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Antifungal ME1111 In Vitro Human Onychopharmacokinetics

Xiaoying Hui*, Eui Chang Jung, Hanjiang Zhu, Howard I. Maibach
Department of Dermatology, University of California San Francisco
Surge Building, Room 110, 90 Medical Center Way
San Francisco, CA 94143

*Corresponding
Xiaoying Hui, MD, MS
Research Dermatologist
Department of Dermatology, University of California San Francisco
Surge Building, Room 110, 90 Medical Center Way
San Francisco, CA 94143
Tel: (415) 502-7761
Fax: (415) 753-5304
E-mail: Xiaoying.Hui@ucsf.edu
ABSTRACT

This study determined ME1111 onychopharmacokinetics and possible topical antifungals’ clinical efficacy in human great toenails using an in vitro finite dose model.

ME1111 topical formulations in 1, 5, 10, or 15% for 3 days observation and 1, 5, or 10% for 14 days observation, respectively were used to determine ME1111 penetration rate and transungual kinetics.

ME1111 concentrations in the deeper nail (ventral/intermediate layers) and a cotton pad/nail bed, were several orders of magnitude greater than MIC\textsubscript{90} and MFC\textsubscript{90} for three major dermatophytes. ME1111 concentrations 3 days after a single and 14 days after multiple dosing of 10% formulation were 253 and 7991 µg/g nail, respectively, and superior to those of 8% ciclopirox control.

ME1111 concentration (µg equivalent/cm\textsuperscript{3}) in the cotton pad following 10% ME1111 multiple applications increased linearly throughout the 336-hour experiment and was significantly greater than that of 8%ciclopirox. Flux rate of ME1111 averaged 50.9 µg/cm\textsuperscript{3}/day, which is ca. two orders of magnitude greater than the MIC\textsubscript{90} values.

The novel antifungal ME1111 penetrated well into human nail plate and its concentrations in the deeper nail and cotton pad after application of 10% formulation were significantly greater than those of ciclopirox.
INTRODUCTION

Onychomycosis, a common fungal infection of the nail plate and bed, affects approximately 13 - 14% of the U.S. population (1, 2) and represents nearly 40% of nail abnormalities (3). The toenail has higher infection rates than that of the finger at 10 to 6 ratio (4). Onychomycosis can cause pain, discomfort, and disfigurement and also produce serious physical and occupation limitations (5).

Oral antifungal therapy has been a mainstay in onychomycosis treatment because of the deep-seated, persistent nature of this infection and as topically applied drugs have not been shown to highly effectively penetrate the nail (5). However, to achieve disease clearance, oral therapy must be taken for several months presumably due to slow nail growth rate.

To achieve an effective chemical concentration into/through the human nail plate, upon which the success of the local therapy of onychomycosis depends, a suitable antifungal drug coupled with an appropriate method of delivery must be chosen. This method should maximize the effect of the active principle by aiding its diffusion into the deep nail layer and/or the nail bed at levels exceeding the minimum inhibitory concentration (MIC) against dermatophytes. Thus, a drug with high antifungal efficacy, good permeability (such as small molecular weight, hydrophilicity, and low keratin affinity) and a suitable carrier may be needed to achieve an effective chemical concentration into/through the human nail plate following topical application (6).

ME1111 (2-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-methylphenol), a new chemical class of antidermatophytic agent (7) inhibits mitochondrial respiratory electron transfer due to its action on succinate dehydrogenase and has relative low molecular weight and low keratin affinity (8) when compared to other antifungal agents such as terbinafine, ciclopirox, etc.

This study examines permeability of a novel topical ME1111 formulation containing trace amount of \(^{14}\text{C}\)-ME1111 penetrating into and through the human great toenail plate using the \textit{in vitro} finite dose
model. The great toenail represents the thickest nail plate with an average thickness of 1.38 in women and 1.65 mm in man (9) and is more commonly to be affected (10). The results should help evaluate ME1111 topical formulation permeability to deep layer of toenail plate and nail bed and estimate antifungal efficacy for topically applied ME1111 treatment, prior to clinical trials.

MATERIALS AND METHODS

Test Article

ME1111 and [14C] - ME1111 were provided by Meiji Seika Pharma Co., Ltd. (Tokyo, Japan). Specific activity of [14C] - ME1111 was 0.29 mCi/mg and radiochemical purity, 98.0% measured by HPLC. Non-radiolabeled ME1111 was prepared in alcohol-based solution to form 1, 5, 10, or 10% (w/v) topical formulations.

