Transungual Penetration and Antifungal Activity of Seven Prescription and Over-the-Counter Topical Antifungals: In Vitro Comparison

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SYNOPSIS

- Toenail onychomycosis in the United States is most often caused by the dermatophytes *Trichophyton rubrum* and *T. mentagrophytes*[1]
- Topical treatments must penetrate the nail to reach the site of infection, but this may be inhibited by drugs binding to keratin within the nail
- Three topical antifungals have been approved by the United States Food and Drug Administration (FDA) for the treatment of onychomycosis, and there are many over-the-counter (OTC) topical antifungals that are used off-label for onychomycosis treatment

OBJECTIVES

- The objective of these *in vitro* experiments was to evaluate the ability of commercially available topical antifungals to inhibit growth of *T. rubrum* and *T. mentagrophytes* via penetration of human cadaverous toenails and keratin-free cellulose disks

METHODS

- Three prescription and four OTC topical antifungals were tested (**Table 1**)
- Products were applied to human cadaverous toenails and allowed to dry prior to punching five 4-mm disks from the center of each nail; disks were placed in the center of an agar plate (85 mm radius) seeded with a clinical isolate of *T. rubrum* or *T. mentagrophytes* (two strains of each species were used; n=5 replicates for each product against each fungal strain) prior to incubation (2-7 days)
- In a second experiment assessing antifungal activity in the absence of human nails, each product was applied to a 6-mm cellulose disk and placed on a seeded agar plate (n=3 replicates of each product against each strain) prior to incubation (2-7 days)
- In both experiments, antifungal activity was assessed using zone of inhibition (ZI), defined as the radius of the area of no fungal growth, disregarding any feathering; results were averaged across the two strains of each fungal species, and untreated disks/nails served as negative controls
  - Differences in ZI were analyzed using 2-tailed Tukey tests for multiple comparisons; the threshold for significance was $P<0.05$

TABLE 1. **Antifungal Products Tested**

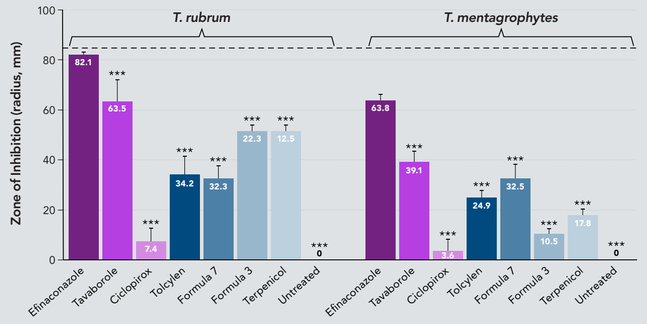
Product	Formulation	FDA Status
Ciclopirox	Ciclopirox 8% lacquer	Approved[a]
Efinaconazole	Efinaconazole 10% solution	Approved[a]
Tavaborole	Tavaborole 5% solution	Approved[a]
Formula 3	Tolnaftate 1% solution	OTC[b]
Formula 7		
Tolcylen	Tolnaftate 1% and undecylenic acid 25% solution	OTC[b]
Terpenicol	Undecylenic acid 25% solution	OTC[b]

[a]Indicated for the treatment of toenail onychomycosis.
[b]OTC topical antifungals used off-label for the treatment of onychomycosis.
FDA, United States Food and Drug Administration; OTC, over the counter.

RESULTS

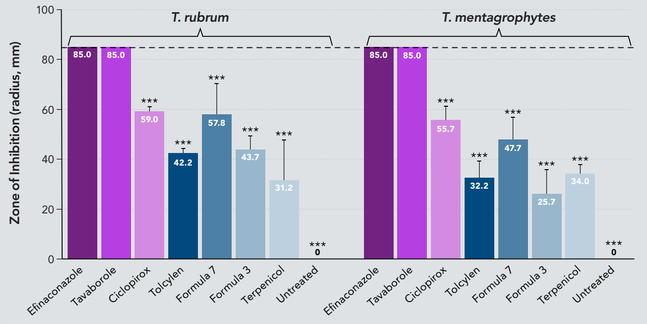
- In the cellulose nail penetration assay, average ZIs for FDA-approved antifungals against both *T. rubrum* and *T. mentagrophytes* were greatest for efinaconazole; ZIs for tavaborole and ciclopirox were significantly lower ($P<0.001$, all; **Figure 1**)
  - Against both species, average ZIs for all OTC products ranked between tavaborole and ciclopirox and were significantly lower than for efinaconazole ($P<0.001$, all)
- In the cellulose disk diffusion assay, average ZIs among FDA-approved antifungals were maximal (85 mm) against both species for efinaconazole and tavaborole; average ZIs for ciclopirox were significantly lower ($P<0.001$, all; **Figure 2**)
  - Against both species, average ZIs for all OTC products were similar to or less than for ciclopirox and significantly lower than for efinaconazole ($P<0.001$, all)
- Among FDA-approved products, comparison of antifungal activity against both species via human nail penetration as a percentage of activity via disk diffusion demonstrated that efinaconazole > tavaborole >> ciclopirox (**Figure 3**)
- Overall, ZIs were generally not affected by nail thickness (data not shown)
- Representative images of fungal inhibition with FDA-approved antifungals are shown in **Figure 4**

