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PRACA DOKTORSKA

Właściwości proangiogenne mezenchymalnych komórek zrębu izolowanych z ludzkiej tkanki tłuszczowej (ADSC)

Proangiogenic properties of mesenchymal stromal cells isolated from human adipose tissue (ADSC)

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STRESZCZENIE ROZPRAWY DOKTORSKIEJ

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Mesenchymal stromal cells (MSCs) are multipotent non-hematopoietic cells derived from mesodermal tissues such as bone marrow or adipose tissue. These cells secrete many growth factors and cytokines, that stimulate blood vessels formation and regeneration of damaged tissues. Despite many preclinical and clinical studies utilizing MSCs in damaged tissues repair, the mechanisms involved in these processes are still insufficiently understood.

The aim of this study was to evaluate the ability of human adipose tissue-derived mesenchymal stromal cells (hADSCs) to repair damaged muscle.

Human mesenchymal stromal cells isolated from subcutaneous adipose tissue were used in the study. The isolated cells had the characteristic features of mesenchymal cells: 1) they were adherent to plastic culture surfaces; 2) possessed the proper phenotype CD29+/CD44+/CD73+/CD90+/CD105+/CD31-/CD33-/CD45-/CD146-/HLA-DR-/LIN-/KDR-; 3) showed the ability to differentiate into adipocytes, chondroblasts and osteoblasts.

The research was performed on a mouse model of ischemic limb. The procedure of femoral artery ligation induced ischemia in the gastrocnemius muscle. One hour after femoral artery ligation 1·106 hADSCs were administered intramuscularly. The administered cells persisted in the limb up to 14 days. In mice which received hADSCs, faster improvements in damaged limb function and skeletal muscle regeneration were observed. After hADSCs administration an increase in the influx of pro-angiogenic and anti-inflammatory macrophages with the M2 phenotype (7-AAD−/CD45+/F4/80+/CD206+) and a greater number of new blood vessels in the ischemic limb were also observed.

The role of macrophages in the repair of damaged muscle following administration of mesenchymal stromal cells was then investigated. For this purpose, macrophages depletion with the use of liposomes containing disodium clodronate was performed. In mice that lack macrophages despite the administration of hADSCs, no regeneration of the damaged muscle and no increase in the number of blood vessels was observed.

hADSCs secrete large amounts of Interleukin 6 (IL-6), which among others polarizes macrophages to pro-angiogenic M2 phenotype. In order to investigate whether IL-6 was responsible for macrophages influx into the ischemic limb following hADSC administration, IL-6 was blocked using Siltuximab (an antibody that specifically blocks human IL-6). After IL-6 blockade there was a reduction in the influx of macrophages with the M2 phenotype and a reduction in the number of blood vessels formed in the injured limb.

The addition of deferoxamine to hADSC in vitro cultures increases the secretion of IL-6, but does not improve the pro-angiogenic properties of the tested cells.

Therefore, it has been shown that hADSCs mediated repair of damaged muscle is facilitated by IL-6 secretion which stimulates macrophages with M2 phenotype, which in turn promote the formation of new blood vessels.