lek. Łukasz Adrian Poniatowski

Wpływ domózgowych podań Atsttrin na procesy neurodegeneracyjne i rozwój reakcji zapalnej w doświadczalnym modelu choroby Parkinsona wywołanym 1-metylo-4-fenylo-1,2,3,6-tetrahydropirydyną (MPTP) u myszy

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu
w dyscyplinie nauki medyczne

Promotor: dr hab. n. med. Ilona Joniec-Maciejak
Promotor pomocniczy: dr n. med. Adriana Wawer

Katedra i Zakład Farmakologii Doświadczalnej i Klinicznej
Warszawski Uniwersytet Medyczny

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STRESZCZENIE W JĘZYKU ANGIELSKIM

Parkinson's disease is one of the most common chronic neurodegenerative disease affecting the central nervous system (CNS). It is estimated that approximately 10 million people worldwide are diagnosed with Parkinson's disease. The occurrence of the disease is largely associated with deterioration in the quality of life and, despite conservative and surgical treatment, leads to a gradual loss of independence and disability, mainly in the elderly population. The classic definition of Parkinson's disease assumes the occurrence of cardinal motor symptoms, such as slowness of movement, resting tremor, muscle stiffness and comprehensive disorders of postural reflexes, expressed comprehensively in the form of hypertonic-hypokinetic syndrome. Non-motor disorders of the disease include, among others, autonomic disorders, neuropsychiatric disorders associated with deterioration of cognitive functions, sensory dysfunctions and sleep disorders, the severity of which usually does not correlate with the current state of the patient's motor skills. The occurrence of neurological symptoms is the result of comprehensive imbalance between neurotransmitter systems, not only in the basal ganglia, but in the entire neuronal system controlling motor functions. Subsequent projection disturbances in other neurotransmitter systems cause the appearance of non-motor symptoms that go beyond typical axial symptoms. Currently, several groups of etiopathological risk factors for Parkinson's disease with different significance have been described and characterized. A number of identified modifiable and non-modifiable factors that predispose to a high degree to the occurrence of the disease, based on the multifactor theory, include advanced age, gender, environmental factors, genetic factors, as well as lifestyle and diet. The essence of the pathogenesis of Parkinson's disease constitutes a cascade of events leading to the loss of dopaminergic neurons controlling motor functions with subsequent depigmentation of the substantia nigra (SN), accompanied by the consolidation of dysfunctional neurofibrillary protein compounds containing alpha (α)-synuclein (ASN) and decrease in the level of dopamine (DA) within the structures of the nigrostriatal system. Other pathogenic factors include mitochondrial dysfunction, oxidative stress, glutamate (GLU) excitotoxicity, proteasome dysfunction, as well as the inflammatory reaction. It is currently believed that the local and generalized response of the immune system plays a significant role in the pathogenesis of Parkinson's disease, as well as other neurodegenerative diseases. Moreover, it is also known that the neuroinflammatory

reaction within the CNS is a process that, on the one hand, may intensify the phenomenon of neurodegeneration, as well as constitute a protective, compensatory mechanism activated in the area of damaged neurons. The analysis of the ever-increasing number of reports draws attention to the special role of the neuroinflammatory reaction in the pathogenesis of Parkinson's disease. Currently, many independent authors focus their work on the identification and precise description of inflammatory mechanisms responsible for the development of neurodegenerative processes, indirectly pointing to the particular importance of this issue and the development of this field of research. The complex course of Parkinson's disease, as well as the pursuit of optimization and obtaining the best treatment effects, requires multidisciplinary care and an interdisciplinary approach. The model of comprehensive, coordinated therapy and care includes pharmacological treatment, rehabilitation, psychotherapy, surgical treatment, as well as other non-pharmacological therapies. The main goals of treatment in Parkinson's disease include slowing the progression of the disease, reducing the severity of motor symptoms, preventing or delaying the onset of complications, and controlling non-motor symptoms. The most important goal of Parkinson's disease treatment is to permanently replenish DA deficiency and, as yet unachieved, to rebuild normal cortical-subcortical pathways in the brain, which is currently the subject of ongoing research in the experimental phase. Currently, a parallel research direction in the treatment of Parkinson's disease is the enrichment of the nervous tissue with specific compounds and proteins through direct intracerebral administration using stereotaxic methods of active forms of growth factors, neuromorphogens, cytokines, neuroprotective compounds and recombinant forms of derivatives of these compounds to the deep structures of the brain or the ventricular system. The essence of the currently set modern directions and challenges in the treatment of Parkinson's disease is to improve and optimize the pharmacological treatment, as well as the application of the achievements of regenerative medicine in the field of possibly permanent replenishment of DA deficiency by rebuilding normal cortical-subcortical pathways and connections in the brain, resulting in clinical improvement in treated patients. Unfortunately, despite enormous progress in understanding the pathomechanisms underlying Parkinson's disease, there is still no causal therapy available that would effectively stop or slow down its natural course. Therefore, research and search for new effective pharmacological compounds, methods of potentiating the action of previously known drugs and innovative surgical methods of

