An Updated Review on the Therapeutic Potential of Stem Cells for Treating Cardiovascular Disorders

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Review Article

ABSTRACT

Despite significant progress in the field of medicine, cardiovascular illnesses continue to be the primary cause of mortality on a global scale. The increasing prevalence of cardiovascular illnesses necessitates the exploration of novel and potential therapeutic strategies to address the escalating risk associated with CVDs. Stem cell therapy has emerged as the central focus of regenerative cardiovascular medicine, surpassing all other treatments and therapies. Multiple clinical trials and studies have demonstrated that stem cell therapy, particularly mesenchymal stem cells, is the most appealing approach for treating cardiac disorders due to their significant therapeutic potential. Recent research have shown that mesenchymal stem cells have a positive impact on several cardiac muscle diseases, such as heart failure, problems with blood vessel lining, damage caused by lack of blood flow, and high blood pressure in the lungs. This study specifically examines the potential, preconditioning techniques, methods of administration, and mechanisms of mesenchymal stem cell (MSC) therapy for the treatment of cardiovascular diseases (CVDs).

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1. INTRODUCTION

With more than 17 million deaths in 2016, cardiovascular diseases (CVDs) remain a prevalent cause of high mortality worldwide [1] and are projected to cross 25 million annual deaths till 2030 [2]. Generation of a large number of free radicals (ROS) leading to oxidative stress in the area of ischemic necrosis in ischemic heart failure [3] exacerbates cardiac myopathies due to an increase in apoptotic/necrotic death of cardiomyocytes [4]. Developed countries reportedly have lower CVDs than developing Asiatic regions, including Pakistan, which poses a threat to their economies with increased pressure on human resources and health budgets [5,6]. This situation demands novel therapeutic approaches for decreasing the CVD epidemic. The most promising emerging therapies include artificial heart transplant and cardiovascular regeneration (biological hearts) approaches which have an advantage over currently used palliative strategies [7,8]. Now, stem cell therapy is a major focus in regenerative cardiovascular medicine. Except for embryonic-derived stem cells, others are isolated from patients with the same phenotypic and genotypic characteristics useful in transplant rejection studies and for developing effective patient-specific therapies [9].

Recent studies have demonstrated the beneficial use of stem cell therapy in various cardiogenic myopathies, including heart failure [10], pulmonary hypertension [11], endothelial dysfunction [12], atherosclerosis [13], and peripheral artery disease [14]. Based on origin, stem cells are classified as adult stem cells and embryonic stem cells [15]. Adult stem cells are further divided into tissue-specific and bone marrow-specific cells (BMCs). BMCs have endothelial progenitor cells, mesenchymal stem cells, and hematopoietic stem cells [16]. Transplantation of progenitor stem cells through infusion or intra-myocardial injection is currently used for treating CVDs as it positively influences cardiac regeneration and improves heart function [17]. Despite this, several shortcomings stills exist, including high costs, immune rejection, infusional toxicity, ectopic tissue formation, safety challenges, ethical concerns of the community in clinical practice, poor survival and differentiation [18,19]. The cardioprotective effect of stem cells is usually achieved by paracrine interaction between donor and recipient stem cells. (Paracrine mechanisms in adult stem cell signaling and therapy Massimiliano Gnecchi1).

Mesenchymal stem cells (MSCs), first isolated in 1970 [20], are pluripotent progenitor stem cells with the ability of self-renewing as defined by the International Society for Cellular Therapy and can be differentiated into various mesodermal origin cell types like chondrocytes, osteocytes, and adipocytes [21]. They are also named as signaling cells, stromal stem cells, and adult progenitor multipotent cells [22]. Under optimum culture conditions, MSCs demonstrate plastic adherence capability with the expression of CD105, CD90, CD73 surface molecules in absence of HLA-DR, CD79a, CD45, CD34, CD19, and CD14 [23,24]. MSCs have been isolated from amniotic fluid [25], placenta [26], umbilical cord blood [27], human urine [28], the dental pulp [29], adipose tissue [30], and synovial fluid [31]. MSCs of multiple origins show variations in organ morphology, isolation protocols, and growth conditions on cell culture [32]. They have been investigated as an alternative therapeutic substance in different injury and disease models such as Alzheimer's disease, myocardial infarction, acute renal failure, hepatic injuries, orthopedic injuries, acute lung injury, autoimmune diseases, corneal damage, cerebral ischemia, and cardiovascular diseases [33-37].The use ofMSCs in medical therapies have several advantages over other stem cells including their encouraging anti-inflammatory properties, low immunogenicity, easy *in vitro* cultivation and expansion [38]. MSCs are strong mediators of cardiogenic tissue regeneration in damaged heart and effects fibrosis, cardiac remodeling, and proliferation of cardiogenic stem cells. Moreover, ease of systemic administration of MSCs through IV injections (intramayocardial) without heart catheterization and their ability to differentiate in to smooth muscle cells, cardiomyocytes, and endothelial cells make them a promising candidate in heart regeneration therapies since their first use incardiomyoplasty by Tomita and co-workers in 1999 [39,40].

