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Authors

Kubota-Ishida, Natsuki
Takei-Masuda, Naomi
Kaneda, Kaori
et al.

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PHARMACOLOGY

In Vitro Human Onychopharmacokinetic and Pharmacodynamic Analyses of ME1111, a New Topical Agent for Onychomycosis

Natsuki Kubota-Ishida,[a] Naomi Takei-Masuda,[a] Kaori Kaneda,[a] Yu Nagira,[a] Tsubasa Chikada,[a] Masahiro Nomoto,[a] Yuji Tabata,[a] Sho Takahata,[a] Kazunori Maebashi,[a] Xiaoying Hui,[b] Howard I. Maibach[b]

[a]Meiji Seika Pharma Co., Ltd., Tokyo, Japan

[b]University of California, San Francisco, San Francisco, California, United States

ABSTRACT ME1111 is a novel antifungal agent currently under clinical development as a topical onychomycosis treatment. A major challenge in the application of topical onychomycotics is penetration and dissemination of antifungal agent into the infected nail plate and bed. In this study, pharmacokinetic/pharmacodynamic parameters of ME1111 that potentially correlate with clinical efficacy were compared with those of marketed topical onychomycosis antifungal agents: efinaconazole, tavaborole, ciclopirox, and amorolfine. An ME1111 solution and other launched topical formulations were applied to an *in vitro* dose model for 14 days based on their clinical dose and administration. Drug concentrations in the deep layer of the nail and within the cotton pads beneath the nails were measured using liquid chromatography-tandem mass spectrometry. Concentrations of ME1111 in the nail and cotton pads were much higher than those of efinaconazole, ciclopirox, and amorolfine. Free drug concentrations of ME1111 in deep nail layers and cotton pads were orders of magnitude higher than the MIC_{90} value against *Trichophyton rubrum* ($n = 30$). Unlike other drugs, the *in vitro* antifungal activity of ME1111 was not affected by 5% human keratin and under a mild acidic condition (pH 5.0). The *in vitro* antidermatophytic efficacy coefficients (ratio of free drug concentration to MIC_{90}s against *T. rubrum*) of ME1111, as measured in deep nail layers, were significantly higher than those of efinaconazole, tavaborole, ciclopirox, and amorolfine ($P < 0.05$). This suggests that ME1111 has excellent permeation of human nails and, consequently, the potential to be an effective topical onychomycosis treatment.

KEYWORDS ME1111, dermatophyte, nail permeability, onychomycosis, topical agent

Onychomycosis is a progressive fungal infection of nails mainly caused by dermatophytes, in particular, *Trichophyton rubrum* and *Trichophyton mentagrophytes* (1). This disease can cause nail destruction and deformity and affects up to 23% of adults worldwide (1–4). Although oral antimycotics (such as terbinafine and itraconazole) are available, these agents have disadvantages, namely, liver toxicity and drug-drug interactions (5), which raises qualms in prescribing them for elderly patients, especially those taking multiple medications. Recently, new topical agents, efinaconazole and tavaborole, were launched and exist beside the conventional topical drugs, ciclopirox and amorolfine (6–9). In general, topical medications are safer than oral treatments, which make them more attractive to prescribe. Unfortunately, the clinical efficacy of topical therapeutics is significantly lower than that of oral medications. As such, a highly effective topical treatment for onychomycosis would be valuable.

Onychomycosis is classified into subtypes, with each class based on a specific pattern of nail plate invasion (10, 11). The most common type is distal-lateral-subungual onychomycosis (DLSO), characterized by fungal invasion of the hyponychium. In DLSO,

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Address correspondence to Natsuki Kubota-Ishida, natsuki.ishida@meiji.com.

