Prepare Cells to Repair the Heart: Mesenchymal Stem Cells for the Treatment of Heart Failure

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Key Words

Mesenchymal stem cell • Heart failure • Neovascularization • Tissue regeneration • Translational research • Gene expression • Paracrine effect

Abstract

Heart failure is one of the most important cardiovascular diseases, with high mortality, and invasive treatment such as mechanical circulatory support and cardiac transplantation is sometimes required for severe heart failure. Therefore, the development of less invasive and more effective therapeutic strategies is desired. Cell therapy is attracting growing interest as a new approach for the treatment of heart failure. As a cell source, various kinds of stem/progenitor cells such as bone marrow cells, endothelial progenitor cells, mesenchymal stem cells (MSC) and cardiac stem cells have been investigated for their efficacy and safety. Especially, bone marrow-derived MSC possess multipotency and can be easily expanded in culture, and are thus an attractive therapeutic tool for heart failure. Recent studies have revealed the underlying mechanisms of MSC in cardiac repair: MSC not only differentiate into specific cell types such as cardiomyocytes and vascular endothelial cells, but also secrete a variety of paracrine angiogenic and cytoprotective factors. It has also been suggested that endogenous MSC as well as exoge-

nously transplanted MSC migrate and participate in cardiac repair. Based on these findings, several clinical trials have just been started to evaluate the safety and efficacy of MSC for the treatment of heart failure.

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Introduction

Heart failure is a major cardiovascular health problem worldwide. About 5.2 million patients in the US have heart failure, and the lifetime risk of developing chronic heart failure for both men and women is 1 in 5 [1]. Coronary artery disease is the most common cause of heart failure, followed by idiopathic dilated cardiomyopathy and valvular heart disease [2, 3]. Myocardial infarction causes necrosis of the myocardium, followed by infiltration of inflammatory cells. Then a scar forms, leading to the loss of cardiac function, ventricular remodeling and progressive dysfunction, and, finally, congestive heart failure [4–6]. Drugs commonly used for the treatment of chronic heart failure include loop diuretics, angiotensin-converting enzyme inhibitors, β -adrenergic receptor blockers, aldosterone antagonists, angiotensin II receptor blockers and digitoxin; however, patients with severe heart failure require invasive treatment such as mechan-

Table 1. Characteristics of cells used for the treatment of heart failure

	Ease of harvest	Expand-ability	Differentiation into cardiomyocytes	Ethical issues	Arrhythmo-genesis
Embryonic stem cells		√	√	√	
Cardiomyocytes			√	√	
Skeletal myoblasts	√	√	√		√
Cardiac stem cells		√	√		
Endothelial progenitor cells	√	√			
Mononuclear cells	√				
Mesenchymal stem cells	√	√	√		

ical circulatory support, continuous inotropic infusion, or cardiac transplantation [7–9]. Therefore, there is a need to develop more effective, less invasive therapeutic strategies for heart failure.

Cell therapy for heart failure has the potential to restore cardiac function by inducing neovascularization, and regenerating and protecting cardiomyocytes [4]. Bone marrow-derived mononuclear cells (MNC) and endothelial progenitor cells (EPC) have been applied for ischemic cardiovascular disease in human studies [10–12]. Recently, mesenchymal stem cells (MSC), a subpopulation of MNC, have emerged as a new therapeutic cell source because they possess multipotency and can be easily expanded in culture [13]. This article reviews cell therapy for heart failure, and focuses on the therapeutic potential of MSC for heart failure.

Cell Sources for Cardiac Repair

Several kinds of cells have the potential to be applied for cardiac repair; embryonic stem (ES) cells and somatic stem or progenitor cells (skeletal myoblasts, MNC, MSC, EPC, cardiac stem cells) (table 1). Because ethical problems may limit the immediate application of ES cells, we herein describe the features and characteristics of somatic cells.

Skeletal myoblasts, which are derived from cultured satellite cells, were the first to be tested in clinical trials. Satellite cells are muscle progenitor cells which normally mediate the regeneration of skeletal muscle [14]. They can be easily expanded in culture in an undifferentiated state, and are highly resistant to tissue ischemia. In vivo studies have demonstrated that the administration of myoblasts improved cardiac function [15–18]. However, in early clinical studies, myoblast transplantation was associated with sustained ventricular tachycardia [19], which requires defibrillator implantation and/or amiodarone therapy.

