Enhanced Econazole Penetration into Human Nail by 2-*n*-Nonyl-1,3-dioxolane

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ABSTRACT: This study determines the enhancing effects of 2-*n*-nonyl-1,3-dioxolane on the penetration of econazole, an antifungal drug, into the deeper layers of the human nail where fungal infection resides. Aliquots (10 μL) of Econail® lacquer formulation containing 0.45 mg of [^{14}C]-econazole with 18% 2-*n*-nonyl-1,3-dioxolane (test group) or without 2-*n*-nonyl-1,3-dioxolane (control group) were applied twice daily for 14 days to human nails that had been washed with ethanol before each morning's application. The hydration of the nail sample was well controlled to simulate normal physiological conditions. After 14 days of dosing, the inner ventral section of the nail plate was assayed for absorbed drug content, using a micrometer-controlled drilling and nail powder removal system. The mass balance values of [^{14}C]-econazole in this study were 90.8 and 96.4% for the test and control groups, respectively. The weight-normalized econazole content in the ventral/intermediate nail plate center in the test group was 6-fold greater than that in the control ($p = 0.008$). The total econazole absorbed into the supporting bed cotton ball in the test group was nearly 200-fold greater than that in the control group ($p = 0.008$) over the 14-day period. The amount of econazole after dosing in the inner part of the human nail (potential diseased area) was 11.1 ± 2.6 (SD) μg/mg of nail powder with 2-*n*-nonyl-1,3-dioxolane in the lacquer and 1.78 ± 0.32 μg/mg without 2-*n*-nonyl-1,3-dioxolane ($p = 0.008$). The surface nail contained more econazole ($p = 0.004$), that is, nonabsorbed drug, where 2-*n*-nonyl-1,3-dioxolane was not part of the dosing solution. Econazole in the support bed under the nail (the total absorbed dose) was 47.5 ± 22.0 mg in the lacquer with 2-*n*-nonyl-1,3-dioxolane and 0.2 ± 0.1 mg in the lacquer without 2-*n*-nonyl-1,3-dioxolane ($p = 0.008$). Moreover the concentration in the deep nail layer in the test group is 14,000 times higher than minimum inhibitory concentration (MIC) believed necessary to inhibit the growth of infecting fungi (*Dermatophytes* species). In a subsequent study, [^{14}C]-dioxolane did not penetrate the nail well. Therefore, the mechanism of enhancement of econazole penetration is at the formulation/nail interface. J Pharm Sci 92:142–148, 2003

Keywords: human nail; onychomycosis; SEPA; lacquer; econazole; antifungal; nail incubation; sampling

INTRODUCTION

Traditionally, the topical treatment of onychomycosis has been less than desirable because of the deep-seated nature of the infection and to the ineffective penetration of the deep nail plate by

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topically applied drugs. Many topical antifungal formulations are aimed at the skin surface rather than the nail. To deliver a therapeutically sufficient quantity of an antifungal drug to the fungus-infected sites, such as nail plate, nail bed, and matrix, a suitable carrier is needed to enhance drug penetration through the nail barrier. Nail lacquer formulation is a popular choice for topical antifungal treatment. It contains a film-forming agent, solvent, antifungal drug, and possibly a penetration enhancer. Once the lacquer is applied, it forms a thin, water-insoluble film containing supersaturated antifungal drug. This film provides chemical gradient to drive drug flux as the drug is released. Thus, a lacquer formulation is suitable for topical treatment of nail diseases.

In this study, the efficacy of econazole nail delivery from a lacquer formulation, EcoNail™ with a penetration enhancer, 2-*n*-1,3-nonyl-dioxolane was examined. Econazole is a broad spectrum antimycotic tested in animals artificially infected with *Dermatophytes*.[1] 2-*n*-Nonyl-1,3-dioxolane is a skin penetration enhancer.[2] To emulate the normal physiological nail condition, the experiment was conducted using a unique hydration-controlled nail incubation system, and econazole penetration into the nail was sampled by a unique micrometer-controlled nail sampling/powder removal instrument.[3]

To examine the function of dioxolane on the nail plate, the level and rate of [^{14}C]-dioxolane nail penetration was also studied for nail penetration. [^{14}C]-Salicylic acid was selected as a positive control to determine if mechanism of action of dioxolane was within the nail or at the nail–lacquer interface.