TABLE 1 Weight-normalized mass equivalent of drugs in upper nail layer and deep nail plate and volume-normalized mass equivalent of drugs in cotton pad[a]

Drug	Upper nail layer (μg/g)	Deep nail plate (μg/g)	Cotton pad (μg/g)
10% (wt/vol) ME1111	11,896 ± 2,892	2,884 ± 924	121 ± 27
10% (wt/wt) efinaconazole	1,031 ± 272	67.7 ± 55.6	1.54 ± 0.83
5% (wt/wt) tavaborole	20,890 ± 3,417	6,000 ± 748	726 ± 176
8% (wt/vol) ciclopirox	12,459 ± 3,048	1,202 ± 563	5.60 ± 5.54
5% (wt/vol) amorolfine	3,455 ± 494	31.2 ± 26.6	0.867 ± 0.263

[a]Each number represents the mean ± SD of 5 or 8 samples for ME1111, efinaconazole, tavaborole, amorolfine ($n = 5$), and ciclopirox ($n = 8$).

the fungi usually invade the nail bed and deep nail plate from the outer (distal) edge of the nail. New topical antionychomycotics are administered not only to the nail surface but also into the gap between the nail and nail bed to reach the subungual infection site (12). To be effective, a topical drug should be delivered to the deeply infected subungual site. The permeability of a topical antifungal drug determines whether a sufficient quantity of drug can reach the nail and its bed. In order to have good nail permeability, drug should have low molecular weight (5) and low affinity to keratin (a major nail component) (5, 13). Thus, a topical antifungal drug with low molecular weight and affinity to keratin would be a promising antimycotic.

ME1111, a novel antidermatophytic drug synthesized by Meiji Seika Pharma Co., Ltd. (Meiji, Tokyo, Japan) and currently under clinical development (14, 15), is a low-molecular-weight compound with a demonstrably low affinity for wool keratin (16). Antidermatophytic ME1111 activity is mediated by the inhibition of succinate dehydrogenase (complex II), a critical enzyme involved in the mitochondrial respiratory electron transfer chain (17, 18). ME1111 penetration through the human nail is greater than that of both ciclopirox and amorolfine, which has been demonstrated using TurChub (19), a Franz cell assay (15), and a nail penetration assay with radiolabeled drugs (20).

There are numerous reports relating the nail permeation capabilities of topical antifungal agents with clinical efficacy (20–25). In addition, pharmacokinetic-pharmacodynamic (PK/PD) parameters, such as the *f*AUC/MIC (*f*AUC, area under the concentration-time curve for the free, unbound fraction of a drug) and efficacy coefficient, have also been suggested to predict antifungal clinical efficacy (26–30). Higher efficacy coefficients (concentration in the nail plate divided by the MIC), compared 8% ciclopirox with 5% amorolfine, were also correlated with higher clinical efficacy (26). Moreover, Matsuda et al. (24) and Shimamura et al. (29) described that parameters similar to the efficacy coefficient (MIC in keratin-containing media, ratio of drug concentration to MIC) are the effective indexes to predict clinical efficacy based on data of 10% efinaconazole, 8% ciclopirox, 5% amorolfine (24), and 5% luliconazole (29). However, further investigation is required for the method and parameters to predict clinical efficacy. Here, we established an *in vitro* nail permeation assay, consisting of a one-chamber diffusion cell, a cotton pad, and human nail mounted in the chamber, and nonradiolabeled drugs used in conjunction with liquid chromatography-tandem mass spectrometry (LC-MS/MS) (31, 32). The nail permeation abilities of ME1111 and other commercialized topical antifungal agents were evaluated. We also examined the human keratin-binding rate and antidermatophytic efficacy. This was performed at pH 5.0, since the pHs at the nail plate surface and interior are approximately 5.0 and 4.1, respectively (33). From these data, we calculated the *in vitro* antidermatophytic efficacy coefficient, which is useful for predicting the clinical efficacy of ME1111 and other antifungals.

