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Stimulated Raman Scattering (SRS) microscopy and Deep Learning: Novel pharmacokinetic approach for evaluation of topical bioequivalence

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PURPOSE

The bioequivalence of pharmaceutical formulations is typically demonstrated by assessing the pharmacokinetics (PK) of a generic product relative to a reference listed drug (RLD). However, for topical products applied to the skin, it has been historically challenging to quantify the drug concentration at the target site of action, and therefore the applicability of the PK-based bioequivalence (BE) approach has been limited. The development of new methods capable of determining the amounts of drug at or near the site of action has been a key area of research in the recent past.

OBJECTIVES

The aim of this work was to develop a novel approach for evaluating dermal delivery of drug following topical application to the skin. This method is based on stimulated Raman scattering (SRS) imaging and computational image analysis based on machine learning.

METHODS

Two commercially available topical cream formulations containing tazarotene (Taz), 0.1% were evaluated, which are the RLD (Tazorac® cream) and approved generic drug product (Tazarotene cream; Taro Pharmaceuticals). A third treatment group consisting of Tazorac® cream was also used to evaluate the data from the RLD against itself. Taz uptake was additionally evaluated from two alternative formulations, *i.e.*, Tazorac® gel and Taz in neat PEG-200, to examine the capability of the method to distinguish between different products. Finite doses of the formulations (5μL/cm^2) were applied to human skin *ex vivo*, and drug permeation was monitored over ~7 hrs by SRS imaging. The SRS signal intensity values were normalized against a concentration standard that was scanned at the time of the experiment. Data processing was performed via deep learning using a U-Net convolutional neural network for image feature extraction and automated analysis of cutaneous PK parameters in the *stratum corneum*.

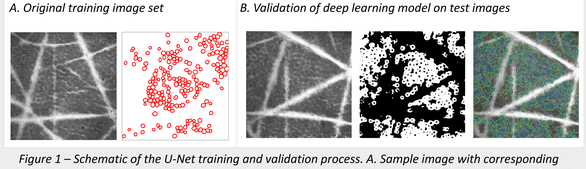


Figure 1 – Schematic of the U-Net training and validation process. A. Sample image with corresponding hand-drawn annotations; B. Test image and machine generated output: probability image ranging from 0 to 1 and probability image as overlay over the original image.

RESULTS

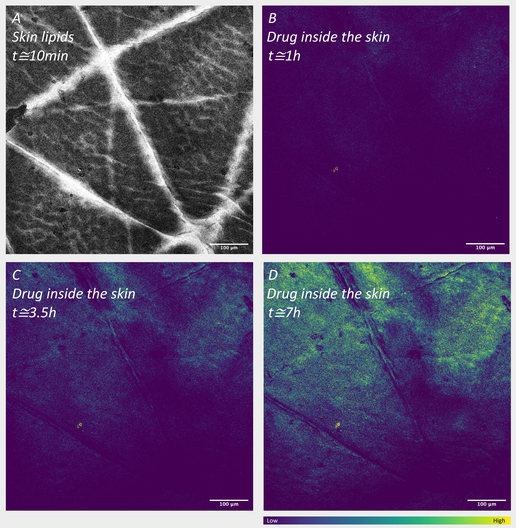


Figure 2 – Monitoring drug delivery into human skin (~8μm depth) by SRS microscopy ex vivo. (A) SRS image (gray-scale) of stratum corneum structure by tuning to the skin lipid CH_2 stretching frequency (2,860 cm^{-1}). (B-D) SRS images showing Taz penetration over time. Images suggest a predominant route of drug permeation via the intercellular lipids. SRS contrast obtained at 1590 cm^{-1}. The images are color-coded with the 'viridis' lookup table to display the regions of low (blue) and high (yellow) Taz uptake.

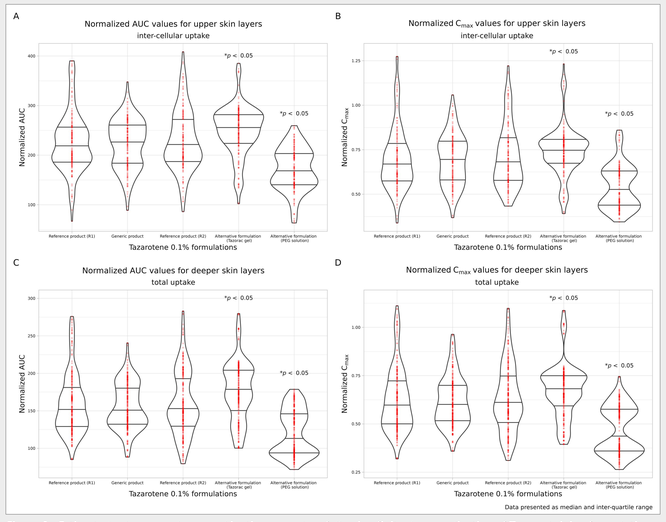
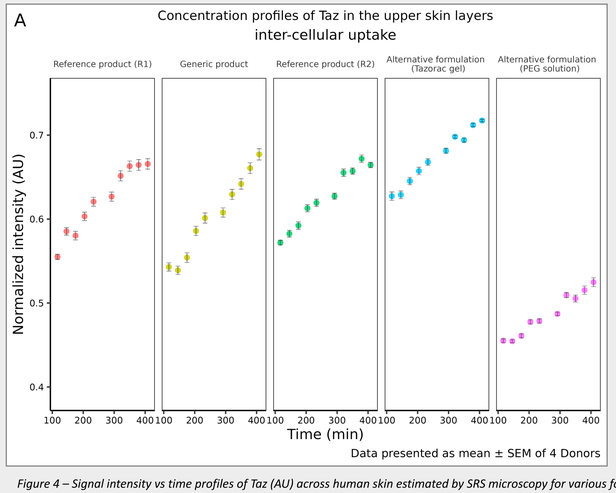