MATERIALS AND METHODS

Chemicals and Topical Formulations

Econazole [chlorophenyl benzyl-^{14}C]-(D,L)-econazole (chemical name: 1-[2-[(4-chlorophenyl)-methoxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole) was obtained from Perkin-Elmer Life Sciences, Inc. (Boston, MA). The radiochemical purity and specific activity of the chemical were determined by high-performance liquid chromatography (HPLC) as >99% and 43.87 mCi/mmol, respectively. [^{14}C]-Dioxolane (dioxolane methylenes-^{14}C) was also synthesized by the Perkin-Elmer Life Sciences. The radiochemical purity and specific activity was determined by the manufacturer by gas chromatography (GC) as >99% and 55 mCi/mmol, respectively. Salicylic acid, [7-^{14}C] was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). The radiochemical purity and specific activity of the chemical were determined by the manufacturer by HPLC as >99% and 55 mCi/mmol, respectively. Nonradiolabeled econazole, 2-*n*-nonyl-1,3-dioxolane, and Eudragit® RL/PO were supplied by MacroChem Corp. (Lexington, MA). The test topical formulation contains 5% (w/w) nonlabeled econazole, Eudragit® RL/PO, ethanol, and 2-*n*-nonyl-1,3-dioxolane (18% w/w). The control topical formation contains the same components as the test formulation, but with no 2-*n*-nonyl-1,3-dioxolane (using ethanol instead). After the nail lacquer was formed, an aliquot of [^{14}C]-econazole was added. The formulation used for dioxolane penetration study was the same as the aforementioned econazole nail lacquer. An aliquot of [^{14}C]-dioxolane was then added to the mixture to make a final solution containing radioactivity of 17.68 μCi/g. The composition (weight %) of the dosage formulation for the [^{14}C]-salicylic acid group followed our previous study.[3] An aliquot of [^{14}C]-salicylic acid was added into 5 mL of normal saline to obtain a final solution containing 14.58 μCi/g.

Human Finger Nails

Healthy human finger nail plates were collected from adult human cadavers with a range of thickness from 0.500 to 0.997 mm and a median thickness of 0.853 mm. The nail samples were randomly selected, and allocated to the two groups, test and control, for the econazole penetration study or to the two groups, [^{14}C]-dioxolane and [^{14}C]-salicylic acid, for the dioxolane penetration study.

Dosing and Surface Washing Procedures

Aliquots of the Econail formulations (0.45 mg of econazole in 10 μL) with and without 2-*n*-nonyl-1,3-dioxolane were applied to the surface of a nail plate with a HPLC microsyringe twice daily, ~8 h apart, for 14 days. Starting on the 2nd day, each morning, 10 min prior to dosing, the surface of the nail was washed with cotton tips (new one for each application) in a cycle, as follows: a tip wetted with absolute ethanol, then wetted with absolute ethanol, then wetted with distilled water, then a final dry tip. Washing samples from each cycle of each nail were pooled and collected by breaking

off the cotton tip into scintillation glass vials. The radioactivity of each sample was measured in a liquid scintillation counter. The experimental procedure of dioxolane penetration study was the same as the aforementioned econazole penetration study. Aliquots (10 μL) of the [^{14}C]-dioxolane or [^{14}C]-salicylic acid formulations were applied to the surface of a nail plate with a HPLC microsyringe twice daily, ~8 h apart, for 14 days.

Nail Incubation

The nail incubation procedure was the same as that published previously.[3] A nail plate was placed in a Teflon one-chamber diffusion cell (Permegear, Inc., Hellertown, PA). A small cotton ball wetted with 0.1 mL of normal saline was placed in the chamber beneath the nail plate to serve as a "nail bed" and provide moisture for the nail plate.

Nail Sampling

The nail sampling instrument was a modified version of that previously reported.[3] It has two parts, a nail sample stage and a drill. The nail sampling stage consists of a copper nail holder, three adjustments, and a nail powder capture. The three adjustments control vertical movement. The first coarse adjustment (on the top) is for changing the copper cell and taking powder samples from the capture. The other two adjustments (lower) are used in sampling. The second coarse adjustment allows movement of 25 mm, whereas the fine adjustment provides movement of 0.20 mm. The nail powder capture is located between the copper cell and the cutter. The inner shape of the capture is an inverted funnel, with the end connected to a vacuum pump. By placing a filter paper inside the funnel, nail powder samples can be captured on the filter paper during the sampling process.

Sampling Procedure

After completion of the incubation phase, the nail plate was transferred from the diffusion cell to a clean copper nail holder for sampling. The nail plate was inverted so that the ventral (nail bed) surface faced up and the dorsal (outer) dosed surfaced faced down. The copper cell was secured on the top of the stage. When sampling was initiated, the coarse adjustment moved the position of the stage until the nail plate just touched the cutter tip. The drill was then turned on and the fine adjustment moved the stage to the cutter tip, removing a nail core sample. After completion of this process, nail powder samples ~0.30–0.40 mm in depth and 7.9 mm in diameter were harvested from the center of the ventral (nailbed) surface of the nail.

