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Optimizing Stem Cell Function for the Treatment of Ischemic Heart Disease

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Abstract

Background—Stem cell-based therapies for myocardial ischemia have demonstrated promising early clinical results, but their benefits have been limited in duration due to impaired donor cell engraftment and function. Several strategies have emerged for enhancing stem cell function prior to their therapeutic use particularly with regard to stem cell homing, paracrine function, and survival. This review discusses current understandings of stem cell-mediated cardioprotection as well as methods of enhancing post-transplantation stem cell function and survival through hypoxic preconditioning, genetic manipulation, and pharmacologic pretreatment.

Materials and Methods—A literature search was performed using the MEDLINE and PubMed databases using the keywords “stem cell therapy,” “myocardial ischemia,” “hypoxic preconditioning,” “paracrine function,” and “stem cell pretreatment.” Studies published in English since January 1990 were selected. In addition, studies were identified from references cited in publications found using the search terms.

Results—All included studies utilized animal studies and/or *in vitro* techniques. Stem cell modifications generally targeted stem cell homing (SDF-1, CXCR4), paracrine function (VEGF, angiogenin, Ang-1, HGF, IL-18 binding protein, TNFR1/2), or survival (Akt, Bcl-2, Hsp20, HO-1, FGF-2). However, individual modifications commonly exhibited pleiotropic effects involving some or all of these general categories.

Conclusion—These strategies for optimizing stem cell-mediated cardioprotection present unique potential sets of advantages and disadvantages for clinical application. Additional questions remain including which are most efficacious in terms of magnitude and duration of benefit as well as whether combinations may yield greater benefits in both the preclinical and clinical settings.

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Keywords

stem cell therapy; myocardial ischemia; hypoxic preconditioning; paracrine signaling; stem cell survival

INTRODUCTION

Ischemic heart disease remains a major cause of morbidity and mortality in developed countries in part due to irretrievable loss of functional myocardium. The limited potential of endogenous myocardial repair even with revascularization has led to the emergence of cell based-therapies for myocardial regeneration and repair in the setting of acute and chronic ischemia [1–3]. As a recent meta-analysis showed, the intracoronary delivery of autologous bone marrow mononuclear cells following acute myocardial infarction was associated with modest improvements in left ventricular ejection fraction, infarct size and remodeling at a mean follow-up of 6 months [4]. However, these benefits did not persist at the 18-month follow-up of the BOne marrOw transfer to enhance ST-elevation infarct regeneration Trial (BOOST), the longest follow-up study to date [5]. Significant barriers to successful stem cell therapies for myocardial ischemia include diminished donor cell function and survival following delivery to an ischemic environment characterized by hostile inflammatory reactions, lack of trophic support, and substrate and oxygen deprivation which may induce cellular apoptosis. Donor cell attrition may be rapid with observed retention rates as low as 3–6% 14 days after infusion [6]. Accordingly, multiple strategies have been undertaken to enhance stem cell function and optimize survival by augmenting resistance to ischemia and oxidative stress.

To date, clinical trials of cell-based therapies have primarily utilized autologous bone marrow-derived mononuclear cells, a semi-enriched population of cells that includes hematopoietic and mesenchymal stem cells (MSCs), endothelial progenitor cells, fibroblasts and other stromal cells. Of these cell types, MSCs have emerged as a particularly well-suited cell for therapeutic applications in part due to their relative ease of isolation and expansion, multipotency, and immunoprivilege [7–9]. Whereas MSCs comprise a small fraction of all bone marrow cells *in vivo*, their ability to be expanded and maintained in culture in addition to their immunomodulatory properties may make them suitable for “off-the-shelf” allogeneic transfers [10]. It is because of these characteristics that MSCs have been the focus of strategies for optimizing the efficacy of stem cell therapies for the ischemic heart.

This review will address mechanisms of stem cell-mediated cardioprotection and explore current strategies targeting the modification and enhancement of stem cell homing and migration, paracrine function, and survival. Specific methods for enhancing these stem cell properties include hypoxic preconditioning, targeted genetic manipulations, and pharmacologic pretreatment during *ex vivo* expansion.

