IMMEDIATE RESULTS OF THREE METHODS OF SURGICAL MYOCARDIAL REVASCULARIZATION IN MULTIVESSEL LESION OF THE CORONARY BED

MILICA MASLOVARIC[1], NIKOLA FATIC[2] , EMILIJA DELEVIĆ[3]

[1] *Prona-Montenegrin Science Promotion Foundation,*
[2] *Department of Vascular Surgery, Clinical Centre of Montenegro,*
[3] *Medical Faculty in Podgorica, University of Montenegro, Podgorica, Montenegro*

Ischemic cardiomyopathy is becoming a leading cause of morbidity and mortality in the whole world. Stem cell-based therapy is emerging as a promising option for treatment of ischemic cardiomyopathy. Several stem cell types, including cardiac-derived stem cells, bone marrow-derived stem cells, mesenchymal stem cells, skeletal myoblasts, CD34+ and CD133+ stem cells have been used in clinical trials. Clinical effects mostly depend on transdifferentiation and paracrine factors. One important issue is that a low survival and residential rate of transferred stem cells blocks the effective advances in cardiac improvement. Many other factors associated with the efficacy of cell replacement therapy for ischemic cardiomyopathy mainly including the route of delivery, the type and number of stem cell infusion, the timing of injection, patient's physical conditions, the particular microenvironment onto which the cells are delivered, and clinical conditions remain to be addressed. Here we provide an overview of modern methods of stem cell delivery, types of stem cells and discuss the current state of their therapeutic potential.

Key words: *mesenchymal stem cells, cardiac-derived stem cells, skeletal myoblasts, bone marrow-derived stem cells, intramyocardial injection, transvascular cell delivery.*

INTRODUCTION

Reduced blood flow in a myocardial infarction affected area is the leading cause of morbidity and mortality in patients with ischemic cardiomyopathy [1, 2]. Although averagely 1% of adult cardiomyocytes appear to possess the ability of self-renewal, they cannot provide recovery of heart tissue after an infarction or some serious heart damage [3–5]. Thus, ischemia-induced apoptosis and necrosis of cardiomyocytes damage the geometry of the left ventricle undergoing progressive remodeling, hypertrophy and proliferation of fibroblasts, which results in cicatrization and poor contractility of the left ventricle [6–8]. Such common treatment strategies as pharmacotherapy, coronary artery bypass grafting and coronary artery stenting allow the recovery of blood supply to the ischemic regions and relative pain relief, but they fail to treat pathophysiological changes after ischemic injuries, and regenerate the muscle tissue of the heart. Therefore, the essential effect of treatment is to enable regeneration of myocardial cells using cardiac progenitor cells or other exogenous multipotent stem cells [9]. Stem cell implantation for the treatment of ischemic cardiomyopathy brought a new age to patients, and at the same time it faces numerous challenges. A lot of evidence suggests that the stem cells perform regeneration of the damaged part of the heart by differentiating into cardiac muscle cells, promoting angiogenesis, proliferation of endogenous cardiac stem cells and secretion of cytokines, chemokines and growth factors that activate endogenous reparative responses, inhibit cellular apoptosis and fibrosis, and improve myocardial contractility [10]. In the last decade, many clinical trials have been conducted to assess the safety, feasibility and efficacy of stem cell administration in patients with ischemic cardiomyopathy. Different types of cells, including bone marrow-derived stem cells, mesenchymal stem cells, cardiac-derived stem cells, skeletal myoblasts and hematopoietic stem cells, have been used to evaluate the potential therapy based on the stem cells. However, promising results from numerous clinical studies, in improvement of functional parameters, have shown several ineffective treatments. Cell transport modes and their doses, cell isolation procedures and transplantation time can determine effects on improving heart function. This paper presents the status of previous clinical trials and future perspectives for the use of stem cell therapy in patients with ischemic cardiomyopathy.

RESULTS

ENDOGENOUS/PROGENITOR CARDIAC STEM CELLS

In 2003, cardiac stem cells (CSCs) were discovered by Nadal-Ginard and his colleagues, who outperformed the traditional understanding that the heart is a terminal-differentiated organ [11]. Multipotential and self-renewal characteristics of these cells have been identified on animal models, proving their ability to differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells, indicating the potential regenerative

capacity of the adult heart [12]. Experimental studies have reported that cardiomyocytes can be derived from different types of cardiac stem cells, including c-kit+ cells, isl-1+ cells, and sca-1+ cells (limited to the mouse heart) and cardiosphere-derived cells (CDC) [13].

The CDCs are extracted with a biopsy of the heart and grown in the form of clusters possessing heterogeneous cell populations positive for c-kit (endogenous stem cell), CD105 and CD90 (cardiovascular MSC), but negative for CD45 (hematopoietic stem cells), thus indicating their capacity for clonogenic, self-renewing and multigenic differentiation [13]. The CSCs are mainly located at the top of the left ventricle and the atrial tissue. To date, several clinical studies (in different clinical stages) have been approved and/or completed to demonstrate the feasibility, safety and efficacy of CSC cell transplantation therapy in patients with ischemic cardiomyopathy.

The first clinical phase I study involving 16 patients after endured coronary artery bypass grafting and postinfarction left ventricular dysfunction (ejection fraction ⩽40%), suggested that an intracoronary infusion of one million autologous CSCs (c-kit+ cells) increased left ventricular ejection fraction (LVEF) from 30.3 to 38.5% at four months and to 42.3% at one year. In addition, it reduced the size of the area affected by infarction in seven patients, from 32.6 to 24.8 g at four months and to 22.8 g at 1 year [14]. Later, a randomized phase I clinical trial conducted by Makkar, et al. evaluated the safety of intracoronary infusion of cardiosphere-derived cells (CSCs) in patients with left ventricular dysfunction after myocardial infarction (with left ventricular ejection fraction of 25–45%) [15]. The obtained results demonstrated a decrease in the mass of damaged tissue, an increase in the weight of healthy heart tissue and regional contractility, during the six-month trial in the group of patients treated with CDCs, but did not show significant differences in changes of end-systolic volume, end-diastolic volume or LV ejection fraction, compared with patients undergoing conventional therapies. Fortunately, there were no serious adverse effects during the six-month trial, thus indicating the safety of CDC implantation treatment for myocardial infarction.

