Mesenchymal stem cells as the near future of cardiology medicine – truth or wish?

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Cardiac damage is one of major cause of worldwide morbidity and mortality. Despite the development in pharmacotherapy, cardiosurgery and interventional cardiology, many patients remain at increased risk of developing adverse cardiac remodeling. An alternative treatment approach is the application of stem cells. Mesenchymal stem cells are among the most promising cell types usable for cardiac regeneration. Their homing to the damaged area, differentiation into cardiomyocytes, paracrine and/or immunomodulatory effect on cardiac tissue was investigated extensively. Despite promising preclinical reports, clinical trials on human patients are not convincing. Meta-analyses of these trials open many questions and show that routine clinical application of mesenchymal stem cells as a cardiac treatment may be not as helpful as expected.

This review summarizes contemporary knowledge about mesenchymal stem cells role in cardiac tissue repair and discusses the problems and perspectives of this experimental therapeutical approach.

Key words: mesenchymal stem cells, cardiomyocytes, cardiology, cardiac regeneration, results translation

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INTRODUCTION

Cardiac diseases remain a major cause of worldwide morbidity and mortality[1]. In the United States of America every 34 seconds somebody suffers a coronary event[2]. Cardiac function of these patients is increasingly compromised with the progression of adverse cardiac remodeling and many patients eventually develop a fatal end-stage cardiac failure[3].

Progress in cardiovascular pharmacotherapy, cardiosurgery and interventional cardiology decreased mortality rate in cardiac diseases, but patients still remain at high risk of cardiac failure, especially when the damaged cardiac mass is large and the extensive cardiac cell loss is not compensated properly[4]. Only known effective treatment of cardiac diseases is heart transplantation, where donor shortage is a great problem[5]. New alternative approaches of endogenous repair have been investigated in adult mammalian hearts[6], consisting of mechanisms that involve mobilization of bone marrow and blood-derived progenitor cells[7], *in situ* turnover of regular cardiomyocytes[8], and the presence of resident cardiac stem cells having the ability to differentiate into vascular and mature cardiac cells[6]. However, all these mechanisms are not sufficient to prevent deleterious re-modeling of cardiac tissue.

Stem cells based therapies are now in worldwide interest as a promising treatment of various diseases[9], including cardiovascular diseases, where intensive research is performed[10]. Initially, the goal of stem cell based therapies was to provide a source of proliferating and functional cardiomyocytes, which will substitute cardiac cell loss and minimize damaged area. This aim has not been achieved to date. For clinical application various stem cell types are relevant, but their capability of creating mature cardiomyocytes *in vivo* is limited[11]. Therefore, stem cell based therapy aims have been expanded to more areas, including prevention of myocardial inflammatory and stress responses, improvement of myocardial perfusion via neovascularization, prevention of myocardial apoptosis and correction of metabolic and electromechanical disturbances[4].

Many cell types were investigated for cardiovascular repair properties and the most of attention was payed on stem cells exhibiting self-renewal, high replicative potential (Table 1) (ref.[12-18]). Promising results were obtained in animal models by application of human embryonic stem cells[19] or cardiomyocytes derived from induced pluripotent stem cells[20]. However, it was not possible to verify these results in patients, because of ethical concerns and high oncogenic risks[21]. More easily available stem cell types for effective clinical application include hematopoietic stem cells, adipose tissue derived cells or mesenchymal stem cells (MSCs), which undergone both preclinical and clinical testing successfully[22,23]. Therefore, this review focuses on current knowledge, achievements and failures of MSCs application in cardiac tissue repair.

In vitro and animal studies were searched in Web of Science database and key words “mesenchymal stem cell” and “cardio” were used. Clinical trials were searched at clinicaltrials.gov and key words “mesenchymal stem cell” and “heart” were used.

Table 1. Overview of stem cell types investigated for cardiac tissue repair.

