Abstract

Neurodegenerative diseases (e.g. Alzheimer's disease, Parkinson's disease) are associated with impaired functioning and gradual death of neurons. In recent years, the number of people affected by these diseases has increased significantly, which is why neurodegenerative diseases are considered the plague of the 21st century. It is estimated that the number of patients with the Alzheimer's disease will triple by 2050, mainly as a consequence of increasing life expectancy. Due to the lack of effective treatment, difficulties in early detection of the disease, and the costs of care, neurodegenerative diseases constitute a serious global socio-economic problem.

A characteristic feature of neurodegenerative diseases is the deposition of damaged proteins (tau protein and amyloid β in Alzheimer's disease, α-synuclein in Parkinson's disease), which are removed in healthy cells by proteolytic systems.

In physiological conditions, the degradation of incorrectly shaped protein structures is performed, among others, by 20S proteasome - a multiprotease consisting of 28 subunits forming four heptameric, coaxially arranged rings. The α rings located outside are not proteolytically active. Their main function is to control access to active sites located inside the catalytic channel. Proteolytic activity in eukaryotes is demonstrated by three β subunits of each ring: β1 (caspase-like), β2 (trypsin-like), β5 (chymotrypsin-like).

Unfortunately, over time, the enzymatic efficiency of the proteasome decreases, which facilitates the development of neurodegenerative diseases. The results of recent studies demonstrate that the use of small-molecule modulators to activate the proteasome can prevent the accumulation of damaged proteins and may be an effective therapeutic strategy.

The aim of this doctoral dissertation was the synthesis of proteasome stimulators capable of penetrating cell membranes and stable under proteolytic conditions. These modulators were designed based on the Blm-pep activator sequence. In order to obtain modulators with increased resistance to the action of proteolytic enzymes, modulators with modifications such as peptoid bonds or N-methylated amino acids were introduced. Activators having duplicated the C-terminal fragment of the parent modulator were also synthesized in order to obtain compounds capable of binding in two adjacent pockets of the 20S proteasome.

In the first stage, the activity of the obtained activators was tested. It was confirmed that the introduction of modifications in the N-terminal region of the sequence did not negatively affect

the ability to activate the 20S proteasome, unlike changes in the C-terminal fragment. In the next stage, proteolytic stability tests in human plasma were performed, which showed that the obtained peptidomimetic analogues are characterized by increased stability compared to the starting compounds. MS analysis verified that there is no degradation at the site of modification. Then, in order to obtain activators capable of penetrating cells, a cell penetrating peptide fragment (CPP): tat or 6r was added to the selected sequences. Using the activator-CPP sequences labeled with the NBD fluorophore, the cellular permeability of the obtained compounds was assessed by fluorescence microscopy. The obtained images showed fluorescence coming from NBD fluorophore inside HEK293T cells. In the subsequent stages of the research, it was demonstrated that the compounds were not cytotoxic to cells, which was verified using the MTT cytotoxicity assessment test.

The activators with the CPP sequence were able to stimulate the proteasome in cell culture, which was confirmed by testing the enzyme activity in cell lysate and inside the cells, using the 20S proteasome-specific, cell-permeable, fluorescent TAS3 probe.

The effectiveness of selected activators was tested using model protein substrates. The tested compounds were able to stimulate the 20S proteasome to digest tau protein and α-synuclein more efficiently. Activators with the CPP sequence attached were also able to cross the blood-brain barrier, while the permeability of the starting compound reached the level of the negative control. In the next stage of the research, studies using the MST technique were carried out, which confirmed the existence of interactions between the peptidomimetics and the 20S proteasome.

For selected activators, tests were performed on a mouse embryonic cell model. Compounds A1-tat, A1-6r and Bis1-tat were able to stimulate the proteasome to efficiently degrade α-synuclein in hippocampal cells. Moreover, after seven days of incubation, these compounds did not reduce the survival of neuronal cells.

Due to the high level of proteasome activation, stability in proteolytic conditions and the ability to penetrate cells, the obtained activators have desirable features that enable their therapeutic use. Blm modulators could restore proteostasis in patients suffering from age-related diseases characterized by the accumulation of damaged proteins.