Spectrally resolved lifetime imaging of intracellular polarity for a quantitative biological assessment of lipid metabolism in PC12 neuronal cells

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Many neurodegenerative diseases are characterized by a progressive neuronal degeneration (ND), combined with a reduction in the antioxidant capability and/or an increase in oxidative stress. Oxidative stress, that can result from mitochondrial disorders or from glutamate excitotoxicity, is responsible for DNA and protein injuries as well as for lipid peroxidation. All these factors, promoting neuronal and glial degeneration, contribute to the demise of neurons. Importantly, signs of oxidative stress precede the neurological symptoms of ND in cellular models of the disease.

One key consequence of reactive oxygen species (ROS) is the accumulation of lipid droplets (LD) in glial cells. LDs are dynamic intracellular organelles that constitute partitioned reservoirs in which excess lipids are stored in an inert form and, under normal conditions, they are mostly found in liver and adipose tissues. Cells other than adipocytes can form lipid droplets as a response to cellular inflammation and stress, thus proving their key role also in more complex systems. However, how they actively play in the onset and consequent course of neural death is still under investigation [1].

The main aim of this study is to determine whether lipid aggregates normally exist in nervous system and, hence, clarify how the complex pathways of reactions constituting the turnover of lipids and fatty acids are regulated in neurons.

PC12 is a cell line derived from a pheochromocytoma of the rat adrenal medulla, that have an embryonic origin with neuroblastic cells. This allows an easily differentiation into neuron-like cells when treated with nerve growth factor (NGF), making them a useful model system for studying neuronal mutual interaction. Nile Red (9-diethylamino-5H-benzo [α] phenoxazine-5-one) is a fluorescent lipophilic dye characterized by a solvatochromic shift of emission from red to yellow according to the degree of hydrophobicity of lipids. Here, we present a high spatial and temporal resolution-based method for the quantification of the turnover of fatty acids into tryglicerides in live cells. We performed fluorescence lifetime imaging (FLIM) of intracellular micropolarity in PC12 cells treated with NGF to induce differentiation into a neuron-like phenotype to detect micropolarity variations as they occur in time and in different cellular microdomains. Spectrally-resolved changes in Nile Red's fluorescence lifetime are analysed using a global phasor approach. By giving different phasor signature in a metabolic plane, this technique provides a metabolic parameter which quantitatively assesses fatty acids-triacylglycerols turnover and the activation of storage pathways [2]. Moreover, it enables real time unmixing of the contribution of hyperpolar, polar and non-polar classes of lipids at a submicrometer resolution, thus revealing the spatial localization of lipids in different cell types. This method, allowing for a fine-tuned monitoring of intracellular processes responsible for energetic homeostasis, such as LDs formation and lipid remodelling, can constitute a valuable tool to detect imbalances between lipid storage and usage, which may lead to metabolic impairment in neurons [3].

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