Parkinson's disease therapy are currently underway. Current knowledge and experimental data on individual concepts of the mechanisms of death of dopaminergic neurons located within the SN are the result of significant development of research techniques using animal experimental models of Parkinson's disease. The starting point for their development was, to a large extent, the detection of a number of compounds with strong neurotoxic properties in relation to dopaminergic neurons located within the nigrostriatal system. The basic experimental models of Parkinson's disease that are currently most often used in experimental research are based on compounds that exhibit a high degree of neurotoxicity and at the same time permanently damage dopaminergic neurons. One of the representatives of such substances is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which is a by-product of the synthesis of heroin derivatives, the pharmacological effect of which is related to the repetitive and specific degeneration of dopaminergic neurons and their endings in the striatum (ST), as well as the occurrence of typical parkinsonian symptoms in primates and in selected rodent species. Models assuming the use of MPTP hydrochloride (MPTP-HCL) constitute a gold standard for experiments and research on the pathophysiology of dopaminergic neuron death in the course of Parkinson's disease, most fully reflecting both the symptoms of the disease and the resulting loss of function of the nigrostriatal pathway.

Recently, the endogenous protein acting as growth factor, progranulin (PGRN) has attracted special attention of researchers due to its strong and specific neurotrophic, anti-inflammatory and immunomodulatory effects. Analyzing the features of the evolutionary and structural classification, PGRN seems to be a unique molecule that cannot be clearly classified into any known family of growth factors, where it may simultaneously act as a growth factor, an anti-inflammatory molecule, an adipokine and be the source of individual granulin (GRN) domains with potentially inflammatory effect. Preclinical studies conducted using transgenic mice and post-mortem analyzes in humans using proteomic, transcriptomic and immunofluorescence methods allowed to provide insight into the expression pattern of the PGRN gene and the degree of induction of its protein products within the nervous tissue. Assessment of individual CNS cell populations shows that PGRN expression is constitutively present on the surface of neurons and inactive microglia, while it is not observed on astrocytes and ependymocytes. Studies conducted so far in experimental models and in clinical conditions in other organ systems have firmly established the role of PGRN as a factor involved in the inflammatory response. PGRN constitutes an important factor involved

in the neuroinflammatory reaction, playing an immunomodulatory role, demonstrating a broad-spectrum anti-inflammatory effect by inhibiting the activity of microglia. In this case, the loss of PGRN function within the nervous tissue leads to the general disturbances of lysosomal function, excessive production of complement components, as well as deregulation and excessive neuroinflammatory response, ultimately resulting in complex neuropathological changes. The implicated role of PGRN should be collectively considered as one of the components of a coordinated and multifactorial neurophysiological mechanism. The analysis of available studies and research indicates that the identification of mutations in the PGRN gene is potentially associated with the predisposition to neurodegenerative diseases, including Parkinson's disease. Atsttrin is a modified protein molecule which structure is based on the structure of the PGRN polypeptide chain. The analyzes and studies carried out so far have allowed us to observe a number of structural and functional properties of Atsttrin, which are related to the molecular mechanisms of its interaction with receptors dedicated to tumor necrosis factor alpha (TNF-α), such as the type 1 receptor (TNFR1) and type 2 (TNFR2). It was observed that the PGRN polypeptide chain includes three domains that can bind to TNFR1 and TNFR2 receptors independently, which seems potentially possible through the internal folding of their linker sequences, which results from the secondary and tertiary structure of the protein. Unlike PGRN, Atsttrin was not found to be degraded and release inflammatory GRN domains when exposed to proteolytic enzymes that cleave PGRN. This same structural property allows Atsttrin to retain its affinity for binding to TNFR1 and TNFR2 receptors and potentially avoid biological effects characteristic of action on cytokines and growth factors. Experimental studies conducted indicate that Atsttrin may currently be one of the more promising and innovative therapeutic agents in the treatment of arthritis-related conditions, such as osteoarthritis (OA) and rheumatoid arthritis (RA) by directly binding to TNFR1 and TNFR2, as well as a parallel antagonistic effect against TNF-α, demonstrating its anti-inflammatory properties in this mechanism.

The assumption and aim of the doctoral thesis was to evaluate the effect of direct bilateral intracerebral administration of Atsttrin using stereotaxic methods in an experimental model of Parkinson's disease induced by intraperitoneal intoxication of MPTP-HCL in C57BL/6 mice. The experimental and research procedures carried out were aimed at clarifying whether the use of Atsttrin could be potentially effective in the treatment of Parkinson's disease, demonstrating the assumed neuroprotective effect, as

