# EVALUATION AND VALIDATION OF TRANSDERMAL MICRODIALYSIS TECHNIQUE FOR IN VIVO MONITORING OF SKIN PENETRATION OF THE P-GP SUBSTRATE ERYTHROMYCIN, AFTER TOPICAL ADMINISTRATION IN RATS

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## Introduction

Skin is the largest organ of the body. It has a complex barrier function against mechanical, chemical and physical insults. Its barrier feature includes the compact cell layers of stratum corneum, the tight junctions between the keratinocytes in the granular layer of epidermis and also the ABC and SLC transporters located at almost all cell types of the skin. However, there are only a few data available on the role of membrane transporters in the dermal barrier. Hashimoto and coworkers provided evidence for the first time in a recent paper on the direction of P-glycoprotein (P-gp) transport in the skin by *ex vivo* investigation of dexamethasone, a well- known P-gp substrate. The aim of our study was to introduce *in vivo* transdermal microdialysis technique to monitor skin penetration of topically applied erythromycin (ERY), another widely known P-gp substrate.

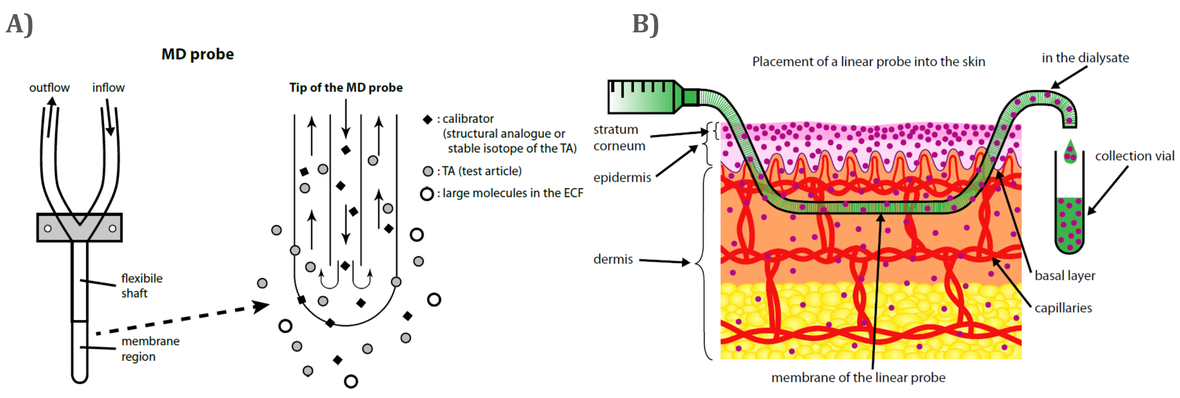



**Figure 1** (A) The concept of microdialysis presented in a microdialysis probe with concentric design . (B) Location of a linear microdialysis probe in the skin in a human experiment .

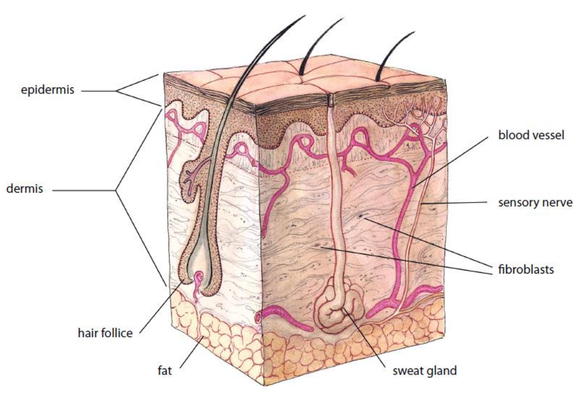



**Figure 2** Schematic representation of anatomical structure of the human skin.

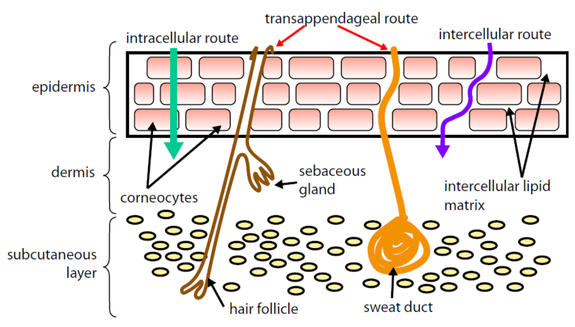



**Figure 3** Routes of percutaneous absorption.

## Methods

Male Wistar rats (250-320 g bodyweight) were used in the experiments. In the first series of experiments the optimal method was searched for barrier perturbation to reach well-detectable skin penetration of topical ERY. In the second series of experiments the optimal drug formulation was searched for the best drug absorption into the subcutis. For verification and standardization of the position of microdialysis probes in the skin ultrasound scanning was used. The dialysate samples were collected 30 min prior to and 240 min after the application of ERY containing patches on the abdominal surface of the skin. The absorption of ERY from different drug formulations was also tested in Franz diffusion cells using artificial membranes, for in vivo - in vitro correlation (IVIVC). ERY content of samples was determined by LC-MS/MS method.