The drilling removed the nail sample as a powder from the intermediate and ventral layers of the nail. The upper part (dorsal), including the area of dose application, was cut to the same diameter as the sampled area. The powdered nail samples, the upper part and the remainder of the nail samples, were individually collected into a glass scintillation vial and weighed. The nail samples were then dissolved by adding 5.0 mL of Packard Soluene-350 (Packard Instrument company, Meriden, CT). The total mass of nail collected was measured by the difference in weight of the plate before and after drilling.

Radioactivity Measurement

All radioactivity measurements were conducted with a Model 1500 Liquid Scintillation Counter (Packard Instrument Company, Downer Grove, IL). Nail samples pretreated with Packard Soluene-350 were incubated at 40°C for 48 h, followed by the addition of 10 mL of scintillation cocktail (HIONIC-FLUOR, Packard Instrument Company, Meriden, CT). Other samples (standard dose, surface washing, and cotton balls) were mixed directly with Universal ES scintillation cocktail (ICN Biomedicals, Costa Mesa, CA). Background control and test samples were counted for 3 min each for radioactivity.

Data Analysis

The value from the test group was compared with the corresponding value of the control group with a t test. The normalized econazole concentration (μg/cm^3) in the dorsal/intermediate layer was calculated by multiplying the raw parameters by the nail density (1.33 g/cm^3, determined under the current experimental conditions). Flux (μg/cm^2/h) was computed from the average of the cumulative amount of econazole permeated into the deeper layer of the nail (dorsal/intermediate layer and cotton ball supporting bed). The efficacy coefficient, E, is defined as the ratio of flux of econazole through the nail plate to the minimum inhibitory concentration.

Table 1. Summary of Mass Balance and Normalized Econazole Concentration in Nail and Supporting Bed Samples after 14-Day Treatment[a]

	Carbon-14 Recovery (% of Dose)		Normalized Econazole Concentration (μg Equivalent/mg Nail)		
Sample	Test Group	Control Group	Test Group	Control Group	*p* Value (*t* Test)
Ventral/intermediate center	1.35 (1.10)	0.22 (0.08)	11.15 (2.56)	1.78 (0.32)	0.0079
Dorsal/intermediate center	11.38 (3.59)	20.11 (2.95)	0.25 (0.03)	0.37 (0.06)	0.0038
Remainder nail	5.63 (3.86)	3.22 (2.32)	0.16 (0.14)	0.06 (0.04)	0.2222
Supporting bed	0.72 (0.33)	0.00 (0.00)	47.55[b] (21.99)	0.24[b] (0.09)	0.0079
Surface washing	71.75 (12.48)	72.81 (5.12)	—	—	—
Total	90.83 (16.42)	96.36 (7.31)	—	—	—

[a]The data represents the mean (SD) of each group ($n = 5$). The test group contains 18% 2-*n*-nonyl-1,3 -dioxolane and the control group has no 2-*n*-nonyl-1,3-dioxolane.
[b]The unit of supporting bed cotton ball is μg/ball.

RESULTS

The mass balance of total radioactivity recovery of [^{14}C]-econazole as measured from surface washing, nail samples, and supporting bed cotton ball samples, shown in Table 1, was $90.8 \pm 16.4\%$ in the test group and $96.4 \pm 7.3\%$ in the control group. The summarized weight-normalized econazole equivalents in the nail and the supporting bed cotton ball in the test group and the control group are also shown in Table 1. After 14 days of topical dosing, the concentration of econazole in the center of the deeper layer (ventral/intermediate) nail samples in the test group was 6.3-fold greater than that in the control group ($p = 0.008$). The total amount of econazole in the supporting bed cotton ball in the test group was 200-fold greater than that in control group ($p = 0.008$). On the top surface of the dosed site (dorsal/intermediate center), the concentration of econazole in the control group was slightly but perceptibly higher than that in the test group ($p = 0.004$).

After 14 days and 28 topical applications, [^{14}C]-dioxolane had poor penetration into and through the human nail plate when compared with that of [^{14}C]-salicylic acid (see Table 2). After weight normalization, carbon-14 contents as percent dose of the ventral/intermediate center (beneath dosed side) of the nail samples in the [^{14}C]-dioxolane group was as low as 5% of those in the [^{14}C]-salicylic acid group ($p = 0.002$). For the dorsal/intermediate center in the [^{14}C]-dioxolane group, it was only 4% of that of in the [^{14}C]-salicylic acid group ($p = 0.002$). The carbon-14 content in the supporting bed cotton ball in the [^{14}C]-dioxolane group was $2.30 \pm 0.61\%$ of dose, which was only one third of that in the [^{14}C]-salicylic acid ($p = 0.002$). Thus, the enhancing nail penetrating effect of dioxolane occurs at the nail–formulation interface not within the interior of the nail plate.

