Preventing the accumulation of cytotoxic oligomers that are causative agents of neurodegenerative diseases and Type 2 Diabetes, by activation of the 20S proteolytic system

Conformational diseases are an extremely serious and growing social problem. They affect not only patients, but also their caregivers and companions, and generate high costs for both individuals and society. A rapidly aging population, the sedentary lifestyle and changes in a diet constitute the reasons for which an increasing number of individuals suffers from both metabolic diseases and neurological and cognitive dysfunctions, characterized by progressive neurodegeneration and dementia. Of all dementing disorders, Alzheimer's disease (AD) is the most common form, comprising 50-70% of all cases. Worldwide, it is estimated that nearly 50 million people live with AD or a related form of dementia. This number will more than triplicate by 2050. In parallel to the global increase in neurodegenerative disorders, the number of people with a metabolic disease, Type 2 Diabetes mellitus (T2DM), has reached more than 415 million in 2015. The number is expected to increase to 642 million by 2040. A key role in Alzheimer's disease plays the amyloid β peptide (Aβ) that forms insoluble aggregates in the brain. The peptide shares its amyloidogenic properties with amylin (islet amyloid polypeptide, IAPP) which forms aggregates in the pancreas. Accumulation of these aggregates results in β-cell death and a deficiency in insulin production, which causes development of Type 2 Diabetes. T2DM is responsible for a number of secondary complications such as stroke, heart attack, blindness and renal failure.

Recent findings indicate that the most toxic to cells are not amyloid fibrils, but soluble oligomers, which precede fibrillar deposits on the aggregation pathway. These oligomers could be removed from cells and their toxicity would be limited if intracellular proteolytic systems were working properly. Unfortunately, the main enzyme responsible for the degradation of abnormal proteins, 20S proteasome, is inhibited by the pre-fibrillar soluble oligomers. This has been confirmed in the case of selected biomolecules associated with neurodegenerative diseases, namely the Aβ peptide associated with Alzheimer's disease, α-synuclein, the aggregation of which is associated with Parkinson's disease, and the mutated huntingtin, whose deposits were found in people suffering from Huntington's disease. Therefore, **the main goal of this proposal is to discover if it is possible to prevent the accumulation of oligomeric forms of proteins by activating the 20S proteolytic system.**

In our studies, we are going to investigate if proteasome inhibition by soluble oligomers is a general mechanism, applicable also to aggregating polypeptides/proteins which are not related to neurodegenerative diseases. To verify this hypothesis we plan to carry out tests using human amylin, associated with the development of T2DM. We will synthesize amylin and subject it to oligomerization. Then, we will test the ability of oligomers of different order to inhibit the 20S proteasome. Next, we will use oligomers of both amylin and Aβ with a photo-crosslinking residue incorporated to the sequence to identify the oligomer-proteasome interface. Separation of the complexes by means of 2D electrophoresis, and subsequent enzymatic digestion and mass spectrometry analysis will allow to determine the interacting regions in both the oligomer and the proteasome. Further questions to which we want to get the answer is the ability of small molecule activators to counteract the inhibition of the proteasome by oligomers, and whether it is possible to use these activators to increase the efficiency of the oligomers degradation by proteasome. The modulators we are going to test were obtained during the implementation of our previous grant. We also plan to obtain and test new modulators of 20S.

Currently, there is no effective pharmacological treatment for conformational diseases, not only affecting central nervous system, but also other tissues and organs throughout the body. The approach proposed by us to counteract cytotoxic processes in the course of diseases related to protein aggregation, is absolutely novel. Studies we are going to perform in the presented project should allow to develop small molecules to counteract proteasome impairment via soluble oligomers. Such interventions have the potential to restore proteostasis in patients suffering from both neurodegenerative and metabolic diseases associated with oligomerization of proteins.