Figure 3 –Estimated cutaneous pharmacokinetic parameters following finite dose application of Taz-containing formulations to human skin ex vivo. Reference product (R1): Tazorac® cream; Generic product (cream): Taro Pharmaceuticals U.S.A., Inc; Reference product (R2): Tazorac® cream; Alternative formulations: Tazorac® gel & Taz in PEG-200 solution (median and inter-quartile range of data from 4 donors; n=4 replicates per donor; 4 regions of interest (ROI) per replicate. Statistical significance determined by Kruskal-Wallis Test and Dunn test for multiple pairwise comparisons. The family-wise error rate was controlled using Dunn's Bonferroni adjustment). (A-B) Peak drug concentration (C_{max}) and area under the drug penetration curve (AUC) values in the upper skin layers (0 – 16μm); (C-D) C_{max} and AUC values in the deeper skin layers (24 – 64μm).



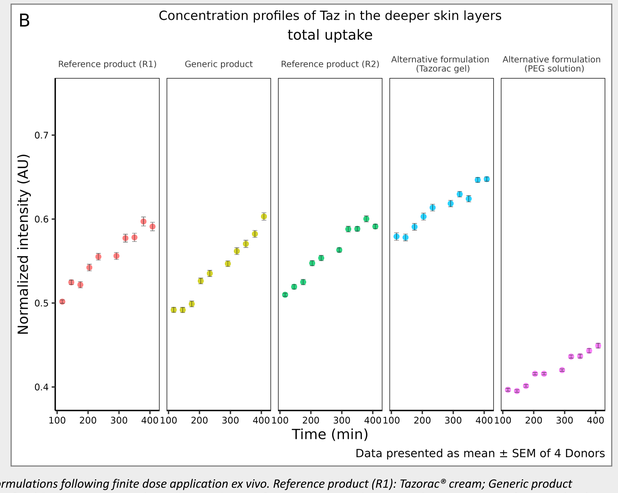


Figure 4 – Signal intensity vs time profiles of Taz (AU) across human skin estimated by SRS microscopy for various formulations following finite dose application ex vivo. Reference product (R1): Tazorac® cream; Generic product (cream): Taro Pharmaceuticals U.S.A., Inc; Reference product (R2): Tazorac® cream; Alternative formulations: Tazorac® gel & Taz in PEG-200 solution (mean ± SEM of 4 donors; n=4 replicates per donor; 4 regions of interest (ROI) per replicate). (A) Inter-cellular Taz uptake in the upper skin layers (0 – 16μm); (B) Taz uptake in the deeper skin layers (24 – 64μm).

CONCLUSIONS

Taz-containing products are typically indicated for the topical treatment of plaque psoriasis and acne vulgaris. Taz exerts its anti-inflammatory and immuno-modulating actions by targeting the keratinocytes in the epidermis, and the bioavailability of the active ingredient from such formulations could be potentially assessed by measuring the local concentration of drug inside the tissue. Here, Taz penetration to the skin was evident from all formulations evaluated. Statistical analysis indicated that the RLD resulted in similar cutaneous PK parameter values of AUC and C_{max} compared to both itself (R1 vs R2; p>0.05) and the generic product (R1 vs Generic; R2 vs Generic; p>0.05). With regards to the alternative formulations, the small dataset suggests that permeation of Taz from Tazorac® gel was slightly higher compared to the other products in the study over ~7h (p<0.05), within the constraints of the current study design. In contrast, PEG-200 solution resulted in significantly lower amounts of Taz uptake by the tissue. (p<0.05).

The findings of this proof-of-concept study demonstrate that SRS is a powerful tool that allows reliable assessment of drug inside the skin and is able to detect differences in the rate and extent of dermal drug absorption from different products. The proposed approach also enables the imaging of drug distribution across the tissue in real time, which can additionally provide insights into drug permeation pathways. Studies are ongoing with additional drug products to examine the sensitivity and robustness of SRS and further explore this method as a novel cutaneous PK-based approach for evaluation of BE.

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