ASSESSMENT OF BIO(IN)EQUIVALENCE OF METRONIDAZOLE TOPICAL FORMULATION USING STIMULATED RAMAN SCATTERING MICROSCOPY

W0930-11-70

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PURPOSE

It has been shown recently that confocal Raman spectroscopy (RS) can track metronidazole (MTZ) permeation into the epidermal skin layers beneath the stratum corneum ex vivo [1].

Bioequivalence of marketed MTZ gels was established, while two different laboratory-made formulations were clearly inequivalent to one another.

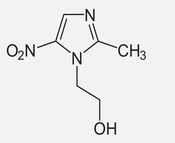
Correlating RS data with independent spectroscopic imaging modalities may support the use of the former as a robust non-invasive tool to assess topical bioavailability/bioequivalence.

OBJECTIVES

To exploit the faster acquisition time of stimulated Raman scattering (SRS) microscopy, to confirm the RS results, and to shed light on the transformation of the MTZ formulations at the drug product-skin interface post-application.

METHODS

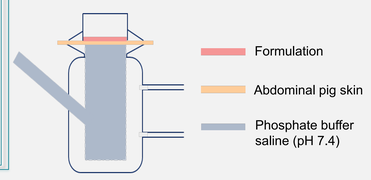
Formulations



Metronidazole (MTZ)

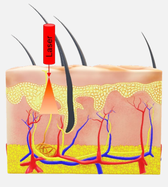
Saturated MTZ solutions in 90:10 or 30:70 v/v water/ propylene glycol (PG) were used.

***In vitro* permeation testing (IVPT)**



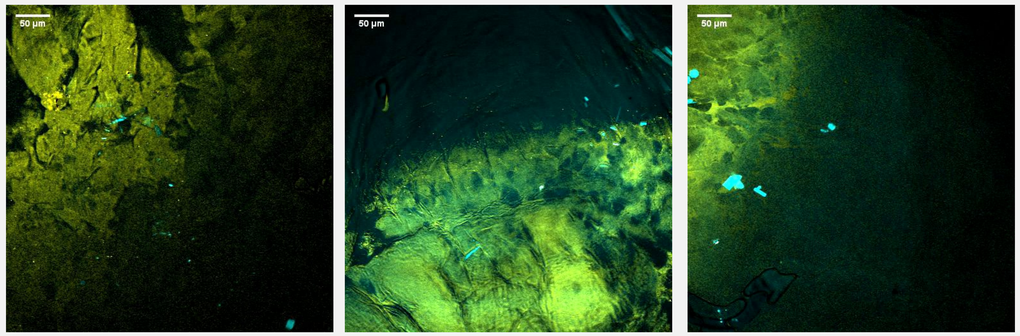
IVPT was performed in Franz cells (32ºC). Each formulation (300 µL) was applied for 6 hours without occlusion. The skin surface was then gently cleaned, and the tissue was stored at -80ºC before analysis.

Stimulated Raman Scattering Microscopy (SRS)



Performed with a Leica SP8 microscope. The sample stage was maintained at 10ºC. Spurious signals were subtracted by data acquisition at off-resonance frequencies. λ scans were performed to identify frequency shifts between dissolved and crystalline drug at or near the skin surface. Then, signals from MTZ (1195 cm^{-1}) and Amide I (1666 cm^{-1}) were recorded as a function of skin depth. MTZ signals were normalised, as before [1]. Experiments were performed in duplicate.

RESULTS



Animation 1: *Skin surface of three different skin pieces after treatment with the 90:10 v/v water/PG formulation for 6 hours. MTZ crystals in cyan are clearly visible against the signal from Amide I in yellow.*

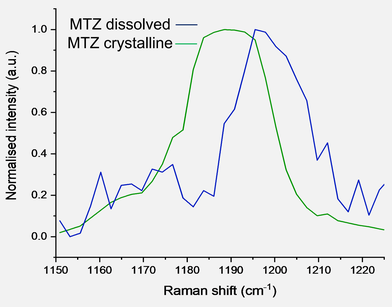


Figure 1: *SRS spectra of MTZ in treated skin areas where crystals are either absent (blue) or present (green).*

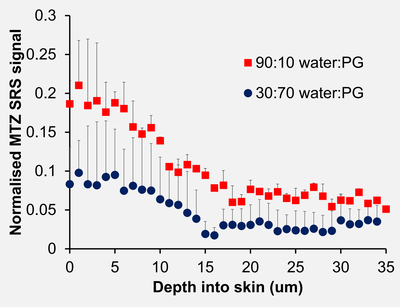
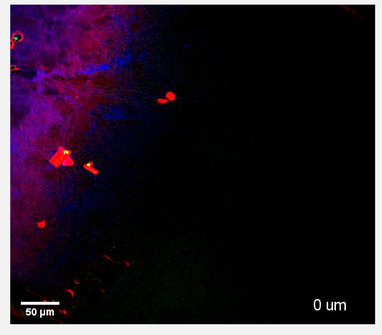


Figure 2: *MTZ signal (normalised by that from Amide I) as function of skin depth after a 6-hr application of two water/PG formulations (mean ± SD, n=12 from each of two skin samples).*



Animation 2: *SRS top-down imaging of the distribution of MTZ (shown in red) in the SC post application of the 90:10 water/PG vehicle. Amide I and the second harmonic generation signals are shown in blue and green, respectively.*

- The earlier confocal Raman data showed that, at 6 hours, the 90:10 v/v water: PG vehicle (but not the 30:70) had evaporated and/or absorbed into the skin [1]. SRS imaging confirmed the resulting, substantial MTZ crystallization (**Animation 1**); furthermore, a characteristic shift in the peak MTZ signal frequency clearly differentiated between dissolved and crystalline drug (**Figure 1**).
- SRS imaging tracked drug distribution in the tissue and (as seen before) showed that, at 6 hours, the 90:10 vehicle delivered more MTZ into the skin compared to the 30:70 v/v (**Figure 2**).
- It is possible that the faster evaporation/absorption of the 90:10 vehicle created a transient state of MTZ supersaturation that temporarily enhanced drug uptake, unlike the slower metamorphosis of the 30:70 formulation.
- Finally, image analysis confirmed the appearance of MTZ in the intercellular lipids of the SC independent of the formulation used (**Animation 2**).

CONCLUSIONS

SRS imaging confirmed that, as observed with RS, there are differences in the amount of drug in the skin when the two laboratory-made MTZ formulations are applied to the skin ex vivo and suggested a mechanism by which this observation might be explained. The added value of SRS microscopy is that the transformation of the vehicle at the interface with the skin can be clearly visualized and that the greater axial resolution permits both skin topology, microanatomy and drug localisation to be identified.

FUNDING / REFERENCE

Reference: [1] P Zarmpi, D. Tsikritsis et al., Skin@Bath Symposium, UK, 2022: https://skinatbath.org/abstracts-oral-poster-presentation/

Acknowledgements: This research is supported by the U.S. Department of Health & Human Services, Food & Drug Administration (1-U01-FD006508 and 1-U01-FD004947). The views expressed do not reflect the official policies of the U.S. FDA or the U.S. DHHS; nor does any mention of trade names, commercial practices, or organization imply endorsement by the U.S. Government.