The CADUCEUS (CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction) randomized and controlled trial was performed on patients with left ventricular dysfunction in order to examine the effectiveness of this therapeutic method [16].

EXOGENOUS STEM CELLS

BONE MARROW-DERIVED MONONUCLEAR CELLS

Bone marrow-derived mononuclear cells (BM-MNCs) are a mixed population of different types of non-differentiated cells that contain primary cells, hematopoietic stems (HSCs), endothelial progenitor cells (EPCs) and a fractional part of about 0.01% of mesenchymal stem cells (MSCs) [17]. Even though most BM-MNCs are not stem cells, the effects of cardiac recovery are considered and are mainly dependent on a significant source of hematopoietic stem cells (HSCs) that contribute to the development of new heart tissue and blood vessels. It is generally accepted that cardiac recovery with HSC therapy may depend on the secretion of growth factors and other proteins that promote angiogenesis and stimulate the proliferation and migration of endogenous cardiac stem cells and cardiomyocytes [18, 19]. Several clinical trials have shown that the intracoronary administration of adult/progenitor bone marrow stem cells in patients with acute myocardial infarction enhances the contractile function of the left ventricle, which was reported by REPAIR-AMI and TOPCARE-AMI clinical studies [20, 21].

It has been shown that the treatment of stem cells separated from the bone marrow significantly reduces unwanted clinical manifestations. Therefore, clinical experimental responses confirmed the efficacy and feasibility of BM-MNSC therapy in the treatment of ischemic cardiomyopathy, although detailed mechanisms of the use of these cells have not been confirmed. Furthermore, much more convincing results on the improvement of ejection fraction of the left ventricle, cardiac contractility and exercise capacity have been suggested by numerous clinical studies [22–24].

To date, the largest meta-analysis conducted by Jeevanantham, et al. has also reviewed the systemically classified clinical results of 50 studies, involving 2562 patients with acute or chronic ischemic cardiomyopathy [25]. Regardless of the detailed plan of this study (e.g. pathway and time of cell transplantation) and specific types of ischemic cardiomyopathy (acute or chronic), patients treated with mononuclear stem cells separated from the bone marrow showed a moderate increase in the ejection fraction of the left ventricle (~3.96%), ESV, EDV and a moderate reduction in the area affected by the infarction (~4.03%), compared with control subjects. This analysis also showed that mononuclear stem cells of the bone marrow significantly reduced the occurrence of some manifestations, including death, myocardial infarction rate and stent thrombosis. This progress is consistent with another recent meta-analysis released in 2014 [26].

The aggregate results from 32 trials, which included 1300 patients treated with BM-MNC therapy and 1006 patients who went to regular controls (both groups with acute coronary syndrome or stable coronary disease) demonstrated a statistically significant increase in LVEF (~ 4.6%) and reduction of the damaged area (~9.5%). However, another

recent meta-analysis has exposed the contradictory conclusion of 22 randomized trials [27]. In this study, improvement in the cardiac function of MRI parameters or clinical outcome was not demonstrated despite a slight increase in the left ventricular ejection fraction (~2.1%), although the safety of BM-MNC therapy for patients with ischemic cardiomyopathy was again demonstrated. The lack of improvement of the ventricle using BM-MNC therapy enhancement was parallel in a recent clinical study, which included 28 patients with advanced ischemic cardiomyopathy [28]. After 6 months, patients treated with BM-MNC therapy did not show significant improvement in LVEF, ESV, and in the volume of affected left ventricular infarction, indicating ineffective treatment results with this type of stem cells.

Another placebo-controlled study, which included 65 patients with ischemic cardiomyopathy and a LVEF of less than 50%, showed no statistically significant increase in the ejection fraction and the reduction of affected infarction area [29].

MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs) mainly express cell surface antigens: CD73, CD90, CD105, CD44 and CD133, as well as CD105 surface molecules [30, 31]. They have many advantages: the ability to self-renew, multi-cellular differentiation potential, low immunogenicity, immunosuppressive properties and low carcinogenicity, and do not face the risk of immune rejection [32]. Under appropriate stimulation, the mesenchymal stem cells can be differentiated into cardiomyocytes [32], or they may develop some growth factors for heart recovery. However, the survival rate and migration abilities in the target tissue are two key factors for determining the therapeutic effects of mesenchymal stem cells. Many clinical studies have shown improvement in cardiac function after the treatment of ischemic cardiomyopathy by using mesenchymal stem cells, despite several ineffective MSC therapy results [33].

A placebo-controlled trial included 69 patients with acute myocardial infarction after percutaneous coronary intervention, who were randomly assigned to receive intracoronary injection of BMSCs (n=34) and saline (control group, n= 5). Three months after transplantation, there was a significant improvement of the left ventricular ejection fraction (LVEF) and the ESV/EDV ratio as compared with the control group, thus suggesting efficacy of MSC in recovery of the cardiac function [34]. Intravenous injection of allogeneic mesenchymal stem cells, which reduced episodes of ventricular tachycardia and increased LVEF, produced a similar effect as in the previous study for a period of six months [35].

Later, another clinical study of intramyocardial injection of autologous mesenchymal stem cells in patients with myocardial infarction showed a reduction in the myocardial infarction area and an improvement in left ventricular function after one year [36]. These conclusions were confirmed by subsequent clinical trials that suggested that patients with acute myocardial infarction showed improvement in LVEF, in the group of patients treated with mesenchymal stem cells compared to the control group [24, 37].

COUNTERARGUMENTS

But the work published in the Journal of the American Medical Association in 2014 revealed that although the transendocardial injection of mesenchymal stem cells improved the six-minute walk, the function of the myocardium, and reduced the area affected by the infarct, no changes in left ventricular chamber volume and ejection fraction were observed [29].

Two meta-analyses evaluated the efficacy of MSC therapy in the treatment of ischemic heart disease and concluded that MSC treatment significantly reduces the risk of death, reduces weekly angina episodes and leads to a better quality of life [38, 39].