Table 2. Normalized [^{14}C]-Dioxolane and [^{14}C)-Salicylic Acid in Nail and Supporting Bed Samples after 14-Day Treatment

	μg Equivalent/mg[a]	
Sample	[^{14}C]-Dioxolane[b]	[^{14}C]-Salicylic Acid[b]
Ventral/intermediate center (beneath dosed side)	0.001 (0.000)	0.012 (0.003)
Dorsal/intermediate center	0.002 (0.001)	0.043 (0.011)
Remainder nail	0.001 (0.000)	0.018 (0.003)
Supporting bed cotton ball	0.398[c] (0.107)	0.782[c] (0.200)

[a]Each data entry represents the mean value (SD) of 6 samples.
[b]Mean drug penetration in each group from the [^{14}C]-dioxolane group is statistically different ($p < 0.05$) from that in the [^{14}C]-salicylic acid group.
[c]The unit is μg equivalent/cotton ball bed sample.

DISCUSSION

The ability of 2-*n*-nonyl-1,3-dioxolane to enhance the penetration of econazole into the human nail from the EcoNail™ lacquer containing 18% 2-*n*-nonyl-1,3-dioxolane was determined and compared with that of an identical econazole lacquer without the enhancer. As previously reported,[3] the *in vitro* nail incubation device was hydration controlled to approximate normal physiological conditions. The modified nail sampling instrument improved the ability to capture nail powder during the sampling process. It enables well-controlled, accurate, and reproducible sampling of the inside of the nail and a higher mass balance efficiency than in the previous study.[3]

Onychomycosis involves the nail and the nail bed. To optimize topical treatment, it is important to know if there is significant drug concentration in the diseased tissues after topical application. This study measured econazole concentration in the different layers of the plate, especially in deeper layers, including the bed. The results in Table 1 shows that after weight normalization, the concentration of econazole in the center of the deeper layer (ventral/intermediate) nail samples in the test group was 6.3-fold greater than that in the control group ($p = 0.008$). The total amount of econazole in the supporting bed cotton ball in the test group was 200-fold greater than that in control group ($p = 0.008$). The results indicate that econazole has a greater nail penetration when formulated in the lacquer containing 2-*n*-nonyl-1,3-dioxolane than in an identical lacquer without enhancer. 2-*n*-Nonyl-1,3-dioxolane is also known as SEPA® (soft enhancement of percutaneous absorption), which has been shown to be effective in increasing transdermal drug delivery.

A mechanism study of 2-*n*-nonyl-1,3-dioxolane in skin penetration shows that 2-*n*-nonyl-1,3-dioxolane can reversibly fluidize stratum corneum lipids and alter the stratum corneum barrier function.[4] During fluidization, drugs can diffuse through stratum corneum at a higher than normal rate. The enhancement function of 2-*n*-nonyl-1,3-dioxolane on nail penetration has not previously been determined. Generally, skin penetration enhancers are thought to be ineffective in accelerating nail penetration if they act only on the lipid domains of the stratum corneum.[5] A significant difference between the human nail and the stratum corneum is that the lipid content of the nail plate is much less than that of stratum corneum. A hydrated nail plate could therefore behave more like a hydrogel membrane in its barrier properties.[6,7] Most enhancement methods of nail penetration, such as chemical alteration of disulfide linkages in the keratin matrix, physically thinning out, or chemically softening the nail plate, are eventually meant to increase nail hydration.[5] Because this study suggests that [^{14}C]-dioxolane had minimal penetration into and through the human nail plate when compared with that of [^{14}C]-salicylic acid (Table 2), 2-*n*-nonyl-1,3-dioxolane possibly has little direct effect on the nail plate. Studies found that in the Econail lacquer formulation, 2-*n*-nonyl-1,3-dioxolane is not only effective in facilitating diffusion of the active agent(s) transungually but also functions as an adhesion promoter and as a plasticizer for the film-forming polymer of the nail lacquer.[8] 2-*n*-Nonyl-1,3-dioxolane can soften the Eudragit film in the lacquer to release more econazole per unit time. When gradually increasing the amount of 2-*n*-nonyl-1,3-dioxolane in the lacquer from 0 to 18%, the hardness of the lacquer was reduced (personal communication). Econazole penetration into the human toenail was increased by 23% (at 15% 2-*n*-nonyl-1,3-dioxolane) and 56% (at 18% 2-*n*-nonyl-1,3-dioxolane) when compared to a 0% 2-*n*-nonyl-1,3-dioxolane lacquer.[8] These data suggest that the effect of 2-*n*-nonyl-1,3-dioxolane on econazole nail penetration is through the unique composition of the nail lacquer.