RESULTS

***In vitro* human nail permeation of ME1111 and other drugs.** Human nail permeation of ME1111 and other topical agents was compared in in-line one-chamber diffusion cells for 14 days. After 14 days, the ME1111 concentration in deep nail layers (ventral/intermediate layers) was 2.4 to 92 times higher than those of ciclopirox, efinaconazole, and amorolfine (Table 1). ME1111 showed greater permeation to the

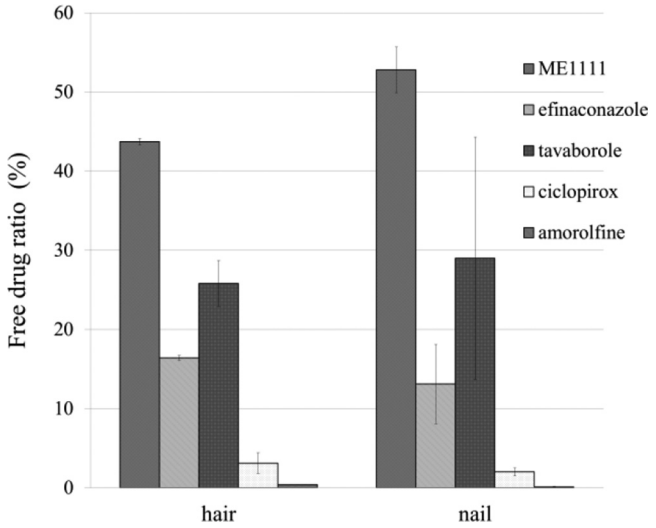

FIG 1 Free drug ratio of ME1111 from human hair and human nail powders. Each drug was added at 1 mg/liter to PBS-containing 5% powdered human hair or nail, and the suspension was incubated with shaking (100 rpm) at 37°C for 1 h. After centrifugation, the free drug ratio was calculated by measuring the concentration of the test drugs in the supernatant by using LC-MS/MS. All data are shown as mean ± standard deviation of 3 samples.

deep nail layer than all the tested drugs, except for tavaborole. The ME1111 concentration in the cotton pads was also 20-fold higher than that of efinaconazole, ciclopirox, and amorolfine. Among the drugs tested, tavaborole showed highest permeation ability with the deep nail plate and cotton pad.

Binding affinity of ME1111 to human hair and nail powder. The free drug ratios of the drugs are shown in Fig. 1. The free drug ratios of ME1111, efinaconazole, tavaborole, ciclopirox, and amorolfine from hair powder were 43.7 ± 0.4, 16.4 ± 0.3, 25.8 ± 2.9, 3.1 ± 1.3, and 0.4 ± 0.0, respectively, and those from nail powder were 52.8 ± 2.9, 13.1 ± 5.0, 29.0 ± 15.3, 2.0 ± 0.5, and 0.1 ± 0.1, respectively. The free drug ratios of ME1111 to human hair and nail powder were the highest among the drugs tested. The binding rates of all drugs were similar for hair and nail powder. Therefore, we used human hair powder instead of nail powder to determine the effect of keratin on the antifungal activity of drugs, because nail powder was difficult to obtain in sufficiently large amounts.

Keratin effect on antidermatophytic activity. The effect of keratin on the antidermatophytic activities of antifungals against *T. rubrum* and *T. mentagrophytes* was determined in RPMI 1640 medium (with or without 5% human hair powder). The antidermatophytic activities of ME1111 were not affected by the presence of 5% human hair powder; however, those of efinaconazole, tavaborole, ciclopirox, and amorolfine against both strains increased. The values of efinaconazole, tavaborole, ciclopirox, and amorolfine against *T. rubrum* in medium with hair increased by 8-, 2-, 16-, and 16-fold, respectively, and against *T. mentagrophytes* in medium with hair by 8-, 4-, 8-, and 32-fold, respectively (Table 2). The ratio of drug that bound well to the human hair reflected a decrease in antifungal activity in the presence of human keratin.

