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Title of doctoral dissertation: *Identification of the molecular mechanisms of action of amorfrutin B in cellular models of hypoxic-ischemic brain damage: studies conducted on primary mouse neuronal cultures and human microglial cell line*

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Summary

Each year, 12.2 million people worldwide experience a stroke, making it the second most common cause of death. The current pharmacotherapy for ischemic stroke involves thrombolysis with rtPA, which must be administered within 4.5 hours of symptom onset. Another significant challenge for modern medicine is perinatal asphyxia, which affects 4 million newborns annually. Despite the use of hypothermia, which has a therapeutic window up to 6 hours, the mortality rate of newborns remains 25%. Due to the low effectiveness, narrow therapeutic window, and contraindications associated with the use of current therapies, there is a need for new strategies to treat hypoxic-ischemic brain damage. An interesting target for new drugs is the peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ agonists, thiazolidinediones (TZDs), show neuroprotective effects in the treatment of central nervous system disorders. Unfortunately, they also cause severe side effects, leading to the partial withdrawal of TZDs from the pharmaceutical market. Available data suggest that, unlike full PPARγ agonists, selective PPARγ modulators (SPPARγMs), such as amorfrutin B, induce a partial transcriptional response and may be a safer alternative to traditionally used TZDs. Therefore, the concept of my research assumes that selective modulation of PPARγ by amorfrutin B could represent a novel approach to treat neurodegeneration caused by hypoxia or ischemia.

To determine the neuroprotective mechanisms of action of this substance and its microglia-dependent anti-inflammatory potential, the primary cultures of mouse cortical neurons as well as human microglial HMC3 cells were subjected to oxygen deprivation (hypoxic model) or oxygen and glucose deprivation (ischemic model). I demonstrated that amorfrutin B, administered 6 hours after the onset of hypoxic-ischemic injury, exhibits strong neuroprotective

effects and microglia-dependent anti-inflammatory action. Amorfrutin B protects neuronal cells from hypoxia- and ischemia-induced damage by activating the PPARγ receptor and reducing oxidative stress and free radical-dependent DNA damage. The neuroprotective effects of amorfrutin B also include the inhibition of hypoxia- and ischemia-induced apoptosis and autophagy, manifested by an increase in mitochondrial membrane potential, a reduction in autophagolysosome formation and apoptotic heterochromatin foci, and normalization of the expression of genes and/or proteins associated with apoptosis and autophagy. Amorfrutin B regulates the methylation levels of promoter regions of genes involved in apoptosis and autophagy processes, suggesting that this substance exerts its neuroprotective effect through epigenetic mechanisms. Amorfrutin B exhibits characteristics of an epigenetic modulator, as it increases the levels of histone acetyltransferases (HAT), decreases sirtuin levels, and normalizes the expression of miRNAs that are markers of ischemic stroke in hypoxic-ischemic conditions. In the final stage of the research, I showed that amorfrutin B inhibits hypoxia- and ischemia-induced microglial activation and transforms the cell phenotype from pro-inflammatory "M1" to anti-inflammatory "M2." More specifically, amorfrutin B suppresses inflammatory processes, changes microglial cell morphology to a more branched phenotype, and inhibits the proliferative potential and metabolic activity of microglia. These changes are accompanied by increased expression of the PPARγ receptor and its coactivator PGC1α, suggesting the involvement of these signaling pathways in the anti-inflammatory properties of the studied substance.

The neuroprotective and anti-inflammatory properties of amorfrutin B demonstrated in this doctoral dissertation provide strong evidence for using amorfrutin B as a model substance for developing new stroke pharmacotherapies that target various cell types and possess a wide therapeutic window. In the longer term, my research may improve the pharmacotherapy of nervous system diseases and help identifying new therapeutic targets based on the selective modulation of the PPARγ receptor.