Stem cell type	Animal model / disease	Injection of cells	Effect	Outcome	Ref.
Mesenchymal stem cells	minipig / MI	PIM	very positive	Improved heart perfusion, higher cell density, lower heart wall damage	18
	rat / MI	IM	positive	Improved cardiac function, decreased fibrosis	20
	rat / IHF	IM	positive	Improved left ventricular function, scar size reduction	14
	mice / cardiomyopathy	IC	positive	Reduced heart dilatation, reduced inflammation	13
Hematopoietic stem cells	rat / MI	IM	positive	Improved left ventricular ejection fraction, reduced scar size	12
BM derived mononuclear cells	minipig / MI	PIM	positive	Improved heart perfusion	18
	human / chronic heart failure	IM	positive	Reparative effect on myocardium	16
AT derived stem cells	pig / MI	IC	positive	Increased neovascularization	17
	rat / IHF	IM	no effect	No improvement	14
Human embryonic stem cells	pig / MI	IM	positive	Full restoration of ventricle function	19
iPSCs derived cardiomyocytes	rat / MI	IM	very positive	Improved cardiac function, decreased fibrosis	20
Endothelial progenitor cells	rat / MI	IM	positive	Improved left ventricular ejection fraction, scar size decrease, increased neovascularization	15
	human / chronic heart failure	IM	positive	Improved myocardial perfusion and left ventricular ejection fraction	16

BM – bone marrow, AT – adipose tissue, iPSCs – induced pluripotent stem cells, MI – myocardial infarction, IHF – ischemic heart failure, PIM – percutaneous intramyocardial, IM – intramyocardial, IC – intracardial injection.

MSCs CHARACTERISTICS AND SOURCES

According to the minimal criteria of Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, MSCs are defined as adherent fibroblast-like cells expressing CD105, CD73 and CD90, not expressing CD34, CD45, CD14 or CD11b, CD79a or CD19, and HLA-DR and with the ability to differentiate into adipocyte, osteoblast and chondrocyte cell types[24]. They can be found in different tissues including bone marrow[25], cord blood[26], placenta[27], fat[23], skin[28], muscle[29], tendon[30], synovium fluid[31] or teeth[32]. In opposite, MSCs are rarely detected in peripheral blood[33]. Further more, some studies indicate that the whole body MSC distribution can originate in a perivascular origin of MSCs. Perivascular $CD146^{pos}$ cells isolated from many tissues (muscles, pancreas, fat, etc.) express MSC surface markers (CD73, CD90, CD105) and differentiate into osteo-, chondro- and adipolineage[34]. These findings suggest that distribution of MSCs in adult organism is related to their existence in the perivascular niche[33].

MSCs source variability

Although MSCs from different sources express the same set of surface markers and differentiate into three mesodermal lineages, various abilities are reported.

Bone marrow is a rich source of cells and the success rate of MSC isolation from it is nearly 100%. Bone marrow derived MSCs are able to proliferate *in vivo* and also *in vitro*, where their growth is reported to be arrested around 11-12 passage. According to colony forming unit-fibroblast assay (CFU-Fa), these MSCs form 16.5 ± 4.4 colonies in third passage[35]. In heart regeneration research, bone marrow MSCs improve heart regeneration after myocardial infarction in many species, reduction in scar size together with improved heart function was reported[14,18,20]. As they were discovered first[36], majority of MSCs characteristics was found through experiments with bone marrow derived MSCs. Those characteristics represent standards for comparison up to date.

Adipose tissue contains 500 times more stem cells in 1g of fat than in 1g of bone marrow. Many people undergo liposuction voluntarily, so it is easy to obtain material for adipose tissue MSCs isolation. Adipose tissue derived MSCs showed similar cardio protective potential as bone marrow MSCs when applied to doxorubicin treated diabetic rat model[37]. On the other hand it has been found that proliferation potential, growth rate and culture time of adipose tissue derived MSCs is lower. CFU-Fa showed only 6.4 ± 1.6 formed colonies in third passage, cell growth was arrested around passage 11 (ref.[35]). Evenmore, it has been shown that adipose derived MSCs possess different abilities according to the tissue of origin[38,39]. Comparison of MSCs from abdominal fat, mesodermal origin, eyelid adipose tissue MSCs, and ectodermal origin, showed different phenotypes of cells together with variety in CD90 expression, suggesting higher abdominal fat MSCs response to angiogeneic factors[38]. Comparison of cardiac adipose tissue derived MSCs and abdominal fat MSCs, both of mesodermal

origin, showed that cells were phenotypically identical, but cardiac MSCs constituted intristic properties toward myogenesis and vasculogenesis in significantly higher percentage and therefore have much better regenerative potential, especially for cardiac therapy[39].

Umbilical cord blood is a rich source of cells, whith MSCs also being present. MSCs isolation and cultivation from this source is complicated and the success rate of isolation is 63% (ref.[35]). If successfull, their culture lasts for long time periods, their proliferation is arrested at passage 14-16. CFU-Fa showed highest ability to form colonies (23.7 ± 5.8) (ref.[35]) compared to others, but there are also evidence that in culture these MSCs display very low proliferation ability[39]. It was shown that MSCs from umbilical cord together with MSCs from amniotic membrane posses higher immunomodulatory capacity, based on gene expression profiling[40] than bone marrow MSCs.

