REASONS FOR ATTEMPTING A PARTICULAR RESEARCH TOPIC

The WHO has established that 15 million people experience stroke worldwide each year. Aside from the stroke, perinatal asphyxia caused by oxygen deprivation during the birth process is the most common cause of death in fetuses and newborns. Each year, 1 million children die due to hypoxia and up to 25% of survivors have permanent neurologic deficits. Despite numerous experimental studies and clinical trials, the basic therapies against acute stroke and perinatal asphyxia i.e., thrombolysis and hypothermia, respectively, cure effectively small number of people suffering from these pathologies. There is no strategy that possess wide therapeutic window and effectively treats stroke and perinatal asphyxia in majority of patients. Globally, there is a strong need to provide chemicals exhibiting wide therapeutic window and acting on complex processes accompanying brain hypoxia/ischemia and reperfusion. It is generally accepted that estrogen receptor-mediated signaling plays key roles in neuroprotection and its impairment during the course of hypoxia/ischemia may predispose for cerebral infarction. Recent research demonstrated that membrane non-nuclear estrogen receptors (ERs) are potential therapeutic targets to evoke neuroprotection without inducing hormonal side effects attributed to activation of classical nuclear ERs. To effectively and safely target non-nuclear ERs, we propose to use a newly designed Pathway Preferential Estrogen-1 (PaPE-1) which activates the membrane-associated subpopulations of ERα and ERβ i.e., mERα and mERβ. It is assumed that PaPE-1 will selectively up-regulate non-nuclear ER signaling without evoking adverse hormone effects via nuclear ERs. The unique properties of PaPE-1 would account for high selectivity and a safe pharmacological profile that gives perspectives for successful therapeutic use of the compound against stroke and perinatal asphyxia. To address the clinical aspect and to extend the therapeutic window, neuroprotective properties of PaPE-1 will be tested in the post-treatment paradigms.

THE PROJECT GOAL

This study proposes a novel therapeutic approach for stroke and perinatal asphyxia that relies on a selective activation of non-nuclear ERs with PaPE-1. The research hypotheses assume that: **I.** Post-treatment with PaPE-1 possesses neuroprotective potential and causes neuroprotection in mouse and human models of stroke and perinatal asphyxia. **II.** The mechanism of neuroprotection is associated with up-regulation of non-nuclear ER signaling that is accompanied by an inhibition of neurotoxicity and microglia-related neuroinflammation, restoration of endothelium integrity as well as by normalization of epigenetic statuses of neuronal cells and restoration of locomotor and cognitive function.

DESCRIPTION OF RESEARCH

Effects of PaPE-1 will be determined in context of neurotoxicity (referred to necrosis, apoptosis, and ferroptosis), microglia-related neuroinflammation, endothelial cells integrity, PaPE-1-dependent signaling pathways, microRNA profiling and epigenetic modifications in terms of e.g. DNA methylation, as well as locomotor and cognitive deficits. To address translational and developmental aspects, neuroprotective capacity of PaPE-1 will be estimated in the mouse and human models of stroke and perinatal asphyxia *in vitro* and/or *in vivo*. To take into account clinical aspects, the compound will be administered as post-treatment starting not earlier than 6 h after the initial damage.

SUBSTANTIAL RESULTS EXPECTED

The unique combination of *in vitro* and *in vivo* models of stroke and perinatal asphyxia, including primary mouse neurons, human iPSC-derived excitatory neurons, microglia and endothelial cells as well as animal models of perinatal asphyxia and stroke will provide complex and adequate platform for assessment of neuroprotective capacities and mechanisms of actions of post-treatment with PaPE-1. To address a ‘Precision Medicine’ approach i.e., stratification of subgroups of populations for the treatment or prevention of stroke and perinatal asphyxia, the sex- and age-dependence as well as vulnerability of microglia and endothelial cells to proposed therapy will also be assessed. The special value of this project relies on the therapy that selectively targets signaling pathways which are essential for neuroprotection and has wide therapeutic window but does not exert serious adverse effects.