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Tytuł rozprawy doktorskiej:

Ocena roli ligandów receptora ALX/FPR2 w wyciszaniu procesów zapalnych w ośrodkowym układzie nerwowym: badania w doświadczalnych modelach immunoaktywacji

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Summary

Despite many years of research, the background of alterations leading to the development of central nervous system (CNS) diseases still remains not fully understood. Recently, the mechanisms involved in the course of inflammatory processes, and the resolution of inflammation (RoI) have become a major focus of research. The short-term inflammatory response is a beneficial process that leads to the elimination of pathogens, combating infections, regeneration, and in consequence, restoring homeostasis. However, the long-term inflammation has an unfavorable effect, which results from the dysfunction of endogenous processes that control the resolution of the acute inflammatory process.

Therefore, the aim of the research presented in this thesis was to determine whether enhancing RoI, through the activity of new exogenous ALX/FPR2 ligands with plausible pharmacokinetic properties and superior bioavailability than endogenous ligands, may provide a new strategy for alleviating the inflammatory process. The research was conducted in several stages: *in vitro* using primary microglia cultures, *ex vivo* with organotypic hippocampal cultures (OHC), and *in vivo* to verify the obtained results in the immunoactivation model in selected brain structures.

In the first part of the presented thesis, the *in vitro* research was conducted using primary microglia cultures stimulated with lipopolysaccharide (LPS; a non-specific immunoactivator with bacterial origin), that were obtained from 0-2-day-old offspring of Spraque-Dawley rats. The results have shown the time-dependent protective and anti-inflammatory effects of all tested ALX/FPR2 receptor agonists lipoxin A4 (LXA4), its analog aspirin-triggered lipoxin A4

(AT-LXA4), and the new exogenous ligand, the compound MR-39. Noteworthy, MR-39 revealed the broadest spectrum of beneficial effects and the longest activity, which lasted 24 hours after microglia culture treatment. Importantly, the observed effects of the new ureidopropanamide ligand MR-39 were mediated by the same intracellular signaling pathways as endogenous ALX/FPR2 ligands (including ERK1/2, kinase p38, and NF-κB).

In the next stage, the research was carried out using organotypic hippocampal cultures (OHC) which constitute an excellent *ex vivo* model for analyzing not only the correlation between the nervous, immune, and endocrine systems but also enabling analysis of

physiological interactions between brain cells because they maintain functional neuronal-glial connections. Therefore, OHC model is crucial for assessing the effects of compounds with anti-inflammatory and pro-resolving potential in the course of inflammatory processes (including LPS-induced ones). This model was used as a tool for analysis of new ALX/FPR2 agonists - AMS21 and CMC23 designed and synthesized by the research group from the University of Bari (Italy). The adjustments made in the structure of these compounds (compared to the reference compound MR-39) suggested their better pro-resolving and immunomodulatory potential. Therefore, the assessment of their neuroprotective, anti-inflammatory, and pro-resolving effects in OHC was carried out at nanomolar concentrations.

The new compounds CMC23 and AMS21 normalized the increased levels of proinflammatory cytokines (IL-1β, IL-6) in LPS-stimulated OHC in an ALX/FPR2 dependent manner. The beneficial effect of CMC23 was conveyed via the STAT3/SOCS3 signaling pathway since the inhibition of the active phosphorylated form of STAT3 protein stimulated by LPS administration, was inhibited. However, AMS21 enhanced RoI disturbed by LPS via limiting excessive activation of the NLRP3 inflammasome complex. Moreover, using the OHC model the key role of the ALX/FPR2 receptor present on microglial cells in the modulation of the beneficial effects of AMS21 was demonstrated for the first time because in microglia-depleted OHCs the beneficial effect of this ligand was not observed.

In the final phase of research, the assessment of the impact of new synthetic ALX/FPR2 agonists and the endogenous ligand LXA4 on behavioral disturbances and immunoactivation factors was assessed in homogenates of the frontal cortex and hippocampus of adult 3-month-

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old males using an *in vivo* model of sickness behavior (a single intraperitoneal (i.p.) administration of LPS). The study carried out using the forced swimming test revealed deficits in the animals' behavior, manifested as an increase in the immobility time with a simultaneous decrease in the swimming and climbing time after a single administration of LPS. The normalizing effect of intraventricular (icv) administration of LXA4 on the changes described in the Porsolt test was observed only 1 hour after the treatment, while CMC23 has shown a longer biological activity. At the same time, the biochemical analysis indicated the anti-inflammatory effect of LXA4 manifested as a reduced level of proinflammatory cytokines (IL-1β, TNF-α) only 1 hour after administration, while the effect of CMC23 was prolonged and

also present 4 hours after icv drug injection. The obtained data indicate a time-dependent modulation of behavioral deficits and the reduction of proinflammatory activation by the tested ligands in the in vivo immunoactivation model. Long-lasting effects of new ligands allow for their potentially more effective use in RoI-targeting strategies.

The results presented in the thesis positively verified the objectives of the undertaken research. Moreover, according to the presented multi-level studies, it can be suggested that an innovative approach to enhancing endogenous mechanisms of RoI regulation, in the future may lead to the development of a new path for modulating inflammatory processes in the course of many CNS diseases based on pharmacotherapy of resolution.

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