A recent meta-analysis, involving 1255 patients, produced moderate-grade evidence suggesting that MSC therapy improves the LVEF [40]. Therefore, many of these studies have shown improvement in cardiac function, reduced left ventricular dilatation, and a faster recovery of patients suffering from acute myocardial infarction or ischemic cardiomyopathy [41]. Although the effects of treatment with MSC grafting do not always reach the target site, significant prospects have been demonstrated in the treatment of acute myocardial infarction or ischemic cardiomyopathy.

SKELETAL MYOBLASTS

Skeletal myoblasts (SMs) are a small population of undifferentiated and inactive stem cells found in mature skeletal muscle fibers. When injuries occur, SMs are activated, rapidly proliferated and differentiated into muscle fibers to replace the injured or muscle cells that are extinct [42]. Experimental results have demonstrated their ability to migrate in the infarct-affected area, to differentiate into skeletal muscle myotubes [43] and to improve angiogenesis, thereby improving heart function and accelerating the recovery of the heart muscle [44].

Several pilot studies have shown to some extent an improvement in LVEF and recovery of the ventricular chamber following the use of SM therapy.

Patients with severe ischemic cardiomyopathy, whose heart was injected with autologous SM, showed improvement in LVEF, as well as the New York Heart Association functional class after 6 or 11 months

after the surgery [45, 46]. Although these studies have demonstrated the feasibility, safety and modest effects of SM grafting for the treatment of ischemic cardiomyopathy, the high incidence of ventricular arrhythmia attacks following myoblast transplantation is drawing attention [47]. In addition, a recent six-year study, conducted after SM cell transplantation in 7 patients with heart ischemia, implies that SM therapy did not improve left ventricular function [48]. Moreover, in the group of patients treated with SM therapy, a higher incidence of interventions was observed in those patients who had an implantable cardioverter defibrillator (ICD) compared to the control group [49]. Therefore, SM therapy has shown some therapeutic effects in patients with chronic ischemic cardiomyopathy, which will appeal to many scientists to investigate the therapeutic effects of SM therapy in patients with acute myocardial infarction.

OTHER TYPES OF STEM CELLS

Several clinical trials, that were completed, focused on the recovery of the heart, using angiogenesis and neovascularization in ischemic tissue [50]. Transendocardial injection of CD34+-cells from the peripheral blood into the heart of patients with ischemic cardiomyopathy (LVEF ⩽40%) increased LVEF and the six-minute walk test, demonstrating improved left ventricular function and better exercise capacity [51]. In addition, CD34+-cell therapy in patients with various-severity angina and myocardial ischemia showed a significant reduction in the incidence of angina and tolerance to exercise [52–54]. The main adverse effects were not observed in these CD34+ treatment studies. It has also been reported that CD133+-cell therapy has positive effects on moderate improvement in cardiac function, without serious adverse effects, in patients with chronic ischemic cardiomyopathy, using intramyocardial injection [53–55]. However, a clinical trial called «Cardio133» showed no effect on left ventricular function and clinical symptoms in patients with chronic ischemia and impaired left ventricular function, treated with intramyocardial injection of CD133+-cells [56].

DISCUSSION

Numerous results have shown the effects of the treatment of ischemic cardiomyopathy using several types of stem cells. Although the number of CSC cells is limited (one cell at 8,000 to 20,000 cardiomyocytes), they can generate enough cardiomyocytes a day in order to preserve the balance of recovery of myocardial cells in a healthy heart. These cells cannot tolerate major acute damage to the heart tissue [57].

The CSC therapy is mainly focused on patients with myocardial infarction or left ventricular dysfunction, mainly due to cardiac fibrosis, when the affected area did not have enough blood flow and this therapy proved to be more effective in treating myocardial infarction than ischemic cardiomyopathy [24]. However, these limited clinical findings cannot fully demonstrate the practical abilities of CSC therapy in the treatment of ischemic cardiomyopathy.

To date, it is known that BM-MNC therapy has shown very different and disaggregated results, which have reported modest effects or that there are no positive effects in the function and left ventricular geometry. Fortunately, no major adverse effects were found in clinical trials. All in all, BM-MNC therapy has been widely used in patients with acute or chronic ischemic cardiomyopathy. However, contradictory results have been presented, due to the effects of numerous factors, which mainly involve the methods of isolating the bone marrow-derived mononuclear cells, as well as the type and total number of injected cells. Cell mechanisms that affect the efficacy of therapy include effective homing, proliferation and differentiation of the injected bone marrow-derived mononuclear cells [58].

These results are expected to be an initiative for larger randomized and controlled clinical studies, which is related to the improvement in the cell isolation procedure and the selection plan for transplantation. As for the SM cells, they have several unique advantages, such as autologous origin, they are easily accessible, easy to isolate, have a high level of flexibility, high resistance to ischemia, and a lack of carcinogenicity [55].

However, these vague and conflicting results have issued an order to initiate large randomized trials and the use of SM cells for further assessment of efficacy in the treatment of acute/chronic cardiomyopathy. However, what is worth keeping in mind is that ventricular arrhythmias can be a potential risk during SM treatment [55].

Of the several ongoing studies, it is expected to give more effective results. Further research is needed to develop new technologies in the treatment of ischemic cardiomyopathy. Thus, more preclinical and clinical trials are needed, which will illuminate the effects of cell infusion on the progression of cardiac recovery. Many other types of stem cells will be used to assess their practical abilities in the treatment of ischemic cardiomyopathy in the future.

FACTORS INFLUENCING THE STEM CELL TREATMENT OF ISCHEMIC CARDIOMYOPATHY

So far, it is obvious that the current status of the stem cell therapy is very promising, but it faces several challenges. First, many clinical results have demonstrated positive effects of stem cell therapy on the recovery of the heart, regardless of cell type and transplantation methods. Secondly, the current

results of stem cell therapy in the treatment of ischemic cardiomyopathy with high variability and diversity have shown mild to modest or even conflicting results in the function and left-ventricular geometry. Lastly, some types of stem cells and injection routes have shown severe side effects, such as cardiac arrhythmias and vascular restenosis. Many important factors affect the efficacy of cellular infusion, in patients with acute myocardial infarction or ischemic cardiomyopathy, including: cell transplant pathways, type and number of donor cells, as well as medical conditions of the patient [59–61].

