

THE ROLE OF MESENCHYMAL STEM CELLS IN THE THERAPY OF MYOCARDIAL INFARCTION

Zorana Antonijević^{1,2}, Aleksandra Vuletić³

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into osteoblasts, chondrocytes, adipocytes. During the early 21st century, *in vivo* studies demonstrated that human MSCs can transdifferentiate into endoderm-derived cells and cardiomyocyte phenotype. Without blood to supply cardiomyocytes (CMs), as in myocardial infarction, the loss of functional CMs progresses as an imbrication of necrosis, apoptosis and autophagy. Besides progressing through different stages of inflammation and healing, the dynamic microenvironment in the infarcted tissue also express cardiac cytokines that promotes stem cell migration and homing. Given the uncertainty of myocardial salvage, dictated by the degree of necrosis from the sentinel event, it soon became clear that in order to change the long term outcomes of acute myocardial infarction, it's needed to search for a therapy that takes the time to presentation out of the equation. A possible solution to that dilemma appeared in the form of targeted stem cell therapy.

Acta Medica Medianae 2019;58(4):xx-xx.

Key words: mesenchimal stem cells; myocardial infarction; therapy

¹Clinical Centre Kragujevac, Clinic for Cardiology, Kragujevac, Serbia

²Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia

³Health Center Kragujevac, Kragujevac, Serbia

Contact: Zorana Antonijević
34206 Gornja Sabanta, 34000 Kragujevac, Serbia
E-mail: antonzjnet@gmail.com

Introduction

Mesenchymal stem cells (MSCs) are adult, multipotent cells that have the ability to differentiate into tissues of mesodermal origin. Also, they are self-renewable, fibroblast-like cells (1,2,3).

First knowledges about stem cells are given by Friedenstein et al in the 1970s. (4) They showed that the bone marrow contains a population of hemathopoetic stem cells (HSCs) and an infrequent population known as mesenchymal stromal cells and displayed the capability of MSCs to differentiate into mesoderm-derived tissue and their significance in regulating hematopoiesis (4, 5). In the 1980s is established that MSCs can differentiate into osteoblasts, chondrocytes, adipocytes (6, 7), and into a myogenic phenotype in the 1990s (8). Pittenger et al. demonstrated that adult human MSCs can be

expanded to colonies while retaining their multilineage potential (9). In later years, *in vivo* studies demonstrated that human MSCs transdifferentiate into endoderm-derived cells and cardiomyocytes (10, 11) dna *in vitro* coculturing of ventricular myocytes with MSCs induced transdifferentiation into a cardiomyocyte phenotype (12), and discovered their imunomodulatory functions (13). MSCs also suppress T-lymphocyte proliferation, so can be used for allogeneic transplantation and as a potential immunomodulatory therapy (13) (Figure 1).

MSCs are found in bone marrow, amniotic membrane, synovial fluid, cord blood, adipose tissue, Wharton's jelly, placenta, umbilical vein, amniotic fluid, skeletal muscles, liver and, cord or peripheral blood (14,15). This wide variety of origins, methodologies, and acronyms prompted standardization in 2005 by the International Society for Cellular Therapy, which set the minimum requirements for MSC definition(16). The minimum criteria for MSCs included plastic adherence, *in vitro* trilineage differentiation to osteoblasts, adipocytes and chondroblasts, cell surface expression of CD105 (endoglin, SH2), CD73 (ecto-5'-nucleotidase, SH3/4), and CD90 (Thy1) and the absence of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR (17).

MSCs have high expansion potential in culture, givinge the large numbers of cells within a short period of time, which is of importance in transplant medicine (18, 19, 20). MSCs are characteristic by genetic stability, compatibility with tissue

engineering principles, reproducibility of features between different bone marrow isolates, their potential to trigger regeneration in various fundamental tissues including the myocardium and neovascularization, their ability to home to the damaged tissue or inflammatory sites, and their immuno-

regulatory properties and so their use as an allogenic treatment (18, 19, 20). It has been shown that MSC transplantation may give benefits in various disease (21), and one of those diseases in which their ability has been researched a lot was heart attack (MI).

