

## RhoA inhibition by C3 prevents dopaminergic neuronal degeneration in rotenone induced neurotoxicity

Letizia Mattii<sup>1</sup>, Antonietta Rm Sabbatini<sup>2</sup>, Chiara Ippolito<sup>1</sup>, Carla Pardini<sup>3</sup>, Francesca Vaglini<sup>3</sup>

<sup>1</sup>Dept of Clinic and Experimental Medicine, Sect. Histology, University of Pisa, Pisa, Italy - <sup>2</sup>Dept of Surgical, Medical, Molecular Pathology and of Critical Care, University of Pisa, Pisa, Italy - <sup>3</sup>Dept of Translational Research on New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

Parkinson disease (PD) is a complex neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons. Mitochondrial dysfunction, oxidative stress or protein misfolding may underlie this process. Rotenone, a natural substance widely used as a pesticide, produces selective degeneration of dopaminergic neurons. Rotenone is a membrane-permeable compound that has two known molecular targets in the cell: it inhibits complex I in the mitochondrial respiratory chain and depolymerizes microtubules. Microtubule depolymerization would disrupt vesicular transport and cause the accumulation of vesicles in the soma. RhoA, a member of the Rho GTP-binding proteins, activates several effector proteins which target actin, actomyosin and microtubules. Here, we use an *in vitro* rotenone-induced PD mouse model to verify the neuroprotective effects of C3, an exoenzyme produced by *Clostridium botulinum* that inactivates RhoA by ADP-ribosylation. Mesencephalic cell cultures from CD1 mice were performed, at D.I.V. 6 the cells were treated with or without 2.5  $\mu\text{g}/\text{ml}$  of C3; 4 hours later 25 nM of Rotenone was added. 24 hours later [<sup>3</sup>H] dopamine uptake and immunocytochemistry with anti-TH and anti-RhoA antibodies were performed. In TH<sup>+</sup> neurons Rotenone reduced dopamina uptake to 45% and activated RhoA that was found at level of the cell membrane. Pre-treatment with C3 was able to protect mesencephalic cells from rotenone toxicity to about 40% preventing the translocation of RhoA from the cytoplasm to the plasma membrane as observed by confocal analysis of immunostained cells. Our data indicate that the inhibition of RhoA by C3 is able to partially rescue the rotenone induced damage by probably preventing microtubules depolymerization.

### Keywords

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Parkinson disease; Rotenone; RhoA; C3; PD mouse model.