



Antioxidant and Anti-Inflammatory Action of Stem Cells in Cardiac Disease

S. Pulavendran^{1*}, G. Thiagarajan¹ and V. Ramakrishnan²

^{1*}Department of Biotechnology, Central Leather Research Institute, Adyar, Chennai, India.

²Genetics Division, Central Research Laboratory, Chettinad University, Kelambakkam, Chennai, India.

Abstract: Cardiac diseases are the consequence of blockage of blood vessels, scar formation and ultimate loss of terminally differentiated cardiomyocytes. Immune cells and oxidative stress easily slow down the cardiac functions by manipulating the cardiac tissue matrix. Stem cell-based therapies, especially mesenchymal stem cells (MSCs), multipotential nonhematopoietic progenitor cells compensate the cardiac diseases by differentiating into multiple lineages of mesenchyme including cardiomyocytes and vascular endothelial cells. Antioxidant and anti-inflammatory action of MSCs has been explored recently by various research groups. Secretion of biomolecules by MSCs perturbs and prevents the initiation, development and the function of the inflammatory cascade. These molecules mainly act through Paracrine mode. Anti-inflammatory action of MSCs mediates the cardiac diseases and the current progress in elucidating the mechanism and clinical use will be focused in detail in this article.

Keywords: Cardiomyocytes, Mesenchymal stem cells, Multipotential nonhematopoietic progenitor cells, Vascular endothelial cells.

1. Introduction

Millions of people all over the world suffer from cardiac diseases and still, the most perfect drug was not found out to safely treat the disease. The reasons behind the regress of treatment are the minimal symptoms, complicate molecular mechanism and risk factors like diabetes, smoking, alcoholism, food habits and lack of exercise. Unlike earlier mechanisms where accumulation of lipid molecules in the arteries causes acute cardiac damage, we now understand better the mechanisms responsible for the initiation and development of cardiac diseases. Recent studies claim that inflammation plays a key role in developing atherosclerosis, myocardial infarction (MI), endocarditis, inner layer of the heart, especially heart valves, myocarditis, inflammation of the muscular part of the heart (Lippy *et al.*, 2002). Inflammation leads the progression of complex plaque from fatty streaks. As the plaque evolves, T cells activate macrophages by either cyto-signaling or contact through CD40 ligation

to secrete panoply of molecules, including cytokines and matrix metalloproteases that make up the collagen that forms the fibrous cap, which ordinarily protects the plaque. As a result, the fibrous cap becomes thin and friable and can rupture, thus creating a thrombus that can lead to MI or other complications.

MSCs was first characterized by Friedenstein and colleagues, who identified an adherent fibroblast-like population in the adult bone marrow that could regenerate rudiments of, bone *in vivo* [Friedenstein *et al.*, 1968; Friedenstein 1976; Friedenstein *et al.*, 1987; Owen and Friedenstein, 1988]. Since then, MSCs has been isolated from many other tissues including adipose, cord blood, fetal liver, blood, bone marrow and lung [Erices *et al.*, 2000; Campagnoli *et al.*, 2001; Noort *et al.*, 2002]. Furthermore, MSCs display genetic stability reproducible characteristics in widely dispersed laboratories and compatibility with tissue engineering principles [Pittinger *et al.*, 1993; Lodie *et al.*, 2002; Gronthos *et al.*, 2003 (6–8)]. Gene therapy, growth factor application, and tissue engineering in

*Corresponding author:

Email: pulav28@gmail.com; Tel: 044 2443 7206.

combination with stem cell transplantation are thought to improve the effectiveness of stem cells. However, the specific mechanism remains controversy followed by stem cell transplantation. The current data on the anti-inflammatory actions and mechanisms of MSCs should be discussed in a narrowed manner to display the dearth of studies and to explore and intensify for the application of MSCs for the treatment of cardiac diseases.

2. Cardiogenesis from MSC

Myocardium tissue has limited intrinsic regenerative capacity because of its terminally differentiated cardiomyocytes and once it is affected, the soft myocardium is typically replaced with noncontractile scar tissue, often initiating congestive heart failure. Heart failure ensues when contractile reserve is depleted below a critical threshold. In many instances, the only effective therapy for end-stage ischemic heart disease is cardiac transplantation. Unfortunately, the number of available donor hearts is well below that of recipients. Stem cell therapies offer tremendous possibilities for curative approaches toward restoring lost myocardium and cardiac function through multiple strategies. As one of the least regenerative organs in the body, the heart would greatly benefit by the regenerative therapy. MI results in large-scale loss of myocardial muscle. Where, other cardiac diseases such as hypertension, valve disease and genetic disorder cause sporadic lose of myocytes.