Bone marrow cells include several types of stem/progenitor cells such as hematopoietic stem cells and MSC, although the vast majority of bone marrow cells are hematopoietic cells [20, 21]. Because of the extensive clinical experience in bone marrow transplantation for hematological diseases over the past decades and the fact that large numbers of bone marrow cells can be easily obtained, the study of bone marrow cell transplantation for treating heart failure has moved quickly from small animals to human studies. Direct or intracoronary injection of bone marrow cells appears to be safe and beneficial for the treatment of acute myocardial infarction and chronic ischemic heart disease [11, 22–25]. However, it is not clear whether the optimal bone marrow cell population for transplantation is hematopoietic stem cells, MSC, or abundant committed cells. The major goal of studies using bone marrow cells will be to identify the most effective cell population from these complex mixtures.

EPC, a rare subpopulation of bone marrow cells with a similar phenotype and function to those of fetal angioblasts, have been demonstrated to be involved in neovascularization after myocardial infarction [26]. In addition, there was a significant reduction in collagen deposition and apoptosis of cardiomyocytes and an improvement in cardiac function. It has been demonstrated that granulocyte colony-stimulating factor (G-CSF) and stem cell factor can mobilize EPC [27, 28]. Clinical trials studying the ability of G-CSF to mobilize stem/progenitor cells in patients with coronary artery disease did not reach a conclusion on efficacy, while concerns have been raised on safety in relation to arterial restenosis and plaque destabilization. HMG-CoA reductase inhibitors [29], estrogens [30], exercise [31], and nonsmoking [32] are more practical than growth factor or chemokine administration to enhance the number of circulating EPC in patients. Currently, clinical trials of EPC therapy for neovascularization and myocardial re-

generation using CD34-positive cells from BM are in progress.

In the past few years, the search for stem or progenitor cells in the heart has intensified. These would be comparable to the satellite cells in skeletal muscle. Evidence is accumulating that cardiac stem cells reside at specific locations in the adult heart. Beltrami et al. [33] described the isolation of lineage-negative, c-kit-positive cells from the adult rat heart and showed that these cells were able to differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells. In addition, Sca-1-positive cells were isolated from the adult mouse heart [34]. This Sca-1-positive subpopulation differentiated in vitro into cardiomyocytes in response to 5-azacytidine, and expressed several cardiac specific markers. In addition, Sca-1-positive cells differentiated into cardiomyocytes after intravenous injection. Messina et al. [35] identified a subpopulation of cardiosphere-forming cells after culturing human atrial or ventricular biopsies and embryonic, fetal or postnatal mouse hearts. The mouse cardiospheres started to beat spontaneously, whereas human cardiospheres only started to beat after co-culture with rat neonatal cardiomyocytes. Furthermore, cardiospheres expressed endothelial markers and were positive for Sca-1 and c-kit. Recently, another subpopulation of cardiac stem cells has been described [36]. These cardiac stem cells were Isl1-positive and were identified in rat, mouse and human adult hearts. Isl1-positive cardiac stem cells were positive for cardiac transcriptional factors Nkx2.5 and GATA4, while transcriptional factors associated with mature cardiomyocytes were absent. However, Sca-1 and c-kit were not expressed on these cells. When co-cultured with neonatal rat cardiomyocytes, 30% of the Isl1-positive cardiac stem cells differentiated into cardiomyocytes.