2. PATHOPHYSIOLOGY OF CARDIOVASCULAR DISEASES (CVDS)

CVDs has a wide range of clinical manifestations such as restenosis, cardiomyopathy, aortic

aneurysms, valvular heart disease, coronary heart disease and hypertensive heart failure [41,42]. CVDs has multiple pathogeneses such as natriuretic peptide ligand-receptor complex-guanylyl cyclase (cGMP) [43], endothelial senescence mediated production of altered extracellular vesicles [44], defective redox status with enhanced superoxide stress leading to atherosclerosi, hypertension [45], altered microRNAs associated with defective proteolysis, lipid metabolism, cell mitosis, and dysregulated inflammatory mediators generation [46], low concentrations of Adiponectin [47] and cell injury associated with inappropriate activation of mineralocorticoid receptors through Rac1-signaling pathways [48]. Recently reported risk factors of CVDs in humans to include age, gender, alcohol consumption, physical inactivity, familial prevalence, depression, nutrient deficiency, hypertension, obesity, diet, and high blood pressure [49-54]. Congenital-based secondary hypertension increases the risk of myocardial infarction (MI), stroke, and heart failure [55]. Similarly, Vitamin D deficiency leads to derangements in cardiac myocyte contraction, abnormal cardiac relaxation with ultimate heart failure [56,57]. Among cardiovascular disorders, ischemic heart disease is the most common type and is the leading cause of death worldwide with estimated 9 million deaths in 2016 [58]. The Problem in cardiovascular diseases is related to severally limited repair capacity after injury. Heart transplantation is the only ultimate approach to cope with end-stage cardiac failure. But it has limitations of being costly and there are limited organs for transplantation [59]. Therefore, researchers look forward to determining the best and minimally invasive cure for CVDs. For this purpose, stem cells gained special interest due to their unique self-renewal properties, varied potency, and ability to differentiate into multilineage [60].

3. INTERACTION WITH IMMUNE SYSTEM

Human MSCs lack expression of MHC-II and costimulatory molecules such as CD40 ligand and B7 [61,62]. Immunogenic tolerance of MSCs is attributed to their specific immunophenotype and strong immunosuppressive properties [63]. They positively influence both humoral and cell-mediated immune responses. However, their interaction with cells of the immune system is under investigation. From 2001 to 2014, PubMed and ScienceDirect data showed 149 and 495 peer-reviewed research publications based on animal models to study MSCs directed immune-modulation with promising results [64]. MSCs interact with adaptive (dendritic cells, T & B lymphocytes) and innate (natural killer cells) immune components. Cells of innate immunity (natural killer cells, mast cells, neutrophils, eosinophils, macrophages, and dendritic cells) modulate the nonspecific immune response to infections and most of these cells are suppressed by MSCs. Neutrophils are the first line of defense against microbial infections with the production of respiratory bursts [65]. MSCs release IL-6 which suppress respiratory burst [66]. Reversible inhibition of monocytes differentiation into dendritic cells (DCs) with upregulation of HLA-DR, CD86, CD80, CD40, and CD1a surface molecules cause suppression of their antigen-presenting ability [67-69]. PD-L1/PD-1 mediated contact between B-lymphocytes and MSCs causes inhibition of B-cell proliferation, largely depending on culture conditions [70,71]. Differentiation activity of MSCs is mainly controlled by the TGF-β superfamily and Wnt canonical pathways [72].