Differentiation of MSCs into cardiomyocytes followed by improvement in myocardial performance attracted many investigators [Orlic *et al.*, 2001; Jackson *et al.*, 2002; Yoon *et al.*, 2005]. Various sources of stem cells have been explored to differentiate into cardiomyocytes. To identify a source of stem cells capable of restoring damaged cardiac tissue, highly enriched hematopoietic stem cells the so-called side population (SP) cells, were transplanted into lethally irradiated mice subsequently rendered ischemic by coronary artery occlusion for 60 minutes followed by reperfusion. The SP cells (CD34⁻/low, c-Kit⁺, Sca-1⁺) or their progeny migrated into ischemic cardiac muscle and blood vessels, differentiated to cardiomyocytes and endothelial cells and contributed to the formation of functional tissue. Donor-derived cardiomyocytes were found primarily in the peri-infarct region at a prevalence of around 0.02 % and were identified by expression of lacZ and α -actinin and lack of expression of CD45. Donor-derived endothelial cells were identified by expression of lacZ and Flt-1, an endothelial marker shown to be absent on SP cells. In another study, CD133⁺ cells were transplanted into ischemic heart (Rafii and Lyden, 2003). CD133⁺ cells integrated into sites of neovascularization and differentiated into mature endothelial cells. Less than 1% of nucleated BMCs are CD133⁺ positive and

however, this source is limited because only limited numbers of CD133⁺ cells can be obtained for therapeutic purposes and cannot be easily expanded *in vivo*. The presence of resident cardiac stem cell (CSC) population(s) capable of differentiating into cardiomyocyte or Vascular lineages suggests that these cells could be used for cardiac tissue repair (Beltrami *et al.*, 2003; Oh *et al.*, 2003). It has been reported that intramyocardial injection of these cells after AMI in mice promotes cardiomyocyte and vascular cell formation and leads to an improvement in systolic function (Messina *et al.*, 2004). However, invasion for biopsy is tedious and cannot be forwarded easily in clinical level.

To improve the potential of stem cells towards vascularization, angiogenesis and myocytes regeneration, stem cells were genetically modified using various genes. MSCs were transfected with the VEGF gene responsible for angiogenesis (Cao *et al.*, 2007) and increased angiogenesis was found out. To reduce cell loss after transplantation Song *et al.*, (2005) introduced the fibroblast growth factor-2 (FGF-2) gene *ex vivo* before transplantation. Viable cells persisted 4 weeks after implantation of 5.0×10^5 FGF-2-MSCs into infarcted myocardia. Expression of cardiac troponin T and a voltage-gated Ca²⁺ channel (CaV2.1) increased and new blood vessels formed. These studies suggested that genetic modification of MSCs before transplantation could be useful for treating cardiac diseases and end-stage cardiac failure.

3. Antioxidant and Anti-Apoptotic Effects

Oxidative stress and immune cells are the major backbone in the reperfusion and ischemia injury (Kaminski *et al.*, 2002). It could easily be predicted the role of oxidants and simultaneous activation of the inflammatory cascade in the development of cardiac diseases. Many pharmacological drugs have been approved to reduce the levels of antioxidants and subsequent nullification of cardiac diseases (Nuttall *et al.*, 1999). However, each has owned its disadvantages and especially they target single molecules or pathway. Stem cells from various sources are used to repair the MI and Cardiovascular diseases (CVD) etc. Stem cells can not only regenerate the diseased organs but also can cure the diseases by multiple therapeutic strategies. One of the earliest and most striking examples was provided by Mangi *et al.*, (2003) who predicted that overexpression of the anti-apoptotic signaling protein Akt in MSCs would improve myocardial repair following AMI through pro-survival effects. As shown in this and subsequent studies, Akt overexpression by retroviral transduction of MSCs was associated with significantly reduced infarct size and myocardial remodeling and improved left ventricular function. In another study to improve the MI, MSCs was subjected to genetic engineering with plasmid causing secretion

of anti-apoptotic and angiogenic peptide. Spermine introduced dextran of cationized polysaccharide (spermine-dextran) a non-viral vector was internalized into MSCs by way of a sugar-recognizable receptor to enhance the expression level of plasmid deoxyribonucleic acid (DNA). When genetically engineered by the spermine-dextran complex with plasmid DNA of adrenomedullin (AM). MSCs secreted a large amount of AM an anti-apoptotic and angiogenic peptide. Transplantation of AM gene engineered MSCs improved cardiac function after myocardial infarction significantly more than MSCs alone (Jo *et al.*, 2007).