PHENOTYPE AND STEM CELL NUMBER

The phenotype of transplanted cells has many correlations with safety and efficacy. MSC transplantation in patients with ischemic cardiomyopathy refers to more promising results and less side effects, contrary to SM therapy. The effects of random meta-analysis, performed on 888 animals, through 52 studies, reported that BMMNC therapy showed poorer results compared to MSC therapy [62]. Sensitivity analysis described that efficacy was more relevant in a large number of cells and subsequent injection of cells (⩾ one week) [52].

A recent comparative clinical trial supported higher efficacy of MSC therapy compared to BMMNC therapy [60]. In addition, the number of injected cells in patients with varying levels of ischemic cardiomyopathy varies from study to study. A single clinical trial showed that a low dose of mesenchymal stem cell concentration (20 million) resulted in an increase in LVEF, compared to a group of patients injected with a higher cell dose (100 million and 200 million) [52], consistent with other comparative results, that say a low dose of intramyocardial injection of CD34+-cells shows a greater improvement in exercise tolerance and reduction in the incidence of angina compared to a group of patients with a high dose of therapy [63]. These cases show the importance of the nature and dose of injected cells, which requires further research in detailed clinical studies.

ISOLATION PROCEDURE

Cell preparation techniques are basically developed, but are to some extent variable due to different periods of incubation. Monoclonal stem cells isolated from the bone marrow are available and injected into patients on the same day after isolation, but other types of stem cells go through the selection, culture and expansion process, which can take several days or weeks. The usual methods for isolating mesenchymal stem cells and stem cells isolated from the heart include a large number of open procedures.

Different centrifugation speeds and the composition of the wash buffer during treatment of cells correlate with the therapeutic effects of ischemic heart disease [64]. Different protocols and components for isolating stem cells and their storage maintain great differences in response to the functional ability to return normal blood flow after BM-MNC transplantation [65]. Contamination of erythrocytes during the isolation process of bone marrow mononuclear cells also reduces cellular viability, migratory function, colony formation capacity, and neovascularization capacity [66]. Also, studies on the development of new technology are ongoing, in order to improve the quality and quantity of cells. A combination of preclinical and clinical studies will produce good results, in terms of developing the best and easiest way to generate the best cells in the body [67].

LOW VIABILITY AND HOUSING RATE OF TRANSPLANTED STEM CELLS AND STRATEGIES FOR INCREASING EFFICACY

Although stem cell therapy imposes a promising approach to heart treatment and cardiac failure after a myocardial infarction, the problem of low efficacy imposes a number of issues. This occurs not only because of the significant loss of transplanted cells due to blood circulation, myocardial contraction and leakage from the site of the sting, but also because of the low level of survival of host cells due to environmental damage, arising after ischemic, hypoxic, inflammatory response or oxidative stress [68]. Various methods have been used to improve cell survival rates and to improve the functional capabilities of these stem cells. An improvement project involves the pre-treatment of cells by environmental conditions and pharmacological, genetic manipulation of stem cells prior to transplantation, combined transplantation of stem cells with molecules of extracellular matrix, nanofibers, hydrogel or fibrous adhesive; as well as a combination of therapy using two types of stem cells [69].

First, pre-treatment cells can maintain the standby state by activating cellular signaling pathways to make them resistant to the enemy environment. Hypoxia or pre-induction of shock waves has been effectively demonstrated to prevent extensive transplant cell transplantation through several processes, including modification of cell phenotype, secretion of different cytokines, and an increase in transcription and translation of antiapoptotic gene [68, 70, 71]. Preclinical studies have shown that treatment of mesenchymal stem cells with transforming growth factor-α (TGF-α), basic fibroblast growth factors, interleukins-1β (IL-1β) or transforming growth factor-β (TGF-β) improves myocardial protection and stimulates angiogenesis [72, 73]. It has also been proven that lysophosphatidic acid and atorvastatin can save rat mesenchymal cells from hypoxic apoptosis [74].

Secondly, the genetic engineering of mesenchymal stem cells has become a promising approach to 'improving' these cells in order to protect themselves from apoptosis in order to increase their retention rates and improve their ability to migrate and differentiate. Excessive expression of antiapoptotic, progenitor or homing receptors causes a significant improvement in the survival rate of mesenchymal stem cells and improves their migratory ability [75, 76].

Thirdly, subsequent transplantation of cells with extracellular matrix molecules, nanofibers, hydrogels or fibrin glues, can directly increase the retention rate of cells that are subjected to certain treatments and also stimulate cell survival and their differentiation [77].

An encapsulation of the cardiac stem cells within the matrix-enriched hydrogel capsule prevents cell death and improves retention of the cardiac stem cells [77]. The last two studies reported that the transplantation of mesenchymal stem cells, cultured on the nanomatrix or on the 3D extracellular matrix, can successfully improve the retention rate of mesenchymal stem cells and stimulate cell proliferation, adhesion and migration, which again demonstrated the potential application in regenerative ischemic cardiomyopathy therapy [78, 79].

Another experimental study demonstrated the formation of a thick layer and the angiogenesis of transplanted single-layer mesenchymal stem cells by paracrine effects. However, the grafting of genetically modified cells has not been performed in undergraduate studies due to unpredictable consequences of rejection of foreign cells and arrhythmias [80].

Finally, combined transplantation of autologous skeletal myoblasts and stem cells separated from the bone marrow, demonstrated the feasibility and efficacy of patients with severe ischemic cardiomyopathy, without generating fatal arrhythmias and complications [81]. After combined transplantation of SM and bone marrow cells, patients have shown significant progress in cardiac function and angiogenesis, as well as reduced fibrosis. In addition, subsequent injection of human cardiac stem cells and human mesenchymal stem cells by intramyocardial infusion caused a greater reduction in infarction affected zone, greater progress in contractility of the left ventricle and improvement of stem cell survival rates, compared with each group of injection of separate type of cells and the placebo group, which reflects an important biological interaction between c-kit+-stem cells of the heart and mesenchymal stem cells.