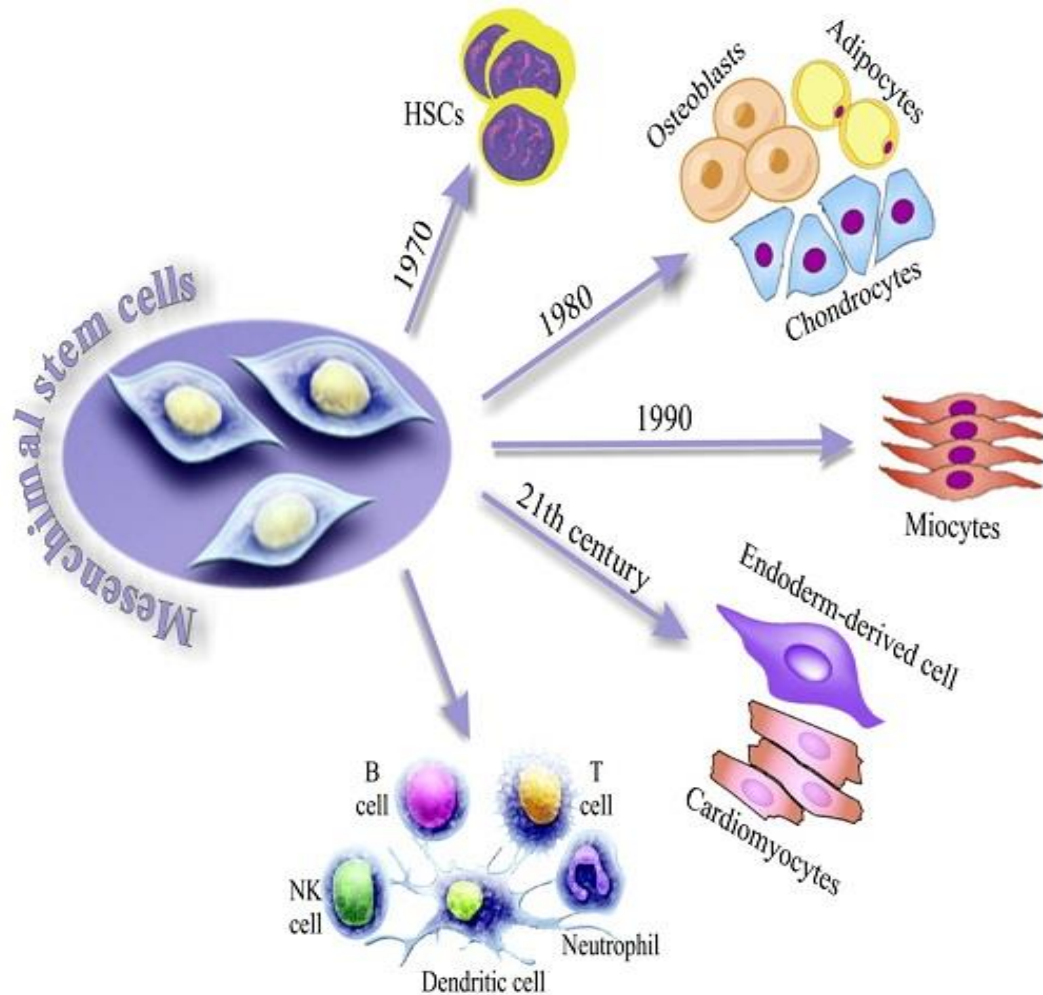


Figure 1. Roles of MSCs

Myocardial infarction and homing endogenous MSCs

Myocardial infarction (MI) is a consequence of the irreversible damage of heart muscle cells, when prolonged ischemia exceeds a critical threshold and overwhelms myocardial cellular repair mechanisms. On this way, functional cardiomyocytes (CMs) pass through processes of necrosis, apoptosis, and autophagy (22, 23).

Besides progressing through different stages of inflammation and healing, the dynamic micro-environment in the infarcted tissue also express

cardiac cytokines that promotes stem cell migration and homing (24).

Stem cells, whether endogenous or exogenous, may reach to the myocardial inflammation, as it has been shown in many studies (25). Also, it has been shown that MSCs homing depend on nature of MSC and on time of their application (25) (Figure 2).