[14C]-Ciclopirox (pyridinone-6- [14C]-ciclopirox) was synthesized by PerkinElmer Life and Analytical Sciences (Boston, MA). Radiochemical purity and specific activity of the chemical was >95% by HPLC and 12.5 mCi/mmol, respectively. Cyclopirox topical solution, 8% (w/v) nail lacquer was manufactured by Perrigo (Bronx, NY).

Final dosing solution was prepared by mixing trace amount of [14C]-ME1111 or [14C]-ciclopirox with corresponding non-radioactive topical formulations. Before topical application, homogeneity of each to

Single Topical Application Study

ME1111 topical formulation concentrated at 1, 5, 10, and 15% (w/v) were tested and compared to ciclopirox, 8% (w/v) in commercial nail lacquer to determine their nail penetration rate. Aliquots (10 µl) of the dose formulations were singly applied to human toenails. The cotton pad/nail supporting bed was collected from the receiving chamber and replaced with a new one at 24 hours after dosing. Seventy-
two (72) hours after dosing, the dorsal/intermediate, ventral/intermediate and remaining nail samples, as well as the cotton pad/nail supporting bed were collected for analysis.

Multiple Topical Application Study

ME1111 formulations concentrated at 1, 5, and 10% were tested and compared to ciclopirox, 8% in commercial nail lacquer to examine nail penetration rate and transungual delivery. Aliquots (10 µl) of the formulations were applied to human toenails once daily for 14 days. Cotton pad /nail supporting bed was collected from each cell chamber and replaced with a new one at 48, 120, 192, and 264 hours post the first dose. Dorsal/intermediate, ventral/intermediate and remaining nail samples as well as cotton pad were collected after the 14-day dose period (336 hours) for analysis.

Experimental Procedure

Healthy human great toenail plate was collected from adult human cadavers from the Anatomy Laboratory, at the University of California San Francisco. Prior to experiments, nail plates were gently washed with normal saline to remove any contamination, and then re-hydrated by placing them for 3 hours on a cloth wetted with normal saline.

An inline one-chamber diffusion cell (PermeGear, Inc., Hellertown, PA) was used to hold each nail. The toe nail plate, cut to 2.1 x 2.1 cm (L x W) size to fit the donor chamber of the inline cell, was placed on a ledge inside the chamber with the dorsal surface facing the open air. The ventral (inner) surface of the nail was placed face down and rested on the wet cotton pad. To approximate physiological conditions, cotton pad was wetted with approximately 0.3 ml 6% (v/v) polyethylene glycol (PEG) in phosphate buffer solution (0.01 M, pH 7.4) and placed in the receiving chamber to serve as a nail bed and provide moisture for the nail plate. At the defined times, the cotton pad was removed and a new one replaced in the chamber. Diffusion cells were placed inside of a water bath (Forma Scientific 2564
CH/P Digital Heated Shaker Water, Marietta, OH) with temperature at 37°C and relative humidity approximately at 55%.

Surface washing was started in the morning 10 minutes prior to next dosing; the nail was washed with cotton tips in a cycle, as follows: a tip wetted with absolute ethanol, then repeated with absolute ethanol, then 5% IVORY® liquid soap (Proctor&Gamble, Cincinnati, OH), then distilled water, and then repeated with distilled water. Washing samples from each cycle of each nail were pooled and collected by breaking off the cotton tip into scintillation glass vials. Aliquots of 3.0 ml organic solvent mixture were added into each vial to extract test material.

At the end, dosed surface area was washed as the same above procedure and then the area was tape stripped twice with D100 - D-Squame standard sampling discs (CuDerm Corp., Dallas, TX) to remove surface residue.

The nail plate was then transferred from the diffusion cell to a clean copper nail holder for the sampling process. The nail sampling instrument has two parts, a nail sample stage and a drill. The nail sampling stage consisted of a copper nail holder, three adjustors, and a nail powder capture. Three adjustors allow movement in the vertical direction. The first coarse adjustor (on the top) is for changing the copper cell and taking powder samples from the capture, the other adjustors (lower) are for the sampling process. The second coarse adjustor allows movement of 25 mm and the fine adjustor 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 similar to an inverted funnel and the end of funnel connects to vacuum. By placing a circle of filter paper inside the funnel, nail powder samples can be captured on the filter paper during sampling.