Thus, these preclinical and clinical results represent an important basis for further testing, in order to improve the quality of life and the cardiac function. Therefore, many unexplored and unknown fields deserve deeper testing, in order to find promising treatment of ischemic cardiomyopathy.

CONCLUSION AND PROSPECTS

Summing up, it should be mentioned that the safety and feasibility of stem cell-transplant therapy in patients with ischemic cardiomyopathy have been widely studied in numerous clinical studies. MSC and BM-MNC cell therapy have been widely tested in patients with acute or chronic ischemic cardiomyopathy and certain therapeutic effects have been demonstrated. MSC therapy shows more promising results in patients with acute myocardial infarction and is a good basis for clinical applications in the near future. Although SM, CD34+ and CD133+ were used in patients with ischemic cardiomyopathy, they showed less application potential than treatments with MSC and CSC cells. Similar results of transplantation of these types of stem cells have shown modest and controversial improvement. However, the inconsistent results of the efficacy assessment are not surprising, due to the use of various techniques and doses of cellular transplantation, due to the use of several pathways for transplantation and due to different conditions in patients. Overall, the mesenchymal stem cells and stem cells of the bone marrow seem to have greater application potential, based on the results of a comprehensive analysis. As for pathways for cell transplantation, intramyocardial and intracoronary pathways show much greater potential than intravenous injection. Mechanisms of action of cell pretreatment include paracrine effects by activating cytokines, chemokines, and increasing factors that will inhibit cell apoptosis and fibrosis, and activate the endogenous regenerative system.

Other elements need to be deeper examined in order to achieve maximum efficiency in terms of optimal separation process, good stem cells, cellular dose, timing and cell transplant pathways, as well as clinical indications. While a large number of comparative studies provide very important results, they are not sufficient to offer the best choice of therapeutic programs. Large random studies are ongoing, in order to optimize therapeutic effects.

In addition, other cell types, such as human mesenchymal cord stem cells, mesenchymal stem cells from the umbilical cord blood and mesenchymal stem cells separated from the fat tissue, show a good prospect in the application of the treatment of ischemic cardiomyopathy. This detailed conclusion will stimulate great interest in additional and large clinical trials. We believe that through the continuation of research and joint multidisciplinary efforts, the transplantation of multipotent stem cells will produce a satisfactory response to the treatment of patients with ischemic cardiomyopathy.

Since cardiovascular diseases (CVD) are the leading cause of morbidity and mortality in the world, it is necessary to develop new technologies that will

enable the treatment of these diseases. Progress in the field of molecular and cell biology allowed the illumination of genetic causes involved in the development of cardiovascular diseases, highlighting a new point of view in terms of prevention and treatment. Thus, the rapid development and improvement of genome editing techniques, such as CRISPR-Cas9, make it possible to influence the complex genome that controls the onset of the disease. Molecular biologists seek to develop a methodology that can nullify or mitigate the effects of CVD-inducing mutations, and it is necessary to establish molecular mechanisms for the regulation of mutated genes and to overcome the problems of transferring therapeutic genetic material into mutated cells.

Stem cells are considered a revolution in regenerative medicine, and their combination with genomic therapy today suggests great potential for the treatment of various diseases. However, gene therapy is a reality, as well as the fact that they have their place in medicine. It is important to point out that gene therapy should be tackled with a high degree of scientific, professional and ethical responsibility, since it is not possible to exclude the possibility of genetic manipulations that are dangerous to human health.

Conflict of interest: none declared.

ЛИТЕРАТУРА/REFERENCES

1. ***Go A.S., Mozaffarian D., Roger V.L., et al.*** Heart disease and stroke statistics – 2014 update: a report from the American Heart Association. Circulation. 2014; 129: 3: e28–e292. doi: 10.1161/01.cir.0000441139.02102.80.
2. ***Henning R.J.*** Stem cells in cardiac repair. Future Cardiology. 2011; 7: 1: 99–117. doi: 10.2217/fca.10.109.
3. ***Beltrami A.P., Barlucchi L., Torellaetal D.*** Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell. 2003; 114: 6: 763–776.
4. ***Hosoda T.*** C-kit-positive cardiac stem cells and myocardial regeneration. American Journal of Cardiovascular Disease. 2012; 2: 1: 58–67.
5. ***Urbanek K., Torella D., Sheikh F., et al.*** Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102: 24: 8692–8697. doi: 10.1073/pnas.0500169102.
6. ***Zamilpa R., Navarro M., Flores I., Griffey S.*** Stem cell mechanisms during left ventricular remodeling post-myocardial infarction: repair and regeneration. World Journal of Cardiology. 2014; 6: 7: 610–620. doi: 10.4330/wjc.v6.i7.610.
7. ***Pangonyte D., Stalioraityte E., Ziuraitiene R., et al.*** Cardiomyocyte remodeling in ischemic heart disease. Medicina (Kaunas). 2008; 44: 11: 848–854.
8. ***Pangonyte D., Stalioraityte E., Kazlauskaite D., et al.*** Changes of heart geometry in patients with ischemic heart disease. Medicina. 2008; 44: 1: 8–14.
9. ***Michler R.E.*** Stem cell therapy for heart failure. Methodist DeBakey Cardiovascular Journal. 2013; 9: 4: 187–194.
10. ***Gnecchi M., Danieli P., Cervio E.*** Mesenchymal stem cell therapy for heart disease. Vascular Pharmacology. 2012; 57: 1: 48–55.
11. ***Nadal–Ginard B., Kajstura J., Leri A., Anversa P.*** Myocyte death, growth, and regeneration in cardiac hypertrophy and failure. Circulation Research. 2003; 92: 2: 139–150.
12. ***Urbanek K., Cesselli D., Rota M., et al.*** Stem cell niches in the adult mouse heart. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103: 24: 9226–9231. doi: 10.1073/pnas.0600635103.
13. ***Matar A.A., Chong J.J.*** Stem cell therapy for cardiac dysfunction. Springer Plus. 2014; 3: 440.
14. ***Bolli R., Chugh A.R., D'Amario D., et al.*** Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. The Lancet. 2011; 378: 9806: 1847–1857. doi: 10.1016/S0140-6736(11)61590-0.
15. ***Makkar R.R., Smith R.R., Cheng K., et al.*** Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. The Lancet. 2012; 379: 9819: 895–904. doi: 10.1016/S0140-6736(12)60195-0.
16. ***Malliaras K., Makkar R.R., Smith R.R., et al.*** Intracoronary cardiosphere-derived cells after myocardial infarction: evidence of therapeutic regeneration in the final 1-year results of the CADUCEUS trial (CArdiosphere-derived a Utologous stem CElls to reverse ventricular dysfunction). Journal of the American College of Cardiology. 2014; 63: 2: 110–122. doi: 10.1016/j.jacc.2013.08.724.
17. ***Dimmeler S., Zeiher A.M.*** Cell therapy of acute myocardial infarction: open questions. Cardiology. 2009; 113: 3: 155–160. doi: 10.1159/000187652.
18. ***Balsam L.B., Wagers A.J., Christensen J.L., et al.*** Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature. 2004; 428: 6983: 668–673.
19. ***Zhang S., Zhang P., Guo J., et al.*** Enhanced cytoprotection and angiogenesis by bone marrow cell transplantation may contribute to improved ischemic myocardial function. European Journal of Cardio-Thoracic Surgery. 2004; 25: 2: 188–195.
20. ***Dill T., Schächinger V., Rolf A., et al.*** Intracoronary administration of bone marrow-derived progenitor cells improves left ventricular function in patients at risk for adverse remodeling afteracute ST-segment elevation myocardial infarction: results of the Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction study (REPAIRAMI)

