## PHARMACOLOGICAL RESTORATION OF AUTOPHAGIC FLUX IN NEURONAL CULTURES PROTECTS FROM TOXICITY OF PRION PROTEIN FRAGMENT 90-231

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Neurons rely on autophagy to maintain cytoplasm homeostasis through the removal of redundant or damaged organelles and malfunctioning proteins. The occurrence of autophagic defects in association with amyloidosis of central nervous system underscores the relevance of such process to prevent intracellular accumulation of pathogenic proteins and ensure neuronal long term survival. Despite their rarity, transmissible spongiform encephalopathies (TSEs) are paradigmatic of protein conformational disorders of nervous system in which posttranslational alteration of host-encoded proteins triggers processes of amyloidogenesis along with neurotoxic oligomers are generated. Increased autophagosomes and signs of impaired autophagy often hallmarks brain areas in which neuronal rarefaction is prominent indicating that its regulation could represent a valuable target of neurorotective strategies for TSEs and other more common neurodegenerative amyloidosis.

This work was aimed to understand the relevance of autophagic activation in neurons after their exposure to amyloidogenic peptides and to understand if its pharmacological modulation may have relevance in improving neuronal survival.

As a model of neurotoxicity we used a recombinant polypeptide that encompasses the aminoacidic sequence 90 to 231of human prion protein (PrP90-231) that corresponds to C-terminal protease-resistant fragments that are generated by Intracellular process of pathogenic prion protein scrapie ($PrP^{Sc}$) whose aggregation produces neurotoxic oligomers. We used mes c-myc A1(A1) cells that derives from immortalization of mesencephalic neuronal progenitors; they display a high sensitivity to PrP90-231and a remarkably efficient modulation of autophagy.

PrP90-231is cytotoxic in vitro, accumulates as insoluble aggregates in the cytoplasm and perturbs lysosomal and mitochondrial stability. We addressed the hypothesis that PrP90-231could cause a derangement of proteostasis mainly through the alteration of autophagy competence. Electron microscopy analysis showed that A1exposure to the peptide reduced autophagosomes/autolysosomes ratio, leading to a significant increase of vesicles containing electron-dense material. In accordance, PrP90-231stimulated a time-dependent increase of p62accumulation in the cytoplasm, indicating that the digestion of the cargo material within autolysosomes is reduced. The level of autophagy, although already elevated in A1cells under basal conditions, can be furtherly stimulated by deprivation of growth factors or treatment with rapamycin and valproic acid. Growth factor deprivation and both drugs protected cells against PrP90-231toxicity; rapamycin also increased the number of autophagosomes, counteracted p62increase and reduced the amount of intracellular PrP90-231. The neuroprotective effect of rapamycin was not strengthened by growth factors deprivation or by valproic acid, but was reverted by autophagy inhibitor 3metyladenine indicating that the activity of rapamycin passes exclusively through the increase of autophagy.

These data suggest that PrP90-231internalization blocks the autophagic response in A1neurons leading to the accumulation and destabilization of autolysosomes. Rapamycin and other autophagy inducers may be helpful to restore autophagosomes efficiency in cargo digestion thus preventing autolysosomes destabilization and neuronal death.