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Mini Review

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Where are Cell-Based Therapies Heading? Current Limitations and Future Directions

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ABSTRACT

Congestive heart failure (CHF) secondary to chronic coronary artery disease is a major cause of morbidity and mortality worldwide. Its prevalence is increasing despite advances in medical and device therapies. Adult stem cell therapies have emerged as a promising treatment generating new cardiomyocytes and vessels, and are anticipated to reverse functional deterioration in patients with congestive heart failure for whom heart transplantation is the only cure. This field was enthusiastically studied in last two decades, revealing that the major beneficial outcomes from cell therapy are associated with paracrine effects rather than direct differentiation. Accordingly, paracrine factors (e.g., growth factors, cytokines and microRNAs) secreted from stem cells reduce scar volume and myocyte apoptosis, increase myocyte proliferation and activate endogenous cardiac stem cells to produce new myocytes. Moreover, functional efficacy of progenitor cells isolated from the bone marrow, adipose tissue and the heart have shown promising effects in preclinical animal models. These convincing results led to the initiation of clinical trials using autologous and allogeneic stem cells, and progenitor cells. Although clinical trials demonstrated their safety in humans, therapeutic efficacy is still inconclusive. This review discusses the progress and limitations of cell-based therapies and alternative solutions for future advancement.

KEY WORDS: Congestive heart failure (CHF); Myocardial Infarction (MI); Adult stem cells; Mesenchymal stem cells (MSCs); Cardiac stem cells (CSCs); Cardiosphere-derived cells (CDCs).

INTRODUCTION

Congestive heart failure (CHF) secondary to chronic coronary artery disease is a major cause of morbidity and mortality worldwide.[1] Its prevalence is increasing despite advances in medical and device therapies. Currently available medical interventions attenuate neurohormonal activation (e.g., renin-angiotensin-aldosterone system, sympathetic nervous system, and arginine vasopressin), reducing myocyte apoptotic cell death and interstitial connective tissue proliferation, and attenuating the progression of myocyte cellular hypertrophy. However, none of the current therapies are effective in reversing myocyte loss and cellular abnormalities associated with poor myocyte contractile performance which are impaired in the failing heart. Therefore, cardiac transplantation has been the only available cure for people who develop advanced heart failure.

Cell based therapy emerged as an alternative new therapy to restore impaired cardiac function.[2,3] Over the past two decades, cell-based studies have been studied enthusiastically. Experimental studies demonstrated promising effects on generating new cardiomyocytes and vessels, reversing functional deterioration and preventing the progression to CHF.[4] Furthermore, *in vivo* experiments revealed that the major beneficial outcomes from cell therapy are associated with paracrine effects, rather than direct regeneration of new tissue.[5,6] Stem cells secrete paracrine factors (e.g., growth factors, cytokines and microRNAs), which reduce scar volume and myocyte apoptosis, increase myocyte proliferation and activate endogenous car-

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diac stem cells to produce new myocytes.[7-11] Convincing data from preclinical animal models led to several clinical trials using autologous and allogeneic stem cells and progenitor cells to assess their safety in humans.[12-14] However, their clinical relevance is still inconclusive.[9,13] Accordingly, the therapeutic benefits of the majority of clinical studies are modest at most.[14] The discrepancies between the animal studies and multiple clinical studies require reassessment of current strategies of cell-therapies. In the following sections we discuss current therapeutic limitations and alternative solutions (also summarized in Table 1) assessed in the experimental and clinical fields.

CURRENT LIMITATIONS AND ALTERNATIVE SOLUTIONS

Low Cell Retention Associated with Cell Delivery Approach

For now, three major cell delivery approaches have been tried in clinical applications (intravenous, intramyocardial (epicardial or subendocardial) and intracoronary injections). For intravenous injection, cells were injected systemically but only 0.04% of cells reached the heart and the majority of cells were entrapped in other organs (i.e., lung, kidney, liver and spleen).[15] Intramyocardial and intracoronary injection approaches have relatively better outcomes on cell retention. However, within minutes of intramyocardial or intracoronary stem cell injection the majority of cells (~85% of cells) are washed out through the coronary venous system or mechanically ejected from the injection site and only 1-2% of the cells are retained in the heart 1-month post-injection.[16-18] Therefore, to maximize their regenerative effects on the myocardium several approaches are being considered to enhance cell viability, improve functional properties of individual stem cells, and to prolong cell retention.

