Pharmacological activation of autophagy exerts neuroprotective effects against amyloidogenic prion peptide neuronal toxicity

S. Thellung[1,2], B. Scoti[1], A. Corsaro[1], V. Villa[1], M. Nizzari[1], L. Colucci-D'Amato[3], K. Cortese[4], F. Birocchi[4], C. Tacchetti[4], T. Florio[1,2]

[1,2]Sect. of Pharmacology, Dept. of Internal Medicine, University of Genova; Center of Excellence for Biomedical Research (CEBR), V. le Benedetto XV. 2, 16132 Genova, Italy
[3]Dept. of Environmental, Biological and Pharmaceutical Sciences and Technologies, Second University of Napoli, Caserta, Italy
[4]Sect. of Human Anatomy, Dept. of Experimental Medicine, University of Genova, Italy

Macroautophagy (often simply named as autophagy) is a evolutionarily conserved mechanism by which mammalian cells survive nutrient deprivation through the digestion and recycling of intracellular organelles. A growing body of evidence indicates that activation of autophagy represents a novel mechanism to promote neuronal survival. In particular in neurodegenerative diseases in which misfolded amyloidogenic proteins alter by intracellular aggregation, organelle functioning autophagy activation can cause their clearing from the cytoplasm. Importantly, failure of autophagic clearance of amyloidogenic proteins has been described in association with several neurodegenerative disorders, such as Parkinson and Alzheimer's diseases. Our study was headed to demonstrate the efficacy of pharmacological strategies which modulate autophagy to contrast the neurotoxicity of amyloidogenic peptides. We induced neurotoxicity in c-myc immortalized murine mesencephalic nurons (cell line A1) using the amyloidogenic C-terminal fragment of the prion protein (PrP90-231), whose neurotoxicity in vitro is associated with its propensity to be internalized and form cytoplasmic insoluble and protease-resistant aggregates. PrP90-231 induced A1 cell death and stimulated the increase in number and size of acidic vesicles. PrP90-231 treatment also increased the expression and the cytosolic distribution of hydrolytic enzyme cathepsin D (CatD) indicating that intracellular aggregation of the peptide impairs lysosomal integrity. Analysis of protein expression showed that PrP90-231 increased the expression of autophagic markers such as LC3-II and beclin1; moreover, by transmitted electron microscopy, we observed that PrP90-231 condensates within autophagic vacuoles, whose number was significantly increased in treated cell as compared to vehicle-treated control cells. Activation of autophagy flux by either by nutrients deprivation, or by pharmacological tools including rapamycin inhibition of mTOR kinase or treatment with valproic acid (a mTOR-independent activator of autophagy), significantly reduced A1 cell death and the amount of intracellular PrP90-231 aggregates. Altogether, these data indicates that PrP90-231 internalization produces a defensive response in A1 neurons characterized by the induction of an autophagic response, that can to be effective as neuroprotective mechanism when is pharmacologically enhanced. Thus the activation of neuronal autophagic flux could represent a promising tool to develop innovative neuroprotective pharmacological strategies.