Enhanced Human Nail Drug Delivery: Nail Inner Drug Content Assayed by New Unique Method

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ABSTRACT: The purpose of this study was to develop an assay method of the human inner nail plate and to compare nail drug penetration by a penetrating enhancing formulation (the test carrier formulation). The test carrier and saline formulations were tested using radiolabeled urea, ketoconazole, and salicylic acid. After twice dosing daily for 7 days on human nail plates, the under inner section of the nail plate was assayed for absorbed drug content using a unique drilling/removal system. Results show that the weight-normalized radioactivity contents of three chemicals in the inner intermediate nail plate center in the carrier formulation were two fold higher than those from saline ($p < 0.05$). Total radioactivity recovery of dosed [^{14}C]-salicylic acid was $89 \pm 2\%$ in the carrier formulation and $88 \pm 5\%$ in saline. In saline formulation, salicylic acid showed greater binding to the outer nail, making it less bioavailable for the inner nail area. This didn't occur with carrier formulation. In conclusion, topical treatment of nail diseases such as onychomycosis is not yet sufficiently effective, likely because of minimal drug penetration into the inner nail plate where the disease perpetuates. The assay system has the unique characteristic of being able to assay the inner part of the nail where the disease resides. J Pharm Sci 91:189–195, 2002

Keywords: enhanced drug delivery; human nail plate; drilling/removal system; nail inner drug content

INTRODUCTION

Onychomycosis is a fungal infection of the nail plate and bed. It is common, affecting 14% of the population.[1] Aging increases the incidence significantly, with the rate estimated to be 48% in persons aged 70 years.[1] Onychomycosis results in discoloration, thickening, hardening, or crumbling of the nail and may result in pain while wearing shoes. To cure the infection, the patient is obliged to take oral systemic medication for months.[1]

The existing clinical evidence suggests that a key to successful treatment of onychomycosis by a topical antifungal product lies in it effectively overcoming the nail barrier. Current topical treatments have limited effectiveness, possibly because they cannot sufficiently penetrate the nail plate to transport a therapeutically sufficient quantity of antifungal drug to the target sites[2] to eradicate the infection.

In this study, a previously described percutaneous drug-enhancing formulation[3,4] containing the absorption enhancer dimethylsulfoxide (DMSO) was studied as the test carrier formulation to enhance nail plate penetration. The penetration of ketoconazole, salicylic acid, and urea was studied after repeated applications under *in vitro* conditions. The assay problem,

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how to sample the inner surface of the nail plate to determine drug delivery, was solved using a precise flat drilling process that removed the sample of nail as a powder, which was then collected and assayed for drug content.

MATERIALS AND METHODS

Model Compounds

[^{14}C]-Urea (specific activity 55 mCi/mmol, 99% purity), [7-^{14}C]-salicylic acid (specific activity 55 mCi/mmol, 99% purity), and [^{3}-H(G)]-ketoconazole (specific activity 5 Ci/mmol, 99% purity) were purchased from American Radio-labeled Chemicals, Inc. (ARC, St. Louis, MO).

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.

Preparation of Formulation

A formulation (Dimethaid Research Inc., Markham, Ontario, Canada) had previously been shown to enhance skin penetration formulation (test carrier formulation).[3] The test formulation contains DMSO and other excipients.[4] Normal saline (ABBOTT Laboratories, North Chicago, IL) was the control. Concentrations (w/v) of [^{14}C]-urea, [^{14}C]-salicylic acid, and [^{3}H]-ketoconazole were 0.002%, 0.068%, and 0.154% in the test carrier formulation, and 0.002%, 0.066%, and 0.098% in normal saline control, respectively. Measured pH values for test carrier and saline formulations were 6.8 and 5.7, respectively. pK_a values for urea, ketoconazole, and salicylic acid were 0.1, 2.9 (and 6.5 as pK_a2), and 2.9, respectively. Thus, neither vehicle increased ionization or changed the solubility of these test chemicals in the vehicles. A 5 μL aliquot was removed from each vial and radioactivity was measured in a Packard Liquid Scintillation Counter (model 1500). A 5 μL aliquot carrier formulation contained 0.1 μCi/0.1 μg Eq. of [^{14}C]-urea, or 0.1 μCi/3.4 μg Eq. of [^{14}C]-salicylic acid, or 0.07 μCi/7.7 μg Eq. of [^{3}H]-ketoconazole. For controls, a 5 μL aliquot saline formulation contained 0.1 μCi/0.1 μg Eq. of [^{14}C]-urea, or 0.09 μCi/3.3 μg Eq. of [^{14}C]-salicylic acid, or 0.04 μCi/4.9 μg Eq. of [^{3}H]-ketoconazole.