Nail plate was inverted so that the ventral (nail bed) surface faced up and the dorsal (outer) dosed surface faced down. The copper nail holder has an opening as it sits on top of the stage. When the
sampling process was initiated, the coarse adjustment was adjusted to move the position of the stage until the nail plate was just touching the tip of the cutter. Then the drill was turned on and the fine adjustment was turned to push the stage closer to the drill, removing a nail core sample. After the above process, approximate half thickness in depth and 7.9 mm in diameter nail pulverized samples were harvested from the center of the ventral (nail bed) surface of the nail.

The powdered nail samples were collected into a glass scintillation vial and weighed. Aliquots of 3.0 ml Solvable™ (PerkinElmer Life and Analytical Sciences, Boston, MA) were added to the scintillation vial to dissolve the powder. The upper part, the dorsal/intermediate 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 and then placed into a glass scintillation vial with 3.0 ml Solvable™. Remaining nail was also placed in a glass scintillation vial with 5.0 ml Solvable™(6).

Mass of removed nail sample was measured by the difference in weight of the nail plate before and after drilling, and collected for subsequent analysis.

Radioactivity Measurement

All radioactivity measurements were conducted with a Tri-Carb 2900TR Liquid Scintillation Counter (PerkinElmer Life and Analytical Sciences, Downer Grove, IL). Nail samples pre-treated with Solvable™ were incubated at 40°C for 48 hours followed by the addition of 10 ml Ultima Gold™ scintillation cocktail (PerkinElmer Life and Analytical Sciences, Boston, MA). Other samples (standard dose, surface washing, tape strips, and cotton pad material) were mixed directly with Ultima Gold™ scintillation cocktail. Background control and test samples were counted for 3 minutes each for radioactivity.

Data calculation and analysis

Individual and mean (± S.D.) amount of test chemical equivalent in nail, cotton pad material, wash samples and tape strips are presented as µCi and percent administered dose at each time point.
Radioactivity of the applied dose, nail and cotton pad samples were determined as µg ME1111 (or ciclopirox) mass equivalent.

Dose calculations: For single dose application, immediate before and after dosing, a standard dose (10 µl) was weighed and radioactivity was measured for each formulation. For multiple dose applications, 5 minutes before first dosing, and 48, 120, 192, 264, 336 hours after the first dosing, a duplicated standard dose (10 µl) was weighed and the radioactivity was measured. Weight normalized standard dose was expressed as the radioactivity versus net dose weight (µCi/g standard dose).

Based upon the ratio of radioactivity and dose weight of the standard dose, radioactivity (µCi) of each individual sample was then calculated from its dose weight and normalized standard dose using the calculating formula below.

Radioactivity per gram weight (µCi/g) of the standard dose X sample dose weight (g) = radioactivity of sample dose (µCi)

ME1111 mass equivalent calculations: The equivalent per dose was calculated from the standard doses. Each dose contains non-radiolabeled ME1111 (cold) and [14C]-ME1111 (hot). The amount of cold ME1111 was calculated from the concentration of the formulation (1, 5, 10, and 15%). Information of [14C]-ME1111 was provided by the manufacturer that each ml of [14C]-ME1111 solution contains 3.41 mg of ME1111 mass equivalent or 990 µCi radioactivity. Therefore 0.0034 mg/µCi is the mass equivalent ratio of its radioactivity.

This number was then used to convert sample radioactivity and calculate the amount ME1111 mass equivalent in each sample using the calculating formula below.

µg eq/µCi of the standard dose X µCi of sample radioactivity measured = µg eq of sample

Ciclopirox mass equivalent calculations: The procedure is the same as ME1111 mass equivalent calculations. Each dose contains non-radiolabeled ciclopirox (cold) and [14C]-ciclopirx (hot). The a
Amount of cold ciclopirox was calculated from the concentration of the formulation (8%).

Information of $^{14}$C-ciclopirox mass equivalent was provided by the manufacturer that each gram of $^{14}$C-ciclopirox contains 12.5 µCi radioactivity. The final topical dosing solution contains 0.008 mg/µCi of $^{14}$C-ciclopirox per single dose or 0.78 mg/µCi of total multiple doses.