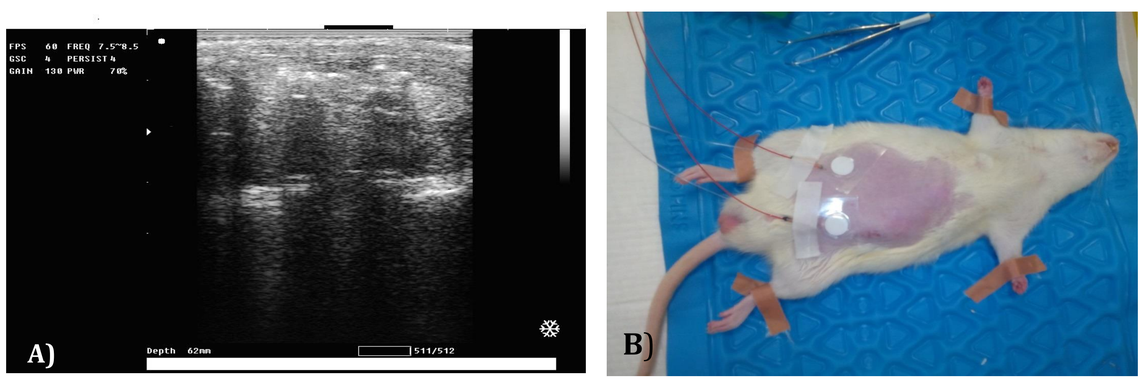



**Figure 4** (A) Verification and standardization of the position of microdialysis probes with ultrasound scanning (B) Schematic representation of TDM to collect dermal interstitial fluid containing test drug.

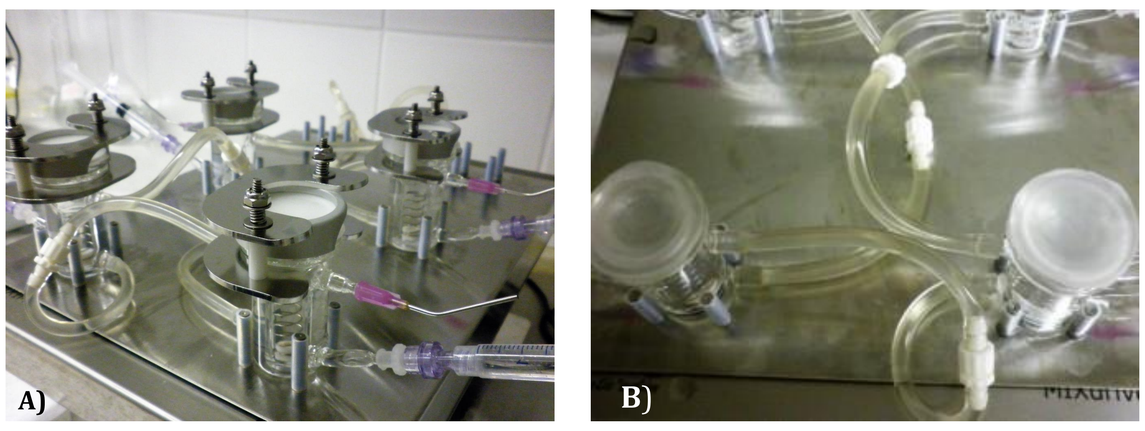
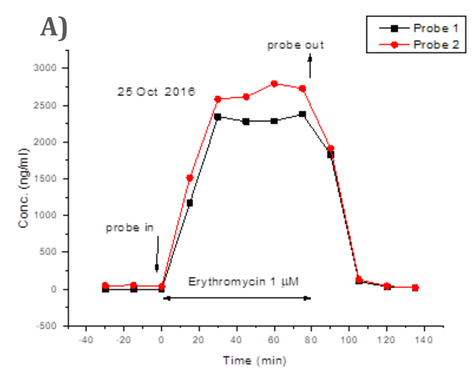



**Figure 5** (A-B) Franz diffusion cells using artificial membranes, for in vivo - in vitro correlation (IVIVC).

## Results and discussion

1. On the basis of the ERY concentrations detected in dialysate samples, we found, that shaving by electric razor followed by application of an epilatory cream and tape stripping repeated 10 times is the most effective combination for barrier perturbation to reach high drug penetration into the subcutis.

2. As for the optimal drug formulation, compounded ERY cream 2% and 4% and Aknemycin cream 2% (Almirall Hermal) were compared. Further *in vivo* and *in vitro* studies are needed to decide which formulation is the best for our purposes. After receiving appropriate drug absorption data in the skin, ERY treatment will be combined with intravenous cyclosporine A (specific P-gp inhibitor) administration. ERY levels will be determined both in the skin and in the plasma by triple- probe microdialysis. Our aim is to verify the P-gp related drug-drug interactions in the skin *in vivo* using transdermal microdialysis technique and *in vitro* in Franz cells on human skin.



