ABSTRACT

Stem cells have the ability to self-renew and differentiate into specialised cells. Among stem cells (SCs), there is a group of adult stem cells that are considered to be a specific regenerative reserve of cells for particular organs. They became a hope for regenerative medicine. Myogenic stem cells are adult stem cells capable of differentiating into functional muscle fibres. They exhibit potential to stimulate and support endogenous repair mechanisms in skeletal muscle, but also in the heart, thus forming the basis for full regeneration of damaged heart after myocardial infarction. This type of adult stem cells, due to the relatively safety in their use, becomes less controversial in clinical application. The risks associated with the transplantation of adult SCs are rare, usually manageable and similar for all cell products. Availability, ease of isolation and propagation of cells *in vitro* culture become an important advantage.

In order to obtain the best possible therapeutic effects, native biological material such as adult stem cells has been proved, however, to be insufficient to fulfill all the requirements, and regenerative medicine is now placing its hopes on modifications, adaptations and preconditioning of stem cells to improve their therapeutic effects.

The research included in this several presented papers involved the use of variety of modifications in preparation of skeletal muscle derived stem/progenitor cells (SKMS/PCs) such as: *in vitro* culture under hypoxic conditions, genetic modification by inducing overexpression of the antioxidant gene- *SOD3,* preconditioning with the exogenous chemical compound-PBN and inhibition of micro RNA-195.

(i) Culturing *in vitro* of stem cells of myogenic origin under hypoxic conditions, enhanced endogenous cell defence strategies and mechanisms, thereby improving their survival *in vivo*. The *in vitro* culture conditions corresponded to those present in post-infarcted heart, which influenced such *in vitro* adaptation to be stress resistant after their administration into the post-infarcted scar region.

ii) DNA modifications are widely used to induce overexpression of therapeutic factors physiologically released; for various signalling pathways regulating e.g. the cell aging or apoptosis processes. SOD3 is the main factor among a group of antioxidant enzymes that detoxify superoxide radicals to hydrogen peroxide and oxygen. It belongs to the extracellular matrix, prevents inactivation of NO released from the endothelium, and is of particular interest because of its longer half-life then other antioxidants and lack of epitopes for binding

immunoglobulin (Ig) E. There are currently no reports on the reactivity of human SOD3 with immune cells and their potential reactivity, suggesting the safety of human SOD3 overexpression in the clinical setting.

(iii) One of the simplest and least costly approaches to modify cell *in vitro* culture is its preconditioning with pharmacological agents. It may leads to an improvement in the therapeutic ability of the stem cells used, by stimulating the intensive release of paracrine factors, beneficial in the regeneration of the post-infarction heart. PBN, i.e. N-tert-butyl-α-phenylnitrone, belongs to the so-called spin-traps, whose mechanism of action follows different pathways to balance the redox environment by scavenging ROS or reactive nitrogen species (ROS/RNS).

(iv) Micro RNAs (mi-RNAs) are small, 20-25-nucleotide non-coding RNAs that play key roles in various cellular processes including: development, cell destiny, proliferation and cell signalling. Comparing to modifications at the DNA level, the use of mi-RNAs offers the possibility of inducing transient effects that improve the therapeutic properties of stem cells on epigenetic level. Since no genomic changes are required, this modification becomes potentially advantageous.

In the present study, beneficial effects of the different modifications on myogenicity, *in vitro* culture potential, myotube formation capacity, ageing of the cell population, apoptosis levels, expression of selected antioxidant, anti-aging, anti-apoptotic, and myogenic genes and the same factors at the protein level were documented. The significant effect of the applied modification with PBN on telomere length was also observed. The aim of the study was to prepare the most optimal *in vitro* culture protocol, for subsequent cell application *in situ* in preclinical scenario. The optimized protocol was to be evaluated in a mouse model of post-infarction heart, which was then performed with promising result but not yet published.

In conclusion, the studies here presented contribute to the expected protocol of skeletal muscle-derived cells preparation, stably transfected with *SOD3* preconditioned with PBN and *in vitro*, cultured under hypoxia condition which would serve to better adaptation to stress conditions. The simultaneous application of several conditioning approaches modification may be a longtime awaited protocol optimalization of cellular therapy with the use of myogenic cells in post-infarction heart treatment.