MSCs from all three sources mostly used in research possess promising abilities for regenerative medicine, but, as it was mentioned before, they all have limits. Low-yielding isolation and complicated cultivation of umbilical cord MSCs makes them a not reliable source of cells. Easy isolation and cultivation of adipose tissue derived MSCs is very promissing, but tissue specific effect of MSCs from different adipose tissue sites makes them too variable. Human cardiac fat MSCs, showing the best qualities for cardiac regeneration, are hard to obtain and not convenient for detailed research. Therefore bone marrow-derived MSCs, a well documented type of MSCs, are in center of interest in cardiac regeneration research and are further discussed in more details.

Aging of MSCs

Important issue about therapeutical MSCs application is their aging. *In vitro* cultured MSCs obtained from older individuals, are larger, broader, flatten and show no spindle-formed morphology contrary to younger spindle shaped MSCs (ref.[41]). Aged MSCs contain more stress actin fibres, form small colonies and show telomerase deficiency[42]. Young MSCs are capable to reach 30-40 times maximal population doubling, aged MSCs have significant decline in replicative lifespan[43]. Aged MSCs also express different levels of various regulatory molecules. MSCs emission of pro-inflammatory interleukin 6 (IL6) increases with age[44], whereas production of anti-inflammatory and cell protective interleukin 11 (IL11) decreases with age[45]. Finally, aged MSCs have lower differentiation ability and proliferation potential[46,47] and the age related loss of regenerative potential of MSCs is even dependent on the source of MSCs (ref.[47]).

IN VITRO STUDIES

Based on both *in vitro* and *in vivo* studies, three main mechanisms of action of MSCs implementation in cardiac tissue reparation process are suggested – differentiation into functional cardiomyocytes (CMCs), paracrine and immunomodulatory effect (Fig. 1).

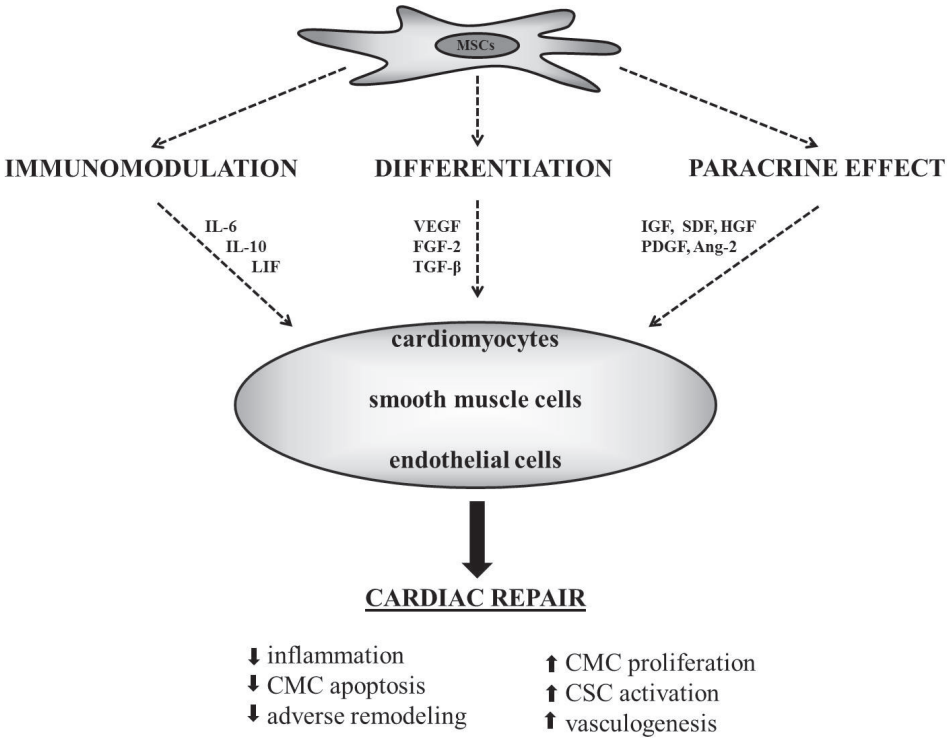

Fig. 1. Scheme of MSCs effect on CMCs and cardiac repair.
Differentiation, paracrine effect and immunomudulation – three postulated mechanisms of action of MSCs on damaged cardiac tissue. Cardiomyocytes, smooth muscle cells, endothelial cells and fibroblasts are influenced by MSCs and participate in cardiac tissue regeneration. MSCs-mesenchymal stem cells, CMCs-cardiomyocytes, CSCs-cardiac stem cells, ILs – interleukins, VEGF-vascular endothelial growth factor, FGF-2-fibroblast growth factor-2, TGF-β-transforming growth factor-β, IGF-insulin-like growth factor, SDF-stromal cell-derived factor, HGF-hepatocyte growth factor, PDGF-platelet-derived growth factor, Ang-2-angiopoietin-2.