Enhancement of Cell Viability and Functional Properties with Genetic Modification

Genetic modification has been mostly assessed using bone marrow derived mesenchymal stem cells (MSCs). Since MSCs lack the expression of major histocompatibility complex (MHC) class II antigen, allogeneic MSCs can escape direct recognition of helper T-cells and are deemed immunoprivileged. The safety and efficacy of MSCs has been demonstrated by clinical work and there is increasing interest in enhancing the benefits of MSC therapy. For example, combining MSC and pharmacotherapy,[19] genetically modifying MSCs[20-22] and pre-conditioning MSCs[23,24] are approaches that are being explored to augment MSC-mediated cardiac repair. MSCs transfected to overexpress Akt or cell survival protein promote myocardial protective function.[6,21] Furthermore, MSCs engineered to express combinations of gene products such as Akt and angiopoietin-1 (Ang1) have also shown functional benefits in experimental animal models.[25] MSCs overexpressing vascular endothelial growth factor (VEGF) and Stromal cell-derived factor-1 (SDF-1) improve cardiac function by activating the Akt pathway.[20] MSCs transfected to express heme-oxygenase 1 (HO-1), an enzyme that improves MSC tolerance to hypoxia, infused into a cardiac ischemia-reperfusion model improve EF and lower end systolic volume compared to controls.[26] Although, these preconditioned MSCs improve engraftment and survival of transplanted cells, due to safety concerns of genetic modification on stem cell nucleus, clinical application is unwarranted.

Table 1: Alternative Approaches to Overcome Current Limitation of Cell-based Therapy.

Enhancement of cell survival, Mobilization and paracrine secretion
- Pharmacology (Statins, etc.)
- Genetic modification (Akt and Ang1, VEGF and SDF-1, HO-1)
- Non-genetic modification (Hypoxia, bFGF/IGF-1/BMP2, poly(I:C), microRNAs)
Enhancement of cell retention/survival
- Biomaterials (hyaluronic acid, collagen, fibrin, ECM, peptide, polymer)
- Cell patch (Cell sheet, scaffold or scaffold-free bioprinting)
Synergistic or accumulating effects of cell
- Synergistic effects: MSCs and CSCs
- Accumulating effects: Repeated cell infusion (MSCs, CSCs, CDCs)

MSCs: Mesenchymal Sstem Cells; CSCs: Cardiac Stem Cells; CDCs: Cardiosphere-derived cells; Ang1: Angiopoieten 1; VEGF: Vascular endothelial growth factor; SDF: Stromal cell-derived factor; HO-1: Hemeoxygenase-1; bFGF: Basic fibroblast growth factor; IGF: Insulin-like growth factor; BMP2: Bone Morphogenetic protein 2; Polyinosinic polycytidylic acid; ECM: Extracellular matrix.

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Enhancement of Cell Viability and Functional Properties without Genetic Modification

Because genetically engineered stem cells may have unwanted long-term side-effects, pre-treatment of stem cells without genetic modification are considered more practical and relevant approaches. One method includes hypoxia preconditioning. Since cells are exposed to a harsh hypoxic environment after injection into the ischemic area, preconditioning of cells in a hypoxic chamber (1-3% O_2) prior to transplantation is reasonable and, in fact, can improve cell survival in this environmental stress scenario.[27] Hypoxia stimulation activates pro-survival pathways *via* phosphorylation of Akt and p38 resulting in HIF-1alpha activation.[23,28] Hypoxic preconditioning of MSCs[20] or cardiac stem cells (CSCs)[29] enhanced therapeutic effects in an ischemia model.