Chen *et al.*, (2008) examined the changes of oxidative stress levels and mitochondria in over confluence culture-induced replicative senescence of MSCs. The results showed that human umbilical cord blood-derived MSCs (HUMSCs) and bone marrow-derived MSCs (BMSCs) reached replicative senescence at the 40th and 38th population doublings respectively. Viability assay showed that HUMSCs were more resistant to exogenous ROS than BMSCs. This study showed the antioxidant properties of the primarily isolated MSCs as well as few passaged cells. However, over the period of repeated culture, the antioxidant properties of stem cells decrease. Tang *et al.*, (2005) proved that hypoxia-regulated heme oxygenase-1 (HO-1) plasmid modification of MSCs enhanced the tolerance of engrafted MSCs to hypoxia-reoxygenation injury *in vitro* and improves their viability in ischemic hearts.

Circulating progenitor cells with an endothelial phenotype (EPCs) can be isolated from peripheral blood and contribute to neovascularization and endothelial regeneration. Whether EPCs are equipped with an antioxidative defense or to provide resistance against oxidative stress were evaluated. EPCs exhibited a significantly lower basal reactive oxygen species (ROS) concentration as compared with mature human umbilical vein endothelial cells (HUVECs). Consistent expression of the intracellular antioxidative enzymes such as catalase, glutathione peroxidase and manganese superoxide dismutase (MnSOD) was significantly higher in EPCs compared to HUVECs. Altogether it was concluded that EPCs have the potential to secrete antioxidative enzymes and thereby to protect against oxidative stress consistent with their progenitor cell character (Dembach *et al.*, 2004). Tolerance of oxidative stress of human endothelial progenitor cells (EPCs) due to the intrinsically high expression of manganese superoxide dismutase was also reported (He *et al.*, 2004).

Few reports have shown that muscle-derived Stem cells (MDSCs) have lower rates of stress-induced cell death, and it was speculated that the MDSCs' increased regenerative capacity which may relate to an increased resistance to oxidative and inflammatory stress (Oshima *et al.*, 2005; Deasy *et al.*, 2007). Evidence of increased enzymatic and nonenzymatic antioxidant capacity of

MDSCs was observed in terms of higher levels of superoxide dismutase and glutathione which appeared to confer a differentiation and survival advantage. In addition, when glutathione levels of the MDSCs are lowered to that of myoblasts, the transplantation advantage of MDSCs over myoblasts was lost when transplanted into both skeletal and cardiac muscles. These findings elucidated an important cause for the superior regenerative capacity of MDSCs and provided functional evidence for the emerging role of antioxidant capacity as a critical property for MDSCs survival post-transplantation (Urish *et al.*, 2009). Later, increase in the proliferation of induced pluripotent stem cells was reported followed by the treatment of vitamin C (Esteban *et al.*, 2010).

As the age progresses, the number of MSCs and their regenerative potential decreases (Raucher *et al.*, 2003). Antioxidants also decrease during the aging process which causes muscular cell damage (Presant *et al.*, 2007). Aging of stem and progenitor cells was suggested to account for aging of tissue and whole organisms (Sharpless and Depinho, 2007). In principle, MSCs from aged individuals may be altered in quality or quantity (for details see the review: Sethe *et al.*, 2006). It was demonstrated not only that MSCs concentration in bone marrow declines with age but also that their function is altered, especially their migratory capacity and susceptibility toward senescence. A functional annotation clustering study revealed that age-affected molecular functions were associated with cytoskeleton organization and antioxidant defense. These proteome screening results were supported by lower actin turnover and diminished antioxidant power in aged MSCs respectively. The reasons for the compromised cellular function of aged MSCs were postulated that:

- a) Declined responsiveness to biological and mechanical signals due to a less dynamic actin cytoskeleton and
- b) Increased oxidative stress exposure favoring macromolecular damage and senescence.