cardiac magnetic resonance imaging substudy. American Heart Journal. 2009; 157: 3: 541–547. doi: 10.1016/j.ahj.2008.11.011.

21. ***Mills J.S., Rao S.V.*** REPAIR-AMI: stem cells for acute myocardial infarction. Future Cardiology. 2007; 3: 2: 137–140.
22. ***Assmus B., Rolf A., Erbs S., et al.*** Clinical outcome 2 years after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction. Circulation: Heart Failure. 2010; 3: 1: 89–96. doi: 10.1161/CIRCHEARTFAILURE.108.843243.
23. ***Leistner D.M., Fischer-Rasokat U., Honold J., et al.*** Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI): final 5-year results suggest long-term safety and efficacy. Clinical Research in Cardiology. 2011; 100: 10: 925–934. doi: 10.1007/s00392-011-0327-y.
24. ***Williams A.R., Trachtenberg B., Velazquez D.L., et al.*** Intramyocardial stem cell injection in patients with ischemic cardiomyopathy: functional recovery and reverse remodeling. Circulation Research. 2011; 108: 7: 792–796. doi: 10.1161/CIRCRESAHA.111.242610.
25. ***Jeevanantham V., Butler M., Saad A., et al.*** Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis. Circulation. 2012; 126: 5: 551–568. doi: 10.1161/CIRCULATIONAHA.111.086074.
26. ***Sadat K., Ather S., Aljaroudi W., et al.*** The effect of bone marrow mononuclear stem cell therapy on left ventricular function and myocardial perfusion. Journal of Nuclear Cardiology. 2014; 21: 2: 351–367. doi: 10.1007/s12350-013-9846-4.
27. ***De Jong R., Houtgraaf J.H., Samiei S., et al.*** Intracoronary stem cell infusion after acute myocardial infarction: a meta-analysis and update on clinical trials. Circulation: Cardiovascular Interventions. 2014; 7: 2: 156–167. doi: 10.1161/CIRCINTERVENTIONS.113.001009.
28. ***Santoso T., Siu C.W., Irawan C., et al.*** Endomyocardial implantation of autologous bone marrow mononuclear cells in advanced ischemic heart failure: a randomized placebo controlled trial (END-HF). Journal of Cardiovascular Translational Research. 2014; 7: 6: 545–552. doi: 10.1007/s12265-014-9580-6.
29. ***Heldman A.W., Di Fede D.L., Fishman J.E., et al.*** Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: the TAC-HFT randomized trial. Journal of the American Medical Association. 2014; 311: 1: 62–73. doi: 10.1001/jama.2013.282909.
30. ***Dominici M., Le Blanc K., Mueller I., et al.*** Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8: 4: 315–317. doi: 10.1080/14653240600855905.
31. ***Wang X.-J., Li Q.P.*** The roles of mesenchymal stem cells (MSCs) therapy in ischemic heart diseases. Biochemical and Biophysical Research Communications. 2007; 359: 2: 189– 193.
32. ***Ryan J.M., Barry F.P., Murphy J.M., Mahon B.P.*** Mesenchymal stem cells avoid allogeneic rejection. Journal of Inflammation. 2005; 2: 8.
33. ***Yu Q., Li Q., Naetal R., et al.*** Impact of repeated intravenous bone marrow mesenchymal stem cells infusion on myocardial collagen network remodeling in a rat model of doxorubicin-induced dilated cardiomyopathy. Molecular and Cellular Biochemistry. 2014; 387: 1–2: 279–285. doi: 10.1007/s11010-013-1894-1.
34. ***Chen S.L., Fang W.W., Qianetal J., et a***l. Improvement of cardiac function after transplantation of autologous bone marrow mesenchymal stem cells in patients with acute myocardial infarction. Chinese Medical Journal. 2004; 117: 10: 1443–1448.
35. ***Hare J.M., Traverse J.H., Henry T.D., et al.*** A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. Journal of the American College of Cardiology. 2009; 54: 24: 2277–2286. doi: 10.1016/j.jacc.2009.06.055.
36. ***Wang X., Xi W.C., Wang F.*** The beneficial effects of intracoronary autologous bone marrow stem cell transfer as an adjunct to percutaneous coronary intervention in patients with acute myocardial infarction. Biotechnology Letters. 2014; 36: 11: 2163–2168. doi: 10.1007/s10529-014-1589-z.
37. ***Lee J., Lee S., Youn Y., et al.*** A randomized, open-label, multicenter trial for the safety and efficacy of adult mesenchymal stem cells after acute myocardial infarction. Journal of Korean Medical Science. 2014; 29: 1: 23–31. doi: 10.3346/jkms.2014.29.1.23.
38. ***Fisher S.A., Dorée C., Brunskill S.J., et al.*** Bone marrow stem cell treatment for ischemic heart disease in patients with no option of revascularization: a systematic review and meta-analysis. PLoS ONE. 2013; 8: 6: 64669. doi: 10.1371/journal.pone.0064669.
39. ***Nowbar A.N., Mielewczik M., Karavassilis M., et al.*** Discrepancies in autologous bone marrow stem cell trials and enhancement of ejection fraction (DAMASCENE): weighted regression and meta-analysis. The British Medical Journal. 2014; 348: 2688. doi: 10.1136/bmj.g2688.
40. ***Fisher S.A., Brunskill S.J., Doree C., et al.*** Stem cell therapy for chronic ischaemic heart disease and congestive heart failure. Cochrane Database of Systematic Reviews. 2014; 4: CD007888. doi: 10.1002/14651858.CD007888.pub2.
41. ***Amado L.C., Saliaris A.P., Schuleri K.H., et al.*** Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. Proceedings of the National Academy of Sciences. 2005; 102: 32: 11474–11479.
42. ***Minguell J.J., Erices A.*** Mesenchymal stem cells and the treatment of cardiac disease. Experimental Bi-

