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Final Project: *Stem Cell Therapy post Acute Myocardial Infarction*

Acute Myocardial Infarction (AMI), more commonly known as a heart attack, occurs when the flow of oxygen-rich blood to a section of heart muscle suddenly becomes blocked and the heart can't get oxygen. If blood flow isn't restored quickly, the section of heart muscle begins to die (Wikipedia). Current management strategies cannot solve the problem of cardiomyocyte loss and consequent progression of heart failure. In this respect, stem-cell therapy has shown potential benefits for repairing the damaged myocardium. Mesenchymal stem cells (MSCs) (derived from the bone marrow) have been considered to be attractive therapeutic candidates because of their “high capacity for replication, paracrine effect, ability to preserve potency, and because they do not cause adverse reactions to allogeneic versus autologous transplants” (Lee et al. 2011).

The current preferred approach of using autologous stem cells aims to avoid immune rejection of donor cells, which can be expected after allogeneic or xenogeneic transplantation (Atoui et al. 2013). However, despite the promising early results, deriving autologous cells from individual patients still poses significant logistic, economic, and timing

constrains. Most importantly, most of the patients who could benefit from such therapy are elderly patients with multiple medical problems. Recent studies have found that MSCs obtained “from elderly donors, and those with diabetes, renal failure, or severe ischemic heart disease, demonstrate significantly reduced capacity for proliferation, differentiation, and neovascularization, with increased levels of apoptosis in vitro and in vivo” (Atoui et al. 2013). Nonetheless, further research has identified the unique immunomodulatory property of the MSCs both in the in vitro and in vivo settings. One intriguing property of MSCs is their ability to “escape immune recognition and even actively inhibit immune responses”.

The potential importance of these findings for the treatment of ischemic heart disease is apparent. In addition to their powerful replicative capacity, “MSCs can easily be harvested from bone marrows, expanded ex vivo, and differentiated into many cell type lineages, if desired” (Atoui et al. 2013). Due to their immunotolerance property, the establishment of MSCs as effective “universal donor cells” could then dramatically expand the therapeutic potential for cellular cardiomyoplasty. From a clinical perspective, these cells could be isolated and expanded from donors “irrespective of their MHC haplotype, tested for their functional capabilities well in advance, and stored as an ‘off-the-shelf’ cell population for immediate use when needed on any patient after an acute myocardial infarction” (Atoui et al. 2013). More importantly, since allogeneic MSCs

can be obtained from young healthy donors, they could be extremely useful to patients with genetic cardiomyopathies and in elderly patients with multiple medical comorbidities whose own MSCs could be dysfunctional.

In 2009, Hare et al. set out to "investigate the safety and efficacy of intravenous allogeneic human mesenchymal stem cells (hMSCs) in patients with myocardial infarction (MI)." The primary end point was incidence of treatment-emergent adverse events within 6 months in his double-blind, placebo-controlled study. "Ejection fraction and left ventricular volumes determined by echocardiography and magnetic resonance imaging were exploratory efficacy end points." His derived data showed that "adverse event rates were similar between the hMSC-treated (5.3 per patient) and placebo-treated (7.0 per patient) groups, and renal, hepatic, and hematologic laboratory indexes were not different." Furthermore, the data also showed "reduced ventricular tachycardia episodes ($p = 0.025$), and improved forced expiratory volume in 1 s ($p = 0.003$)" in the hMSC-treated patients. "Global symptom score in all patients ($p = 0.027$) and ejection fraction in the important subset of anterior MI patients were both significantly better in hMSCs versus placebo subjects." Finally, Hare was also able to observe that in the hMSC treatment group, but not placebo, there was "increased left ventricular ejection fraction and led to reverse remodeling" (Hare et al. 2009). From this study, it was concluded that intravenous allogeneic hMSCs are safe in

patients after acute MI. The study demonstrated safety both with regard to acute infusions of hMSCs as well as long-term absence of ectopic tissue formation. Additionally, results from cell-treated patients showed improved outcomes “with regard to cardiac arrhythmias, pulmonary function, left ventricular function, and symptomatic global assessment” (Hare et al. 2009).

