ESCULETIN PROTECTS AGAINST MUTANT HUNTINGTIN-INDUCED TOXICITY IN HUNTINGTON'S DISEASE MODELS

Letizia Pruccoli[1], Carlo Breda[2], Flaviano Giorgini[2], Gabriella Teti[3], Mirella Falconi[3], Andrea Tarozzi[1]

[1]Department for Life Quality Studies - University of Bologna, Rimini - Italy, [2]Department of Genetics and Genome Biology - University of Leicester, Leicester - United Kingdom, [3]Department of Biomedical and Neuromotor Sciences - University of Bologna, Bologna - Italy

Introduction: Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the coding region of the HD gene. An increase in the size of the CAG segment leads to the production of an abnormal expanded huntingtin (HTT) protein that is responsible of significant impairments in the proteostasis network, dysregulated transcription, mitochondrial toxicity, cellular energy imbalance, synaptic dysfunction and axonal transport impairment. Numerous *in vivo* and in vitro studies have documented the protective role of various natural products including phenolic compounds with multiple cellular targets in the prevention of neurodegenerative disorders, including HD. In our research work, we evaluated the potential neuroprotective effects of the phenolic coumarin esculetin (ESC) against the toxic events occurring in HD pathogenesis using different HD models.

Methods: We used an inducible cell model (PC12HD-Q74) and a transgenic Drosophila melanogaster model of HD (HTT93Q, pan neuronal expression), both of which express mutant HTT exon 1fragments. The expression of HTT exon 1fragment with 74glutamines in PC12HD-Q74cells was induced by 72hours of incubation with doxycycline (DOX) (1μg/mL). ESC (5μM) was added during the last 24hours of incubation with DOX. We evaluated the ability of ESC to counteract the formation of mutant HTT protein aggregates and the neurotoxicity in terms of cytostatic effect and necrosis. In parallel, we investigated the antioxidant effects of ESC against the oxidative damage induced by mutant HTT. Finally, we focused on the ability of ESC to ameliorate the mitochondrial activity, in terms of mitochondrial area and ATP levels. Further, several neurodegenerative parameters were evaluated in Drosophila such as the progressive loss of rhabdomeres, the lethality of larvae and the reduced survival time in adult flies. Appropriate crosses were carried out in order to obtain the desired genotype and ESC was mixed in fly food at 10 and 100 μM for growing experimental larvae or flies.

Results: The treatment with ESC partially modulated the aggregation of mutant HTT protein in PC12HD-Q74cells, ameliorated the cell proliferation and counteracted the necrosis induced by mutant HTT. Further, ESC showed to counteract the oxidative stress as well as to increase the intracellular glutathione levels in PC12HD-Q74cells. ESC also ameliorated the mitochondrial activity of PC12HD-Q74cells, restoring the mitochondrial area and increasing the ATP levels. In addition, ESC significantly counteracted photoreceptor neurodegeneration in both newly emerged flies (day 0) and adult flies (day 7post eclosion). In parallel, ESC enhanced in a dose-dependent manner the emergence of adult HD flies from the pupal cage and improved the median life span in HD flies.

Conclusion: Our results show that ESC ameliorated several disease-related neurodegenerative dysfunctions in both PC12HD-Q74and Drosophila models, suggesting its potential development as a novel neuroprotective agent for the treatment of HD.