Differentiation of MSCs to CMCs

MSCs show ability to differentiate into CMCs *in vitro*[48]. This differentiation can be induced by addition of 5-azacytidine, retinoic acid and dimethyl sulfoxide (DMSO) into cultivation media[49,50]. When MSCs are treated by 5-azacytidine they start to be positive for desmin and α-sarcomeric actin. Later, they show presence of sarcoplasmic reticulum, T-tubules and intercalated disc-like structures[51]. When MSCs are stimulated to CMC diffrentiation the expression of nesprin-1 protein is higher, which suggests it plays an important role in mediating MSCs differentiation[50]. MSCs can differentiate into CMCs also without 5-azacytidine, but the presence of insulin-like growth factor 1 (IGF-1), fibroblast growth factor 4 (FGF-4), hepatocyte growth factor (HGF), transforming growth factor β (TGF-β1) and bone morphogenic protein 2 (BMP-2) is required[52]. Even only cell-to-cell contacts of MSCs and isolated CMCs are able to support MSC differentiation into new CMCs (ref.[53]). It is further documented that N-cadherin (CD325) negative fraction of MSCs has lower CMCs differentiation potential than N-cadherin positive fraction of MSCs, which expresses significantly elevated mRNA levels of cardiomyogenic progenitor-specific transcription factors, including Nkx2.5, Hand1, and GATA4 (ref.[54]).

Paracrine and immunomodulatory effect of MSCs on CMCs

Bioactive molecules released by MSCs can positively modulate the functions of CMCs by paracrine and trophic mode. Cytokines from interleukin 6 (IL6) family secreted by MSCs bind to receptor glycoprotein 130 (gp130) and activate the JAK-STAT3 signaling pathway which results in increased expression of STAT3 targets hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) (ref.[55]). Conditioned media from MSCs can also protect CMCs from apoptosis when it inhibits caspase-3 activation and the release of cytochrome C from the mitochondria. These findings suggest that MSCs paracrine signalling helps to protect CMCs by interfering with mitochondria-mediated apoptotic pathway[56]. Factors released by MSCs can also protect CMCs from ischemia, when MSCs conditioned media decreases the numbers of apoptotic cells, the numbers of dead cells and improves CMCs metabolic activity. These improvements are regulated via Akt, ERK1/2 and STAT3 signaling pathways[57].

ANIMAL STUDIES

The large number of studies investigating MSCs influence on variety of heart diseases on animal models has been done. Small animal models as rat, mouse and rabbit were used mostly and approximately 56% of studies were performed on rat heart disease models. Large animal models as swine or sheep were used in 29% of all preclinical studies[5].

Majority of studies investigate the effect of MSCs on heart function or explore the role of MSCs in the repair of acute as well as chronic heart failure such as myocardial infarction[58,59], dilatation cardiomyopathy[59] or Chagas disease[1].

Differentiation of MSCs to CMCs

As both cell types, MSCs and CMCs are of mesenchymal origin, it can be easily expected that MSCs retain the ability of differentiation into CMCs. This is evidenced in many studies with various experimental designs. In the rat model of myocardial infarction (MI) MSCs are infected via tail vein and at four days MSCs engraft MI injured area. Engrafted MSCs expresses cardiac troponin T, endothelial CD31 and smooth muscle major histocompatibility complex (sm-MHC) suggesting MSCs differentiation into all major cells of cardiovascular lineage. Moreover, hearts treated by MSCs show improved cardiac features such as left ventricular ejection, end diastolic and end systolic volume or left ventricular myo-mass[60]. Another study, where green fluorescent protein (GFP) labeled MSCs (GFP-MSCs) are injected into mouse model of MI shows that over 60% of GFP-MSCs co-expressed collagen type IV and troponin T or myosin heavy chain, characteristic for MSCs and cardiomyocytes, respectively, and were $CD45^{neg}$. This study further demonstrates that MSCs can differentiate into CMCs in various extent when nearly 25% of GFP-MSCs express one of two cardiomyocyte markers in the absence of MSCs characteristics. Despite the lower differentiation properties of GFP-MSCs, myocardium treated by these cells shows improved left-ventricular and end-diastolic pressure[61]. In the study employing porcine model of MI, MSCs overexpressing integrin-linked kinase (ILK-MSCs) improve ventricular remodelling and cardiac function by increased CMCs proliferation, cardiac angiogenesis and reduced apoptosis[10].

