

ANNOTATION

on the dissertation for scientific degree of PhD in specialty 6D060700 – Biology

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Study of the combined application of plant polyphenols and mesenchymal stem cells in ischemic brain injury

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This thesis is a complex research devoted to the pre-clinical study of the effectiveness of new treatment strategy against the consequences of ischemic brain damage based on combined application of stem cell therapy and extract of plant polyphenols isolated from roots of *Limonium Gmelinii* (*L.gmelinii*).

The relevance of research. Ischemic stroke is a third cause of death after heart disease and cancer, and the leading cause of long-term disability in aging adults in the most countries. In Kazakhstan, 2.5-3.7 cases of stroke per 1000 members of the population with mortality rate of 1-1.8 cases are registered annually. Now, the only approved therapy for this devastating condition is a local or systemic thrombolysis of the occluded vessels by using “clot-dusting” recombinant tissue plasminogen activator (tPA), effectiveness of which is limited by a time window of 3-4 hours after symptom onset. Thus, development of alternative, more flexible, and clinically relevant therapies are in high demand. One of the promising areas of post-stroke rehabilitation is the application of mesenchymal stem cell therapy. On the other hand, there is a number of data indicating that natural antioxidants, in particular plant polyphenols, capable of exerting pronounced antioxidant and neuroprotective effects. In this regard, an integrated approach based on the study of the neuroprotective properties of plant polyphenols and the regenerative potential of mesenchymal stem cells (MSCs) in the treatment of the consequences of ischemic cerebral stroke is very promising approach.

The objects of the research. The objects of research were primary human astrocytes, neurons, and mouse cerebral endothelial cell line (CECs, bEnd3, ATCC), extract of polyphenols from roots of *L.gmelinii*, Wistar rats and rat mesenchymal stem cells.

The subject of research: *in vitro* and *in vivo* evaluation of the antioxidant, anti-inflammatory and neuroprotective properties of polyphenols extract isolated from the roots of *L.gmelinii*; *in vivo* evaluation of the therapeutic potential of the

combined application of plant polyphenols and mesenchymal stem cells transplantation on animal model of ischemic brain injury.

The purpose of the dissertation research is the development and preclinical testing of a new treatment strategy against ischemic brain damage based on combined application of stem cell therapy and plant polyphenols.

Three tasks have been completed in order to achieve this goal:

- *In vitro* evaluation of the antioxidant and anti-inflammatory properties of polyphenol extract isolated from *L.gmelinii* on cultures of primary human neurons, astrocytes and mouse CECs;
- *In vivo* evaluation of the neuroprotective properties of the polyphenol extract isolated from *L.gmelinii* in Wistar male rats, subjected to middle cerebral artery occlusion (MCAO) and oral administration of *L.gmelinii* extract;
- *In vivo* evaluation of the effectiveness of oral administration of *L.gmelinii* extract in combination with transplantation of mesenchymal stem cells for the treatment of the consequences of experimentally induced ischemic brain damage in Wistar male rats subjected to MCAO

The methodological basis of the research are the following cytological and histological methods: *in vitro* cell cultivation, isolation and culturing of neurons, astrocytes and MSCs, cryopreservation and thawing, cell transplantation, light and fluorescent microscopy, histology and immunohistochemistry, protein immunoblotting, microsurgery methods (MCAO), functional behavioral tests, *in vivo* optical imaging.

The scientific novelty of the research. There are three points considered as the most significant scientific outcomes in the thesis:

- The antioxidant, anti-inflammatory and neuroprotective properties of the extract from roots and rhizomes of *Limonium gmelinii* have been found;
- The new features of the distribution and homing of mesenchymal stem cells in the body upon transplantation in conditions of ischemic brain damage have been found;
- An integrated approach based on the use of plant polyphenols and MSCs is a more effective way of treating the consequences of ischemic cerebral stroke in comparison with monotherapy, due to their wider spectrum of therapeutic action.