ology and Medicine. 2006; 231: 1: 39–49.

43. ***Dowell J.D., Rubart M., Pasumarthi K.B., et al.*** Myocyte and myogenic stem cell transplantation in the heart. Cardiovascular Research. 2003; 58: 2: 336–350.
44. ***Nomura T., Ashihara E., Tateishietal K., et al.*** Skeletal myosphere derived progenitor cell transplantation promotes neovascularization in delta-sarcoglycan knockdown cardiomyopathy. Biochemical and Biophysical Research Communications. 2007; 352: 3: 668–674.
45. ***Ince H., Petzsch M., Rehders T.C., et al.*** Percutaneous transplantation of autologous myoblasts in ischemic cardiomyopathy. Herz. 2005; 30: 3: 223–231.
46. ***Menasché P., Hagége A.A., Vilquin J.-T., et al***. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. Journal of the American College of Cardiology. 2003; 41: 7: 1078–1083. doi: 10.1016/S0735-1097(03)00092-5.
47. ***Brickwedel J., Gulbins H., Reichenspurner H.*** Long-term follow-up after autologous skeletal myoblast transplantation in ischaemic heart disease. Interactive Cardiovascular and Thoracic Surgery. 2014; 18: 1: 61–66. doi: 10.1093/icvts/ivt434.
48. ***Veltman C.E., Soliman O.I.I., Geleijnse M.L., et al.*** Four-year follow-up of treatment with intramyocardial skeletal myoblasts injection in patients with ischaemic cardiomyopathy. European Heart Journal. 2008; 29: 11: 1386–1396. doi: 10.1093/eurheartj/ehn171.
49. ***Wang J., Zhang S., Rabinovich B., et al.*** Human CD34+ cells in experimental myocardial infarction: long-term survival, sustained functional improvement, and mechanism of action. Circulation Research. 2010; 106: 12: 1904–1911. doi: 10.1161/CIRCRESAHA.110.221762.
50. ***Poglajen G., Sever M., Cukjati M., et al.*** Effects of transendocardial CD34+Cell transplantation in patients with ischemic cardiomyopathy. Circulation: Cardiovascular Interventions. 2014; 7: 4: 552–559. doi: 10.1161/CIRCINTERVENTIONS.114.001436.
51. ***Wang S., Cui J., Peng W., Lu M.*** Intracoronary autologous CD34+ stem cell therapy for intractable angina. Cardiology. 2010; 117: 2: 140–147. doi: 10.1159/000320217.
52. ***Losordo D.W., Henry T.D., Davidson C., et al.*** Intramyocardial, autologous CD34+ cell therapy for refractory angina. Circulation Research. 2011; 109: 4: 428–436. doi: 10.1161/CIRCRESAHA.111.245993.
53. ***Perinand E.C., Willerson J.T.*** CD34+ autologous human stem cells in treating refractory angina. Circulation Research. 2011; 109: 4: 351–352. doi: 10.1161/CIRCRESAHA.111.245993.
54. ***Povsic T.J., Junge C., Nada A., et al.*** A phase 3, randomized, double-blinded, active-controlled, unblinded standard of care study assessing the efficacy and safety of intramyocardial autologous CD34+ cell administration in patients with refractory angina: design of the renew study. American Heart Journal. 2013; 165: 6: 854–861. doi: 10.1016/j.ahj.2013.03.003.
55. ***Nasseri B.A., Ebell W., Dandel M., et al.*** Autologous CD133+ bone marrow cells and bypass grafting for regeneration of ischaemic myocardium: the Cardio133 trial. European Heart Journal. 2014; 35: 19: 1263–1274. doi: 10.1093/eurheartj/ehu007.
56. ***Hu S., Yan G., He W., et al.*** The influence of disease and age on human cardiac stem cells. Annals of Clinical Biochemistry. 2014; 51: 5: 582–590.
57. ***Capogrossi M.C.*** Cardiac stem cells fail with aging: a new mechanism for the age-dependent decline in cardiac function. Circulation Research. 2004; 94: 4: 411–413.
58. ***Fujita T., Sakaguchi T., Miyagawa S., et al.*** Clinical impact of combined transplantation of autologous skeletal myoblasts and bone marrow mononuclear cells in patients with severely deteriorated ischemic cardiomyopathy. Surgery Today. 2011; 41: 8: 1029–1036. doi: 10.1007/s00595-010-4526-3.
59. ***Heldman A.W., Hare J.M.*** Cell therapy for myocardial infarction: special delivery. Journal of Molecular and Cellular Cardiology. 2008; 44: 3: 473–476. doi: 10.1016/j.yjmcc.2007.10.024.
60. ***Loughran J.H., Elmore J.B., Waqar M., et al.*** Cardiac stem cells in patients with ischemic cardiomyopathy: discovery, translation, and clinical investigation. Current Atherosclerosis Reports. 2012; 14: 5: 491–503. doi: 10.1007/s11883-012-0273-9.
61. ***Vander Spoel T.I.G., Jansen of Lorkeers S.J., Agostoni P., et al.*** Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease. Cardiovascular Research. 2011; 91: 4: 649–658. doi: 10.1093/cvr/cvr113.
62. ***Hare J.M., Fishman J.E., Gerstenblith G., et al.*** Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. The Journal of the American Medical Association. 2012; 308: 22: 2369–2379.
63. ***Beem R.T., Hirsch A., Lommerse I.M., et al.*** Recovery and functional activity of mononuclear bone marrow and peripheral blood cells after different cell isolation protocols used in clinical trials for cell therapy after acute myocardial infarction. Euro Intervention. 2008; 4: 1: 133–138.
64. ***Seeger F.H., Tonn T., Krzossok N., et al.*** Cell isolation procedures matter: a comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction. European Heart Journal. 2007; 28: 6: 766–772.
65. ***Assmus B., Tonn T., Seeger F.H., et al.*** Red blood cell contamination of the final cell product impairs the efficacy of autologous bone marrow mononuclear cell therapy. Journal of the American College of Cardiology. 2010; 55: 13: 1385–1394. doi: 10.1016/j.jacc.2009.10.059.