In contrary, some studies indicate that the differentiation of MSCs into functional CMCs hardly or even not at all occurs. MSCs overexpressing Akt (Akt-MSCs) injected into mouse model of infarcted myocardium engraft the infarcted area at higher extent than MSCs, but only rare differentiation of both types of MSCs into functional CMCs is observed. Despite this, Akt-MSCs restore early cardiac function and decrease infarct size indicating another mechanism of MSCs facilitated tissue repair[62]. In another study is demonstrated that MSCs injected into mouse infarcted myocardium migrate into site of injury and survive there for 14 days, but no significant differentiation into functional CMCs or improvement of cardiac function is detected. Evenmore, same MSCs treated by pro-cardiomyogenic agents or by co-culture with beating CMCs do not differentiate into new CMCs (ref.[63]).

Paracrine and immunomodulatory effect of MSCs on cardiac tissue

Despite the inconsistent results in the ability of MSCs to differentiate into CMCs, some studies show that MSC transplantation improves cardiac functions. In these cases, the paracrine and immunomodulatory effect of MSCs on cardiac repair is more likely.

In many animal experiments, the application of MSCs after MI had a possitive effect on cardiac functions in comparison with controls. Improvement in left ventricu-

lar ejection fraction, reduction in infarct scar size and inhibition of left ventricle remodeling was observed together with decrease in end-systolic and end-diastolic volumes[64-66]. Anyway, the particular cellular mechanisms and regulating molecules or signalig pathways responsible for the cardiac function improvement remain undetailed. The decrease in CMCs apoptosis rate, decrease in inflammation and scar formation and increase in CMCs proliferation and cardiac tissue neovascularization are described as the most probable cellular mechanisms[67]. Particulary, diabetic rats treated by anti-cancer drug doxorubicin (DOX) possesing cardiotoxicity were co-treated by MSCs. MSCs prevented DOX-induced myocardial damage and significantly induced angiogenesis and reduced immune cell infiltration and collagen deposition[37]. In MI rat model, injected MSCs increased levels of angiogenic factors FGF-2, VEGF and stem cell homing factor (SDF-1α) in infarcted hearts. This was followed by declined CMCs apoptosis, increased capillary density and improved left ventricular contractility[68]. In another study, mice suffered from insuline resistence and MI and treated by MSCs showed improved cardiac function connected with enhanced glucose uptake by peripheral tissues and mitochondrial oxidative phosphorylation efficiency. Moreover, MSCs improved insulin signaling via Akt phosphorylation and maintaining of glucose transporter type 4 (ref.[69]). Some investigators stimulated paracrine function of therapeutically applied MSCs by over-expression of VEGF (ref.[70]) or by over-expression of miRNA-126 (ref.[71]), which led to improved cardiac function after MI. The Akt molecule was identified responsible for the protective role of MSCs in cardiar repair function[71,72].

Homing of MSC into damaged cardiac tissue

The ability of MSCs to home into damaged cardiac tissue is documented in some studies[60,61] but it is still a very limited factor of MSCs cardiac therapy. Recent study demonstrated that up to 70% of MSCs applicated into rat peripheral blood stream was trapped in lungs and some cells were detected in heart, kidney, spleen and bladder. The fraction of MSCs homed to the ischemic heart was only around 6% (ref.[73]). Even the ability of MSCs to circulate in blood stream is limited[74]. Therefore, the extensive investigation is performed to describe MSCs homing mechanisms and to improve this process.

As in the homing of other cell types, the homing of MSCs is based on the process of chemotaxis. Ischemic myocardium is rich in many chemokines and adhesion molecules including chemokine (CC motif) ligands (CCL) 2, 6, 7, 9, chemokine (CXC motif) ligands (CXCL) 1, 2, SDF-1, IL-6, TGF-β, VEGF, intercellular adhesion molecule (ICAM), vascular adhesion molecule (VCAM) or fibronectin[75], thus the expression of particular receptors on MSCs' surface should govern the process of homing.