Practical significance of the research. The results of the conducted studies showed that the extract of polyphenols from the roots of *Limonium Gmelinii* possesses antioxidant and neuroprotective properties, and combined therapy with extract of *Limonium Gmelinii* and mesenchymal stem cells is a more effective approach in comparison to monotherapy. Since the dry extract from the roots of *Limonium Gmelinii* “Limonidine” is already authorized by the Medical and Pharmaceutical Control Committee of the Ministry of Health of the Republic of Kazakhstan for medical use as a hepatoprotective and anti-inflammatory agent, it can be recommended for further clinical trials as a rehabilitation therapy after a stroke, either alone or in combination with MSCs. The obtained results can be included into the lecture courses for undergraduate and graduate students of higher educational institutions of medical and biological profile.

The main results of the study. In accordance with the goals and objectives, the following research tasks performed:

- *In vitro*, an oxidative and inflammatory models induced by, H₂O₂, and TNF- α in human primary neurons and astrocytes, and mouse cerebral endothelial cell (CECs) line have applied. The levels of ROS generation, NADPH oxidase activation, P-selectin expression, and activity of ERK1/2 were evaluated by quantitative immunofluorescence analysis, confocal microscopy, and MAPK assay.
- *In vivo* model of temporary MCAO was developed by introducing a nylon monofilament with a silicone tip 4/0 (Docol Corp, USA) followed by reperfusion.
- The analysis of the locomotor function of the laboratory animals subjected to MCAO was performed using a balance test "Beam walk".
- Mesenchymal stem cells were isolated from the compact bones of laboratory animals (rats); the purity of the obtained MSC population was confirmed by immunofluorescent stain with markers CD90, CD105, CD34, and CD19.
- The method of transfection of MSCs with lentiviral vector was optimized; the method and dosages of intravenous injection of MSCs into laboratory animals and the intravital imaging of transfected MSCs within the rat organism were developed.
- Histological slicing of the brain tissue and the analysis of brain slices of experimental animals have been conducted.

Thus, the conducted studies and the scientific results obtained on their basis allow us to draw the following conclusions:

1. It has been shown that the extract from *L.Gmelinii* has no toxic effect on neurons, astrocytes and brain endotheliocytes at a dosage of 0.001-0.1 mg/ml.
2. In neurons, H₂O₂ induced overproduction of ROS, in astrocytes TNF- α initiated ROS generation, NADPH oxidase activation, and phosphorylation of ERK1/2. In CECs, the exposure by TNF- α induced oxidative stress and triggered the accumulation of P-selectin on the surface of the cells. In turn, pre-treatment of the cells with the extract of *L.gmelinii* suppressed oxidative stress in all cell types and pro-inflammatory responses in astrocytes and CECs.
3. *In vivo*, daily oral treatment of rat with 200 mg/kg of *L.Gmelinii* extract for 28 days significantly improved impaired by stroke motor activity of rats and partially normalizes the histological structure of the brain damaged from MCAO, thereby confirming the neuroprotective properties of the extract.
4. The distribution of MSCs in the body of animals with focal ischemic stroke was shown to differ from that in intact rats: in healthy animals, MSCs are localized mainly in the thoracic and abdominal regions of the body; in animals with MCAO most of the cells were found in the damaged regions of the brain.

5. Daily oral treatment of rats with 200 mg/kg of *L.gmelinii* extract for 28 days and single transplantation of MSC (5x10⁶ cells) completely restored motor activity of the rats after stroke by 28th day thus confirming that combined therapy with plant polyphenols and MSCs is a more effective approach in restoring neurological functions after the development of a stroke compared to monotherapy.

The main results of the dissertation research were published in 10 publications, of which one paper was published in an international scientific journal included in the Thompson Reuters and Scopus databases with an impact factor of 6.313; 4 papers were published in the journals recommended by Committee for Control in the Sphere of Education and Science (CCSES) MES RK; 5 conference abstracts were published in the proceedings of the international conferences. In addition, a patent of the Republic of Kazakhstan No. 34466 dated September 11, 2020 has been obtained.

The Thesis consists of an introduction, three sections, a conclusion and a list of cited literature. The total volume of the Thesis is 98 pages.