MECHANISMS OF STEM CELL PROTECTION

Whether delivered exogenously or mobilized from bone marrow endogenous following the onset of myocardial ischemia, stem cells are home to and integrate into sites of injury along chemotactic gradients, specifically that of stromal-derived factor 1 (SDF-1; Figure 1) [11]. Following relocation to ischemic myocardium whether following bone marrow mobilization or direct transfer, MSCs may acquire a cardiomyocyte phenotype characterized by the expression of cardiac-specific proteins and contractile properties [12–13]. However, the true magnitude of donor cell differentiation remains unknown and is likely insufficient for bolstering cardiac function alone [14].

The observations that media from hypoxia-conditioned MSCs protected cultured rat cardiomyocytes from hypoxic injury and that intramyocardial injection of conditioned media reduced infarct size led to the formulation of the “paracrine hypothesis” as another mechanism of stem cell protection [15]. This concept holds that stem cells secrete growth factors, cytokines, and other molecules that, in turn, regulate local inflammatory processes, decrease cardiomyocyte apoptosis, promote neoangiogenesis, and mitigate ventricular remodeling in the injured myocardium [16–17]. This hypothesis is further supported by the observation that delivery of cell-free conditioned media from MSCs conferred cardioprotection during ischemia [15,18–19]. Furthermore, donor cell-mediated post-ischemic functional recovery following stem cell transplantation begins to occur within 72 hours of cell transfer, thereby precluding differentiation as a possible mechanism in the acute setting [15].

The list of known mediators of paracrine protection continues to grow, and the current body of work investigating the expression of individual growth factors reflects the diversity of this process. In addition, the various general strategies for enhancing stem cell-mediated cardioprotection share the overlapping effect of altering stem cell paracrine function (Figure 2). Furthermore, it may be possible for a single *ex vivo* modification or treatment to confer multiple benefits to donor and host cells through pleiotropic paracrine effects.

HYPOXIC PRECONDITIONING

The concept that exposing cells to brief episodes of nonlethal ischemia alternating with periods of reperfusion could afford protection during subsequent prolonged ischemia was first observed in cardiomyocytes [20]. Early studies demonstrated that hypoxic or ischemic preconditioning decreased cardiomyocyte apoptosis, limited infarct size, and improved neoangiogenesis through upregulation of survival signaling pathways involving hypoxia-inducible factor 1 α (HIF-1 α) [21–23]. In addition, vascular endothelial growth factor (VEGF) was found to be an important mediator of hypoxia-induced protection in part through HIF-1 α -mediated upregulation [24].

The first donor cell type to be preconditioned with hypoxia was the skeletal myoblast, which was found to be more resistant to oxidative stress post-transplantation after hypoxic preconditioning [25]. This treatment was then applied to stem cells, which exhibited augmented pro-survival and pro-angiogenic effects following myocardial infarction in rodent models [26–28]. Further *in vitro* characterization revealed that hypoxic preconditioning induced MSC expression of pro-survival factors including HIF-1 α , Bcl-2, and Bcl-xL as well as decreased caspase 3 activation [28]. These protective effects also appeared to involve upregulation of the PI3K/Akt and ERK pathways, which are known to be involved in cell survival and anti-apoptotic mechanisms [27,29].

In addition to directly promoting cell survival, hypoxic preconditioning enhances MSC paracrine function. As with cardiomyocytes, hypoxia induces MSC VEGF production and in part via a signal transducer and activator of transcription 3 (STAT3)-mediated mechanism [28]. Moreover, in a mouse hind limb ischemia model, transplanted hypoxia-preconditioned MSCs were associated with increased local hepatocyte growth factor (HGF) expression and an earlier increase in neovascularization [27]. The preconditioned cells also displayed increased expression of the HGF receptor, cMET, as well as increased migration rates [27].

The advantages of this treatment strategy include the ease of its application and the ability to treat quantities of cells sufficient for therapeutic applications. The duration of these effects as well as the optimal pattern of hypoxia/reperfusion remain unknown. It has been observed that two 30 min ischemia/reperfusion (I/R) cycles were associated with decreased cellular

injury and apoptotic signaling *in vitro* compared to one cycle, but whether there is a limit to the number of cycles that yield additional benefit is unclear [29].

