OVEREXPRESSION OF PINK1 PROTECTS CAD CELL AGAINST C2-CERAMIDE: RELATIONSHIP WITH MITOCHONDRIAL FUNCTION AND THE PI3K/AKT PATHWAY

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Introduction: The etiology of Parkinson's disease (PD) remains unknown. Mutations in several genes, including PINK1, have provided an understanding of the molecular mechanisms of this pathology.

Aim: To analyzed the role of overexpression of PINK1 (wt or mutations G309D - L347P) on the neurotoxicity associated to C2-ceramide exposure in CAD cells.

Methodology: CAD cells were transiently transfected with PINK1 (wt or mutated) or with empty vector and then treated with 25 µM C2-ceramide for 6 hours. Cell viability and mitochondrial membrane potential was analyzed by flow cytometry. Expression of Bax and Bcl2 was determined by Real-Time PCR and AKT phosphorylation was analyzed by Western blot.

Results: Cells with overexpression of PINK1 wt and treated with C2-ceramide showed a decrease percentage of depolarized mitochondria, decrease expression of Bax and increase expression of Bcl2, in comparison to non-transfected cells. In addition, PINK1 rescued the C2-ceramide-induced inhibition of AKT phosphorylation. Overexpression of PINK1 G309D mutation caused an increase in depolarized mitochondria, decrease in Bax and increase in Bcl2 expression levels. PINK1 L4347P mutation was associated with a higher drop in mitochondrial membrane potential, increased expression of Bax with minimal variation in the expression of Bcl2. The effect of PINK1 mutations on AKT phosphorylation showed no variations.

Conclusion: PINK1 confers a neuroprotective effect against the endogenous neurotoxin C2-ceramide by regulating mitochondrial function and through reinforcement of anti-apoptotic and neuronal survival pathways such as Bcl2 and PI3K/AKT. These effects were abrogated by PINK1 mutations.

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