Ex Vivo Assessment of FMX101 & FMX103 Human Skin Permeation and Penetration

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Introduction

- Acne vulgaris is a prevalent, chronic, inflammatory skin disorder affecting approximately 85% of the population at some point in their lifetime[1]
- Oral antibiotics, such as minocycline and doxycycline, are mainstays of treatment for moderate-to-severe acne but are associated with potentially serious systemic side effects[2]
- Two novel foam formulations of minocycline, FMX101 4% and FMX103 1.5% differing only in their antibiotic concentration, have been developed for potential use in acne vulgaris and papulopustular rosacea
- Understanding the permeation and penetration of active agent into skin structures, particularly the epidermis, dermis, and sebaceous appendage, is critical to characterizing these formulations

Objective

- To perform an *ex vivo* permeation and penetration assessment for 2 prototype foam formulations of minocycline, FMX101 4% and FMX103 1.5%

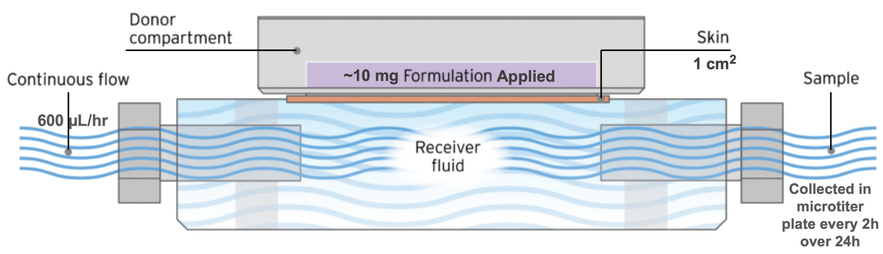
Methods

- An *ex vivo* permeation and penetration experiment using flow through diffusion cells (MedFlux-HT™) was performed to assess foam formulations developed by Foamix Pharmaceuticals Inc.
- The *ex vivo* experiments assessed two active formulations, FMX101 4% and FMX103 1.5%, and 2 placebo formulations
- Minocycline penetration was examined by extracting drug from epidermis (n=5), dermis (n=5), and the sebaceous appendage (n=5)
- Full scale skin permeation and penetration investigations were performed (n=5, one skin donor)

Ex vivo assessment of drug delivery across human skin to the sebaceous appendages assessed using a flow-through diffusion cell

- The flow-through diffusion cell (**Figure 1**) was used to assess drug delivery across human skin and to the sebaceous appendages
- Human skin from a single donor was initially stored at –80^0 C and thawed at room temperature. Next, the human skin was placed between the donor compartment and receptor compartment of the diffusion cell

Figure 1: Schematic representation of flow-through diffusion cell



Development of a suitable receptor solution and extraction diluent for *ex vivo* drug permeation and penetration experiments in human skin

- Three receptor solutions were assessed for their ability to minimize degradation of minocycline during *ex vivo* permeation and penetration
  - 165 mM sodium acetate pH 5.0 with 0.01% Brij-O20
  - 50 mM MgCl2 in 115 mM sodium acetate pH 5.0 with 0.01% Brij-O20
  - 35 mM EDTA in 130 mM sodium acetate pH 5.0 with 0.01% Brij-O20
- Three different diluents were assessed for their suitability as extraction fluids for the recovery of minocycline from the surface of the skin, the skin layers (epidermis and dermis), and from a single drop of 2-octyl cyanoacrylate glue (SurgiSeal®)
  - 90/9/1 acetonitrile/water/formic acid
  - 90/9/1 methanol/water/formic acid
  - 25 mM MgCl2 in 90/9/1 methanol/water/formic acid)
- Method development tests determined 35 mM EDTA in 130 mM sodium acetate pH 5.0 with 0.01% Brij-O20 was suitable as the receptor solution and that 90/9/1 methanol/water/formic acid should be used as the extraction solvent

Skin Penetration and Permeation

- Foam samples were collapsed in a glass container an approximately 10 mg of the collapsed foam was dosed onto the skin (n=10)
- Flow was set at 10 µl/min and the formulation was allowed to penetrate through the skin for 12 hours
- Subsequently, two treatment paradigms were implemented:
  1. **Sebaceous Appendage Intact:** Five skin samples were cleaned and heated at 60°C (140°F) for 2 minutes in order to manually separate the dermis from the epidermis
  2. **Sebaceous Appendage Separation:** The other five skin samples were cleaned and then treated with SurgiSeal® to extract sebaceous appendages. Minocycline was analyzed for sebaceous appendage, dermis, and remaining epidermis