Bone marrow derived stem cells have displayed the potential for myocardial regeneration in animal models as well as in clinical trials. Unfractionated bone marrow mononuclear cell (MNC) population is a heterogeneous group of cells known to include a number of stem cell populations. Sadek et al. conducted a study in 2009 to investigate the role of murine and human bone-marrow-derived side population cells in myocardial regeneration. Their studies showed “that mouse bone-marrow-derived SP cells expressed the contractile protein, alpha-actinin, following culture with neonatal cardiomyocytes and after delivery into the myocardium following injury. Moreover, the number of green-fluorescent-protein-positive cells, of bone marrow side population origin, increased progressively within the injured myocardium over 90 days” (Sadek et al. 2009). Further analysis of these bone marrow cells revealed that their pattern of expression was consistent with that of the immature cardiomyocytes. Additionally, “the differentiation capacity of human granulocyte colony-stimulating factor stimulated peripheral blood stem cells were assessed following injection into injured rat myocardium.” Bone

marrow mononuclear cell and side population cells could be identified after 1 month of injection in the rat's myocardium. These human cells were expressing human-specific cardiac troponin as well as other cardiac transcripts. "Both human bone marrow mononuclear cells and human side population cells augmented cardiac systolic function following a modest drop in function as a result of cryoinjury" (Sadek et al. 2009).

The increase in cardiac function after injection of side population cells "occurred earlier than with bone marrow mononuclear cells despite the fact that the number of side population cells used was one tenth that of bone marrow mononuclear cells" (Sadek et al. 2009). These results were able to support the hypotheses bone-marrow derived side population cells can acquire cardiac fate as well that human bone-marrow-derived side population cells are "superior to unfractionated bone marrow mononuclear cells in augmenting left ventricular systolic function following cryoinjury" (Sadek et al. 2009).

In 2013, Jeevanantham et al. performed clinical trials of cardiac repair with adult bone marrow-derived cells (BMCs). These trials included patients with acute myocardial infarction (MI) as well as chronic ischemic heart disease (IHD) and utilized different types of BMCs with "variable numbers, routes of administration, and timings after MI". However, because of differences in methods, the "outcomes from these trials have been often disparate and controversial" (Jeevanantham et al. 2013). However, analysis of pooled data suggests that "BMC injection enhances

left ventricular function, reduces infarct scar size, and improves remodeling in patients with acute MI as well as chronic IHD." Furthermore, data demonstrated that BMC therapy also helped improve the clinical outcomes during follow-up without any increase in adverse effects. Jeevanantham goes on to state, "Although the underlying mechanisms of heart repair are difficult to clarify in human studies, valuable insights may be gleaned from subgroup analysis of key variables. This information may be utilized to design future randomized controlled trials to carefully determine the long-term safety and benefits of BMC therapy."

The safety and efficacy of BMC therapy for cardiac repair have been evaluated in more than 50 clinical trials that used different BMC types and injected widely variable cell numbers via several distinct routes at variable intervals after MI in diverse patient populations. Despite these differences, "several meta-analyses of pooled data consistently show that BMC therapy improves LV structure and function without any significant increase in adverse effects" (Jeevanantham et al. 2013). BMC therapy also improves "patient-important outcomes, including total and cardiovascular mortality and recurrent MI." The data that has been acquired from these trails have helped identify "methodological variables of BMC therapy. Optimization of these methodological aspects is likely to further improve the benefits of this highly promising approach of myocardial repair" (Jeevanantham et al. 2013).

The importance of stem cell therapy in helping improve the outcome of patients who have suffered from myocardial infarctions is no longer a question. Researchers are now focusing on ways to improve stem cell therapy for better results. Recent observations that adult cardiomyocytes can reenter the cell cycle and form new myocytes after MI have challenged long-held dogma that the adult heart is a "terminally differentiated organ" (Williams et al. 2012). The identification of stem cell niches in the adult heart and the "ability to isolate c-kit$^+$ cardiac stem cells (CSCs) from a small heart biopsy have generated enormous enthusiasm for the potential to develop safe and effective cell-based therapies to treat ischemic cardiomyopathy". Because mesenchymal stem cells (MSCs) induce proliferation and differentiation of c-kit$^+$ cardiac stem cells (CSCs) in vivo and in vitro, combining human MSCs with c-kit$^+$ hCSCs produces "greater infarct size reduction compared with either cell administered alone after myocardial infarction (MI)" Williams et al. 2012).

