Attenuation of mitochondrial damages by dimethyl fumarate, monomethyl fumarate and Biotin in 7β-hydroxycholesterol-treated 158N murine oligodendrocytes

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Background: The biological activity of oxysterols, which are cholesterol oxidation products, depends on the oxidation site. Thus, whereas those oxidized on the lateral chain are involved in the regulation of cholesterol homeostasis, those oxidized at C7 have numerous side effects. Among these oxysterols, 7β-hydroxycholesterol (7β-OHC) is able to trigger oxidative stress leading to a mode of cell death by apoptosis which is associated with morphological, topographical and functional mitochondrial changes. So, it is of interest to identify drugs capable to prevent 7β-OHC-induced mitochondrial damages. Mitochondrial damages are observed in numerous neurodegenerative diseases including multiple sclerosis. In this context, we evaluated on oligodendrocytes (myelin synthetizing cells) the effects of dimethylfumarate (DMF; marketed as Tecfidera®), monomethylfumarate (MMF) (the main metabolite of DMF), and Biotin (marketed as MD1003), which are used as therapeutic molecules in the treatment of Multiple Sclerosis.

Materials/Methods: Murine oligodendrocytes 158N were cultured in the absence or presence of 7β-OHC (50 µM, 24 h) without or with DMF (25 µM), MMF (25 µM) and Biotin (10 nM; 100nM). The effect of 7β-OHC without or with DMF, MMF and Biotin on mitochondria was determined with different criteria: measurement of succinate dehydrogenase activity (MTT test); flow cytometric quantification of transmembrane mitochondrial potential (ΔΨm), mitochondrial mass and mitochondrial superoxide anions ($O_2^{\bullet -}$) production using $DiOC_6(3)$, Mitotracker Red and MitoSOX, respectively; evaluation of the morphological aspect of mitochondria by transmission electron microscopy; characterization and quantification of cardiolipins, organic acids, and fatty acids by GC-MS;.

Results: With 7β-OHC, an alteration of mitochondrial enzyme activity (succinate deshydrogenase) associated with an increase of ΔΨm (reflecting a mitochondrial hyperpolarization and/or an increased of mitochondrial mass) as well as an increase of mitochondrial superoxide anions ($O_2^{\bullet -}$) production were observed. In addition, modifications of fatty acid profile, organic acid levels and cardiolipins content were detected. Mitochondrial

shape and size were also modified. DMF, MMF and Biotin attenuate these mitochondrial changes. They restore the mitochondrial functions, normalize the fatty acid profile, the cardiolipins and organic acids levels and they also prevent 7β-OHC-induced morphological mitochondrial alterations.

Conclusion: DMF, MMF and Biotin, which are used in the treatment of multiple sclerosis, attenuate 7β-OHC-induced mitochondrial damages leading to cell death on 158N murine oligodendrocytes. These data reinforce the interest of DMF and Biotin in the treatment of multiple sclerosis, and open new perspectives to use these molecules in diseases associated with increased levels of 7β-OHC and/or mitochondrial damages.