Quantification of Minocycline

- The LC-MS/MS analytical method was developed and used to determine amount of drug delivered to the sebaceous appendages, dermis and epidermis
- This was determined to be a sensitive quantification of minocycline from receptor solution and skin tissue homogenates

LC-MS/MS=Liquid chromatography – mass spectrometry/mass spectrometry

Post-Hoc Data Analysis for Tissue and Appendage Concentrations

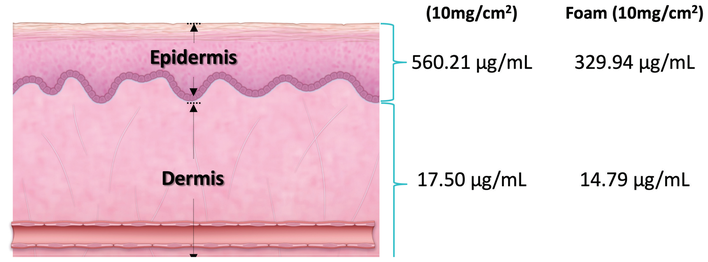
- In order to convert the amounts of minocycline in epidermis and dermis to concentrations on a volume of skin (ie, per mL), the following assumptions were made:
  - Historical average weights from over 438 skin samples were used. The average weight of epidermis was assumed to be 8.6 mg and the average weight of dermis was assumed to be 96 mg
  - The density of skin was assumed to be 1 g/mL
- The amount of drug in the sebaceous appendage (amount of drug per volume of appendage) was calculated using the following assumption:
  - The volume of the infundibula (sebaceous appendage separated by cyanoacrylate glue) was calculated based on a volume of 0.09 mm3 (Lademann et. al, Section 10). This was calculated as the average of the volume of the infundibula on the forehead (0.19 mm3) and that on the forearm (0.01 mm3) as no determination of the volume of the infundibula on the abdomen was conducted

Results

Penetration: Distribution of Minocycline in the Skin and in Plasma (tissue distribution)

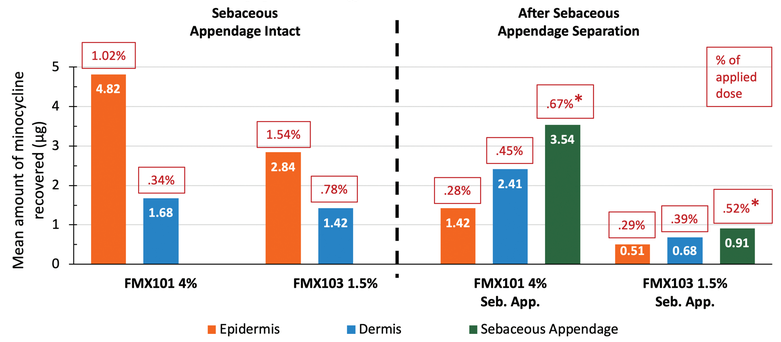
- FMX101 delivered 3539 ng of minocycline to the sebaceous appendages (0.67% of applied dose) and FMX103 delivered 909 ng of minocycline to the sebaceous appendages (0.52% of applied dose) (p<0.001)
- After a single application of either FMX101 4% or FMX103 1.5%, the concentration of minocycline in the skin was as follows:

Figure 2: Concentrations of Minocycline in the Skin and Plasma



*These data were converted into minocycline concentrations (µg/mL) accounting for average weight and density of skin, volume of sebaceous appendages, and cellular volume corrections.

Figure 3: Penetration: Distribution of Minocycline in the Sebaceous Appendage



*Approximately half of minocycline delivered to the epidermis was recovered from the sebaceous appendages for both formulations

Conclusions

- Both FMX101 4% and FMX103 1.5% were shown to deliver high concentrations of minocycline to the epidermis and sebaceous appendage, while much lower concentrations were delivered to the dermis skin layer
- There were no statistical differences between FMX101 and FMX103 in the amount of minocycline that permeated across the skin into the receptor solution over 12 hours
- Approximately half of minocycline delivered to the epidermis was recovered from the sebaceous appendages

Limitations

- Derived calculations based on *ex vivo* skin penetration studies are intended to approximate, but may not precisely reflect, the permeation and penetration of active molecules in patients with a dermatologic condition

References

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Disclosures
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