Effect of pH on antidermatophytic activity. As the nail plate is slightly acidic, the antifungal activities of ME1111 and comparators against *T. rubrum* were compared at pHs of 7.0 and 5.0. The MIC_{90}s of ME1111 and ciclopirox remained unchanged between a pH of 7.0 and 5.0. However, the MIC_{90} of tavaborole decreased by 4-fold, and those of efinaconazole and amorolfine increased by 8- and 2-fold, respectively, at a pH of 5.0 (Table 3).

***In vitro* antidermatophytic efficacy coefficients of ME1111 and other drugs.** Free drug concentrations in nail plates were calculated from its binding affinity to human nail powder, which consists of keratin as a main component. The free drug

TABLE 2 Antidermatophytic activity in the presence of human hair keratin

Species (no. of strains)	Drug	MIC_{90} (μg/ml)		Ratio
		RPMI	RPMI with 5% human hair	
T. rubrum ($n = 30$)	ME1111	0.5	0.5	1
	Efinaconazole	0.016	0.125	8
	Tavaborole	8	16	2
	Ciclopirox	0.5	8	16
	Amorolfine	0.5	8	16
T. mentagrophytes ($n = 30$)	ME1111	0.5	0.5	1
	Efinaconazole	0.03	0.25	8
	Tavaborole	8	32	4
	Ciclopirox	0.5	4	8
	Amorolfine	0.25	8	32

concentration of ME1111 in nail plates was 1,523 ± 488 μg/g. This concentration equaled that of tavaborole and was higher than those of efinaconazole, ciclopirox, and amorolfine. The concentration of each free drug in the deep nail plate was orders of magnitude greater than the MIC_{90} values against *T. rubrum*. The *in vitro* antidermatophytic efficacy coefficients were calculated as the ratio of free drug concentration in nail plates to the MIC_{90}s against *T. rubrum* at pH 5.0 and 7.0. The antidermatophytic efficacy coefficients of ME1111, efinaconazole, tavaborole, ciclopirox, and amorolfine at pH 5.0 were 6,091 ± 1,952, 148 ± 121, 870 ± 108, 96.2 ± 45.1, and 0.250 ± 0.213, respectively, and at pH 7.0, they were 6,091 ± 1,952, 1,109 ± 910, 218 ± 27, 96.2 ± 45.1, and 0.521 ± 0.443, respectively (Fig. 2). ME1111 penetrated well into the human nail plates, and the *in vitro* antidermatophytic efficacy coefficient of ME1111 in the deep nail plate was significantly higher than that of efinaconazole, tavaborole, ciclopirox, and amorolfine ($P < 0.05$). The antidermatophytic efficacy coefficient of ME1111 with total or free drug concentration at pH 5.0 was 2.4 to 46 times and 7.0 to 24,000 times greater than those of other antifungals, respectively, and at pH 7.0, they were 1.4 to 22 times and 5.5 to 12,000 times greater than those of other antifungals, respectively.

DISCUSSION

We established a novel *in vitro* nail permeation assay using nonradiolabeled drugs and evaluated PK/PD parameters to attempt to predict the clinical efficacy of topical onychomycosis treatments. Current topical drugs suffer from low clinical efficacy, presumably due to minimal permeability, localization, and nonuniformity of the drugs in the nail. Our analysis of topical treatments sought to identify drugs that would help combat these issues.

We previously demonstrated that ME1111 showed good penetration by using a Franz cell assay, a TurChub assay (15), an *in vitro* infinite dose model assay with radiolabeled drugs (20), and an *in vivo* guinea pig nail penetration study. Here, we established a novel *in vitro* nail permeation assay using nonradiolabeled drugs in order to compare human nail penetration and the kinetics of ME1111 with those of other topical agents used for onychomycosis. Our assay setup consisted of a one-chamber diffusion cell with cotton pads and human nails mounted inside the chamber. ME1111

TABLE 3 Effect of pH on MIC_{90} for *T. rubrum*

Species (no. of strains)	Drug	MIC_{90} for *T. rubrum* (μg/ml)		Ratio
		pH 7.0	pH 5.0	
T. rubrum ($n = 30$)	ME1111	0.25	0.25	1
	Efinaconazole	0.008	0.06	8
	Tavaborole	8	2	0.25
	Ciclopirox	0.25	0.25	1
	Amorolfine	0.06	0.125	2