FIGURE 1. **Fungal Inhibition via Penetration Through Human Cadaverous Toenail**



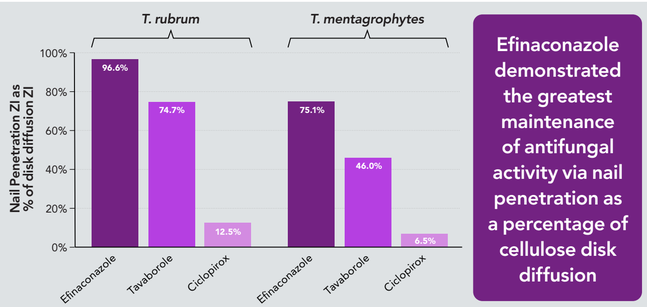
***P<0.001 vs efinaconazole.
Purple bars denote United States Food and Drug Association-approved topical antifungals indicated for onychomycosis. Blue bars denote over-the-counter topical antifungals used off-label for the treatment of onychomycosis. Bars represent average data from 5 replicates of each product against 2 strains of each fungal species. Zone of inhibition was defined as the radius of the area of no fungal growth, disregarding any feathering. Dashed line indicates maximum zone of inhibition (85 mm).

FIGURE 2. **Fungal Inhibition in the Cellulose Disk Diffusion Assay**



***P<0.001 vs efinaconazole.
Purple bars denote United States Food and Drug Association-approved topical antifungals indicated for onychomycosis. Blue bars denote over-the-counter topical antifungals used off-label for the treatment of onychomycosis. Bars represent average data from 3 replicates of each product against 2 strains of each fungal species. Zone of inhibition was defined as the radius of the area of no fungal growth, disregarding any feathering. Dashed line indicates maximum zone of inhibition (85 mm).

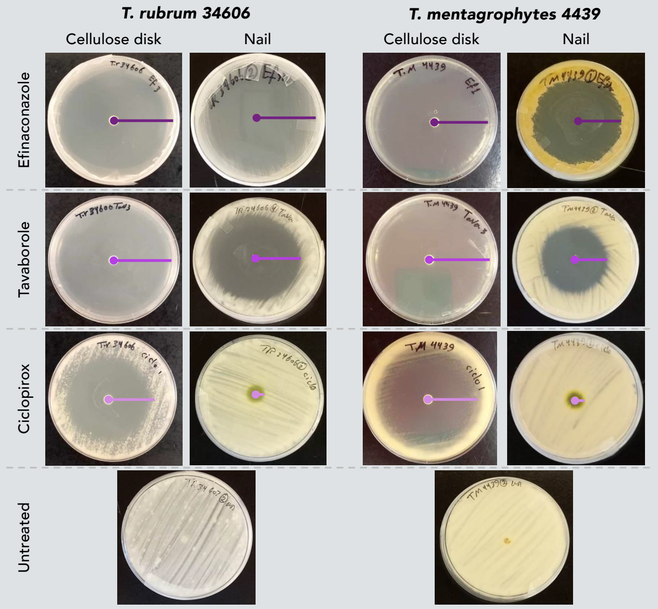
FIGURE 3. **Maintenance of Antifungal Activity via Nail Penetration**



Efinaconazole demonstrated the greatest maintenance of antifungal activity via nail penetration as a percentage of cellulose disk diffusion

Data shown only for United States Food and Drug Association-approved topical antifungals indicated for onychomycosis. ZI was defined as the radius of the area of no fungal growth, disregarding any feathering.
ZI, zone of inhibition.

FIGURE 4. **Antifungal Inhibition via Cellulose Disk Diffusion and Nail Penetration**



Representative images shown only for topical antifungals approved by the United States Food and Drug Administration and indicated for toenail onychomycosis. One of two strains tested for each fungal species is shown. Purple lines indicate zone of inhibition, defined as the radius of the area of no fungal growth, disregarding any feathering.

CONCLUSIONS

- Efinaconazole demonstrated superior transungual penetration compared to other FDA-approved topical antifungals for onychomycosis and all OTC topical antifungal products tested
  - Efinaconazole and tavaborole demonstrated maximal antifungal activity against two strains each of *T. rubrum* and *T. mentagrophytes* in a cellulose disk diffusion assay, significantly greater than with ciclopirox and OTC antifungals
  - However, when required to penetrate through human toenail, antifungal activity of efinaconazole was significantly greater than all other products tested
- The greater nail penetration and fungicidal activity of efinaconazole is likely due to its low keratin affinity relative to other topical antifungals[2-4]

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AUTHOR DISCLOSURES

Ali Elabbasi and Ahmed Eltokhy have nothing to disclose. Warren Joseph has served as consultant and speaker for Ortho Dermatologics. Boni Elewski has provided clinical research support (research funding to University) for AbbVie, Anaptys-Bio, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Incyte, LEO Pharma, Lilly, Merck, Menlo, Novartis, Pfizer, Regeneron, Sun Pharma, Ortho Dermatologics, Vanda; and as consultant (received honorarium) from Boehringer Ingelheim, Bristol Meyers Squibb, Celgene, LEO Pharma, Lilly, Menlo, Novartis, Pfizer, Sun Pharma, Ortho Dermatologics, Verrica. Mahmoud Ghannoum has acted as a consultant or received contracts from Scynexis, Inc, Bausch & Lomb, Pfizer, and Mycovia.

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