Frequently investigated ligand/receptor pair is SDF-1/CXC chemokine receptor 4 (CXCR4). The level of surface CXCR4 in MSCs is low and unstable[76] and their expression needs to be stimulated to facilitate cardiac function repair[77,78]. Also over-expression of CC chemokine receptor 1 (CCR1) promoted migration of MSCs and their homing to injured heart[79]. Another studies detected integrin β1 (ref.[80]), hyaluronic acid/CD44 (ref.[76]), N-formyl peptide receptor (FPR) and the formyl peptide receptor-like-1 (FPRL1) (ref.[76]) or platelet-derived growth factor-AB (PDGF-AB)/PDGF receptor alpha and beta and insulin-like growth factor 1 (IGF-1)/IGF receptor[76] as crucial ligands and receptors for MSCs homing into injured cardiac tissue.

The importance of cell delivery routes

Current routes for MSCs delivery in heart treatment include intravenous injection (IV), where the MSCs are applied into the peripheral blood stream, intramyocardial injection (IM), where the MSCs are applicated by surgeons directly to heart perioperatively, or alternatively using percutaneous endoventricular injection using dedicated catheters, and intracoronary injection (IC), where percutaneous cathether delivers MSCs into coronary artheries.

Systemic IV injection is used because of low invasiveness, low cost and reported MSCs homing ability[81]. In the case of heart damage, local and systemic chemo-attractants are upregulated, including various interleukines, stromal cell-delivery factors and adhesion molecules[4] However, this homing signal seems to be not sufficient. It has been demonstrated that after IV injection of MSCs, only few of them were accumulated in infarcted myocardium of mice, majority of the cells was found in lungs[82].

Purpose of IM injection is to deliver MSCs directly to the damaged heart area via epicardial, endocardial or transvascular application. Advantages of this method are that it is similar to routine cardiac surgery, for surgeons it is easy to perform, and there is no risk of coronary embolism like in other application forms[5]. Also there is no need to rely on up-regulation of homing signal particles, because of MSCs delivery directly to the site of damage[4]. However, there is also a disadvantage, MSCs have tendency to form islet-like clusters consisting of donor cells and host inflammatory cells generating electrical and biological heterogenity in the host myocardium, which potentially results in arrhythmia occurrence[83].

IC injection method achieves higher first-pass delivery of MSCs into the heart and more homogenous cell distribution in target area with less inflammatory response[4]. Unfortunately donor MSCs engraftment is similarly poor as after IM injection. It has been demonstrated that initial retention of applicated cells is 15%, but after one hour only 5% of donor MSCs have been detected in damaged heart area[84]. Also it has been reported that IC applicated MSCs are relatively large which may result in microvascular obstruction and ischemia[85]. Elevation of cardiac infarct markers and changes on electrocardiogram after IC injection of MSCs has been reported[86].

Despite extensive research, MSCs engraftment in damaged cardiac tissue is still poor and several explanations have been suggested. First, the injection of MSCs by thin needle can cause damage to MSCs, which could lead to their apoptosis or death[87]. Second, MSCs harvested for application by trypsin can lose their surface proteins and

reduce cell-cell affinity, which can cause quick flush out of MSCs (ref.[88]). Third, MSCs in late passages can lose their surface expressions of chemokine receptors, which can disrupt their chemotactic ability[76]. Despite low MSCs retention in the damaged heart site, the majority of experiments show improvement in cardiac function and damaged area size after MSCs treatment.

CLINICAL STUDIES

Till today, 21 clinical trials are registered at clinicaltrials.gov when searching for the keywords "mesenchymal stem cells" AND heart, which have been completed. From those, results were published from 9 trials and are summarized in Table 2 and the following text.

Patients with left ventricule disfunction, ischemic cardiomyopathy, acute or chronic myocardial infarction, idiopathic dilated cardiomyopathy or ischemic heart failure were included into clinical trials. At 6 trials out of 9, autologous MSCs isolated from bone marrow were applied by intramyocardially, intracoronary or transendocardially. The general effect of MSCs application was the improvement of left ventricule ejection fraction (LVEF) and reduction of infarcted tissue area (Table 2).

