AN IN VIVO-IN VITRO CORRELATION STUDY FOR EVALUATION OF P-GLYCOPROTEIN (P-GP) FUNCTION AT THE DERMAL BARRIER – A PILOT STUDY

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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 investigate if the modulation of P-gp by PSC-833 has an impact on skin penetration of topically applied erythromycin (ERY) *in vivo* and *ex vivo*.

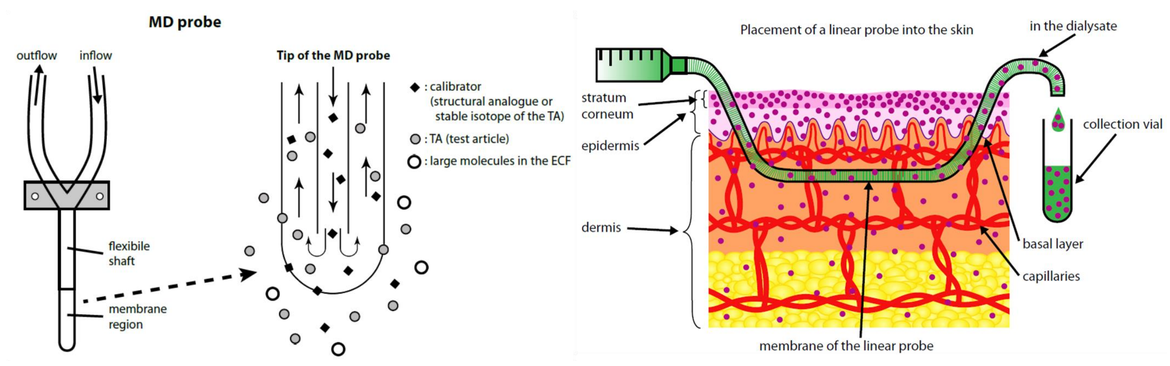

Figure 1 (A) The concept of microdialysis presented on a microdialysis probe with concentric design . (B) Placement of a linear microdialysis probe in the skin in a human experiment .

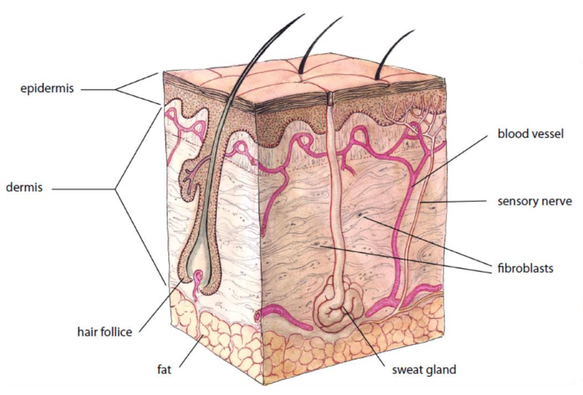

Figure 2 Schematic representation of anatomical structure of the human skin.

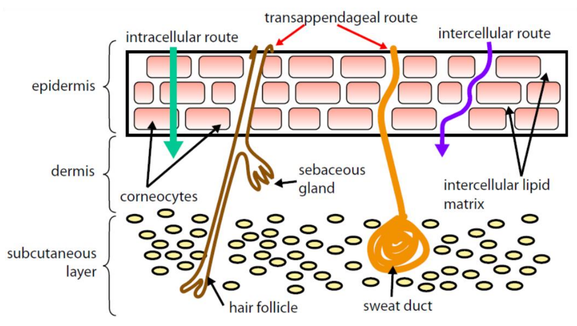

Figure 3 Routes of percutaneous absorption.