Dosing and Surface Washing Procedures

A 5 μL dosing aliquot of each of the six test solutions was applied to the surface of a nail plate with a microsyringe twice daily, approximately 8 h apart for 7 days. Starting 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 Ivory soap, 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 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 test material. The radioactivity of each sample was measured in a liquid scintillation counter.

Nail Incubation System

A Teflon one-chamber diffusion cell (AMIE Systems) was used to hold each nail. To approximate physiological conditions, a small cotton ball wetted with 0.1 mL normal saline was placed in the chamber to serve as a "nail bed" and provide moisture for the nail plate. On day 5, 0.1 mL normal saline was injected through the inlet into the chamber to keep the cotton ball wet. A 1.5×1.5 cm aluminum sheet (0.24 mm thickness) with a hole in the center (1.0 cm diameter) was centered and glued to the top (dorsal) surface of the nail as a "frame" for the seal. The aluminum sheet frame flattens the curved nail and extends the surface area of the nail plate to fit the size of the top cover, so that potential leaking is prevented. The nail plate was placed on a ledge inside the receptor (1.0 cm in diameter and 0.5 cm high), sheet side up. The ventral (inner) surface of the nail was placed face down and rested on the wet cotton ball. Hydration of the nail plate and the supporting cotton bed was measured with a relative humidity/temperature meter (Corneometer CM 820; Courage & Khazaka, Cologne, Germany). Hydration was expressed digitally in arbitrary units (AU).

The rim of the cell base was covered with silicon gel and the top cover was set into the gel to prevent leaking (Figure 1). The metal holder was then tightly screwed into place.

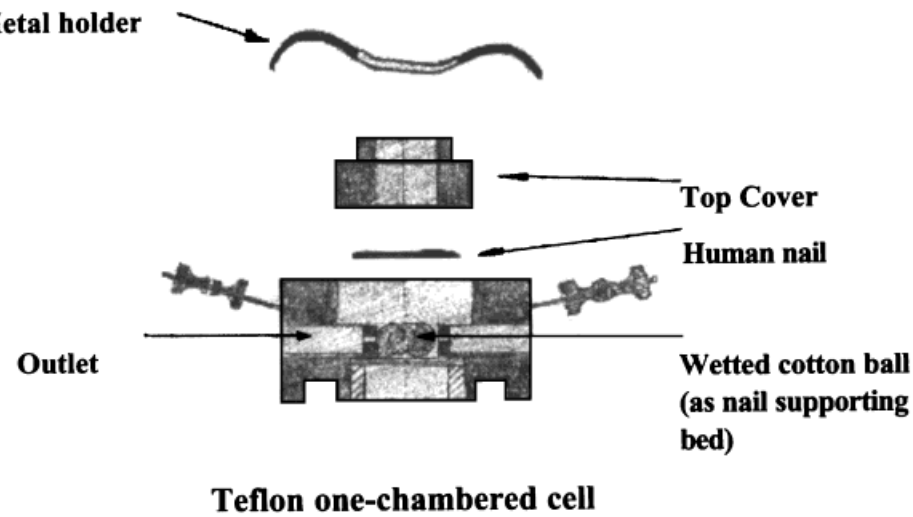

Figure 1. Nail support and incubation system.

The cells were placed on a platform in a large glass holding tank filled with saturated sodium phosphate solution. A digital relative humidity/temperature meter was used for monitoring room temperature, the chamber temperature, and humidity. The holding tank was then covered, thereby maintaining the cells at a constant humidity of 40%.

Sampling Instrument and Procedure

The sampling instrument consisted of a drill (Dremel model 2850; Dremel Inc., Racine, WI), a drill press (Dremel), an adjustable stage (Fisher, Pittsburgh, PA) with an adjustable holder, and a high-speed cutter (5/16 inch or 7.9 mm in diameter) (Figure 2).

After completion of the incubation phase, the nail plate was removed from the diffusion cell. The aluminum sheet frame was peeled off and placed in a glass scintillation vial. The nail plate was transferred to a clean Teflon diffusion cell for processing. The nail plate was inverted so that the ventral (nail bed) surface faced up and the dorsal (outer) dosed surface faced down. A top cover was tightly screwed into place. The cell was placed in the middle of the holds on the adjustable stage. The stage was pushed to its lowest position. The adjustable stage was then raised until the nail plate was just touching the tip of the cutter. The drill press was raised, and, without moving the Fisher stage, one piece of aluminum sheet (0.24 mm in thickness) was placed under the bottom of the diffusion cell to elevate it. The drill was turned on and the press was again pushed to its lowest position, removing a nail core sample of approximately the thickness of the aluminum sheet, 0.24 mm in depth and 7.9 mm in diameter, from the center of the ventral (nail bed) surface of the nail. The depth of the core thus removed by the drill was confirmed by measuring the