This number was then used to convert sample radioactivity and calculate the amount of ciclopirox mass equivalent in each sample.

Antifungal efficacy calculation: Determined by the ratio of the drug concentration –weight (or volume) normalized mass equivalent in the test sample (µg equivalent per ml) to MIC90 or MFC90. (MIC90 is defined as the lowest concentration of the drug at which 90% of the strains were inhibited; MFC90 is defined as the minimum fungicidal concentration of drug at which 90% of the strains tested).

Statistical Analysis

Statistical analysis was performed using Sigma Stat version 11 (Systat Software Inc., San Jose, CA). Normal distributions were tested before calculating comparisons. When 3 or more experimental groups were compared, ANOVA was performed followed by Kruskal-Wallis test. Flux of cotton pad samples versus time was determined with linear regression. Level of significance for all the cases was set at p<0.05.

Terminology

**Ventral / intermediate center:** Powdered nail sample drilled from the center of the inner surface (facing the nail bed) approximately 0.3 - 0.5 mm in depth to the surface. Area is beneath the dosed site of the nail place but does not include dosed surface (dorsal nail surface).

**Dorsal / intermediate center:** Immediate area of dosed site.

**Surrounding nail:** Remaining part of the nail that has not been dosed.
**Cotton pad – nail supporting bed**: Cotton pad placed within the receiving chamber of the diffusion cell to provide moisture to the nail plate and also to receive chemicals penetrating through the nail plate.

**Surfacing washing**: Ethanol and soap/water washing on the surface of the dosed site.

**Upper cell washing**: Manually removed formulation residue, washing and soaking and soap/water wash from the top part of the diffusion cell (the part above the nail plate and inside of the donor chamber).

**Tape stripping**: Using D100 - D-Squame standard sampling discs two times to remove the dose residue from the superficial surface of the nail plate.

**Inner cell washing**: Manually removed formulation residue, washing and soaking and soap/water wash from the inside of the receiving chamber part of the diffusion cell (the part that has been in contact with the inner surface of the nail plate and the cotton pad/nail supporting bed).
RESULTS

An average of 0.99 mm in thickness (± 0.15 mm S.D., ranged from 0.60 to 1.34 mm, n = 45) of human great toenail plates were selected for study. No statistical differences (P > 0.05) were observed between each ME1111 group and the 8% ciclopirox in nail thickness.

Approximately 0.01 gm of ME1111 by weight and 1 µCi of [14C]-ME1111 radioactivity in average of topical formulations was dosed for all single dose groups. For multiple dose groups, the average dose weight was 0.01 gm per application and 0.12 gm in total, and radioactivity, 0.2 and 3 µCi, respectively. Mass balance - radioactive recovery from all collected samples in these groups reached approximately 100 percent of applied dose.

In the single dose study, nail permeation and disposition of four ME1111 formulations (1, 5, 10, and 15%) and ciclopirox nail lacquer was compared. Seventy-two hours post a single dose application, the weight normalized ME1111 mass equivalent from the 5%, 10%, and 15% formulations penetrated to the ventral/intermediate layer (µg/gm) and cotton pad/nail supporting bed (µg/cm³) to a greater extent than those in the control with statistical significance (P < 0.05) (Table 2). From the 24 – 72 hour period, amounts of ME1111 (as well as ciclopirox) in the cotton pad increased 2 to 7 fold than those of the 0 – 24 hour period from each ME1111 groups and 8% ciclopirox (Table 2). The 1% ME1111 group has similar amount mass equivalent in the cotton pad sample to that of 8% ciclopirox.

For the multiple dose application study, penetration rates and kinetics of ME1111 and ciclopirox were determined and compared. After a 14-day multiple topical application, average of weight normalized ME1111 mass equivalent accumulated in the ventral/intermediate layer of the dosed nail plate center was 831, 5281, and 7991 µg/gm sample for 1%, 5%, and 10% ME1111 dose groups, respectively (Table 3). Values increased approximately 100, 30, and 30 times when compared with values from corresponding single dose groups (Table 2), respectively. In comparison, weight normalized
ciclopirox mass equivalent in the ventral/intermediate layer increased 24 times in average from 32 (µg/gm) of the single dose application to 772 (µg/gm) of the multiple dose application.

