In Vitro Efficacy of Tavaborole Topical Solution, 5% after Penetration through Nail Polish on *Ex Vivo* Human Fingernails

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What's already known about this topic? (70 word max) Clinical studies have demonstrated tavaborole's efficacy and safety in the treatment of onychomycosis. Using cadaveric nails, tavaborole has been shown to penetrate up to 4 layers of nail polish without disrupting the appearance and integrity of the polish.

What does this study add? (70 word max) The current study investigates the efficacy of tavaborole in the presence of nail polish using an *ex vivo* healthy human distal nail model. Although previous studies have suggested that tavaborole may be compatible with nail polish, this is the first study to demonstrate fungicidal effects of tavaborole when applied to human nails with up to 4 layers of nail polish.

What is the translational message? (70 word max) Many patients suffering from onychomycosis have a strong desire to mask their infection with nail polish. Until now, there was no evidence to suggest that any topical treatment for onychomycosis is efficacious in the presence of nail polish. The current study provides evidence that tavaborole penetrates polished nails and subsequently kills *T rubrum* in an *ex vivo* model.

Abstract

Background: Topical antifungal treatments for onychomycosis are applied to clean, unpolished nails for 48 weeks or longer. Patients often wish to mask their infection with nail polish yet there is no evidence to suggest antifungal efficacy in the presence of nail polish.

Objective: To determine if tavaborole retains the ability to penetrate the nail plate and inhibit fungal growth in the presence of nail polish.

Method: Tavaborole was applied to human fingernails painted with 2 or 4 coats of nail polish, and unpainted nails in an *ex vivo* model. Nails were mounted on TurChub® chambers seeded with *Trichophyton rubrum* and allowed to incubate for 7 days. Antifungal activity was assessed by measuring zones of inhibition.

Results: Tavaborole exhibited antifungal activity in all experimental groups. The zones of inhibition of *T. rubrum* for all experimental groups (2 or 4 coats of polish, unpolished) were significantly greater than infected controls (polished and unpolished), $p_s < 0.001$.

Conclusion: Tavaborole penetrates polished nails and kills *T. rubrum* in this *ex vivo* model.

Introduction

In 2014, the FDA approved two new topical antifungals for the treatment of onychomycosis, tavaborole and efinaconazole.[1,2] For patients where oral antifungal medications are not an option or are undesired, the new topical antifungals present an alternative that circumvents concerns with oral antifungals (i.e., drug-drug interactions, hepatotoxicity). In particular, patients requiring multiple medications (e.g., elderly), or with diabetes and/or autoimmune diseases are likely unable to use oral antifungal medications and will benefit from the availability of topical antifungals.[3]

There are two primary objectives when treating onychomycosis. The first is the eradication of the fungal infection or mycological cure. The second is clearance of the infection to produce a normal appearing nail or clinical cure. This objective is likely the primary concern for patients.[4] Unsightly nails showing discoloration or structural deformity can be a source of distress and distraction for patients and lead to avoidance of social situations where nails will be seen. Nail polish is one solution for these patients; however, while nail polish may be applied while taking oral antifungals, product inserts do not provide guidance on their use in combination with nail polish.[1,2] A topical treatment that could be used in the presence of nail polish would be advantageous and desirable to many patients.

Tavaborole topical solution, 5% was shown to be effective in treating onychomycosis in two Phase 3 studies conducted over 52 weeks on 1191 patients who did not use nail polish.[5] *Ex vivo* work has also demonstrated that tavaborole 10% can penetrate the nail plate, having been detected in significantly greater concentrations as compared to ciclopirox 8% following daily application to cadaveric human fingernails for 5, 10 and 15 days.[6] Tavaborole 10% applied to *ex vivo* fingernails penetrated the nail to inhibit the fungal growth of *Trichophyton rubrum* in a TurChub® chamber model. Conversely, no inhibition of fungal growth was observed for ciclopirox 8% and amorolfine 5%.[6]

These *ex vivo* penetration studies were extended with investigation into the ability of tavaborole to penetrate the nail plate in the presence of nail polish. Tavaborole 5% was applied once daily for 14 days to human cadaveric nails painted with 1 to 4 layers of nail polish. Nail polish penetration was greater in painted nails than in unpainted nails, and overall accumulation of tavaborole increased over time.[7] Nail polish appearance on cadaveric nails was evaluated over 7 days of consecutive application with tavaborole 5%, with tavaborole-treated polished nails showing no loss of nail polish integrity including lack of discoloration.[8] It is yet to be determined if tavaborole can inhibit fungal growth in the presence of nail polish. The aim of the current study was to determine if a clinically relevant dose of tavaborole 5% applied over nail polish maintains fungicidal activity. To date, this is the first study of its kind to assess the ability of a topical antifungal to penetrate a nail and inhibit subungal growth of fungus in the presence of nail polish.