Table 3. Econazole Concentration and Relative Antifungal Efficacy with and without 2-*n*-Nonyl-1,3-Dioxolane after 14-Day Dosing[a]

Parameter	Test Group	Control Group	*p* Value (*t* Test)
Econazole in the deeper layer (μg/cm^3)[b]	14,830 (341)	2,371 (426)	0.0079
Efficacy coefficient *E* (MIC = 1 μg/mL)	14,830 (341)	2,371 (426)	0.0079
Efficacy coefficient *E* (MIC = 100 μg/mL)	148 (3.4)	23.7 (4.3)	0.0079

[a]The data represent the mean (SD) of each group ($n = 5$). The test group contains 18% 2-*n*-nonyl-1,3-dioxolane and the control group contains no 2-*n*-nonyl-1,3-dioxolane.
[b]The deeper layer is the center of the ventral/intermediate layer of the nail plate.

Table 4. Comparison of Relative Antifungal Efficacy with and Without 2-*n*-Nonyl-1,3-Dioxolane[a]

Parameter	Test Group	Control Group	*p* Value (*t* Test)
Flux of the deeper layer ($\mu g/cm^2/h$)[b]	1.58 (0.32)	0.21 (0.04)	0.0001
Efficacy coefficient *E* (MIC = 1 μg/mL)	1.58 (0.32)	0.21 (0.04)	0.0001

[a]The data represent the mean (SD) of each group ($n = 5$). The test group contains 18% 2-*n*-nonyl-1,3-dioxolane and the control group contains no 2-*n*-nonyl-1,3-dioxolane.
[b]The deeper layer is the center of the ventral/intermediate layer of the nail plate. The flux ($\mu g/cm^2/h$) was computed from the average of the cumulative amount of econazole permeated into the deeper layer of the nail (dorsal/intermediate layer and cotton ball supporting bed).

The MIC is a laboratory index used in the determination of antifungal potency. For econazole, the range of MIC for *Dermatophytes* species is 0.1–1.0 μg/mL and that for yeasts species is 1.0–100 μg/mL.[9] The results in Table 1 show that after 14 days exposure, the econazole content measured in the test group was 11.15 ± 2.56 μg/mg for the ventral/intermediate layer. This content multiplied by the density of the nail sample (1.332 mg/cm^3, measured under current experimental conditions), yields $14{,}830 \pm 340$ $\mu g/cm^3$ of econazole, almost 15,000 times the MIC for most *Dermatophytes* species and 150 times that for most yeasts species (Table 3). Mertin and Lippold[10] introduced an efficacy coefficient, *E*, to better estimate and compare the relative efficacy of antifungal agents. The efficacy coefficient *E* is the ratio of the flux of an antimycotic drug through the nail plate to the minimum inhibitory concentration. As shown in Table 4, the flux of econazole into the deep layer of human nail is 1.58 ± 0.32 $\mu g/cm^2/h$ in the test group, compared with only 0.21 ± 0.04 $\mu g/cm^2/h$ in the 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 with the enhancement of 2-*n*-nonyl-1,3-dioxolane, the amount of econazole in the ventral/intermediate layer and supporting bed dramatically exceeds the inhibitory concentration of econazole for most common onychomycosis organisms.

In conclusion, the addition of 2-*n*-nonyl-1,3-dioxolane to econazole nail lacquer delivered six times more antifungal drug through human nail than an identical lacquer-drug formulation without enhancer. Concentrations of econazole in the deep nail layer and nail bed were significantly higher in the test group than in the control group. Moreover, the concentration in the deep nail layer in the test group is 14,000 times greater than the MIC believed necessary to inhibit the growth of infecting fungi (*Dermatophytes* species). [Note that this study and the previous study[3] are the first to examine drug concentration within the nail. Calculation of current MIC values derived in a test tube may not equate to the nail matrix. *In vivo–in vitro* correlations will further define the clinical relevance of this *in vitro* method.] These results suggest that 2-*n*-nonyl-1,3-dioxolane-enhanced econazole lacquer has the potential to be an effective topical treatment for onychomycosis.

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