That implied that MSC-based therapeutic approaches for the elderly should focus on attracting the cells to the site of injury and oxidative stress protection rather than merely stimulating differentiation (Kasper *et al.*, 2009). Thus, the results of these studies confirm the treatment effect of MSCs by antioxidant as well as anti-apoptotic effects in addition to the differentiation potential.

4. Anti-Inflammatory Effects

Immune cells or proinflammatory cytokine IFN γ alone or together with TNF α , IL-1 α or IL-1 β should activate the MSC for the initiation of immunosuppression properties (Krampera *et al.*, 2006; Ren *et al.*, 2008). The necessity of this activation has also been proved *in vivo* in a model of graft versus host

disease (GVHD) since recipients of IFN γ -/- T cells did not respond to MSC treatment and succumbed to GVHD (Polichert *et al.*, 2008). Indeed, MSCs from mice deficient for the IFN γ receptor 1 do not have immunosuppressive activity, highlighting the important role of IFN γ in this process (Ren *et al.*, 2008). Although target cell-MSC interactions may play a role, the MSC-mediated immunosuppression mainly acts through the secretion of soluble molecules that are induced or upregulated following cross-talk with target cells.

Induction of inducible nitric-oxide synthase (iNOS) by murine MSCs and the production of nitric oxide were suggested to play a major role in T-cell proliferation inhibition (Sato *et al.*, 2007). The expression level of iNOS mRNA in human MSCs was minimal (Ren *et al.*, 14) and the secretion of nitric oxide by human MSCs was undetectable. Indeed, different mechanisms of immunosuppression exist in different species since human MSCs employ indoleamine 2,3-dioxygenase (IDO) as a major effector molecule whereas nitric oxide plays a critical role in mouse MSCs (Ren *et al.*, 2008). One of the first *in vivo* studies showed that systemic infusion of MSCs isolated from bone marrow prolonged the survival of allogeneic skin grafts from 7 to 11 days in baboons receiving MSCs (Bartholomew *et al.*, 2001). Although regeneration via MSC differentiation into cardiomyocytes was initially considered, further studies demonstrated that the predominant beneficial mechanism was through enhanced production of anti-inflammatory, pro-repair factors (Noiseux *et al.*, 2006). MSC-Akt decreased infarct size at 3 days and restored early cardiac function. In conclusion, MSC-Akt improved early repair despite transient engraftment, low levels of cellular fusion and differentiation. These new observations further confirmed the recently reported data by the same group that early paracrine mechanisms mediated by MSC are responsible for enhancing the survival of existing myocytes and that Akt could alter the secretion of various cytokines and growth factors (Noiseux *et al.*, 2006).

MSCs secrete number of molecules which play different kinds of activities during the pathological conditions since it is difficult to explain the timing and extent of improvement by only the effect through cardiac regeneration or cell fusion from MSCs. The repair function of MSCs is also involved with the secretion of paracrine factors, which can help to prevent apoptosis, promote angiogenesis, assist in matrix reorganization and augment circulating MSCs recruitment (Caplan *et al.*, 2006). Transplanted MSCs could upregulate multiple growth factors such as vascular endothelial growth factor [angiogenic, increase blood flow and decrease apoptosis (Tang *et al.*, 2005)], fibroblast growth factor [angiogenic, antifibrotic and anti-apoptotic factors (Ono *et al.*, 2004)], hepatocyte growth factor [not only angiogenic but also cardioprotective, including anti-apoptotic, mitogenic

and antifibrotic activities (Nakamura *et al.*, 2000)], adrenomedullin [angiogenic, anti-apoptotic and antifibrotic activities (Okumura *et al.*, 2004; Tsuruda *et al.*, 1999; Yoshihara *et al.*, 2003)], and insulin-like growth factor-1 [enhancing myocardial growth, anti-apoptotic and positive inotropic effects (Fuller *et al.*, 1992; Florinie *et al.*, 1996)]. The use of cytokine-mobilized autologous MSCs for infarcted myocardium (alone or in conjunction with currently used therapies) has the potential to significantly reduce morbidity and mortality associated with cardiac remodeling. If paracrine factors are the key agents, isolating and delivering such factors at high concentrations or engineering MSCs to secrete larger amounts could result in more significant protection.