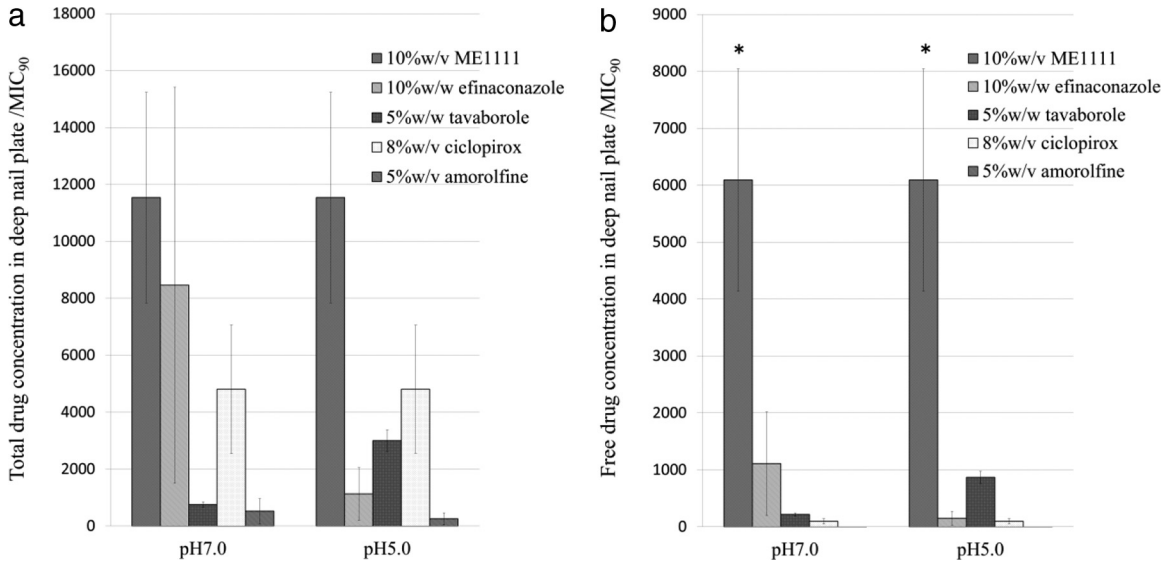

FIG 2 *In vitro* antidermatophytic efficacy coefficients (total or free drug concentrations/MIC_{90}s against *T. rubrum* at pH 7.0 and pH 5.0) of each drug in the deep nail plate. The *in vitro* antidermatophytic efficacy coefficient was calculated as the ratio of total drug concentration (a) and free drug concentration (b) in the nail plates to the MIC_{90}s against *T. rubrum* at pH 5.0 and 7.0. The free drug concentrations in the nail plates were calculated from its binding affinity to human nail powder. Each bar represents the mean ± SD of 5 or 8 data points. ME1111, efinaconazole, tavaborole, amorolfine ($n = 5$), and ciclopirox ($n = 8$). Data were analyzed using one-way ANOVA and Dunnett's test; P values of <0.05 were considered statistically significant and are labeled with an asterisk (*).

was also able to penetrate deep into human nail in this assay: its penetration factor and the concentration in the cotton pad were greater than those of current commercial formulations of efinaconazole, ciclopirox, and amorolfine. While tavaborole was found at a concentration six times higher than that of ME1111 in the cotton pad, its concentration was only twice as much as that of ME1111 in the deep nail layer. This may be because a cotton pad containing polyethylene glycol (PEG)-phosphate-buffered saline (PBS) was used to mimic the nail bed, and the solubility of tavaborole in PEG-PBS is much greater than that of ME1111. Solubility of drugs in the solution within the cotton pad, therefore, presents a possible method limitation. In addition, the disappearance of the drugs from nail bed is not accounted for in this assay method. As a result, for this assay, it may be better to predict the clinical efficacy based on drug concentration in deep nail rather than in the cotton pad.