They cause immunoregulation through cellular contacts via the PD-1 pathway [73] and secretion of various chemokines [74], cytokines [75], growth factors [76], and biologically active agents [77]. They contribute to immune response homeostasis by creating a tolerogenic environment and preventing untimely T-lymphocytes activation, especially during wound repair or healing [78]. T-cell activation is characterized by secretion and expression of surface molecules such as tumor necrosis factor (TNFα), CD69, CD25, CD38, IL2, HLA-DR, and CTLA-4 [79]. Some studies reported inhibition of CD69 and CD25 expression by bone marrow-derived MSCs in T-lymphocytes treated with phytohemagglutinin [80], while some revealed no direct effect on the expression of surface molecules [81]. This contraindication may be due to the difference in the T-lymphocytes population studied in each experiment. Some studies revealed that activated T-cells can reduce [82] or increase [79] IFNγ secretion in the presence of MSCs; however, this effect depends on the source of the T-cells population under investigation [83]. CD3/CD28 mediated activation of CD3$^+$ T-cells in MSCs presence from adipocytes stimulates IFNγ secretion. MSCs interfere with the antigen-presenting property of T-cells by suppressing CD34$^+$ progenitor cells [84].

Recent studies have demonstrated that MSCs can survive and differentiate in immunocompatibility-mismatchedxenogeneic or

allogeneic transplant recipients [85] with reported ability to induce immunological tolerance in immunocompetent xenotrans plant or allotransplant recipients [86]. This unique immunotolerance mechanism is under intensive research and three possible interrelated mechanisms have been proposed. MSCs evade host immune defense by (a) immunosuppression of local environment, (b) modulating T-cell phenotype, and (c) being immune privileged (hypoimmunogenic) [87]. Being hypoimmunogenic enables transplantation of mesenchymal stem cells across histocompatibility barriers and devising therapeutic approaches based on MSCs growth in culture [88].

4. MSCs IN CARDIOVASCULAR REGENERATIVE THERAPY

Recently, stem cell therapy gave new insights into cardiovascular disease treatment and clinical operations. MSCs are now considered an attractive candidate for MI treatment; because MSCs can transdifferentiate into cardiomyocytes with complete replacement of damaged cardiomyocytes [89]. Various studies have demonstrated MSCs differentiation into cardiomyocytes and engrafting to host tissue after directly injecting into the myocardium (Mesenchymal Stem Cell-Based Therapy for Cardiovascular Disease: Progress and Challenges LuizaBagno). *In vitro* and *in vivo* treatment of MSCs with 5-azacytidine stimulate their differentiation into beating cardiomyocytes. Directly injected MSCs into infarcted heart induce myocardial regeneration and improve cardiac function. The cardiac function improvement in the rat model of DCM through MSC transplantation was due to myogenesis or angiogenesis and inhibition of myocardial fibrosis. The clinically beneficial effect of MSCs might not be due to their differentiation into cardiomyocytes but also due to their ability to provide a large

number of angiogenic, anti-apoptotic, and mitogenic factors [90]. In a study, Konstantinos and coworkers concluded that MSC derived from bone marrow (BM-MSCs) facilitates substantial cardiac recovery and engraftment and differentiation when injected into the porcine heart. Differentiation actually occurs after transplantation [91].

Preconditioning of MSCs: Convenient isolation and ex-vivo expansion, along with the greater potential of differentiation into myocardial cells with low rejection rates, have made bone marrow-derived stem cells (BMSCs) a preferred choice for cardiac regenerative therapy [92,93]. Despite of initial hype and promising results, stem cell therapy is of little clinical usage due to low retention, differentiation, and survival of transplanted MSCs in hostile tissue surroundings. In diseased myocardium, low pH, overexpression of inflammatory mediators, deprivation of oxygen and nutrients cause poor post-transplant survival of MSCs. Aggregation of macrophages and neutrophils in response to myocardial insult leads to reactive oxygen species (ROS) and cytokines, which intensify cell death. This emphasizes the importance of identifying new methods for improving post-transplantation survival and differentiation of MSCs. Optimizing, pre-treating, reprogramming, or preconditioning stem cells by genetic manipulation, pharmacological, environmental/physical, or using cytokines can help transplanted cells to withstand the hostile microenvironment of injured tissue. [94, 95]. Preconditioning of stem and progenitor cells before transplantation with various preconditioning triggers is an emerging research area with established promising results. Preconditioning of MSCs results in increased cell survival by reducing immune responses, enhanced homing to target tissue, and improved neuronal differentiation [96].
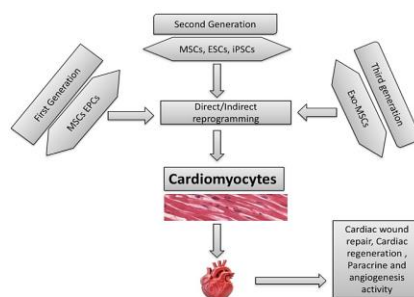