Among these factors, indoleamine 2,3-dioxygenase (IDO) has consistently been reported (Meisel *et al.*, 2004; Maby *et al.*, 2009). On stimulation with IFN γ , this enzyme metabolizes tryptophan to kynurenine, causing depletion of local tryptophan and accumulation of toxic breakdown products. IDO however, exerts its effects mainly through the local accumulation of tryptophan metabolites rather than through tryptophan depletion (Ryan *et al.*, 2007). Whereas the majority of studies indicate a potentially important function for the IDO, human MSCs lacks both IFN γ receptor 1 and IDO still exerted important immunomodulatory activity (Gieseke *et al.*, 2007). This observation may be explained at least in part by a recent study reporting that Toll-like receptors expressed on MSCs augment their immunosuppressive activity in the absence of IFN γ through an autocrine IFN β signaling loop, which was dependent on protein kinase R and able to induce IDO (Opitz *et al.*, 2009). Contrary to human MSCs, lack of IDO activity was constantly reported for murine MSCs (Djouad *et al.*, 2007; Ren *et al.*, 2009).

Akt overexpression in MSCs of tumor necrosis factor receptor (TNFR) *via* AAV was associated with reduced cardiac inflammation and Apoptosis and improved LV function in rat AMI (Bao *et al.*, 2008).

Prostaglandin E2 (PGE2) has also been involved in the immunosuppressive activity of MSCs. PGE2 is a product of arachidonic acid metabolism that acts as a powerful immune suppressant, inhibiting T-cell mitogenesis and IL-2 production and is a cofactor for the induction of T-helper type 2 lymphocyte activities. Production of PGE2 by MSCs is enhanced following TNF α or IFN γ stimulation and its inhibition using specific inhibitors resulted in restoration of T-lymphocyte proliferation (Aggarwal and Pittinger, 2005). MSC-derived PGE2 was shown to act on macrophages by stimulating the production of IL-10 and on monocytes by blocking their differentiation toward dendritic cells (DCs) (Nemeth *et al.*, 2009; Spaggian *et al.*, 2009). IL-6 secreted by MSC was reported to be involved in the inhibition of monocyte differentiation toward DCs, decreasing their stimulation ability on T cells (Jiang *et al.*, 2005). Bone marrow

MSCs improved function by attenuating post-MI inflammation and repair.

Inflammatory cytokines and cells were measured and their impacts on the (myo) fibroblastic repair response, angiogenesis, and scar formation were determined. BMC implantation reduced the decline in fractional shortening and ventricular dilation. Interestingly, BMC implantation caused a 1.6-fold increase in the number of macrophages infiltrating the infarct but did not affect neutrophils. This increase was associated with a 1.9-fold higher myocardial TNF- α level. The heightened inflammatory response was associated with a 1.4-fold induction of transforming growth factor- β and a 1.3-fold induction of basic FGF. These changes resulted in a 1.6-fold increase in α -smooth muscle actin and a 1.9-fold increase in total discoidin domain receptor 2-expressing cells in the BMC group. Consistent with a more robust repair-mediated scar contracture the final scar size was 0.7-fold smaller in the BMC group. In conclusion, after myocardial infarction, BMC therapy induced a more robust inflammatory response that improved the priming of the (myo) fibroblast repair phase (Sun *et al.*, 2009).

5. Conclusion

Molecular understanding displayed a different perspective of cardiac diseases as a result of inflammatory events rather than lipid storage problem. Our assessment and management of cardiovascular disease risk must evolve in step with a deepened understanding of pathophysiologic mechanisms. Lifestyle modification and proven medical therapies must join stenting and coronary bypass surgery. Chronological data indicate that MSCs represents a promising alternative strategy not only for the cardiomyocytes regeneration but also in the immunomodulation of cardiac diseases. However number of studies published on the viability of MSC for cardiac repair through preclinical and clinical tests, bench to bed still remains scarce. Transfer of MSCs into clinical formulation depends upon the mode of injection, number and times of injection, techniques to retain cells at the injured areas to furnish autocrine and paracrine effects. Genetic engineering of MSC with the factors that will augment the secretion of soluble factors should be explored while maintaining the regenerative potential to completely utilize the potential of multipotent MSCs.

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