Homing depend on the chemokine receptor, CXCR4 which is present on a subpopulation of MSCs, and its binding partner that is stromal derived factor-1 CXCL12 (25). Freshly isolated BM MSCs and cultured MSCs also express CCR1, CCR4, CCR7, CCR10, CCR9, CXCR5, and CXCR6 which also participate in MSC migration (25). Adipose-derived

MSC-like cells express integrins, cell surface molecules that participate in migration of variety of cells, while neutralizing antibodies against integrins (integrin-beta1 integrin, but not integrin-alpha4

which is involved in MSC migration) inhibit MSC homing to infarcted myocardium (25).

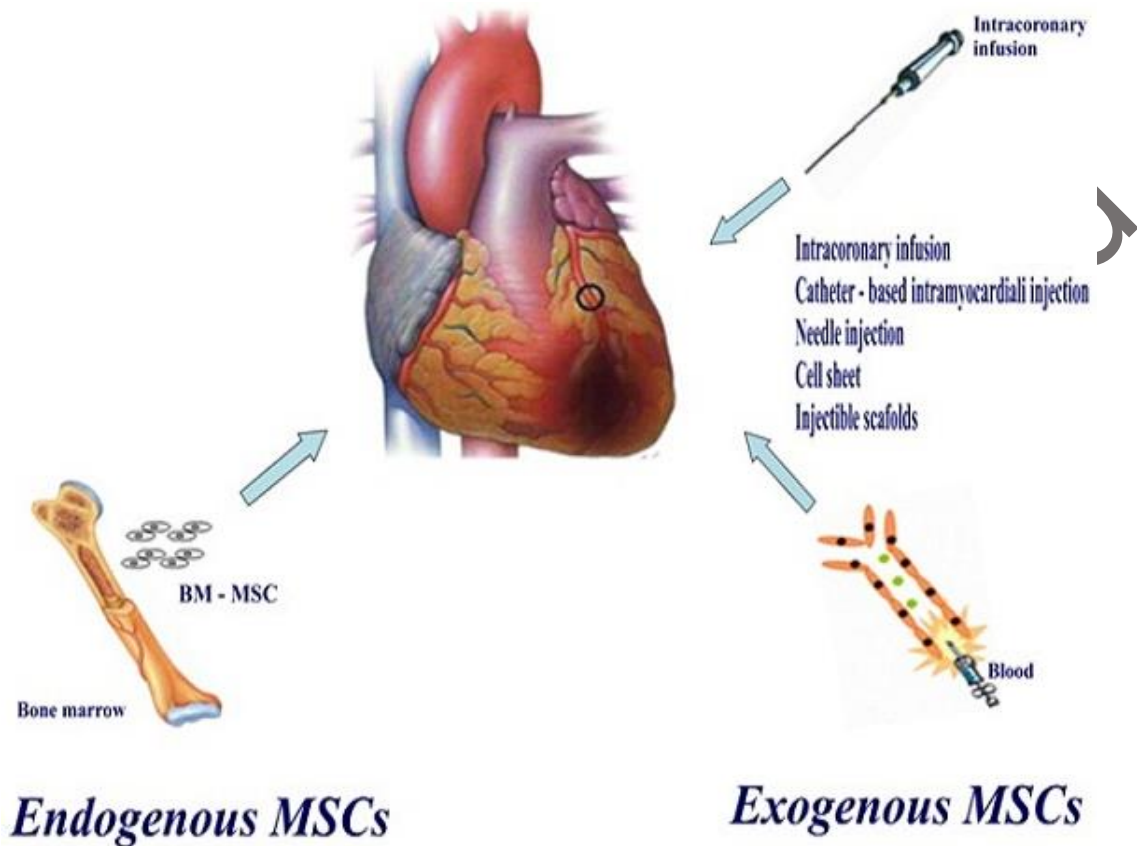


Figure 2. MSCs therapy in myocardial infarction

MSCs transplanting to the post-infarct myocardium

Transplanting stem cells to the post-infarct myocardium augment the cytokine effect to attract endogenous stem cells, via their paracrine mechanisms (anti-inflammatory and immunomodulatory mechanisms) and on that way modulate the regenerative environment (26). MSCs secrete an array of growth factors and anti-inflammatory proteins in response to inflammatory molecules such as interleukin-1 (IL-1), IL-2, IL-12, tumor necrosis factor- α (TNF- α) and interferon-gamma (INF- γ). These factors have feedback mechanisms among the many types of immune cells (27). The key immunomodulatory cytokines include prostaglandin 2, TGF- β 1, HGF, SDF-1, nitrous oxide, indoleamine 2,3-dioxygenase, IL-4, IL-6, IL-10, IL-1 receptor antagonist and soluble tumor necrosis factor- α receptor (27). MSCs prevent proliferation and function of many inflammatory immune cells,

including T cells, natural killer cells, B cells, monocytes, macrophages and dendritic cells (27).

