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Visualization of penetration of topical antifungal drug substances through mycosis-infected nails by MALDI-MSI

Fernanda Endringer Pinto[1], Charlotte Bagger[1], Hanan Osman-Ponchet[2], Christian Janfelt[1], Nicolas Joly-Tonetti[3]

[1]University of Copenhagen, Faculty of Health and Medical Sciences, Department of Pharmacy – Blegdamsvej 3B, DK-2200 Copenhagen N, Denmark
[2]PKDerm – Grasse BIOTECH, Parc d'activités ArômaGrasse, 45, boulevard Marcel Pagnol, F-06130 Grasse, France
[3]Galderma SA, Rue d'Entre-Deux-Villes 10, CH-1814 La Tour-de-Peilz, Switzerland

Introduction

Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) is a mass spectrometry-based technique, which can be applied for compound-specific imaging of pharmaceuticals in tissues samples[1]. It combines the spatial resolution of classical histology with the molecular specificity of mass spectrometry to create molecular distribution profiles in tissues[1,2]. The mass spectrometry image (MSI) is acquired by a laser rastering across the specimen, in a systematic fashion such that a mass spectrum is recorded in every point on the sample. Subsequently, for each detected compound a specific image may be generated by plotting the ion intensity of the compound peak as a function of position. Therefore, numerous images may be generated from the same MSI experiment, including endogenous as well as exogenous compounds. By use of different colors, images of drug substances may be overlaid on images of endogenous compounds, which are characteristic for a specific tissue type, revealing how the drug substance is localized in the tissue[3]. MALDI-MSI has been successfully used to visualize drug penetration in tissues[4–6] and cell cultures[7]. However, to the best of our knowledge, this is the first time that MALDI-MSI is used on nail tissue, therefore a method for cryo-sectioning of nails and MALDI imaging of nail sections had to be developed. In the present study, MALDI-MSI was used to analyze the distribution profile and penetration into human mycosis-infected nails of three antifungal actives in different topical galenical formulations: Amorolfine (5% in an non-water soluble methacrylic acid copolymer formulation), Ciclopirox (8% in an water soluble HPCH-formulation) and Naftifine (10% in an water soluble propylene glycol solution).

Objectives

Verification and proof of concept to use MALDI-MSI technology to visualize different distribution profiles and penetration into ex-vivo human mycosis-infected toenails of three antifungal active substances contained in different, widely used topical galenical formulations.

Methods

The following antifungal topical preparations were investigated:

- Amorolfine 5% nail lacquer (Loceryl®, Galderma SA, Lausanne, Switzerland)
- Naftifine hydrochloride 1% solution (Exoderil®, Sandoz GmbH, Kundl, Austria)
- Ciclopirox 8% nail lacquer (Ciclopoli®, Almirall Hermal GmbH, Germany)

Mycosis-infected human cadaver toenails (big toe) were obtained from Tissue Solutions Ltd (Glasgow, UK). The identification of donors with onychomycosis was based on image guide. Human nail samples were obtained in agreement with ethical and legal regulations for the use of biological material of human origin applicable in France. A total of 14 healthy toenails and 24 mycosis-infected toenails were used. The nails were stored individually at -20°C.

Prior to one single application of the topical preparations under investigation, the nails were thawed at room temperature, immersed in 70% ethanol for 15 minutes and then washed twice with deionized water. The thickness of each nail sample was measured using a C110 electronic external measuring gauge (Kroeplin GmbH, Schlüchtern, Germany). At least three different measurements were performed on different zone of each nail, and the average of the measurement were reported. Six nails were filed across the surface using an emery board (four forward and backward motions, *i.e.* a total of eight repetitions). The filed nails were reserved for topical application exclusively with amorolfine 5% nail lacquer in accordance with its Summary of product characteristics (SmPC).