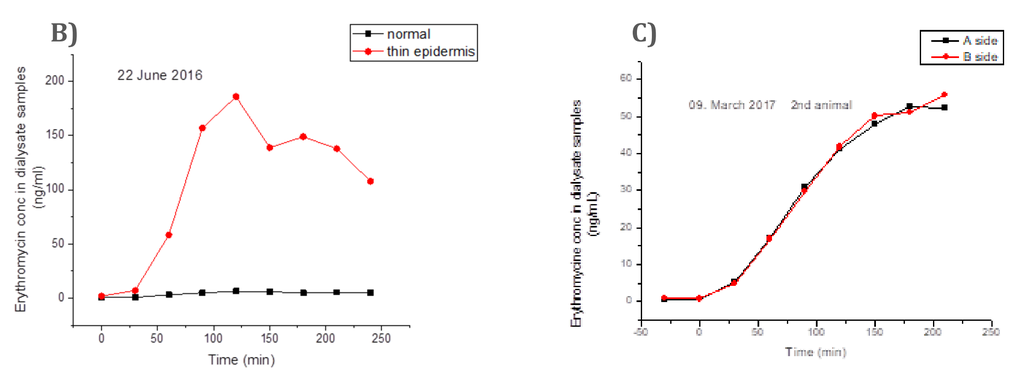




**Figure 6** (A) In vitro recovery of MAB11.8.10 probes for Erythromycin (B) Comparison of the thin and normal epidermis penetration of erythromycine from 4% cream formulanion tested by transdermal microdialysis technique in rats Skin penetration of erythromycine from Aknemycin cream tested by transdermal microdialysis technique in rats.

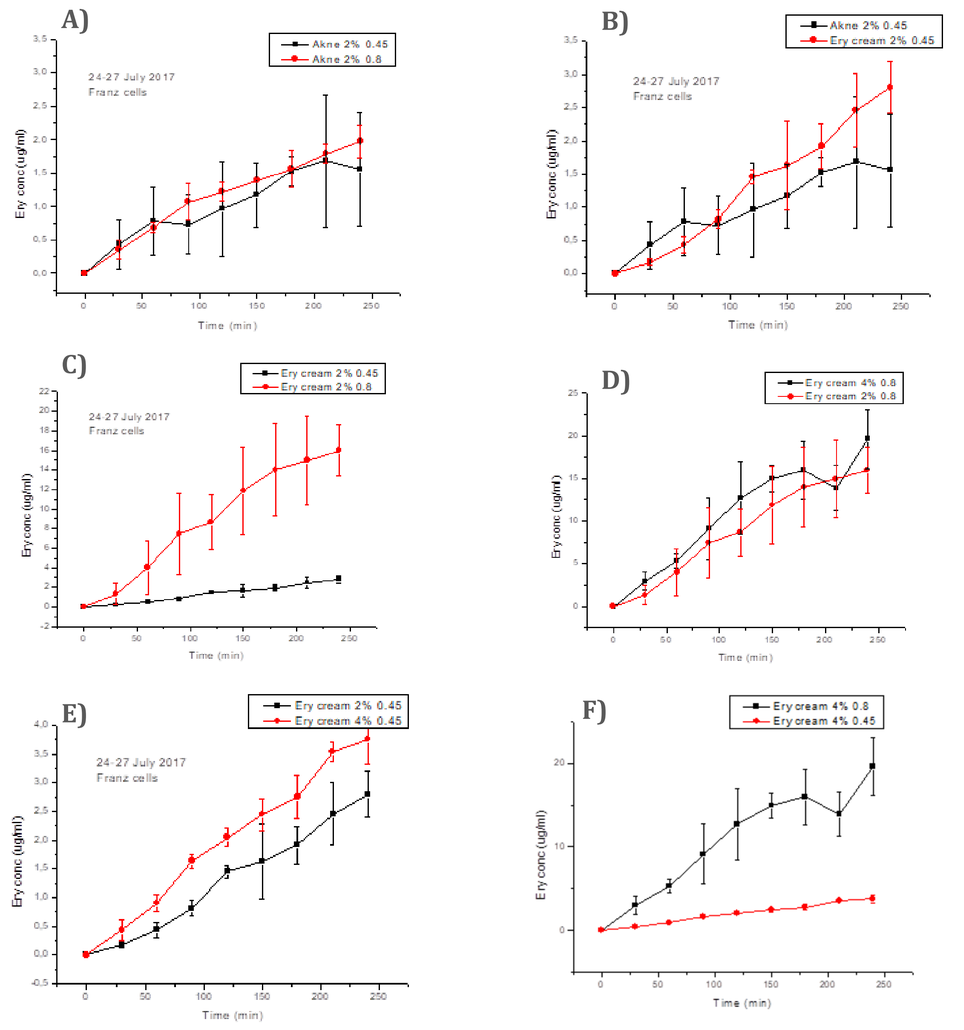



**Figure 7** (A-C) Comparison of the release of erythromycin from Aknemycin 2% cream and Erythromycin 2% cream using d=0.45 um and 0.8 um artificial membrane in Franz cells (D-E) Comparison of the release of erythromycin from Erythromycin 2% and 4% cream using d=0.45 um and 0.8 um artificial membrane in Franz cells.

## References

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Hashimoto N et al, Int J Pharm. 2017, 15;521(1-2):365-373.

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