Methods

Following informed consent, fingernail clippings from healthy human volunteers were obtained. Inclusion criteria for nails were clean, disease-free, and unpainted in the last 6 months. Nails were frozen until ready to use. Upon thawing, nails were washed and cut into 3 mm x 3 mm sections before undergoing one of the following treatments with commercially available products (OPI):

- Full salon: OPI base coat (2 μL), 2 coats of OPI nail lacquer (2 μL each, "An affair in red square"), OPI top coal (2 μL)
- Partially polished: 2 coats of OPI nail lacquer (2 μL each, "An affair in red square")
- Unpainted

TurChub® cell chambers (MedPharm Ltd) were calibrated and the compartment partially filled with potato dextrose agar (PDA). Chambers were seeded with 50 μL *T. rubrum* suspension. Prepared nails were mounted to the chambers and a single application of 2 μL of tavaborole, 5%, was applied to the nail surface and allowed to dry for 10 minutes. TurChub® chambers were then covered and incubated

The experimental groups and controls are summarized in **Table 1**. The groups of interest were unpolished nails, full salon, and partially polished nails with tavaborole applied on top. Additionally, tavaborole was applied to nails prior to a full salon treatment. Infected and non-infected controls with polished and unpolished nails were included. The variable of interest was the growth of *T. rubrum* in the TurChub® chambers. The zone of inhibition was recorded for each chamber, measured centrally in a straight line from the underside of the nail (i.e., where the nail and agar are in contact) to the point of first signs of microbial growth.

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 19.0 (IBM, New York, USA). To determine if significant differences in the zone of inhibition were present between experimental groups, a one-way analysis of variance (ANOVA) with Tukey's post-hoc test was used. Significance was set to $\alpha = 0.05$.

Results

The maximum zone of inhibition (ZOI) that is possible in the chambers is > 3.2 cm and represents complete inhibition of *T. rubrum* growth. The zones of inhibition for the experimental and control groups are depicted in **Figure 1** and representative TurChub® chambers in **Figure 2**. Of the experimental groups, tavaborole applied to unpolished nails produced the largest ZOI at 3.17 ± 0.12 cm, followed by tavaborole applied prior to full salon polish, 2.90 ± 0.1 cm. Tavaborole applied on top of partial polish and on top of full salon polish produced zones of inhibition of 2.67 ± 0.08 cm and 2.15 ± 0.24 cm, respectively.

There were significant differences in the zones of inhibition among the experimental groups. The ZOI from tavaborole applied to unpolished nails ($p < 0.001$) and tavaborole applied prior to full salon polish ($p < 0.05$) were significantly greater than the ZOI seen with tavaborole applied on top of full salon polish. Tavaborole applied on top of partial polish did not produce a ZOI significantly different from the other 3 experimental groups: unpolished, prior to full salon polish, or full salon polish ($p_s > 0.05$). ZOI for all 4

experimental groups were significantly greater than infected controls (polished and unpolished), $p_s < 0.001$. The ZOI of the tavaborole applied to unpolished nails and the tavaborole applied prior to full salon polish groups were not significantly different from non-infected controls ($p_s > 0.05$).

Discussion

The present study investigated the ability of tavaborole, 5%, solution to penetrate *ex vivo* human fingernails and inhibit dermatophyte growth when applied in the presence of nail polish. A single, clinically relevant dose of tavaborole was able to penetrate both a salon polish (4 coats) and a partial polish (2 coats) in order to inhibit growth of *T. rubrum*, inhibition that was significantly greater than that of untreated controls. This is the first study to demonstrate the fungicidal ability of a topical antifungal in the presence of nail polish.

Previous *ex vivo* work has used various methods to demonstrate that topical antifungals penetrate nails in the presence of nail polish. Penetration of tavaborole was quantified by measuring tavaborole in the receiving medium found in the chamber below the cadaveric nail.[7] Radioactively-labeled efinaconazole was measured in solubilized nails.[9] In short, tavaborole penetrated through cadaveric nails whereas efinaconazole penetrated into the nail plate. This suggests that both can penetrate and fight infection within the nail plate but that additionally, tavaborole may reach the site of deep seated infection in the nail bed.