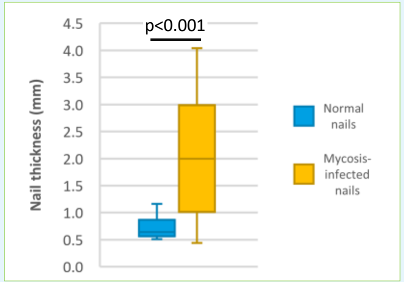

Figure 1. Mycoses-infected nails are three times thicker than healthy nails. Statistics: Mann-Whitney Rank Sum Test. Normality Test: Passed (P = 0.244) Equal Variance Test: Failed (P < 0.050)

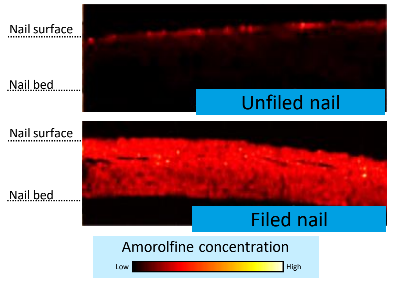

Figure 2. The effect of the filing procedure on the penetration of the nail plate by amorolfine. Sagittal section of healthy toenails, analysed 6 hours after topical application. Amorolfine is shown as a gradient of concentration from the lowest to the highest. Representative images of 3 samples.

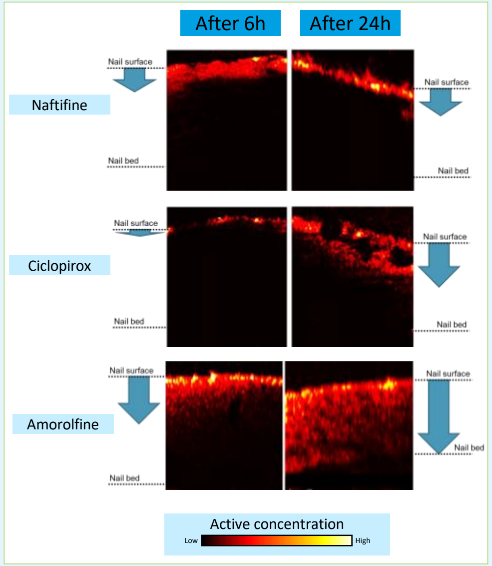

Figure 3. Comparison of the penetration of the nail of the 3 antifungal active substances after 6h (left) and 24h (right) of one single topical application according to their respective SmPCs. Nail plate limits are indicated by dotted lines. The arrows highlight the depth of the active penetration, from the surface toward the nail bed. Active substances are shown as a gradient of concentration from the lowest to the highest. Nails used for amorolfine penetration are filed, according to its respective SmPC posology. Representative images of 3 samples.

Results & Discussion

Nail thickness and the benefit of nail filing

- Mycosis-infected nail generally are thicker than normal nails[8]. We measured the thickness of all the nails collected for this study: we found that mycosis-infected nails are significantly thicker than normal nails. By mean and medium, they are three times thicker (Fig. 1).
- We filed mycosis-infected nails and measured their thickness after the procedure. We observed that a back-and-forth movement is able to decrease the thickness of the samples (data not shown). We compared the nail penetration of amorolfine 5% on both unfiled and filed according to the SmPC. We observed that the filing procedure dramatically induced the nail penetration of the active substance (Fig. 2).
- Clinical studies have demonstrated that the physical removal of the affected parts of the nail plate prior to the application of topical therapeutic agents contributes to treatment success[9,10]. Permeation studies have shown that filing of the dorsal nail plate was associated with improved penetration compared with that of an intact, full-thickness nail plate[11]. Here again, we demonstrated the efficacy of this procedure.

Mass-spectrometry imaging (MSI)

- Naftifine presented a similar distribution into the nail in both remanence time points, seemingly being highly localized in the dorsal layer of the nail plate (Fig. 3, bottom panel).
- Ciclopirox was localized mainly in the dorsal layer at 6h remanence period and kept penetrating the intermediate layer of the nail plate after 24 h of remanence. The compound did not however reach the nail bed limit (Fig. 3, middle panel).
- Amorolfine showed a more homogeneous distribution through the nail already after 6h, where it seemed to penetrate the intermediate layer, in contrast to the other topical drugs evaluated. After 24 h, a significant fraction of this active substance was also present in the lower layers of the nail plate, at the limit of the nail bed. This confirms previous ex vivo data showing better penetration and a higher antifungal growth inhibition for amorolfine after one single application[12].

Conclusions

This study shows for the first time the distribution and penetration of antifungal drug substances in mycosis-infected human nails using MALDI-MSI analysis. The results highlight a deeper penetration of amorolfine through the infected nails than ciclopirox and naftifine. Amorolfine is the only active able to reach the nail bed limit within 24h after one application.

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DISCLOSURES

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