GENETIC MANIPULATIONS

The ability to directly target the expression of specific genes has not only enabled investigators to elucidate mechanisms involved in stem cell-mediated cardioprotection but to also augment stem cell function and/or survival in a highly focused manner. In addition, genetic manipulations may offer more durable phenotypic changes than either hypoxic preconditioning or pharmacologic pretreatment [30]. These manipulations typically involve altering the genome to upregulate expression of pro-survival and pro-angiogenic genes or silencing expression of receptors or other molecules that may contribute adversely to stem cell function or survival. This approach is potentially limited, however, by practical considerations of timing and generating an adequate quantity of cells for therapeutic use. While these manipulations are collectively diverse, they will be considered under the general categories of Homing, Paracrine Function, and Survival (Table 1).

Homing

Stem cells migrate to injured myocardium following SDF-1 gradients recognized by the cell surface receptor, CXCR4 [31]. SDF-1 is constitutively expressed in the myocardium and is upregulated immediately after acute myocardial infarction [32]. SDF-1 is also expressed by MSCs and may regulate stem cell proliferation and survival [33]. MSCs engineered to overexpress SDF-1 demonstrated increased cell viability and proliferation *in vitro* and further improved infarct size, ventricular remodeling, and cardiac function *in vivo* [34]. In addition, SDF-1-overexpressing MSCs injected by tail vein one day after AMI in rats improved cardiomyocyte survival possibly through direct inhibition of cardiomyocyte apoptosis and promotion of neovascularization in the infarct zone [35].

Upregulation of CXCR4 may also enhance MSC homing. CXCR4-overexpressing MSCs homed to infarcted myocardium in greater numbers and were associated with greater preservation of post-ischemic myocardial function and remodeling than unmodified MSCs [36–37]. In addition, MSCs overexpressing insulin-like growth factor-1 (IGF-1) also exhibited increased migratory capacities through increased CXCR4 expression as well as greater engraftment, improved remodeling and vascular formation [37–38].

Paracrine Function

Given that exogenously administered angiogenic growth factors could induce neovascularization within ischemic myocardium [39–40], it followed that delivering cells to ischemic tissue that secrete these same factors could offer more sustained growth factor delivery *in vivo*. One of the most widely studied growth factors for this purpose is VEGF, an important regulator of angiogenesis and vascular maintenance [41]. VEGF is also cytoprotective and mitogenic for cardiomyocytes [42] and can mobilize progenitor cells from the bone marrow [43]. Furthermore, VEGF has been shown to promote myocardial protection in the short-term by decreasing cardiomyocyte apoptotic signaling and in the long-term by increasing neovascularization and tissue perfusion [44–45]. Exogenous VEGF, when delivered via the coronary circulation, may also augment myocardial function during acute I/R [46].

MSCs increase VEGF production in response to oxidative stress and other injurious stimuli including hypoxia, thereby making them favorable donor cell candidates [47–48]. VEGF production is a crucial component of stem cell-mediated cardioprotection as evidenced by a reduction in post-ischemic myocardial functional recovery following intracoronary infusion of MSCs with targeted VEGF suppression using siRNA [49]. Conversely, transplantation of

bone marrow-derived progenitor cells overexpressing VEGF were found to enhance post-ischemic myocardial neovascularization, decrease infarct size, and improve functional recovery to a greater extent than unmodified cells in rat models of coronary artery ligation [50–51]. Interestingly, transplantation of MSCs overexpressing VEGF resulted in greater post-infarction functional recovery than delivery of unmodified MSCs or VEGF plasmid alone [51]. In addition, in one of the few studies combining separate treatments for AMI, intramyocardial injection of MSCs overexpressing VEGF was associated with the greatest post-infarction functional recovery and improvement in vessel density and ventricular remodeling when combined with pharmacologic mobilization of bone marrow progenitor cells [50].

Other pro-angiogenic growth factors have been targeted for genetic manipulation for augmenting stem cell-mediated cardioprotection (Table 1). Angiogenin is a basic heparin-binding angiogenic factor that, like VEGF, is upregulated in the heart in response to hypoxia [52–53]. MSCs transfected to overexpress angiogenin further increased myocardial perfusion and function in a pig model of chronic ischemia and were superior to delivery of unmodified cells or adenovirus containing the angiogenin gene alone [54]. Ang-1 regulates vascular maintenance and maturation as well as neovascular formation and induction of endothelial cell sprouting [55–56]. Intramyocardial injection of MSCs co-overexpressing Ang-1 and Akt improved vascularization and cardiac function following coronary artery ligation in rats [57]. Finally, MSCs overexpressing HGF, another potent angiogenic factor which also displays antifibrotic and antiapoptotic properties, further improved myocardial function, reduced ischemic area, and improved neovascularization following infarction in rats [58].