Hare et al.[89] performed a series of clinical trials focused on evaluation of safety and efficacy of MSCs application into patients with ischemic cardiomyopathy. POSEIDON, one of the first clinical trials in this field, compared the effect of autologous and allogeneic MSCs applicated transendocardially into 30 patients with ischemic cardiomyopathy. After 1year follow-up it was shown that this application is safe and beneficial for patients. Application of both auto- and allo- MSCs had low rate of serious adverse events (SAE), reduction in infarcted size area was observed, but no improvement in LVEF was shown. Only autologous MSCs application led to significant improvement in a 6 min walking test. However, lack of placebo control prevented additional comparisons[89]. In the following TAC-HFT clinical trial, autologous bone marrow MSCs application was compared with bone marrow mononuclear cells application and placebo group, also focused on safety and efficacy of cell application. In this trial, 65 patients with ischemic cardiomyopathy were enrolled. Transendocardial injection in 10 left ventricle sites showed that application is safe, with no SAE, but only MSCs improved myocardial functions, including contractility. No change in LVEF was observed[90].

In PROMETHEUS, the clinical trial where autologous bone marrow MSCs were injected into infarcted site of myocardium of 6 patients not eligible for bypass surgery, it was shown that MSCs reduce scar mass size for 48%

Table 2. Clinical trials of MSCs application for cardiac function improvement.

Codename	Years of performance	Disease	MSCs source	Application form	Number of patients	Follow up (months)	Outcome	Ref.
PROMETHEUS	2007-2011	LV dysfunction/CHMI	BM (auto)	IM	6	18	Significant improvement in LVEF; reduced scar formation	91
CHART-1	2012-2017	LV dysfunction	BM (auto)	IM	315	12	Decreases in LVEDV and LVESV	95
TAC-HFT	2008-2013	LV dysfunction/CHMI	BM (auto)	TE	65	12	Low rates of treatment-emergent SEAs; reduced infarcted tissue area; improved cardiac parameters	90
–	2007-2010	AMI	BM (auto)	IC	58	6	Low rates of treatment-emergent SEAs; improvement in LVEF	92
MSC-HF	2008-2015	IHF	BM (auto)	IM	60	6	Improvement in LVEF, LVESV, stroke volume, myocardial mass	94
POSEIDON	2010-2012	LV dysfunction/ICM	BM (auto/allo)	TE	30	13	No difference between auto/allo; low rates of treatment-emergent SEAs; positive improvement of LV remodeling	89
STEMPEUCEL	2009-2012	AMI	BM (allo)	IV	20	24	Safe application of allogenic MSCs; no difference between treated and placebo group	97
–	2011-2012	AMI	WJ (allo)	IC	116	18	No harmful effects; significant increase in LVEF	93
RIMECARD	2012-2014	LV dysfunction	UC	IV	30	12	LVEF improvement	96

TE – transendocardial application, IM – intramyocardial application, IV – intravenous application, IC – intracoronary application, auto – autologous transplantation, allo – allogeneic transplantation, LVEF – left ventricule ejection fraction; ICM – ischemic cardiomyopathy; SAEs – serious adverse evetns; CHMI – chronic myocardial infarction; IDCM – idiopathic dilated cardiomyopathy; IHF – ischemic heart failure; LVESV – left ventricular end-systolic volumes; LVEDV – left ventricular end-diastolic volumes.

compared to baseline. Also improvement in contractility and perfusion in these patients was shown together with improved LVEF (ref.[91]).

Effect of clinical application of MSCs was also tested on accute myocardial infarction (AMI) patients a few days and up to a month after AMI. Lee et al.[92] applied bone marrow MSCs into infarcted site of myocardium of 80 patients and followed them for 6 months. 58 patients completed the trial and it was shown that application of MSCs is safe and even effective when performed month after AMI. LVEF, measured by SPECT, was improved for 6% in the 6th month of follow-up, in comparison to control group, receiving regular treatment only[92].

As a possible treatment for accute myocardial infarction also Wharton jelly MSCs (WJ-MSCs) application was tested. Intracoronary application of WJ-MSCs into 116 patients in 5-7 days after reperfusion treatment showed increased myocardial viability, measured by PET, and improved heart perfusion in 4 months. In the end of study, after 18 months of follow-up, LVEF was significantly improved (7%) in comparison to controls[93].

Autologous bone marrow MSCs were shown to be beneficial also for patients with ischemic heart failure where no more therapeutic options are available. 40 patients, out of 60 involved in study called MSC-HF, received intramyocardial injection of MSCs, follow-up for 6 months was performed. In the end of the clinical trial, LVEF of patients who received MSCs was improved for 6% and their left ventricle end-systolic volume was reduced for 7%, in comparison to placebo control, suggesting improved myocardial function[94]. Similary, in CHART-I study 164 patients with symptomatic advanced heart failure secondary to ischaemic heart disease were intramyocardialy transplanted with bone-marrow-derived, lineage-directed, autologous cardiopoietic mesenchymal stem cells which resulted in the significant decreases in LVEDV and LVESV in 12 months of follow-up[95].