Molecular weight (MW) and keratin-binding affinity are important factors for nail penetration (5). Drugs with either high keratin affinity or a molecular weight of over 300 have difficulty penetrating the nail plate and reaching the nail bed (11). Similarly, this study showed that drugs with a molecular weight of over 300, such as efinaconazole (MW, 348.4) and amorolfine (MW, 317.5), were found at a drug concentration of less than 4,000 μg/g in the upper nail layer. This value is less than one-third of the concentration observed when testing drugs with a lower molecular weight, such as ME1111 (MW, 202.2), tavaborole (MW, 151.9), and ciclopirox (MW, 207.3). These lighter drugs were present in the upper nail layer at a concentration of more than 11,000 μg/g. However, while ME1111 and tavaborole both demonstrated high drug concentrations in the deep nail plate, ciclopirox did not. This may be due to the differences in their keratin-binding affinity.

We evaluated the human keratin-binding rate of antifungals by using human nail and hair powders. Nails and hair are composed of hard keratin and have similar components (34, 35). Therefore, the binding rates of each antifungal were similar for the two powders. The keratin-binding rates of ME1111 and tavaborole were between 50 and 60%, and that for ciclopirox was around 96.9%. These results suggest that drugs with low molecular weight can permeate the nail surface. However, it is difficult for these drugs to permeate the deep nail and penetrate the full thickness of the nail plate

if they also have a high keratin-binding affinity. Thus, a low molecular weight and low keratin-binding affinity seem to be necessary for nail permeability. ME1111 and tavaborole share these properties and are able to permeate deeply into the nail.

Some antifungals show decreased antifungal activities in the presence of keratin (24). We previously reported that the MIC of ME1111 was not affected by the existence of wool keratin powder (15). In this study, the MIC of ME1111 remained unchanged in the presence of human keratin powder from hair, whereas the MICs of efinaconazole, tavaborole, ciclopirox, and amorolfine increased in correlation with their keratin-binding affinities. These results indicate that the concentration of free non-keratin binding is important for determining antifungal activity in the presence of keratin. Therefore, the clinical efficacy of drugs can be predicted by determining the antifungal activity in the deep nail plate, and they can be computed based on the MIC in the presence of keratin or the free drug concentration obtained from the keratin-binding rate. Here, the keratin concentration in the liquid medium for testing MIC was only 5% to support dermatophyte growth; however, the nail is primarily composed of keratin (33). Therefore, the influence of keratin on antifungal activity in the nail plate is likely much greater than can be determined from the liquid medium used in this test. The release rate from keratin, binding mode (i.e., reversible, covalent, and so on), binding strength of the drug, and binding rate are all also important in determining the available free drug that can exert antifungal activity in the nail. It is necessary to estimate these factors well in order to predict their clinical efficacy accurately and precisely.

pH is also an important factor in determining MIC and antifungal activity in nails. The pH of the nail ranges between 4.1 and 5.0 (33). The MIC of azole antifungals increases under mildly acidic conditions (36). However, in this study, the MIC of ME1111 at pH 5.0 remained unchanged compared to the MIC at pH 7.0. This invariance may be due to the fact that ME1111 possesses acidic and basic functional groups.