In addition to promoting neoangiogenesis, MSCs may modulate local ischemia-induced inflammatory responses through paracrine effects. IL-18 is a proinflammatory cytokine that suppresses myocardial function and is upregulated during ischemia [59–61]. Its natural inhibitor, IL-18 binding protein (IL-18BP), exhibits a greater affinity for IL-18 than its cell surface receptor and is able to scavenge and neutralize IL-18 [61]. We have observed that MSCs overexpressing IL-18BP secrete more VEGF and better preserve ventricular function and size while reducing improved myocardial inflammation during chronic myocardial ischemia compared to unmodified MSCs [17].

Modifying cell surface marker expression may also mitigate deleterious effects of inflammatory cytokines. TNF- α is also a proinflammatory cytokine upregulated during myocardial ischemia that mediates myocardial dysfunction and cardiomyocyte apoptosis [62]. TNF- α signals via two receptors, TNFR1 and TNFR2, both of which are expressed by MSCs. TNFR1 activation results in detrimental suppression of progenitor proliferation, growth factor production, generation of reactive oxygen species, and induction of apoptosis [63–65]. Accordingly, we observed that ablation of TNFR1 resulted in increased VEGF production and decreased pro-inflammatory IL-6 production by MSCs [64,66]. Conversely, TNFR2 activation may be beneficial for survival, proliferation, and growth factor production [67–69], and MSCs overexpressing TNFR2 were found to further decrease myocardial inflammation and improve left ventricular (LV) function two weeks after AMI [70].

Survival

Several strategies have been employed to optimize pro-survival signaling pathways in response to adverse stimuli. Akt is a serine threonine kinase that functions as a powerful pro-survival signal in response to hypoxia and other stimuli [71]. MSCs transfected to overexpress Akt exhibit decreased apoptosis and augmented paracrine function *in vitro* [15,47]. Following delivery to ischemic myocardium, Akt-overexpressing MSCs demonstrated increased engraftment and decreased apoptosis in association with decreased

myocardial infarct size and increased LV functional recovery *in vivo* [72–73]. As mentioned previously, the combination of Ang-1/Akt overexpression increased MSC resistance to anoxia *in vitro*, and cotransfected cells further improved neoangiogenesis and cardiac function *in vivo* [57].

In addition, MSCs have been modified to be more resistant to oxidative and other cellular stresses to improve survival and function. Heat-shock protein 20 (Hsp20) is a small heat-shock protein associated with cellular resistance to oxidative stress in part through interaction with the Akt pathway [74]. In addition, Hsp20 has been hypothesized to regulate folding and processing of VEGF thereby prolonging VEGF half-life [75]. Overexpression of Hsp20 by MSCs doubled their engraftment following post-infarction transplantation and further improved associated LV function, remodeling, and neovascularization compared to unmodified MSCs [76]. These effects were associated with increased Akt activation and increased production of VEGF, fibroblast growth factor 2 (FGF-2), and IGF-1 [76]. Similar findings were observed following treatment of ischemic hearts with MSCs overexpressing Hsp70 [77]. Heme oxygenase 1 (HO-1) is a heme-degrading enzyme with anti-apoptotic and anti-oxidative properties that has also been found to be cytoprotective in ischemic environments [78–79]. Accordingly, overexpression of HO-1 by MSCs was associated with decreased MSC apoptosis in response to hypoxia-reoxygenation *in vitro* [80]. HO-1-overexpressing MSCs also demonstrated increased engraftment, increased capillary density, and improved cardiac function up to 28 days following coronary ligation [80–81].

Bcl-2 is a regulator of apoptotic pathways and inhibitor of cell death [82–83], and MSCs genetically modified to overexpress Bcl-2 exhibited decreased apoptosis and increased VEGF production in response to hypoxia *in vitro* [84]. In addition, the survival of Bcl-2-overexpressing MSCs in infarcted rat myocardium was twice as great as that of unmodified cells 6 weeks later. Lastly, FGF-2 exhibits several protective effects in the heart including cardioprotection, mitigation of inflammation, promotion of neoangiogenesis, and remodeling [85]. MSC overexpression of FGF-2 improved MSC survival as well as Bcl-2 activation under hypoxic conditions *in vitro* and viability in infarcted myocardium *in vivo* [86].

