Simultaneous NAD(P)H and FAD Fluorescence Lifetime Microscopy reveals long UVA–induced metabolic stress in reconstructed human skin

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ABSTRACT

Solar ultraviolet longwave UVA1 exposure of human skin has short-term consequences at cellular and molecular level, leading at long-term to photoaging. Following exposure, reactive oxygen species (ROS) are generated, inducing oxidative stress that might impair cellular metabolic activity. However, the dynamic of UVA1 impact on cellular metabolism remains unknown because of lacking adequate live imaging techniques. Here we assess overtime the UVA1-induced metabolic stress response in reconstructed human skin with multicolor two-photon fluorescence lifetime microscopy (FLIM). Simultaneous imaging of the two endogenous biomarkers nicotinamide adenine dinucleotide (NAD(P)H) and flavin adenine dinucleotide (FAD) by wavelength mixing allows quantifying cellular metabolism in function of NAD(P)H/NAD(P)H and FAD/FADH2. We measure NAD(P)H and FAD fluorescence lifetime and fraction of bound coenzymes both in keratinocytes in the epidermis basal layer and in fibroblasts in the dermis superficial layer. After UVA1 exposure, we observe an increase of fraction of bound NAD(P)H and decrease of fraction of bound FAD indicating a metabolic switch from glycolysis to OXPHOS or oxidative stress possibly correlated to ROS generation. NAD(P)H and FAD biomarkers have unique temporal dynamics and sensitivities to skin cell types and UVA1 dose. While FAD biomarker is UVA1 dose-dependent in keratinocytes, NAD(P)H biomarker shows earlier time points modulation in fibroblasts, thus reflecting different skin cells sensitivities to oxidative stress. Finally, we show that a sunscreen including a UVA1 filter MCE prevents UVA1 metabolic stress response from occurring.

Keywords: FLIM, endogenous biomarkers, metabolic imaging, multicolor two-photon

1. INTRODUCTION

Solar ultraviolet (UV) rays constitute one of the most important environmental stress to which human skin is constantly exposed. While UVB rays penetration is limited to the epidermis, UVA rays penetrate deeper within skin dermis and can cause dermal damage with long-term consequences resulting in the appearance of photoaging clinical signs such as wrinkles, loss of skin elasticity and dyspigmentation and photocarcinogenesis [1]. Longwave UVA (UVA1, 340-400 nm) exposure has several important consequences both at cellular and molecular level as the photoexcited states of skin endogenous photosensitizers generate reactive oxygen species (ROS) [2]. Despite light-driven ROS formation are now widely accepted as contributors to skin photoaging and photocarcinogenesis [3, 4], the dynamic of the metabolic stress response in the skin as well as its modulation with UV exposure remains largely unknown due to the lack of non-invasive techniques to monitor the energetic metabolism in live tissues. Multiphoton microscopy of endogenous biomarkers has shown important potential for label-free and non-invasive monitoring of cellular metabolic processes in living tissues [5]. NAD(P)H and FAD are the most important metabolic cofactors of redox (reduction/oxidation) reactions in the cell and central regulators of mitochondrial ATP production and
antioxidant defense [6]. The fluorescence lifetimes of NAD(P)H and FAD are exquisitely sensitive to enzyme binding during the cycling of the electron transport chain [7]. Therefore, multiphoton FLIM imaging of the metabolic coenzymes NAD(P)H and FAD can provide functional information on cellular redox ratios (NAD(P)H/NAD(P)+ and FAD/FADH2) and on the complexity of several metabolic pathways (glycolysis, oxidative phosphorylation, oxidative stress, fatty acid oxidation and synthesis) [8-13]. Although quantitatively associating NAD(P)H and FAD lifetime changes to different metabolic cellular pathways remains challenging [14] the two endogenous fluorophores are emerging as complementary biomarkers highlighting metabolic phenotypic heterogeneity [15]. Our group recently implemented simultaneous two-photon excitation of NAD(P)H and FAD by wavelength mixing to acquire FLIM data of the two biomarkers at the same time and perform multiparametric metabolic imaging in dynamic biological system [16].

2. RESULTS

The goal of this study is to assess at different time scales the effects of UVA1 light exposure on the cellular metabolic activity of different cell types in reconstructed human skin using simultaneous fluorescence lifetime imaging of NAD(P)H and FAD [17]. We used a reconstructed human skin in vitro model which includes a dermal equivalent with living fibroblasts and a fully differentiated epidermis. The 3D architecture of such skin model has been shown to be suitable for studying the effects of UV exposure in both epidermal keratinocytes and dermal fibroblasts, especially regarding the oxidative stress response [18]. The skin metabolic state was assessed before and following UVA1 exposure with physiological doses (25 J/cm² and 40 J/cm²), at 30 min and 2 h, in basal epidermal and superficial dermis layers. We present measurements of NAD(P)H and FAD fluorescence lifetimes changes in keratinocytes and fibroblasts after short-term UVA1 exposure. We show that the metabolic response is biomarker, cell type and dose-dependent and appears at very early time points when no visible changes are detected by histology. Finally, we evidence that a sunscreen including a UVA1 filter prevents UVA1 metabolic stress response from occurring [17].

Figure 1: Multicolor two-photon excitation of NAD(P)H and FAD by wavelength mixing to assess temporal dynamics of UVA1 dose effects in reconstructed human skin. (a) Schematic of two synchronized pulse trains from dual-output femtosecond laser used to generate one-color (2PEF) and two-color (2c-2PEF) nonlinear signals epi-detected in two separate spectral channels. The reconstructed human skin sample is kept in a temperature and CO₂ controlled chamber. (b-c) Wavelength mixing (WM) excitation enables simultaneous and efficient 2P excitation of NAD(P)H and FAD by a virtual equivalent wavelength (dotted line) at 880 nm that optimally excites FAD biomarker independently of NAD(P)H. (d) Workflow of UVA1 exposure, FLIM imaging and histological analysis in reconstructed human skin. (e-f) Representative images of NADH and FAD fluorescence intensities (e) and fB fractions of bound NAD(P)H and FAD (f) acquired before (T0) and 2h (Texpo+2 h) post UVA1 exposure at 25 J/cm². (g - h) Representative phasor plot scatters of NAD(P)H and FAD fluorescence lifetime data.
3. REFERENCES

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