Assessing Dermatological Product Bioequivalence With Raman Spectroscopy

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Background

- Raman spectroscopy is under examination as a tool with which to assess topical drug bioavailability (BA) and dermatological product bioequivalence (BE).
- Particular challenges relate to the at least semi-quantification of drug levels within the viable epidermis below the stratum corneum (SC) and how to correct for Raman signal attenuation as a function of skin depth.

Objectives

- Having recently described a strategy to correct for signal attenuation [1], this research explores the extent to which confocal Raman spectroscopy (RS) and stimulated Raman scattering microscopy (SRS) are able to detect an active moiety within the living layers of the skin and close(r) to the site of pharmacological action.

Methods

4-Cyanophenol (CP) was chosen as a model compound for its strong Raman signal (C≡N vibration) at 2230 cm^{-1} where the skin is infrared-transparent.

In vitro Permeation Testing (IVPT)

Abdominal pig skin samples were prepared for Raman assessment in an *in vitro* skin permeation set-up following a 1, 2 or 6-hour application of saturated 4-cyanophenol (CP) in 50:50 v/v water/propylene glycol (PG). For correlation purposes, CP was also quantified in the SC, viable tissue and IVPT receptor phase (RP) by HPLC-UV.

Confocal Raman Spectroscopy (RS)

RS used a Renishaw inVia microscope. C≡N signals as a function of depth into the skin were normalised by the corresponding Amide I intensity (from keratin) at 1650 cm^{-1} as previously reported [1].

Stimulated Raman Scattering Microscopy (SRS)

SRS images were acquired with a Leica SP8 microscope following a similar to the RS approach

Results

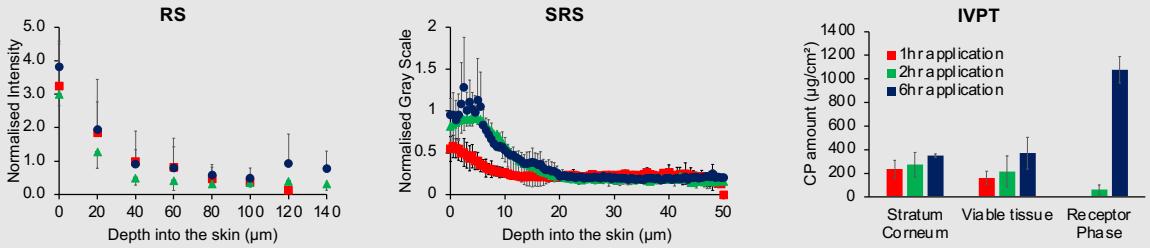

Figure 1: Normalised, "top-down" RS and SRS signals as function of skin depth following application of the CP formulation for 1 (red), 2 (green) or 6 hr (blue) (left and central panels) compared with CP distribution conventionally determined *in vitro* (right panel).

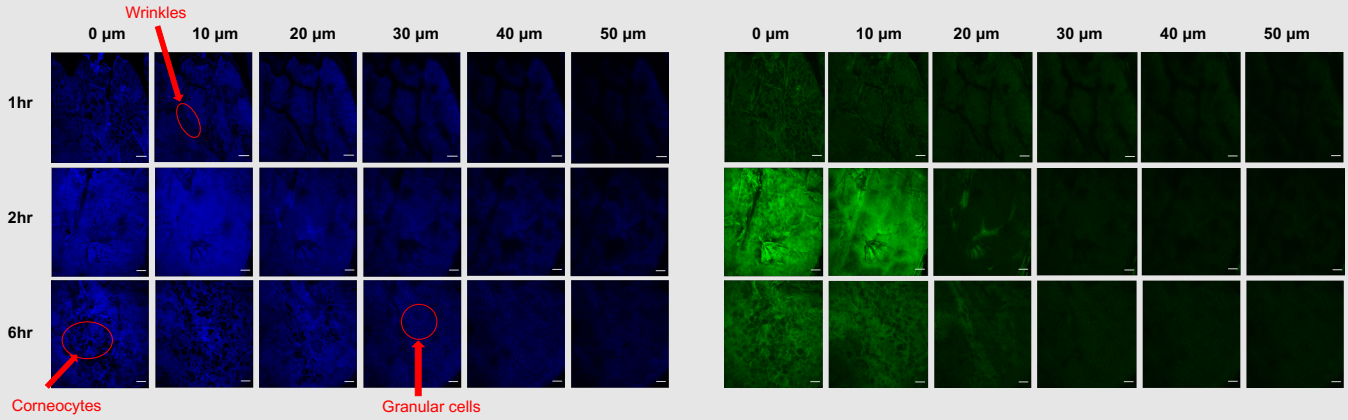

Figure 2: 'Top down' SRS imaging showing Amide I (left) and CP (right) signals after application of the CP formulation for 1, 2 and 6 hr (scale bar = 50 μm)

- Good correlation between the three sets of experimental results was observed (Fig. 1) and both spectroscopic methods were able to detect CP at depths in the skin below the SC.
- Structural features of skin (corneocytes and wrinkles at skin surface, granular cells at deeper layers) were evident with SRS imaging (Fig 2). The Amide I and CP gray scales reduce as a function of skin depth due to signal attenuation from scattering and absorption of the light by the tissue.
- The image stacks generated reveal the progressively deeper penetration of CP with longer times of application (Fig. 2).
- The apparently higher Amide I and CP gray scale intensities seen after the 2-hr application of the formulation cannot be attributed to higher CP penetration as is clearly observed when the normalized data are considered; rather, the differences are due to variability in the thickness of skin samples used which directly affect signal intensity.

Conclusions

Raman spectroscopy and imaging can assess *ex vivo* the rate and extent at which a topically administered drug becomes available in the epidermis below the SC. Further work aims to provide evidence that the routine, facile and non-invasive measurement of drug pharmacokinetics in the skin *in vivo* is achievable and has potential for application in regulatory science and decision-making.

Reference: [1] P Zarmpi et al., AAPS PharmSci 360 annual meeting, USA, 2021: https://www.eventscribe.net/2021/PharmSci360/

Acknowledgements

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