MSC: Distribution and Behavior

MSC reside not only in bone marrow [37] and adipose tissue [38], but also in other tissues such as synovium [39], periosteum [40], muscle [41], dental pulp [42], periodontal ligament [43], placenta [44] and umbilical cord blood [45]. A recent study suggests that MSC reside in virtually all postnatal organs and tissues, and may be localized to vessel walls [46]. MSC can differentiate not only into osteoblasts, chondrocytes and adipocytes, but also into cardiomyocytes and vascular endothelial cells [13, 47]. Bone marrow-derived MSC, which are most investigated, are a rare subpopulation of bone marrow cells

(approximately 0.001–0.01%) [13]. It has been demonstrated that bone marrow MSC can be mobilized and differentiate into cardiomyocytes in a murine model of myocardial infarction, suggesting the importance of bone marrow MSC in cardiac regeneration [48]. Moreover, when cultured MSC were intravenously administered to rats with myocardial infarction, they were preferentially engrafted into the infarcted, but not the non-infarcted, myocardium, and a small fraction of the transplanted MSC differentiated into cardiomyocytes and vascular endothelial cells [49].

Differentiation of MSC into Myocardial Lineage

In vitro studies have demonstrated that MSC can differentiate not only into adipocytes and osteocytes, but also into cardiomyocytes and vascular endothelial cells [13, 47, 50–53]. The differentiation of MSC into cardiomyocytes in vitro has been induced in cultures containing either MSC alone treated with 5-azacytidine or a cocktail of growth factors, and in co-culture with cardiomyocytes, and has been demonstrated by evidence of spontaneous beating or cardiomyocyte specific markers [50–52, 54–61], although there is a lack of a clear definition of which markers are evidence for full differentiation into functional cells [62]. After direct injection of MSC into infarcted myocardium in a pig model, engrafted MSC differentiated toward a myogenic lineage, expressing muscle specific proteins, and attenuated cardiac function and pathologic thinning [63]. Furthermore, we and others have demonstrated that directly or intravenously injected MSC differentiated into endothelial cells, and were involved in angiogenesis as well as myogenesis in animal models of myocardial infarction [49, 64, 65].

Paracrine Effects Produced by MSC

MSC exert their effect on cardiac regeneration not only by differentiation into specific cell types, but also through paracrine actions. In vitro studies have demonstrated that MSC can secrete a variety of angiogenic, anti-apoptotic and mitogenic factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), adrenomedullin (AM) and insulin-like growth factor-1 (IGF-1) [66, 67]. Interestingly, administration of conditioned medium obtained from MSC culture exerted cytoprotective effects on the myocardium in an animal model of myocardial infarction [68]. We have recently

demonstrated that cultured cardiomyocytes were injured in response to monocyte chemoattractant protein-1 (MCP-1), which plays an important role in myocarditis, whereas this effect was significantly attenuated by conditioned medium derived from MSC culture [69]. These results suggest a cardioprotective effect of MSC acting in a paracrine manner, demonstrating the importance of secreted factors in cardiac repair.

Gene Expression in MSC

MNC have been shown to induce therapeutic neovascularization in critical limb ischemia and myocardial infarction in human studies [10, 11]. Similarly, many studies have demonstrated the therapeutic potential of MSC; however, the underlying mechanisms contributing to cardiovascular protection might be different between MSC and MNC. The gene expression profiles under normoxia and hypoxia are largely different between MSC and MNC [70]. MNC express a number of genes involved in inflammatory response and chemotaxis. On the other hand, MSC express a number of genes involved in development (e.g. transgelin, actin- γ 1), morphogenesis (e.g. bone morphological protein-2, transforming growth factor- β 3), cell adhesion (e.g. neural cell adhesion molecule-1, cadherin-11) and proliferation (e.g. connective tissue growth factor, platelet-derived growth factor-A) under normoxia. Furthermore, focusing on genes encoding secretory proteins in response to hypoxia, the upregulated genes in MSC include several molecules involved in cell proliferation and survival such as VEGF-D, placenta growth factor (PGF), pre-B cell colony-enhancing factor 1 (PBEF1), heparin-binding epidermal growth factor-like growth factor (HB-EGF) and matrix metalloproteinase-9 (MMP-9), while the upregulated genes in MNC under hypoxia include proinflammatory cytokines such as chemokine (CXC motif) ligand 2 (CXCL2) and interleukin-1 α (IL-1 α) [70]. These results suggest that transplanted MSC may act to promote cell proliferation, including angiogenesis, and cell survival in response to hypoxia, while MNC may induce an inflammatory response, followed by angiogenesis. In fact, implantation of MNC into ischemic limbs has been reported to lead to local inflammatory reactions [71].