Methods

Male Wistar rats (250-320 g bodyweight) were used in the experiments. In the first part of the study 2% Erythromycin (ERY) cream and 2% Aknemycin (AKNE) cream were compared both in vivo and in vitro for skin penetration. In the second series of experiments topical PSC-833 (a specific P-gp inhibitor) pretreatment was applied before the abdominal placement of substrate (ERY) containing pathes on the skin. For verification and standardization of the position of microdialysis probes in the skin ultrasound scanning was used. The dialysate samples were collected from 30 min prior to, until 240 min after the application of ERY containing patches. The absorption of ERY was tested in Franz diffusion cells using artificial membranes (0.45 and 0.8 μm pore size) and rat skin preparation and also in vivo by transdermal microdialysis. In vivo - in vitro correlation (IVIVC) was calculated. ERY content of samples was determined by LC-MS/MS method.

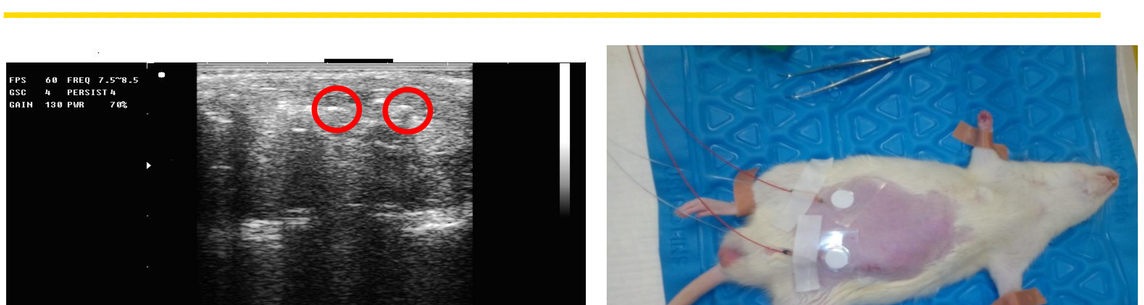

Figure 4 (A) Verification and standardization of the position of microdialysis probes with ultrasound scanning (B) Dual-probe transdermal microdialysis experiment in a Wistar rat

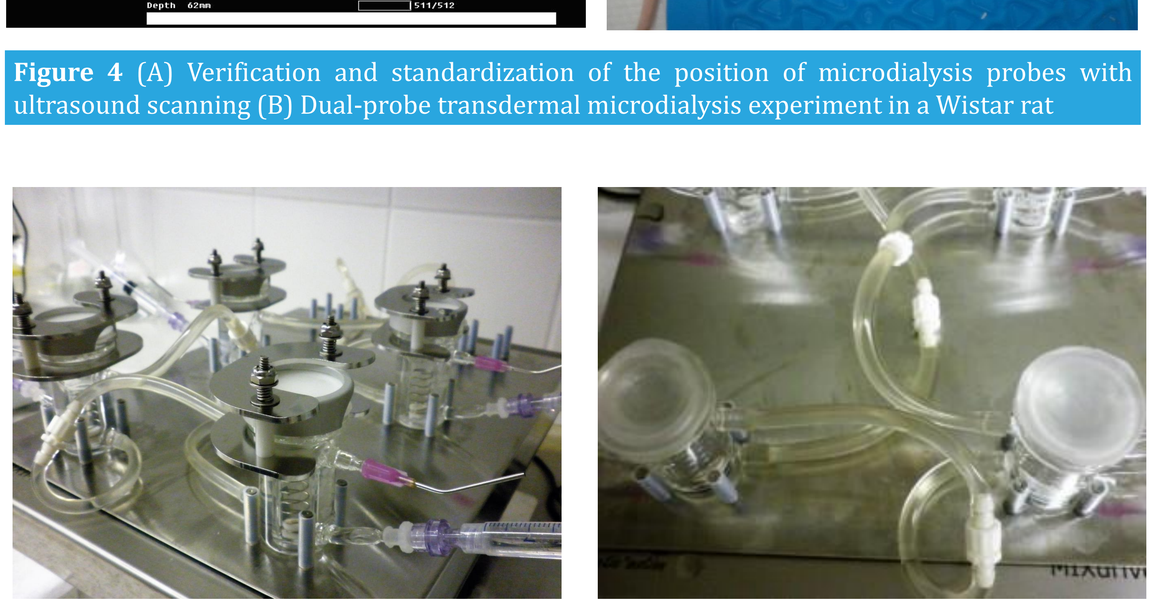

Figure 5 (A-B) Franz diffusion cell instrumentation for *in vitro* permeation study

Results

The results of *in vitro* Franz diffusion cell experiments are summerized in Figure 6. The initial *in vivo* results gained in transdermal microdalysis experiments are presented in Figure 7.