Another approach is pretreatment with growth factors. MSCs pretreated with Basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF)-1 and bone morphogenetic protein 2 (BMP2) improved myocardial repair in a rat model of myocardial infarction (MI).[30] Behfar and Terzic et al pretreated MSCs with growth factors to enhance their cardioprotective functions. They demonstrated the ability of a "cardiogenic cocktail" (consisting of TGFβ1, BMP-4, Activing-A, retinoic acid, IGF-1, FGF-2, α-thrombin and IL-6) to enhance the therapeutic benefits of autologous MSCs. Subsequently the same group initiated clinical trials in patients with class 2 or 3 heart failure (C-CURE) trial.[31] Also, the Safety and Efficacy of Autologous Cardiopoietic Cells for Treatment of Ischemic Heart Failure (CHART-1) trial is currently ongoing.

We demonstrated that stimulation of Toll-Like Receptor 3 (TLR3) produced many trophic factors without induction of inflammatory-related cytokines.[26] Poly (I:C) is structurally similar to double-stranded RNA and is known to interact with TLR3, which is expressed on the membrane of B-cells, macrophages, dendritic cells, bone marrow and heart-derived stem cells (MSCs and CDCs). Poly (I:C) directly reacts with the TLR3 receptor on the endosome of MSCs/CDCs. After stimulation with poly (I:C) MSCs/CDCs are collected and washed and since the poly (I:C) does not reside within the cells, it does not affect the heart environment after injection of cells. Interaction of poly (I:C) with TLR3 on MSCs causes secretion of the growth factor VEGF and the cytokine IL-6 without upregulation of the inflammatory cytokines IL-1 and tumour necrosis factor-α (TNFα). Injection of TLR3 activated MSCs (TLR3-MSCs) in a non-ischemic cardiomyopathy model improved cardiac function more than standard MSCs along with increased myocyte proliferation, reduced fibrosis and reduced myocyte apoptosis.[32] Also activation of TLR3 on Cardiosphere-derived cells (CDCs) (TLR3-CDCs) stimulated the secretion of HGF, IGF1 and IL-6 without up-regulation of inflammatory cytokines.[33] Thus, TLR3-MSCs or TLR3-CDCs are safe and feasible to use in the human heart. Further investigation is necessary to confirm long-term safety and feasibility in a preclinical animal model. Transient genetic modulation of cellular therapies may minimize unwanted side effects in the heart environment and can be considered clinically relevant approaches.

Recently, it was reported that exosomes secreted from stem cells play important roles for cardiac regeneration.[8,11,30] Exosomes transfer microRNAs from cell-to-cell and inhibit inflammation[8] (miR146a, miR155) and apoptosis[34] (miR21, miR22, miR24), and increase angiogenesis (miR210) and myocyte proliferation[35] (miR1, miR133a, miR294). Therefore, new methods of treatment are focusing on modulating microRNAs in stem cells. For example, Hu et al showed that a cocktail of three miRNAs (miR21, miR24, and miR22) was able to substantially improve the engraftment of CSCs by targeting apoptotic activators.[34] Similarly, in MSCs, miR133 was shown to act as a pro-survival agent to mitigate ischemic insult on the cells and improve subsequent engraftment.[35]

Enhancement of Cell Retention by Bio-Injectable Materials and Cell Patch

The use of biomaterials is an effective approach to enhance cell retention and survival after implantation into damaged myocardium. Biomaterials physically support the cells to improve retention directly after administration and create a protective environment for the survival of the cell. Mainly bio-injectable materials are injected into myocardium or cell patch is placed on the surface of myocardium.

Recently, injectable biomaterials combined with cell-based therapies for cardiovascular disease are gaining more attention because they have shown therapeutic potential in preclinical models for MI. Natural (e.g., hyaluronic acid,[36] collagen[37,38], fibrin[39-41] or extracellular matrix-based[42]) or synthetic (e.g., peptide[43] or polymer-based[44]) materials can enhance stem cell survival and retention *in vivo*, prolong growth factor release from hydrogel or particle constructs and stimulate endogenous cardiac regeneration. Although, there is promising preclinical data, the therapeutic potential of biomaterial-based products for cardiovascular disease has yet to be proven in a clinical setting.