Vlahovic *et al.* reported that tavaborole penetrated nails with 1-4 coats of polish in greater amounts than the penetration observed for unpolished controls over 14 days.[7] This differs from the current study where a larger ZOI, and presumably greater penetration, was observed with unpolished nails. Only one dose of tavaborole was used in the present work, while penetration was previously measured following 14 days of consecutive application of tavaborole; therefore, it is difficult to make parallels between these two studies. The Vlahovic et al. study suggests that repeated use of tavaborole over nail polish will result in continued

penetration. Additional research is needed to understand why additional layers of nail polish may contribute to increased penetration of tavaborole. Theoretically, nail polish may serve as a form of occlusion, increasing nail plate hydration and improving drug penetration.[10] Alternatively, the nail polish may serve as a reservoir that allows tavaborole to accumulate and remain in contact with the nail for an extended period of time.

In terms of translating this data into clinical practice, the data suggests that patients who want to wear nail polish while using tavaborole 5% to treat their onychomycosis have good evidence to support this practice. Tavaborole appears to successfully penetrate the nail, reaching the site of the infection, and maintain its antifungal activity. Though this study compared typical combinations of nail polish, extrapolating the efficacy of a single dose as compared to consecutive daily doses is difficult. In addition, new topical treatments (tavaborole 5% and efinaconazole 10%) are applied transungually (on the dorsal nail surface) and subungually at the hyponychium. Accessing the site of infection by multiple routes likely contributes to the increased efficacy seen with newer topical antifungals.[11] Likewise, subungual application may bypass the need to interfere with nail polish. Further, patients need not always wear nail polish, but could be more receptive to topical treatment if the option is available to wear nail polish in social situations. Importantly, it has been shown that tavaborole applied over nail polish does not alter the appearance of the polish[8] and this should also encourage use.

The current studies and previous investigations of penetration of tavaborole with and without nail polish have been performed on healthy nails. Recent research suggests that penetration of caffeine, a small hydrophilic molecule (194 Da), through onychomycotic nails is increased as compared to healthy nails. Interestingly, the presence of onychomycosis did not influence penetration of terbinafine (328 Da) or amorolfine (354 Da), both significantly larger molecules with differing physicochemical properties.[10] Considering that tavaborole is also a small molecule (152 Da) and slightly water soluble, there may be an

increase in penetration of tavaborole in onychomycotic nails as compared to healthy nails. This should be kept in mind when interpreting this and other studies with healthy nails as it is a potential limitation.

Males are disproportionately affected with onychomycosis, yet females are generally more likely to seek out treatment for onychomycosis. Particularly during warmer months, and regardless of sex, footwear that exposes the feet is common and expected. To avoid seeming out of place, nail polish provides a way to cover-up any unsightly nails to allow for wearing such footwear. The current study is the first to demonstrate the efficacy of a topical antifungal for onychomycosis in inhibiting fungal growth when applied over nail polish. Tavaborole remained efficacious in the presence of up to 4 layers of nail polish. How this *ex vivo* work with healthy nails will translate to clinical efficacy in diseased nails remains to be seen. However, this work presents an opportunity for topical antifungals to reach a population concerned with nail appearance but traditionally resistant to adhering to the long treatment durations of currently available topical treatments.

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Table 1. Summary of experimental and control groups