It is postulated that not only exogenously administered MSC, but also endogenous MSC migrate and participate in wound repair. In healthy animals, intravenous administration of MSC preferentially engraft in the bone marrow cavity; however, when rats were subjected to ischemia/reperfusion, a significant number of xenogene-

ic MSC could be identified in the circulation, and subsequently in the infarcted region of the heart [72]. Taking these findings together, MSC are considered to exert cardiac repair by a variety of mechanisms.

MSC for Treatment of Heart Failure

Myocardial injection of MSC improved cardiac function in a rat model of dilated cardiomyopathy, a myocardial disease characterized by a loss of cardiomyocytes and an increase in fibroblasts [73, 74], possibly through induction of angiogenesis and myogenesis, as well as by inhibition of myocardial fibrosis [67]. In this study, MSC transplantation significantly increased capillary density and decreased the collagen volume fraction in the myocardium. In addition, intravenous administration of MSC improved inflammatory changes and cardiac function in rats with acute myocarditis, suggesting an anti-inflammatory effect of MSC [69]. Recently, we have demonstrated that adipose tissue-derived monolayered MSC, generated by cell sheet technology using temperature-responsive culture dishes, repair scarred myocardium after myocardial infarction in rats [75]. Interestingly, the engrafted MSC sheet gradually grew after transplantation to form a thick stratum that included undifferentiated MSC as well as newly formed vessels, which were composed of graft-derived cells, host-derived cells or both. Unlike a fibroblast cell sheet, the monolayered MSC reversed wall thinning in the scar area, contributing to a reduction in left ventricle wall stress and improvement of cardiac function. Therefore, this new technology may overcome problems associated with needle injection of bone marrow cells into the scar area, i.e. difficulty to reconstruct sufficient cardiac mass.

There are few reports describing the results of clinical studies on the treatment of heart failure, but several studies are on going. It has been reported that intracoronary administration of autologous bone marrow-derived MSC improved cardiac function in patients with acute myocardial infarction [76] and chronic ischemic cardiomyopathy [77]. Katritsis and co-workers reported that intracoronary transplantation of bone marrow-derived MSC may contribute to regional regeneration of myocardial tissue following myocardial infarction [78], and intracoronary transplantation of these cells did not appear to be arrhythmogenic [79]. The National Institute of Health in the United States provides information on current clinical trials using MSC (www.clinicaltrials.gov). In December 2005, the Rigshospitalet in Denmark started a phase

I/II safety and efficacy study (NCT00260338) to evaluate the clinical effect of autologous bone marrow-derived MSC therapy in patients with severe chronic myocardial ischemia. In October 2006, Helsinki University started a prospective double-blind trial (NCT00418418) of intra-operative transmyocardial autologous bone marrow-derived MSC transplantation versus placebo in patients with heart failure scheduled to undergo coronary bypass operation. In October 2006, the National Heart, Lung, and Blood Institute in the United States started a phase II study (NCT00383630) to evaluate the effect of injected autologous bone marrow cells to improve heart function in individuals with a left ventricular assist device (LVAD) awaiting heart transplantation. This study is enrolling individuals undergoing surgery to receive an LVAD, and patients are randomly assigned to one of the following three groups: group 1 patients receive injected MSC while undergoing LVAD implantation; group 2 patients receive injected immunoselected CD34-positive hematopoietic stem cells while undergoing LVAD implantation; group 3

patients undergo LVAD implantation. We have also started a pilot study of intramyocardial injection of autologous bone marrow-derived MSC in patients with end-stage heart failure. In this study, 20 ml of bone marrow cells are aspirated from the ilium, and MSC are cultured and expanded with medium containing autologous serum for 3 weeks. A large number of MSC are directly injected into the myocardium at 40 sites, using a catheter.

Conclusions

MSC have emerged as a new therapeutic tool for cell therapy in heart failure, and exert their effects by differentiating into specific cell types as well as through paracrine actions such as angiogenic, cytoprotective, anti-inflammatory and anti-fibrotic effects. Whether autologous MSC have significant value in the treatment of heart failure is currently being explored in clinical trials.

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