Streszczenie w języku angielskim

The influence of selected parameters on the metabolism of human mesenchymal stem cells and their regenerative potential in wound therapy

Introduction

The objective of this doctoral dissertation is a comprehensive analysis of the impact of selected in vitro culture conditions on the metabolism of human mesenchymal stem cells (MSC), along with the evaluation of their therapeutic efficacy in a clinical model of chronic wounds, exemplified by diabetic foot ulcer (DFU). Given the dynamic advancement in regenerative medicine and the necessity of developing effective methods to enhance tissue repair processes, understanding the mechanisms underpinning the beneficial effects of MSC on tissue regeneration becomes critical.

MSC are characterized by self-renewal capacity, multilineage differentiation potential (including osteoblasts, chondrocytes, and adipocytes), and effective paracrine activity manifested by modulation of inflammatory responses and stimulation of angiogenesis. Existing research suggests potential applications for statins, pleiotropic medication, which may enhance MSC functions under specific environmental conditions. Additionally, oxygen concentration in the culture environment (normoxia versus hypoxia) is an essential factor influencing MSC metabolism, proliferation, and differentiation.

Understanding the mechanisms that lead to improved wound healing under conditions of increased metabolic demand and impaired perfusion (as observed in diabetes) is crucial for the implementation of innovative therapies in everyday clinical practice. This series of publications contributes to expanding knowledge on the effects of statins and hypoxia on MSC, as well as clinical protocols for MSC application in chronic wounds.

To date of publication, no study has previously assessed the combined influence of statins and reduced oxygen concentration in MSC cultures. Study no. 1 investigated the effects of atorvastatin and rosuvastatin on MSC under varying oxygen conditions, also considering donor age. Study no. 2 conducted in vivo experiments: adipose-derived mesenchymal stem cells (ADSC) were applied to diabetic foot ulcer to verify the efficacy of cellular therapy in treating chronic wounds. Study no. 3 reviewed literature

and previous study results to assist future researchers in optimizing the design of cellular therapies for wounds, such as DFU and other chronic wounds.

Materials and methods

In the initial stage of the study, human mesenchymal stem cells derived from bone marrow (BM-MSC) were obtained from 12 patients after receiving their written consent. Ethical approval was granted by the Local Bioethics Committee. The study was conducted at the Department of Immunology, Transplantology and Internal Diseases of the Medical University of Warsaw. Standard phenotypic characterization of cells (expression of CD73, CD90, CD105 antigens, and absence of markers such as CD45, CD34) and differentiation assays (osteogenic, chondrogenic, and adipogenic lineages) were performed.

Two culture environments were tested to evaluate the impact of oxygen conditions. The first group involved normoxic conditions (21% O_2, standard laboratory conditions), while the second employed hypoxic conditions (2% O_2, reflecting cellular hypoxia). Cells were incubated with atorvastatin at 0.05 μM (close to physiological therapeutic concentrations in patients) and rosuvastatin at 0.04 μM. Cytotoxicity tests were additionally performed using lower concentrations and a higher concentration (1 μM). Proliferation was assessed by BrdU (5-bromo-2'-deoxyuridine) assay, and cytotoxicity was evaluated by measuring LDH (lactate dehydrogenase) activity in the culture medium.

Study no. 2 summarized a clinical trial involving patients with diabetic foot ulcers. Ethical approval (KB/128/2019 24.06.2019 and KB/3/A2021 18.01.2021) was obtained, and the study was registered on ClinicalTrials.gov (NCT03865394). The study group comprised 47 patients divided into two subgroups: patients receiving ADSC embedded in fibrin carriers and those receiving fibrin glue alone (control group). ADSC isolated from adipose tissue of healthy donors were applied to wound surfaces, and healing progression and the time to 50% ulcer size reduction were monitored. Additionally, wound samples underwent proteomic analysis and assessment for donor-derived DNA to determine cell survival duration post-application.

Study no. 3 consisted of a literature review summarizing:

- MSC mechanisms of action (paracrine effects, immunomodulation, etc.),
- results of key preclinical and clinical MSC studies,
- potential therapeutic protocol modifications such as MSC preconditioning, genetic modifications, or scaffolds to enhance MSC survival and integration within wounds,
- limitations and perspectives of MSC therapy development for chronic wounds, particularly in diabetes.

The review also emphasized MSC application safety concerns, particularly immunogenicity risks under allogeneic conditions and possible inflammatory reactions.

Results

Observations from study no. 1 identified optimal preconditioning conditions using statins and variable oxygen levels for effective MSC performance. Key findings included:

- Physiological concentrations of atorvastatin significantly increased MSC proliferation under both normoxic and hypoxic conditions without substantial cytotoxicity;
- Rosuvastatin stimulated proliferation in hypoxic conditions without significant toxic effects, while slight dose-dependent cytotoxicity increases were observed under normoxia;
- Hypoxia alone enhanced MSC proliferative capacity, suggesting cells better adapt to low oxygen environments typical in ischemic tissues;
- Donor age did not significantly affect MSC response to statins, although generally older donors showed lower MSC proliferative activity.

Study no. 2 observed the following results from long-term patient monitoring with DFU:

- Faster wound size reduction in the ADSC-treated group compared to controls, demonstrated by shorter times to 50% ulcer reduction and higher complete healing rates;
- Proteomic analysis indicated increased expression of regenerative and angiogenesis-related proteins (GAPDH, CAT, etc);
- Donor DNA detection at wound sites persisted for approximately 2-3 weeks post-application, suggesting adequate MSC survival duration for initiating beneficial wound healing effects;
- No serious adverse events directly linked to MSC administration occurred, confirming clinical safety.

The findings of studies 1 and 2 significantly contributed to the review paper (study no. 3), summarizing previous observations and highlighting future research directions:

- MSC wound healing mechanisms involve immunomodulation, angiogenesis stimulation (e.g., VEGF secretion), and direct tissue regeneration support;
- Culture parameters (oxygen concentration, pleiotropic drugs, donor age) optimize MSC therapeutic potential;
- Therapeutic enhancement strategies include genetic modifications, MSC activation by stimuli (hypoxia, growth factors), and cell-scaffold integration (hydrogels or tissue constructs);
- Safety issues and standardization: microbiological purity, immunological reaction risks in allogeneic transplantation, and standardized MSC production protocols.

Conclusions

This series of publications demonstrates that hypoxic conditions and appropriate statin concentrations positively impact human MSC proliferation and functionality in vitro. Optimized MSC, combined with suitable administration techniques (tissue adhesives, hydrogels), can improve wound healing in clinical models, particularly challenging diabetic foot ulcer patients.

Clinically, allogeneic ADSC therapy accelerated diabetic foot wound healing, increasing complete recovery frequency without significant adverse events. Proteomic analysis and cellular survival observations confirm MSC survival in challenging local environments, actively modulating inflammatory responses and stimulating repair via proangiogenic and immunomodulatory protein expression.

The review provides guidance for future researchers, highlighting MSC regenerative potential and challenges transitioning from laboratory research to clinical practice. Essential development areas include precise dosage and administration protocols, personalized approaches (considering patient age or ulcer severity), and MSC modification strategies enhancing tissue survival.

In summary, this publication series offers comprehensive evidence of MSC benefits under hypoxia and statins, confirming cellular therapy's efficacy and safety in treating chronic diabetic wounds, laying the foundation for regenerative medicine advancements and innovative clinical applications.