After the transplantation to the post-infarcted myocardium, MSCs decrease protein production and gene expression of inflammation cytokines TNF- α , IL-1 β and IL-6, inhibit deposition of type I and III collagen and gene and protein expression of MMP-1 and TIMP-1 (27). So MSCs preventing myocardial remodeling after MI, due to their capability to attenuate LV cavity dilation and transmural infarct thinning, and to increase EF, FS, LVESP and dp/dtmax, decrease LVDd, LVEDV, LVEDP (27). MSCs also have the cardiac protective effect in ischemic heart disease, thanks to their anti-inflammatory role (28).

Endogenous stem cells, beside the paracrine signaling effects, have the potential to differentiate into functional myocardium (24). Together, the delivery and differentiation of stem cells replenishes the lost cardiomyocytes from MI and provides increased vascularity in the post-injury zone to prevent further ischemic tissue damage (29).

MSCs as novel therapeutics

In response to acute MI, MSCs, as novel therapeutics, can be developed to promote CMs survival and improve cardiac function after MI (30, 31). Actually, gold standard for resolving acute MI is percutaneous coronary intervention (PCI) (32). The aim of any medical or surgical therapy is to establish revascularization and limit the degree of myocardial injury, so targeted stem cell therapy is a possible solution (33, 34).

In comparison with the other cell types, MSC therapy can be promising option among in AMI treatment (11, 35). The border zone between necrotic myocardium and viable myocardium is part of interest, and depends on the reaction of viable myocardium to the area of infarct (20). It is need stem cells home to the injured myocardium, in order to produce a therapeutic response. They adhere to myocardium and transmigrate through the endothelium, invade the interstitium and at the end engraft the damaged myocardium (36).

Goals of stem cell therapy

The main goal of cell-based therapies for cardiac diseases is stopping damage of myocardial tissue and establishing its revascularisation by accelerating the normal healing process, improving vascularization, inhibiting apoptosis and potentially regenerating cardiac muscle (37,38). MSC therapy has found application in myocardial repair wheather in ischaemic heart diseases or in heart failure patients (20).

Limitation and improvements of cell-based therapy

Because of the low retention of cardiac stem cells regardless of the delivery method used, in future it is need to improve theirs engraftment and differentiation.

There is several limitations based on most previous clinical trials of cell-based therapies: low engraftment of BMCs, poor survival of transplanted cells in ischaemic tissue, failure of adult stem cells to differentiate efficiently into mature and functional

cardiomyocytes, inadequate recruitment of circulating or resident cardiac stem cells, anomalous electromechanical coupling between the transplanted cells or between the transplanted and host cells with consequent arrhythmias (39).

There exists some difficulties that interfere evaluation the effect of cell-based therapy, like the use of LVEF for assessing the effects of cell therapy, incorrect target population of not very sick patients with baseline LVEF 50% and existence alternative therapeutic strategies like PCI and standard medicament treatment (39).

Homing of stem cells can be improved by using extracorporeal shockwaves of the target organ or tissue. Hydrogels, cell sheets, prefabricated matrices, microspheres and injectable nanomatrix have been used for improving retention of transplanted cells (39). Also, correction of enviroment conditions can help in cell retention as well as using genetic engineering tools, including overexpression of pro-survival genes or by transplanting the cells together with pro-survival or pro-angiogenic factors or transplantation of preconditioned cells that suppress inflammatory factors and immune responses, and promoted heart function (39).

Questions remain about whether adult stem cells in the heart truly undergo functional and electrical integration and whether this may have hypocontractile and proarrhythmic consequences.

Conclusion

Many preclinical and clinical studies performed on animal and human models, have showed that MSC therapy is safe and effective therapy for cardiac regeneration, although theirs helling mechanism is not precise defined. MSCs do not appear to be rejected by the immune system, have great growing potential and so availability to use as allogeneic tissue repair therapeutics. Understanding adult stem cells such as MSCs provides further development of this field and eventually use of other stem cells in treatment of many other human diseases.