Fig. 1. Direct reprogramming of cells into cardiomyctes

Heat shock treatment is one of the several physical preconditioning treatments. Thermal exposure of cardiomyocytes to high temperature (39°C-45°C) triggers HSP70 expression, protecting MSCs from *in vivo* and *in vitro* oxidant stress [97]. Thermal treatment of MSCs to 43°C results in HSP70 and HSP27 secretion, which are associated with increased cell survival [98]. Oxygen tension is crucial for stem cell survival and differentiation in both *in vivo* and *ex vivo* environments. *In vivo*, hypoxia can cause apoptosis, but it can be reduced with MSCs hypoxic preconditioning and overexpression of some important pro-survival genes like Akt. In cell culture, hypoxia can enhance proliferation and differentiation of stem cell lineages by modulating their paracrine activity, resulting in upregulation of IL-6 and angiogenic factors. Hypoxia-induced overexpression of stromal cell–derived factor-1 plays an important role in homing of MSCs [99,100]. Autophagy is promoted in BMSCs after hypoxic preconditioning and it serves as a protector for MSCs apoptosis under H/SD [101]. Transplantation of hypoxic preconditioned stem cells shows the improved expression of angiopoietin-1, hypoxia-inducible factor 1, and vascular endothelial growth factors along with its receptors i.eBcl-xL, Flk-1, Bcl-2, and erythropoietin. Preconditioned stem cells also show decreased caspase-3 activation and increased angiogenesis after myocardial infarction (MI) [102]. The protective effect of hypoxic preconditioning on cultured cells remains approximately for six days [103].

Pharmacologically active substances also cause increased cell survival. Conditioning mimetics triggers the release of cytokines and growth factors (IGF, VEGF, HGF, Ang-1, and SDF-1α) with angiogenic effects [104]. Priming stem cells with Pitocin (a synthetic analog of Oxytocin) enhances differentiation into vascular cells and cardiomyocytes with increased protection against oxidative stress [105,106]. Oxytocin treatment cause upregulation of HSP70, HSP27, VEGF, MMPs, TIMPs, and HSP32 with cardiac anti-apoptotic and anti-remodeling properties [107]. In recent years, the protective role of hydrogen sulfide signaling in cardiac myopathies in mammals is under investigation [108]. H_2S plays a critical role in vasorelaxation, angiogenesis, cardioprotection, atherosclerosis inhibition, and lowering of blood pressure [109,110]. Transplantation of H_2S-treated MSCs improves left ventricular heart function (LVHF), reduces the infarct size, and enhanced cell survival 4-days post-transplantation in the myocardium of MI patient [111]. Nitric oxide is an essential factor of chemical signaling for cardiovascular homeostasis. NO can be generated from a family NO synthase (NOS). The endothelial NOS (e-NOS) produces nitric oxide from L-arginine amino acid. A study showed that overexpression of NOS could improve the therapeutic potential of MSCs in cardiac repair [112].

