Fingolimod phosphate protection against mitochondrial damage in neuronal cells.

Martín-Montañez Elisa[1], Gil Antonio[1], Valverde Nadia[2], Lara Estrella[2], de Luque Vanessa[1], Boraldi Federica[3], Urbano María[1], García-Fernández María[2], Pavía José[1].

[1]*Department of Pharmacology and Paediatrics, Faculty of Medicine, Malaga University, Malaga, Spain.*
[2]*Department of Human Physiology, Faculty of Medicine, Malaga University, Malaga, Spain.*
[3]*Department of Life Sciences, University of Modena e Reggio Emilia, Modena, Italy.*

Background: Major role of oxidative stress in the pathogenesis of neurodegenerative diseases have been suggested, being mitochondria one of the main sources of ROS.

Aim: In the present work, we have studied the antioxidant effect of fingolimod phosphate (FP) on neuronal mitochondrial function and morphology using a model of mitochondrial oxidative damage induced by menadione (Vitk3).

Methods: SN4741 neuronal cells were grown (70-80% confluence) and used as control (non-treated cells) or treated cells with Vitk3 15 µM alone or in presence of FP 50 nM during 4 hours. Mitochondrial membrane potential (MMP), cytochrome c oxidase (COX) activity, mitochondrial oxygen consumption rate (OCR), mitochondrial distribution (MTG) and morphology (EM) were analysed. Statistical differences were determined using one-way ANOVA.

Results: Vitk3 incubation produces a dramatical decrease in MMP compared to control (43.7 %); this can be almost totally reverted by the co-incubation of Vitk3 in presence of FP ($p<0.05$). A 20.7 % decrease in COX activity has been found after Vitk3 incubation, again this effect was counteracted when Vitk3 and FP are combined, restoring COX activity to control levels ($p<0.05$). Vitk3 incubation triggers initially an increase in OCR, decreasing dramatically (61%) after 4 hours. In experiments co-incubating Vitk3 in presence of FP, the OCR decrease found was reduced to only 17% ($p<0.05$). In experiments with MitoTracker™ Green, we found a change in the network pattern distribution after Vitk3 administration that partially disappears when co-incubated in presence of FP. Almost all the mitochondria treated with Vitk3 show ultrastructural alterations at the electron microscopy level while normal mitochondria can be found when Vitk3 and FP are combined.

Conclusion: FP protects against the mitochondrial damage induced by Vitk3, as seen by the results obtained in mitochondrial functional markers, distribution and morphology.

Keywords: Fingolimod phosphate, Mitochondria, Oxidative stress.