References

1. Friedenstein AJ, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luriá EA et al. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. *Exp Hematol.* 1974;2(2):83-92.
2. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999 Apr 2;284(5411):143-7.
3. Brighton CT, Hunt RM. Early histologic and ultrastructural changes in microvessels of periosteal callus. *J Orthop Trauma.* 1997 May;11(4):244-53.
4. Friedenstein AJ, Chailakhyan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet.* 1970 Oct;3(4):393-403.
5. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation.* 1974 Apr;17(4):331-40.
6. Caplan AI. Molecular and cellular differentiation of muscle, cartilage, and bone in the developing limb. *Prog Clin Biol Res.* 1986;217B:307-18.
7. Piersma AH, Brockbank KGM, Ploemacher RE. Characterization of fibroblastic stromal cells from murine bone marrow. *Experimental Hematology.* 1985;13(4):237-243.
8. Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve.* 1995 Dec;18(12):1417-26.
9. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999 Apr 2;284(5411):143-7.
10. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc Natl Acad Sci U S A.* 1999 Sep 14;96(19):10711-6.
11. Sato Y, Araki H, Kato J, Nakamura K, Kawano Y, Kobune M et al. Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. *Blood.* 2005 Jul 15;106(2):756-63.
12. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation.* 2002 Jan 1;105(1):93-8.
13. Xu W, Zhang X, Qian H, Zhu W, Sun X, Hu J et al. Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype in vitro. *Exp Biol Med (Maywood).* 2004 Jul;229(7):623-31.
14. da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci.* 2006 Jun 1;119(Pt 11):2204-13. Epub 2006 May 9.
15. Caplan AI. Mesenchymal stem cells. *J Orthop Res.* 1991 Sep;9(5):641-50.
16. Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ et al. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells.* 2004;22(7):1330-7.
17. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy.* 2006;8(4):315-7.
18. Pittenger MF, Martin BJ. Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res.* 2004 Jul 9;95(1):9-20.
19. Psaltis PJ, Zannettino AC, Worthley SG, Gronthos S. Concise review: mesenchymal stromal cells: potential for cardiovascular repair. *Stem Cells.* 2008 Sep;26(9):2201-10. doi: 10.1634/stemcells.2008-0428.
20. Boyle AJ, McNiece IK, Hare JM. Mesenchymal stem cell therapy for cardiac repair. *Methods Mol Biol.* 2010;660:65-84.
21. Li W, Ren G, Huang Y, Su J, Han Y, Li J et al. Mesenchymal stem cells: a double-edged sword in regulating immune responses. *Cell Death Differ.* 2012 Sep;19(9):1505-13.
22. Tölli MA, Ferreira MP, Kinnunen SM, Rysä J, Mäkilä EM, Szabó Z et al. In vivo biocompatibility of porous silicon biomaterials for drug delivery to the heart. *Biomaterials.* 2014 Sep;35(29):8394-405.
23. Cotran RS, Kumar V, Robbins SL, Schoen FJ (eds): *Inflammation and repair: in Robbins Pathologic Basis of Disease.* Philadelphia. WB Saunders Company, 1994, pp 51-93.
24. Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M et al. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet.* 2003 Aug 30;362(9385):697-703.
25. Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ et al. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells.* 2004;22(7):1330-7.
26. Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. *Exp Mol Med.* 2013 Nov 15;45:e54.
27. Guo J, Lin GS, Bao CY, Hu ZM, Hu MY. Anti-inflammation role for mesenchymal stem cells transplantation in myocardial infarction. *Inflammation.* 2007 Aug;30(3-4):97-104.
28. LaFramboise WA, Bombach KL, Dhir RJ, Muha N, Cullen RF, Pogoselski AR et al. Molecular dynamics of the compensatory response to myocardial infarct. *J Mol Cell Cardiol.* 2005 Jan;38(1):103-17.
29. Hsieh PC, Segers VF, Davis ME, MacGillivray C, Gannon J, Molkenstein JD et al. Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. *Nat Med.* 2007 Aug;13(8):970-4.
30. Abbate A, Bussani R, Biondi-Zoccai GG, Santini D, Petrolini A, De Giorgio F et al. Infarct-related artery occlusion, tissue markers of ischaemia, and increased apoptosis in the peri-infarct viable myocardium. *Eur Heart J.* 2005 Oct;26(19):2039-45.
31. Kanamori H, Takemura G, Goto K, Maruyama R, Ono K, Nagao K et al. Autophagy limits acute myocardial infarction induced by permanent coronary artery occlusion. *Am J Physiol Heart Circ Physiol.* 2011 Jun;300(6):H2261-71.
32. Kushner FG, Hand M, Smith SC Jr, King SB 3rd, Anderson JL, Antman EM et al. American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. 2009 Focused Updates: ACC/AHA Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction

