Visualization of Cutaneous Distribution of Minocycline of a Topical Gel in Human Facial Skin with Two-Photon Excited Fluorescence Lifetime Imaging Microscopy (FLIM) and Phasor Analysis

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Introduction

To limit systemic exposure to antibiotic, and to achieve localized delivery into the skin, a topical minocycline (BPX-01) is being developed clinically to address acne vulgaris by targeting the lesions directly. Knowing the efficiency of topical delivery could translate to a better understanding of clinically effective dose. We have previously demonstrated temporal and transdermal penetration of minocycline with conventional fluorescence microscopy. However, because of poor signal-to-noise (SNR) due to high endogenous tissue fluorescence, this approach is limited for detailed pharmacokinetic analysis. We have therefore developed a novel technique that improves the endogenous tissue fluorescence lifetime imaging microscopy (FLIM) signal-to-noise ratio (SNR) by using confocal line scanning, and two-photon excitation fluorescence (2PEF) microscopy in identifying minocycline with the equivalent of about 3.5 daily doses.

In the current study, we introduced a novel method of visualization and quantification of minocycline within human skin tissue by utilizing a phasor approach to fluorescence lifetime imaging microscopy (FLIM) to further enhance the SNR of the system. We chose to use the subcutaneous region of the face as our model tissue, as most acne vulgaris lesions are present in this area. Skin samples were obtained from 3 patients, with 2 patient’s having lesions of acne vulgaris, and 1, with no skin lesions. Phosphorescence signals from the skin samples were captured using an imaging FLIM system, and were then used to generate phasor plots. From these plots, we can identify the specific molecule by its position in the phasor plot.

Results:

Pharmacokinetic Tomography of a Single Daily Dose of BPX-01

The FLIM experimental setup enabled co-registration of a series of images acquired in the bright field and 2PEF, as shown in Figure 3. Previously, our team has shown that the 2PEF approach alone was adequate in demonstrating minocycline uptake and distribution in an ex vivo human facial skin model with approximately 2.5x daily dose or higher of BPX-01 at 4.5 hrs and 24 hrs.

In the current study, only a single daily dose of BPX-01 was applied. As a result, we found it difficult to determine the 2PEF images if minocycline fluorescence was present when compared to the blanks (negative controls) and the vehicle controls, as endogenous tissue fluorescence appeared to be overwhelming any fluorescence from the drug substance.

However, with the Phasor approach to time-correlated single photon counting fluorescence lifetime microscopy (FLIM), we revealed the presence of minocycline distribution within all anatomical structures of interest in the skin – the epidermis, hair follicle and sebaceous gland – in both the 1% and 2% BPX-01 treatment groups. We observed a general trend among the FLIM images in which minocycline distribution was most concentrated in the epidermis, followed by the hair follicle, and with noticeable trace amount in the sebaceous gland, in a single daily dose treatment after 24 hrs of incubation time. These observations were supported by the amount of identifiable data points associated with minocycline fluorescence among the corresponding Phasor plots from different areas of the skin. The Phasor approach also enabled quantification of local concentrations of minocycline as shown in the far right column in Figure 3.

In conclusion, with FLIM analysis, tissue samples treated with a single daily dose of both 1% and 2% BPX-01 exhibited substantial uptake in the epidermis and the hair follicle. Trace amount of minocycline was also found in the sebaceous gland where it would have otherwise been difficult to identify with 2PEF microscopy.

Quantitative estimation of minocycline local concentrations appeared viable with FLIM-Phasor analysis. This is the first such study designed to demonstrate the potential of FLIM-Phasor analysis in understanding the absorption and distribution of a drug substance, minocycline, as part of a translational research program.

References

1. References

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