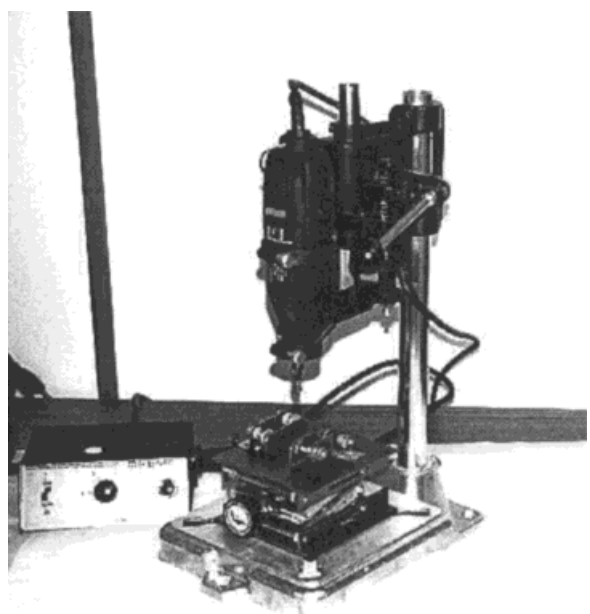

Figure 2. The sampling system. The drill instrument for nail sampling consisted of a Dremel drill, a Dremel drill press, a Fisher adjustable stage with adjustable holder, and a high-speed cutter (5/16 inch or 7.9 mm in diameter).

difference in the thickness of nail before and after sampling with a digital micrometer (Sony M-30; Sony Magnescale Inc., Japan).

The drilling removed the nail sample as a powder. The powered nail samples were collected with a small paint brush and aluminum funnel into a glass scintillation vial and weighed. An aliquot of 5.0 mL Packard soluene-350 was added to the scintillation vial to dissolve the powder.

The upper part, the intermediate and dorsal layers of the center of the nail, including the area of application of the dose, was cut in the same diameter as the sampled area. The sample was then placed into a glass scintillation vial and dissolved by adding 5.0 mL Packard soluene-350. The rest of the nail was also dissolved in 5.0 mL Packard soluene-350. All samples were incubated at 40°C for 48 h, followed by the addition of 10 mL scintillation cocktail (Hionic-fluor; Packard Instrument Company, Meriden, CT).

The radioactivity of each sample was counted by a liquid scintillation counter (Packard model 1500; Packard Instrument Company, Downer Grove, IL). ^{3}H efficiency of the scintillation counter is equal to or larger than 60% and ^{14}C efficiency is equal to or larger than 95%.

The amount of nail sample removed was also measured by the difference in weight of the nail plate before and after drilling and collecting the core of powder.

RESULTS

Experimental Conditions

During the experiment, the holding tank temperature was 25 ± 2°C and relative humidity was 44 ± 8%. Hydration (AU) of the dorsal nail surface was 8.5 ± 2.4 for saline formulation and 11.2 ± 3.6 for test carrier formulation. The supporting cotton bed hydration (AU) was 115.9 ± 9.9 for saline formulation and 118.0 ± 9.4 for test carrier formulation.

Characteristics of Nail Samples

Table 1 shows for each experiment the thickness of whole nail plate, the depth of the ventral surface core sample removed by the cutter, the percentage of the whole nail thickness, the total weight of the nail sample removed, and the actual weight of powered nail sample collected. The weight of the nail samples collected was consistent for all experiments.

Comparison of Chemical Penetration of Two Formulations

Table 2 shows weight normalized drug penetration (μg Eq. of ketoconazole, urea, and salicylic acid/g nail sample) into the combined dorsal and

Table 1. Nail Core Sampled from the Ventral (Inner) Surface Center of the Human Nail Plate