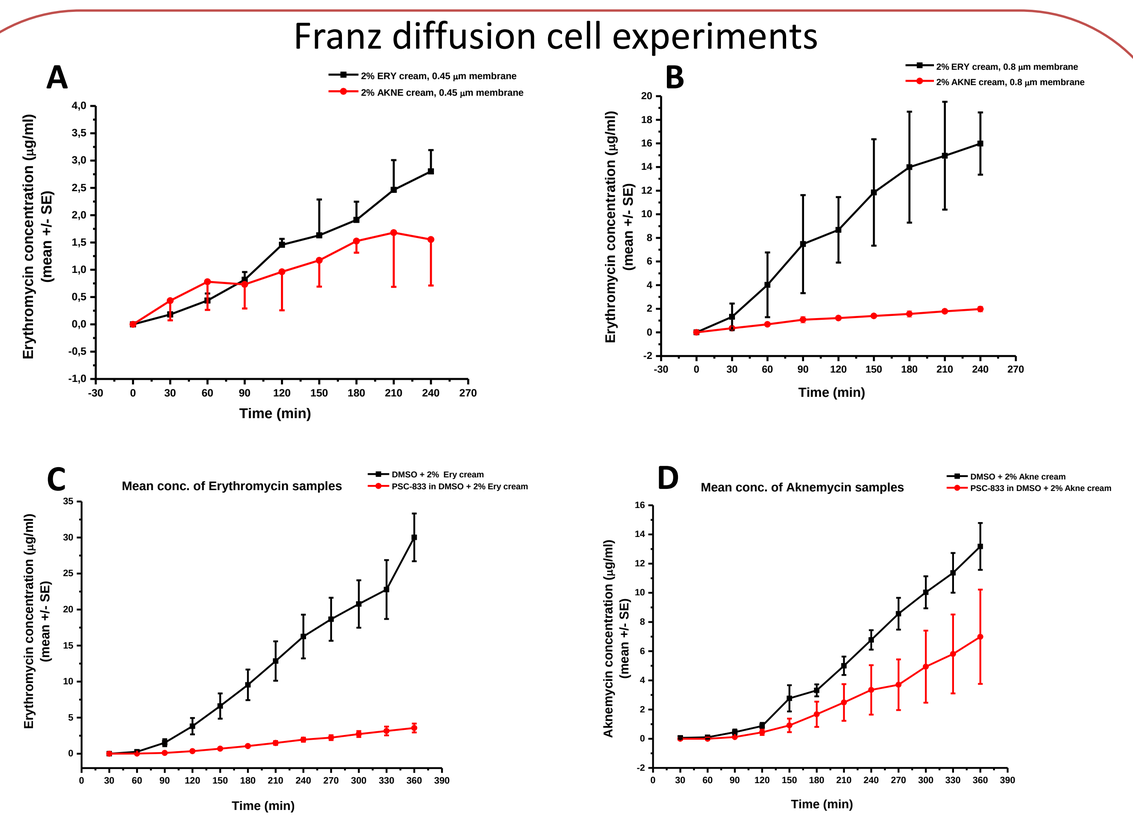

Figure 6 Diffusion of erythromycin from two formulations through arteficial membrane (A: d=0.45 μm, B: d=0.8 μm) and rat skin preparation (C, D). In skin absorption experiments (C, D) control and P-gp inhibitor pretreated skin absorptions were compared.