- (updating the 2004 Guideline and 2007 Focused Update) and ACC/AHA/SCAI Guidelines on Percutaneous Coronary Intervention (updating the 2005 Guideline and 2007 Focused Update): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2009 Dec 1;120(22):2271-306.
33. Reffelmann T, Könemann S, Kloner RA. Promise of blood- and bone marrow-derived stem cell transplantation for functional cardiac repair: putting it in perspective with existing therapy. *J Am Coll Cardiol*. 2009 Jan 27;53(4):305-8.
 34. Dauwe DF, Janssens SP. Stem cell therapy for the treatment of myocardial infarction. *Curr Pharm Des*. 2011 Oct;17(30):3328-40.
 35. Schaper J. Ultrastructural changes of the myocardium in regional ischaemia and infarction. *Eur Heart J*. 1986 Jul;7 Suppl B:3-9.
 36. Mummery CL, Davis RP, Krieger JE. Challenges in using stem cells for cardiac repair. *Sci Transl Med*. 2010 Apr 14;2(27):27ps17.
 37. Reimer KA, Jennings RB. The "wavefront phenomenon" of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. *Lab Invest*. 1979 Jun;40(6):633-44.
 38. Hale SL, Kloner RA. Ranolazine, an inhibitor of the late sodium channel current, reduces postischemic myocardial dysfunction in the rabbit. *J Cardiovasc Pharmacol Ther*. 2006 Dec;11(4):249-55.
 39. Madonna R, Van Laake LW, Davidson SM, Engel FB, Hausenloy DJ, Lecour S et al. Position Paper of the European Society of Cardiology Working Group Cellular Biology of the Heart: cell-based therapies for myocardial repair and regeneration in ischemic heart disease and heart failure. *Eur Heart J*. 2016 Jun 14;37(23):1789-98.

AMM Paper Accepted

ULOGA MEZENHIMALNIH MATIČNIH ĆELIJA U TERAPIJI INFARKTA MIOKARDA

Zorana Antonijević^{1,2}, Aleksandra Vuletić³

¹Klinički centar Kragujevac, Klinika za kardiologiju, Kragujevac, Srbija

²Fakultet medicinskih nauka Univerziteta u Kragujevcu, Kragujevac, Srbija

³Dom zdravlja Kragujevac, Kragujevac, Srbija

Kontakt: Zorana Antonijević
34206 Gornja Sabanta, 34000 Kragujevac, Srbija
E-mail: antonzjnet@gmail.com

Mezenhimalne matične ćelije (eng. *Mesenchymal stem cells* (MSCs)) su multipotentne stromalne ćelije koje mogu da se diferenciraju u osteoblaste, hondroците i adipocite. Početkom 21. veka, *in vivo* studije su pokazale da humane MSCs mogu da transdiferenciraju u ćelije dobijene od endoderma i kardiomiocitni fenotip. Kada kardiomiociti nisu snabdeveni krvlju, kao što je u infarktu miokarda, gubitak funkcionalnih kardiomiocita se odigrava putem nekroze, apoptoze i autofagije. Pored prolaska kroz različite faze inflamacije i ozdravljenja, dinamično mikrokruženje u infarciranom tkivu takođe eksprimira citokine koji promovišu migraciju matičnih ćelija i njihov *homing*. S obzirom na neizvesnu sudbinu miokarda diktiranu stepenom nekroze, postaje jasno, da u cilju boljeg ishoda infarkta miokarda, je neophodno pronaći adekvatnu terapiju. Moguće rešenje ove dileme je ciljana terapija matičnim ćelijama.

Acta Medica Medianae 2019;58(4):xx-xx.

Ključne reči: Mezenhimalne matične ćelije; infarkt miokarda; terapija