		Nail Core Sampled from the Ventral (Inner) Surface Center of the Nail Plate[a]			
Test Number	Whole Nail Thickness (mm)	Depth of Core (mm)	% Whole Nail Thickness	Total Core Sample Removed (mg)	Powder Sample Collected (mg)
Urea	0.65	0.25	39.52	16.4	5.2
(saline)	(0.09)	(0.03)	(8.05)	(4.3)	(0.8)
Urea	0.71	0.27	37.97	17.6	6.4
(test)	(0.07)	(0.03)	(2.69)	(4.3)	(1.3)
Ketoconazole	0.68	0.28	41.88	14.3	6.7
(saline)	(0.05)	(0.03)	(1.16)	(6.7)	(2.6)
Ketoconazole	0.73	0.28	38.62	14.1	4.3
(test)	(0.03)	(0.02)	(2.69)	(5.1)	(1.6)
Salicylic acid	0.77	0.25	32.62	12.1	6.0
(saline)	(0.07)	(0.08)	(9.38)	(2.4)	(0.5)
Salicylic acid	0.60	0.21	35.03	23.4	4.7
(test)	(0.12)	(0.06)	(6.45)	(8.3)	(0.8)
Average	0.69	0.26	37.61	16.3	5.5
	(0.09)	(0.05)	(6.20)	(6.2)	(1.6)

[a]Nail sample, approximately 0.24 mm in depth and 7.9 mm in diameter, was drilled from the center of the ventral surface of the nail. The amount of nail sample removed was measured by difference in weight and depth of the drilled area before and after sampling. Each number represents mean ± SD of five tests.

Table 2. Drug Penetration into Human Nail

Drug	Radioactive Content (μg Eq./g Nail Sample)		
Nail Layer	Saline Formulation[a]	Test Carrier Formulation[a]	p Value
Ketoconazole			
Dorsal/intermediate center[b]	444.1 (121.0)	289.2 (209.4)	0.190
Ventral/intermediate center[c]	34.0 (15.9)	53.9 (10.6)	0.048
Urea			
Dorsal/intermediate center[b]	0.4 (0.3)	0.8 (0.5)	0.054
Ventral/intermediate center[c]	0.2 (0.09)	0.35 (0.15)	0.039
Salicylic acid			
Dorsal/intermediate center[b]	71.6 (10.6)	16.3 (4.2)	0.000
Ventral/intermediate center[c]	7.0 (1.1)	10.2 (0.6)	0.008

[a]Each number represents mean ± SD of five tests.
[b]7.9 mm diameter core sample cut from the dosed area in the center of the dorsal surface of the nail.
[c]7.9 mm diameter core powdered nail sample removed by the drill from the ventral (inner) surface center of the nail, beneath the dosed area.

intermediate layers of the center of the nail plate with the test carrier formulation and saline formulation. Both descriptive and statistical analysis (student's t test, $p < 0.05$) show a greater penetration of drug into the ventral (inner) layer of the nail plate with the test carrier than with saline control: ketoconazole, $p = 0.048$; urea, $p = 0.039$; salicylic acid, $p = 0.008$.

Radioactive Content Recovery of [^{14}C]-Salicylic Acid

During the experiment, a "pooling effect" was observed. The drying process of topically applied salicylic acid or urea in the carrier formulation was much slower when compared with saline controls. Thus, the determination of the distribution of radioactive content is of great interest. Table 3 shows the radioactivity recovery and mass balance for dosed salicylic acid in the two formulations. Note that after topical dosing in test carrier formulation, the dorsal, dosed surface of the nail remained visibly wet, with an apparent limited penetration of the nail plate, leading us to conclude that the surface wash contained most of the recovered drug. After topical dosing of urea in the test carrier, again some surface pooling was observed. In contrast, with each of these two chemicals the saline formulation dried within 60 min. There was no visible wetness observed with ketoconazole.

DISCUSSION

Topical therapy for onychomycosis is not yet sufficiently 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.[5] The concentration of an applied drug across the nail dropped about 1000-fold from the outer surface to the inner surface.[6] As a result, the drug concentration presumably had not reached a therapeutically effective level in the inner ventral layer.

The human nail plate consists of three layers: the dorsal and intermediate layers derived from the matrix, and the ventral layer from the nail bed. The intermediate layer is three-quarters of the whole nail thickness and consists of soft keratin. The upper layer, dorsal, is only a few cell layers thick but consists of hard keratin.[7] The upper layer of the nail is the main barrier for drug diffusion into and through the nail plate.[8] The ventral layer consists of soft hyponychial in which many pathological changes occur.[7] Thus, in the treatment of these nail diseases, an effective drug concentration in the ventral nail plate would be of great importance.