MSCs regenerative therapy can be made more effective by genetic modifications. There are four main strategies adopted for genetic modification, including gene editing (CRISPR/Cas9), gene silencing (RNAi), protein overexpression through DNA delivery, and miRNA-based modifications [113]. Currently, viral transduction is a widely used method for DNA delivery. However, it has several reported safety issues like tumorigenesis and mutagenesis [114]. CRISPR/Cas9 gene-editing tool is a novel technique for the precise insertion of required genes without activation of an oncogene [115].CRISPER/CAS9 technology derives from the adaptive immunity of bacteria and archaea against invading nucleic acids by using CRISPER RNAs which guides the silencing of invading nucleic acids [116].CAS 9 is an enzyme produced in bacteria naturally which initiates anti-phage activity by combining with CRISPER loci which are short repetitive sequences of about 30 to 40bp. These loci are transcribed into long RNAs, which are broken down into small CRISPER RNA (crRNA) by the activity of CRISPR-associated nucleases. Cas-RNA complex is formed by these crRNAs, which helps recognize invading nucleic acids and cleaves them. The crisper cas9 technology is applied to develop mouse models of cardiomyopathy in a shorter time than traditional techniques [117,118]. CRISPER technology helps to generate the genetically modified cells and model organisms to study CVDs more efficiently [119]. Some gene modifications are reported till now which includes AkT and Bcl-2 (apoptosis-regulating protein) modifications. Bcl-2 gene is involved in apoptosis. It reduces apoptosis and promotes cardiac regeneration. A study showed the effect of exosomes derived from Akt on MSC's role in CVDs. They use human umbilical cord stem cells and AKt genes were transfected into hucMSCs and extracted exosomes from control (hucMSCs) and Akt (hucMSC). They studied the effect in MI rat model and demonstrated that platelet-derived growth factor D was upregulated in Akt-exo which increased cardiac repair [120,121].

5. MODES OF MSCs DELIVERY TO CARDIAC TISSUES

Different methods are used to successfully implant mesenchymal stem cells to the infected or injured area. Following are some methods which are commonly used nowadays.

5.1 Direct Surgical Inframyocardial (IM) Injection

This procedure is the most precise, direct, and authentic approach for injecting mesenchymal stem cells into damaged/infracted regions of the heart [122]. It can be done either during thoracotomies for open-heart surgeries or as a separate method without cardiac arrest [123,124]. Infra-myocardial infraction involves the injection of therapeutic agents directly into the myocardium into LV either directly or by using a catheter-based approach [125]. The location for IM method can be identified by using RCG or nuclear imaging. It offers an extra advantage of targeting localized myocardium without perturbing neighboring tissues [126]. Wang et al performed experiments in which Mesenchymal stem cell sheet fragments injected by direct IM method shows increased vascular density, more cell retention in infected zone and increased graft/host cell communication, thus improving left ventricle functions [127]. Such direct IM techniques of stem cells have been used when trans-vascular cell delivery becomes limited due to occluded coronary artery in cardiomyopathy patients [128].

5.2 Intracoronary Delivery

This approach includes the infusion of MSCs inside the coronary artery. In this procedure, cells can be injected while maintaining coronary flow or following flow interruption with balloon occlusion to minimize rapid cell washout. An intracoronary approach allows for selective delivery of cells to the myocardial area of interest and theoretically limits risks of systemic administration [128]. This procedure has some advantages in that there is no need for special equipments for injecting cells and the injected cells are uniformly distributed in the infracted region. Some disadvantages are also associated with this technique and among them, the most important is the low immediate retention of cells which may cause microvascular occlusion [129,130].

5.3 Mechanism of Action

For the development and improvement of MSC therapy, it is utterly important to know the mechanism of action of MSC in cardiac tissue regeneration and repair. MSCs follow two main mechanisms known as Direct and Indirect/paracrine. Numerous *in-vitro/in-vivo* studies showed that paracrine signaling is the major and fundamental mechanism of action of MSCs [131].
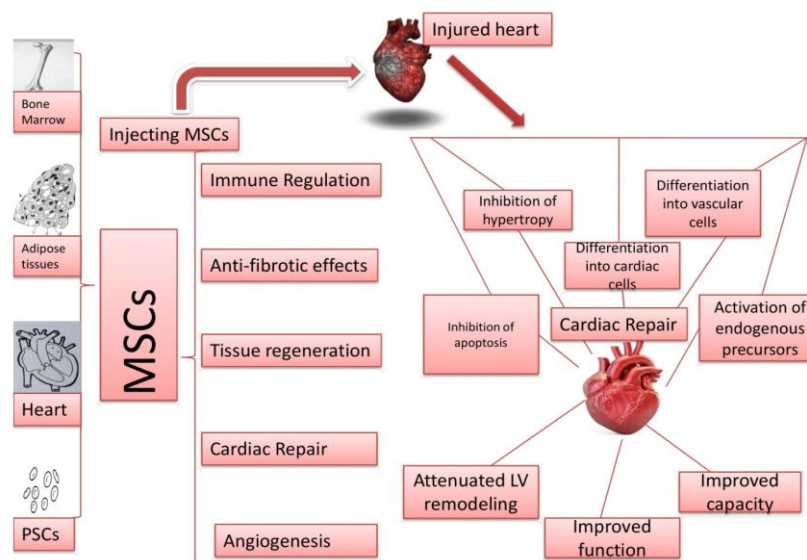