In Williams et al.'s study from 2012, they were able to show that show that hMSCs and c-kit$^+$ hCSCs helped reduce infarct size and improve left ventricular (LV) function in post-MI. "When both cells are injected together, there is a 2-fold-greater reduction in scar size [and] substantial recovery in cardiac diastolic and systolic function, which was not as robust when either cell was administered alone" (Williams et al. 2012). In terms of functional improvement in LV performance, "all stem cell–treated animals had recovery of ejection fraction (EF) to near-baseline

levels, whereas the placebo-treated animals had persistently depressed LV function." To assess more detailed analysis of LV function, Williams et al. conducted further measurements to assess the impact of stem cell therapy on hemodynamic performance. Although all stem cell–treated groups had improved measures of integrated cardiac performance (EF, stroke work, and cardiac output), specific measures of contractility such as "preload recruitable stroke work and dP/dtmax were preferentially improved in the group receiving combination hMSC/hCSC therapy. In addition to systolic impairment, diastolic dysfunction is an additional hallmark of the postinfarction LV" (Williams et al. 2012).

Williams et al.'s study has many implications for cell-based therapy in post-MI patients. hMSCs and c-kit$^+$ hCSCs are adult stem cells easily isolated from "a minimally invasive BM biopsy and cardiac endomyocardial biopsy, respectively". Combining hMSCs and hCSCs as a cell therapeutic enhances "scar size reduction and restores diastolic and systolic function toward normal after MI." Clearly, these results emphasize important biological interactions between c-kit$^+$ CSCs and MSCs that enhance cell-based therapeutic responses.

In 2013, Carvalho et al. performed a study that showed "priming mesenchymal stem cells (MSC) towards cardiomyogenic lineage enhances their beneficial effects *in vivo* as treatment option for acute phase myocardial infarction." MSC were primed using cardiomyogenic media for 4 days, after which "peak expression of key cardiomyogenic genes are

reached and protein expression of Cx-43 and sarcomeric α-actinin are observed" (Carvalho et al. 2013). MSC and primed MSC (pMSC) were characterized *in vitro* and were utilized to treat rats with infarctions "immediately after left anterior descending (LAD) occlusion" (Carvalho et al. 2013). Detailed analysis of the data indicated that MSC-treated myocardium presented improvement in function, but it also showed that pMSC treatment lead to "superior beneficial results, compared with undifferentiated MSC." Moreover, MSC and pMSC could still be detected in the myocardium even after seven days of cell injection. Connexin-43 expression was quantified through "immunoblotting, and was superior in pMSC", indicating that this could be a possible explanation for the superior performance of pMSC therapy (Carvalho et al. 2013).

In 2010, Zhang et al. hypothesized that "the combination of overexpression of CXCR4 in mesenchymal stem cells (MSC) with diprotin A would enhance MSC recruitment and penetration into ischemic myocardium, leading to an improvement in heart function after myocardial infarction (MI)". In their study, male rat MSCs were genetically engineered with "adenoviral vectors coexpressing CXCR4 and enhanced green fluorescent protein (EGFP) (MSCCXCR4), GFP alone (MSCNull, control), or siRNA-targeted CXCR4 (MSCsiRNA). Cell sheets were applied over the surface of infarcted left ventricle (LV) in female rats 7 days after ligation of the left anterior descending coronary artery (LAD) pretreated with either vehicle (VEH) or diprotin A (DIP)" (Zhang et al. 2010).

Echocardiography was performed after 28 days of the implantation and the hearts were harvested for histological analysis 7 days after LAD ligation (or 28 days after cell sheet implantation). The researchers also analyzed DPP-IV and stroma-derived factor-1α (SDF-1α) in the LV. Tge presence of Y chromosome in nuclei (Ych+) helped the researchers establish the efficacy of engraftment. LV blood vessel density and apoptosis were also analyzed. "Myocardial SDF-1α was elevated before placement of the cell sheet in the DIP group compared with vehicle group on *day 7* after LAD. On *day 28* after cell sheet transplantation, the number of Ych+ was increased in the MSCCXCR4 + VEH group compared with the MSCNull + VEH group and further increased in the MSCCXCR4 + DIP treated group" (Zhang et al. 2010). This enhanced response was also associated with increased angiogenesis in both sides of epicardium and improvement of LV function. Thus, Chang et al. were able to conclude that "combination of gene-manipulated MSCCXCR4 patch with DIP pretreatment inhibits myocardial ischemia-induced apoptosis, promotes tissue angiogenesis, and enhances cell engraftment, leading to improved LV mechanical function after MI".

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