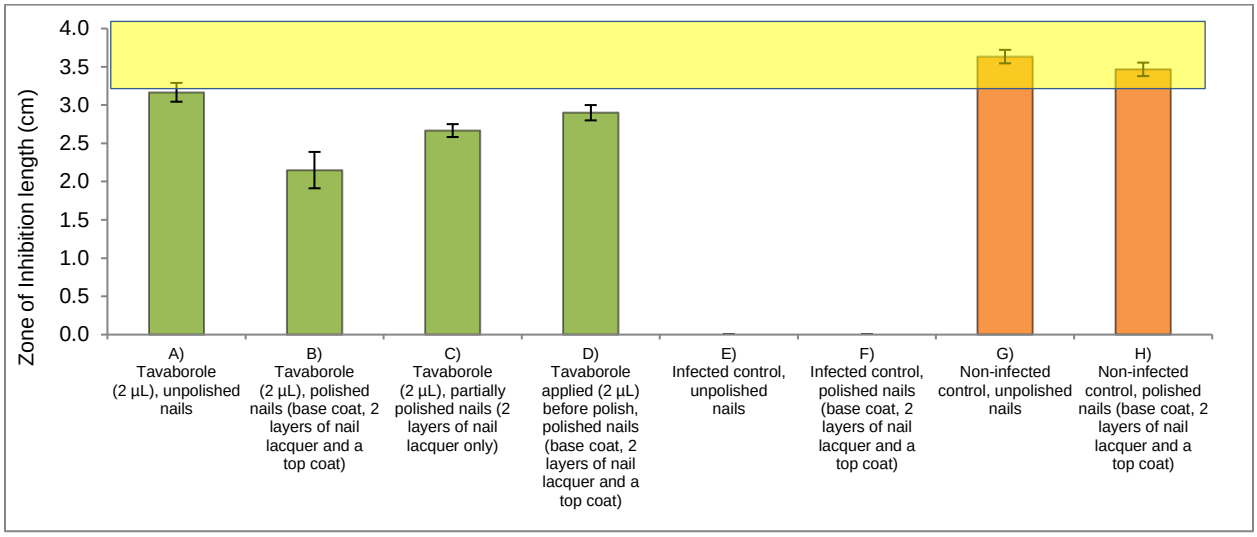
	Experimental Group	Tavaborole dosing	Replicates (n)
A	T. - Unpolished Nails	2 μL applied to nail surface	6
B	T. - Salon Polished Nails	2 μL applied to nail surface after polishing	6
C	T. - Partial Polished Nails		6
D	T. - Salon Polished Nails	2 μL applied to nail surface prior to polishing	3
E	N/A - Unpolished Nails*	N/A	6
F	N/A - Polished Nails*		6
G	N/A - Unpolished Nails**		3
H	N/A - Polished Nails**		3

T. = Tavaborole; N/A = not applicable

* Infected controls were seeded with *T. rubrum*, but not treated with nail polish or tavaborole

** Non-infected controls were not seeded with *T. rubrum*, and not treated with polish or tavaborole

Figure 1. Zone of inhibition assay mean distance (± SEM) of *T. rubrum* on PDA in chambers mounted with full thickness human nails. Polished and unpolished nail surfaces were treated with tavaborole, 5%, (2 µL) and incubated at 20-25°C for 7 days (n=6 per group, with the exception of groups D, G, and H where n=3 per group). The yellow bar represents the maximum range of length of agar (3.2-4.0 cm) within the TurChub® chambers. See text for statistical analysis.



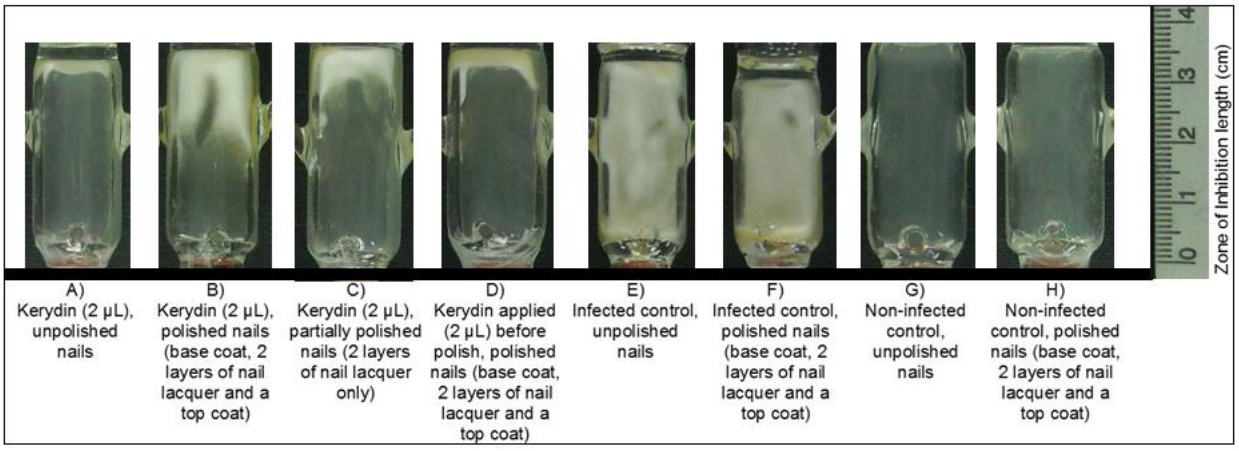


Figure 2. Representative TurChub® chambers showing the zone of inhibition (ZOI) following tavaborole, 5%, applied to nail surfaces and incubated at 20-25°C for 7 days.