Both the human nail and stratum corneum are rich in keratin. However, the two barriers behave differently. The human nail is like a hydrophilic

Table 3. Radioactivity Recovery as Percent Applied Dose of [^{14}C]-Salicylic Acid

	Percent Applied Dose	
	Saline Formulation[a]	Test Carrier Formulation[a]
Nail		
Dorsal/intermediate center[b]	47.10	4.96
	(7.76)	(2.13)
Ventral/intermediate center[c]	2.53	7.04
	(0.51)	(2.67)
Remainder	14.20	3.87
	(5.10)	(1.26)
Subtotal	63.83	15.87
	(9.54)	(3.76)
Surface washing	20.69	70.86
	(5.97)	(2.62)
Cotton supporting bed	3.66	2.23
	(2.02)	(0.75)
Total	88.18	88.96
	(5.52)	(2.29)

[a]Each number represents mean ± SD of five tests.
[b]7.9 mm diameter core sample cut from the dosed area in the center of the dorsal surface of the nail.
[c]7.9 mm diameter core powdered nail sample removed by the drill from the ventral (inner) surface center of the nail, beneath the dosed area.

gel membrane rather than a lipophilic partition membrane as is the case with the stratum corneum. The rate of chemical penetration into/through the human nail depends upon its water solubility,[9] and its molecular size.[10] To increase an effective chemical concentration into/through the human nail plate, penetration enhancers, which tend to promote diffusion through the skin's horny layer, have been studied. Walters et al.[11] found that DMSO retarded the permeation of methanol and hexanol across the nail plate, and isopropyl alcohol had little influence on penetration of methanol. They considered that these penetration enhancers have little promise as accelerators of nail plate permeability. However, Stüttgen and Bauer[6] found that DMSO appears to facilitate the penetration of some topical antimycotics. Franz[12] also reported that pretreatment of nail with DMSO resulted in a large increase in an antifungal agent (amorolfine) penetration.

We had previously shown[4] that this percutaneous drug-enhancing formulation enhanced the permeability of human skin to diclofenac sodium. With multiple dosing (four doses/day for 2 days) *in vivo*, human skin penetration of diclofenac in the carrier continually increased over the 48 h of study, as compared with a saline control. An *in vivo* human study[3] found that after a single topical application of radiolabeled diclofenac in that formulation on the human knee area, there was a continuous delivery of diclofenac from the carrier through the skin and into the urine, until the dosed site was washed at 24 h postdosing. In this study, the efficacy of the carrier on the permeability of human nail plate to three radiolabeled chemicals, urea, salicylic acid, and ketoconazole was measured using a new assay technique. Three chemicals were chosen as model compounds because they are frequently found in formulations. Salicylic acid and urea are often used topically for a variety of conditions, and ketoconazole is a widely used synthetic imidazole antifungal. After topical application of each chemical twice daily for 7 days, the radioactivity content in the dorsal/top center dosed area, the intermediate center (beneath the dosed area), and ventral layer of the nail plate was determined. Results (Table 2) show that the carrier formulation increased radioactivity significantly in the ventral surface of the nail plate by approximately 50% compared with saline control ($p < 0.05$).

There is a large difference in the mass balance ratios of salicylic acid (Table 3) between nail content and surface wash. With the saline formulation the top of the nail retains a large portion of the dosed drug after washing. The salicylic acid may interact and bond with the nail surface and

thus be unavailable for absorption further into the nail. The test carrier formulation secures the salicylic acid and makes it available for further nail absorption.

The absolute rate of water transpiration from the nail is faster than intact skin. Thus, if using the methods described previously for nail study[9,13], the human nail sample has to be in contact with an aqueous solution on either one or both sides during the incubation. As a result, the human nail plate is artificially kept totally hydrated. In this study, a novel experimental system was used to simulate *in vivo* conditions of therapeutic, nonoccluded application of drug to a human nail. In the new incubation device, the human nail surface (top center) was open to air and the inner surface made contact with a small cotton ball as a nail supporting bed. The cotton ball was wetted by normal saline and the degree of hydration was monitored and controlled during the experiment. The averages of hydration of the wetted cotton balls, 118 ± 9.4 AU, resemble the average hydration of a human nail bed, 99.9 ± 8.9 AU, measured from fresh human cadavers. The advantage of this incubation device is that it is nonocclusive and hydration controlled. Another advantage of this system is the accuracy of the sampling process. In this study design, the average depth of nail sampling from the inner center surface was well controlled at 0.26 ± 0.05 mm (Table 1), which was close to the expected depth of 0.24 mm.

In conclusion, the test carrier formulation enhanced penetration of radiolabeled urea, salicylic acid, and ketoconazole into the intermediate nail center as much as 50% higher than with saline controls ($p < 0.008–0.05$) after 7 day repeated topical dosing. The disadvantage of the carrier formulation found in the experiment is that after mixing with urea and salicylic acid, the drying process of a topically applied dose is much slower compared to saline controls. At present, the clinical significance of these data cannot yet be generalized until more tests are done using other compounds with different physical-chemical properties, including *in vivo* dermatopharmacokinetic comparisons or clinical and patient data.

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