Penetration flux of ME1111 was determined with the cotton pad samples collected at 48, 120, 192, 264, and 336 hours after the first dose application. Results showed that 1% ME1111 and 8% ciclopirox groups slowly increased penetration rates and reached a similar level at each time point. 5% ME1111 group quickly increased its concentrations up to 192 hours and then kept a relatively flat level until the end. 10% ME1111 however increased linearly from 0 to 336 hours (Figure 2).

The potential antifungal efficacy of ME1111 was determined by the ratio of the ME1111 concentration in the test sample (µg equivalent per ml or cm³) to MIC₉₀ and MFC₉₀. MIC₉₀ and MFC₉₀ values against three dermatophytes, *Trichophyton rubrum* (*T. rubrum*), *Trichophyton mentagrophytes* (*T. mentagrophytes*), and *Epidermophyton floccosum* (*E. floccosum*) are from Ghannoum et al, (2015).

Antifungal efficacy of ME1111 of the ventral/intermediate nail center was analyzed and compared to different concentration groups following multiple dose applications (Table 4). Results show that antifungal efficacies to *T. rubrum*, *T. mentagrophytes*, and *E. floccosum* from 5% and 10% ME1111 groups are statistically different from those of 8% ciclopirox (P < 0.05).

Antifungal efficacy of [¹⁴C]-ME1111 of the cotton pad/nail supporting bed samples was analyzed and compared to different concentration groups following multiple dose applications (Table 4). ME1111 groups, especially in the 10% ME1111 group were several orders greater than the MIC₉₀ for tested dermatophytes species; whereas the 8% ciclopirox group, *in vitro* antifungal efficacy was approximately the same, or sometimes lower, than that of the 1% ME1111 group.
DISCUSSION

The efficacy of topical treatment of onychomycosis differs from that of skin fungal infection in selecting antifungal agents and designing formulations. The human nail possesses high sulfur content (cysteine) in its hard domain, whereas the stratum corneum does not, and its thickness is approximately 100 times greater than the stratum corneum (11). The upper (dorsal) layer, even though only a few cell layers thick, constitutes the main barrier to drug diffusion into and through the nail plate. Therefore, a topically applied chemical must penetrate into the dense, hydrophilic keratin fabric network containing fewer pores, and pass through a longer distance compared to the skin to reach the deeper layers of the nail and the bed. As a result, concentration of an applied drug across the nail can drop about 1000-fold from the outer surface to the inner surface (12). Certain diseases such as onychomycosis of the nail can produce hyperkeratosis or alterations of the shape of the nail plate. These conditions may be painful, and may decrease the penetration of topical medicaments. Hence, it is important to consider an antifungal drug with low molecular weight, hydrophilicity or low lipophilicity, and good permeability through the plate.

Keratin affinity is an important physicochemical property affecting the efficiency of antifungal agents’ permeability into/through the nail plate following topical or systemic administrations. Beneficial or deleterious effects of drug-keratin affinity depend upon the location in the plate and release rate of the bound drug (13). If a high keratin affinity drug is administered systemically, it diffuses from the capillary in the nail bed to the lower part of the plate – ventral layer and forms high concentration reservoir which favors local treatment. But if applied topically, the drug may accumulate in the nail surface- dorsal layer and has insufficient release and diffusion to the deep part – ventral layer and the bed. In addition, the drug needs to be an unbound form to exert its therapeutic effects. In such a case, high drug concentration in the plate and high calculated antifungal efficacy (nail concentration to MIC<sub>90</sub>) may not yield efficacy. Sugiura et al (14) investigated nail penetration and antifungal activity of
efinaconazole *in vitro* and compared to other commercial topical antifungal drugs – ciclopirox and amorolfine, and suggested that the high nail permeability of efinaconazole and its potent antifungal activity in the presence of keratin are related to its low keratin affinity, which may contribute to its efficacy. Gupta and Paquet (15) also confirmed the effect of low keratin affinity on efinaconazole on nail penetration.

ME1111 has relatively low molecular weight, lower keratin affinity (56%) than ciclopirox (16) and strong antifungal activity (Table 4) which aids permeability through the plate and to reach significant concentration in the treated (deep nail) tissues after topical application.