well as potentially implying the development and subsequent use in the future of new targeted therapies based on this compound in clinical practice. An additional aim of the study was to deepen the knowledge regarding the pharmacological mechanisms of action of Atsttrin, as well as to optimize and validate an alternative method of administering the compound, including direct stereotaxic intracerebral injection in an experimental model in C57BL/6 mice. In the first, preliminary stage, an analysis of the overall kinetics of the effect of increasing doses of Atsttrin applied during direct bilateral intracerebral administration to the ST was performed using stereotaxic methods in an experimental model of Parkinson's disease in C57BL/6 strain mice subjected to intraperitoneal intoxication with MPTP-HCL. This stage included determining the dose-response and dose-effect relationships by plotting a standard curve necessary to identify a potentially therapeutically effective and safe dose of Atsttrin within selected brain structures such as the hippocampus (CA), cortex (CX), cerebellum (CM) and ST. In the second, appropriate stage, the effect of direct bilateral intracerebral administration of an empirically determined dose of Atsttrin to the ST or SN was assessed using stereotaxic methods in an experimental model of Parkinson's disease in C57BL/6 strain mice subjected to intraperitoneal intoxication with MPTP-HCL. This stage included the assessment of the potentially neuroprotective effect of Atsttrin on the course of neurodegenerative processes and the development of the inflammatory reaction within selected brain structures such as the CA, CX, CM and ST. Predefined range value of five increasing Atsttrin doses of 0.1 μg (0.025 μg/μl), 0.5 μg (0.125 μg/μl), 1 μg (0.25 μg/μl), 2 μg (0.5 μg/μl), and 5 μg (1.25 μg/μl) was extrapolated and determined on the basis of literature data from previously conducted studies on the musculoskeletal system in analogous animal models in mice. The panel of analyzed parameters included the expression of cytokines and inflammatory mediators (IL-1α, TNF-α, IL-6, IFN-γ and COX-2), the expression of anti-inflammatory cytokines and mediators (IL-10), and the expression of stress parameters oxidative and nitrative (iNOS and nNOS), the expression of growth and neurotrophic factors (TGF-β and BDNF), as well as the expression of enzymes related to the metabolism of neurotransmitters (TH and TG2) measured using the Real-time PCR method. In addition, the central neurochemical profile was analyzed by assessing the concentrations of monoamines (DA, DOPAC, 3-MT, HVA, NA, MHPG, 5-HT and 5-HIAA), as well as amino acids (GLU, GABA, ALA, ASP, TAU, HIS and SER) measured using the high performance liquid chromatography (HPLC) method.

According to the data obtained from the analysis of the general kinetics of the impact of increasing doses of Atsttrin on the dose-response and dose-effect relationships and the results read from the plotted standard curve, in order to conduct and complete the further, appropriate part of the study, a dose of 0.5 μg (0.125 μg/μl) was selected, which showed in this case, an optimal pharmacological effect and was characterized by a sufficiently high level of safety in the brain tissue microenvironment. The observed changes in the values of the analyzed parameters after the use of this dose of Atsttrin in an experimental model of Parkinson's disease caused by intraperitoneal intoxication of MPTP-HCL in mice of the C57BL/6 strain are in this case subjectively similar reflected in the observed anti-inflammatory effects demonstrated during previous studies of this compound on other models of units. diseases and organ systems. In the second, relevant part, the panel of test and control groups was successively increased, taking into account the extended scope of procedures and interventions performed to include animals injected into the SN. At this stage, stereotaxic administration of Atsttrin at a dose of 0.5 μg (0.125 μg/μl) was also performed into the ST or SN structures in mice not subjected to other interventions. The panel of parameters analyzed in the second part of the study included the expression of selected mediators, factors and enzymes at the transcriptional level and the analysis of changes in the central neurochemical profile to a similar extent as during the first part of the study. It was found that direct bilateral intracerebral administration of Atsttrin at a dose of 0.5 μg (0.125 μg/μl) using stereotaxic methods to the ST and SN is associated with the inhibition of neurodegenerative processes and the inflammatory reaction in the ST, CA, CX and CM in an experimental model of Parkinson's disease in C57BL/6 strain mice subjected to intraperitoneal intoxication with MPTP-HCL. Additionally, it was observed that direct bilateral intracerebral administration of Atsttrin at a dose of 0.5 μg (0.125 μg/μl) using stereotaxic methods to the ST and SN is associated with changes in the expression levels of selected mediators, factors and enzymes at the transcriptional level as well as changes in the central neurochemical profile of amino acids. and monoamines in C57BL/6 strain mice not subjected to any additional experimental procedure.

As demonstrated, the obtained results allowed us to collectively verify whether the use of Atsttrin may constitute a new, potentially promising pharmacological element of the therapeutic strategy for the treatment of Parkinson's disease in the future. In this case, direct intracerebral administration of Atsttrin has a neuroprotective effect on damaged neurons that are part of the nigrostriatal pathway. One of the basic

mechanisms of the neuroprotective effect of Atsttrin is the inhibitory and modulatory effect on the kinetics of the expression of selected mediators of the inflammatory reaction. The presented results encourage further research to deepen knowledge about Atsttrin regarding the pharmacological mechanisms of action of this compound, optimization of its administration methods as well as its further potential use in clinical conditions.