Drugs with a higher efficacy coefficient (concentration in the nail plate divided by the MIC) may demonstrate enhanced clinical efficacy (26). We maintain that clinical efficacy can be accurately predicted by correlation with relevant parameters, so long as the appropriate parameters are used. In this study, we proposed a revised efficiency coefficient which uses the ratio of total or free drug concentration (calculated from keratin-binding rate in the deep nail plate) and the antifungal activity (measured at pH 5.0). The total and free drug concentrations of ME1111 in the deep nail plate were more than several thousand times greater than the MIC_{90} of ME1111 at pH 5.0. This concentration should be sufficient to exert antifungal activity in the deep nail plate. Efinaconazole showed enhanced efficacy compared to other topical antifungals (ciclopirox and amorolfine), although approved antifungal agents were not directly evaluated in clinical trials (6, 30, 37, 38). Tavaborole demonstrated clinical efficacy (9). From these clinical trials, free drug concentration/MIC_{90} may be a better indication of clinical efficacy. The revised efficacy coefficients of ME1111 at pH 5.0 and 7.0 were greater than those of other antifungals. Thus, ME1111 has a strong potential for clinical efficacy when used as a topical treatment.

There are current topical treatment options for onychomycosis which deviate from our criteria of low molecular weight and low human keratin-binding affinity; however, these treatments are useful only under fairly restrictive conditions. In the case of superficial white onychomycosis (SWO) or tinea pedis, the infecting dermatophytes populate the surface of the nail or skin. Therefore, drugs with relatively high molecular weight, proper keratin-binding affinity, and good antifungal activity may exhibit good clinical efficacy in this case, because the drug cannot permeate the nail as well and is constantly exposed to the pathogens on the surface. However, drugs with high nail permeability accumulate at a higher concentration within the deep nail layer and are more suitable for the treatment of DLSO, which is triggered by a dermatophyte infection at the nail bed. Although ciclopirox did not penetrate the deep nail plate, its clinical efficacy has been reported to be higher in clinical trials for pediatric onychomycosis than in the adult condition (39). This may be because it is easier for drugs to

permeate the child nail plate than the adult plate. However, because the treatment of onychomycosis requires the penetration of a topical drug to a deep infected subungual site, any potential drug needs to be able to penetrate the nail. Thus, nail permeation activity is an important factor for predicting clinical efficacy of a drug for onychomycosis.

In conclusion, by having a relatively low molecular weight and a low human keratin-binding affinity, ME1111 can penetrate the human nail plate. It is possible that a significant amount of ME1111 can reach the deep nail plate tissues after topical application. ME1111 also demonstrated high levels of antifungal activity in the presence of keratin and under mildly acidic condition. Therefore, we predict that these parameters imply its clinical efficacy, and that ME1111 will be an effective topical treatment for onychomycosis. We await *in vitro* and *in vivo* correlation and well-controlled clinical data to confirm these hypotheses.

MATERIALS AND METHODS

Chemicals. ME1111, efinaconazole, and tavaborole were synthesized at Meiji. Ciclopirox olamine and amorolfine hydrochloride were obtained from Sigma-Aldrich Co. (St. Louis, MO) and LKT Laboratories, Inc. (St. Paul, MN), respectively. ME1111 alcohol-based solution (10% [wt/vol]) and tavaborole solution (5% [wt/wt]) were dissolved in ethanol-propylene glycol (4/1 [vol/vol]) and were manufactured by Meiji. Efinaconazole 10% (wt/wt) solution (Jublia) and ciclopirox 8% (wt/vol) nail lacquer (Penlac) were purchased from Valeant Pharmaceuticals International, Inc. (Quebec, Canada). Amorolfine 5% (wt/vol) nail lacquer (Loceryl) was purchased from Galderma S.A. (Lausanne, Switzerland). Imipramine hydrochloride, as an internal standard (IS) for quantitative analysis, was obtained from Sigma-Aldrich Co.

Preparation of powdered human nail and hair. Human nails were purchased from Analytical Biological Services, Inc. (Wilmington, DE) and Science Care, Inc. (Phoenix, AZ). Human hair was obtained from Tissue Solutions Ltd. (Glasgow, UK) and volunteers from the Pharmaceutical Research Center of Meiji. The human nail plates were first immersed in 70% ethanol for at least 30 min and then further in distilled water for 3 h or longer. The human hair was washed with water, followed by acetone. After cutting with scissors, the human nails and hair were pulverized to powders using a Multi-beads shocker (Yasui Kikai Corporation, Osaka, Japan). Powdered nail and hair were defatted by washing with ethanol and diethyl ether (1:1) three times, followed by acetone three times. Ethical approval for the use of human samples in this study was obtained from the research ethics committee at the Meiji Pharmaceutical Research Center.

