporting bed, which is an effect of the drug delivery enhancer. Now notice that at the sampling area dorsal nail plate, there is more econazole from the saline control because the dose remained on the nail surface.

Discussion

Topical therapy for onychomycosis is not yet maximally effective, and this failure may be due to poor penetration of drugs into the nail plate. The nail's unique properties, particularly its thickness and relatively compact construction, make it a formidable barrier to the entry of topically applied agents. The concentration of an applied drug across the nail drops about 1000-fold from the outer surface to the inner surface. As a result, the drug concentration presumably does not reach a therapeutically effective level in the ventral/intermediate layers.

Minimum inhibitory concentration (MIC) is a laboratory index in the determination of antifungal potency. For econazole, the range of MIC for *Dermatophytes* species is 0.1 to 1.0 µg/ml and for yeast species it is 1.0 to 100 µg/ml.[8] After 14 days of exposure, the econazole content measured in the test group was 11.15±2.56 µg/mg for the ventral/intermediate layers. This content multiplied by the density of the nail sample (1.332 mg/cm^3, measured under current experimental conditions) yields 14,830±340 µg/cm^3 of econazole, almost 15,000 times the MIC for most *Dermatophytes* species, and 150 times that for most yeast species.

Mertin and Lippold[9] introduced an efficacy coefficient E to better estimate and compare the relative efficacy of antifungal agents. The efficacy coefficient E is the ratio of

[f] *Packard Soluene-350, Packard Instrument Company, Meriden, Connecticut*

the flux of an antimycotic drug through the nail plate to the MIC. The flux of econazole into the deep layer of human nail is 1.58±0.32 μg/cm²/h in test group, compared to only 0.21±0.04 μg/cm²/h in control group.

If the MIC value is 1.0 μg/ml, the efficacy coefficient E calculated from the test group is 1.58, which is 6 fold greater than that in the control group. The results suggest that the enhanced level of econazole in the ventral/intermediate layers and supporting bed dramatically exceeds the minimum inhibitory concentration of econazole for most common onychomycosis organisms.

The intermediate nail plate has remained a sanctuary against drugs, as exhibited by the failure of topical nail therapy for nail infections. The intermediate nail plate can now be scientifically studied, and with proper formulation one can deliver a variety of chemicals, be they drugs or nail modifiers (cosmetics). The nail is now ready for serious attention and treatment just as hair and skin have been in the past. The nail barrier can be breached.

These findings presumably relate to delivery of cosmetic agents for the management of nail abnormalities, such as nails that are peeling or fragile. R&D on these agents will be simplified when the rules describing the relationship of physical chemistry to flux are developed for the nail, as they have been in part for the skin.

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