Fig. 2. MSCs applications in CVDs

5.3.1 Direct pathway (direct trans-differentiation of MSCs into cardiac cells)

Despite the fact that MSCs can differentiate into different cell types e.g cardiomyocytes and endothelial cells, it is not the primary mechanism of action of cardiovascular regeneration [132]. Direct trans-differentiation of MSCs into cardiac tissue is still a major controversy in cardiac regeneration. Some studies suggest that MSCs transdifferentiate into functional cardiomyocytes and some studies suggest a fusion of MSCs with host cardiomyocytes as a prevalent mechanism [133].

5.3.2 Paracrine pathway (secretion of complex biological compounds)

Recent studies suggested that the ability of MSCs to regenerate or repair cardiac tissues is mediated by paracrine factors secreted by MSCs [134]. In paracrine signaling, MSCs positively influence the surroundings of cardiovascular tissue by the activation of several other signaling pathways. MSCs secrete biological active molecules like cytokines, remolding factors, and differentiation signals such as interleukine-6, a granulocyte, and macrophage colony-stimulating factors. These molecules secrete anti-apoptotic and angiogenic factors to inhibit cardiomytic apoptosis around the area of administration and induce cardioprotection. The release of various cytokines e.g. transforming growth factor β, vascular endothelial growth factor (VEGF), stromal cell-derived factor (SDF)-1, and epidermal growth factor (EGF) promotes various processes e.g. neovascularization, activation of tissue intrinsic progenitor cells, etc. Neovascularization which is referred to as the formation of new blood vessels is an important factor of the healing process as it re-supplies nutrients and oxygen to the damaged tissue. Studies showed 20% enhanced neovascularization in the MI mouse model due to the release of paracrine factors [135]. MSCs not only secret cytokines but they are also able to secrete exosomes. Exosomes are small extracellular vesicles containing important bioactive constituents with a diameter ranging from approximately about 40 to 160 nm containing microRNAs and they induce biological effects even at distant locations. Exosomes work by decreasing infarct size in a mouse model of myocardial ischemia. MSC-derived exosomes have growingly proved their cardiac repair effects through stimulating cardiomyocyte proliferation, vascular angiogenesis, immune-regulation, and inhibiting the progression of scar formation [136,137]. Exosomes plays important role in regulating CVD progression and act as a bioactive ingredient that stimulates repairing of cardiac injury via transpose and exchange of signal molecules. Exosomes delivered via intravenous route prove to effectively alleviate myocardial ischemia/reperfusion injury [138,139].

6. CONCLUSION

Cardiovascular diseases remain the leading cause of death worldwide, despite tremendous advancements in medical science. The rising incidence of cardiovascular diseases requires the investigation of new and promising treatment approaches to manage the rising risk of CVDs. Regenerative cardiovascular medicine now centres around stem cell therapy, superseding all other forms of therapy and treatment. Because mesenchymal stem cells have such a large therapeutic potential, numerous clinical trials and studies have shown that stem cell therapy—and especially mesenchymal stem cells—is the most promising treatment option for cardiac diseases. Recent studies have demonstrated the beneficial effects of mesenchymal stem cells on a number of cardiac muscle disorders, including heart failure, vascular lining issues, damage from blood flow restriction, and elevated blood pressure in the lungs. The potential, preconditioning strategies, administration approaches, and processes of mesenchymal stem cell (MSC) therapy for the treatment of cardiovascular diseases (CVDs) are specifically examined in this work.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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