Human nail plate is generally considered to be composed of three layers: dorsal (surface), intermediate, and ventral (inner) layers (17, 18). In most cases, the ventral nail plate and the subungual keratin (nail bed) are more likely to be infected by fungi such as *Trichophyton rubrum* and *Trichophyton mentagrophytes* (18). Therefore, it is important to determine nail penetration rate and kinetics of topical applied antifungal agents in these tissues. This study focused on determination of ME1111 concentrations (as µg equivalent per gm or cm³) of the ventral/intermediate layers and cotton pad/nail supporting bed samples. Following a 14-day multiple doses application, ME1111 concentrations in the ventral/intermediate nail center layers increase proportionally as the formulation concentration increasing (Table 3). Concentration of ME1111 (µg equivalent/ cm³) in the cotton pad/nail supporting bed samples following multiple application of 10% ME1111 topical solution increased linearly throughout the 336 hours of experiment, while other ME1111 groups had relatively slower increasing levels (Figure 3).

The data provide useful information that sufficient amount of ME1111, as “free” unbound molecules diffused to the deep nail layer and penetrated through into the nail bed, especially when the
concentration of ME1111 increase to be 10%. These values are further used to estimate real ME1111 antifungal efficacies and select concentrations of the topical formation.

This study showed the amount of ME1111 after 14 days exposure of 10% topical solution achieved to $7991 \pm 4476 \, \mu g/gm$ in the ventral/intermediate layer samples (Table 3), 10,000 times greater than the MIC$_{90}$ for three dermatophytes species or three orders of magnitude greater than the MFC$_{90}$ for *T. rubrum* and *T. mentagrophytes* (Table 4). Flux of $[^{14}\text{C}]$-ME1111 penetrating from 10% topical solution into the cotton pad/nail supporting bed averaged $50.9 \pm 8.2 \, \mu g/ cm^3$/day throughout study (Figure 3). This daily flux is ca. two orders of magnitude greater than the MIC$_{90}$ values of three dermatophytes, or approximately twice as high as the MFC$_{90}$ value for *E. floccosum* or five times higher than that for *T. rubrum* and *T. mentagrophytes*. Results suggest that the amount of ME1111 in the ventral/intermediate layer and supporting bed dramatically exceed the inhibitory concentration of ME1111 for most common onychomycosis organisms.

Extent of antifungal drug penetration into/through the human nail plate depends on the design of the topical formulation: how to alter the chemical's solubility, how to select an appropriate vehicle and the incorporation of penetration enhancers (19). Recent research of molecular diffusion in the human nail measured by stimulated Raman scattering microscopy demonstrated that optimization of delivery platforms to the nail must prolong and sustain exposure of the barrier to excipients. Common solvents like water, propylene glycol and dimethyl sulfoxide can facilitate drug and their own transport (20). The nature of the nail plate is a hydrogel, which swells when hydrated (21). Walters and Flynn (22) reported that the permeability coefficients of alcohols diluted in saline through nail plates were five times greater than that of neat alcohols. Monti et al (23) demonstrated that using 8% ciclopirox hydrosoluble nail lacquer showed higher human nail penetration compared to the use of a water–insoluble 5% amorolfine lacquer. Onchomycotic nail results a thicker but more porous barrier, and its eroded intracellular matrix renders the tissue more permeable to topically applied chemicals when an aqueous vehicle was used.
ME1111 nail formulation prepared on alcohol/water-based vehicle possibly can soften the nail plate, increase nail hydration, and enhance penetration.

Tables 2 and 3 showed weight normalized ME111 mass equivalent in non-dosed surrounding nail area, dosed dorsal (or ventral)/intermediate layer center areas that suggest penetration of tested antifungal agents diffused not only vertically but laterally as well. This is important for developing clinical treatment methodology. Similar observations were reported previously in terbinafine HCl (19), AN2690, and ciclopirox (6) in vitro nail penetration studies. From our preliminary studies (unpublished data) we observed that some antifungal agents retained in the nail plate can be slowly released to the deep layer – cotton pad/nail supporting bed and kept antifungal efficacy for 3 – 5 days post 14 days multiple dose applications in vitro. This phenomenon did not occur in experiments with a single or 3 days multiple dose applications. We cannot conclude that all these are related to the testing antifungal agents’ keratin binding affinity or other physicochemical properties but will be interested to explore in future studies.