Umbilical cord-derived MSCs (UC-MSCs) are described as efficient in animal studies of heart failure treatments. In clinical set-up, 15 patients with LVEF dysfunction were intravenously transplanted by allogenic UC-MSCs which was followed by LVEF improvement and harmless of UC-MSCs application[96].

Contrary, in order to make MSCs application as less invasive for patients as it is possible, intravenous application was tested. In clinical trial STEMPEUCEL bone marrow derived MSCs were injected into antecubital vein of 10 patients with AMI two days after coronary intervention. After a 2 year follow-up and in comparison with placebo control it was shown that this application does not cause SAE and is safe for patients, but no beneficial effect was observed and no significant differences between MSCs and placebo group has been found in any tested parameter[97].

Meta-analyses of performed clinical studies showed confusing correlation between discrepancies and positive results. More methodical discrepancies have been found in research, where better results were reported. According to meta-analyses, studies with no discrepancies showed negative results[98], which is disturbing.

PROBLEMS AND PERSPECTIVES

MSCs and their influence on heart have been studied intensively. It has been reported that MSCs possess ability to home into site of cardiac damage and support damaged myocardium by differentiation into CMC, by paracrine signaling and immunomodulation properties. MSCs differentiation into CMC was shown to be not significant *in vivo*, but all other properties were confirmed *in vitro* and also *in vivo*.

In preclinical studies MSCs showed to be a safe and promising treatment for variety of cardiac tissue damages. Some clinical trials performed on patients also showed positive effect of MSCs application, but no all of them. All performed clinical studies agreed that the application of MSCs is safe for patients. However, new evidence is questioning the effect of MSCs in patients with cardiac diseases and therefore implementation of MSCs treatment as a regular therapy in the clinic might be further away than expected/hoped for. It has been also shown that application of MSCs may not be beneficial enough to use it as a standart treatment. This could depend on several factors including age and source of MSCs, their manipulation after isolation and the route of application when e.g. IC application of MSC could be associated with the risk of microembolism.

As discussed earlier, age of MSCs is a very important factor. Usually, patients are older and therefore autologous transplantation of MSCs might not be efficient enough. In respect to therapy efficiency, use of MSCs from young and healthy donors, more active and capable of regenerative potential, should be considered. Especially after repeated prove, that allogeneic and autologous transplantation are both safe and have a similar effect.

Another consideration should be the source of MSCs. Bone marrow MSCs are the best investigated ones known to improve heart functions, but also cardiac adipose tissue derived MSCs, which are rarer and harder to harvest, show promissing and even better cardio specific abilities.

Any MSCs chosen for application need to be cultured in order to achieve satisfactory numbers for application. Cultivation conditions as well as cell harvesting are well described, but there is room for improvement. MSCs have documented homing ability into site of injury, but a large number of researchers reported minimal homing to the cardiac damaged sites in human. The final harvesting procedure before application may destroy surface receptors of the MSCs, so they are unable to find the cardiac site of damage, instead they are trapped elsewhere.

In consideration of previous discussed challenges, the chosen form of application is crucial. Peripheral application is the cheapest and the most comfortable for patients and medical personal, but the risk that MSCs will be trapped outside of the heart is big. Intracoronary or transmyocardial application is more reliable, but possesses risk of microembolism. All these facts need to be taken into consideration together with application speed, application number and number of application doses.

The most important question for the future of MSCs therapeutical application is what should be considered

as a positive result of application. Should it be any positive effect which is statistically significant or is it better to agree on a general evaluation protocol? Many facts are well known, but many more questions need to be answered, before the MSCs application will become a real treatment option for cardiac patients.

SEARCH STRATEGY AND SELECTION CRITERIA

Our research strategy was focused on the studies dealing with mesenchymal stem cells (MSCs) effects on cardiac repair. First, we aimed to summarize MSCs characteristics, sources and effect of aging. Afterwards, we wanted to cover the knowledge about MSCs cardiac repair potential obtained in in vitro, animal and clinical studies. Web of Science database was used for the search of in vitro and animal studies. Web page https://clinicaltrials.gov/ was used for the search of clinical trials. Publications from years 1990-2017 and only completed clinical trials were taken into account. Search terms were mesenchymal stem cell AND cardiomyocyte for in vitro studies; mesenchymal stem cell AND cardiac repair OR failure for animal studies; mesenchymal stem cells AND heart for clinical trials.

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