***In vitro* permeation studies with in-line diffusion cells.** *In vitro* human nail permeation was measured using a modification of a method described previously (20). After immersion in 70% ethanol and distilled water, human fingernails ($n = 5$ or more per group), each in a square of approximately 100 mm^2, were set on one-chamber in-line diffusion cells (PermeGear, Inc., Hellertown, PA). Cotton pads wetted with 0.6 ml of 6% (vol/vol) polyethylene glycol (PEG) in PBS (pH 7.4) were placed in a receptor compartment and incubated at 37°C, with humidity over 60%. Then, 3 μl each of 10% ME1111, 10% efinaconazole, 5% tavaborole, and 8% ciclopirox solution was applied to a dorsal nail surface once daily for 14 days. Mirroring clinical usage, 5% amorolfine lacquer was applied twice weekly. The wetted cotton pads beneath the nail were collected from each cell chamber at 2, 5, 8, 11, and 14 days and replaced with new pads prior to the next dosing. After 14 days, nail samples were drilled and divided into two equal parts, the deep layer and upper layer. Nail samples of the respective drugs were dissolved in 1 N sodium hydroxide-methanol (1:1) solution or Soluene-350 (PerkinElmer, Inc., Waltham, MA)-methanol (1:1) solution. After incubation at 60°C for 4 h or 37°C for 24 h, drug concentrations were determined by using an LC-MS/MS system with negative- or positive-ion electrospray ionization (Waters Corporation, Milford, MA, and/or Applied Biosystems, Foster, CA).

Measurement of keratin-binding affinity. Each drug was added at 1 mg/liter to PBS-containing 5% powdered human hair or nail, and the suspension was incubated while shaking (100 rpm) at 37°C for 1 h. After centrifugation, the free drug ratio and binding rate to keratin were calculated by measuring the concentration of test drug in supernatant by using LC-MS/MS.

Antifungal susceptibility of *T. rubrum*. Antifungal susceptibility testing against *T. rubrum* clinical isolates ($n = 30$) was performed according to the CLSI M38-A2 standard broth microdilution method (40). The lowest concentration required for macroscopically inhibiting 80% of the fungal growth compared to the growth control was defined as the MIC. The MIC was determined in RPMI 1640 medium (Life Technologies, Grand Island, NY) buffered with MOPS [3-(*N*-morpholino)propanesulfonic acid] (Nacalai Tesque, Ltd., Kyoto, Japan) at pH 7.0. The MIC_{90} was defined as the MIC at which 90% of the isolates tested were inhibited. The MIC was also measured in medium adjusted to pH 5.0.

Antidermatophytic activity in the presence of human hair powder. The susceptibilities of clinical isolates of *T. rubrum* ($n = 30$) and *T. mentagrophytes* ($n = 30$) to ME1111 and comparators were evaluated in RPMI 1640 medium (with or without 5% [wt/vol] human hair powder) by using a method described previously (15). MICs were determined according to the CLSI M38-A2 broth microdilution method, with the modification that conidial suspensions were inoculated at a final concentration of 2×10^4 CFU/ml.

Statistical data evaluation. The data are shown as the mean ± standard deviation (SD) and analyzed by calculating the concentration of drug in the upper and deep nail layers (in micrograms per gram), cumulative penetration (in micrograms per milliliter), and *in vitro* antidermatophytic efficacy

coefficients (micrograms per gram per MIC_{90}). Statistical analysis was performed using SAS version 9.3 (SAS Institute Japan Ltd., Tokyo, Japan). The data were analyzed using one-way analysis of variance (ANOVA) and Dunnett's test; *P* values of <0.05 were considered statistically significant and are labeled with an asterisk (*).

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