CONCLUSION

In conclusion, ME1111 possesses excellent penetration activity into/through human nail. Cumulative concentrations of ME1111 after 14 days in the deep nail layer and the nail bed were several orders of magnitude greater than the MIC₉₀ (and/or MFC₉₀) believed necessary to inhibit the growth of infecting fungi (dermatophyte species), respectively. Comparison of a commercial product of 8% ciclopirox nail lacquer, ME1111 penetrated well into human nail plate and its concentrations in the deeper nail and cotton pad after application of 10% formulation were significantly greater than those of the ciclopirox lacquer. Thus ME1111 topical formulation has the potential to be an effective topical treatment for onychomycosis. In vivo–in vitro correlations will further define the clinical relevance of this in vitro method. Although a bioequivalent comparison between the oral in vivo nail clipping data to our
onychopharmacokinetic (topical) data requires direct comparative studies, the current in vitro data
appears sufficient to justify controlled clinical trials.

Acknowledgement: This study is supported by Meiji Seika Pharma Co., Ltd. (Tokyo, Japan).

DECLARATIONS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing
of this paper

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171:937-58

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10 U.S. NIM Mediline Plus: Fungal nail infection – fungal nail infection is a fungus growing in and around your fingernail or toenail (updated 11/14/2014).


Figure 1. Cross-section of one-cell diffusion chamber showing sections of nail sampled.
Figure 2. Time course of ME1111 concentrations in cotton pad/nail supporting bed samples following multiple dose applications and comparison with the corresponding values from ciclopirox. Each bar represents the mean (S.D.) of 5 samples. ME = ME1111 and CPX = ciclopirox.
Figure 3. Flux rate (observed individual and estimated mean value) of cotton pad/nail supporting bed samples (µg eq/cm³/hr) following multiple dose applications of 10% ME1111 topical solution. 8% Ciclopirox topical solution applied with the same methodology as the control. Each line represents the mean of 5 samples.

Table 1 Physicochemical properties of ME1111

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>2-(3,5-Dimethyl-1H-pyrazol-1-yl)-5-methylphenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical structure</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C₁₂H₁₄N₂O</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>202.25</td>
</tr>
<tr>
<td>Log P</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Table 2 Weight normalized ME1111 mass equivalent in different nail plate layers and cotton pad/nail supporting bed following a single dose application

<table>
<thead>
<tr>
<th>Samples</th>
<th>Weight normalized drug mass equivalent following a single dose application (mean ± S.D., n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME1111 1%  5%  10%  15%  Ciclopirox 8%</td>
</tr>
<tr>
<td>Dosed dorsal/intermediate layer</td>
<td>699 ± 185*  2526 ± 542*  6348 ± 1181  5730 ± 2077  15278 ± 10902</td>
</tr>
<tr>
<td>center</td>
<td></td>
</tr>
<tr>
<td>Dosed ventral/intermediate layer</td>
<td>8 ± 2  183 ± 92*  253 ± 88*  241 ± 121*  32 ± 13</td>
</tr>
<tr>
<td>center</td>
<td></td>
</tr>
<tr>
<td>Non-dosed surrounding area</td>
<td>123 ± 55  723 ± 231  1353 ± 432*  1507 ± 789*  409 ± 158</td>
</tr>
<tr>
<td>Cotton pad/nail supporting bed</td>
<td></td>
</tr>
<tr>
<td>In 24-hr</td>
<td>0.7 ± 0.2  6 ± 6*  5 ± 1*  8 ± 1*  0.5 ± 0.3</td>
</tr>
<tr>
<td>In 72-hr</td>
<td>2 ± 1  14 ± 6*  27 ± 5*  56 ± 20*  1.0 ± 0.4</td>
</tr>
<tr>
<td>Total **</td>
<td>3 ± 1  26 ± 6*  38 ± 2*  72 ± 25*  3 ± 3</td>
</tr>
</tbody>
</table>

*After a single topical application for 72 hours, ME1111 released from 5, 10 and 15% formulations diffused more amount of drug (µg/gm with weight normalization) in the deep nail part (ventral/intermediate layer) and the bed, which are statistical significant (P < 0.05) than the corresponding 8% cicloprox (control).

