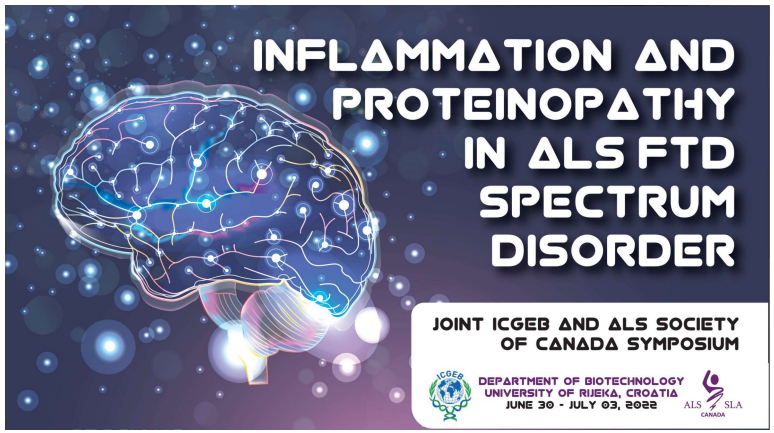


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## PROGRAM

**Mechanism of paroxetine-mediated autophagic induction in cell models of ALS/FTD**

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Protein aggregation is a hallmark of neurodegenerative diseases (NDs), including Amyotrophic Lateral Sclerosis/Frontotemporal dementia (ALS/FTD). The *C9ORF72*-hexanucleotide expansion G4C2 represents one of the most frequent genetic causes of ALS/FTD diseases. One molecular mechanism underlying *C9ORF72*-pathology is the accumulation of dipeptide repeats (DPRs) encoded by G4C2 expansion. Also, TDP-43 inclusions are present in the 98% of ALS and 50% of FTD cases. It has been postulated that aggregates represent a protective response of the cells, aimed to compartmentalize harmful substrates for subsequent removal. However, persistent aggregates cause physical damage on intracellular components, and sequester key factors of the protein quality control (PQC) system. PQC enhancement for aggregates clearance represents a therapeutic approach under investigation in ALS/FTD. Here, we aimed to dissect the mechanism of autophagic induction of the antidepressant paroxetine in NeuroblastomaXSpinal Cord 34 (NSC-34) motoneuron-like cells. We show that paroxetine induced the expression of autophagic markers LC3, SQSTM1/p62 and LAMP1, by RT-qPCR. Also, an enhancement of the autophagic flux was observed, with appearance of LC3 and SQSTM1/p62 puncta in immunofluorescence, increased protein levels and LC3-I/LC3-II conversion in western blot. Paroxetine is a cationic amphiphilic drug, known to induce lysosomal membrane permeabilization (LMP). Indeed, we observed an induction of lysosomal damage upon paroxetine treatment using galectin-3 puncta assay. We found that LMP triggered the activation of Transcription Factor EB (TFEB), a master regulator of autophagy, suggesting lysophagy induction for damaged lysosomes turnover. Therefore, we tested if paroxetine-mediated autophagic induction favoured the clearance of protein aggregates. Indeed, we observed a decrease in high molecular weight insoluble species and aggregates of ALS/FTD substrates, such as the toxic fragment of TDP-43 (TDP-25) and the DPR polyGA.

In conclusion, these results suggest that paroxetine, by inducing a cell protective response, may be beneficial in the removal of harmful aggregates in ALS/FTD.