66. ***Hanley P.J., Mei Z., Durett A.G., et al.*** Efficient manufacturing of therapeutic mesenchymal stromal cells with the use of the Quantum Cell Expansion System. Cytotherapy. 2014; 16: 8: 1048–1058. doi: 10.1016/j.jcyt.2014.01.417.
67. ***Kurtz A.*** Mesenchymal stem cell delivery routes and fate. International Journal of Stem Cells. 2008; 1: 1: 1–7.
68. ***Chen J., Baydoun A.R., Xu R., et al.*** Lysophosphatidic acid protects mesenchymal stem cells against hypoxia and serum deprivation-induced apoptosis. Stem Cells. 2008; 26: 1: 135–145.
69. ***Liu X., Hou J., Shi L., et al.*** Lysophosphatidic acid protects mesenchymal stem cells against ischemia-induced apoptosis in vivo. Stem Cells and Development. 2009; 18: 7: 947–954. doi: 10.1089/scd.2008.0352.
70. ***Zhang Q., Wang H., Yang Y.J., et al.*** Atorvastatin treatment improves the effects of mesenchymal stem cell transplantation on acute myocardial infarction: the role of the RhoA/ROCK/ERK pathway. International Journal of Cardiology. 2014; 176: 3: 670–679. doi: 10.1016/j.ijcard.2014.07.071.
71. ***Herrmann J.L., Wang Y., Abarbanell A.M., et al.*** Preconditioning mesenchymal stem cells with transforming growth factor-alpha improves mesenchymal stem cell-mediated cardioprotection. Shock. 2010; 33: 1: 24–30. doi: 10.1097/SHK.0b013e3181b7d137.
72. ***Luo Y., Wang Y., Poynter J.A., et al.*** Pretreating mesenchymal stem cells with interleukin-1 and transforming growth factor synergistically increases vascular endothelial growth factor production and improves mesenchymal stem cell-mediated myocardial protection after acute ischemia. Surgery. 2012; 151: 3: 353–363. doi: 10.1016/j.surg.2011.09.033.
73. ***Park J.S., Suryaprakash S., Lao Y.H., Leong K.W.*** Engineering mesenchymal stem cells for regenerative medicine and drug delivery. Methods. 2015; 84: 3–16. doi: 10.1016/j.ymeth.2015.03.002.
74. ***Yin P.T., Han E., Lee K.B.*** Engineering stem cells for biomedical applications. Advanced Healthcare Materials. 2015; 5: 1: 10–55. doi: 10.1002/adhm.201400842.
75. ***Mayfield A.E., Tilokee E.L., Latham N., et al.*** The effect of encapsulation of cardiac stem cells within matrix-enriched hydrogel capsules on cell survival, post-ischemic cell retention and cardiac function. Biomaterials. 2014; 35: 1: 133–142. doi: 10.1016/j.biomaterials.2013.09.085.
76. ***Schmuck E.G., Mulligan J.D., Ertel R.L., et al.*** Cardiac fibroblast-derived 3D extracellular matrix seeded with mesenchymal stem cells as a novel device to transfer cells to the ischemic myocardium. Cardiovascular Engineering and Technology. 2014; 5: 1: 119–131.
77. ***Santhakumar R., Vidyasekar P., Verma R.S.*** Cardiogel: a nano-matrix scaffold with potential application in cardiac regeneration using mesenchymal stem cells. PLoS One. 2014; 9: 12: 114697. doi: 10.1371/journal.pone.0114697.
78. ***Miyahara Y., Nagaya N., Kataoka M., et al.*** Monolayeredmesenchymal stem cells repair scarred myocardium after myocardial infarction. Nature Medicine. 2006; 12: 4: 459–465.
79. ***Miyagawa S., Matsumiya G., Funatsu T., et al.*** Combined autologous cellular cardiomyoplasty using skeletal myoblasts and bone marrow cells for human ischemic cardiomyopathy with left ventricular assist system implantation: report of a case. Surgery Today. 2009; 39: 2: 133–136. doi: 10.1007/s00595-008-3803-x.
80. ***Williams A.R., Hatzistergos K.E., Addicott B., et al.*** Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. Circulation. 2013; 127: 2: 213–223. doi: 10.1161/CIRCULATIONAHA.112.131110.
81. ***Belojević J.*** Stem ćelije – Biološki transformer. Zimska škola nauke. 2016; 1: 10–17.

Адрес для корреспонденции:
Эмилия Делевич
Тел.: (+382) 69-48-24-12
E-mail: emilija.delevic@gmail.com

Correspondence to:
Emilija Delevic
Tel.: (+382) 69-48-24-12
E-mail: emilija.delevic@gmail.com