**Combined with 24-hr, 72-hr cotton pad samples and inner cell washing after the experiment was finished.
Table 3. Weight normalized ME1111 mass equivalent in different nail plate layers and cotton pad/nail supporting bed following multiple dose applications

<table>
<thead>
<tr>
<th>Samples (µg eq./ml or cm³)</th>
<th>Weight normalized ME1111 mass equivalent following multiple dose applications (mean ± S.D., n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosed dorsal/intermediate layer center</td>
<td>7354 ± 2595*</td>
</tr>
<tr>
<td>Dosed ventral/intermediate layer center</td>
<td>831 ± 607</td>
</tr>
<tr>
<td>Non-dosed surrounding area</td>
<td>357 ± 165*</td>
</tr>
<tr>
<td>Cotton pad/nail supporting bed**</td>
<td>70 ± 13</td>
</tr>
</tbody>
</table>

*After multiple once daily topical application for 14 days, ME1111 released from 5 and 10% formulations and diffused more amount of drug (µg/ with weight normalization) in the deep nail part (ventral/intermediate layer) and the bed, which are statistical significant (P < 0.05) than the corresponding 8% ciclopirox (control).

**Cumulative total cotton pad samples (0 - 336 hours) and inner cell washing after the experiment was finished.
Table 4. Antifungal efficacy of ME1111 in deep nail plate – ventral/intermediate layer center and cotton pad/nail supporting bed following multiple dose applications

<table>
<thead>
<tr>
<th>Organism</th>
<th>T. mentagrophytes</th>
<th>E. floccosum</th>
<th>T. rubrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nail samples</td>
<td>Ventral/intermediate layer center</td>
<td>Cotton pad – nail supporting bed</td>
<td>Ventral/intermediate layer center</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;90&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% ME1111</td>
<td>0.25 µg/ml</td>
<td>0.25 µg/ml</td>
<td>0.25 µg/ml</td>
</tr>
<tr>
<td></td>
<td>3323 ± 2430</td>
<td>282 ± 52</td>
<td>3323 ± 2430</td>
</tr>
<tr>
<td>5% ME1111</td>
<td>0.25 µg/ml</td>
<td>0.25 µg/ml</td>
<td>0.25 µg/ml</td>
</tr>
<tr>
<td></td>
<td>21124 ± 2823</td>
<td>1796 ± 173</td>
<td>21124 ± 2823</td>
</tr>
<tr>
<td>10% ME1111</td>
<td>0.25 µg/ml</td>
<td>0.25 µg/ml</td>
<td>0.25 µg/ml</td>
</tr>
<tr>
<td></td>
<td>31965 ± 17902</td>
<td>3157 ± 362</td>
<td>31965 ± 17902</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;90&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8% Ciclopirox</td>
<td>0.25 µg/ml</td>
<td>0.5 µg/ml</td>
<td>0.5 µg/ml</td>
</tr>
<tr>
<td></td>
<td>3087 ± 1239</td>
<td>217 ± 75</td>
<td>1544 ± 619</td>
</tr>
<tr>
<td><strong>MFC&lt;sub&gt;90&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% ME1111</td>
<td>8 µg/ml</td>
<td>32 µg/ml</td>
<td>8 µg/ml</td>
</tr>
<tr>
<td></td>
<td>104 ± 76</td>
<td>8.8 ± 1.6</td>
<td>26 ± 19</td>
</tr>
<tr>
<td>5% ME1111</td>
<td>8 µg/ml</td>
<td>32 µg/ml</td>
<td>8 µg/ml</td>
</tr>
<tr>
<td></td>
<td>660 ± 88</td>
<td>56 ± 5</td>
<td>165 ± 22</td>
</tr>
<tr>
<td>10% ME1111</td>
<td>8 µg/ml</td>
<td>32 µg/ml</td>
<td>8 µg/ml</td>
</tr>
<tr>
<td></td>
<td>999 ± 559</td>
<td>99 ± 11</td>
<td>250 ± 140</td>
</tr>
<tr>
<td><strong>MFC&lt;sub&gt;90&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8% Ciclopirox</td>
<td>16 µg/ml</td>
<td>16 µg/ml</td>
<td>8 µg/ml</td>
</tr>
<tr>
<td></td>
<td>48 ± 19</td>
<td>3.4 ± 1.2</td>
<td>48 ± 19</td>
</tr>
</tbody>
</table>

* The results demonstrated that amount of ME1111 in the ventral/intermediate layer and supporting bed dramatically exceed the inhibitory concentration of ME1111 for most common onychomycosis organisms, especially for 10% ME1111 formulation. MIC<sub>90</sub> and MFC<sub>90</sub> were determined by Ghannoum et al, (2015). Calculation method of antifungal efficacy is determined in Data Calculation and Analysis.