PHARMACOLOGIC PRETREATMENT

As with hypoxic preconditioning, treating cells with exogenous agents during *ex vivo* expansion may be an alternative method for rapidly enhancing stem cell function and in quantities sufficient for therapeutic use. One promising agent for stem cell pretreatment is transforming growth factor α (TGF- α), a ligand of the epidermal growth factor receptor tyrosine kinase [87]. TGF- α is produced by macrophages and other cell types during wound healing and in response to inflammation [88–89]. In addition, activation of the EGF receptor, which is expressed by MSCs, promotes stem cell survival, proliferation, and migration [90–91]. We have found that TGF- α induces MSC VEGF and HGF production *in vitro* [92–94]. In addition, TGF- α additively increases MSC VEGF production in combination with TNF- α and hypoxia making it a potentially useful agent for optimizing MSC VEGF delivery to ischemic tissue [94]. Furthermore, we have found that the intracoronary infusion of MSCs pretreated with TGF- α was associated with greater myocardial functional recovery as well as decreased inflammatory and apoptotic signaling during acute I/R [94].

In addition to promoting stem cell migration through regulation of CXCR4, IGF-1 induces cell proliferation and inhibits apoptotic signaling. Pretreating MSCs with IGF-1 improved their resistance to oxygen deprivation and survival via PI3K/Akt-dependent caspase 3

downregulation [95]. Furthermore, IGF-1 pretreated cells injected into the myocardium following acute myocardial infarction were associated with greater engraftment, myogenic differentiation, and neoangiogenesis [75].

While pharmacologically pretreating MSCs may hold practical advantages, it remains unclear what the duration of these treatments on stem cell paracrine function are and whether these treatments affect other MSC properties such as engraftment and differentiation. In addition, pretreating with multiple agents may offer additional benefit, but this has not been extensively studied. In particular, MSCs pretreated with the combination of FGF-2, IGF-1, and bone morphogenetic protein 2 (BMP-2) further reduced native cell apoptosis, enhanced cardiomyocyte Akt expression, reduced infarct size and better preserved cardiac function following myocardial infarction in rats compared to untreated cells [96]. However, comparisons with individual growth factor treatments were not performed.

COMMENT

Stem cell therapy for myocardial ischemia, despite early promising clinical results, remains challenged by high rates of donor cell loss and impaired function. Multiple strategies have emerged to overcome these challenges including targeted genetic manipulations and global treatment of MSCs with hypoxia or other agents such as TGF- α that optimize their homing ability, paracrine function, and/or survival. The ability to administer hypoxic or pharmacologic pretreatments to large numbers of cultured cells in less than 24 h may make these applications more suitable for the setting of acute myocardial ischemia. However, if targeting intracellular signaling pathways through genetic manipulation yields beneficial phenotypic changes of longer duration, an additional cell pretreatment time of 48–72 h may be tolerable. Future clinical trials will need to address in a randomized, controlled fashion the optimal donor cell type; the optimal time for cell transfer; the expected duration of these modifications; whether multiple applications can sustain improvements; and whether strategies can be combined to achieve additive benefits.

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Figure 1. Putative mechanisms of mesenchymal stem cell (MSC)-mediated cardioprotection during ischemia include homing to sites of injury, differentiating into terminal cell types (e.g., endothelial cells), and releasing growth factors in a paracrine fashion with subsequent inhibition of apoptosis and promotion of neoangiogenesis.

Figure 2. General strategies for optimizing stem cell function include hypoxic preconditioning, genetic manipulation, and pharmacologic pretreatment which may affect stem cell homing, paracrine function, and survival.

Table 1

Molecular targets of stem cell modifications.

Homing	Paracrine Function	Survival
SDF-1	VEGF	Akt
CXCR4	Angiogenin	Bcl-2
	Ang-1	Hsp20
	HGF	HO-1
	IL-18BP	FGF-2
	TNFR1,2	

Abbreviations: SDF-1, stromal cell-derived factor 1; VEGF, vascular endothelial growth factor; Ang-1; angiopoietin 1; HGF, hepatocyte growth factor; IL-18BP, IL-18 binding protein; TNFR, tumor necrosis factor receptor; Hsp20, heat-shock protein 20; HO-1, heme oxygenase 1; FGF-2, fibroblast growth factor 2.