Cell Patch technology generates a tissue-like structure *in vitro* and transplants it, typically onto epicardial myocardium. The main advantage of this approach is that the cells are cultivated under precise culture conditions. Therefore, cell proliferation, differentiation, and tissue structure can be well-controlled. Nevertheless, there are several limitations of this approach *in vivo*. 1) This procedure is more invasive than catheter-based approaches since open chest surgery is required. 2) Poor nutrient diffusion and vascularization immediately after transplantation usually limits the thickness of the constructs and long-term cell survival in the heart.[16] 3) Patch-based transplantation provides inadequate integration of the graft with the host myocardium. Although paracrine factors secreted from cell patches can eas-

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ily cross the barrier and be effective, poor vascularization and improper coupling of cultured cardiomyocytes with the native myocytes may limit remuscularization and therapeutic efficacy. Recently, 3D bioprinting technology has been introduced in the cardiovascular field.[45] This uses 3D structured cell spheroids rather than a monolayer of cells since 3D structure is known to enhance hypoxia resistance and encourage vascularization.[46,47] The application of this technology may overcome current limitations associated with patch-based therapy.

Combination of Mesenchymal Stem Cells and Cardiac Stem Cells

Another approach is combined MSC and CSC to enhance the therapeutic effects of each cell type. Recent work by Williams et al demonstrated that the combined use of 1 million human CSCs with 200 million human MSCs provided greater recovery, almost to baseline, in a swine model of anterior wall MI.[48] While all stem cell treated animals demonstrated improved left ventricular ejection fraction compared to placebo controls, notably, animals receiving dual cell therapy had a 2-fold greater reduction in scar size (21.1% for CSC/MSC *versus* 10.4% for CSC alone or 9.9% for MSC alone) and had improved rates of pressure change during diastole. Overall left ventricular chamber dynamics were improved in both the dual therapy and CSC or MSC alone treated groups. Interestingly, CSC alone treated animals demonstrated better isovolumic relaxation as compared to controls, while MSC alone treated animals exhibited improved diastolic compliance, indicating that the enhanced effect of dual therapy on both systolic and diastolic function may be due to a synergistic effect between CSC and MSC targeted mechanisms. A current clinical trial has been initiated to assess the therapeutic effects (CONCERT-HF: ClinicalTrials.gov #NCT02501811).

Repeated Stem Cell Injection

Allogeneic MSCs and CDCs are immunoprivileged and can escape from direct recognition of helper T-cells due to the lack of expression of MHC class II antigen.[49,50] Based on these observations, a recent clinical trial was initiated using allogeneic human MSC/CDC treatment in patients with chronic myocardial infarction (POSEIDON[51], ALLSTAR[59]). Since a single injection of MSCs or CDCs has moderate influence on cardiac function and reduced scar volume[52,53], it was thought that repeated injections of stem cells would be more effective in regenerating myocardial tissue.[54,55] However, the initial infusion of cells activates and enhances the immune response[49,50] and subsequent injected cells are quickly eliminated and ineffective. This quick reaction is mainly associated with acquired/adaptive rather than innate immunity. Thus, development of efficacious MSC/CDC platforms administered with optimal immune suppression could circumvent barriers related to multiple injections of stem cells and allow the widespread application of "off-the-shelf" cell therapy to treat the large number of patients in need.[50,56,57] Gene-editing technology could also be applied to minimize acquired immunity creating immune-tolerant MSCs or CDCs.

CONCLUSION

Promising data derived from experimental models indicate the potential success of using cell based therapy in clinical applications. However, early stage clinical trials are revealing therapeutic limitations. We need to reassess the current problems and find alternative solutions. Feedback from clinical outcomes is providing more information for the development of the second stage of cell-based therapy research. In light of their proven safety profiles, adult stem cells (i.e., bone marrow mononuclear cells, adipose-derived stem cells, MSCs, CDCs and CSCs) are prime candidates for cell based therapies. Genetic modification, preconditioning, biomaterials, bioengineering, combination of cells and repeated injection approaches will further improve the efficacy of stem cell therapy. Taken together, the current understanding of stem cell based therapy and the emerging approaches and discoveries will definitely advance cell-based therapy and cure many CHF patients.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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