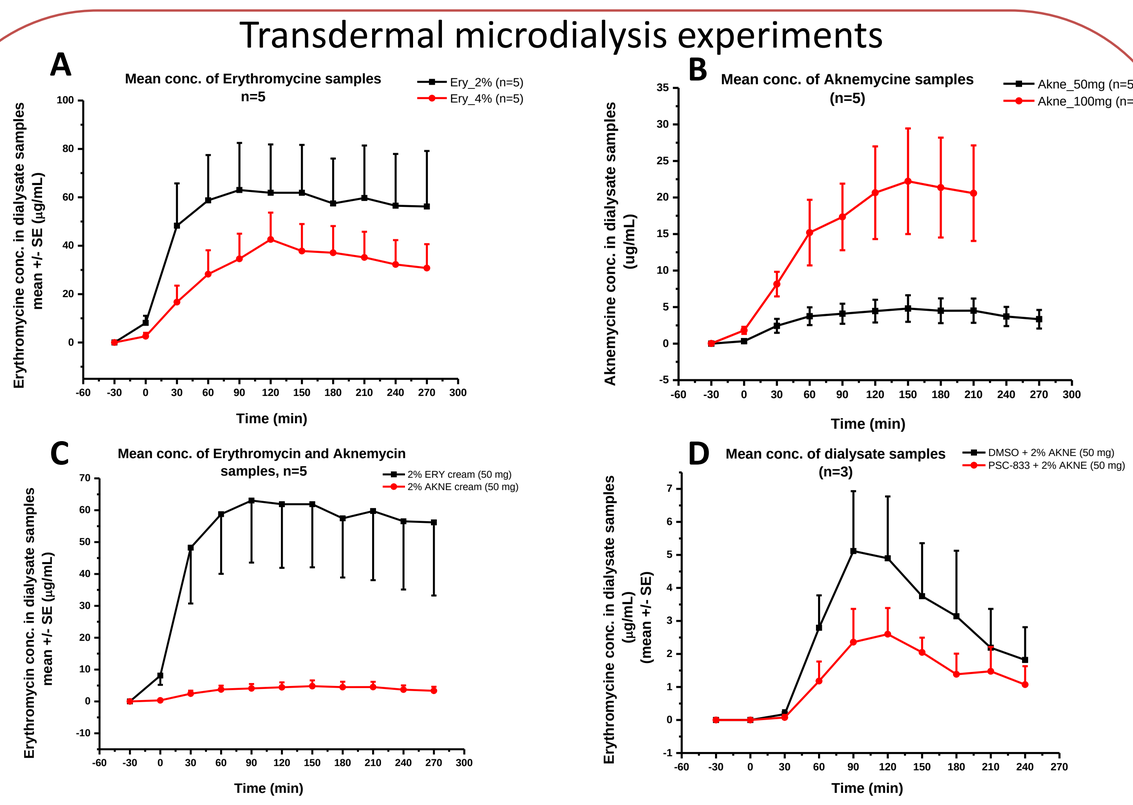

Figure 7 Skin penetration of erythromycin from two formulations. I*n vivo* experiments in rat abdominal skin. Two cream concentrations (A), two amount of creams (B) and two formulations (C) were compared for absorption into the subcutis by transdermal microdialysis technique. Erythromycin release and skin absorption from Aknemycin cream formulation was also tested in presence and absence of a topically applied P-gp inhibitor (D).

Conclusions

Erythromycin showed higher release and absorption from the emulsion-cream formulation, than from the Aknemycin cream formulation. The drug penetration was dose-dependent if higher amount of the cream was applied on the patch, but showed no dose-dependent absorption if the concentration of the cream was increased from 2% to 4% (saturation). P-gp inhibition by a topical pretreatment with PSC-833 resulted in lower penetration of erythromycin into the subcutis *in vivo* in accordance with the recent findings of Hashimoto and coworkers (2017). To make stronger conclusions and statistical analysis the number of animals should be further increased.

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References

Erdő F et al, J Control Release. 2016, 10;233:147-61.
Hashimoto N et al, Int J Pharm. 2017, 15;521(1-2):365-373.

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